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Pharmacology of Itch

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Pharmacology of Itch

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Preface

Itching is a remarkably common symptom of dermatologic and systemic diseases and seriously impacts a patient's quality of life. The good news is that there has been a tremendous upsurge in basic itch research over the past decade, galvanized by an influx of molecular biologists and neurophysiologists, and reflected in excellent cutting-edge reviews (e.g., Biró et al. 2007; Han et al. 2013; Roberson et al. 2013; Kremer et al. 2014) and timely books (Yosipovitch et al. 2004; Misery and Ständer 2010; Carstens and Akiyama 2014). The current volume in Springer's well-established Handbook series updates the findings of key researchers and summarizes progress in the itch field through 2014. The scope is comprehensive, encompassing pharmacological advances at all levels from receptor to clinic, and includes historical and veterinary aspects.

Itch is an enigmatic sensation quite distinct from pain and yet, in many ways, still connected. It is multimechanistic and unraveling its molecular underpinnings provides a real challenge for intrepid "itch doctors". The most recent research describes pruritoceptive messages being transmitted by at least two populations of spinal interneurons (natriuretic peptide receptor-A-expressing neurons followed by gastrin-releasing peptide receptor-expressing neurons) before being conveyed to the brain. These academic breakthroughs may prompt the launch of much needed, new antipruritic drugs into the dermatology field.

We wish to express our sincere thanks to the international group of experts who, collectively, have energized this venture with their insightful chapters, enthusiasm, and collegiality. A special nod of appreciation goes to Dr. James Barrett, Chair of the Department of Pharmacology and Physiology, Drexel University College of Medicine in Philadelphia, and champion of all things pharmacological, for initiating the project in his role as Board Member of the venerable Handbook series. Additionally, we acknowledge the professionalism of Susanne Dathe and Wilma McHugh from the supportive Springer team in bringing the volume to completion.

Philadelphia, PA

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Historical Background of Itch

Elke Weisshaar, Wolfgang U. Eckart, and Jeffrey D. Bernhard

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Abstract

Itch as a disease, and especially as a symptom, was the object of medical and scientific curiosity for centuries. The reluctance of historians to focus on the history of itch relates to its nature as a subjective symptom. After all, how can historians have known what itch really felt like in previous centuries? Since the establishment of dermatology as an independent discipline of medicine in the middle of the nineteenth century, itch has become a subject of investigation in its

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own right. This chapter summarises research we conducted on the medical history of itch in ancient medicine and up through the twentieth century.

Keywords
Itch • Medical history • Prurigo • Pruritus

1 Introduction

Although itch is the most frequent symptom of the skin, surprisingly little is known about its medical history. This chapter offers a selection of information from ancient and more recent medical texts which document characteristic ways of dealing with itch as well as cultural aspects in the centuries leading up to the development of dermatology in Europe. This chapter is based on research described in three previous publications (Weisshaar et al. 2008, 2009a, b). Some of the contents of this chapter are direct quotations from those publications.

As recently as 1979, there were only a few paragraphs about itch in the second edition of Fitzpatrick's *Dermatology in General Medicine*, at that time the most important American textbook on dermatology. One might have expected an entire chapter about the most prominent symptom of skin diseases. It may be of some interest to know that when one of the authors (JDB) complained about this to Prof Fitzpatrick, he (TBF) immediately replied: 'OK, you write the chapter'. And so it came to pass (Bernhard 1987).

The exact evolutionary moment at which itch appeared is not known. Suffice it to say that experts believe that all vertebrates experience what we would call itch. This belief is based on the notion that where there is scratch there must be itch. The essential references on the history of dermatology include Pusey's *History of Dermatology* (published in 1933), *Classics in Clinical Dermatology* by Shelley and Crissey (published in 1953) and Crissey and Parish's *The Dermatology and Syphilology of the Nineteenth Century* (published in 1980). To a large extent, the history of itch is intertwined with the biographical history of dermatology, and for that reason, Shelley and Crissey's book is of particular interest, especially if one is sympathetic to the biographical approach to history. Table 1 lists some of the people and some of the major events in the history of itch in the twentieth century.

2 Starting from Scratch: A Note on Terminology

It is important to note that itch is usually not described in a straightforward manner in medical literature composed before the nineteenth century, but is usually found in the context of a localised disease with which it was associated, or sometimes in a chapter dealing specifically with dermatoses. Often one has to rely on textual searches, which makes terminology a major issue.

Table 1 A selection of important events and discoveries in the history of itch in the twentieth century

1922	Von Frey suggests that itch is transmitted by the same network of fibres as pain and may be induced by mechanical, thermal, chemical and thermal stimuli
1939	Bickford proposes that itch is transmitted by fine C fibres (as pain) and is attenuated by cooling
1948	Bishop describes the itch pathway from skin to excitation of sensory nerves and the transfer from spinal cord to thalamus and up to the cerebral cortex
1951	Graham and colleagues describe different sensations of itch and that pain can inhibit itch
1952	Cormia provides a detailed description of histamine-induced itch and a lower itch threshold for histamine in dermatitis or pruritic disorders
1954	Stephen Rothman includes a chapter on pruritus in his landmark book, "The Physiology and Biochemistry of the Skin" (Univ of Chicago Press)
1955,1957	Shelley and Arthur publish results of their studies on the neurophysiology of itch. They identify nerves terminating at the dermo-epidermal junction and determine that mucanian protease(s) is involved in the itch sensation induced by cowhage
1977	Gilchrest and colleagues discover that ultraviolet phototherapy is effective in the treatment of the pruritus of chronic kidney disease
1979	Fjellner and Hagermark publish their work on serotonin and itch
1979	Bernstein and Swift publish the first report on reducing intractable itch with naloxone
1991	Bergasa and Jones employ finger nail transducers to measure scratching and report their work on cholestatic itch and its attenuation with naloxone
1991	Handwerker and colleagues report results of their studies on nociceptors. These results become increasingly important for research on the electrophysiology of itch
1991	LaMotte's group defines alloknesis and performs psychophysics on itch (Simone et al. 1991)
1997	Schmelz et al. identify a subset of nerves in humans that appears to be itch specific
1999	Bernhard and colleagues report the beneficial effect of systemic gabapentin for the treatment of brachioradial pruritus (Bueller et al. 1999)

In classical Latin, the term *pruritus* refers to itching, irritation or sexual excitement and is thus nearly interchangeable with *prurigo*, perhaps surprisingly given the modern understanding of 'prurigo' as describing the specific group of papular dermatoses to which the term is applied today. The Greek term *knêsmos* and related words denote itch, in an irritating or pleasurable sense (tickling). Metaphorical meanings reach from irritation to titillation. From an etymological point of view, *knêsmos* and related words are associated with the notion of scraping or scratching (Weisshaar et al. 2009b).

At the same time, the names of clinical conditions used today have often evolved from the terms employed by ancient authors. However, translations can create a misleading picture of ancient clinical observation. For example, a typical translation of a sentence from the *Corpus Hippocraticum* could run as follows: 'Leprosy, pruritus, scabies, lichen, vitiligo and alopecia arise from phlegm and are mere blemishes rather than diseases' (*Affections* 35) (Hippocrates 1988). A more detailed

analysis of the terms used in describing itch and related skin lesions through the centuries remains to be addressed in depth elsewhere. This applies especially to the terms pruritus and itch, which we have used synonymously (Bernhard 1994).

3 Medical Histories: Ancient Descriptions of Itch

Cures for itch were sought from the early days of mankind and are documented in sources even more ancient than those of Greek antiquity (e.g. Papyrus Ebers, dating from approximately 1550 BC). The earliest attempt at understanding the mechanisms underlying the complaint, however, was made in Greece around the fifth century BC. Hippocrates is generally seen as the first to introduce rational science into medicine. The *Corpus Hippocraticum* was compiled from the fifth century BC onwards and completed around the tenth century AD. Itch is mentioned in various places in the *Corpus Hippocraticum*. The development of a certain skin lesion is vividly described as follows: 'Serous fluids appeared in the skin. Having coalesced, they became warm and caused itching. Then they erupted like burn blisters caused by fire, and they seemed to smoulder underneath the skin' (*Epidemics* 2.1.1). Elsewhere it is noted that a symptom only seen in the Perinthian women suffering from 'summer fevers' was 'roughness of the skin, furfuraceous, similar to the bites of gnats, not very itchy at all' (*Epidemics* 2.3.1). An extensive case history is documented for a certain Andreas, who 'before the rising of the Pleiades, displayed shivering, fever, and vomiting', until among other symptoms, 'efflorescences appeared on his skin, itching a little, but hot as if caused by fire' (*Epidemics* 7.43) (Hippocrates 1994). In the *Aphorisms*, a collection of concise clinical observations probably intended for teaching, Hippocrates states that 'extensive efflorescences' generally cause less itching (*Aphorisms* 6.9).

In the competitive milieu of Ancient Greek medicine, it was a crucial skill for a doctor to predict the development of a disease. In this, itch served as a prognostic criterion, as can be read in the *Coan Prenotions* containing an extensive sketch of bedside prognostics. Itching of the body preceded by constipation in a patient with consumption is said to bode ill (*Coan Prenotions* 432). Likewise, 'in all patients, itching will be followed by black stools, and vomiting of curdled masses' (*Coan Prenotions* 626) [Weisshaar et al. 2008). As the *Corpus Hippocraticum* is based on the concept of humoural pathology, itch was mostly explained from this point of view which also involved, besides the humours of the body, the influence of external factors such as the weather. Thus, in the *Humours*, it is said that 'certain forms of leprosy (*leprê*) and joint pain are itchy when rain is impending' (*Humours* 17).

The Alexandrians succeeded the Hippocratics in the Hellenistic period. The focus of their research shifted to investigative and experimental aspects. Aulus Cornelius Celsus, an encyclopaedist from the first century AD, observed what he calls *scabies*: 'quite hard, with ruddy skin' and causes pustules, some of which are 'rather moist, others more dry'. They emit a serous discharge and proceed to exulcerate, causing itch. The rougher the skin, and the more intense the itch, the

more difficult it is to cure. 'Therefore, the Greeks call this form of scabies "agria", that is, "the savage one"'. For this disease, Celsus prescribed a salve 'composed of sublimed zinc oxide, saffron, verdigris, white pepper and a preparation made from unripe grapes' (Celsus 5.28.16a) (Weisshaar et al. 2009b). Aretaeus of Cappadocia, a Greek physician of the first or second century AD, connected generalised itch to icterus when he discussed two types of jaundice—one 'light and saffron-coloured' and the other 'greyish and dark'—saying that in both, 'the whole body is itchy' (Aretaeus 1958).

In the second century AD, Galen of Pergamon developed a synthesis of the medical knowledge of his time and thus became a crucial figure in medical research and practice until the Renaissance and even later times. In his work, *On the Preservation of Health*, Galen explained how to assess a patient's medical history as an important part for differential diagnosis. Among other things, 'it is essential to determine whether the patient is one of those who by nature tend to accumulate unhealthy humours. You can find this out by asking him whether he has suffered from a predisposition to scabies (*psôrôdês diathesis*), leprosy (*leprôdês*), whiteness (*alphôdês*) or itching (*knêsmôdês*), or from erysipelas, herpes, the elephant disease (*elephas*), the snake disease (*ophiasis*), the fox disease (*alôpekiasis*), many blisters, ulcerous efflorescences, night rash or any one at all of the symptoms that arise from, or are worsened by, an unbalanced state of the humours' (Galen *On the Preservation of Health* 4.4.5) (Galen 1823).

4 The Byzantine Period

The Byzantine period (fifth to fifteenth century AD) is marked by a tendency to compile ancient sources. Alexander of Tralles, writing in the sixth century AD, recommended a remedy for scabby and serous conditions of the head: 'Crush rue and alum in honey and use them to anoint the shaven head. When the skin has peeled off, boiled leaves of the olive tree should be applied with honey as a poultice' (Alexander Trallianus *Therapeutics* 1.6). In a fascinating testimony to the broadness with which terms like scabies (*psôra*) were used before systematisation in dermatology, Alexander states that 'bladder scabies' (*psôriasis kysteôs*) is present if 'furfuraceous sediment appears in the urine fluid. This must be distinguished from that carried in the blood vessels' which arises from excess heat in the body. 'When the urine is of thin consistency and very acrid, conclude that the furfuraceous sediment has come from the blood vessels. But when the urine is of a thick consistency, conclude that it is bladder scabies' (Alexander Trallianus 11.5).

In terms of leprosy (*lepra*) and scabies (*psôra*), Paul of Aegina from the seventh century AD states that 'these diseases have in common the rough appearance together with itching and scratching of the body, which originates from a proneness to black bile' (Paulus Aegineta 4.2.1). In comparison with leprosy (*lepra*), scabies (*psôra*) is said to be 'more superficial, adopting various forms, and rendering furfuraceous particles. It is necessary to resort to phlebotomy, if the patient seems strong enough; if not, cleansing should nevertheless be achieved by purging with

substances that expel black bile'. Later, he adds an extensive description of drugs for external application (Paulus Aegineta 4.2.3–5), listing plants, other organic ingredients, metallic components and also some ingredients less familiar for the modern reader, such as goat droppings, which were not uncommon in ancient pharmacopoeias.

Both Paul and Theophanes Nonnos report that 'the itch that comes about in old age' cannot be cured but can be alleviated by a number of procedures and remedies including phlebotomy, baths and salves (Paulus Aegineta 4.4.2–3; Theophanes Nonnos 237) (Paulus Aegineta 1921; Theophanes Nonnus 1794). As these chapters are devoted specifically to itch (*knêsmos*), they may be viewed as an early attempt to define pruritus as a disease in its own right.

5 Medieval Medicine

After the end of the Byzantine empire, ancient medical knowledge continued to be transmitted in the Muslim world and in the Latin West. The tradition of handbooks in Arabic medicine reached its climax in the tenth and eleventh century AD. The physician, Haly Abbas, owes much to the characteristic blend of Byzantine tradition with Persian influences. Scabies and itching arise from 'a mixture of salty phlegm with bilious blood'. If this mixture is 'thin and fine', it soon brings about an itch to promote healing, but if it is coarse, it will 'cause persistent dry itching and scabies'. Especially 'those who do not take warm baths and on whose skin soot accumulates' suffer from itching. Also, 'itch in old age often arises from the weakness of the skin and because salty liquids come about in their bodies'. Haly Abbas adds that 'the characteristic of scabies are intense itch and small nodules, which are first reddened, then suppuration occurs. Scabies is found between the patients' fingers, on their hands, elbows, and on the coccyx' (Weisshaar et al. 2009b).

Another influential author is Avicenna whose medical knowledge is contained in *Kitāb al-Qānūn fi at-Tibb*. He assumes that salty water causes itch and scabies, arguing that it dehydrates the body, draining it of all vigour. First, its effect is cleansing, but later on, it leads to constipation, as it is dry by nature. It spoils the blood and produces itch and scabies. Furthermore, southern winds can promote the curing of itch, for 'they relax the body and open the pores, stimulating the humours and expelling them', and at the same time, however, 'they cause paucity of spirit, festering of wounds, and recidivism. They drain the body of all vigour, promoting ulcers, gout, headaches, dizziness and itching' (Avicenna 1562).

The *Lorscher Arzneibuch* (Lorsch Book of Remedies) was written in Latin in the monastery of Lorsch around 795 AD and is the earliest of its kind in Germany. It documents the role of monasteries in the transmission of medical knowledge. For example, instructions for the preparation and application of drugs for external and internal use in itch and scabies are given: an ointment for 'itching and scabby scalp' based on stinging nettle seeds is recommended, next to the suggestion of inexpensive local medicinal plants (Stoll 1992). Hildegard of Bingen (twelfth century AD),

mystic and medic, is also a typical figure of the monastic phase of medieval medicine. For scabies and ulcers, she recommended, in her treatise *Causae et Curae*, a decoction of herbs and lard which is applied to the skin. Hildegard emphasised that 'the warmth of the lard, as far as it is still fresh, is a gentle remedy for ulcers and scabies' (Schulz 1990).

6 From Paracelsus to Plenck

In Renaissance medicine, ancient sources were critically taken over and adapted. This led to a re-evaluation of traditional doctrines and an ambition to search for new explanations. One of the most extreme critics of other medical writers and practitioners is Paracelsus (1493–1541). His belief that diseases arise from lack or excess of chemical principles and that remedies should thus be based on an understanding of chemistry (the 'Arcanes') is summarised in his quote 'dosis facit sola venenum'. In expressing his opinion on the subject of phlebotomy as a remedy for mange and scabies, Paracelsus employs no small measure of irony: 'Truly, Doctor Quack has used the Avicennian doctrine to make an invention suitable for the kitchen when he recommends blood-letting to cure mange and scabies'. Somewhat cryptically, he continues to say that 'whoever aspires to cure and counsel those afflicted by mange, must not use blood-letting, but rather other things, that is: the Arcanes. Behold what blood-letting be in truth. It is no more than if someone has a scab on his scalp, and scratches it, and it bleeds in the morning, of what use is the bleeding? It is of no use at all, for soon another scab is in its place. Thus, blood-letting is nothing but the bleeding for tomorrow's scabs when a scab has been scratched' (*Über Aderlassen und Schröpfen* 2) (Paracelsus 1928).

Girolamo Mercuriale, philologist and physician, distinguished between pruritus as 'a sensation accompanying some skin diseases, such as the *scabies* of the Latin authors', and the condition called *psôra* in Greek, displaying visible skin lesions.

The first German text on skin diseases was Samuel Hafenreffer's *Nosodochium cutis* ('Skin Clinic', 1660), published in 1630 under the Greek title *Pandocheion ailodermon* ('Speckled Hide Inn' in Greek). Hafenreffer uses pulse and urine as diagnostic parameters and supplements them, for example, with astrological observations. He is credited as the first to define itch as an unpleasant sensation that provokes the desire to scratch—a definition still valid today.

In the eighteenth century, dermatology made its first hesitant steps onto the stage of modern medicine when treatises with the title *De morbis cutaneis* were published by Daniel Turner, Charles-Anne Lorry and Joseph Jacob Plenck, who introduced a consistent classification system for skin diseases in 1776 (Weisshaar et al. 2009b). To appreciate Plenck's achievement, it is interesting to read Zedler's encyclopaedia published in the mid-eighteenth century for comparison. Here, *pruritus* and *prurigo* are said to be synonymous, without further definition. The term *cnismus* is likewise followed by a reference to *pruritus*, and so is *cnesis*, which is however illustrated by the additional remark 'the itch of the skin as felt by those who suffer from scabies'.

By contrast, Plenck distinguished 14 classes of dermatoses. Among them, scabies was grouped in the category of pustules or purulent eruptions (*Eiterblattern*). According to Plenck's definition, 'small, very itchy suppurative blisters occur, discharging pus when ripped open by scratching'. Caused by mites, scabies is seen between the fingers, on the knees and elbows. However, it was not before the mid-nineteenth century, when the classification of dermatoses was well on its way, that pruritus was truly seen as an illness in its own right, and prurigo was described as a distinct condition.

7 Scratching the Surface: Classification

In the nineteenth century, some very radical changes in the history of medicine occurred, including the differentiation of specialty disciplines such as dermatology. As the differentiation proceeded, new systems of disease classification developed from chemistry, biology and pathological anatomy. The Austrian, Ferdinand Hebra (1816–1880), abandoned humoural pathology in favour of a new understanding of pathogenesis. His Att*empt at a Classification of Skin Diseases on the Basis of Pathological Anatomy* (1845) is often quoted as the foundation of modern dermatology. Robert Willan of London is also accorded recognition for his foundational work in the description and categorisation of the basic lesions of the skin and is considered the father of modern English dermatology. In his 1808 textbook, Willan provided striking descriptions of psoriasis and 'senile pruritus'. Because our patients assume that 'senilis' refers to dementia, it has been suggested that this form of itching be called 'Willan's itch' (Ward and Bernhard 2005).

Still, itch and the related skin diseases did not really fit into any category. In 1898, Max Joseph (1860–1932) provides the following definition of pruritus: 'The appearance of the skin is fully normal and without pathological findings, but there is hyperesthesia accompanied by intense itching' (Weisshaar et al. 2009b). Three years later, Joseph Jadassohn (1863–1936) emphasises that when itching is the only cutaneous symptom, 'we can merely state that a patient suffers from itching, while we are unable to discover the nature and the cause of this condition'. He draws attention to the fact that the term 'pruritus' is 'of course not quite satisfactory, as on one occasion it is taken to denote no more than a symptom of, for example, diabetes, and on another, it denotes a disease in its own right, as in pruritus senilis' (Jadassohn 1901). Erhard Riecke (1869–1939) calls pruritus 'a sensory neurosis of the skin' and stresses that 'itch is not only concomitant to some dermatoses, but rather it may occur as a disease in its own right' (Weisshaar et al. 2009a, b). In his 1933 textbook, Walther Krantz (1891–1970) points out that as pruritus is defined as 'skin itch with no obvious cause', one should be aware of the fact that 'as a diagnosis it will always be less than satisfactory, because first and foremost it is designed to express our own ignorance, indicating that we have taken note of a disease state without fully understanding it and without truly gaining an insight. On the contrary, we have done nothing but labelling a subjective complaint with a Latin term' (Weisshaar et al. 2009a, b).

In practice, pruritus or 'pruritus cutaneous' was subdivided into 'pruritus localis' or 'pruritus universalis' to distinguish localised/circumscribed itch from generalised itch. For further differentiation, 'primary' pruritus, which was seen as arising independently from an underlying disease, was distinguished from 'secondary', or concomitant, pruritus which occurs in scabies, urticaria and lichen. In addition, localised forms were specified as pruritus ani, pruritus vulvae, pruritus of the palms of the hands and the soles of the feet, or the mucosa. Generalised forms of pruritus often diagnosed were pruritus hiemalis and pruritus senilis.

8 An Itch for Science

In accordance with the growing interest in scientific explanations, Hebra suggested that itch could arise from a slowing down of circulation in the capillaries of the papillary body. The following scratching and bleeding was seen to enable its reperfusion. Isidor Neumann (1832–1906) saw itch as a 'neurosis', more specifically a 'paresthesia', and a 'hyperesthesia', suggesting that those lesions cause itch which 'only irritate the papillary body', as opposed to 'wounds and ulcers which cause pain, rather than itch, as they penetrate into the subcutaneous tissue' (Weisshaar et al. 2009a, b).

Jadassohn discusses aetiopathogenetical theories in the early twentieth century, including the idea that oedema and hyperaemia could lead to compression of the 'fibres of the nerve endings'. Scratch lesions would then allow the fluid to drain, reducing stress on the tissue and alleviating the itch. Also, parasites were suspected to be a possible cause for itch. Furthermore, it was thought that toxic chemicals circulating in the body could lead to gout, uremia and icterus and consequently pruritus, either by directly influencing the nerve endings or by interfering more generally with the body fluids, as it was seen especially in diabetes. Similarly, autointoxication originating from the digestive tract was considered. Exogenous substances such as morphine, caffeine and alcohol were also suspected of provoking itch. Last but not least, in some cases, 'itching may occur without a connection to any cause whatsoever in terms of time, place, function or anatomy, quite similar to the way in which hysterical or psychotic symptoms come about' (Jadassohn 1901).

In 1920, Riecke conceptualised itch as a specific form of nervous irritation, which 'should not be seen as identical with pain, or paresthesia'. The trigger point could be anywhere along the 'sensory pathway', or external stimulation of 'the periphery', which is to say of the skin. Walther Schönfeld (1888–1977) took a different approach in a 1938 textbook, arguing that a stimulation of either the 'target organs' or 'conducting pathways' of pain could lead to itch. However, he does not rule out the possibility of specific 'itch nerves', as 'occasionally, patients with intact pain sensation experience an isolated loss of the ability to feel itch, while other patients feel itch even when pain sensation is lost'. While discussing the properties of the skin, he remarks that 'certain sensations are singular to the skin, for example tickling and itching'. Special attention was directed at pruritic disorders

where ‘we do not know as yet how to proceed in discovering the underlying causes’ (Weisshaar et al. 2009a, b).

There is a strong discrepancy between the many new hypotheses on the aetiology of pruritus and the actual advances in understanding its pathogenesis. Krantz summarises the state of research as follows: ‘Straightforward though it may seem to identify “abnormal” sensations that can be labelled itching, the true nature and causes of itch have not been sufficiently understood, nor what itching essentially is’ (Weisshaar et al. 2009b).

9 Modern Cures

The *Deutsche Medizinische Wochenschrift* (*DMW*; German Weekly Journal of Medicine) was a platform for dermatology at a time in which some of the most progressive contributions to dermatology originated from Germany and Austria.

Dietary interventions were among the most popular recommendations, including cutting down on hot spices, coffee, alcohol and nicotine. A diet low in sodium and protein was also recommended occasionally. According to Paul Gerson Unna (1850–1929), ‘itching caused by tobacco abuse will subside soon after smoking has ceased. Monochlorbenzene (in 1–5 % spirituous solution) is recommended as the most suitable symptomatic drug’. Travels to a different climate were also thought to have a positive impact on the symptoms of the patients. Max Winkler (1875–1952) suggests that ‘the Southern winds in the valleys of the alps, notably the Foehn, have been reported to cause a notable increase in itching. In contrast, high altitude climate (1,600–1,800 m above sea level) has a toning effect which sometimes works very well indeed’ (Weisshaar et al. 2009a, b). Unna (1915) notices a connection between itch and the change of seasons: ‘During the winter, some patients with varicose veins suffer from exacerbated itching in bed at night’. Therapeutic baths were another popular treatment approach, either in the form of simple cold baths or combined with additives such as carbon, sulphur, iron, menthol, vinegar, ethyl alcohol, bran, starch, tar and oak bark (Joseph 1920; Weisshaar et al. 2009a, b). For pruritus vulvae, for example, the recommended treatment consisted of dietary adjustments, exercise, going to sleep late, cold ablutions for five minutes in the morning and in the evening and ablutions with carbolic acid. Drugs for external use contained carbolic acid, salicylic acid, ichthyol, naphthol, tar, chloral hydrate, menthol and thymol and were mostly applied as salves. Alcohol, concentrated acetic acid, chloroform, opium and belladonna were also prescribed. For internal use, carbol, salicylic acid and iron are mentioned. In addition to this, medication for ‘internal calming’ is reported which consisted of valerates and bromides combined with antipyrine, salicylic acid and arsenic. A new treatment approach was hormone replacement, including injections of progynone (estradiol valerate) and ovocycline (estradiol) for pruritus vulvae, and substitution of thyroid hormones and hypophysin.

Soon after the discovery of ionising radiation in 1895, its therapeutic potential was tested on pruritus, especially pruritus ani and vulvae. Due to its heavy side

effects, however, it never became a general treatment choice for pruritic conditions. 'Notable and lasting successes' with electric current therapy were reported, including treatment of pruritus vulvae with constant current pulses. In a total of six sessions per week over a period of 6 weeks, a current with an amperage of 15–20 mA was applied for 10–15 min (Weisshaar et al. 2009a, b). Jadassohn (1901) considers the possible use of 'localised application of high-voltage and high-frequency electrical currents (d'Arsonval-Tesla)'.

In the early twentieth century, surgical treatment of pruritus became a discussed approach. Küttner argues that 'when all other methods of treatment have been exhausted, it is often possible to offer successful surgical treatment for symptomatic as well as severe essential pruritus' (Weisshaar et al. 2009a, b). While most authors saw surgery as a last resort, others, like Frank, considered surgical intervention as a therapeutic possibility, including nerve block anaesthesia, resection of the relevant peripheral nerve or sacral anaesthesia in pruritus vulvae and pruritus ani. For 'desperate cases', even the 'resection of the posterior radices', 'cauterisation of the entire affected area' and 'total extirpation of the affected tissue' are mentioned (Frank 1928). However, Rave points out, 'I have once allowed myself to be induced to extirpate. Well, this is the least appropriate thing to do, for even in the absence of any visible skin signs after surgery, pruritus will reappear in the intact skin' (Weisshaar et al. 2009b).

The National Socialist regime brought about a disruption in pruritus research. Many of Germany's most prominent academics, including about six hundred dermatologists (among them Siegfried Bettmann, Erich Hoffmann, Joseph Jadassohn, Erhard Riecke and Leo von Zumbusch), were persecuted for racial or political reasons and went into exile. Others remained, working more or less in accordance with the regime, among them Walther Krantz and Walther Schönfeld who, however, did not join the National Socialist party. As Germany isolated itself from the international scientific community, the focus of dermatological research, which may well be said to have been in Germany in the 1920s, shifted to the United States. For example, Bickford (1939) found that itching is sensitive to cooling, as is pain, and suggested that itch is transmitted by C nerve fibres, again similar to pain, and Bishop (1948) studied skin sensation and proposed that itch is transferred via sensory fibres to cerebral cortex and thalamus.

Graham et al. (1951) performed experiments indicating that itch sensation has two distinguishable components (burning, transmitted by C fibres and pricking, by A fibres) and postulated that the spinal cord has interneuronal circuits and that painful stimuli can inhibit itch. These workers argued that itchy skin is not merely a low-threshold type of pain, this being a common school of thought at the time.

Shelley and Arthur (1955, 1957) published pivotal studies demonstrating that proteinases from a wide variety of sources are pruritogenic when inserted into the superficial layers of the skin and induce itch via a non-histaminergic pathway.

In the 1990s, a new era of itch research has been evolving highlighted by critical studies conducted by Handwerker et al. (1991) and Schmelz et al. (1997) in Erlangen on the role of C nerve fibres dedicated to transmitting itch and by

LaMotte's group at Yale University who performed psychophysics on itch sensations (Simone et al. 1991).

10 Itching for Revolution

To conclude, it may be interesting to consider briefly the cultural meaning of pruritus. The most famous patient in the history of itch was Jean Paul Marat (1743–1793). Having practised as a doctor, he was appointed personal physician to the Earl of Artois, the younger brother of Louis XVI. In 1780, he abandoned his profession as a doctor, henceforth devoting his energies to the cause of the revolution. The tone of his speeches became increasingly radical. On July 13 1793, Jean Paul Marat was assassinated in his bathtub by the counter-revolutionary Charlotte Corday.

From 1788 onwards, but very likely even earlier, he suffered from severe pruritus and often took baths to alleviate it. As the condition persisted, he increasingly spent time in a medical bath, even editing his newspaper there. Many speculations abound about the possible differential diagnoses of his pruritic condition, among them syphilis, scabies, diabetic candidosis, atopic eczema, seborrheic dermatitis and pemphigus vegetans. Recent studies suggest Morbus Duhring as the most likely cause. It has been suggested that unbearable itch was one of the reasons for Marat's personal and political unrest, causing him to leave his profession as a doctor and scientist to engage in revolutionary activities. Chronic pruritus, then, would have advanced radical thinking and acting, thus influencing the course of the revolution.

However, throughout cultural history, itching is often associated with perceptions of diseases arising from lack of personal hygiene and social inferiority. Thus, in Shakespeare's *Troilus and Cressida,* Thersites, who is portrayed as an unsightly and vulgar social critic, abuses his 'scurvy lord' Ajax with the words: 'I would thou didst itch from head to foot and I had the scratching of thee; I would make thee the loathsomest scab in Greece' (Shakespeare 1982). A critical perspective on these matters is essential, for the social stigma of itch is no less an issue in clinical practice than the research about its causes and its treatment.

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Pruritus Epidemiology and Quality of Life

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Abstract

The burden of chronic pruritus is increasingly recognized as significant worldwide. As wet-laboratory researchers investigate the pathophysiology of chronic pruritus, epidemiologists and health services researchers are quantifying the impact of pruritus by incidence, prevalence, and quality of life measures. Outcomes researchers are also investigating factors that may predict chronic pruritus incidence and severity. Such efforts will direct resources for research, public health intervention, and clinical care.

Keywords

Pruritus • Epidemiology • Incidence • Prevalence • Quality of Life

Abbreviations

ESRD	End-stage renal disease
IFSI	International Forum for the Study of Itch
QoL	Quality of Life

1 Definition and Classification of Pruritus

In 1660, German physician Samuel Hafenreffer defined pruritus as "an unpleasant cutaneous sensation that provokes the desire to scratch" (Binder et al. 2008). This description may seem rather imprecise, but the myriad of parameters characterizing pruritus (e.g., severity, location, frequency, duration) and the absence of any consistent physical signs make defining pruritus difficult. These same factors have also historically complicated and impeded research into pruritus. To overcome this challenge, in recent years, physicians have worked to better classify pruritus. The International Forum for the Study of Itch (IFSI) has been a leader in these efforts. The IFSI has divided pruritus into acute (less than 6 weeks) and chronic (lasting 6 weeks or longer) pruritus (Ständer et al.). The latter is associated with many diseases, most commonly chronic renal failure, atopic dermatitis, and cholestatic liver diseases (Mettang et al. 2002; Talwalkar et al. 2003; Zucker et al. 2003). The IFSI has also categorized patients using two complementary taxonomies. The first taxonomy is based on the clinical picture and history allowing a patient to be assigned to one of three groups. Group I represents pruritus on diseased (inflamed) skin, group II represents pruritus on non-diseased (noninflamed skin), and group III represents pruritus presenting with severe chronic secondary scratch lesions, such

as prurigo nodularis. The IFSI's second taxonomy categorizes pruritus by etiology; four of these categories are dermatologic, systemic (including pregnancy and drug induced), neurologic, and psychiatric. Patients with multiple causes of pruritus are classified as mixed, and patients with no identified underlying disease are classified into an "other" category. The latter category is also referred to as "pruritus of undetermined origin" (Ständer et al. 2007).

2 Epidemiology of Pruritus

Numerous studies have been conducted to assess the prevalence and incidence of chronic pruritus. A handful of these studies have attempted to assess general population parameters, while many more studies have focused on disease-specific populations. While results have varied, the literature on the epidemiology of chronic pruritus as a whole reveals a substantial burden of disease.

2.1 Chronic Pruritus in the General Population

Most research (Table 1) into the incidence and prevalence of pruritus in the general population has been undertaken in Europe. These studies produced insightful epidemiologic data and also found associations of pruritus with various demographic and disease factors.

In London, a population study using mailed questionnaires found the point prevalence of prurigo and allied conditions to be 8.2 % (Rea et al. 1976). In France, a survey was conducted to assess the prevalence of dermatologic disorders (Wolkenstein et al. 2003). Of those with skin diseases, 28.7 % claimed that it caused real impairment, mostly from chronic pruritus.

A cross-sectional population-based study in Norway using mailed questionnaires found 8.4 % of individuals had itchy skin in the past week (Dalgard et al. 2004). In a subsequent analysis by Dalgard et al. of the same study population, the authors looked at the prevalence of itch by severity as opposed to the prevalence of itch of skin complaints. In this analysis, they asked individuals about itchy skin in the last week with the following options: no, yes (a little), yes (quite a lot), and yes (very much), finding 27 % of individuals reported at least some itch in the last week (Dalgard et al. 2007).

In Germany, multiple studies examining the prevalence of chronic pruritus have been conducted. A smaller study of 199 individuals found that 16.5 % of individuals had experienced chronic pruritus within the past year (Matterne et al. 2009). A cross-sectional study of 2,540 subjects taken from the general population yielded a 12-month prevalence of 16.4 % and an estimated lifetime prevalence of 22 % (Matterne et al. 2011). Matterne and colleagues conducted a separate study of 1,135 individuals with 1-year follow-up using a questionnaire assessing for chronic pruritus as well as medical, lifestyle, and psychosocial factors. The incidence of chronic pruritus over 12 months was 7.0 % and the lifetime prevalence was 25.5 %

Table 1 Chronic pruritus in the general population

Author	*N*	Population	Age	% Female	12-month cumulative incidence	Point prevalence	12-month prevalence	Lifetime prevalence
Rea (1976)	1,979	Residents of North Lambeth with skin diseases	15–74	49.3 %	N/A	8.2 %	N/A	N/A
Wolkenstein (2003)	25,441	Households in France	0 to >75	51.4 %	N/A	28.7 %	N/A	N/A
Dalgard (2004)	18,747	Residents of Oslo	30–76	55.2 %	N/A	8.4 %	N/A	N/A
Dalgard (2007)	18,747	Residents of Oslo	30–76	55.2 %	N/A	27 %	N/A	N/A
Matterne (2009)	199	Residents of Heidelberg and Ludwigshafen	21–93	65.8 %	N/A	13.9 %	16.5 %	22.6 %
Stander (2010)	11,730	Employees of 144 German companies	16–70	46.8 %	N/A	16.8 %	N/A	N/A
Matterne (2011)	2,540	Residents of Heidelberg and Ludwigshafen and six surrounding rural communities in Southwest Germany	51.7 ± 17.8	55.3 %	N/A	13.5 %	16.4 %	22.0 %
Misery (2012)	1,703	Representative national sample of the French population age ≥ 15 years	Not reported	Not reported	N/A	32.1 %	N/A	N/A
Matterne (2013)	1,135	Residents of Heidelberg and Ludwigshafen and six surrounding rural communities in Southwest Germany	56.0 ± 16.5	58.0 %	7.0 %	15.4 %	18.2 %	25.5 %
Shive (2013)	Seven million visits	National Ambulatory Medical Care Survey (United States)	<15 to ≥75	65.7 %	N/A	1 % outpatient visits	N/A	N/A
Carr (2014)	1,075	US Veterans	60.7 ± 13.0	7 %	N/A	38 %	N/A	N/A

(Matterne et al. 2013). Another large cross-sectional study in Germany with 11,730 adults aged 16–70 years demonstrated similar results, with a 16.8 % point prevalence of chronic pruritus. The prevalence of chronic pruritus increased with age, from 12.3 % in the 16–30 age group to 20.3 % in the 61–70 age group (Ständer et al. 2010). In France, a cross-sectional study of 1,703 people found 32.1 % of people itched in the last 7 days (Misery et al. 2012).

In the United States, two recent studies have also demonstrated the substantial presence of pruritus. Shive and colleagues used retrospective data from the National Ambulatory Medical Care Survey from 1999 to 2009 to describe ambulatory care visits to clinicians for which itch was coded as a patient symptom. They found that 1 % of all outpatient visits, approximately seven million visits per year, included a code for itch. Approximately one-third of the visits were considered chronic (>3 months) (Shive et al. 2013). Carr and colleagues (2014) utilized the Veterans Administration (VA) National Patient Care Database to randomly select 6,000 veterans who had at least one encounter with the VA hospital system. Of the 1,075 who agreed to participate, 403 (37 %) reported pruritus lasting for at least 6 weeks.

2.1.1 Predictors of the Incidence of Itch

Matterne and colleagues' (2011) study additionally revealed an association of ethnic origin and female gender with chronic pruritus. Subsequent studies have also found ethnicity and female gender to be significant predictors of pruritus. Shive and colleagues' retrospective study from the National Ambulatory Medical Care Survey from 1999 to 2009 found patients seen for itch were more likely to be black or Asian than other patients (20 % vs. 14 %) (Shive et al. 2013). Additionally, the study by Carr et al. (2014) of veterans found race was a significant predictor of itch severity. African-Americans had increased itch severity compared to Caucasian patients, and the greatest pruritus severity was experienced by Asians, Native Hawaiians, Pacific Islanders, and those who are identified as "other." American Indians and Alaskan Indians experienced the least pruritus severity of all ethnic groups (Leader et al. 2013b).

Other studies finding gender as an important factor in the epidemiology of chronic pruritus include a recent study of 1,037 patients with chronic pruritus in Münster, Germany (54.8 % female). Ständer et al. (2013) identified gender-specific differences in the quality, location, triggers, etiology, and associated scratching of chronic pruritus. Women had more neuropathic and psychosomatic diseases underlying their chronic pruritus compared to men. Women experienced greater exacerbation of chronic pruritus by emotional and psychosomatic factors ($p < 0.05$), greater localization of pruritus ($p = 0.016$), more episodic attacks, and more stinging, warm, and painful sensations ($p = 0.046$). Women more commonly had prurigo nodularis compared to men ($p < 0.01$).

In Matterne and colleagues' (2013) study, age was found to significantly contribute to the incidence of chronic itch. Disease predictors of the prevalence of chronic pruritus in multivariate analysis were hepatic disease, asthma, dry skin and eczema, elevated BMI, and anxiety. The VA study found that allergic diseases,

autoimmune or inflammatory diseases, and neurological and/or psychiatric conditions were also predictors of chronic pruritus (Leader et al. 2013a). Subsequent studies are needed to further characterize the predictors of the incidence of itch.

2.2 Disease-Specific Epidemiology of Chronic Pruritus

2.2.1 Dermatologic Disease

For many skin diseases, pruritus is a significant symptom. The diseases featuring pruritus include autoimmune, genetic, infectious, inflammatory, neoplastic, and pregnancy-related dermatoses. Epidemiologic statistics have been reported for some of these diseases. Pruritus is one of the major diagnostic criteria for atopic dermatitis, a disease which afflicts 11 % of people under 18 years of age (Shaw et al. 2011). In elderly patients with xerosis, 30–60 % experience pruritus (Beauregard and Gilchrest 1987). Another common cause of pruritus is urticaria, which has a lifetime prevalence of 15–20 % in the general population (Soter 1998; Bakker et al. 2013). In patients with psoriasis involving more than 30 % of their skin, 80 % experience itch (Yosipovitch et al. 2000; Krueger et al. 2001). Cutaneous T-cell lymphoma, although rare, is associated with significant pruritus with one study reporting 88 % of their 100 patients with pruritus in the preceding 4 weeks and 46 % indicating that it was often or always a problem (Wright et al. 2013). Contact dermatitis, keloids, and scars are also associated with itch, but the prevalence of itch in these conditions is unknown (Herman 1994; Yosipovitch 2003). Rare skin diseases are also anecdotally reported with pruritus as a significant symptom such as bullous pemphigoid (Bakker et al. 2013), dermatitis herpetiformis (Powell et al. 2004; Passe et al. 2008), and lichen planus (Welz-Kubiak and Reich 2013). However, epidemiologic studies in such rare entities are very difficult to perform.

2.2.2 Systemic Diseases

Chronic pruritus is frequently present in systemic disease (Cassano et al. 2010). Studies have found that 10–50 % of patients with pruritus and no skin findings have an underlying systemic disease, and up to 70 % of these patients have a psychiatric disease (Ferm et al. 2010). Different studies identified systemic causes of chronic pruritus in 14–50 % of patients (Rajka 1966; Beare 1976; Kantor and Lookingbill 1983; Zirwas and Seraly 2001; Afifi et al. 2004). Three studies of patients presenting with pruritus found that 24 % to 57 % of cases were due to dermatoses (Lyell 1972; Weisshaar et al. 2006; Sommer et al. 2007). When the patients with dermatoses were excluded, 31 % of the patients of Lyell and 82 % of the patients of Weisshaar et al. had an underlying systemic disease. A retrospective case review of patients with chronic pruritus revealed that the most severe and long-lasting pruritus occurred in patients with multiple systemic diseases and in those patients where the etiology of pruritus was unknown. Of 139 patients with itch, 47 had one systemic disease, 9 had two or more internal diseases, 24 had neuropathic itch,

31 had psychiatric disease, and 37 had pruritus of unknown origin. Scalp and face pruritus was most common in patients with psychogenic pruritus. The authors concluded that work-up of a patient with chronic pruritus rarely reveals an underlying systemic disease which is responsible for their pruritus (Ferm et al. 2010).

2.2.3 Renal Disease

In end-stage renal disease (ESRD), pruritus occurs independent of the etiology of the renal failure. All races, ages, and both genders are susceptible to ESRD pruritus (Senturk et al. 2008). The pathogenesis is unknown but is associated with an elevated C-reactive protein as well as other inflammatory cytokines. The renal failure must be severe to be associated with pruritus and resolves after renal transplant (Berger and Steinhoff 2011). Narita and colleagues found pruritus in 15–49 % of patients with chronic renal failure and 50–90 % of patients undergoing dialysis (Narita et al. 2008). Zucker and colleagues similarly found 66 % of patients undergoing hemodialysis experienced pruritus at some point (Zucker et al. 2003). The ITCH National Registry Study was a prospective, multicenter, longitudinal study of 103 hemodialysis patients. In this group, daily or almost daily itching was reported by 84 % of patients (Mathur et al. 2010). The Observational Dialysis Outcomes and Practice Patterns Study collected data from over 29,000 hemodialysis patients in 12 countries and found that 42 % of these patients experienced moderate to extreme itch during the year they were followed (Pisoni et al. 2006). Other studies reported a pruritus prevalence of 25–86 % in ESRD patients (Young et al. 1973; Bencini et al. 1985; Szepietowski and Schwartz 1998; Zucker et al. 2003; Duque et al. 2006). Differences in pruritus prevalence were found between countries: 38 % in France, 45 % in Japan and the United States, 48 % in the United Kingdom, 49 % in Germany, and 55 % in Italy (Pisoni et al. 2006; Wikström 2007). Tessari et al. saw that 52 % of dialysis patients experience pruritus, with no difference in prevalence between hemodialysis and peritoneal dialysis patients (Tessari et al. 2009). Those patients dialyzed with less permeable and less biocompatible membranes have a lower incidence of pruritus (Murphy and Carmichael 2000; Pauli-Magnus et al. 2000). Additionally, patients on statins are significantly less likely to suffer from pruritus ($p = 0.02$) (Duque et al. 2006). In patients undergoing hemodialysis, chronic pruritus was associated with poor outcomes (Narita et al. 2008).

2.2.4 Hepatic Disease

The reported prevalence of chronic pruritus in hepatic disease ranges from 15 % to 100 % (Weisshaar and Dalgard 2009). Itching is a frequent symptom of cholestasis occurring in 80–100 % of cases (Bergasa et al. 2000). Pruritus is seen in 5 % of patients with chronic hepatitis (Chia et al. 1998) and 15 % of patients with chronic hepatitis C infection (Maticic et al. 2008). Pruritus is the presenting symptom of primary biliary cirrhosis in 25–70 % of patients, and 10 years after, diagnosis is present in at least 70 % of patients (Heathcote 1997; Bergasa et al. 2000; Mela et al. 2003; Talwalkar et al. 2003). In an online survey, 69 % of women with primary biliary cirrhosis experienced pruritus, and of these women, 75 %

experienced itch prior to their diagnosis (Rishe et al. 2008). A case control study of 49 patients with primary biliary cirrhosis echoed these results with a pruritus prevalence of 69 % (Koulentaki et al. 2006).

2.2.5 Hematologic Disease

Hematologic abnormalities can engender pruritus. In a meta-analysis of 10 studies, Saini and colleagues (Saini et al. 2010) found pruritus in 42 % of 821 patients with polycythemia vera. Other investigators found that half of patients with polycythemia vera experience pruritus (Diehn and Tefferi 2001; Vannucchi et al. 2007). Lower mean corpuscular volume and a higher leukocyte count are significantly associated with the presence of pruritus in patients with polycythemia vera (Diehn and Tefferi 2001). In a recent study of 441 polycythemia vera patients, 68.2 % reported aquagenic pruritus (Siegel et al. 2013).

Iron deficiency may play a role in chronic pruritus (Diehn and Tefferi 2001). In a prospective study assessing the frequency of systemic disease in patients with generalized pruritus ($n = 55$), iron deficiency anemia was found to be the most common cause of generalized pruritus. The mean serum hemoglobin, iron, and cyanocobalamin were significantly lower in the patients with generalized pruritus as compared to the control group (Polat et al. 2008). Additional studies have similarly reported iron deficiency in association with chronic pruritus (Bharati and Yesudian 2008).

2.2.6 Neoplasms

Pruritus is associated with hematologic malignancies and less commonly with solid tumors. Thirty percent of Hodgkin's lymphoma patients report itch (Goldman and Koh 1994). Patients with chronic lymphocytic leukemia, multiple myeloma, and non-Hodgkin lymphoma also report pruritus; occasionally, pruritus is the presenting symptom (Daponte et al. 2007; Robak and Robak 2007). Solid tumors, including breast, lung, colon, and prostate neoplasms, represent a rare cause of chronic pruritus (Kleyn et al. 2006). While malignancy-associated itch is usually generalized, in some cases, it is associated with the location of the tumor, which may be due to direct activation of the nerves (McMichael 2004). In contrast, Yosipovitch (2010) described paraneoplastic itch as a distinct entity which is not caused by tumor invasion or compression, occurs early in the natural process of the malignancy, and subsides after the removal of the tumor.

2.2.7 Other Diseases

Infectious diseases are also known to engender pruritus, including viral and parasitic infections, as well as skin diseases in HIV patients such as eosinophilic folliculitis and papular pruritic eruption of HIV (Bonacini 2000; Rodwell and Berger 2000; James et al. 2005). Pruritus is also associated with endocrine disorders including hyperthyroidism and diabetes (Jabbour 2003).

Pruritus is a common symptom after burns. In a multicenter cohort study of adult burn survivors, the prevalence of itch at discharge, 6, 12, and 24 months after injury, was 93 %, 86 %, 83 %, and 73 %, respectively. In a group of patients burned

4–10 years ago, 44.4 % reported itching at the area of previous injury (Carrougher et al. 2013).

Pruritus occurs in psychiatric diseases. In a study of 100 inpatient psychiatric patients, 42 % suffered from idiopathic pruritus. Idiopathic pruritus was related to psychosocial stressors, with 29.5 % and 48.5 % prevalences in patients with and without adequate social support, respectively ($p = 0.02$). Additionally, 48.5 % of patients not regularly employed experienced pruritus compared to 16.7 % of those with regular employment ($p = 0.01$). This study also found that tricyclic antidepressants reduce pruritus prevalence from 48 % to 14 % ($p = 0.09$) (Kretzmer et al. 2008). In a similar study, 111 inpatient psychiatric patients at an Israeli hospital were administered a validated itch questionnaire; 32 % of the patients reported pruritus despite few seeking treatment for their pruritus (Mazeh et al. 2008).

Neurologic disease can engender pruritus. Patients who suffer from postherpetic neuralgia following a shingles infection may additionally report postherpetic itch or may only experience itch instead of pain. Oaklander et al. (2003) reviewed three previously collected data sets of patients with recent shingles infection or postherpetic neuralgia from Finland, Seattle, and Liverpool for a sample of 586 individuals to better characterize the epidemiology of postherpetic itch. They found pruritus commonly occurs with postherpetic neuralgia as well as with acute zoster infections. Patients who had shingles on their head, face, and neck were more likely to suffer from postherpetic itch compared to those who only had singles on the torso. In a study looking at the point prevalence of pruritus in people with recent shingles, 17 % of adults from Finland reported itch, 36 % from Liverpool, and 58 % from Seattle (Oaklander et al. 2003). Neuromyelitis optica has also been associated with pruritus; in a small study of 44 patients, 27.3 % reported pruritus (Elsone et al. 2013).

Collagen diseases such as scleroderma and dermatomyositis have a relatively high prevalence of chronic pruritus. Of 959 patients in the Canadian Scleroderma Research Group Registry, 42.6 % reported pruritus during the past month on most days (Razykov et al. 2013). Similarly, a retrospective 30-year chart review of 16 patients with juvenile dermatomyositis revealed pruritus was a symptom in 38 % of patients (Peloro et al. 2001).

2.2.8 Pharmacologic

Many medications cause pruritus. This pruritus may be secondary to a cutaneous drug reaction (e.g., urticaria), but medications can also provoke pruritus without signs of skin irritation (Reich et al. 2009). For example, 10–50 % of patients receiving intravenous opioids experience pruritus, as do 20–100 % of patients receiving intraspinal or epidural opioid injections (Ganesh and Maxwell 2007). While the exact mechanism for opioid-induced pruritus is not fully understood, μ opioid receptor agonists play a significant role, while serotonin and dopamine D2 receptors appear to play a role as well. Pruritus is also estimated to affect 5–27 % of patients in palliative care. The etiology is complex and possibly due in part to medications (Kleyn et al. 2006).

2.3 Considerations of Epidemiologic Estimates of Pruritus

Several factors should be considered when reviewing the published literature regarding the incidence and prevalence of pruritus. First, the definition of chronic pruritus must be transparent. Several earlier studies neglected to report their definition of pruritus. While reporting the pruritus severity of the preceding week, it may not be clear whether the pruritus was >6 weeks, as defined by the IFSI criteria, or shorter. Secondly, several studies utilized proxies for patients with chronic pruritus. For instance, several early studies attempted to determine the prevalence of skin conditions that are known by experience to be itchy. Additionally, several of the epidemiology studies in the published literature consist of convenience samples and thus may not be representative of the general populations.

3 Impact of Chronic Pruritus on Quality of Life

Quality of life (QoL) is a patient-reported metric that reflects how an individual perceives and reacts to their health status and to other nonmedical aspects of life (Gill and Feinstein 1994). QoL encompasses physical and emotional well-being, as well as satisfaction with social functioning (Croog et al. 1986). Health-related QoL assesses the impact of a disease on all aspects of an individuals' life, including psychosocial, emotional, physical, and functional domains (Chen 2012). Because pruritus is not visible and the secondary changes (scratching, rubbing, picking) may not reflect the intensity of the pruritus, patient-reported outcomes such as QoL are important to the clinician and/or researcher to assess severity.

3.1 Instruments to Measure the Impact of Pruritus on QoL

To understand the QoL impact of pruritus, readers must appreciate the instruments that have been developed to measure such impact. Early researchers utilized proxies to investigate the impact of pruritus such as agitation, poor concentration, anxiety, and depression (Gupta and Gupta 2004; Evers et al. 2005; van Os-Medendorp et al. 2006; Dalgard et al. 2007; Amatya et al. 2008; Zachariae et al. 2008). In an effort to more directly and effectively evaluate the influence of pruritus on QoL, researchers have developed and utilized validated instruments that quantify QoL impact. There are generic, skin-specific, and pruritus-specific instruments used to measure the impact of pruritus. The two commonly used generic tools are the Short-Form 12 and 36. The Short-Form 36 (SF-36) was developed from the Medical Outcomes Study and is a measure of health status consisting of eight domains: physical functioning, limitations due to physical health, limitations due to emotional health, energy level, emotional well-being, social functioning, pain, and general health. The SF-36 has also been condensed into a 12-item version, SF-12, which assesses both physical and mental health. The components of the SF-12 measure general health, daily and social activity

limitations as a result of physical and/or mental health, impact of pain on normal work, feelings of calm and peace, energy, and downheartedness (Chen 2012).

The two most commonly used skin-specific instruments are Skindex and the Dermatology Life Quality Index (DLQI), which both ask questions related to pruritus. Skindex-29 asks the frequency of "My skin itches" and Skindex-16 queries "During the past week, how often have you been bothered by your skin condition itching?" (Chren et al. 1997, 2001). Numerous studies have utilized this instrument to investigate the impact of pruritus on QoL, including research into antihistamines (Murota et al. 2010), chronic venous insufficiency (Duque et al. 2005), and dialysis (Tessari et al. 2009). The DLQI is the most frequently used skin-specific QoL questionnaire. DLQI addresses pruritus by asking "Over the last week, how itchy, sore, painful or stinging has your skin been?" (Chen 2012; Finlay and Khan 1994). Investigators have used DLQI to evaluate the impact of pruritus on QoL in multiple studies, including research into ESRD (Szepietowski et al. 2011) and vitiligo (Silverberg and Silverberg 2013). A pediatric version of the DLQI, the Children's Dermatology Life Quality Index (CDLQI), has been created as well.

Pruritus-specific instruments have been developed and validated to better assess the impact of pruritus. Yosipovitch and colleagues developed the Short-Form Itch Questionnaire, modeled after the Short-Form McGill Pain Questionnaire. The questionnaire includes QoL questions on mood, eating habits, sexual desire and function, and sleep (Melzack 1975; Yosipovitch et al. 2001; Ikoma et al. 2006), but does not fully address all QoL constructs. Studies using the questionnaire include studies of psoriasis patients (Yosipovitch et al. 2000), chronic idiopathic urticaria (Yosipovitch et al. 2002a), and atopic dermatitis (Yosipovitch et al. 2002b). Majeski et al. (2007) modified the Short-Form Itch Questionnaire into the Itch Severity Scale, which also measures the severity and patient burden of pruritus but, unlike the former, does not require interviewer administration.

A more comprehensive validated pruritus-specific QoL instrument is the ItchyQoL, which addresses the symptom, emotional, and functional impact of pruritus. Studies which have utilized ItchyQoL include a small cohort of patients with cutaneous T-cell lymphoma (Chen et al. 2010). ItchyQoL has been translated into German and studied in a cohort of 308 patients with pruritus of diverse etiology: urticaria, atopic eczema, psoriasis, prurigo, renal disease, liver disease, neoplasm, and unknown etiology (Krause et al. 2013).

Other pruritus-specific instruments used in QoL studies include the Eppendorf Itch Questionnaire (Darsow et al. 1997), 5-D itch scale (Elman et al. 2010), and Patient Benefit Index for pruritus (Blome et al. 2009). Weisshaar and colleagues (2011) recently developed a German language questionnaire assessing for chronic pruritus with the goal of usage in epidemiologic studies. The questions assess the course, intensity and quality of pruritus, general health status, sociodemographic data, QoL, and pruritus cognition. Other studies utilize consolidated summary measures of QoL including health economic utilities (Kini et al. 2011) and general questions such as "Has your life changed?" (Weisshaar et al. 2006).

3.2 Quantifying the QoL Impact of Pruritus in General Population Studies

Pruritus has been repeatedly demonstrated to have a significant impact on QoL (Gupta et al. 1994; Yosipovitch et al. 2000, 2002b; Radmanesh 2001; Zucker et al. 2003; Chamlin et al. 2005; van Os-Medendorp et al. 2006; Goon et al. 2007; Wikström 2007; Amatya et al. 2008; Yamamoto et al. 2009). Indeed, the impact of chronic pruritus on QoL has been found not significantly different from the impact of chronic pain on QoL (Kini et al. 2011) with 73 chronic pruritus study patients willing to give up 13 % of their lives to not have pruritus. Halvorsen et al. (2009) found that there was a 3.0 odds (confidence interval: 2.1–4.2) to have suicidal ideation associated in adolescents with chronic itch compared to adolescents without itch, which was comparable to the odds from chronic pain.

A Dutch study found that 13 % of their 167 chronic pruritus patient cohort sought a mental health professional that resulted with 4.8 % prescribed tranquilizers and 6.2 % with antidepressants. Their research suggested that the coping strategy adopted by patients is more responsible for this impact than the pruritus itself; catastrophizing and helpless coping have been found to play a greater role in morbidity from pruritus than itch frequency (van Os-Medendorp et al. 2006). Other studies have also indicated that a support structure may be beneficial as marital status seems to confer a positive impact in pruritus-specific QoL (Kini et al. 2011; Carr et al. 2014).

The deleterious impact of pruritus on QoL has been demonstrated in many different populations. For example, Weisshar and colleagues (2006) compared two populations with pruritus, one in Germany (132 patients) and one in Uganda (84 patients). Patients in both populations exhibited an impaired QoL. Interestingly, while the majority of the etiology of pruritus in these two populations was from a dermatosis, it was the pruritus of unknown origin that led to the greatest QoL impact.

3.2.1 Predictors of Pruritus QoL Impact in General Population Studies

In addition to the coping strategy and support structure's impact on QoL impact of pruritus, studies have examined other factors that might affect the impact of chronic pruritus on QoL. Carr et al. (2014) utilized ItchyQoL in a cross-sectional population-based study from the Veterans Hospital Patient Database. The investigators found the factors that mediate the impact of chronic pruritus on QoL are demographics (age, race, marital status), personality (extraversion and neuroticism), pruritus characteristics (severity, duration, frequency, location), and etiology (cutaneous versus systemic). Notably, gender did not mediate the impact of chronic pruritus on QoL.

Desai et al. (2008) found pruritus etiology and gender influenced the impact of pruritus on QoL: urticaria engendered greater impact on functional aspects of QoL, and women suffered more from chronic pruritus than men. Ständer et al. (2013) also found a greater impact of chronic pruritus on women. Kini et al. (2011) found that unmarried persons suffer more from chronic pruritus than their married

counterparts. Schut and colleagues (2013) as well as Kini and colleagues (2011) have performed preliminary work on the prediction of personality on chronic pruritus. Schut and colleagues (2013) have found that agreeableness and public self-consciousness were significant predictors of induced scratching in an experimental setting in addition to depression. Booker and colleagues (2013) found that neuroticism, irrespective of other personality factors, was significantly associated with a greater itch-specific QoL impact in adults with eczema. As this research has only been undertaken recently, much remains to be understood regarding which pruritus characteristics predict the impact of pruritus on QoL.

3.3 Disease-Specific Impact of Chronic Pruritus on Quality of Life

3.3.1 Dermatoses

Atopic Dermatitis

The symptoms of atopic dermatitis such as scratching, itching, and sleeplessness can be a burden not only to the patient, but their whole family, leading to stress and increased irritability (Fivenson et al. 2002; Ben-Gashir 2003). In the German Atopic Dermatitis Intervention Study, 823 children and adolescents were followed to investigate if there was a correlation between itch severity, QoL, and coping behavior in both children and parents using the Severity Scoring of Atopic Dermatitis (SCORAD). QoL was assessed with the German questionnaire "Quality of life in parents of children with atopic dermatitis" which consists of 26 items to evaluate psychosomatic well-being, effects on social life, confidence in medical treatment, emotional coping, and acceptance of disease. QoL in both children and adolescents showed a significant negative correlation with itch intensity. The authors conclude that, in managing patients with atopic dermatitis, QoL, coping strategies, and itch intensity should all be assessed (Weisshaar et al. 2008).

In another study of patients with atopic dermatitis, health-related QoL was assessed in two visits at 6-month intervals in 101 patients with atopic dermatitis and in 30 controls using DLQI and SF-36. SCORAD and visual analogue scales were used to measure disease severity. Patients with atopic dermatitis had significantly lower QoL compared to healthy controls and the general population. Atopic dermatitis negatively impacted mental health and social, emotional, and physical functioning. SCORAD positively correlated with DLQI. The visual analogue score of patients' assessment of disease severity exhibited the tightest correlation with most of the QoL measures. This suggests that asking the patient "how is your eczema today?" may be an effective means of assessing impact on QoL (Holm et al. 2006).

Psoriasis

Patients suffering from psoriasis frequently experience sensory skin symptoms, sleep impairment, decreased QoL, and psychological distress (Finlay et al. 1990; Fortune et al. 2005; Gowda et al. 2010; Ljosaa et al. 2010). Skin discomfort

(including itching, burning, and sensitivity) has been reported in up to 37 % of patients with psoriasis. Up to 57 % of psoriasis patients report sleep disturbance (Sharma et al. 2001; Zachariae et al. 2008; Callis Duffin et al. 2009). Sleep impairment has been shown to mediate the relationship between itch and psychological distress in patients with psoriasis (Fortune et al. 2005) as well as depressive symptoms and disabilities as measured by the Dermatology Life Quality Index (Zachariae et al. 2008).

Reich and colleagues (2010) also assessed the impact of pruritus on QoL in patients with psoriasis using the DLQI. Out of 102 patients with psoriasis, 91 reported pruritus. Patents with pruritus had significantly decreased QoL compared to those who did not itch ($p = 0.02$). The study also showed that pruritus severity correlated with feelings of stigmatization, stress, and depressive symptoms.

In order to assess the impact of pruritus in patients with psoriasis, Globe and colleagues (2009) utilized physician interviews and patient focus groups. They found dermatologists most frequently cited itch as the major symptom of psoriasis patients, followed by arthralgia/arthritis, flaking, and pain. In focus groups of patients with mild to severe psoriasis, most patients rated itch as the most important symptom (31/39), the most severe symptom (31/39), and the most troublesome symptom (24/39). Patients reported that pruritus impacted daily activities such as sleep, attendance at work and school, concentration, and emotions including embarrassment and anxiety.

Chronic Urticaria

Chronic urticaria is a 6-week or longer history of widespread whealing (Greaves 1995). In a study assessing the impact of urticaria on QoL, 142 patients with chronic urticaria or delayed pressure urticaria completed two self-administered questionnaires: the Nottingham health profile and a disease-specific questionnaire. The results showed urticaria affected many aspects of daily life including home management, personal care, recreational and social activities, mobility, emotions, sleep, rest, and work (O'Donnell et al. 1997). In another study, a modified questionnaire based on the McGill Pain Questionnaire was administered to 100 chronic idiopathic urticaria patients. Sixty-eight patients reported daily pruritus, mostly in the evening, and 62 reported difficulty in falling asleep. Seventy-six patients felt the itch was bothersome, 66 said it was annoying, and 14 reported depression (Yosipovitch et al. 2002a).

Zachariae and colleagues (2012) conducted a study to examine the associations between pruritus severity, QoL, and psychological symptoms in patients with a variety of skin diseases. The authors found patients with urticaria reported significantly greater ($p < 0.05$) pruritus severity as compared to the other patient groups. Severity of pruritus was significantly associated with impaired quality of life, impaired sleep quality, increased depressive symptoms, greater anxiety, and increased nonspecific somatic symptoms. The affective dimension of pruritus was a better predictor on quality of life impairment than the sensory dimension.

Vitiligo

In a prospective study, 1,541 patients with vitiligo were queried online regarding the extent, distribution, duration, and associated symptoms of their vitiligo. The questionnaire evaluated QoL using DLQI. This study found that pruritus or burning skin occurred in 35 % of vitiligo patients and was predicted by an affected body surface area of > 25 % ($p < 0.001$). The investigators concluded that vitiligo extent was associated with QoL impairment (Silverberg and Silverberg 2013). One limitation of this study is that pruritus and burning skin cannot be separated for analysis.

3.3.2 Extracutaneous Disease

Systemic Sclerosis

A study using 578 patients from the Canadian Scleroderma Research Group found 43 % of systemic sclerosis patients reported itch on most days of the prior month. This study assessed QoL using the mental and physical summary scores of the SF-36 and assessed disability using the Health Assessment Questionnaire disability index. Pruritic patients with systemic sclerosis had significantly worse mental and physical function and greater disability. In multivariate analyses controlling for demographic and disease variables, pruritus was independently associated with mental ($p = 0.017$) and physical function ($p = 0.003$) but not disability ($p = 0.112$) (El-Baalbaki et al. 2010).

Other studies examining the impact of pruritus in systemic sclerosis patients have demonstrated similar results. Pruritus in systemic sclerosis can prevent sleep and engender excruciating intermittent pruritus, causing some patients to scratch until they bleed (Maynes 1999; Center 2010). Chularojanamontri and colleagues (2011) conducted a cross-sectional study of 80 systemic sclerosis patients utilizing a Thai version of the DLQI. Twelve patients had limited disease and 68 had diffuse sclerosis. Patients reported that pain/pruritus was the most significant problem to them, whereas the salt-and-pepper appearance was the cutaneous finding associated with worst DLQI scores.

Chronic Venous Insufficiency

Paul and colleagues (2011) studied pruritus in chronic venous disease as part of a larger study on the impact of intravenous drug use on the distribution and severity of chronic venous disorders. The 161-person subgroup completed both a visual analogue scale for pruritus intensity and the SF-12. Eighty-eight participants (54.7 %) reported pruritus, with 74 of them (45.9 %) reporting itch on the legs or feet. Persons with leg or feet itch had poorer scores on the physical component of the SF-12 than those without itch. The two groups did not differ significantly on the mental health component. Compared with the US norms as provided with the SF-12, those with pruritus were greater than one standard deviation below the mean for both their mental and physical health scores. The investigators concluded that pruritus on the legs or feet is a clinically relevant problem that lowers QoL (Paul et al. 2011).

Another study investigated the prevalence of itch, pain, and burning sensation and their QoL impact in 100 patients with mild to moderate chronic venous insufficiency. The prevalence of pruritus was 66 %, concomitant pruritus and burning was 47 %, and concomitant pruritus and pain was 44 %. Pruritic patients were administered a modified Skindex-16 questionnaire and a visual analogue scale for itch intensity. The study found a significant ($p < 0.05$) direct relationship between itch intensity and bother produced by itch, persistence of itch, irritation, frustration, and depression. The investigators concluded that pruritus, pain, and burning sensation are common symptoms of chronic venous insufficiency with a significant impact on QoL (Duque et al. 2005).

End-Stage Renal Disease

In a study of 334 patients with end-stage renal disease, the DLQI and a visual analogue scale were administered to evaluate QoL and pruritus severity, respectively. The patients who reported pruritus experienced a greater impact on QoL compared to non-pruritic ESRD patients ($p < 0.001$). The investigators found that age and pruritus intensity, but not xerosis, independently contributed to DLQI deterioration ($p < 0.001$) (Szepietowski et al. 2011).

In another study, researchers evaluated QoL using Skindex-29 and pruritus intensity using a visual analogue scale in 169 patients undergoing hemodialysis or peritoneal dialysis. Prevalence of poor sleep in pruritic patients was higher than in patients without pruritus (59 % vs. 11 %, $p < 0.001$). Pruritus intensity was significantly correlated with poor scores in all three subscales of Skindex-29. The investigators concluded that pruritus impacted all aspects of QoL and was a predictor of poor sleep (Tessari et al. 2009).

The ITCH National Registry Study was a prospective, multicenter, longitudinal study of 103 hemodialysis patients that assessed the impact of pruritus on QoL over 14 weeks. The investigators developed and utilized uremic pruritus QoL instruments: Skindex-10, Brief Itching Inventory, Itch Medical Outcomes, and the Self-Assessment of Disease Severity. Statistically significant associations were found among pruritus intensity, pruritus severity, and QoL proxies (sleep, social relations, and mood) (Duque et al. 2006; Mathur et al. 2010).

Chemical Exposure

Mustard gas used in wartime causes pruritus. A study of 125 Iranian veterans exposed to mustard gas utilized DLQI and a pruritus visual analogue scale. Patients were classified by their pruritus severity with 11 % having mild, 35 % moderate, and 54 % severe pruritus with corresponding median DLQI scores of 16, 20, and 21, respectively ($p = 0.014$). The DLQI subscores of symptoms and feelings ($p = 0.015$), personal relationships ($p = 0.002$), and daily activities ($p = 0.036$) were worst in patients with severe itching. The investigators concluded that mustard gas victims suffering from severe pruritus had a significantly poorer QoL score than those with milder pruritus (Panahi et al. 2008).

4 Conclusion

Pruritus is a common chronic condition affecting up to 25 % of the population during their lifetime (Matterne et al. 2013) and has a significant impact on QoL (Chen 2012). Pruritus affects many aspects of affected individuals' lives leading to a plethora of problems including sleep impairment, hindered school and work performance, agitation, poor concentration, anxiety, and depression (Yosipovitch et al. 2000; Gupta and Gupta 2004; Evers et al. 2005; van Os-Medendorp et al. 2006; Dalgard et al. 2007; Amatya et al. 2008; Zachariae et al. 2008). Numerous instruments have been created to help capture the impact of pruritus on QoL and improvements as a result of therapy (Chen 2012).

Recent research has been directed at assessing the incidence and prevalence of pruritus (Carr et al. 2013; Matterne et al. 2013; Shive et al. 2013). Pruritus is a symptom of a multitude of diseases, both dermatologic and systemic (Cassano et al. 2010). Therefore, many studies have been conducted to assess the prevalence of pruritus in variety of diseases (Weisshaar and Dalgard 2009). Recent studies have identified predictors of chronic pruritus including ethnic origin, female gender (Matterne et al. 2011; Leader et al. 2013b; Shive et al. 2013; Ständer et al. 2013), and age (Matterne et al. 2013). However, few of these studies are compared against matched populations, so it is unclear which of these variables are true predictors of chronic pruritus. Other recent studies have utilized QoL instruments to assess the burden of pruritus in various diseases (Chen 2012). Factors shown to mediate the impact of chronic pruritus on QoL include demographic, personality, and pruritus characteristics as well as etiology, gender, and marital status (Desai et al. 2008; Kini et al. 2011; Ständer et al. 2013; Carr et al. 2013).

These recent studies are important on two fronts. First, the documentation of the magnitude of the burden of pruritus is important for allocation of resources for clinical care and research. Second, unveiling the predictors of pruritus and the quality of life impact will direct public health interventions, clinical care, and research efforts.

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Neurophysiology and Itch Pathways

Martin Schmelz

Contents

Abstract

As we all can easily differentiate the sensations of itch and pain, the most straightforward neurophysiologic concept would consist of two specific pathways that independently encode itch and pain. Indeed, a neuronal pathway for histamine-induced itch in the peripheral and central nervous system has been described in animals and humans, and recently several non-histaminergic pathways for itch have been discovered in rodents that support a dichotomous concept differentiated into a pain and an itch pathway, with both pathways being composed of different "flavors." Numerous markers and mediators have been found that are linked to itch processing pathways. Thus, the delineation of neuronal pathways for itch from pain pathways seemingly proves that all sensory aspects of itch are based on an itch-specific neuronal pathway. However, such a concept is incomplete as itch can also

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be induced by the activation of the pain pathway in particular when the stimulus is applied in a highly localized spatial pattern. These opposite views reflect the old dispute between specificity and pattern theories of itch. Rather than only being of theoretic interest, this conceptual problem has key implication for the strategy to treat chronic itch as key therapeutic targets would be either itch-specific pathways or unspecific nociceptive pathways.

Keywords
Specificity • Pattern theory • Pain • Chronic itch

1 Teleological Implications of Itch

Traditionally, itch and pain have been separated according to the different sensations and the opposite behavioral consequences. While painful stimuli provoke withdrawal reflexes, itching stimuli provoke the very characteristic scratching reflex. Teleologically, the withdrawal reflex is obviously suited to evade a potentially noxious stimulus. In contrast, scratching will help to remove irritating objects and agents from the skin. One might also describe scratching as a reflex pattern that is used in situations in which the noxious stimulus has already invaded the skin. In this situation, withdrawal would be useless; instead, it appears to be more appropriate to scratch the injured site. Concerning the differentiation between pruritic and nociceptive pathways, the dichotomous reflex pattern appears to rather support the specificity of itch and pain. However, it should be noted that stimuli typically linked to scratching are not limited to "itch-specific" events such as histamine-releasing mosquito bites. Scratching is a promising approach for all threatening events that are highly localized in the epidermis as scratching of the epidermis including the noxious source will be a definitive cure at a "low cost." In this scenario, it is the spatial characteristics of the noxious event rather than its specific mediators that determine the effectiveness of scratching. The spatial characteristics of the noxious event are encoded in the spinal cord as the activation pattern including nociceptive and pruritic afferents. Therefore, the teleological approach is compatible with *specific* pruriceptive pathways that detect the typical mediators of pruriceptive stimuli. On the other hand, unspecific nociceptive pathways that detect pruriceptive stimuli being spatially restricted to the epidermis by its characteristic activation *pattern* are also teleologically sound.

2 Peripheral Pathways for Itch

Functional and structural assessments have been used to characterize classes of neurons. Functional characteristics are directly recorded electrophysiologically or indirectly by behavioral measurements. Ideally, structural characteristics would be

assessed in electrophysiologically identified neurons. However, only a few approaches allow such links using single-neuron recordings in vivo (Acosta et al. 2014) or ex vivo (Vrontou et al. 2013) combined with marker analysis in single identified cells. As the experimental complexity of these approaches is high, we still lack a comprehensive translation between the electrophysiologically defined classes of nociceptors in rodents and their molecular markers. The link between the functionally defined classes of nociceptors in primates and their molecular markers is even more problematic as the rodent markers might not match those in primates such as for the Mrgpr family, and also electrophysiological classes might not completely overlap. The main task for future experimental work is therefore linking functionally defined nociceptor classes to their adequate markers.

2.1 Electrophysiologically Classified Functional Groups of Primary Afferents in Primates

In human and experimental primates, nociceptors have been separated into mechanosensitive (Perl 1996) and mechano-insensitive ("silent") nociceptors (Meyer et al. 1991; Schmidt et al. 1994, 1995). The mechanosensitive nociceptors have been further categorized into those with a rapid ("QC") and a slow ("SC") response to a fast heating stimulus (Meyer and Campbell 1981) (see Fig. 1, left column). Also, Aδ nociceptors have been classified in a similar fashion (Adriaensen et al. 1983; Meyer et al. 1991; Davis et al. 1993). The key question concerning potential roles in itch would be the activation of certain fiber classes by known pruritic agents. Data that support such links in primates exist for the stimulation with histamine and to a lesser degree with the application of cowhage.

C-fibers responding to histamine iontophoresis in parallel to the itch ratings of subjects have been discovered among the group of mechano-insensitive C-afferents (Schmelz et al. 1997), suggesting that there is a specific pathway for itch. In contrast, the most common type of C-fibers, mechano-heat-responsive nociceptors (CMH or polymodal nociceptors), is either insensitive to histamine or only weakly activated by this stimulus (Schmelz et al. 2003). Hence, this fiber type cannot account for the prolonged itch induced by the iontophoretic application of histamine. Yet, when histamine is injected intracutaneously, polymodal nociceptors are also activated for several minutes (Johanek et al. 2008). Thus, a contribution of this fiber class to histamine-induced itch cannot be entirely ruled out.

The histamine-sensitive pruriceptors among the mechano-insensitive C-nociceptors are characterized by a particularly low conduction velocity, large innervation territories, mechanical unresponsiveness, and high transcutaneous electrical thresholds (Schmelz et al. 1997, 2003; Schmidt et al. 2002). In line with the large innervation territories of these fibers, two-point discrimination for histamine-induced itch is poor (15 cm in the upper arm) (Wahlgren and Ekblom 1996). The excellent locognosia for histamine-induced itch in the hand (Koltzenburg

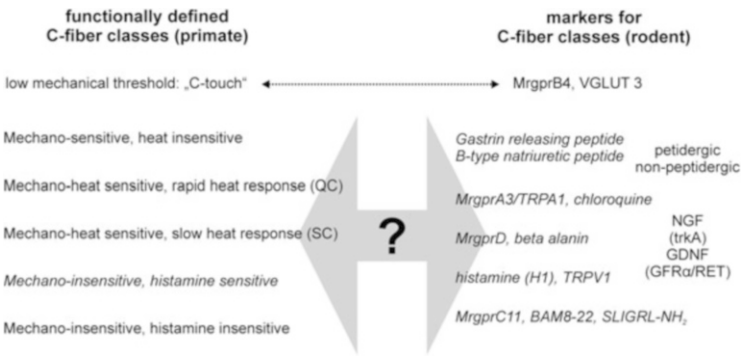

Fig. 1 Lack of corresponding functionally defined C-fiber classes in primates and molecular markers in rodents. Only for the markers of Mas-related G-protein-coupled receptor B4 (MrgprB4) and vesicular glutamate transporter (VGLUT3) has the link to the low-threshold C-fibers ("C-touch") been established. Yet there is no link of identified markers of pruriceptors on the *right* and electrophysiologically defined C-fiber classes in primates

et al. 1993) might therefore be based on central processing compensating for the low spatial resolution in the periphery.

The relative prevalence of the different C-fiber types in human skin nerves has been estimated from recordings in the superficial peroneal nerve (Schmidt et al. 1997). Polymodal nociceptors, which respond to mechanical, heat, and chemical stimuli, are about four times as abundant as the mechano-insensitive nociceptors in young healthy volunteers, but their proportion decreases in the elderly (2.5 times) (Namer et al. 2009). Mechano-insensitive nociceptors (Schmidt et al. 1995) are activated by chemical stimuli (Schmelz et al. 2000b) and can be sensitized to mechanical stimulation in the presence of inflammation (Schmidt et al. 1995; Schmelz et al. 2000b). Among the mechano-insensitive afferent C-fibers, only a subset of units shows a strong and sustained response to histamine. They comprise about 20 % of the mechano-insensitive class of C-fibers, i.e., about 5 % of all C-fibers in the superficial peroneal nerve. Specific activation of histamine-positive chemonociceptors by PgE_2 (Schmelz et al. 2003) in combination with the pruritogenic effects of prostaglandins (Neisius et al. 2002) provides a strong argument for a specific neuronal system for the itch sensation which is separate from the pain pathway.

The axon reflex flare is a neurogenic vasodilation that characteristically surrounds a histamine stimulation site; it is induced by neuropeptide release from mechano-insensitive C-fibers (Schmelz et al. 2000a). The absence of an axon reflex flare therefore suggests that the itch is independent of histamine-sensitive C-fibers. Indeed, itch was induced by papain in an early study in the absence of a flare response indicating a histamine-independent action (Hägermark 1973). Itch without axon reflex flare can also be elicited by weak electrical stimulation (Shelley and Arthur 1957; Ikoma et al. 2005), providing further evidence that the sensation of itch can be dissociated from cutaneous vasodilation.

Cowhage spicules inserted into human skin produce itch in an intensity which is comparable to that following histamine application (LaMotte et al. 2009; Sikand et al. 2009). However, mechano-heat-responsive "polymodal" C-fiber afferents, the most common type of afferent C-fibers in the human skin (Schmidt et al. 1995), can be activated by cowhage in the cat (Tuckett and Wei 1987) and, according to recent studies, also in nonhuman primates (Johanek et al. 2007, 2008) and in human volunteers (Namer et al. 2008) (Fig. 2).

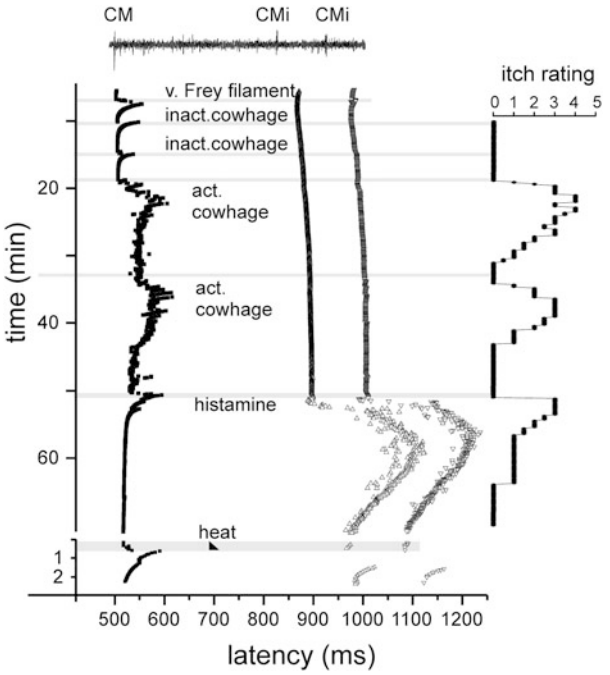

Fig. 2 Specimen of a multifiber recording from a mechano-responsive (CM) and two mechano-insensitive nociceptors (CMi) in human (raw signal with marked action potential on *top*). Conduction latencies of these three marked fibers (*filled square*, *open triangles*) in response to successive electrical stimulation at the receptive field are plotted from *top* to *bottom*. When activated by mechanical (v. Frey filament, inactivated cowhage spicules), chemical (active cowhage, histamine), or heat test stimuli (*black triangle*), C-fibers exhibit an activity-dependent increase of response latency followed by a gradual normalization ("marking"). The mechano-responsive fiber is activated during mechanical stimulation with the v. Frey filament and during application of inactive cowhage, but lasting activation is only seen after application of active cowhage. In contrast, the mechano-insensitive fibers do not respond to cowhage stimulation, but are activated following histamine iontophoresis.

At the *right side* of the panel, the itch ratings of the subject are depicted which were assessed during this experiment. Ratings are given on a numerical rating scale from 0 (0 = no itch) to 10 (10 = maximal imaginable itch). Inactive cowhage does not evoke any itch, whereas active cowhage and histamine evoke itch of similar time course and intensity, mirroring nicely the activation pattern of the fibers. Modified from Namer et al. (2008)

These fibers are unresponsive to histamine and not involved in sustained axon reflex flare reactions (Schmelz et al. 2000b). This is consistent with the observation that cowhage-induced itch is not accompanied by a widespread axon reflex flare (Shelley and Arthur 1955, 1957; Johanek et al. 2007). While in humans the segregation between histamine-positive mechano-insensitive fibers and cowhage-positive mechanosensitive fibers is clear-cut, in monkeys mechanosensitive C-fibers also responded to histamine (Johanek et al. 2008). The different histamine responses might be explained by higher histamine concentrations upon intradermal injection vs. iontophoresis.

Aδ fibers responding to cowhage insertion for several minutes (Ringkamp et al. 2011) suggest an additional role of afferent input from myelinated fibers. Differential block of myelinated afferents does not reduce capsaicin-induced pain and only slightly reduces histamine-induced itch; however, it massively reduces cowhage-induced itch at least in some of the subjects (Ringkamp et al. 2011). The exact role of Aδ fiber input for the cowhage-induced itch is unclear as reduced skin temperature induced by the nerve blocking maneuver in these experiments might also have reduced cowhage-induced activation.

The active compound, the cysteine protease mucunain, has been identified lately and shown to activate proteinase-activated receptor 2 (PAR 2) and even more potently PAR 4 (Reddy et al. 2008). Given that cowhage spicules can activate a large proportion of polymodal nociceptors, we face a major problem to explain why activation of these fibers by heat or by scratching actually inhibits itch, whereas activation by cowhage produces it. On the other hand, data from monkeys suggest that mechano-heat-sensitive C-nociceptors with a fast response to heating (“QC”) might play a more important role in mediating cowhage-induced itch (Johanek et al. 2008). One might therefore still hypothesize that there is a certain selectivity among mechano-heat-sensitive C-nociceptors for cowhage that would allow the central nervous system to separate nociceptive from pruriceptive stimuli (LaMotte et al. 2014).

2.2 Structural Markers for Classification of Primary Afferents

A variety of marker proteins on sensory afferents are currently used to separate classes of primary afferent sensory neurons in rodents. These markers include sensory transduction proteins such as vanilloid receptors (TRPV1, TRPA1) and purinergic receptors (P2X3), neuropeptides such as substance P and calcitonin gene-related peptide (CGRP), receptors for growth factors such as nerve growth factor (NGF) and glia-derived neurotrophic factor (GDNF), receptors of unknown function such as the family of Mas-related G-protein-coupled receptors (Mrgpr), but also certain staining characteristics such as lectin binding (IB4) (see Fig. 1; right column). Among these markers, there are some with particular relevance for neurons involved in the itch sensation (Akiyama and Carstens 2013). These include histamine H1 receptors, the neuropeptides gastrin-releasing peptide and B-type natriuretic peptide, and the several members of the Mrgpr family (A3, D, C11).

Unfortunately, there are only a few examples for a convincing link between the rodent marker and functional neuronal class in primates (see Fig. 1). For a very special subtype of afferent C-fiber, the very low threshold, the so-called C-touch fibers (CT afferents) (Ackerley et al. 2014), links to the expression of MrgprB4 (Vrontou et al. 2013) and to the expression of the glutamate transporter VGLUT3 (Seal et al. 2009) have been described.

In the realm of itch processing, however, we do not have such convincing ties between molecular markers used in *rodents* and fiber classes in the *primate*. There is evidence that cowhage induces itch via the activation of proteinase-activated receptors (Reddy et al. 2008). Thus, the activation of QC-type mechano-heat-sensitive nociceptors by cowhage (Johanek et al. 2008) might be a possible link to MrgprC11 (Akiyama and Carstens 2013). Beta-alanine, the activator of MrgprD, does provoke itch in humans (Han et al. 2012; Liu et al. 2012; Qu et al. 2014), but the responsible fiber class is unknown. This is similarly true for BAM8-22, the activator of MrgprC11, which also provokes histamine-independent itch in humans (Sikand et al. 2011) probably via activating MrgprX1, the human homologue of rodent MrgprC11.

The neuropeptides, B-type natriuretic peptide and gastrin-releasing peptide, have both been suggested to represent an itch-specific cotransmitter of the primary afferent pruriceptive neuron (Sun et al. 2009; Mishra and Hoon 2013). Regardless of the current debate (Liu et al. 2014) about the exact role of these two peptides, both will prove highly important to study function/structure relationships by identifying the neurons being involved in itch. On the other hand, therapeutic implications might not be particularly strong as both gastrin-releasing peptide (Sakamoto 2011) and B-type natriuretic peptide (Zhang et al. 2010) have also functions beyond itch processing.

Although we have increased our knowledge on itch-specific targets enormously over the last few years, we still did not succeed to translate the targets and markers from rodents. This is a highly complex task as the traditional markers such as peptidergic (NGF-dependent) vs. non-peptidergic (GDNF-dependent, IB4-positive) nociceptors work fine in the mouse, but do not completely translate into rat or primate. Ultimately, we have to take into account that the crucial functional unit of nociception or pruriception is not a marker molecule, but the cell (Reichling et al. 2013) with its particular peripheral receptors and central connections. Thus, based on the broad knowledge provided by basic research in rodents, we need to translate mediators, markers, and neuronal classes into humans and thereby identify the crucial targets to treat chronic itch in patients.

2.3 Spatial Specificity for Itch?

The spatial characteristics of an itching stimulus need to be considered as it may functionally convert an algogenic mediator into a pruritic mediator: capsaicin, when injected into the skin, is painful, but when applied very locally on a cowhage

spicule into the epidermis, it causes itch (Sikand et al. 2009). The highly localized stimulation in the epidermis strongly activates some of the local nociceptors, while their immediate neighbors remain silent, resulting in a mismatch signal of activation and absence of activation from this site. It has thus been hypothesized that this mismatch might be perceived by the central nervous system as itch (Namer et al. 2008). Teleologically, it is obvious that scratching behavior in the case of a highly localized superficial noxious focus is an adequate response as it can eliminate the presumed cause. Moreover, scratching activates *all* the mechanosensitive nociceptors in the stimulated area, and thus, the mismatch signal of activated and nonactivated nociceptors at this site is terminated. Therefore, it needs to be pointed out that pruritus cannot only be explained by itch-specific or itch-selective neurons (LaMotte et al. 2014) along the specificity theory. In addition, the pure spatial pattern of activated nociceptors might similarly underlie the itch sensation without any requirement of itch-specific primary afferent neurons (Fig. 3).

It is interesting to note that "specificity" is not only discussed for the neurons but also for mediators (Ross 2011). Capsaicin, the classic algogen, generally provokes pain when applied to the human skin. However, it induces itch when applied on the tip of an inactivated cowhage spicule (Sikand et al. 2009). Thus, the response of neurons to the algogen capsaicin might pose an argument against their specificity for itch. Alternatively, the itch evoked by capsaicin applied via a cowhage spicule might be an argument against the nociceptive specificity of capsaicin.

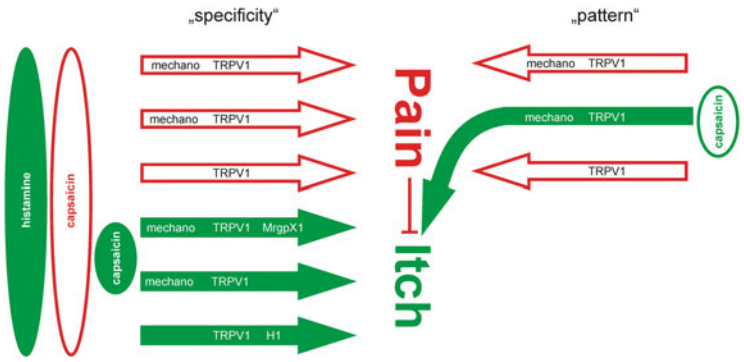

Fig. 3 Specificity versus pattern theory of itch: assuming specific nociceptive primary afferents (*open arrows*) and specific pruriceptive afferent fibers (*filled arrows*), one could easily explain itch induced by pruritogens such as histamine by activation of the specific histamine-sensitive pathway. Capsaicin would activate most of the specific nociceptors and pruriceptors and would cause pain according to the spinal inhibition of itch by pain. Very localized application of capsaicin might preferentially activate pruriceptors and thus could provoke itch. Alternatively, localized stimulation with capsaicin activates only a small number of nociceptors (*right column*: "pattern"). The pattern of activated and nonactivated nociceptors from a given skin site would be interpreted as itch at a spinal level

3 Spinal Processing of Itch

The concept of dedicated pruriceptive neurons has been extended by the results obtained from cat spinal cord recordings. A specific class of dorsal horn neurons projecting to the thalamus, which responds strongly to histamine administered to the skin by iontophoresis, has been demonstrated (Andrew and Craig 2001). The time course of these responses was similar to that of itch in humans and matched the responses of the peripheral C-itch fibers. These units were also unresponsive to mechanical stimulation and differed from the histamine-insensitive nociceptive units in lamina I of the spinal cord. In addition, their axons had a lower conduction velocity and anatomically distinct projections to the thalamus. The itch-selective units in lamina I of the spinal cord form a distinct pathway projecting to the posterior part of the ventromedial thalamic nucleus (VMpo) which projects to the dorsal insular cortex (Craig 2002), a region which has been shown to be involved in a variety of interoceptive modalities like thermoception, visceral sensations, thirst, and hunger.

Thus, the combination of dedicated peripheral and central neurons with a unique response pattern to pruritogenic mediators and anatomically distinct projections to the thalamus provides the basis for a specific neuronal pathway for itch.

This is also supported by studies performed with rodents. Dorsal horn neurons bearing the receptor for gastrin-releasing peptide (GRPR) have been identified as being crucial for the scratch behavior in a variety of itch models. There was some reduction of scratching by constitutively inactivating the gene encoding the GRP-receptor gene or pharmacologically blocking the receptor (Sun and Chen 2007). However, the selective deletion of the GRPR-bearing cells by a toxin linked to the GRPR ligand bombesin (bombesin-saporin) completely abolished the scratching behavior, whereas the nociceptive behavior was virtually unchanged (Sun et al. 2009). This indicates that GRPR-expressing dorsal horn neurons may be indispensable for the itch response in this species, though not necessarily only the GRPR receptor alone. However, data on bombesin-induced itch that could not be blocked via a GRPR agonist have shed some doubts on the GRPR specificity of the bombesin results (Su and Ko 2011). Moreover, the B-type natriuretic peptide has been suggested to be the cotransmitter of the primary afferent pruriceptors with GRP being the transmitter of secondary spinal cord neurons (Mishra and Hoon 2013). The exact role of the two peptides is currently debated (Liu et al. 2014). Another type of spinal interneuron involved in itch processing has recently been found. Inhibitory interneurons dependent on the transcription factor Bhlhb5 were described to be involved in itch processing (Ross et al. 2010). Cell-specific deletion of Bhlhb5 resulted in increased scratching to experimental itch stimuli and spontaneous scratching with skin lesions, whereas pain behavior was grossly unchanged (Ross et al. 2010). These results suggest that Bhlhb5-dependent GABAergic inhibitory interneurons are crucial modulators of scratch behavior.

In contrast to the above evidence for a specific pathway for itch, histamine-sensitive projection neurons in the monkey were found to also respond to mechanical stimuli and to capsaicin (Simone et al. 2004; Davidson et al. 2007). Also, in

rodents, overlapping between nociceptive and pruriceptive neuronal responses was found (Akiyama et al. 2009, 2010). This is not necessarily a contradiction to the concept of a "specific pathway." One has to distinguish between "selectivity" (i.e., only a subgroup of neurons responds to a particular pruritogenic substance) and "membrane specificity" (a subgroup of neurons responds only to a group of pruritogenic agents). "Membrane specificity" is not necessarily required for a "specific" or better "selective" pathway. A "selectivity hypothesis" for itch processing has been discussed before by several authors (McMahon and Koltzenburg 1992; Cevikbas et al. 2010; Davidson and Giesler 2010; Handwerker 2010; Patel and Dong 2010; Ma 2010; Ross 2011).

Interestingly, histamine-induced itch and cowhage-induced itch are not only processed separately in the primary afferent neurons, but the separation is maintained at the spinal level. Spinothalamic projection neurons in the dorsal horn could be separated into a histamine- and cowhage-responsive population without overlap (Davidson et al. 2007). Moreover, the thalamic projections of the two subgroups also differed. The histamine-sensitive pathway can be assumed to be selective for itch albeit the recordings in monkeys; STT neurons also suggest some mechanical and capsaicin sensitivity. In contrast, the cowhage-sensitive pathway may be regarded as unspecific as the activation of mechano-heat-responsive polymodal nociceptors is probably underlying the generation of cowhage-induced itch (Davidson and Giesler 2010).

4 Interactions Between Itch Pathways with Painful and Non-painful Stimuli

The inhibition of itch by painful stimuli has been experimentally demonstrated by the use of various painful thermal, mechanical, and chemical stimuli. Electrical stimulation via an array of pointed electrodes ("cutaneous field stimulation") has also been successfully used to inhibit histamine-induced itch for several hours in an area around a stimulated site of 20 cm in diameter. The large area of inhibition suggests a central mode of action (Nilsson et al. 1997). Consistent with these results, itch is suppressed inside the secondary zone of capsaicin-induced mechanical hyperalgesia (Brull et al. 1999). This central effect of nociceptor excitation by capsaicin should be clearly distinguished from the neurotoxic effect of higher concentrations of capsaicin which destroy most C-fiber terminals, including fibers that mediate itch (Simone et al. 1998). The latter mechanism, therefore, also abolishes pruritus locally, until the nerve terminals are regenerated.

Not only is itch inhibited by enhanced input of pain stimuli, but vice versa, inhibition of pain processing may reduce its inhibitory effect and thus enhance itch (Atanassoff et al. 1999). This phenomenon is not only particularly relevant to the spinally administered μ-opioid receptor agonists which induce segmental analgesia often combined with segmental pruritus (Andrew et al. 2003) but has also been confirmed in animal experiments (Nojima et al. 2003). Recent result suggests that the analgesic and pruritic effects of μ-opioids might be mediated by different

isoforms (MOR1 vs. MOR1D) which would have major therapeutic implications (Liu et al. 2011). Conversely, κ-opioid antagonists have been found to enhance itch (Kamei and Nagase 2001). In line with these results, the κ-opioid agonist nalbuphine has been shown to reduce μ-opioid-induced pruritus (Kjellberg and Tramer 2001) and has already been tested successfully in chronic itch patients using nalfurafine, a newly developed κ-opioid agonist (Kumagai et al. 2010).

Central inhibition of itch can also be achieved by cold stimulation. In addition, cooling has a peripheral inhibitory effect: the histamine-induced activation of nociceptors can be reduced by cooling (Mizumura and Koda 1999). Also in humans, cooling of a histamine-treated skin site reduced the activity of the primary afferents and decreased the area of "itchy skin" or "hyperkinesis" around the application site (Heyer et al. 1995). Paradoxically, histamine-induced itch increases during the cooling process (Valet et al. 2008), and this increase can be used as an experimental model for central imaging (Napadow et al. 2014). Increasing the skin temperature in the noxious range results in a painful stimulus that will reduce itch by central inhibition of pruritus (Schmelz 2002).

Recent work on the antipruritic effects of subpopulations of primary nociceptive afferents indicates that the input from the VGLUT2-positive subpopulation is especially crucial for the inhibition of itch behavior by painful stimuli (Lagerström et al. 2010; Liu et al. 2010). When VGLUT2 release and, thereby, glutamate release were selectively eliminated in NaV1.8-positive nociceptors using conditional genetic knockout techniques, inflammatory and neuropathic pain responses were grossly abolished, but spontaneous scratching behavior and increased experimental itch were massively enhanced (Liu et al. 2010). Most interestingly, capsaicin-induced pain behavior was changed into scratching behavior in these mice suggesting that the lack of noxious input via VGLUT2 positive nociceptors disinhibited itch (Liu et al. 2010). The exact nature of the crucial nociceptor class is still unclear, as another group did not find increased scratching when VGLUT2 was selectively eliminated in TRPV1-positive primary afferent neurons (Lagerström et al. 2010).

5 Sensitization to Itch

Independent of nociceptor or pruriceptor classes, inflammatory mediators such as histamine, bradykinin, serotonin, prostanoids, and low pH are assumed to activate and sensitize neurons for prolonged periods. However, acute effects of inflammatory mediators alone cannot explain the prolonged changes of neuronal sensitivity observed in inflammatory processes. Regulation of gene expression induced by trophic factors, such as nerve growth factor (NGF), has been shown to play a major role in persistently increased neuronal sensitivity and chronic inflammatory pain (Lane et al. 2010). NGF is released in the periphery and specifically binds to trkA receptors located on nociceptive nerve endings. It is then conveyed via the retrograde axonal transport to the dorsal root ganglion where gene expression of neuropeptides and receptor molecules, such as the vanilloid receptor (TRPV1), is

increased. Trophic factors also initiate nerve fiber sprouting and thus change the morphology of sensory neurons. Sprouting of epidermal nerve fibers in combination with localized *pain* and hypersensitivity has been reported before (Bohm-Starke et al. 2001). Thus, similar mechanisms appear to be underlying chronic itch and chronic pain (Yosipovitch et al. 2007; Handwerker and Schmelz 2009).

Increased intradermal nerve fiber density has been found in patients with chronic pruritus (Urashima and Mihara 1998). In addition, increased epidermal levels of neurotrophin 4 (NT_4) have been found in patients with atopic dermatitis (Grewe et al. 2000; Yamaguchi et al. 2009), and massively increased serum levels of NGF and SP have been found to correlate with the severity of the disease in such patients (Toyoda et al. 2002). These similarities between localized painful and pruritic lesions might suggest that on a peripheral level similar mechanisms of nociceptor sprouting and sensitization exist. Interestingly, intradermal injection of NGF in human volunteers not only sensitizes the treated skin to mechanical and heat stimuli for several weeks (Rukwied et al. 2010), but mechanical hyperalgesia also correlates to sensitized itch upon cowhage but not histamine stimulation (Rukwied et al. 2013b). Thus, long-term sensitization of mechanosensitive nociceptors that is underlying the observed hyperalgesia might also contribute to the increased itch sensation. This observation would be compatible with the itch induction via the pattern theory (see Fig. 3). When NGF sensitization is combined with inflammation (sunburn), the subjects even report moderate spontaneous pain (Rukwied et al. 2013a). It will be highly interesting to test whether such a mechanism is also contributing to clinically relevant inflammation itch such as contact dermatitis or atopic dermatitis.

6 Perspectives

We have seen an enormous increase of knowledge on specific pathways for itch including markers of primary afferent and spinal neurons and transmitters. These results have provided numerous candidates that might underlie or contribute to the clinical chronic itch conditions. In order to translate the findings from rodents to primates and finally to patients, we need to correlate the published markers to neuronal populations that are operational in humans. In this attempt we need to be open for results that do not completely follow the specificity theory of itch but rather indicate that nociceptors can also generate itch when activated in the correct spatial or temporal pattern. Thus, future therapy for chronic itch might be based on both itch-specific targets following the specificity theory of itch and nociceptor-related targets following the pattern theory.

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Neuroimaging of Itch as a Tool of Assessment of Chronic Itch and Its Management

Gil Yosipovitch and Hideki Mochizuki

Contents

Abstract

Chronic itch is a multidimensional physical state strongly associated with emotional and cognitive aspects of suffering that causes the urge to scratch. Pathophysiology, psychological stress, and social milieu can influence itch. Here, we review brain neuroimaging research in humans that detects functional and anatomic changes in health and disease states. New data are emerging that are shaping our understanding of itch mechanisms and scratching—the behavioral response as well as the effect of treatments and brain dynamics during itch. Future developments will continue to expand our knowledge of itch mechanisms, allowing translation to clinical assessment and novel therapies focused on the brain, the final relay of itch transmission.

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Keywords
Precuneus • Insula • Claustrum • Craving • Kappa opioids • ESRD • Contagious itch

Abbreviations

ACC	Anterior cingulate cortex
AD	Atopic dermatitis
ASL	Arterial spin labeling
BA6	Brodmann area 6
BA44	Brodmann area 44
DLPFC	Dorsolateral prefrontal cortex
DTI	Diffusion tensor imaging
EASI	Eczema Area and Severity Index
ESRD	End-stage renal disease
fMRI	Functional magnetic resonance imaging
MEG	Magnetoencephalography
NAc	Nucleus accumbens
PAG	Periaqueductal gray matter
PAR2	Protease-activated receptor 2
PFC	Prefrontal cortex
PET	Positron emission tomography
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
VBM	Voxel-based morphometry
VPI	Ventral posterior inferior
VPL	Ventral posterior lateral
VTA	Ventral tegmentum

1 Introduction

Positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and magnetoencephalography (MEG) have been commonly used during itch episodes to objectively document pruritus (Hsieh et al. 1994; Darsow et al. 2000; Drzezga et al. 2001; Mochizuki et al. 2003, 2007, 2009; Herde et al. 2007; Leknes et al. 2007; Papoiu et al. 2012). The majority of the studies focused on healthy subjects. Only a few studies have assessed brain processing of itchy stimuli in patients with chronic pruritus and demonstrated that it differs from

that of healthy controls (Schneider et al. 2008; Ishiuji et al. 2009). As yet there is no clear itch neuromatrix similar to the pain neuromatrix. However, it is clear that there are unique areas that differentiate pain from itch. Here, we review progress in neuroimaging research that is contributing to the development of tools that are clinically relevant to future management of chronic itch patients.

2 Itch Transmission to the Superior Centers of the Central Nervous System

7Two main neuronal pathways have been described for itch transmission: one mediated by histamine and the other by protease-activated receptors PAR2(4) that can be exogenously stimulated by spicules of cowhage (*Mucuna pruriens*). The continuing spinothalamic pathways ascend via the lateral spinothalamic tract and (mostly) maintain their specialization up to their thalamic (third neuron) stations (Davidson et al. 2009, 2012). Only recently with development of higher-resolution fMRI has the thalamus been shown as the first relay in the brain for itch stimuli associated with both histamine and cowhage. It is known that the spinothalamic neurons project in the ventral posterior lateral (VPL), ventral posterior inferior (VPI), and posterior nuclei, while cowhage-sensitive neurons additionally end in the supragenicular and medial geniculate nuclei (Davidson et al. 2012). Functional MRI data demonstrated that cowhage itch activated more strongly than histamine, in particular a thalamic area consistent with the location of the mediodorsal nucleus (Leknes et al. 2007; Papoiu et al. 2012) which is connected with the orbitofrontal area and the limbic system. Of note, a dysfunction of this circuit was suggested to occur in processing itch in atopic patients (Leknes et al. 2007).

3 Itch Transmission from the Thalamus to the Cortex

As yet the exact projections of the thalamic neurons conveying itch information from the thalamus to the cerebral cortex have not been identified. Future studies assessing brain connectivity during itch stimuli will provide us with better insights on these projections.

4 Cortical Regions that Are Important During Itch Processing

Numerous brain imaging studies, irrespective of the investigative modalities, demonstrated that there are several cortical regions in humans that are considered to be important for the perception of itch. The primary and secondary somatosensory cortices (S1, S2), the insula and Anterior cingulate cortex (ACC), and the prefrontal cortex (PFC) are commonly activated, often bilaterally during itch stimuli. These areas are not exclusively involved in itch and are known to be highly activated by pain and other sensory stimuli. The activity of these areas reflects the

multidimensional character of the itch experience and the complexity of itch processing in the brain. Areas such as the ACC and insula are associated with an emotional–affective response recruiting deep-seated areas of the limbic system, areas connected to craving and unpleasantness as well as pleasure and addiction. Itch is a bothersome, intrusive, acute sensation, requiring immediate action; thus, major arms of the cerebral itch response are involved in refocusing attention, planning the motor action, and seeking itch relief. Other areas that have shown high activity during itch are the premotor, motor, and supplementary motor areas as well as the cerebellum which may control the urge to scratch. The precuneus (medial parietal cortex) is prominently activated by the sensation of itch (Mochizuki et al. 2009; Papoiu et al. 2012). This area is related to memory retrieval, visuospatial processing, and self-awareness and has rarely been reported in imaging of pain processing. It is considered part of the default network. The insula has a paramount role in processing itch information; it is a cortical region linked to salience, self-awareness/interoception, and addiction (among others). The insula is considered a major hub for processing viscerоceptive and interoceptive inputs and is significantly involved in the processing of pain and, especially, in assessing stimulus intensity. The bilateral insula is activated in patients with end-stage renal disease (ESRD) pruritus at rest.

The claustrum is a discrete gray matter area whose role has recently been emphasized in itch processing. The functional specialization and connectivity of the claustrum seem very fitting for a region involved with itch sensing since it has the capability to analyze, compare, and integrate sensory information from various inputs; it is connected to almost all areas of the cortex, but (especially) with the somatosensory cortex, thalamus, and limbic structures (cingulate cortex, hippocampus, amygdala). The claustrum is closely linked, functionally and anatomically, with the insula. It is activated more extensively by a complex, transient, fluctuating itch stimulus, such as cowhage, than by a rather constant stimulus like histamine (Papoiu et al. 2012). Activation of the claustrum (as well as of the insula) is largely correlated with the perceived itch intensity, although some discrete areas are activated irrespective of itch stimulus intensity (Papoiu et al. 2012). The insula and claustrum (in particular) are activated continuously, while itch intensity varied and is fully activated bilaterally when histamine and cowhage stimuli are administered at the same time (Papoiu et al. 2012). These features suggest a principal role in itch processing for these regions.

5 Prefrontal and Limbic Control

Numerous imaging studies have demonstrated activation of the PFC by applying itch stimuli, suggesting that this region can regulate the perception and behavioral expression of itch in humans in a manner very similar to pain. The PFC is connected to limbic regions that regulate motivation and emotion. Coactivation of these regions during itch stimuli implies that the motivational and emotional aspects of itch are also regulated by this network. A recent imaging study of the cerebral processing of scratching suggested that the dorsolateral prefrontal cortex (DLPFC) controls the

itch response via possible connections with the amygdala to suppress the itch (Yosipovitch et al. 2008). These areas could be targets for drug therapies and psychological biobehavioral treatments for chronic itch.

Frontal limbic regions reciprocally connected to the brain stem via periaqueductal gray matter (PAG), nucleus accumbens (NAc), and tegmentum can inhibit or exacerbate the itch experience via inhibition or activation of neurons in the spinal cord (Carstens 1997; Millan 2002; Davidson and Giesler 2010).

6 Differential Cerebral Processing of Histamine and Cowhage Itches

In healthy individuals, the cerebral representation of cowhage itch displays a common core of activation with that of histamine itch while showing certain features that are clearly unique (Fig. 1). These activations provide a comprehensive detail of itch processing in the brain of the two major itch pathways. Of note,

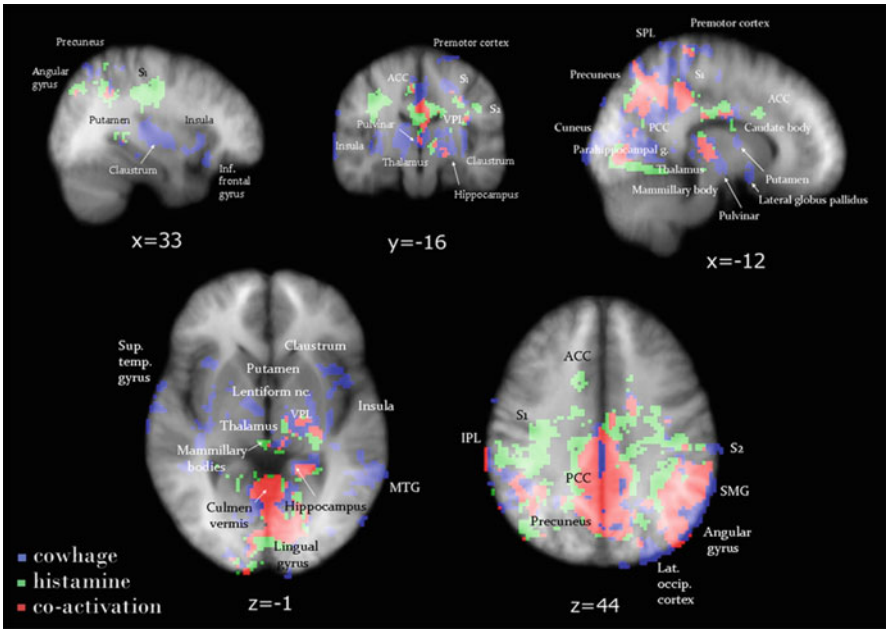

Fig. 1 The overlap of brain activations induced by histamine itch (in *green*) and by cowhage itch (in *blue*) illustrates the regions coactivated (in *red*) and distinct areas activated separately by the two itch pathways. Standard Talairach space coordinates. The color tones displayed correspond to Z score values as shown in the color bar. *ACC* anterior cingulate cortex, *PCC* posterior cingulate cortex, *SPL* superior parietal lobule, *M1* primary motor cortex, *S1* primary somatosensory area, *SMG* supramarginal gyrus, *MTG* middle temporal gyrus, *IPL* inferior parietal lobule, *S2* secondary somatosensory area, *VPL* ventral posterior lateral nucleus (of thalamus) *(can be appended separately; from NeuroImage 59(4) 2012)*

cowhage evokes a more extensive activation of the insula, claustrum, globus pallidus, caudate body, putamen, and thalamic nuclei on the contralateral side of the stimuli (Fig. 1). These differences may be related to not only an intrinsic specificity in cortical projection, but also to the fluctuating quality and associated nociceptive signaling (e.g., stinging, burning) elicited by cowhage. These sensations are frequently reported in many cases of chronic itch (Yosipovitch et al. 2002).

7 Brain Processing of Chronic Itch

A few neuroimaging studies have been performed in patients with chronic itch. These studies have revealed altered function (Schneider et al. 2008; Ishiuji et al. 2009; Papoiu et al. 2014); one study also demonstrated altered structure in the frontolimbic regions compared to healthy controls.

In atopic dermatitis, which is the most common skin disease causing chronic itch, activation of the ACC and DLPFC is directly correlated with disease severity (as measured by the Eczema Area and Severity Index (EASI) score, a standardized validated clinical tool). Intensity of histamine-induced itch correlates with activations in the ACC and insula. Overall, the pattern of association between activation and perceived itch intensity is different in healthy volunteers (Schneider et al. 2008; Ishiuji et al. 2009; Yosipovitch group, unpublished). The distinction between the patterns evoked by histamine and cowhage itches, clearly identified in healthy individuals, appears to be blurred in chronic itch diseases (ESRD and AD). Recently, an investigation of structural and functional perfusion differences between ESRD patients with chronic pruritus and healthy individuals found a significant thinning of the gray matter in the thalamus, insula, ACC, precuneus, and caudate body (areas involved in itch processing) in the ESRD patients. Assessment was by voxel-based morphometry (VBM), a technique that quantitates gray matter densities. These MR-based changes were not demonstrated in two chronic itch states: atopic eczema and psoriasis (unpublished data). In pruritic patients with ESRD, persistent perfusion increases at baseline in the insula, ACC, claustrum, amygdala, hippocampus, and NAc—areas that are known to be highly activated by itch (Fig. 2). Interestingly, baseline brain perfusion in chronic itch of atopics and psoriatics does not differ from that in healthy subjects. These results further suggest that chronic itch states differ in brain imaging patterns. Moreover, the processing of cowhage itch appeared altered in ESRD, while no significant differences could be demonstrated in the processing of histamine-induced itch. In ESRD pruritus, multiple brain activations appear to work either directly or are inversely correlated with perceived itch intensity, suggesting a dual modulation of itch perception. These unique features can be facilitated by the reduced gray matter thickness in ESRD affecting critical areas involved in itch processing, thus revealing a form of neocortical plasticity. As with chronic pain, central neuronal remodeling occurs (Parise et al. 2014). In ESRD, it appears that the PAR2-mediated itch pathway is already overstimulated, in association with an overexpression of PAR2 in the skin.

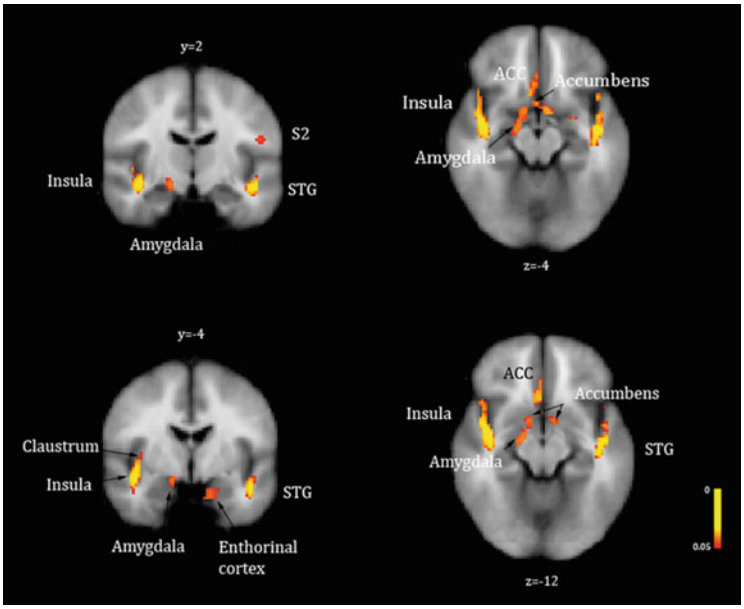

Fig. 2 Baseline state (resting) perfusion increases or brain activations identified in ESRD patients with chronic pruritus in significant contrast with healthy volunteers. Brain perfusion was higher at baseline in ESRD patients with chronic pruritus compared to healthy individuals in the insula, claustrum, ACC, amygdala, entorhinal cortex, and subcallosal gray matter—nucleus accumbens. Arterial spin labeling fMRI; $p < 0.05$. *STG* superior temporal gyrus, *S2* secondary somatosensory area, *ACC* anterior cingulate cortex

This may lead to a tonic inhibition of cortical processing of acute cowhage itch when induced in the preexistent context of ESRD.

A central "top-to-bottom" inhibition of itch is drawn from the parallel descending pathway for suppression of pain and proposes that the PAG modulates the activity of spinal interneurons. According to this model, descending inputs directed toward itch receptive neurons in the dorsal horn exert an inhibitory action, effectively silencing them (Carstens 1997; Davidson and Giesler 2010). Another possibility is that the cortical projection of itch information into S1/S2 may be inhibited via cortico-cortical inhibitory loops, in a similar fashion to mechanisms that have been known to operate in chronic pain (Henry et al. 2011). Our recent findings in ESRD patients possibly suggest that a tonic inhibition may be exerted at the neocortical level to selectively limit the receptive fields for PAR2-mediated itch processing in S1, precuneus, and insula. These findings are of significant interest because they offer insight into mechanisms the brain may employ to process and modulate itch sensation. Contrary to a widely accepted paradigm in the neuroimaging literature, it is thus possible that a higher intensity itch does not necessarily translate into a higher or more extensive activation of the cerebral cortex. Moreover, longitudinal studies from patients with chronic pain, which shares many similarities with chronic itch, suggest that abnormalities in gray matter densities

within brain regions such as the ACC and insula can resolve after treatments that reduce the symptom (Rodriguez-Raecke et al. 2009).

8 Craving for Itch Relief and Its Cerebral Mechanisms

The sensation of itch and the immediate craving for itch relief manifested as the urge to scratch are inseparable. Four recent fMRI studies have investigated the cerebral processing of scratching. In three of them, experimenters scratched the subjects' skins using brushes and copper plates (Yosipovitch et al. 2007, 2008; Vierow et al. 2009; Mochizuki et al. 2014). In the fourth one, visualized active scratching provided a more robust scratch response (Papoiu et al. 2014). The observed brain regions common to these studies were the PFC, ACC, insula, secondary S2, and cerebellum (Fig. 3). The significant activations of the PFC and insula during scratching are interesting since these regions, in particular the PFC, are less sensitive to other tactile stimuli such as a vibrotactile stimulus (Gelnar et al. 1999; Seitz and Roland 1992; Coghill et al. 1994; Golaszewski et al. 2002), Hagen and Pardo 2002; Burton et al. 2004). Several clinical studies on patients with

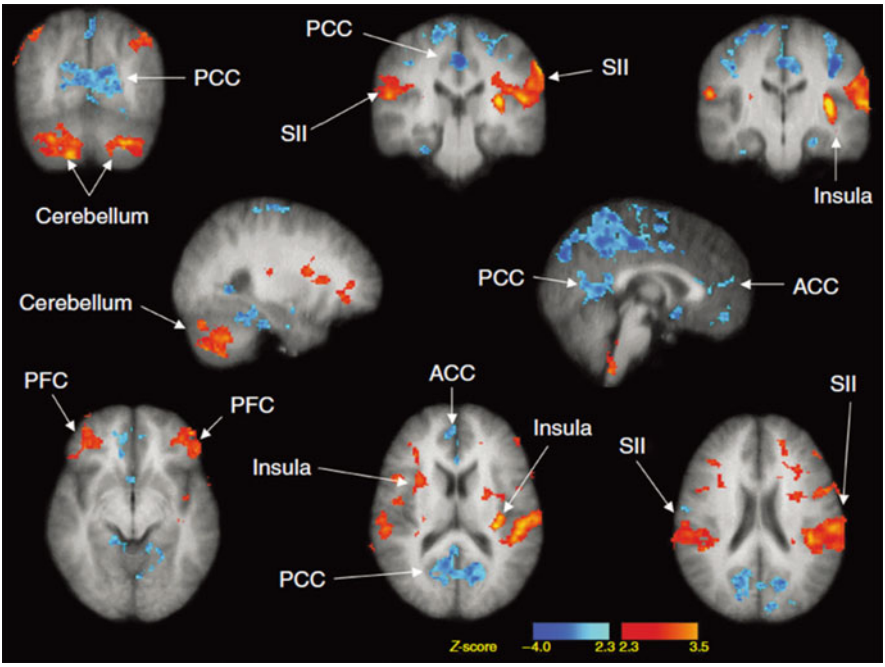

Fig. 3 Brain regions activated by scratching the skin. *Red* and *blue* regions were where neural activity was significantly increased and decreased during scratching, respectively. *PCC* posterior cingulate cortex, *SII* secondary somatosensory cortex, *ACC* anterior cingulate cortex, *PFC* prefrontal cortex

addictive behaviors have also demonstrated the importance of the PFC in motivation and reward. Reward circuits have been linked with the pleasurability of scratching and may play an important role in itch inhibition, as well as in the formation of "vicious" itch–scratch cycles. Recent findings suggest that the reward system of the midbrain, more specifically the ventral tegmentum (VTA), and substantia nigra, as well as the NAc, may play a role in the urge to scratch and the subsequent satisfaction (pleasure) derived from scratching, via connections with the insula, ACC, and striatum (Papoiu et al. 2014; Mochizuki et al. 2013, 2014). The involvement of VTA and NAc underscores the addictive nature of the itch–scratch cycle and also suggests a role for the dopaminergic system in itch relief (Papoiu et al. 2013, 2014).

9 Role of the PAG in Itch Inhibition

The PAG has been considered the major descending inhibitory control of pain (Millan 2002). A PET study showed increased PAG activity, while itch sensation was reduced, by applying pain stimuli (Mochizuki et al. 2003). Thus, it is likely that descending inhibitory control has the potential to inhibit itch sensation. However, our recent fMRI studies of scratching did not show activation of the PAG; in fact, deactivation of the PAG was noted with high Z scores. Additionally, there was significant activation of the ventral tegmentum (which is closely related to the PAG) during scratching an itch. Thus, more precise investigations using electrophysiological techniques with animals, and fMRI with higher spatial resolution (e.g., 7 T), will be necessary to clarify whether descending PAG inhibitory control is associated with itch inhibition by scratching.

10 Modulation of Itch Targeting the Brain

Currently, there are no published studies on the effect of antipruritic drugs against itch-related changes in brain wiring. We recently demonstrated that butorphanol, a kappa opioid agonist and mu opioid antagonist, known to exert antipruritic effects in the spinal cord (Dawn and Yosipovitch 2006) completely suppressed the itch induced experimentally with histamine (Papoiu et al. 2015). The functional MRI data showed that, in comparison with the placebo, butorphanol produced a bilateral deactivation of the claustrum, insula, and putamen, areas described to be activated during itch processing. The inhibition of histamine itch by butorphanol was paralleled by well-defined, significant activations which mapped to nucleus accumbens bilaterally and to a subcallosal gray matter area on the midline consistent with the location of septal nuclei. Our results indicate that the antipruritic action of butorphanol is mediated by these two formations, known to express a high density of κ opioid receptors (Peckys and Landwehrmeyer 1999; Peckys and Hurd 2001) on which it is likely the κ opioid *agonist*, butorphanol, acts. This is the

first clear identification of discrete structures within the human brain capable of exerting itch suppressions upon opioid activation.

Another study assessed the effect of acupuncture on itch-evoked activation and demonstrated reduced activation in the insula, putamen, and premotor and prefrontal cortical areas (Napadow et al. 2012). An overactive limbic system (anterior cingulate cortex–amygdala–nucleus accumbens) may reflect a more intense, unbalanced craving for itch relief, accompanied by activations in the insula (as seen in ESRD pruritus at baseline) as well as an increase in acute itch induction in atopic eczema itch and psoriatic itch. This leads to the amplification of compulsive scratching behavior and more distress. Therefore, addressing the emotional and psychological suffering by targeting these areas is a cornerstone for building a successful therapy for pruritus. Brain areas involved in self-awareness (precuneus) and self-perception (insula) are involved, confirming the observation that itch is a very intrusive and disturbing sensation, perturbing the well-being of the person.

Neuroimaging studies using quantitative arterial spin labeling (ASL) enable us to measure regional changes in cerebral blood flow in itch states associated with slow (tonic) activities and provide insight not only on the physiological responses (or their dysfunction), but also serve as a window into the supramodal functions of the mind and psyche. Cognitive and emotional aspects of the itch experience influence the higher-level integration of physical stimuli. A successful treatment would need to target and interrupt the vicious itch–scratch cycle and offer a solution for the intense, "amplified" craving for relief. This raises the possibility that cognitive behavioral techniques could be helpful in limiting the emotional and affective impact of this bothersome symptom. Refocusing attention on tasks unrelated to itch could be one avenue worth exploring, since these approaches have been shown to be effective in diminishing the perception of pain (Zeidan et al. 2011). These "mindfulness"-reframing techniques may prove to be even more effective for the subjective relief of itch, since itch (contrary to pain) can be easily generated via a central induction mechanism (the "contagious itch" phenomenology). Therefore, if there is a central "source" that is capable of producing an itch sensation (in the absence of external pruritogenic stimuli), there must be a way to reverse the mechanism, turning it into a cure.

11 Contagious Itch

Contagious itch is an intriguing phenomenon in daily life. Thus, observers, while looking at others scratching an itch, themselves feel itchy. It has been studied in humans and primates (Niemeier et al. 2000; Papoiu et al. 2011; Feneran et al. 2012; Holle et al. 2012; Lloyd et al. 2012). The elucidation of central mechanisms underlying contagious itch is of high interest since it could provide invaluable clues for the treatment of itch. A recent brain imaging study pointed toward BA44 and the premotor cortex (BA6) as significant areas activated during the process of "itch induction." These findings can be seen as a first step in attempting to elucidate

the phenomenon (Holle et al. 2012). Another brain imaging study of contagious itch reported that a functional coupling of the insula and basal ganglia likely plays an important role in triggering scratching while viewing and imagining situations associated with itch (Mochizuki et al. 2013) (Fig. 4). Stated bluntly, the existence of a single, specific itch (processing) center remains elusive. It is becoming increasingly clear that the complex neuronal processes involved in processing itch cannot be reduced to a single cortical or subcortical area.

Contagious itch is significantly easier to induce in atopic dermatitis sufferers than in healthy volunteers. Interestingly, the itch induced by visual cues had a scattered, wide body distribution. We suspect that there are several brain centers that can support the generation of a somatic sensation in the absence of peripheral stimulation. Further elucidation of brain mechanisms behind this phenomenon can provide insight on specific central areas that can be targeted and used in the therapeutic approach to relieve itch. A functional coupling between the insula and basal ganglia could be one of the targets to control scratching behavior.

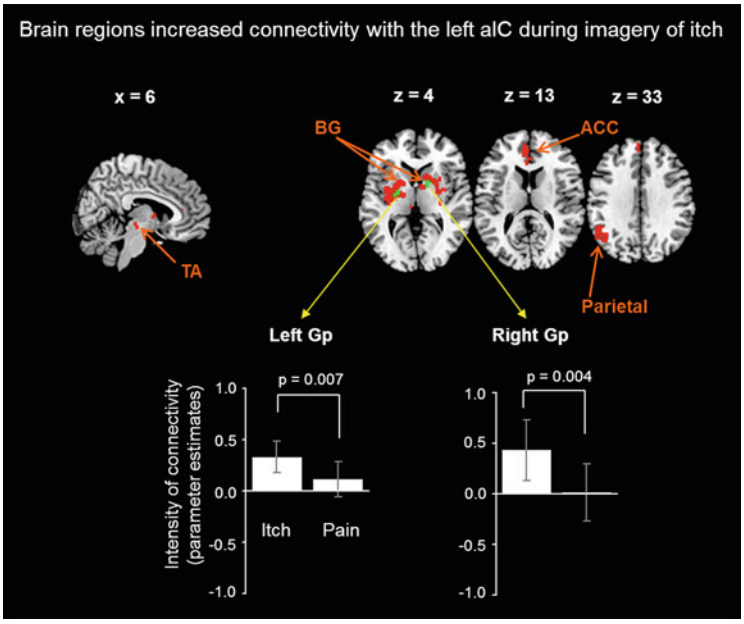

Fig. 4 Increased functional connectivity while imaging the itch sensation. *Red* regions: brain regions in that activity significantly increased functional connection with activity in the insula. *Green* regions: the functional connectivity while imaging the itch sensation was significantly stronger than that while imaging the pain sensation. *TA* the tegmentum, *BG* the basal ganglia, *ACC* the anterior cingulate cortex, *Gp* the globus pallidus. The figure has been reproduced with permission of the International Association for the Study of Pain® (IASP)

12 Future Directions

Linking functional human brain imaging, such as brain perfusion measurements with quantitative arterial spin labeling, to anatomical studies, such as voxel-based morphometry, is a powerful way to gain insight into brain regions associated with the itch–scratch cycle. Arterial spin labeling provides us with techniques that measure tonic events, such as itch, and to identify the neural correlates that underpin unrelenting spontaneous itch that occurs in chronic itch patients. As imaging techniques are refined, we will be able to explore in depth the mechanisms of temporal activation of structures that appear unrelated. Combining techniques such as magnetoencephalography to fMRI will further add to our understanding of the itch–scratch cycle. Techniques assessing functional connectivity using diffusion tensor imaging, that enables the study of white matter and neuronal pathways, will increase our understanding of visualizing the connections between the different areas involved in itch. Spinal cord fMRI is evolving as a new tool and will hopefully enable us to simultaneously assess neural activities from dorsal horn, brain stem, to cortex. Magnetic resonance spectroscopy will enable us to quantify changes in brain metabolites (e.g., dopamine glutamate) that are involved in itch.

The challenge now is to improve the sensitivity and specificity of these techniques so we can use them as tools in the assessment of chronic itch and the effectiveness of treatments.

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The Role of the Mrgpr Receptor Family in Itch

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Abstract

Itch is a complex sensory modality that can be evoked by an extremely diverse set of stimuli and has multiple components of disease etiology. Thus, determining the basic molecular and cellular players is essential before we can tackle the more complex aspects of itch. The identification of novel itch receptors has been extremely fruitful and has uncovered novel signaling pathways and pruritogens. Mrgprs encode a family of G protein-coupled receptors, many of which are expressed specifically in sensory nerves and function as itch receptors in mediating histamine-independent itch. In this chapter, we will review the discovery of the receptor family, their specific expression, their roles as itch receptors, and the itch-inducing agonists. Furthermore, we will summarize the results indicating that Mrgpr-expressing sensory neurons are itch-sensing neurons. In the end we will discuss the role of Mrgprs and Mrgpr-positive neurons in chronic itch.

Keywords

Itch • Mrgprs • GPCR • G protein-coupled receptor • DRG neurons • Chloroquine • BAM8-22 • β-alanine • SLIGRL • Mouse • Knockout • Skin • Labeled line

Abbreviations

CNS	Central nervous system
BAM8-22	Bovine adrenal medulla 8-22 peptide
CGRP	Calcitonin gene-related peptide
DRG	Dorsal root ganglion
γ2-MSH	γ2-melanocyte-stimulating hormone
GABA	Gamma-aminobutyric acid
GFP	Green fluorescence protein
IB4	Isolectin B4
Mrgpr	Mas1-related G protein-coupled receptor
NPFF	Neuropeptide FF
NPAF	Neuropeptide AF
TRPV1	Transient vanilloid receptor 1; the capsaicin receptor

1 Introduction

Organisms use their sensory systems—including vision, audition, olfaction, gustation, and somatosensation—to detect stimuli in the environment. At the cell's surface, receptors and ion channels play an essential role in every sensory system

by acting as the molecular sensors of their respective stimuli. The most famous example is the family of olfactory receptors expressed in olfactory receptor neurons (Buck and Axel 1991; Gogos et al. 2000; Lomvardas et al. 2006).

Itch sensation, a subtype of somatosensation, can be evoked by a wide range of compounds with a variety of chemical structures, including small molecule compounds, peptides, proteases, cytokines, and lipid components (Paus et al. 2006; Patel and Dong 2010). Presumably, these itch-inducing substances—or pruritogens—activate the membrane receptors and/or ion channels that are expressed in sensory nerve endings in the skin, and the activation of these receptors triggers the electrical impulses that are delivered through a series of connected nerves to the brain, where the itch sensation is generated. The best-characterized pruritogen is histamine, which is released from mast cells and activates histamine receptors located in sensory nerves in the skin. In addition to histamine-dependent itch, a wealth of pharmacological, clinical, electrophysiological, and molecular data support the existence of histamine-independent itch (Shelley and Arthur 1957; Schmelz et al. 1997; Ikoma et al. 2005; Namer et al. 2008). Indeed, most chronic itch conditions are not alleviated by antihistamines, suggesting the involvement of other itch mediators and receptors. Therefore, understanding the basic molecular and cellular mechanisms that underlie histamine-independent itch will improve methods for treating chronic itch. In this chapter, we will summarize the recent findings regarding a family of G protein-coupled receptors called Mrgprs, many of which are expressed specifically in sensory nerves and function as itch receptors in mediating histamine-independent itch.

2 Identification of the Mrgpr Family of Receptors

In 1997, Michael Caterina and David Julius cloned the *TRPV1* gene, which encodes a noxious heat sensor and capsaicin receptor (Caterina et al. 1997, 2000; Tominaga et al. 1998). This seminal discovery prompted us to search for additional molecules that are expressed specifically in small-diameter nociceptors in the mouse DRG. Using a cDNA subtractive screening approach, we isolated a cDNA clone that is enriched in small-diameter DRG neurons and encodes a G protein-coupled receptor (Dong et al. 2001). Because this receptor shares sequence homology with the proto-oncogene *Mas1*, we named this new gene *MrgA1* for "Mas1-related gene"; the name was later changed to *MrgprA1* for "Mas1-related G protein-coupled receptor." Subsequent database searches and genomic DNA screening using *MrgprA1* as a probe led to the identification of additional *Mrgpr* member genes. Ultimately, approximately 50 Mrgpr genes have been identified in the mouse genome. Based on their sequence homology, we grouped these genes into the MrgprA, MrgprB, and MrgprC subfamilies (Dong et al. 2001). Genomic and bioinformatics analyses suggest that these Mrgpr subfamilies arose from gene duplication and expansion, likely via retroviral LINE elements present in the mouse *Mrgpr* loci (Zylka et al. 2003). Like the olfactory receptor family (which was also expanded in the mouse genome), approximately half of all MrgprA, MrgprB, and MrgprC genes are

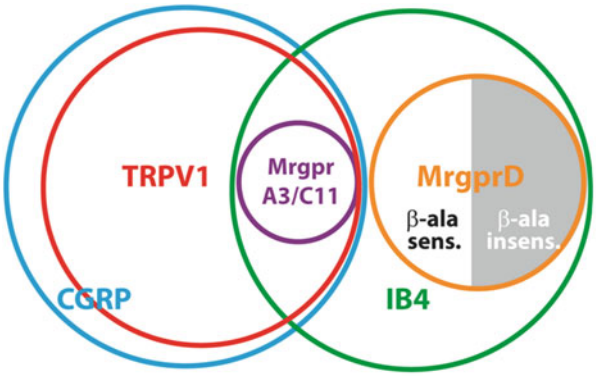

Fig. 1 Venn diagram summarizing the expression of Mrgprs in DRG neurons. The small- to medium-diameter neurons represent the major primary sensory neuron population in the DRG and can be grouped into two partially overlapping subpopulations known as the peptidergic (CGRP positive; *blue*) and non-peptidergic (IB4 positive; *green*) nociceptors. Most—if not all—CGRP-positive nociceptors also express TRPV1 channels (*red*). MrgprA3-positive and MrgprC11-positive neurons largely overlap (*purple*) and are positive for both CGRP and IB4. Finally, MrgprD-positive cells (*orange*) are IB4 positive and CGRP negative. MrgprD-positive and β-alanine sensitive neurons represent a nonoverlapping subpopulation of itch-sensing neurons

pseudogenes, with shortened open-reading frames caused by premature stop codons due to mutations. The human genome contains at least four Mrgpr genes, which are highly homologous with the murine MrgprA through MrgprC genes (Dong et al. 2001). However, because the human proteins do not form orthologous pairs with their rodent counterparts, we named them human MrgprX1 through MrgprX4. In the following section, we will discuss why the human genome contains fewer Mrgprs than the mouse genome. In addition to these multimember subfamilies, the mouse genome also contains several single-member Mrgpr subfamilies called MrgprD through MrgprH, which have clear human orthologs.

A striking feature of Mrgprs is their specific expression patterns. Using in situ hybridization and genetic labeling approaches, we found that many Mrgpr genes are expressed exclusively in subsets of small-diameter DRG neurons (see below for a detailed description; Fig. 1) (Dong et al. 2001). The Mrgpr gene family was also discovered independently by a research group at AstraZeneca, and they introduced the term "sensory neuron-specific receptors" (SNSRs) (Lembo et al. 2002). Consistent with our results, they used RT-PCR and found that several human MrgprXs are expressed selectively in subsets of human DRG neurons.

3 Ligands and Agonists

In contrast to olfactory receptors that are poorly expressed and trafficked in heterologous cells, expression of Mrgprs in most heterologous cells (e.g., HEK293 and CHO cells) is rather straightforward. By adding a C-terminal GFP

tag to Mrgprs, we can easily follow the trafficking of the receptors to the plasma membrane (Dong et al. 2001; Han et al. 2002). Because Mrgprs have the highest sequence homology with G protein-coupled receptors that have peptide ligands (e.g., the formyl peptide receptors), we specifically searched for peptides as the likely ligands for Mrgprs. We used a conventional Ca^{2+} imaging assay to monitor Mrgpr activation in HEK cells expressing Gα15, a promiscuous G protein that couples G protein-coupled receptors to a signal transduction pathway that ultimately triggers the release of intracellular Ca^{2+} (Offermanns and Simon 1995). Using this heterologous system (i.e., Mrgprs expressed in Gα15-expressing HEK293 cells), we screened a panel of peptides from the Sigma catalog and identified several RFamide peptides—including the molluscan peptide FMRFamide and the mammalian peptides NPFF, NPAF, and γ2-MSH—that function as agonists for the mouse MrgprA1, MrgprA4, and MrgprC11 receptors (Dong et al. 2001; Han et al. 2002). Although these agonists share a common C-terminal RF(Y)G or RF(Y) amide motif, they activate MrgprA1, MrgprA4, and MrgprC11 receptors with different sensitivities (Dong et al. 2001; Han et al. 2002).

Interestingly, the AstraZeneca group reported that BAM8-22 (a 15-amino acid C-terminal fragment of pro-enkephalin that ends with the amino acid RYG) and γ2-MSH activate human MrgprX1 and rat MrgprC receptors, respectively (Lembo et al. 2002). We also found that BAM8-22 is a potent activator of mouse MrgprC11 and triggers internalization of the receptor from the plasma membrane to the cytosol (Han et al. 2002). The initial identification of Mrgpr ligands provided us with essential tools to probe the function of Mrgprs. For example, we found that all of the Mrgprs with an identified ligand (e.g., the MrgprC11 and MrgprX1 receptors) signal through the Gαq subunit (Han et al. 2002) and some receptors also couple to the Gαi subunit (Chen and Ikeda 2004; Crozier et al. 2007). Therefore, regular heterologous cells (i.e., without Gα15) can be used to express Mrgprs and monitor the receptor's activation using Ca^{2+} imaging. This approach can also be used to study the activation of native Mrgprs in DRG neurons. In addition, we also identified TRP channels as the downstream effector targets of Mrgpr activation (Wilson et al. 2011).

More recently, we discovered that mouse MrgprA3 and human MrgprX1 are receptors for the antimalarial drug chloroquine (Liu et al. 2009). A Japanese group reported that β-alanine binds to and activates both human and mouse MrgprD receptors (Shinohara et al. 2004). Thus, BAM8-22, chloroquine, and β-alanine are agonists of both mouse and human Mrgprs, and they have been used as probes to study histamine-independent itch (see below). As mentioned above, there are far fewer human Mrgprs than mouse Mrgprs. Furthermore, human MrgprXs do not form orthologous pairs with their mouse counterparts, despite their sequence homology. However, the ligand specificity of human and mouse Mrgprs suggests that the human MrgprX1 receptor is a functional homolog of both mouse MrgprA3 and MrgprC11 receptors. It is therefore tempting to speculate that during the evolution of the mouse genome, multiple copies of Mrgprs arose through duplication, and each copy has its own specific agonist (e.g., chloroquine for MrgprA3, BAM8-22 for MrgprC11), whereas the human genome contains only a few Mrgpr

receptors, each of which serves as a receptor for multiple agonists (e.g., MrgprX1 for both chloroquine and BAM8-22). This broadly tuned mechanism at the receptor level may preserve a functional diversity in a relatively small family of human Mrgprs during evolution.

4 Mrgprs as Itch Receptors

Despite their specific expression patterns, analyzing the in vivo function of Mrgpr receptors has proven to be quite challenging. Because the mouse genome contains so many Mrgpr genes, the conventional single-gene knockout strategy was not feasible due to possible compensation by other genes. In addition, we lacked sufficient information to decide which Mrgpr to study first. Finally, given the lack of orthologs between mouse MrgprA–Cs and human MrgprXs, generating and studying mouse Mrgpr single-knockout mice seemed to have little clinical relevance. Therefore, we chose to genetically delete a group containing several Mrgpr genes from the mouse genome, a viable strategy given that the MrgprA, MrgprB, and MrgprC genes are clustered on mouse chromosome 7. Using the Cre recombinase-loxP strategy, we deleted an 845-kb stretch of genomic DNA containing 12 full Mrgpr genes (including MrgprA3 and MrgprC11) but no additional genes (Liu et al. 2009). Importantly, homozygous mice carrying this large chromosomal deletion (called *Mrgpr-cluster* $\Delta^{-/-}$ mice) survive to adulthood with no detectable developmental or motor function defects. Therefore, *Mrgpr-cluster* $\Delta^{-/-}$ mice are a valuable animal model for studying the role of Mrgprs in itch. Indeed, results obtained from behavioral analyses of these mutant mice have provided key evidence that led us to identify MrgprA3 and MrgprC11 as itch receptors.

4.1 MrgprA3

From all of the pruritogens identified to date, we chose to test the antimalarial drug chloroquine by injection into our *Mrgpr-cluster* $\Delta^{-/-}$ mice (Liu et al. 2009); we selected this pruritogen first because of its medical relevance and wide clinical usage. Most black Africans have a severe itch reaction to chloroquine, and this reaction can be so severe that many patients even refuse to take chloroquine to treat their malaria (Olatunde 1977; Abila et al. 1989; Mnyika and Kihamia 1991; Ademowo et al. 1998); interestingly, other races do not have such an itch response to chloroquine (Sams and Epstein 1965; Spencer et al. 1982). Because patients experience itch when they first ingest chloroquine and because antihistamines do not effectively prevent this side effect, researchers in Africa concluded that chloroquine-induced itch is not an allergic response, nor is it mediated by histamine receptors (Abila et al. 1994). Interestingly, injecting chloroquine into the skin of mice evokes a robust scratching response in the mice, and various inbred strains of mice exhibit different degrees of scratching responses (Green et al. 2006). Based on

this response, we tested whether our *Mrgpr-cluster* $\Delta^{-/-}$ mice respond differently from wild-type following chloroquine injection. The results were immediately obvious—the *Mrgpr-cluster* $\Delta^{-/-}$ mice scratched significantly less than the wild-type mice (Liu et al. 2009). We were quite encouraged by this robust phenotype, as we had been searching for a function for Mrgprs in more than 7 years since they were first identified. This important finding opened a new research path in our laboratory that ultimately led to the functional characterization of Mrgprs and Mrgpr-expressing neurons in mediating itch.

To determine the specific itch-inducing effect of chloroquine, we also tested a synthetic precursor of chloroquine (called quinoline), in which one of the benzene rings lacks a specific side chain. Injecting quinoline did not evoke scratch in either wild-type or *Mrgpr-cluster* $\Delta^{-/-}$ mice (Liu et al. 2009), suggesting that the unique chemical structure of chloroquine is required for inducing itch. Because the majority of Mrgpr genes that are deleted in the cluster knockout mice are expressed specifically in DRG neurons, we hypothesized that the chloroquine itch-resistant phenotype of *Mrgpr-cluster* $\Delta^{-/-}$ mice was likely due to a functional deficit in the DRG neurons in these mice. Using Ca^{2+} imaging in cultured wild-type DRG neurons, we found that a small subpopulation of DRG neurons (representing approximately 5 % of the total population) is indeed activated by chloroquine (Liu et al. 2009). Although this small subpopulation of chloroquine-responding neurons could be easily overlooked or considered to reflect a nonspecific response, cultured DRG neurons from *Mrgpr-cluster* $\Delta^{-/-}$ mice did not respond at all to chloroquine, indicating that the activation of wild-type DRG neurons by chloroquine is both specific and Mrgpr dependent. Similar results were obtained by performing electrophysiological recordings of DRG neurons to monitor their action potentials in response to chloroquine. Taken together, the behavioral and cellular data pointed to the exciting likelihood that Mrgprs are receptors for chloroquine. To test this hypothesis directly, we cloned the cDNAs of each of the 12 deleted Mrgprs and expressed each receptor individually in HEK293 cells (which do not express endogenous Mrgprs). We found that only the MrgprA3-expressing HEK293 cells responded robustly to chloroquine; the remaining 11 lines of Mrgpr-expressing HEK293 cells were completely insensitive to chloroquine (with the exception of MrgprA1-expressing cells, which responded weakly) (Liu et al. 2009). We were quite surprised by this high specificity of activation, particularly given that MrgprA3 is highly homologous to the other MrgprAs that we tested.

4.2 MrgprC11

Our group and others reported that the preproenkephalin peptide BAM8-22 is a specific ligand for both mouse MrgprC11 and human MrgprX1 receptors (Han et al. 2002; Lembo et al. 2002). The precise physiological function of BAM8-22 is currently unclear. However, because both the MrgprA3 and MrgprC11 receptors are expressed in a single subset of small-diameter DRG neurons, we tested whether BAM8-22 is a pruritogen. Indeed, intradermal injection of BAM8-22 evoked a

scratch response in wild-type mice (Liu et al. 2009). In collaboration with Robert LaMotte's group at Yale University, we used cowhage spicules to deliver BAM8-22 to the skin of healthy human volunteers (Sikand et al. 2011). The participants reported an itch response that was similar in intensity to histamine-induced itch. Importantly, the BAM8-22-loaded spicules did not trigger skin flares or wheals (both of which are hallmarks of histamine-mediated itch). Moreover, pretreating the participants with antihistamine cream completely blocked histamine-evoked itch but had no effect on BAM8-22-induced itch. Finally, BAM8-18, a truncated form of BAM8-22 that lacks the Mrgpr-interacting motif, failed to elicit any sensation in the participants. Taken together, these data support the results of our mouse study and suggest that BAM8-22 is likely an endogenous mediator of histamine-independent itch.

SLIGRL is a 6-amino acid peptide cleaved from the *N*-terminus of activated PAR2 following protease cleavage. SLIGRL and its human ortholog SLIGKV evoke itch in mice and humans, respectively (Steinhoff et al. 2003; Shimada et al. 2006). Although it is generally accepted that this type of itch is mediated by PAR2, no study has directly addressed this issue (e.g., using PAR2-knockout mice). Because SLIGRL is used widely as a pruritogen for studying histamine-independent itch, we tested the ability of this peptide to induce itch in *Mrgpr-cluster* $\Delta^{-/-}$ mice (Liu et al. 2011). To our surprise, the *Mrgpr-cluster* $\Delta^{-/-}$ mice scratched significantly less in response to SLIGRL injection compared with their wild-type littermates. Perhaps even more surprisingly, the SLIGRL-induced itch response in PAR2-knockout mice was similar to the response induced in wild-type mice, suggesting that this response is not mediated by PAR2. In vitro, SLIGRL activated a small subset of both wild-type and PAR2-knockout DRG neurons (approximately 3 % of the total neuron population), whereas *Mrgpr-cluster* $\Delta^{-/-}$ neurons did not respond at all. Interestingly, most of the SLIGRL-sensitive DRG neurons also responded to BAM8-22, suggesting functional overlap.

Based on these unexpected yet compelling results, we next tested whether SLIGRL can activate Mrgprs directly by expressing each of the 12 Mrgprs in heterologous cells and monitoring activation with Ca^{2+} imaging. Because HEK293 respond to SLIGRL (presumably because they express endogenous PAR2), we used SLIGRL-insensitive Chinese hamster ovary (CHO) cells for these experiments. Strikingly, among the 12 genes that were tested, only MrgprC11-expressing CHO cells were activated by SLIGRL. In parallel experiments, we also found that SLIGKV (the human version) specifically activated human MrgprX2 but not MrgprX1, MrgprX3, or MrgprX4. Based on a dose-response study, we determined that the EC50 of SLIGRL-induced MrgprC11 activation is on par with the EC50 of SLIGRL-induced PAR2 activation, suggesting that the peptide can activate these two receptors equally well. When we examined the SLIGRL sequence in detail, we hypothesized that the C-terminus of the peptide is required for the activation of MrgprC11, as this region contains a sequence motif that is similar to other MrgprC11-activating peptides such as RYamide and RFamide (Dong et al. 2001; Han et al. 2002). We next generated a truncated SLIGR peptide by removing the C-terminal leucine and found that SLIGR failed

to activate MrgprC11 in heterologous cells. On the other hand, the truncated peptide still activated PAR2, as the PAR2-activating motif resides at the *N*-terminus of SLIGRL (which is intact in the truncated peptide). Therefore, SLIGR provided us with a useful tool to separate MrgprC11 and PAR2 signaling. Unlike SLIGRL, SLIGR cannot directly activate wild-type DRG neurons, nor does it evoke a scratching response in wild-type mice. Taken together, these data strongly suggest that SLIGRL-evoked itch is mediated by MrgprC11 but not PAR2.

Using the truncated peptide SLIGR, we also found that PAR2 plays a role in inflammatory pain by sensitizing TRPV1 channels in the DRG. Moreover, we cannot exclude the possibility that PAR2 mediates itch in other cell types such as keratinocytes. It is also highly conceivable that the function of PAR2 in the DRG switches from pain to itch under chronic itch conditions. For example, human psychophysical studies demonstrated that bradykinin and acetylcholine—two stimuli that are normally painful—evoke an itch response when applied to atopic dermatitis patients (Rukwied and Heyer 1999; Hosogi et al. 2006). Various proteases—including plant-derived proteases such as mucunain and papain and mammalian cathepsin S—evoke an itch response that is likely mediated by PAR2 (or related PARs), as PAR2 can be cleaved and activated by these proteases (Hollenberg and Compton 2002; Ossovskaya and Bunnett 2004). This hypothesis can be tested using PAR2-knockout mice. Our results also raise the interesting possibility that Mrgprs are targets for proteases.

4.3 MrgprD

Both MrgprA3 and MrgprC11 are deleted in our *Mrgpr-cluster* $\Delta^{-/-}$ mice. Thus, is it possible that other Mrgprs that are not within the cluster are also itch receptors? A promising candidate is MrgprD, which is located far from the Mrgpr cluster near the telomere of mouse chromosome 7 and which has a clear human ortholog. Genetic labeling experiments revealed that MrgprD-positive axons only innervate the skin and single-gene MrgprD-knockout ($MrgprD^{-/-}$) mice have been generated (Zylka et al. 2005). Although MrgprD is expressed in 70 % of IB4-positive DRG neurons (which represent approximately 30 % of all DRG neurons), $MrgprD^{-/-}$ mice do not exhibit any sensory (e.g., thermal or mechanical) deficits. Previous studies revealed that β-alanine, a naturally occurring β-form of alanine, directly binds and activates both the human and mouse MrgprD receptors (Shinohara et al. 2004), although a literature search did not yield any published study demonstrating β-alanine-evoked itch. Nevertheless, we noticed that several weblogs by body builders contained many complaints of severe itch experienced immediately after taking oral β-alanine. Interestingly, β-alanine is a popular supplement used to build muscle; β-alanine is a major component of the dipeptide carnosine, a buffer that prevents muscle cells from becoming acidic, thereby decreasing fatigue during exercise (Crush 1970; Derave et al. 2007; Hill et al. 2007). In order to mimic the effect observed in humans, we gave wild-type and $MrgprD^{-/-}$ mice sweetened water containing β-alanine or sweetened water without β-alanine (Liu et al. 2012). The

sweetened water alone (without β-alanine) did not elicit scratching in either wild-type or *MrgprD*$^{-/-}$ mice. However, several minutes after drinking the β-alanine-containing water, the wild-type mice began scratching (primarily at the back of the neck), and this behavior lasted for at least 1 h. In contrast, the *MrgprD*$^{-/-}$ mice scratched significantly less after drinking the β-alanine-containing water; the level of scratching in the *MrgprD*$^{-/-}$ mice was comparable to the scratching response in the mice that were given sweetened water without β-alanine. These data suggest that as in humans, β-alanine induces itch in mice and this effect is solely MrgprD dependent.

We were also excited by the ability to induce itch by delivering a compound orally, as we typically induce itch by injecting a pruritogen into the skin. However, many oral drugs have itch as a side effect. Thus, we can employ this oral delivery model to study the mechanisms of itchy side effects in mice. In addition to the oral delivery assay, we also injected β-alanine into the cheek (Liu et al. 2012). Consistent with oral administration, wild-type mice that were injected with β-alanine exhibited robust scratching with apparent pain response; in contrast, the *MrgprD*$^{-/-}$ mice did not exhibit an itch response. L-alanine itself (which does not activate MrgprD receptors) did not evoke a significant scratch response in wild-type animals, suggesting that only the β-form of the amino acid (i.e., with the amino group located at the β-position from the carboxylate group) can induce MrgprD-mediated itch. At the cellular level, β-alanine directly activated a subset of wild-type DRG neurons, but this response was absent in *MrgprD*$^{-/-}$ neurons (see below) (Liu et al. 2012). To quantify and analyze the site-specific effect of β-alanine-induced itch in humans, Dr. LaMotte's group injected β-alanine into the skin of healthy volunteers (Liu et al. 2012). Upon injection of β-alanine, each participant experienced modest itch that was accompanied by tingling and a mild stinging sensation. Importantly, injecting β-alanine did not generate a typical histamine-mediated itchy skin reaction such as flares or wheals. Finally, as reported in mice, L-alanine did not evoke an itch sensation in humans.

Structurally, β-alanine is similar to the primary inhibitory neurotransmitters GABA and glycine, and β-alanine activates both GABA and glycine receptors (albeit with less potency) (Wu et al. 1993; Rajendra et al. 1997). Furthermore, β-alanine binds to glycine's co-agonist site on NMDA receptors and suppresses glutamatergic signaling (Ogita et al. 1989; Pullan and Powel 1992). Therefore, in the central nervous system, β-alanine acts as an inhibitory neurotransmitter or modulator. Based on these findings, β-alanine-induced itch has been proposed to involve a central mechanism. However, our analysis of knockout mice strongly suggests that β-alanine-induced itch depends solely on MrgprD receptors. Our skin injection experiments in mice and humans combined with the finding that MrgprD receptors are expressed in DRG neurons—but not in CNS neurons—suggest that β-alanine-induced itch is mediated by a peripheral, cutaneous mechanism.

5 Mrgprs as Molecular Markers of Itch-Mediating Neurons

Both itchy and painful stimuli are detected by small-diameter nociceptors in the DRG. Given the similarities between itch-mediating and pain-mediating neurons and the differences between these sensations and their behavioral responses (e.g., pain elicits a withdrawal response and itch elicits a scratch response), several theories have been proposed to explain itch and pain coding. Here, we discuss the two most prominent theories, namely, the intensity and labeled-line theories. The "intensity theory" holds that itch and pain are perceived by a common group of DRG neurons. Neurons discriminate between the two based on the intensity of stimulation received and encode into different patterns of electrical activities and transmit through the same synaptic pathway to the brain. On the other hand, the "labeled-line theory" posits that itch and pain are detected and encoded by discrete groups of DRG neurons and transmitted to the brain via separate synaptic pathway (Patel and Dong 2010, 2011). Other theories such as population coding and contrast coding will not be discussed here due to space constraints, and the reader is referred to published literature for further information regarding these theories (Davidson and Giesler 2010; Patel and Dong 2010, 2011).

5.1 MrgprA3-Positive Neurons

The chloroquine receptor MrgprA3 is expressed in 5–8 % of all DRG neurons (the reported percentage differs depending on the detection method used and the spinal level of the ganglion that is examined) (Liu et al. 2008, 2009; Han et al. 2013). These neurons are positive for both IB4 labeling and CGRP expression and negative for substance P (Han et al. 2013). However, the level of CGRP expression in MrgprA3-positive neurons is lower than in canonical peptidergic neurons. Therefore, the pattern of MrgprA3 expression is not random, but is specific to a subset of DRG neurons. Because MrgprA3-positive neurons can be activated by several pruritogens (including chloroquine, BAM8-22, and histamine) (Liu et al. 2009), they are a promising candidate as the itch-specific neurons. To test this hypothesis, we used the BAC transgenic approach to generate a new mouse line in which a GFP-Cre fusion protein is expressed selectively in MrgprA3-positive neurons (Han et al. 2013). Thanks to recent advances in mouse genetics, we were able to cross this MrgprA3-Cre line with several Rosa26 reporter lines, thus enabling specific expression of various reporter genes in MrgprA3-positive neurons without the need to generate additional mouse lines. With these tools in hand, we first examined the morphology of these DRG neurons using a reporter mouse expressing the red fluorescent protein tdTomato. Strikingly, MrgprA3-positive axons (i.e., red fluorescent fibers) innervate the skin but not any other tissue examined; this finding provides a cellular explanation for why we only feel itch in the skin but not in other, deeper tissues such as muscles and visceral organs (Han et al. 2013). Dr. LaMotte's group then used their powerful in vivo DRG neuron recording technique to study the cellular properties of MrgprA3-positive neurons

(identified clearly by their intrinsic green fluorescence). They recorded soma activation of lumbar DRG neurons in response to various stimuli applied to the receptive fields in the hind limbs and hind paws of live mice. Strikingly, they found that the electrophysiological properties of the MrgprA3-positive neuron population are remarkably homogenous. These neurons are typical polymodal nociceptive neurons, as they have slow conduction velocity (0.5 m/s) and respond to noxious heat, noxious mechanical force, and capsaicin (Han et al. 2013). In addition, MrgprA3-positive neurons can be activated by a variety of pruritogens, including histamine, BAM8-22, chloroquine, and cowhage. On the other hand, MrgprA3-positive neurons do not respond to the MrgprD agonist β-alanine (Han et al. 2013). Interestingly, ablation of MrgprA3-positive neurons specifically affects itch but not pain. Finally, we used a genetic approach to generate mice in which TRPV1 is exclusively expressed in MrgprA3-positive neurons. Strikingly, capsaicin, a normally painful stimulus, caused itch and not a pain response in the MrgprA3-TRPV1 mice. Together, these data strongly suggest that MrgprA3-positive neurons are itch-specific neurons and most fit the labeled-line theory.

5.2 MrgprD-Positive Neurons

Although both MrgprA3-positive neurons and MrgprD-positive neurons stain positive for IB4, these receptors label two discrete subpopulations with both overlapping and nonoverlapping cellular properties (Dong et al. 2001; Zylka et al. 2003). Like MrgprA3-positive neurons, MrgprD-positive neurons also terminate in the most superficial layer of the epidermis (Zylka et al. 2005). However, unlike MrgprA3-positive neurons, MrgprD-positive neurons represent the majority of the IB4-positive neurons (representing approximately 70 % of IB4-positive neurons and 30 % of all DRG neurons). In addition, MrgprD-positive neurons are not CGRP negative (Zylka et al. 2005) (Fig. 1). Based on in vivo DRG recordings in MrgprD-GFP mice, MrgprD-positive neurons are composed of a mixed population of neurons—although all of the neurons responded to noxious mechanical force, only half of the neurons responded to noxious heat (the other half were not sensitive to this stimulus). Furthermore, only the heat-sensitive neurons responded to β-alanine (the heat-insensitive neurons did not respond to β-alanine). Strikingly, none of the MrgprD-positive neurons responded to pruritogens that activate MrgprA3 (Liu et al. 2012). The identification of two distinct itch-responsive neuron populations (*MrgprD*$^+$ and *MrgprA3*$^+$ neurons, Fig. 2) provides us with a unique opportunity to investigate how itch signals are perceived, encoded, and transmitted in the nervous system.

5.3 The Role of Mrgprs-Positive Neurons in Chronic Itch

Chronic pruritus (chronic itch) severely affects both quality of life and productivity. Based on their neuropathophysiological mechanisms and origin, chronic itches can

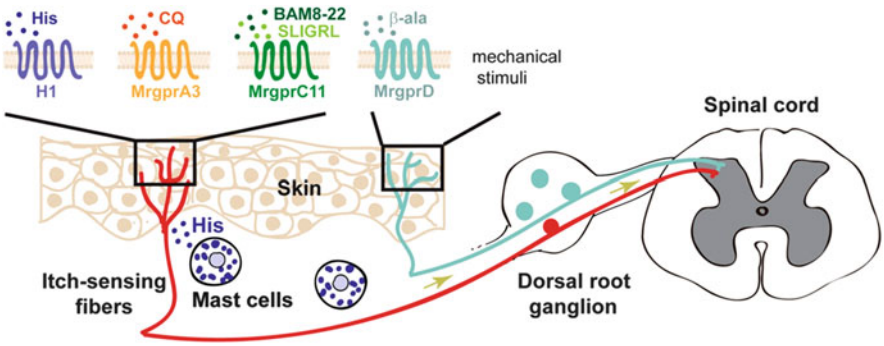

Fig. 2 Two distinct itch-responsive neuron populations ($His^{+}/MrgprA3^{+}$ and $His^{-}/MrgprD^{+}$ neurons) in the DRG. They detect various exogenous or endogenous itch-inducing molecules via itch receptors on their peripheral axons in the skin and transmit the itch signals to the spinal cord via their central axons

be classified into the following four categories: *pruritoceptive itch*, which is mediated by the activation of cutaneous afferent fibers; *neuropathic itch*, which results from diseased or lesioned itch-related neural circuitry; *neurogenic itch*, which is induced by mediators acting centrally in the absence of neural damage; and *psychogenic itch*. Of these four types of chronic itch, only pruritoceptive itch arises exclusively from peripheral mechanisms and is most commonly seen in clinical practice. Because Mrgprs are expressed selectively in primary sensory neurons in the peripheral nervous system, here, we will delineate the role of Mrgprs- and Mrgpr-expressing neurons in mediating pruritoceptive chronic itch.

In pruritoceptive chronic itch, the activity of secondary cell types such as keratinocytes or mast cells is believed to trigger the release of substances that act directly on a group of primary sensory neurons in order to elicit the itch sensation (Paus et al. 2006; Davidson and Giesler 2010; Patel and Dong 2011). However, which substances are released from these neurons and which group of sensory neurons is activated are currently unknown, and our lack of this information has greatly impeded our ability to understand chronic itch. As mentioned above, MrgprA3 selectively labels a subpopulation of itch-sensing neurons (Han et al. 2013). Thus, this begs the question of whether this population of neurons is activated by secondary cells to mediate chronic itch. Indeed, our recent studies revealed that ablating MrgprA3-positive neurons dramatically reduced the severity of both allergy-induced and dry skin-induced chronic itch (Han et al. 2013). These results provided the first evidence of a critical group of sensory neurons in chronic itch and opened new avenues for investigating both allergic and nonallergic chronic itch.

Mast cells are the immune effector cells that play a key role in mediating allergic chronic itch. Mast cells originate in the bone marrow and migrate to many peripheral tissues—including the lungs, intestines, peritoneum, and skin—where they mature and then reside (Metcalfe et al. 1997). We previously reported that Kit^{W-}

$^{sh}/Kit^{W\text{-}sh}$ mice (which lack mast cells due to a chromosomal inversion that affects c-kit gene function) do not scratch in response to allergy, suggesting that mast cells are required for mediating allergy-associated itch (Patel et al. 2011). When mast cells are activated by cross-linking the high-affinity IgE receptor (FcεRI) through an allergen challenge, they release a plethora of preformed mediators—including histamine, serotonin, lipid compounds, proteases, and neuropeptides—to induce an itch sensation (Metcalfe et al. 1997; Metz and Maurer 2009). It is generally accepted that IgE-mediated allergic itch is mediated principally by the action of histamine on its receptor (H1) expressed in skin sensory nerve fibers. However, recent studies revealed that allergic itch is only mildly reduced in H1-deficient mice (Sugimoto et al. 2003), suggesting an involvement of histamine-independent pathways in itch allergy. The identification of MrgprA3-positive neurons as itch-sensing neurons raises an interesting question: Do histamine-independent Mrgprs mediate the communication between mast cells and sensory neurons and induce allergic itch? Future studies will test the function of Mrgprs in allergic itch and will likely provide novel insight into the etiology of allergic itch.

Unlike allergic itch, nonallergic itch lacks antigen specificity. Nonallergic itch can be generated by harmful metabolites, immune factors, or the activity of nonimmune cells such as keratinocytes. Keratinocytes are the most important resident cells in the skin (Metz and Maurer 2009; Raap et al. 2011). In addition to providing an effective barrier against harmful environmental factors, keratinocytes also play an important role in the induction and modulation of cutaneous sensations such as warmth, cold, contact, pain, and itch. Previous studies have shown that keratinocytes can release ATP and prostaglandin E_2 (PGE_2) to activate sensory fibers in the skin and modulate thermal and pain sensations (Koizumi et al. 2004; Huang et al. 2008; Mandadi et al. 2009). However, the specific signal transduction pathways between keratinocytes and sensory fibers in chronic itch remain poorly understood. Studies have shown that TRPV3 channels are expressed in high levels in keratinocytes and mice with the gain-of-function Gly573Ser substitution in the TRPV3 develop chronic itch (Yoshioka et al. 2009). In addition, overexpressing the stratum corneum chymotryptic enzyme in keratinocytes induces chronic itchy dermatitis in mice (Hansson et al. 2002; Ny and Egelrud 2003). These studies support the notion that keratinocytes play a role in chronic itch. However, which population of sensory neurons detects the substances released from keratinocytes—and thus mediates chronic itch—was unclear. Our recent studies revealed that MrgprA3-positive neurons play a key role in dry skin-induced chronic itch (Han et al. 2013), which has cast a new light on the interaction between keratinocytes and itch-sensing neurons. Furthermore, these results led to the intriguing hypothesis that Mrgprs might be the missing link between keratinocytes and itch-sensing sensory fibers, thus providing a new direction for researchers to explore the signaling cascade between keratinocytes and sensory neurons in nonallergic itch.

6 Future Direction

Itch is a complex sensory modality that can be evoked by an extremely diverse set of stimuli (chemical, mechanical, and thermal) and has multiple components of disease etiology (cutaneous, neurological, and immunological). Thus, determining the basic molecular and cellular players is essential before we can tackle the more complex aspects of itch. Although the identification of novel itch receptors has been extremely fruitful and has uncovered novel signaling pathways and pruritogens, much more research is clearly needed to understand all of the mechanisms that underlie the various forms of itch. For instance, MrgprA3-positive neurons express high levels of histamine receptors and many other Mrgprs and can be activated by many pruritogens (Fig. 2), suggesting a "broadly tuned" cellular mechanism. It is distinct from "finely tuned" chemosensations such as olfaction in which each olfactory receptor neuron expresses only one olfactory receptor. This finding begs the question of how the body discriminates between different types of itch evoked by chemically diverse molecules. In addition, what is the functional relevance of Mrgpr divergence in the mouse genome? What are the endogenous ligands for Mrgprs? Efforts to address these questions will greatly advance our understanding of the molecular and cellular mechanisms underlying itch perception and lead to new frontiers for studying itch.

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Transient Receptor Potential Channels and Itch: How Deep Should We Scratch?

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Abstract

Over the past 30 years, transient receptor potential (TRP) channels have evolved from a somewhat obscure observation on how fruit flies detect light to become the center of drug discovery efforts, triggering a heated debate about their potential as targets for therapeutic applications in humans. In this review, we describe our current understanding of the diverse mechanism of action of TRP channels in the itch pathway from the skin to the brain with focus on the peripheral detection of stimuli that elicit the desire to scratch and spinal itch processing and sensitization. We predict that the compelling basic research findings on TRP channels and pruritus will be translated into the development of novel, clinically useful itch medications.

Keywords

Pruritic TRP channels • TRPV1 • TRPA1 • TRPV3 • TRPM8 • Capsaicin • Menthol • Pruriceptive sensory fibers • Spinal itch processing

1 Transient Receptor Potential Channels and Itch: Introduction

Itch (pruritus) has resisted repeated attempts to classify it as a unique sensory experience. Indeed, itch shares many similarities with pain. Like pain, itch can be acutely generated (as exemplified by pruriceptive itch evoked by activation of sensory nerve endings by insect bites), chronic (often neuropathic in origin or part of a clinical syndrome like uremia or liver failure), or even psychogenic (seen in some psychiatric diseases like obsessive-compulsive disorder) (Biro et al. 2007; Ikoma et al. 2006; Toth and Biro 2013).

Traditionally, itch was defined as an "unpleasant sensation that elicits the desire or reflex to scratch" (Rothmann 1941). Although this definition has withstood the test of time, it remained unsatisfactory in that it did not delineate the mechanistic distinction between itch and pain. Generally speaking, both itch and pain are unpleasant sensations associated with protective motor responses, though the purpose of the motor response is clearly different: removal of the itch-producing substance by scratching and avoidance of the nociceptive stimuli by withdrawal, respectively. Acutely, we scratch ourselves until it hurts in order to relieve itch, suggesting a close interaction between the neuronal pathways for pain and pruritus. Indeed, chronic pruritus was referred to as the "skin equivalent of chronic pain." Based on the similarities between itch and pain, older studies regarded itch as "subthreshold pain." In other words, itch sensation was thought to reflect a weak activation of nociceptors (von Frey 1922), a concept known as *the intensity theory*

of itch (Biro et al. 2007; Toth and Biro 2013; Yosipovitch et al. 2003). This theory postulates that the same neurons produce the sensation of itch and pain, respectively, based on their state of activation. If this model holds true, low-intensity painful stimuli should be perceived as itchy, whereas high-intensity itchy stimuli should become painful. These postulates are, however, inconsistent with the human observations, rendering the intensity theory untenable.

An alternative model, *the labeled-line theory* (also known as specificity theory) *of itch*, proposes the existence of an autonomous pruriceptive system organized independently from pain sensation (Biro et al. 2007; Handwerker 2010; Handwerker and Schmelz 2009; Ikoma et al. 2006; Schmelz et al. 1997; Toth and Biro 2013). This model assumes the existence of specialized receptor structures, neural pathways, as well as higher centers that process only itch but not pain. This model is, however, seemingly inconsistent with the observation that chemical ablation of sensory neurons by capsaicin causes deficits in both itch sensation and nociception.

To reconcile the intensity and labeled-line theories, it was suggested that itch-sensitive neurons comprise a specific subset of nociceptive neurons (*selectivity theory if itch*) (McMahon and Koltzenburg 1992; Biro et al. 2007; Handwerker 2010; Ross 2011; Toth and Biro 2013). This model provides a mechanistic explanation for the observations that capsaicin desensitization ameliorates both itch and pain, yet some known activators of capsaicin-sensitive neurons (as exemplified by histamine) cause pruritus, whereas others (like formalin) elicit pain. Based on the experimental results, it is not clear whether the selectivity or the labeled-line theory provides a better explanation (Namer and Reeh 2013). Namely, itch and pain could be transmitted by anatomically different neural pathways, although some pain-evoking compounds, like capsaicin, can activate both systems. Clearly, the nociceptive and pruriceptive systems can form two separate (but not necessarily independent) neural networks that may share common molecular mechanisms like the expression of transient receptor potential (TRP) channels. For example, if both nociceptive and pruriceptive afferents express the capsaicin receptor TRPV1, capsaicin challenge will result in a virtual overlap between the two anatomically distinct systems. This controversy emphasizes the importance of identifying new molecular markers that are selective for itch-transmitting afferents.

2 The Neural Organization of the Pruriceptive System

2.1 Pruriceptive Sensory Fibers in the Skin

The skin is innervated by a dense network of sensory unmyelinated (slow conducting) C-afferent axons. These peripheral fibers arise from pseudo-unipolar neurons with somata in dorsal root (DRG) ganglia. The central fibers of these DRG neurons enter the dorsal horn of the spinal cord where they form synapse at second-order neurons. Approximately 80 % of the dermal C-fibers are mechanosensitive polymodal (i.e., responding to both chemical and thermal stimuli) nociceptors,

whereas the remaining 20 % are mechano-insensitive ones activated by chemical signals only (Schmidt et al. 1995, 1997). In the mechano-insensitive group, there is an "itch-sensitive" subset of neurons, characterized by strong and sustained activation by histamine, the prototypical itch mediator. These histamine-sensitive sensory neurons have (1) large receptive fields, (2) low conduction velocity, (3) high transcutaneous electrical threshold, and (4) they exhibit poor two-point discrimination threshold for histamine-induced itch (Schmelz et al. 1997, 2000, 2003; Schmelz and Schmidt 2010; Wahlgren and Ekblom 1996; Schmidt et al. 2002). Importantly, histamine-responsive C-fibers are thought to be involved in the generation of the axon reflex erythema by releasing vasoactive neuropeptides (e.g., calcitonin gene-related peptide, CGRP) in the vicinity of dermal blood vessels (Schmelz et al. 2000).

The existence of a second, histamine-insensitive (but most likely mechano- and heat-sensitive) class of pruriceptors was also proposed (Steinhoff et al. 2006). Indeed, intradermal insertion of highly pruritic spicules from the pods of the cowhage plant *Mucuna pruriens* activated only mechanosensitive (but not mechano-insensitive) C-fibers (Namer et al. 2008). In addition, Ikoma and colleagues (Ikoma et al. 2005) demonstrated that low-intensity-high-frequency focal electrical stimulation was able to evoke itch sensation without causing erythema. The lack of the axon reflex also indicates that itch sensation can be transmitted by a subset of sensory neurons that are clearly distinct from the histamine-sensitive ones.

Recently, it was shown that some of the mechano- and heat-sensitive nociceptive C-fibers (but not the heat-insensitive ones) can be activated by histamine and other pruritogenic substances (Ma et al. 2012). Furthermore, a recent study demonstrated that nociceptive, myelinated A-fibers may also contribute to the itch sensation evoked by cowhage (Ringkamp et al. 2011). This intriguing diversity of afferent pruriceptive fibers suggests that the various "submodalities" of itch experienced by humans could be due to the selective "encoding" from the very first level of the sensory system. Indeed, it is also suggested that peptidergic and non-peptidergic sensory neurons can detect itch evoked by different stimuli (Akiyama and Carstens 2013; McCoy et al. 2013). Most recently, members of the Mas-related G protein-coupled receptor family (Mrgprs) were identified as molecular determinants of itch sensation. Indeed, it was postulated that these Mrgprs can serve as specific markers for (at least some subsets of) pruriceptive neurons (Han et al. 2013; Liu et al. 2009).

2.2 Spinal Itch Processing

There is good evidence that itch and pain sensations are processed separately at the spinal cord level. In the cat, histamine-responsive/mechano-insensitive neurons were described in the dorsal horn (lamina I) of the spinal cord (Andrew and Craig 2001). Moreover, histamine-responsive spinothalamic tract neurons were identified among the mechano-, heat-, and capsaicin-sensitive ones in primates (Simone et al. 2004). In support of the selectivity theory, it was postulated that spinal projection neurons receive input from both itch-sensitive and pain-transmitting

(nociceptive) primary fibers (Handwerker 2010). By contrast, other studies reported the existence of distinct, nonoverlapping populations of spinothalamic tract neurons that transmit histaminergic and non-histaminergic (cowhage-evoked) itch sensations, respectively (Davidson et al. 2007). Indeed, the two sensations can be selectively inhibited even if they are transmitted by the same spinothalamic tract neurons. For example, it was demonstrated that only the histamine (but not the capsaicin)-evoked activity of the spinal-projecting neurons can be inhibited by eliciting scratching within the respective cutaneous receptive field (Davidson et al. 2009). Importantly, histaminergic and non-histaminergic itch appear to be transmitted by different neurotransmitters. For example, histamine-evoked responses were inhibited by blocking glutamate (AMPA/kainate) receptors, whereas chloroquine-evoked itch can be mediated via glutamate, gastrin-releasing peptide (GRP), and substance P (SP) (Akiyama and Carstens 2013).

Recently, another subset of itch-specific spinal cord neurons was identified. In mice, genetic ablation of gastrin-releasing peptide receptor (GRPR)-expressing neurons in the lamina I of the spinal cord abolished the scratching behavior evoked by diverse pruritogenic stimuli (including histamine and cowhage) without affecting pain sensation and/or motor activity. Of note, the complete loss of the scratching behavior occurred only when these neurons were eliminated (Sun et al. 2009), and loss-of-function GRPR mutations resulted in only slight decrease in the scratching behavior (Sun and Chen 2007). These GRPR-expressing neurons are thought to represent a higher level of itch processing in the spinal cord; they likely function as tertiary neurons in the pruriceptive pathway. Moreover, Mishra and Hoon (2013) showed that the first synaptic transmission between the central processes of both histaminergic and non-histaminergic itch-sensing primary pruriceptors and spinal cord interneurons is mediated by a novel neurotransmitter, natriuretic polypeptide b (Nppb). *Nppb* knockout mice (similar to wild-type animals in which the majority of Nppb receptor (Npra)-expressing neurons were ablated by Nppb–saporin injection) showed impaired scratching behavior in response to various pruritogenic stimuli but still responded to intrathecal administration of GRP (Mishra and Hoon 2013).

Unlike the nociceptive second-order spinothalamic neurons, itch-specific projection neurons are not spontaneously active. This lack of spontaneous activity may reflect an active (tonic) inhibition by pain-processing neurons (Davidson and Giesler 2010; Handwerker 2010; McMahon and Koltzenburg 1992; Ross 2011; Schmelz 2001). This model is supported by the observation that morphine can alleviate pain, but, at the same time, it may evoke itch (Handwerker 2010; Moser and Giesler 2013). However, as an alternative explanation it was proposed that morphine can trigger itch directly via cross-activation of GRPR by the μ-opioid receptor subtype MORD1 (Liu et al. 2011). Furthermore, increasing evidence supports the existence of spinal itch-inhibitory mechanisms which are likely mediated by GABA and glycinergic interneurons (Akiyama et al. 2011). Recently, a subpopulation of inhibitory interneurons that express the transcription factor Bhlhb5 during development was identified as the mediator of the pain that suppresses itch (Ross 2011; Ross et al. 2010). Last, descending adrenergic

pathways were proposed to control the inhibitory interneurons of the spinal cord (Gotoh et al. 2011a, b).

2.3 Higher Itch Centers

From lamina I of the dorsal horn, the itch-sensitive spinal neurons project to the ventrocaudal part of the medial dorsal nucleus of the thalamus which, in turn, has connections to the anterior cingulate and dorsal insular cortex (Andrew and Craig 2001). Functional brain imaging (PET) studies detected alterations in neuronal activities in several brain areas during itch processing; these include the primary and secondary somatosensory cortex, premotor and supplementary motor cortex, inferior parietal lobe, cerebellum, as well as certain temporal regions (Craig 2002; Drzezga et al. 2001; Hsieh et al. 1994; Kleyn et al. 2012; Mochizuki et al. 2003, 2009; Vogt et al. 2006). Although there are slight differences in the pattern of itch-related brain regions among studies (which could be attributed to different experimental designs) (Kleyn et al. 2012), there is emerging consensus that (1) the sensory-discriminative component of itch is likely processed in the primary somatosensory cortex; (2) the activities of the anterior cingulate and insular cortex may be related to the motivational and affective component; (3) the motor component of the goal-directed scratching is attributed to the premotor and supplementary motor areas; and (4) these brain regions are also involved in the central pain processing. In other words, the distinction between itchy and painful sensations may be predominantly based on the differential activation patterns of mostly identical centers (Biro et al. 2007; Davidson and Giesler 2010; Drzezga et al. 2001; Hsieh et al. 1994; Ikoma et al. 2011; Mochizuki et al. 2003; Paus et al. 2006a).

In addition, PET studies have identified specific brain activity patterns related to quantitative and qualitative characteristics of itch sensation. For example, a positive correlation was found between the subjective intensity of histamine-evoked itch sensation and the activity of the insula and the anterior cingulate cortex (Bergeret et al. 2011). Moreover, Papoiu and colleagues (2012) described different cerebral activity patterns during itch evoked by histamine or cowhage.

3 Pruritus: Types and Mediators

A widely accepted classification of pruritus that reflects the hierarchical organization of the pruriceptive system (and is also clinically relevant) is as follows (Toth and Biro 2013; Biro et al. 2007; Ikoma et al. 2006, 2011; Twycross et al. 2003; Yosipovitch et al. 2003):

- *Pruriceptive itch*: The most "trivial" category of itch which involves all peripherally induced pruritus arising from skin injuries (e.g., insect bite, botanical irritation) or diseases such as dry skin, atopic dermatitis (AD), psoriasis, infestations (e.g., scabies, pediculosis), and urticaria.

- *Neurogenic itch*: Caused by systemic disorders such as chronic liver disease (cholestasis), chronic renal failure (uremia), and thyroid dysfunction which directly trigger higher levels (brain nuclei) of the pruriceptive system. The cause of itch is not a primary failure of the itch-processing nervous system, although the pathological activation of the nervous system is a key element in the development of neurogenic itch.
- *Neuropathic itch*: Pruritus develops due to a primary neurological disorder. For example, neuropathic itch can be induced by certain brain tumors, multiple sclerosis, peripheral neuropathy (e.g., postherpetic pruritus), nerve compression, or irritation (e.g., notalgia paresthetica, brachioradial pruritus).
- *Psychogenic itch*: Pruritus is related to psychological or psychiatric disorders such as parasitophobia, obsessive-compulsive disorder, or different psychoses leading to "neurotic or psychotic excoriations."

Although some TRP channels are highly expressed in the brain, they have not yet been implicated in the pathomechanism of neurogenic, neuropathic, or psychogenic itch. Therefore, we will focus on pruriceptive itch where the involvement of TRP channels in the generation and processing of itch is well established.

Pruriceptive itch is induced by various endogenous agents, collectively referred to as pruritogens. These molecules are mostly stored in (and are released from) various cell types of the skin which are in close proximity to (or even in direct physical contact with) sensory nerve endings. Some exogenous substances (e.g., mucunain or chloroquine) can also evoke itch (Akiyama and Carstens 2013; Ansel et al. 1997; Botchkarev et al. 1997; Paus et al. 2006a; Undem et al. 1995). The release of endogenous mediators may be initiated by external effects (e.g., insect bites or poison ivy) or skin diseases. The interaction between pruritogen-containing cells and sensory neurons is, however, not a "one-way street": neuropeptides released from sensory neurons may provide a positive feedback to the pruritogen-containing cells (Ansel et al. 1997). TRP channels play pivotal roles in these epithelial–neuronal interactions (Fig. 1).

3.1 Histamine

Histamine is the central mediator of local (cutaneous) inflammatory reactions and systemic allergic responses. It is mostly released from mast cells and basophils following activating stimuli (Metz and Maurer 2009; Metz et al. 2008). Arguably, histamine is the best known itch-producing substance (Jeffry et al. 2011; Metz et al. 2011). Although histamine can evoke itch by directly activating sensory neurons, its role in the pathogenesis of pruritic skin diseases is yet to be convincingly demonstrated. Indeed, although antihistamines provide relief in urticarial diseases (Ortonne 2012; Zuberbier and Maurer 2007), most pruritic chronic skin disorders (e.g., AD) are resistant to antihistamine therapies (Jeffry et al. 2011; Klein and Clark 1999; Metz et al. 2011). In accord, in a mouse model of AD, histamine failed to evoke scratching behavior (Akiyama et al. 2010).

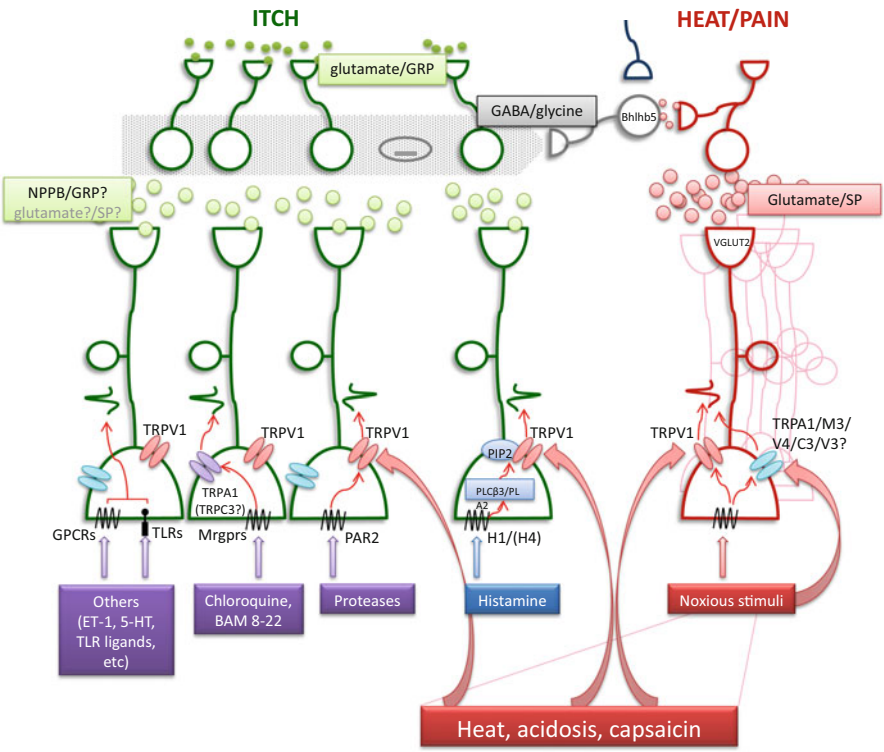

Fig. 1 TRP channels expressed on the peripheral terminals of sensory neurons as molecular targets for itch- and pain-producing agents. Please note that TRPV1 is a direct downstream target for histamine. TRPV1 is also expressed on neurons that mediate non-histaminergic itch: some pruritogens (e.g., proteases) indirectly activate TRPV1 (via PAR-2), whereas others (e.g., chloroquine acting through TRPA1) bypass TRPV1. TRP channel expression is, however, not specific for itch-transmitting afferents: nociceptive neurons also express an overlapping subset of TRP channels. Observe that at the level of the spinal cord, nociceptive neurons exert a tonic inhibition (via Bhlhb5-expressing neurons) on the pruriceptive pathway. This model postulates that itch and pain are transmitted by distinct afferents with overlapping TRP channel expression. Abbreviations are *ET-1* endothelin-1, *5-HT* 5-hydroxytryptamine, *GPCR* G protein-coupled receptor, *TLR* toll-like receptor, *Mrgpr* Mas-related G protein-coupled receptor, *PAR2* protease-activated receptor-2, *H1*/*H4* histamine receptor 1 and 4, *PLC* phospholipase C, *PIP2* phosphatidylinositol 4,5-bisphosphate, *SP* substance P, *VGLUT2* vesicular glutamate transporter 2, *NPPB* natriuretic peptide type-B, *GRP* gastrin-releasing peptide, *GABA* gamma-aminobutyric acid, *Bhlhb5* basic loop helix transcription factor, beta-3 subfamily-5

Histamine receptors belong to the G protein-coupled receptor (GPCR) superfamily. Of the four known histamine receptor subtypes (H1–H4) (Jeffry et al. 2011), two (H1 and H4) are believed to function as pruriceptors (Bell et al. 2004; Jeffry et al. 2011). Unexpectedly, H3 receptor inverse agonist and antagonists were reported to induce scratching behavior in mice (Hossen et al. 2006; Rossbach et al. 2011), whereas H3 agonists were shown to attenuate the symptom of allergic

rhinitis (Yokota et al. 2008). These findings might be explained by the expression of the inhibitory H3 receptor on the sensory nerve endings. These receptors could inhibit substance P (SP) release from sensory terminals (Arrang et al. 1983; Ohkubo et al. 1995; Rossbach et al. 2011; Yokota et al. 2008) and disrupt the sensory nerve–mast cell "itchy circuit" (see below). If this hypothesis holds true, the intimate relationship of histamine to itch might be more complicated than previously thought.

3.2 Proteases and Their Receptors

Serine proteases (including trypsin, chymotrypsin, chymase, and kallikrein) are capable of activating proteinase-activated receptors (PARs) (Steinhoff et al. 2005; Vu et al. 1991). Of PARs, PAR-2 (expressed on sensory neurons) is the major itch mediator (Steinhoff et al. 2000, 2003a; Vergnolle et al. 2003). For example, cowhage spicules contain the protease mucunain that can activate PAR-2. When applied to the human skin, cowhage evokes a histamine-independent itch reaction (Davidson and Giesler 2010; Davidson et al. 2007; Papoiu et al. 2011, 2012; Reddy et al. 2008). In AD patients, a dysregulation of PAR-2 was observed both in sensory fibers and in epidermal keratinocytes (Buddenkotte et al. 2005). In addition, kallikrein activity was increased in pruritic papular eruptions (Reis et al. 1999). In Netherton syndrome, mutations in the serine protease inhibitor Kazal-type 5 can lead to increased PAR-2 stimulation due to epidermal protease hyperactivity; this mechanism may be responsible for the development of AD-like symptoms in these patients (Briot et al. 2009).

3.3 Neuropeptides and Neurotrophins

Sensory neuropeptides released from peripheral terminals of activated sensory neurons can initiate the release of pruritogens from non-neuronal cell types of the skin. For example, the neurokinin SP, a well-known pain mediator, is also able to induce the degranulation of mast cells (Church et al. 1991; Kulka et al. 2008; Thomsen et al. 2002; van der Kleij et al. 2003). [Parenthetically, the mast cell SP receptor is different from the neurokinin NK1 receptor.] In addition to histamine, mast cells also release various proinflammatory mediators which, in turn, may further increase the activity of sensory neurons, creating a positive feedback (an "itchy circuit") (Ansel et al. 1997; Luger 2002; Paus et al. 2006a, b; Steinhoff et al. 2003b). As the initiator of this feedback loop, SP can be considered as an important etiological factor in various "itchy" skin diseases. Indeed, AD patients show significantly elevated plasma SP levels (Salomon and Baran 2008). Furthermore, in prurigo nodularis lesions increased density of SP+ nerve fibers was found compared to non-affected skin areas (Abadia Molina et al. 1992; Haas et al. 2010). Patients with hepatic (cholestasis-associated) pruritus also have elevated plasma SP levels (Trivedi and Bergasa 2010).

The pruriceptive role of CGRP remains controversial. The findings are conflicting. Some studies suggested that CGRP prolonged the latency of SP-induced itch (Ekblom et al. 1993). In keeping with this, decreased CGRP plasma levels were found in AD patients during the exacerbation period. By contrast, significantly higher CGRP values were measured in patients with severe pruritus compared to those with mild pruritus (Salomon and Baran 2008). Increased CGRP levels were also reported in patients with pruritic dermatoses (e.g., AD, nummular eczema, prurigo nodularis) (Jarvikallio et al. 2003; Liang et al. 2000). In anesthetized mice, scratching itself increased the outgrowth of CGRP+ nerve fibers (Yamaoka et al. 2007). Based on these results, one may speculate that decreased CGRP level might be a primary itch-inducing factor, whereas the elevation of CGRP might only be a consequence of scratching.

Neurotrophins (such as nerve growth factor [NGF], neurotrophin-3 and neurotrophin-4, and glial cell line-derived neurotrophic factor [GDNF]) are well-known regulators of cutaneous nerve development and regeneration (Botchkarev et al. 2006). Neurotrophins may also play an etiological role in the development of itch sensation and pruritic diseases. Several cell types of the skin (e.g., keratinocytes, mast cells, and fibroblasts) produce and release neurothrophins (Groneberg et al. 2005). Neurotrophins significantly influence itch sensation in various pathophysiological conditions via modulating the innervation density of the skin, as well as regulating the expression and/or activity of receptors expressed by sensory neurons. For example, expression of NGF is highly increased by inflammation and injuries; this, in turn, initiates acute sensitization and sprouting (leading to chronic sensitization) of C-type afferent fibers via activating specific TrkA receptors (Botchkarev et al. 2006; Hefti et al. 2006; Lazar et al. 2004; Urashima and Mihara 1998). Increased expression and plasma level of NGF were also reported in pruritic skin diseases such as in AD (Toyoda et al. 2002), prurigo nodularis (Johansson et al. 2002), and psoriasis vulgaris (Nakamura et al. 2003). Moreover, increased neurotrophin-4 expression was found in lesional skin of AD patients (Grewe et al. 2000).

Importantly, NGF levels correlated to the symptom severity in AD, and they declined with effective treatment (Yamaguchi et al. 2009). In accord with these findings, atopic human keratinocytes cocultured with porcine sensory neurons were shown to induce increased neurite outgrowth. This effect was mediated by NGF and GDNF (Roggenkamp et al. 2012). NGF not only stimulated the sprouting of itch-sensitive C-fibers but also upregulated the expression of SP, CGRP (Chang et al. 2007; Raychaudhuri and Raychaudhuri 2004; Roggenkamp et al. 2012; Verge et al. 1995), and TRPV1 (Amaya et al. 2004; Ji et al. 2002; Zhang et al. 2005b). Combined, these changes promote itch sensation. Last, neurotrophins may also directly induce degranulation of mast cells with resultant histamine release (Marshall et al. 1999; Metz et al. 2004; Stempelj and Ferjan 2005; Tal and Liberman 1997).

3.4 Inflammatory Mediators as Peripheral Itch Sensitizers

Nociceptive sensory neurons can be sensitized by an array of inflammatory mediators produced during acute or chronic inflammation of the skin, a phenomenon called inflammatory hyperalgesia (Reeh and Kress 1995). Various cell types of the skin are involved in the generation of these mediators. For example, (1) mast cells release histamine, tryptase, serotonin, tumor necrosis factor-α (TNF-α), leukotrienes, and prostaglandins; (2) keratinocytes synthesize prostaglandins, interleukins, and neurotrophins; (3) sebocytes produce prostaglandins, leukotrienes, and interleukins; (4) endothelial cells generate kinins and endothelins; and (5) immune cells contribute interleukins and chemokines. Importantly, the majority of these mediators possess marked pruritogenic potentials (Bickford 1938; Biro et al. 2005; Ikoma et al. 2006; Paus et al. 2006a; Stander et al. 2003; Steinhoff et al. 2006; Toth et al. 2011).

The prostaglandins PGE1 and PGE2 were shown to initiate and/or potentiate itch responses independent of histamine liberation (Boss and Burton 1981; Hägermark and Strandberg 1977; Lovell et al. 1976; Neisius et al. 2002). In addition, intradermal injection of other eicosanoids, such as leukotriene B4 (LTB4) or a stable thromboxane A2 analogue, evoked itch-associated responses in mice (Andoh and Kuraishi 1998; Andoh et al. 2007). Furthermore, PAR-2 activation in keratinocytes resulted in LTB4 (and PGE2) release in vitro and evoked scratching behavior in vivo. These effects were reduced by 5-lipoxygenase inhibition, suggesting that LTB4 may mediate the effect of PAR-2 activation (Zhu et al. 2009). Interestingly, LTB4 is also thought to play a role in mediating SP-evoked itch (Andoh et al. 2001).

By contrast, PGD2 exerts an antipruritic effect (Kaur et al. 2010; Sugimoto et al. 2007). In accord, PGD2 can suppress IgE-induced histamine release from the RBL-2H3 mast cell line (Hashimoto et al. 2005). Both pharmacological inhibition and in vivo silencing of cyclooxygenase-1 (COX1) resulted in decreased PGD2 levels and enhanced the scratching behavior in the AD model, NC/Nga mice (Inoue et al. 2007; Sugimoto et al. 2006).

Bradykinin also induces itch (Cormia and Dougherty 1960; Hägermark 1974; Schmelz et al. 2003) by (1) enhancing histamine release from mast cells (Walker et al. 1995), (2) augmenting histamine-evoked responses (Koppert et al. 2001; Lang et al. 1990), and (3) sensitizing the sensory afferent fibers and their receptors (e.g., TRPV1) (Lang et al. 1990). Bradykinin applied to the skin enhances the release of neuropeptides and prostaglandins (Averbeck and Reeh 2001) and evokes itch on lesional atopic skin via a histamine-independent manner (Hosogi et al. 2006). Both B1 and B2 bradykinin receptors were shown to mediate protease and PAR-2 activation-evoked scratching in mice (Costa et al. 2008, 2010). Bradykinin was also found to evoke scratching responses in complete Freund's adjuvant (FCA)-inflamed skin (Liang et al. 2012).

Immune cells of the skin can produce a variety of cytokines and interleukins (ILs) which may contribute to the pathogenesis pruritic disorders. For example, IL-2 was found to induce itch (Gaspari et al. 1987; Wahlgren et al. 1995) by

activating a histamine- and bradykinin-sensitive subpopulation of sensory C-fibers (Darsow et al. 1997; Martin and Murphy 1995). Interestingly, bradykinin potentiated the responsiveness of polymodal nociceptors to IL-2 (Martin 1996), resulting in a bidirectional augmentation. IL-2 appears to be clinically relevant in that elevated IL-2 serum levels were reported in hemodialyzed patients with uremic pruritus compared to non-pruritic individuals (Fallahzadeh et al. 2011; Martin 1996). Of note, in mice cutaneous overexpression of IL-4 resulted in an AD-like pruritic inflammatory skin disease (Chan et al. 2001). Furthermore, increased IL-6-like immunoreactivity was reported in cutaneous nerve fibers of prurigo nodularis patients (Nordlind et al. 1996).

Recently, additional cytokines (e.g., IL-13 and IL-31) have been linked to AD. Transgenic overexpression of IL-31, as well as skin-specific overexpression of IL-13, resulted in severe pruritic dermatitis in mice (Dillon et al. 2004; Zheng et al. 2009). Furthermore, both AD patients (Bilsborough et al. 2006; Nobbe et al. 2012) and atopic mice (Takaoka et al. 2005) showed increased expression of IL-31, with levels correlating to symptom severity and scratching behavior in humans and mice, respectively (Ezzat et al. 2011; Kim et al. 2011a; Sonkoly et al. 2006). Although IL-31 seems to be an important player in AD (and intradermal IL-31 injection evokes itch both in NC/Nga and BALB/c mice (Arai et al. 2013), the exact mechanism by which IL-31 contributes to itch sensation remains unclear. Of note, in the skin, IL-31 is mostly released by T_h2 lymphocytes and mature dendritic cells (Cevikbas et al. 2013), and IL-31 receptors are also expressed both on a subset of DRG neurons (Bando et al. 2006; Cevikbas et al. 2013) and on normal human epidermal keratinocytes (NHEKs) (Kasraie et al. 2011). IL-31 receptor expression on NHEKs is increased by stimulation of the toll-like receptor, TLR-2. This, in turn, results in enhanced secretion of the proinflammatory chemokine ligand, CCL2 (also known as monocyte chemotactic protein-1). Intriguingly, this effect is absent in epidermal keratinocytes obtained from AD patients where TLR-2 expression is impaired (Kasraie et al. 2011). Finally, it should be mentioned here that in pruritic myeloproliferative disorders mast cells release (among others) increased amounts of IL-31 (Ishii et al. 2009).

4 The "Pruritic" TRP Channels

TRP channels were initially discovered in a blind strain of *Drosophila* (Cosens and Manning 1969): when exposed to prolonged intense light, these mutant fruit flies showed transient calcium influx into their photoreceptor cells (Montell and Rubin 1989), hence the term "transient receptor potential"(Minke 2010). This seminal observation paved the way to the discovery of the first mammalian TRP channels called "canonical" (TRPC) due to their homology to the *Drosophila* channels (Wes et al. 1995; Zhu et al. 1995). Unlike most families of ion channels that have been grouped together based on function, TRP channels are subdivided based on primary amino acid structure (Clapham et al. 2001; Nilius and Voets 2005; Pedersen et al. 2005; Wu et al. 2010). As of today, mammalian TRP channels comprise

28 members and are categorized into six subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin). The mucolipin and polycystin subfamilies were named after the diseases ("TRP channelopathies") they are associated with, mucolipidosis and autosomal dominant polycystic kidney disease, respectively (Nilius et al. 2007). The vanilloid subfamily was named after its founding member, the vanilloid (capsaicin) receptor TRPV1 (Caterina et al. 1997). The first melastatin channel (TRPM1) was discovered as a protein present in benign nevi and absent in malignant melanoma (Duncan et al. 1998). As of today, the ankyrin subfamily has only one member, TRPA1, which, as the name implies, is rich in ankyrin segments at its N-terminus.

As a general rule, TRP channels have six transmembrane-spanning domains (S1 to S6) with a pore-forming loop between S5 and S6 (Wu et al. 2010). Most TRPs form functional channels as homotetramers, but heteromultimerization is frequently observed (Cheng et al. 2010a). Beyond that, few generalities can be made about TRP channels. Consistent with the diversity in primary structure is the diversity observed in functional properties. The TRPs are commonly referred to as calcium-permeable nonselective cation channels, but TRPM4 and TRPM5 are sodium-selective channels, whereas TRPV5 and TRPV6 are inwardly rectifying calcium-selective channels (Owsianik et al. 2006). TRP channels also vary in their tissue expression patterns. For example, TRPM7 is ubiquitously expressed, whereas the expression of TRPA1 is highly restricted. Even the subcellular localization of the proteins varies with family members: for example, TRPML1 is being hypothesized to be an intracellular channel (Bach 2005). Some TRP channels like TRPM2 are unique in that they contain a functional NUDIX/ADP ribose domain along with a kinase domain that bears some resemblance to protein kinase A (Eisfeld and Luckhoff 2007). These TRPs combine features of ion channels and enzymes and are therefore referred to by some as "chanzymes" (Montell 2003).

Despite decades of intensive research, only a few endogenous ligands of TRP channels have been identified. For example, some endogenous lipid mediators, like arachidonic acid derivatives or lipoxygenase products, can activate some TRP channels like TRPV1 (Huang et al. 2002; Hwang et al. 2000; Vriens et al. 2009; Watanabe et al. 2003), whereas other TRPs (e.g., TRPM2) are believed to function as redox sensors (Kozai et al. 2013; Miller and Zhang 2011). In the days of the finalization of this manuscript, the first high-resolution TRP channel structure was published: this pioneering finding can potentiate the discovery of further endogenous ligands and their structure–activity relationships (Cao et al. 2013; Liao et al. 2013). For the time being, most TRP channels, however, remain orphan receptors. How these channels are modulated in vivo is still largely a mystery.

A functional subset of TRP channels (the so-called thermoTRPs) is distinguished by their sensitivity to temperature changes: combined, these channels cover a broad range of temperatures, ranging from noxious hot to ice-cold (Szallasi et al. 2007; Patapoutian et al. 2009; Talavera et al. 2008; Voets 2012; Voets et al. 2004). With respect to pruritus, these thermoTRPs (in particular, TRPV1, TRPV3, and TRPA1) are the most interesting (Figs. 1 and 2). However, some other

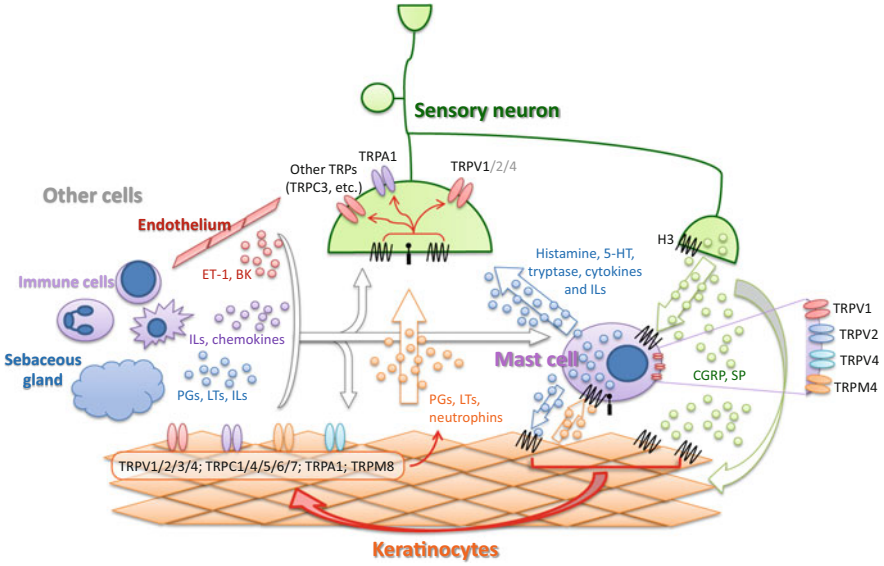

Fig. 2 A cartoon depicting the positive feedback loop between TRP channel-expressing neurons and non-neuronal cells, in particular keratinocytes and mast cells. Please note that both neuropeptides released from sensory afferents (e.g., SP and CGRP) and substances (PGs, LTs, and neurotrophins) generated by skin keratinocytes activate mast cells; in turn, mast cells release substances (histamine, 5-HT, tryptase, cytokines and ILs) that activate sensory afferents and stimulate keratinocytes. TRP channels that are expressed on sensory afferents, mast cells, and keratinocytes are believed to play a central role in these neuro-immune interactions. Abbreviations: *PGs* prostaglandins, *ET-1* endothelin-1, *BK* bradykinin, *ILs* interleukins, *LTs* leukotrienes, *SP* substance P, *CGRP* calcitonin gene-related peptide

members of the TRP family may also contribute to the development of itch and related diseases (Biro et al. 2007; Toth and Biro 2013).

4.1 The Vanilloid (Capsaicin) Receptor TRPV1

Accumulating evidence suggests a crucial role for TRPV1 in pain sensation as a molecular integrator of a plethora of nociceptive stimuli. TRPV1 is a unique channel in that it functions as a polymodal nociceptor with a dynamic threshold of activation that could be significantly lowered under inflammatory conditions (reviewed in Szallasi et al 2007). Indeed, agents in an "inflammatory soup" act in concert to reduce the activation threshold of TRPV1. In addition to capsaicin, TRPV1 can also be activated and/or sensitized by numerous endogenous substances, including changes in pH [both acidosis (Caterina et al. 1997) and alkalosis (Dhaka et al. 2009)]; bradykinin (Chuang et al. 2001; Shin et al. 2002); ATP (Tominaga et al. 2001); lipoxygenase products (Hwang et al. 2000; Shin et al. 2002); prostaglandins (Mohapatra and Nau 2003); various neurotrophins

such as NGF, neurotrophin-3, and neurotrophin-4 (Lazar et al. 2004; Shin et al. 2002); tumor necrosis factor-α (TNFα) (Nicol et al. 1997); and proinflammatory chemokines (Zhang et al. 2005a). In addition, activation of PAR-2 (Amadesi et al. 2004; Dai et al. 2004), metabotropic receptor-coupled hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) by phospholipase C (PLC) (Prescott and Julius 2003), or activation of the ionotropic purinoceptor P2X3 (Saloman et al. 2013) can indirectly lead to TRPV1 activation/sensitization. Some of these agents (like pH) activate TRPV1 directly, whereas others (like bradykinin) influence the phosphorylation state of the protein via protein kinases. There is preliminary evidence that TRPV1 can be operated by voltage, and the size of the pore is dynamically changing during activation (Chung et al. 2008; Voets 2012; Voets et al. 2004). Importantly, a subset of TRPV1-expressing neurons is activated by histamine (Kim et al. 2004; Shim et al. 2007). It has been shown that increases in cutaneous temperatures or exceedingly acidic pH of the skin (known TRPV1 activators) can effectively modulate itch sensation in humans (Paus et al. 2006a; Pfab et al. 2006; Steinhoff et al. 2006; Yosipovitch et al. 2005).

Several lines of evidence suggest that (similar to its role in nociception) TRPV1 may function as a molecular integrator of pruriceptive molecules in the itch pathway (Biro et al. 2005, 2007; Paus et al. 2006a; Steinhoff et al. 2006). Indeed, histamine-induced inward currents in sensory neurons can be blocked by TRPV1 antagonists (Shim et al. 2007). Activation by histamine of TRPV1-expressing neurons is mediated by the synthesis of 12-hydroperoxyeicosatetraenoic acid (12-HPETE), a pruritogenic (Kim et al. 2007) metabolite of 12-lipoxygenase and an endogenous activator of TRPV1 (Hwang et al. 2000; Shim et al. 2007). Moreover, upstream of TRPV1, the phospholipase A2 (PLA2)-coupled signaling pathway, was shown to mediate histamine-induced experimental itch (Kim et al. 2004; Shim et al. 2007). Recently, Imamachi and colleagues (2009) provided evidence for the role of phospholipase C beta3 (PLCβ3) in the downstream signaling pathway that links the histamine receptor to TRPV1. Indeed, histamine-induced scratching behavior was significantly reduced in TRPV1-deficient mice (Imamachi et al. 2009; Shim et al. 2007).

TRPV1 is clearly required for the transmission of histamine-induced scratching behavior but not for serotonin- or endothelin-1 (ET-1)-induced itch. However, chemical ablation by capsaicin of TRPV1-positive sensory neurons leads to deficits in behavioral responses to a variety of itch inductors, suggesting that the molecular targets for these molecules (though distinct from TRPV1) are also present on capsaicin-sensitive neurons (Imamachi et al. 2009).

The pharmacological blockade or genetic deletion of TRPV1 inhibited trypsin-evoked itch in mice (Costa et al. 2008), implying a role for TRPV1 in PAR-2-coupled pruriceptive signaling. In a rat model of hepatic pruritus, not only the expression of TRPV1 was increased in sensory neurons, but the expression of the channel shifted from the small- toward the medium-sized DRG neurons (Belghiti et al. 2013). The pruritogenic substance IL-31 also activated a small subset of TRPV1-positive neurons co-expressing the IL-31 receptor IL-31RA (Cevikbas et al. 2013). Approximately two thirds of the IL-31-responsive DRG neurons

responded to capsaicin, and the majority (~90 %) were activated by mustard oil, an activator of both TRPV1 and TRPA1 (Everaerts et al. 2011). IL-31-evoked scratching behavior was significantly reduced both in TRPV1 and TRPA1 KO animals, suggesting that both molecules play a role in the IL-31-induced itch (Cevikbas et al. 2013). Unexpectedly, chloroquine (a molecule which, as discussed later, causes scratching behavior in mice via TRPA1 signaling) was also found to sensitize TRPV1 utilizing the MrgprA3–phospholipase C–PKC pathway (Than et al. 2013). Moreover, selective silencing of TRPV1+ sensory neurons ameliorates both histamine- and chloroquine-evoked responses (Roberson et al. 2013), providing further proof that TRPV1 can be involved in the mediation of both histaminergic and non-histaminergic itch.

The membrane-associated phosphoinositide-binding protein, Pirt, was recently identified as an important positive regulator of TRPV1 (Kim et al. 2008). Pirt-null mice showed not only impaired responsiveness to noxious heat and capsaicin (Kim et al. 2008) but also a decreased scratching response to both histaminergic and non-histaminergic (e.g., evoked by chloroquine, alpha-methyl-serotonin, ET-1, or PAR-2 activation) itch (Patel et al. 2011). Although the authors speculated that the itch-mediating effect of Pirt may be partly independent of TRPV1, it did not affect formalin-induced TRPA1 activation and the resultant scratching response (Patel et al. 2011). Of note, Pirt is not selective for TRPV1: indeed, it can regulate other TRP channels like TRPM8 (Tang et al. 2013).

Toll-like receptors (TLRs), originally identified as pathogen-pattern recognition receptors, are emerging as intriguing new players in itch sensation (Liu et al. 2012b). TLR7 is co-localized with TRPV1 in mouse sensory neurons where it mediates the imiquimod (a TLR7 agonist)-evoked scratch response. Interestingly, in TLR7-null mice the non-histaminergic itch response evoked by serotonin and ET-1 and PAR-2 activation or chloroquine was impaired. The chemical ablation of TRPV1-expressing DRG neurons by resiniferatoxin (an ultrapotent capsaicin analogue) treatment abolished the imiquimod response, but the inactivation of TRPV1 by genetic manipulation had no effect. Taken together, these findings imply a crucial role for TRPV1+ sensory afferents, but not the TRPV1 channel itself, in mediating TLR7-induced itch (Liu et al. 2010a).

The findings on the role of TLR7 in pruritus are, however, conflicting. Another study (Kim et al. 2011b) concluded that the pruritic effect of imiquimod was not related to TLR7, yet the authors also confirmed the role of TRPV1-expressing neurons (but, again, not of TRPV1) in the itch response. TLR3 (present in a subset of sensory neurons that co-express TRPV1 with GRP) was also reported to mediate both histamine-dependent and histamine-independent itch. Importantly, genetic deletion of TLR3 attenuated itch responses, but it did not affect acute pain sensation. In these animals, the responsiveness of DRG neuronal cell bodies was normal to both pain- and itch-producing agents, but the excitatory synaptic transmission in the spinal cord was impaired (Liu et al. 2012a).

Conditioned genetic deletion of the vesicular glutamate transporter type 2 (VGLUT2) in DRG neurons resulted in a dramatic increase in itch-related behavior, and, at the same time, it caused a significant deficiency in thermal pain

sensation. The absence of VGLUT2 affected both the majority of TRPV1-expressing neurons that are negative for GRP (thought to be responsible for the pain transmission) and a smaller, partially overlapping subpopulation of TRPV1+ neurons that co-express GRP (which are suggested to be responsible for itch sensation). It was proposed that VGLUT2 deletion terminated the negative control that the pain-processing pathway exerts over the itch-processing pathway; this, in turn, resulted in a dramatic increase in spontaneous as well as histamine-dependent and histamine-independent scratching behavior. Indeed, in the VGLUT2-deficient mice, capsaicin evoked mostly itch at doses at which it caused pain in the wild-type littermate controls (Lagerstrom et al. 2010; Liu et al. 2010b). Most recently, in an elegant study, Han et al. (Han et al. 2013) reexpressed TRPV1 only in the MrgprA3-expressing neurons of TRPV1-null animals: in these animals, capsaicin evoked only itch but not pain responses. Combined, these findings provide strong experimental support for the selectivity theory.

The ongoing controversy that surrounds the function (or even very existence) of TRPV1 in non-neuronal cells is beyond the scope of this review. Here it suffices to mention that, albeit at a much lower level than in DRG neurons, widespread TRPV1 expression was reported in the human skin, including keratinocytes, dermal mast cells, dendritic cells, hair follicle (HF) keratinocytes, and sebocytes of the sebaceous glands (SG) (Basu and Srivastava 2005; Bodo et al. 2004, 2005; Denda et al. 2001; Inoue et al. 2002; Southall et al. 2003; Stander et al. 2004; Toth et al. 2009a, b). Moreover, TRPV1 activation (besides affecting the proliferation, differentiation, and apoptosis of keratinocytes) resulted in the release of cytokines and mediators (e.g., ILs, PGs, and growth factors) that may elicit itch sensation (Amantini et al. 2004; Basu and Srivastava 2005; Birder et al. 2001; Biro et al. 1998; Bodo et al. 2005; Southall et al. 2003; Toth et al. 2009b). TRPV1 expression is also increased in epidermal keratinocytes obtained from patients with prurigo nodularis (Stander et al. 2004). The expression of TRPV1 in skin mast cells is of particular interest (Bodo et al. 2004; Stander et al. 2004). One might speculate that TRPV1 (which is expressed on both sensory afferents and non-neuronal cells) may play a crucial bidirectional role in the interaction between neurons and non-neuronal cells that controls itch initiation and augmentation (Biro et al. 2005, 2007; Paus et al. 2006a; Steinhoff et al. 2006). Indeed, TRPV1 was reported to contribute to the LTB4-induced itch via mediating leukocyte infiltration as measured by myeloperoxidase (MPO) activity in rat dorsal skin (Fernandes et al. 2013).

If TRPV1-expressing sensory neurons play a central role in the development of pruriceptive itch, silencing of these neurons by pharmacological manipulation (e.g., capsaicin desensitization) should alleviate pruritus. Although there are anecdotal reports and case studies to report a beneficial effect for topical capsaicin application in different pruritic syndromes [ranging from notalgia paresthetica through psoriasis, prurigo nodularis, aquagenic pruritus, uremic pruritus, and cholestasis to pruritus ani and allergic rhinitis (Stander et al. 2001)] (Blom et al. 1997; Ellis et al. 1993; Lacroix et al. 1991; Lotti et al. 1994; Lysy et al. 2003; Makhlough et al. 2010; Stjarne et al. 1998; Tarng et al. 1996; Wallengren and Klinker 1995; Weisshaar

et al. 2003), the antipruritic effect of topical capsaicin in controlled clinical trials is yet to be confirmed (Gooding et al. 2010). In dogs with a disease resembling human AD, itch-relieving effect was noted by the owners but not the investigators. A word of caution here: capsaicin-containing creams (e.g., Zostrix, 0.075 %) have been in clinical use for decades for the treatment of chronic painful conditions such as diabetic neuropathy. Despite their popularity, controlled clinical trials found no evidence that these creams have superior analgesic compared to placebo (Knotkova et al. 2008). To increase the exposure of cutaneous nerve endings to capsaicin, occlusive patches (Qutenza, 8 %) were developed (Noto et al. 2009). However, in 2012 the US Food and Drug Administration found no evidence that Qutenza relieves HIV-associated neuropathic pain. In summary, the clinical experience with topical capsaicin as an analgesic agent has been disappointing, and we have no reason to believe that topical capsaicin could fair much better in patients with chronic itch. The explanation why capsaicin desensitization has failed to live up to expectation is detailed elsewhere (Szallasi and Sheta 2012). A potential strategy to boost the efficacy of capsaicin desensitization is by using pore-permeable capsaicin analogs (e.g., permanently charged capsaicinoids) which target and desensitize only (hyper)active channels (Li et al. 2011; Moran et al. 2011).

After the cloning of TRPV1, there was a great deal of enthusiasm in the pharmaceutical industry to develop small molecule TRPV1 antagonists as analgesic agents (Szallasi et al. 2007). Indeed, a number of compounds entered clinical trials, but none has been advanced so far beyond Stage II due to a combination of unforeseen side effects (e.g., hyperthermia and impaired heat pain sensation) and lack of clinical efficacy. For further details, interested readers are referred to recent reviews (Brederson et al. 2013; Szallasi and Sheta 2012; Szolcsanyi and Pinter 2013; Trevisani and Szallasi 2010; Wong and Gavva 2009). Pilot studies with the TRPV1 antagonist PAC-14028 reported beneficial effects to alleviate itch in different AD models (Lim and Park 2012; Yun et al. 2011a, b). For example, in a mouse model of AD (induced by *Dermatophagoides farina* and oxazolone), PAC-14028 administration prevented the development of dermatitis-associated barrier damages by suppressing transepidermal water loss, inducing reconstruction of epidermal lipid layers, and normalizing altered expressions of epidermal differentiation markers and, at the same time, improved the AD-like symptoms (clinical severity, skin score, serum IgE levels, mast cell degranulation status). These beneficial effects obtained with TRPV1 antagonists need to be confirmed in AD patients, especially because SB-705498, another TRPV1 antagonist, did not provide any symptomatic relief in patients with seasonal allergic rhinitis (Bareille et al. 2013), although it was effective in guinea pig models (Changani et al. 2013; Delescluse et al. 2012).

The endocannabinoid anandamide (depending on its concentration and other local factors such as acidosis in inflamed tissue compartments (Olah et al. 2001) may also function as an endogenous TRPV1 agonist, that is, an "endovanilloid" (Biro and Kovacs 2009; Di Marzo et al. 2002; Di Marzo and Petrocellis 2006; Pacher et al. 2006). Indeed, it was suggested that TRPV1 doubles as an "ionotropic cannabinoid receptor." Interestingly, various synthetic cannabinoids (e.g., HU210,

WIN 55, 212–2) and certain endocannabinoids (e.g., N-palmitoyl ethanolamine) were shown to ameliorate both histamine-induced and disease-related pruritus (Dvorak et al. 2003; Gingold and Bergasa 2003; Stander et al. 2006a). This effect was accompanied by decreased neuropeptide release from the sensory endings (Dvorak et al. 2003). Furthermore, cannabinoid CB2 receptors have recently been identified on epidermal keratinocytes where they co-localize with TRPV1 (reviewed in (Biro et al. 2009)). The activation of keratinocyte CB2 resulted in the release of β-endorphin (Ibrahim et al. 2005), an analgesic and pruritic pro-opiomelanocortin substance. Taken together, these findings strongly support a role for the endocannabinoid system in the modulation of pruritoception, presumably via both TRPV1 and CB2. If this concept is corroborated by further experimental evidence, compounds that target both TRPV1 and CB2 may represent a novel strategy to relieve itch.

For the sake of completeness, it should be mentioned here that TRPV1 has been implicated in the puritic side effects of various drugs. For example, the antifungal agent clotrimazole was reported to activate both TRPV1 and TRPA1 (and inhibit TRPM8), and it was speculated that this might contribute to the burning–itching sensation that clotrimazole evokes in some patients (Meseguer et al. 2008). Moreover, retinoids (widely used to treat various skin diseases) can also activate TRPV1 and evoke nocifensive behavior (Yin et al. 2013). The antitumor agent paclitaxel was reported to increase TRPV1 expression in the skin (Hara et al. 2013). Burning itch is a well-documented side effect of topically applied calcineurin inhibitors (e.g., pimecrolimus and tacrolimus); this itch is accompanied by the release of SP and CGRP from primary sensory afferents (Stander et al. 2006b). Tacrolimus may influence the phosphorylation state of TRPV1 (Pereira et al. 2010).

4.2 TRPV2

TRPV2 was originally cloned from rodent brain as a "VR1-like protein" (VRL-1), and it was also described in a subset of medium- to large-diameter sensory neurons as a detector of noxious hot temperature (>52 °C) (Caterina et al. 1999). It is also expressed in C-fibers innervating the human skin (Axelsson et al. 2009) in co-localization with its closest relative, TRPV1 (Rutter et al. 2005). Furthermore, TRPV2 is expressed on human epidermal keratinocytes and the human mast cell line HMC1 (Radtke et al. 2011; Zhang et al. 2012a).

The role of the TRPV2 in the noxious heat sensation is controversial. Indeed, TRPV2 KO mice display normal thermal and mechanical nociception (Park et al. 2011). Moreover, unlike its murine homologue, the human TRPV2 is insensitive to noxious heat (Neeper et al. 2007). TRPV2 is still an orphan receptor. The non-psychotropic phytocannabinoid, cannabidiol, was shown to activate TRPV2 expressed on sensory DRG neurons, resulting in CGRP release (Qin et al. 2008). Moreover, activation of TRPV2 by various physical stimuli induced degranulation of human mast cell line HMC1, which was inhibited by the nonselective TRPV2 blocker, SKF96365 (Zhang et al. 2012a). It was also suggested that the potentially

pruritogenic PKA-dependent phosphorylation of TRPV2 can modulate channel activity in mast cells. However, TRPV2 as a potential target for preventing mast cell degranulation remains to be validated.

4.3 TRPV3 and TRPV4

TRPV3, a warm-sensitive channel, is highly expressed in keratinocytes (Nilius and Biro 2013; Nilius et al. 2013; Peier et al. 2002). In mice, a spontaneous gain-of-function (Gly573Ser) mutation in the *trpv3* gene not only results in a hairless phenotype and dermatitis but also causes severe itching (Asakawa et al. 2006; Xiao et al. 2008). Transgenic mice overexpressing this mutant channel in their epidermal keratinocytes exhibit itching/scratching behavior and intracutaneous accumulation of NGF (Yoshioka et al. 2009). Importantly, Olmsted syndrome, a rare genetic disorder (TRP channelopathy) which is partly due to the same gain-of-function (Gly573Ser) TRPV3 mutation, is also characterized by debilitating itch (Lai-Cheong et al. 2012; Lin et al. 2012).

Somewhat unexpectedly, in an experimental dry skin model (evoked by application of acetone–ether–water), no differences were found in histological features of barrier disruption between wild-type and TRPV3-null animals, but the latter group showed less intensive scratching behavior attributed to decreased sprouting of cutaneous sensory C-fibers, suggesting a possible role of TRPV3 in dry skin-associated itch (Yamamoto-Kasai et al. 2012). TRPV3 can also regulate the release of various mediators like ATP, PGE2, or NO (Huang et al. 2008; Mandadi et al. 2009; Miyamoto et al. 2011), which can influence sensory functions, maybe involving itch.

Experimentally induced Mg^{2+} deficiency in mice or rats is a widely used model of AD-like scratching behavior (Chavaz et al. 1984; Neckermann et al. 2000). Intriguingly, both extra- and intracellular Mg^{2+} were shown to tonically inhibit TRPV3 activity in cultured epidermal keratinocytes (Luo et al. 2012). It was, therefore, speculated that hypomagnesemia (which can develop in various human disease conditions such as type 2 diabetes mellitus) may induce the overactivity of TRPV3, resulting in the development of pruritus. The role of TRPV3 in the development of AD is, however, controversial. The pathogenesis of AD is thought to involve compromised skin barrier formation, and the skin barrier is clearly defective in the *trpv3* (−/−) animals. To explain the phenotype of the TRPV3-null mice, it was proposed that TRPV3 forms a complex with ADAM17, EGFR, and TGase1, which is required for normal terminal keratinocyte differentiation. Dysregulation of this complex (by absent TRPV3) may impair barrier function and lead to dry skin (Cheng et al. 2010b). Yet, as discussed above, TRPV3 KO mice showed less scratching than their wild-type littermates in an experimental AD model; conversely, the gain-of-function TRPV3 phenotype was associated with intense itching. To reconcile these seemingly contradictory findings, one might speculate that a certain level of basal TRPV3 activity is essential for normal skin function, with both increased and decreased activities leading to dermatological

disease. Of note, disturbed barrier formation was detected in neonatal *trpv3*−/− mice, but not in adult TRPV3 KO animals with a different genetic background (Miyamoto et al. 2011; Moqrich et al. 2005).

TRPV4 as an itch target is highly controversial since none of the numerous human gain-of-function mutation channelopathies has a pruritic phenotype (Nilius and Voets 2013). Yet, in experimental models certain pruritogens were found to target TRPV4. For example, PAR-2 activation sensitizes TRPV4-coupled cellular responses in DRG neurons, resulting in increased neuropeptide release and mechanical hyperalgesia (Grant et al. 2007). Furthermore, in patients undergoing breast reduction surgery for painful mastopathy, increased expression of TRPV4 (as well as TRPV3) was detected in the basal layers of keratinocytes. This altered expression of TRPV4 was associated with an increase in TRPV1 and NGF expression in sensory fibers (Gopinath et al. 2005): this may intensify not only pain but also itch sensation. Furthermore, mast cell functions also seem to be affected by TRPV4. For instance, laser irradiation of mouse RBL-2H3 mast cells evoked intracellular Ca^{2+} increase and histamine release via TRPV4 (Yang et al. 2007). TRPV4 (like TRPV2) was also expressed by HMC1 human mast cells, and the increase of temperature to 37–39 °C, as well as the TRPV4 agonist 4α-phorbol 12,13-didecanoate, induced a weak outwardly rectifying current with subsequent increase in the intracellular Ca^{2+} concentration (Kim et al. 2010).

4.4 TRPA1

TRPA1 has emerged as a major target for histamine-independent itch. Chloroquine, an "itchy" antimalarial drug, was shown to activate both rodent (mouse and rat) MrgprA3 and human MrgprX1, members of the Mrgpr family that are exclusively expressed in peripheral sensory neurons (Dong et al. 2001). In mice, chloroquine-responsive, MrgprA3-expressing sensory neurons were activated by capsaicin, histamine, and BAM 8–22 (a specific MrgprC11 agonist), suggesting the co-expression of Mrgprs with TRPV1 and histamine receptors. Furthermore, these Mrgprs were also co-expressed with GRP, another neurotransmitter of the "itch pathway" (see above). Importantly, the deletion of the *mrgpr* gene cluster resulted in impaired chloroquine response, leaving the histamine-evoked scratching behavior intact: this observation implies a central role for Mrgprs in non-histaminergic itch (Liu et al. 2009).

TRPA1 is now thought to play a crucial role in the downstream signaling of both MrgprA3 and MrgprC11 (Wilson et al. 2011; Xiao and Patapoutian 2011). Indeed, in TRPA1 KO mice (in contrast to TRPV1 KO and wild-type animals) the activation of neither MrgprA3 (by chloroquine) nor MrgprC11 (by BAM 8–22) resulted in itch-related scratching behavior. Although in wild-type animals these substances evoked an elevation in the intracellular Ca^{2+} concentration and a resultant firing of action potentials in the capsaicin- and mustard oil-sensitive subsets of DRG neurons, respectively, these effects were abolished in the TRPA1 KO mice (but not in the TRPV1 KO animals). Furthermore, in heterologous expression systems,

activation of the Mrgprs was able to evoke Ca^{2+} responses only in cells in which they were co-expressed with TRPA1. Finally, pharmacological experiments suggested that MrgprA3 was coupled to TRPA1 via the βγ subunit of G proteins, whereas MrgprC11 most probably signals via a PLC-dependent pathway (Wilson et al. 2011).

The situation is, however, probably even more complex. A recent study demonstrated that only a slight minority (~43 %) of chloroquine-sensitive DRG neurons respond to the TRPA1 agonist mustard oil, whereas the majority of the mustard oil-insensitive cells were activated by the TRPV1-specific agonist, capsaicin. Intriguingly, a minor population responded to neither TRPA1 nor TRPV1 stimulation (Than et al. 2013). Unexpectedly, these TRPA1-negative neurons seem to respond to chloroquine in a TRPC3-mediated fashion. If confirmed, this observation will furnish TRPC3 with a previously unexpected function in itch pathogenesis.

There is good evidence that TRPA1 is also involved in other pruritogenic mechanisms. For example, hydrogen peroxide-induced itch is clearly mediated by TRPA1, as it is inhibited by the pharmacological blockade of TRPA1 but not by TRPV1. Moreover, the hydrogen peroxide-induced itch was not influenced by histamine receptor blockers (Liu and Ji 2012). By contrast, scratching behavior induced by intradermal LTB_4 administration was attenuated by the TRPA1 antagonist TCS-5861528 and the TRPV1 antagonist SB366791 with comparable efficacies (82 % inhibition and 97 % inhibition, respectively), but their mechanisms likely differ. TRPA1 was found to play a role in increased superoxide production in contrast to TRPV1, the deletion of which resulted in impaired myeloperoxidase release (Fernandes et al. 2013). In addition, TRPA1 might play a role in mediating the activation of sensory neurons by PAR-2, which was shown to sensitize TRPA1 by most likely degrading PIP2 by PLC (Dai et al. 2007). Further, TRPA1 (apparently in concert with TRPV1) seems to be involved in the downstream signaling of itch, generated by the activation of IL-31 receptor (IL-31RA) on DRG sensory neurons (Cevikbas et al. 2013). IL-31-evoked responses were significantly reduced both in TRPA1 and TRPV1 knockout mice. Most recently, TRPA1 was also found to be involved in the itch development in AD. In a mouse model, the thymic stromal lymphopoietin (TSLP) produced by atopic keratinocytes excited sensory neurons via the TSLP receptor–PLC–TRPA1 pathway and resulted in intense scratching behavior. The TSLP production of keratinocytes was stimulated by PAR-2 activation (Wilson et al. 2013).

Unexpectedly, it was reported that TRPA1 blockade enhanced ET-1 (but not histamine)-induced itch responses (Liang et al. 2011), suggesting a paradoxical inhibitory role of TRPA1 in (at least some forms of) histamine-independent itch. This controversy indicates the urgent need for further studies to clarify the contribution of TRPA1 to other forms of itch.

Although TRPA1 expression is considered to be remarkably selective for neurons, TRPA1 expression was also described in non-neuronal cells. Indeed, TRPA1 expressed in lung fibroblasts may play an important role in nonneurogenic inflammatory responses in diseased airways (Nassini et al. 2012). Pertinent to the

topic of this review, TRPA1 expression was reported in epidermal keratinocytes. Exposure of NHEKs to low temperatures (13–15 °C) or TRPA1 agonists (allyl isothiocyanate or cinnamaldehyde) induced elevation of $[Ca^{2+}]_e$, which was prevented by the TRPA1 antagonist, HC-030031. Interestingly, these effects were more pronounced in undifferentiated cells (Tsutsumi et al. 2010). Moreover, treatment of NHEKs with icilin (activator of both TRPA1 and TRPM8, another cold-sensitive channel) caused alterations in the expressions of adhesion and extracellular matrix components, as well as molecules regulating cell cycle, apoptosis, and differentiation (Atoyan et al. 2009; commented in Biro and Kovacs 2009). Taken together, these observations suggest that TRPA1 in keratinocytes may be involved in the regulation of the epidermal barrier. Indeed, in mice following tape striping, topical application of TRPA1 agonists accelerated barrier recovery, and this effect was prevented by pretreatment with HC-030031. Interestingly, HC-030031 alone delayed the barrier recovery which argues for a "constitutive" (tonically active) role of TRPA1 in epidermal barrier homeostasis. Local cooling of the skin (10–15 °C for 1 min) evoked similar effects, most probably via accelerated secretion of (barrier-forming) lamellar bodies at the interface of stratum granulosum and corneum; this action was also inhibited by the TRPA1 antagonist (Denda et al. 2010b).

Stimulation of TRPA1 expressed on NHEKs also induced the synthesis of the proinflammatory interleukins, IL-1α and IL-1β (Atoyan et al. 2009). Moreover, topical application of the TRPA1 agonist, cinnamaldehyde, induced skin inflammation. Interestingly, although the edema component was prevented by aprepitant (an antagonist of the tachykinin NK1 receptor), it was not affected by HC-030031. By contrast, the cinnamaldehyde-induced leukocyte infiltration was effectively suppressed by pharmacological TRPA1 blockade, while the NK1 antagonist was ineffective (Silva et al. 2011). In a mouse contact hypersensitivity model, TRPA1 activation enhanced ear swelling response and migration of dendritic cells to draining lymph nodes, which the effect was antagonized by HC-030031 (Shiba et al. 2012).

A recent study testing various agents known to induce contact dermatitis in humans verified the central role of TRPA1 activation and resultant SP release in the development of contact dermatitis. Genetic deletion or pharmacological blockade of TRPA1 (but not TRPV1) resulted in decreased skin edema, keratinocyte hyperplasia, leukocyte infiltration, and scratching behavior in mice exposed to oxazolone. In oxazolone-challenged skin of TRPA1-deficient mice, a significant decrease was found in the expression of inflammatory cytokines, NGF, and endogenous pruritogens (e.g., SP and serotonin). Oxazolone was also shown to activate recombinant TRPA1, further supporting the causal role of the channel in contact dermatitis. Urushiol, the contact allergen in poison ivy, evoked similar responses which were diminished in TRPA1-null mice (Liu et al. 2013).

4.5 TRPM4

TRPM4 is a Ca^{2+}-activated, nonselective cation channel which negatively regulates the driving force of Ca^{2+} ion influx in mast cells. Indeed, bone marrow-derived mast cells obtained from TRPM4 KO animals showed increased degranulation and mediator release in vitro, as well as a more severe IgE-mediated acute passive cutaneous anaphylactic response in vivo (Vennekens et al. 2007). Genetic deletion of TRPM4 dysregulated the migration of mast cells (Shimizu et al. 2009). Taken together, these observations imply a therapeutic potential for TRPM4 agonists in the management of anaphylactic reactions or local allergic responses. Unfortunately, the widespread expression of TRPM4 and its involvement in various physiological functions (most important, cardiac impulse generation) may strongly limit (or completely prevent) the systemic application of TRPM4 activators in clinical practice.

4.6 TRPM6 and TRPM7

In rats, insufficient dietary Mg^{2+} intake leads to low serum Mg^{2+} concentrations (hypomagnesemia) and subsequent development of dermatitis and intense scratching behavior (Thomsen et al. 2005). Indeed, uremic patients on hemodialysis with low serum Mg^{2+} levels develop itch which disappears after the concentration of Mg^{2+} in the dialysate is normalized (Graf et al. 1979). The emerging link between low Mg^{2+} and itch has brought TRP channels (TRPM6 and TRPM7) that play a role in Mg^{2+} homeostasis (the so-called Mg^{2+}nificent TRP channels) in the center of spotlight.

Epidermal growth factor (EGF), a central growth factor in epidermal differentiation, has a crucial role in controlling TRPM6 functions (Muallem and Moe 2007). Indeed, EGF stimulates Mg^{2+} reabsorption in the renal distal convoluted tubule. A point mutation in pro-EGF was shown to disturb this mechanism, resulting in renal Mg^{2+} loss (Groenestege et al. 2007). In support of a link among EGF, TRPM6, Mg^{2+}, and itch, several studies described itch as a common side effect of cetuximab, a chemotherapeutic monoclonal antibody that inhibits the EGF receptor (Agero et al. 2006; Porzio et al. 2006; Wu et al. 2011).

4.7 TRPM8

Menthol, the classical activator of TRPM8, is an active ingredient in various OTC topical anti-itch lotions. Indeed, several case studies reported a modest clinical benefit for menthol in various forms of pruritus (Frolich et al. 2009; Haught et al. 2008). For example, menthol (as well as causing moderate cooling of the skin) decreased the subjective intensity of histamine-induced itch (Bromm et al. 1995). Furthermore, menthol provided relief in mustard gas-induced pruritus in chemical warfare-injured veterans (Panahi et al. 2007). Controlled clinical trials,

however, found no evidence that menthol is more effective than placebo in relieving pruritus. Indeed, menthol was not only ineffective to suppress histamine-induced itch, but, even worse, it increased the transepithelial water loss, suggesting an irritant nature of the compound (Yosipovitch et al. 1996).

Although TRPM8 is often referred to as the menthol receptor, menthol is not selective for TRPM8. For example, menthol was reported to activate TRPV3 (Macpherson et al. 2006) and to either inhibit or activate TRPA1 in a dose- and species-dependent manner (Karashima et al. 2007; Xiao et al. 2008).

TRPM8 appears not to be involved in the development and/or exacerbation of itch sensation, although it may exert an inhibitory role. Indeed, pruritogenic proinflammatory mediators (such as bradykinin and PGE2) desensitize the cooling-evoked and most likely TRPM8-mediated calcium transients and shift the activation threshold toward colder temperatures (Linte et al. 2007). This inhibitory effect of the proinflammatory mediators was proposed to target TRPM8 via a quite "unusual" signaling mechanism, an α-subunit of a G protein, directly bound to TRPM8 (Zhang et al. 2012b).

Our understanding of the roles that non-neuronal TRPM8 may play in itch sensation is both limited and controversial. For example, TRPM8 expression was described in the RBL-2H3 basophilic leukemia mast cell line. In these cells, activation of TRPM8 by menthol and cold stimuli evoked an elevation in the intracellular Ca^{2+} concentration and induced histamine release. These effects were blocked both pharmacologically and by siRNA-based silencing of TRPM8 (Cho et al. 2010). By contrast, another study could not detect any TRPM8 expression in human mast cells. Moreover, TRPM8 did not appear to play any role in mast cell degranulation (Medic et al. 2011). Recently, TRPM8 expression (both at mRNA and protein levels) was detected in epidermal keratinocytes isolated from hairless mice. In these animals, following epidermal barrier disruption by tape stripping, topical application of menthol or the TRPM8 agonist WS12 potentiated the barrier recovery; this effect was blocked by the general TRP antagonist ruthenium red, as well as the nonselective TRPM8 antagonist, BCTC (Denda et al. 2010a). Chloroquine (a pruritogenic activator of TRPA1 and maybe also TRPC3) was reported to inhibit TRPM8 indirectly via cellular signaling pathways (Than et al. 2013). Last, Han et al. (Han et al. 2012) reported a beneficial effect for the topical TRPM8 agonist icilin in vulvar pruritus secondary to *Lichen sclerosus et atrophicus*.

4.8 Canonical TRP Channels

Calcium signaling is a key mechanism in keratinocyte differentiation (Bikle et al. 2001; Micallef et al. 2009; Tu et al. 2004; Yuspa et al. 1988), and altered differentiation of epidermal keratinocytes may play a role in pruritic diseases such as AD and psoriasis (Hanifin 2009). TRPC channels are important regulators of intracellular calcium homeostasis in several cell types (Clapham 2003; Nilius and Mahieu 2006; Nilius and Voets 2005; Ramsey et al. 2006; Talavera et al. 2008),

including epidermal or mucosal keratinocytes. The expression level of individual TRPC channels (TRPC1, TRPC4, TRPC5, TRPC6, and TRPC7) in keratinocytes fluctuates in a differentiation-dependent manner (Cai et al. 2005, 2006; Fatherazi et al. 2007).

TRPC1 is overexpressed in the epidermis of patients with Darier's disease (DD), a genetic disorder caused by loss-of-function mutations in the SERCA2b gene that encodes the Ca^{2+} pump of the endoplasmic reticulum. This causes a severe differentiation disorder of keratinocytes which is often associated with intense pruritus (Barfield et al. 2002; Pani et al. 2006). In keratinocytes obtained from DD patients, TRPC1-mediated Ca^{2+} influx was significantly higher compared to healthy subjects. Furthermore, DD keratinocytes show enhanced proliferation and apoptosis resistance, suggesting that TRPC1 is involved in the abnormal keratinization in DD epidermis. Importantly, experiments performed in SERCA2b KO mice, as well as on human epidermal HaCaT keratinocytes in which the expression of SERCA2b was silenced by siRNA, yielded similar results (Pani et al. 2006). Other studies showed that TRPC1/TRPC4 heteromers were important for keratinocyte differentiation as siRNA-based silencing of these channels prevented the induction of Ca^{2+}-induced differentiation. In basal cell carcinoma (BCC), the lack of TRPC1/TRPC4 heteromers was coupled to impaired differentiation and enhanced proliferation (Beck et al. 2008).

Furthermore, the activation of TRPC6 expressed in both HaCaT and NHEKs induced differentiation and inhibited proliferation by increasing Ca^{2+} influx (Leuner et al. 2011; Muller et al. 2008; Woelfle et al. 2010). In addition, decreased expression of TRPC1/TRPC3/TRPC4/TRPC5/TRPC6 and TRPC7 was found in keratinocytes obtained from psoriasis patients, another pruritic dermatosis characterized by a disturbed proliferation–differentiation program (Leuner et al. 2011)]. Most recently, TRPC6 has been implicated in the pathogenesis of AD (Sun et al. 2012). Collectively, these data argue for a role of certain TRPCs in itch development. Indeed, as discussed above, TRPC3 was implicated in the chloroquine-induced activation of TRPA1-negative neurons (Than et al. 2013).

5 Targeting TRP Channels for Itch Relief: The Promise and the Challenges

Without doubt, TRP channels (and drugs that target them) have become hot topics in biomedical research during the past three decades as demonstrated by the almost exponential growth in the number of papers in scientific publications (Kaneko and Szallasi 2013). The most studied TRP channel is no doubt TRPV1. Indeed, a number of potent small molecule TRPV1 antagonists have advanced to clinical trials for indications of pain (also chronic cough and overactive bladder), but a combination of unforeseen adverse effects (most important, hyperthermia and impaired heat pain sensation) and disappointing clinical efficacy has prevented any compounds from progressing beyond Phase II (Brederson et al. 2013; Moran et al. 2011). These disappointing results emphasize a number of questions that

already emerged after the failure of attempts to translate the compelling basic research findings into clinical applications. Most important: do currently used pain (itch) models accurately reflect the biological situation in patients suffering from chronic pain (itch)? For example, TRPV1 antagonist block evoked cough (e.g., inhaled citric acid) in study volunteers but are without any clinical benefit in chronic cough patients (Kaneko and Szallasi 2013). It is with these questions in mind that we have to ponder the clinical promise of TRP channel-targeting drugs as novel antipruritic agents.

Chronic itch is a large, unmet medical need. According to a questionnaire-based German study, the lifetime prevalence of chronic itch exceeds 20 % (Matterne et al. 2013). In animal experiments, TRPV1 has been linked to histamine-induced itch, and TRPA1 has emerged as a primary target for chloroquine and urushiol, the contact allergen in poison ivy. TRPV1 and TRPM6/TRPM7 have also been implicated in the pathogenesis of pruritus that develops in patients with kidney (uremia) or liver failure. A gain-of-function TRPV3 phenotype was associated with itch both in experimental animals and in patients (Olmsted syndrome). Last, enhanced TRP channel (e.g., TRPV1, TRPV3, and various TRPCs) expression was described in various pruritic skin disorders. Taken together, these findings imply a clinical potential for targeting TRP channels in patients with pruritus.

However, several outstanding questions about the following remain, including:

- Identification of physiological roles of TRP channels expressed on non-neuronal cells of human skin during inflammation and its related production of pruritogens
- Definition of relationship of TRP channels to peripheral and central itch processing (sensitization, desensitization)
- Determination of the expression patterns of individual TRP channels in various pruritic dermatoses
- Investigation of prurigo nodularis-like lesions in TRP channel gene-deficient mice
- In vivo evaluation of “combination therapies,” using substances that target different TRP channels
- Synthesis of selective drugs targeting TRP channels and their evaluation in vitro as well as in vivo

In conclusion, there is no uniformly effective pharmacological intervention to treat all pruritic diseases because no single target/mediator is responsible for itch. Clearly, we need to accept the need for a combination of drugs. During the past few years, tremendous progress has been made in our understanding of the roles that TRP channels play in itch sensation in preclinical models. Selective and effective TRP channel blockers are indispensible to corroborate the relevance of these compelling basic research findings in the pharmacotherapy of pruritus in patients. For some channels (e.g., TRPV1, TRPV3, and TRPA1) such antagonists are already available. For others (e.g., TRPC3, TRPM4, and TRPM6/TRPM7) the development of potent and selective agonists/antagonists is still awaited. It remains to be seen if

TRP channel drugs will become components of a clinically effective itch therapy regimen.

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Itch Control by Toll-Like Receptors

Sarah Taves and Ru-Rong Ji

Contents

Abstract

Toll-like receptors (TLRs) are cellular sensors designed to recognize molecular danger signals associated with exogenous or endogenous threats. Their activation leads to initiation of the host's immune responses in order to remove or contain the danger. However, one of the most effective methods of defense against invading pathogens and parasites is itch. The perception of itch elicits the rapid defensive action to scratch, which can remove the offending pathogen or parasite before infection can occur. Recent findings show that TLRs such as TLR3, TLR4, and TLR7 are expressed in a subset of pruriceptive/nociceptive neurons in the dorsal root and trigeminal ganglion providing a direct link between TLR activation and itch. Activation of neuronal TLRs can initiate itch sensation by coupling with ion channels. Furthermore, TLRs are expressed in skin cells and glial cells in the spinal cord to regulate inflammation and neuroinflammation in chronic itch. Thus, identification of the role of TLRs in

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neurons, skin cells, and glial cells may provide new targets for the treatment of chronic itch, a common clinical problem associated with skin diseases, systemic diseases, and metabolic disorders, for which current treatments are far from sufficient.

Keywords
Toll-like receptor • Itch • Pruritus • Glia • Dorsal horn • Dorsal root ganglia • Primary sensory neurons

Abbreviations

5HT	Serotonin
BDNF	Brain-derived neurotrophic factor
CCL2	Chemokine ligand 2 (MCP-1)
CGRP	Calcitonin gene-related peptide
CNS	Central nervous system
COX-2	Cyclooxygenase 2
CXCL	Chemokine (C-X-C motif) ligand
DAMPs	Danger-associated molecular patterns
DRG	Dorsal root ganglia
dsRNA	Double-stranded RNA
ERK	Extracellular signal-regulated kinase
ET-1	Endothelin-1
GRP	Gastrin-releasing peptide
GRPR	Gastrin-releasing peptide receptor
IL-1β	Interleukin-1beta
IRAKs	IL-1R-associated kinases
IRFs	Interferon regulatory factors
JNK	c-Jun N-terminal kinase
LPS	Lipopolysaccharide
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein 1 (CCL2)
miRNA	Microribonucleic acid
mRNA	Messenger ribonucleic acid
NF-kB	Nuclear factor-kappa B
NGF	Nerve growth factor
NO	Nitric oxide
Nppb	Natriuretic polypeptide b
PAMPs	Pathogen-associated molecular patterns
PAR2	Protease-activated receptor 2
PGE2	Prostaglandin E2
Poly I:C	Polyinosinic:polycytidylic acid
RIP-1	Receptor-interacting protein 1

sEPSCs	Spontaneous excitatory postsynaptic currents
ssRNA	Single-stranded RNA
TIR	Toll–interleukin-1 receptor
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor alpha
TRAF6	Tumor necrosis factor receptor-associated factor 6
TRPV1	Transient receptor potential vanilloid subtype 1

1 Introduction to Toll-Like Receptors

Toll-like receptors (TLRs) are pattern recognition receptors that are involved in the initiation of the innate immune response. Firstly, they recognize molecular patterns that are broadly shared by exogenous pathogens but that are distinguishable from host molecules. These are collectively referred to as pathogen-associated molecular patterns (PAMPs). Secondly, a subset of TLRs also recognize endogenous danger-associated molecular patterns (DAMPs) released in response to cellular stress or tissue injury. Thus, TLRs play a key role in initiating the immune responses to both foreign and endogenous threats.

When microbes were first recognized as the cause of infectious diseases, it was immediately clear that organisms must be capable of identifying when infected in order to initiate an immune response. Over a century ago, Richard Pfeiffer discovered "endotoxins" now known as lipopolysaccharide (LPS) which is produced by most Gram-negative bacteria (Westphal et al. 1978). Administration of LPS alone could provoke fever and shock in experimental animals. Additional molecules such as lipopeptides, flagellin, and unmethylated DNA could also provoke host immune responses; however, their receptors remained elusive.

The Toll receptor was first identified in *Drosophila melanogaster* and was reported for its role in dorsal–ventral patterning in the development (Anderson et al. 1985a, b). In 1996, Hoffmann and colleagues found that Toll also participated in the fly's immune response to fungal infection (Lemaitre et al. 1996), and in 1997 Charles Janeway and Ruslan Medzhitov showed that activation of human Toll-like receptor 4 (TLR4) could promote an adaptive immune response (Medzhitov et al. 1997). Beutler and colleagues demonstrated that mice that could not respond to LPS had mutations that abolished the function of TLR4 and proved that TLR4 was in fact the receptor for LPS (Poltorak et al. 1998). Notably, Drs. Beutler and Hoffman were awarded the Nobel Prize in Medicine or Physiology for their pioneer work on TLRs in 2011. To date, 10 (TLR 1–10) and 12 (TLR 1–9; TLR 11–13) functional TLRs have been identified in human and mouse, respectively (Kawai and Akira 2010).

1.1 Structure

TLRs are type I transmembrane proteins comprised of an ectodomain responsible for detecting PAMPs or DAMPs, a transmembrane region consisting of a single α-helix, and a cytosolic Toll–interleukin-1 receptor (TIR) domain that activates downstream signaling pathways (Kawai and Akira 2010). The extracellular domain binds either directly to ligands or to cofactors that can modulate their sensitivity to their natural ligand. In the case of TLR4, recognition of LPS requires the presence of MD-2, while cofactors CD14 and LPS-binding protein facilitate the presentation of LPS to MD-2. Upon stimulation, most TLRs form homodimers; however, some TLRs such as TLR1–TLR2 and TLR2–TLR6 also form heterodimers, each with a different ligand specificity (Triantafilou et al. 2007; Oosting et al. 2011).

Structurally, TLRs may be classified into two groups. The first has structural transitions that divide the proteins into three subdomains: N terminal, central, and C terminal (Jin et al. 2007; Kim et al. 2007; Kang et al. 2009; Kang and Lee 2011). The ligand-binding pockets of TLR1, TLR2, and TLR6 are located close to the transition site between N terminal and central (Jin et al. 2007; Kang et al. 2009; Kang and Lee 2011), and the MD-2-binding pocket of TLR4 is located between the transition from central to C terminal (Kim et al. 2007). The second group has a single-domain configuration such as TLR3 (Choe et al. 2005; Bell et al. 2006). While the structures of TLR5, TLR7, TLR8, TLR9, and TLR10 have not yet been reported, sequence analysis suggests that the first four should belong to the single-domain group, while TLR10 should belong to the three-domain group (Kang and Lee 2011). These structural observations agree with functional analyses that have demonstrated that the three-domain TLRs interact with lipid-containing molecules such as LPS and lipoproteins. Accordingly, all three-domain TLRs (1, 2, 6, and 10) are expressed on the cell membrane for the detection of microbial membrane components. Conversely, the single-domain TLRs interact with hydrophilic ligands, such as vial or endogenous nucleic acids, and are expressed intracellularly on endosomes and the endoplasmic reticulum (TLR3, TLR7/TLR8, and TLR9). However, some TLRs (TLR3 and TLR7) are localized both on membrane and in intracellular compartments (Akira et al. 2006) (Table 1).

1.2 Ligands

Each type of TLR detects distinct molecular patterns specific to pathogenic threats to the organism such as viruses, bacteria, mycobacteria, fungi, and parasites. For example, TLR4 recognizes LPS (Poltorak et al. 1998; Shimazu et al. 1999), a component of Gram-negative bacterial cell membranes; TLR5 detects flagellin, a protein in bacterial flagella (Hayashi et al. 2001); and TLR11 senses profilin-like protein (Yarovinsky et al. 2005). TLR2 heterodimers bind to specific lipopeptides, another component of bacterial cell membranes. The ligand specificity of TLR2 is modulated by its heterodimeric partner. The TLR1–TLR2 complex binds to triacyl lipopeptides with only a weak affinity for diacyl lipopeptides, while the TLR2–

Table 1 Subcellular localization and ligands of TLRs

TLR	Subcellular localization	Exogenous ligands	Origin of exogenous ligand	Endogenous ligands
TLR1/ TLR2	Cell surface	Triacyl lipopeptides	Bacteria, viruses	Unknown
		(Pam3CSK4)		
TLR2/ TLR6	Cell surface	Diacyl lipopeptides PGN	Bacteria, viruses	HSP-60, HSP-70, HSP-90
		LTA		HMGB1
		Zymosan		
TLR3	Intracellular	dsRNA	Viruses	mRNA
	Cell surface	Poly I:C		Stathmin
TLR4	Cell surface	LPS	Bacteria, viruses	HSP-22, HSP-60, HSP-70
		Lipid A derivatives		HMGB1, fibronectin
				Defensin 2, oxLDL
				Tenascin C
TLR5	Cell surface	Flagellin	Bacteria	Unknown
TLR7	Intracellular	ssRNA Imidazoquinoline	Bacteria, viruses	Self-RNA
	Cell surface	Loxoribine		MicroRNA
		Bropirimine		
TLR8	Intracellular	ssRNA	Viruses	Self-RNA
		Imidazoquinoline		MicroRNA
TLR9	Intracellular	Unmethylated CpG DNA	Bacteria, viruses, protozoa	Self-DNA
		CpG ODNs		HMGB1
TLR10	Intracellular	Unknown	Unknown	Unknown
TLR11	Cell surface	Uropathogenic bacteria	Bacteria	Unknown
		Profilin-like molecules	Protozoa	

CpG ONDs CpG-containing oligodeoxynucleotides, *dsRNA* double-stranded RNA, *LPS* lipopolysaccharide, *LTA* lipoteichoic acid, n.d., *oxLDL* oxidized low-density lipoprotein, *PGN* peptidoglycan, *poly I:C* polyinosinic-polycytidylic acid, *ssRNA* single-stranded RNA

TLR6 complex interacts with diacyl lipopeptides but not to triacyl lipopeptides (Takeuchi et al. 2001, 2002; Alexopoulou et al. 2002; Yamamoto et al. 2002; Jin et al. 2007; Kang et al. 2009). TLR3 senses double-stranded RNAs and TLR7/TLR8 single-stranded RNAs, present in retroviruses and viruses, respectively (Alexopoulou et al. 2001; Diebold et al. 2004; Heil et al. 2004; Town et al. 2006). TLR9 senses unmethylated CpG DNA (Hemmi et al. 2000; Krieg 2002). Importantly, TLRs also recognize endogenous markers of cell necrosis and tissue injury, termed DAMPs. Activation of TLRs by DAMPs induces sterile

inflammatory responses (Okamura et al. 2001; Biragyn et al. 2002; Vabulas et al. 2002; Kariko et al. 2004; Jiang et al. 2005; Tian et al. 2007; Imai et al. 2008; Midwood et al. 2009; West et al. 2010). Thus, TLRs can recognize both pathogen invasion through their recognition of PAMPs and tissue injury through their recognition of endogenous DAMPs (summarized in Table 1).

1.3 Signaling Pathways

Activation of TLR signaling leads to a tightly regulated intracellular signaling pathway that initiates the production and secretion of proinflammatory mediators. The intracellular signaling domains of TLRs have a high sequence similarity with the interleukin-1 receptor and are termed Toll/IL-1R (TIR) homology domains. Signaling adaptor proteins MyD88, TRIF, and TRAM also contain TIR domains and interact with the TIR domains of the TLR receptors through heterotypic TIR–TIR interactions (Watters et al. 2007; Kenny and O'Neill 2008). TRIF and TRAM are also referred to as TICAM-1 and TICAM-2, respectively. All TLRs, with the exception of TLR3, signal through MyD88. TLR3 signals through a TRIF-dependent pathway discussed later. TLR4 is also capable of signaling in a MyD88-independent manner by recruiting TRAM (Yamamoto et al. 2003). TLR1/TLR2, TLR4, and TLR6 recruit the additional adapter protein TIRAP (Akira and Takeda 2004). Upon activation of the MyD88-dependent pathway, MyD88 recruits the IL-1R-associated kinases (IRAKs), which interact with tumor necrosis factor receptor-associated factor 6 (TRAF6), leading to the phosphorylation and degradation of the inhibitor of nuclear factor-κB (NF-kB) IκB. Degradation of IκB releases NF-κB which translocates to promote transcription of proinflammatory genes. The MyD88-dependent pathway also activates the mitogen-activated protein kinase (MAPK) signaling pathways, such as p38, and c-Jun N-terminal kinase (JNK), leading to the activation of AP-1 and interferon regulatory factors (IRFs) (see Fig. 1).

TLR3 signals through a TRIF-dependent pathway which activates two signaling pathways. First, TRIF recruits TRAF3 to activate IRF3 and IRF7 and initiate the production of type I interferons (e.g., IFN-α/IFN-β), which are the first line of defense produced by the innate immune system. Second, TRIF interacts with receptor-interacting protein 1 (RIP-1) and TRAF6 to activate NF-κB and/or MAPK pathways (Fig. 1). This is involved in the late-phase induction of proinflammatory genes.

The signaling molecules that comprise each TLR signaling pathway are also subject to a high degree of regulation through physical interactions, conformational changes, phosphorylation, ubiquitination, and proteasome-mediated degradation (Carpenter and O'Neill 2009). Some miRNAs are capable of regulating TLR signaling through targeting the 3′ untranslated regions of mRNAs encoding components of the TLR signaling system (O'Neill et al. 2011). Activation of TLR signaling produces a wide array of proinflammatory mediators, such as cytokines, chemokines, and reactive oxygen/nitrogen intermediates including nitric

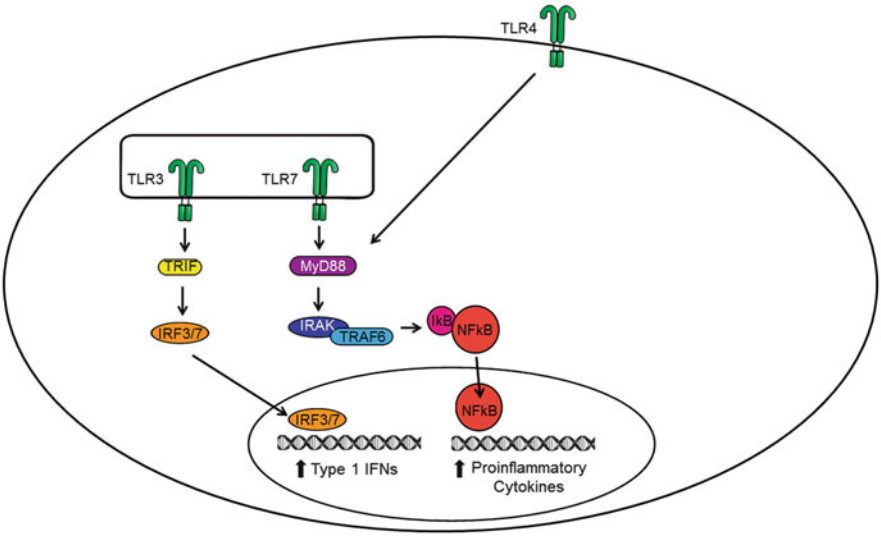

Fig. 1 Conventional signaling of TLRs. Activation of extracellular TLRs (e.g., TLR4) and intracellular TLRs (e.g., TLR3 and TLR7) initiates canonical TRIF- and MYD88-dependent signaling pathways resulting in the transcription of proinflammatory genes. Specifically, the activation of the transcriptional factors IRF3/IRF7 and NF-κB induces the production of inflammatory mediators such as cytokines (e.g., IL-1β, interferons), chemokines (e.g., CCL2), PGE_2 via the enzyme cyclooxygenase 2 (COX-2), and NO via the enzyme inducible nitric oxide synthase (iNOS). These inflammatory mediators act in concert to initiate the host's innate immune response

oxide (Takeda and Akira 2004). Upregulated activity of TLR signaling may promote chronic itch (Liu and Ji 2013).

2 Introduction to Itch

Itch, or pruritus, is defined as an unpleasant sensation that elicits the desire or reflex to scratch. While acute itch is an adaptive mechanism to warn an organism of potential chemical or parasitic danger (Ikoma et al. 2006), chronic itch is a common clinical problem associated with skin diseases (Reich and Szepietowski 2007; Bieber 2008), liver and kidney diseases (Cassano et al. 2010; Kremer et al. 2010), and metabolism disorders (Yamaoka et al. 2010). Additionally, itching itself leads to scratching which can cause inflammation, skin degradation, and secondary infection.

While some itch-sensing circuitry may overlap with existing nociceptive circuits, there are features that have recently been shown that are specific to itch. Itch sensation arises from $MrgprA3^+$ fibers, derived from a specific subset of primary sensory neurons that reside in the skin, but not in deeper tissues, muscles, organs, or bones where itch sensation does not occur (Han et al. 2013). Their cell bodies are located in the dorsal root ganglia (DRG) or trigeminal ganglia, while

their peripheral terminals, free nerve endings, reach to the stratum granulosum of the epidermis, and their central projections terminate in the superficial horn of the spinal cord. Itch-specific neurons terminate in lamina I–II and release glutamate as well as gastrin-releasing peptide (GRP), a neuropeptide known to elicit itch sensation via activation of GRP receptors (GRPRs) on superficial dorsal horn neurons (Sun and Chen 2007; Liu et al. 2009; Sun et al. 2009). A major source of GRP in the spinal cord may arise from interneurons (Alemi et al. 2013; Mishra and Hoon 2013). In addition, the neuropeptide natriuretic polypeptide b (Nppb) and substance P are also implicated in spinal cord itch transmission (Akiyama and Carstens 2013; Mishra and Hoon 2013). In the dorsal horn, itch signals can be processed and modulated before ascending to the brain where activation of specific brain regions then results in the perception of itch. Notably, in acute conditions, pain can suppress itch sensation via spinal cord inhibitory neurons (Liu et al. 2010b; Ross et al. 2010; Liu and Ji 2013).

The most characterized mediator of itch is histamine. The released histamine from local mast cells binds to H1/H4 receptors on nerve terminals (Shim and Oh 2008) which is followed by activation of PLCbeta3 and transient receptor potential vanilloid subtype 1 (TRPV1) (Han et al. 2006; Imamachi et al. 2009). TRPV1-positive C-fibers are required for both histamine-dependent and histamine-independent itch (Imamachi et al. 2009). Histamine-independent itch can be induced by the activation of transient receptor potential cation channel, subfamily A, member 1 (TRPA1), chloroquine (an antimalarial drug and MrgprA3 agonist), BAM8–22 (an endogenous MrgprC11 agonist), and oxidative stress (Liu et al. 2009; Wilson et al. 2011; Liu and Ji 2012), respectively.

3 Control of Itch by Peripheral TLRs

The skin is the body's largest organ and first line of defense against microbial and parasitic invaders. The skin expresses every type of known TLR receptor; however, each cell type has a unique expression pattern and distinct contribution to the skin's immune response (Ermertcan et al. 2011). In particular, two TLR-expressing cell type keratinocytes and mast cells have been implicated in chronic itch (Ikoma et al. 2006) (see Fig. 2).

Epidermal keratinocytes express TLRs 1–6, 9, and 10, which are upregulated in pruritic skin diseases, such as psoriasis and atopic dermatitis (Baker et al. 2003; Ermertcan et al. 2011). Mast cells, predominantly in the dermis, express TLRs 1–7 and 9 and play a key role in IgE-mediated allergic inflammation. Activation of these TLRs results in the synthesis and release of cytokines and chemokines to recruit immune cells from the circulation and mount an adaptive immune response. In particular, keratinocytes and mast cells are the major sources of nerve growth factor (NGF) (Ikoma et al. 2006). Intradermal administration of NGF enhances itch sensation in humans (Rukwied et al. 2013), and NGF is upregulated in dry skin models of itch in mice (Tominaga et al. 2007). Of interest, this increase is suppressed in *Tlr3* knockout animals (Liu et al. 2012). Therefore, TLR3-mediated

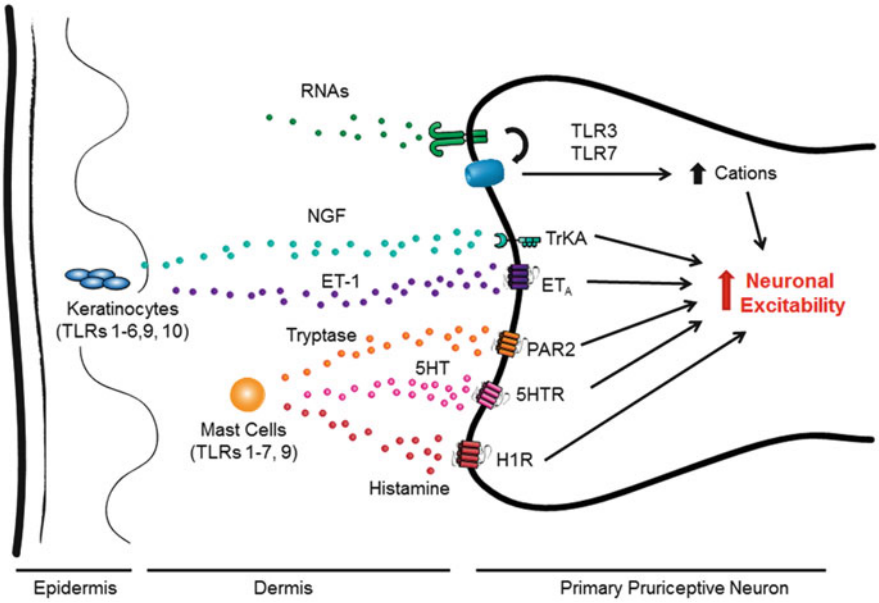

Fig. 2 Involvement of peripherally expressed TLRs in itch sensation via different mechanisms. Cells residing in the skin such as keratinocytes and mast cells express TLRs. Their activation by exogenous ligands (PAMP) and endogenous ligands (DAMP) results in the release of multiple pruritogens including NGF, ET-1, 5HT, tryptase, and histamine, all of which can activate G protein coupled receptors (GPCRs) or TrkA receptor (in the case of NGF) on pruriceptive neuronal terminals to increase neuronal excitation and trigger itch sensation. TLRs such as TLR3 and TLR7 are also expressed by pruriceptor terminals, which can be activated by DAMP such as double-strand and single-strand RNAs. Activation of TLR3 and TLR7 elicits rapid inward currents and action potentials, due to the direct coupling with ion channels in pruriceptors, leading to increased neuronal excitation and itch sensation. A second late-phase response to the activation of TLRs may also involve the increased transcription of proinflammatory mediators which can maintain neuronal hyperexcitability in chronic itch conditions

release of NGF from skin cells may contribute to peripheral sensitization in chronic itch conditions.

While TLRs have mainly been studied on immune cells, we have recently demonstrated the expression of TLR3 and TLR7 on a subset of primary sensory neurons that are responsible for itch. Single-cell Real-time PCR (RT-PCR) and immunohistochemistry demonstrated that TLR7 is expressed in small TRPV1-expressing DRG neurons (Liu et al. 2010a, 2012) and is completely colocalized with $MrgprA3^{+}$. TLR3 partially colocalizes with TLR7 and is also expressed in TRPV1-positive DRG neurons as shown by in situ hybridization, immunocytochemistry, single-cell RT-PCR, and electrophysiology (Liu et al. 2012).

TLR7 responds to synthetic ligands such as imidazoquinoline derivatives [e.g., imiquimod and resiquimod (R848)] and guanine analogs (e.g., loxoribine) (Hemmi et al. 2002). Intradermal injection of these synthetic compounds produces

scratching behavior in wild-type mice, which is reduced in *Tlr7*$^{-/-}$ mice, suggesting that these responses are, at least partially, TLR7 dependent (Liu et al. 2010a). *Tlr7*$^{-/-}$ mice also show a significant reduction of scratching behavior in response to non-histaminergic pruritogens such as chloroquine, endothelin-1 (ET-1), and SLIGRL-NH2, an agonist of protease-activated receptor 2 (PAR2) (Liu et al. 2010a). Interestingly, *Tlr7*$^{-/-}$ mice exhibited normal thermal and mechanical pain sensitivity. These findings show that TLR7 may serve an important role as a non-histaminergic itch receptor.

Activation of TLR7 in immune cells initiates signaling cascades leading to a variety of transcriptional changes to promote inflammation. However, in small DRG neurons, application of TLR7 agonists elicits rapid inward currents and action potentials, which are abolished in *Tlr7*$^{-/-}$ knockout mice (Liu et al. 2010a). This immediate increase in neuronal excitability suggests that TLR7 may be coupled to ion channel activation in primary sensory neurons. Our recent study shows that TLR7 is functionally coupled to TRPA1 but not TRPV1 (Park et al. 2014).

TLR3 responds to dsRNA as well as its synthetic analog, polyinosinic–polycytidylic acid (poly I:C), in which one strand of RNA is replaced by a polymer of inosinic acid. Activation of TLR3 by its ligands poly I:C or purified total RNAs also elicits rapid inward currents and the generation of action potentials from DRG neurons in wild-type but not *Tlr3*$^{-/-}$ mice (Liu et al. 2012). Furthermore, intradermal application of poly I:C produced dose-dependent scratching behavior in wild-type mice which was abolished in *Tlr3*$^{-/-}$ mice (Liu et al. 2012). Similar to TLR7, TLR3 also seems to serve as an itch receptor/co-receptor on pruriceptive neurons. In contrast to *Tlr7*$^{-/-}$ mice, which showed a partial reduction in histamine-independent itch, *Tlr3*$^{-/-}$ mice displayed significant reductions in both histamine-dependent and histamine-independent itch (Liu et al. 2012). Knockdown of TLR3 by intrathecal injection of *Tlr3* antisense oligonucleotides significantly reduced TLR3 expression in the DRG and reduced both histamine-dependent and histamine-independent itch in wild-type mice (Liu et al. 2012). This corroborates the results found using *Tlr3* knockout animals.

TLR3 and TLR7 expression by primary sensory neurons seems to serve as itch receptors to detect foreign pathogens and endogenous ligands (e.g., ds- and ssRNA, respectively), leading to a rapid defensive response: scratching. As neuronal excitation occurs within minutes of agonist application, the neuronal signaling pathway must have a distinct component from the traditional TLR signaling pathway in immune cells; however, the details remain to be determined. We postulate that TLR3 and TLR7 are expressed on the cell surface and are coupled to unidentified ion channels capable of inducing inward currents and action potentials (see Fig. 2). Of note, it was also demonstrated that the activation of TLRs, including TLR3, TLR7, and TLR9, in DRG neurons by their respective ligands may indirectly influence the excitability of DRG neurons by inducing the expression of proinflammatory mediators such as prostaglandin E2 (PGE2), calcitonin gene-related peptide (CGRP), and interleukin-1beta (IL-1β) (Qi et al. 2011) (see Table 2). Thus, TLRs expressed by DRG neurons may regulate neuronal excitability by both transcriptional and non-transcriptional mechanisms.

Table 2 Distribution of TLRs in the spinal cord and DRG

TLR	Cellular localization	Adapter proteins	Signaling pathway	Response
TLR2	Microglia	MyD88	NF-kB	TNF-α, IL-1β, BDNF, PGE2, NO
TLR3	Microglia	TRIF	p38	TNF-α, IL-1β, BDNF, PGE2, NO
	Astrocyte	TRIF	NF-kB	IL-1β, CCL2, CXCL1, CXCL10
			JNK	
	DRG neurons	Unknown	ion channel coupling	Increased excitation
	Primary afferents	Unknown	ion channel coupling	Increased excitation
TLR4	Microglia	MyD88	NF-kB	TNF-α, IL-1β, BDNF, PGE2, NO
			ERK	
			p38	
	Astrocyte	MyD88	NF-kB	IL-1β, CCL2, CXCL1, CXCL10
			ERK	
			JNK	
TLR7	DRG neurons	MyD88	Ion channel coupling	Increased excitation

4 Control of Itch by Central TLRs

While both TLRs play a role in eliciting itch sensation from peripheral stimuli, only TLR3 was found to play a critical role in spinal synaptic transmission and the central sensitization underlying itch processing in the spinal cord (Liu et al. 2012). *Tlr3*$^{-/-}$ mice displayed decreased spontaneous excitatory postsynaptic currents (sEPSCs) in spinal lamina II neurons, while activation of TLR3 by poly I:C resulted in an increase in sEPSCs in wild-type animals (Liu et al. 2012). Furthermore, knockout of *Tlr3* abolished spinal long-term potentiation (LTP) following tetanic stimulation of the sciatic nerve (Liu et al. 2012). In contrast, both sEPSC activity and LTP remained intact in *Tlr7*$^{-/-}$ mice (Liu et al. 2012). Given the important role of spinal synaptic plasticity in itch hypersensitivity (Ross et al. 2010), impairments in central sensitization are likely to contribute to the profound itch deficit in *Tlr3*$^{-/-}$ mice.

Within the spinal cord, glial cells also express TLRs. Microglia, the resident immune cell of the central nervous system (CNS), express almost all known members of the TLR family, while astrocyte expression of TLRs is limited (Farina et al. 2007) (see Table 2). Similar to the activation of TLRs on peripheral immune cells, the activation of TLRs on glia results in the production and secretion of proinflammatory mediators including cytokines (e.g., TNF-α), chemokines [e.g., chemokine ligand 2 (CCL2)], and enzymes [e.g., cyclooxygenase 2 (COX-2)], as well as other inflammatory mediators (e.g., prostaglandins) (Basbaum et al. 2009;

van Noort and Bsibsi 2009; Lehnardt 2010; Nicotra et al. 2011). While these mediators are known to generate central sensitization and pain hypersensitivity, their role in chronic itch is unknown.

5 Clinical Significance and Future Perspectives

Chronic itch is a common and significant clinical problem. Chronic itch may involve the entire skin or be localized to a specific area or dermatome. It is more common in women than in men (Ständer et al. 2013), and its incidence increases with age (Weisshaar and Dalgard 2009; Ständer et al. 2010). It can be broadly categorized into four major etiologies: dermatologic causes (e.g., atopic eczema, psoriasis, or scabies), systemic causes (e.g., liver and kidney disease or metabolism disorders), neuropathic causes (such as spinal nerve impingement), and psychogenic causes (Yosipovitch and Bernhard 2013). However, regardless of the cause, it is associated with a marked reduction in the quality of life. In fact, a recent study showed that chronic itch was as debilitating as chronic pain (Kini et al. 2011). Clinically, the current treatments for chronic itch are far from sufficient (Yosipovitch and Bernhard 2013); however, targeting TLRs may offer new therapy options for treating debilitating itch-related problems.

TLRs are emerging as important players in the regulation of acute and chronic itch. They are expressed by many components of the itch signaling pathway including the cells of the skin, resident and infiltrating immune cells, peripheral and central neurons, as well as central glia. However, the precise contribution of TLR signaling in each cell type remains to be determined. Additionally, our work has raised the exciting possibility that TLR-mediated neuronal excitation occurs in a non-transcriptional manner. Again, much work remains to be done in order to elucidate the details of this pathway. These distinctions may provide targets for developing new therapies that block the detrimental effects of persistent unregulated TLR signaling, while leaving their beneficial effects intact.

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Transmission of Pruriceptive Signals

Santosh K. Mishra and Mark A. Hoon

Contents

Abstract

In this chapter we discuss the many recent discoveries of the mechanisms by which itch is transmitted: the neurotransmitters and the responses they trigger, the mechanisms by which specific neuronal targets are activated, and the specificity of the pathways. Current data reveal that DRG neurons and spinal cord cells use a remarkably selective set of transmitters to convey pruritic information from the periphery to the brain: glutamate and Nppb are released from primary itch-sensory cells; these molecules activate secondary spinal cord pruriceptive-specific neurons, which in turn utilize Grp to activate tertiary pruriceptive-selective neurons. Intersecting this basic linear excitatory pathway, inhibitory input from dynorphin and neurons that express the somatostatin receptor modify itch sensation. Cumulatively, these studies paint an elegantly simple picture of how itch signals are transformed and integrated in the spinal cord and open new avenues for research efforts aimed at understanding and better treating itch.

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Keywords
Pruritic • Itch • Neurotransmitters • Receptors • Nppb • Grp • Grpr • Dynorphin • Somatostatin

Abbreviations

Bhlhb5	Basic helix-loop-helix transcription factor 5 inhibitory neurons
DRG	Dorsal root ganglion
Grp	Gastrin-releasing peptide
Grpr	Gastrin-releasing peptide receptor
NK-1	Neurokinin 1
Nmb	Neuromedin b
Nppb	Natriuretic polypeptide b
Npr1	Natriuretic peptide receptor 1
Npr2	Natriuretic peptide receptor 2
PLC	Phospholipase C
Sst	Somatostatin
Sstr2	Somatostatin receptor 2
Tlx3	T-cell leukemia homeobox 3
TR4	Testicular orphan nuclear receptor 4
TRPV1	Transient receptor potential subfamily vanilloid member 1
VGlut2	Vesicular glutamate transporter 2

1 Transmission of Itch Signals from Primary Itch-Receptor Neurons

Historically, it was thought that, like all other classes of somatosensory neurons, the excitatory transmitter glutamate was the primary neurotransmitter used by pruritic sensory neurons. More recently, the number of molecules reported to be involved in the initial step of itch transmission has expanded to include gastrin-releasing peptide (Grp), natriuretic polypeptide b (Nppb), and neuromedin b (Nmb) (Koga et al. 2011; Mishra et al. 2012; Mishra and Hoon 2013; Sukhtankar and Ko 2013; Sun and Chen 2007; Sun et al. 2009).

The role for a potential glutamate co-transmitter released from primary itch neurons is supported by studies in which mice were engineered to lose glutamate transmission from selective subsets of somatosensory neurons. This was accomplished by eliminating the gene responsible for the uptake of glutamate into synaptic vesicles (vesicular glutamate transporter 2, VGlut2) in TRPV1 or Nav1.8 neurons. The resultant mutant animals exhibit spontaneous itch behavior (Lagerström et al. 2010; Liu et al. 2010). This result suggests that in the absence of glutamate, some other transmitter is used at the first itch synapse (and that glutamate-mediated transmission also contributes to the inhibition of itch; see

later). The co-transmitter for glutamate was initially thought to be Grp, because gastrin-releasing peptide receptor (Grpr) and Grpr neurons are required for itch, and Grp immunostaining was reported to be present in DRG (Sun and Chen 2007; Sun et al. 2009; Zhao et al. 2013). Furthermore, the intrathecal administration of Grp (into the spinal cord) directly induces scratching behavior. Therefore, Grp was postulated to be a co-transmitter with glutamate in primary itch-responsive neurons (Sun and Chen 2007). However, a number of reports have called into question the expression of Grp in DRG neurons (Fleming et al. 2012; Mishra et al. 2012; Mishra and Hoon 2013), and it was suggested that Grp is instead expressed in spinal cord interneurons. The neuropeptide Nmb is expressed at high levels in DRG neurons (Mishra et al. 2012; Wada et al. 1990). Nmb was suggested to act as a potential modulator of itch in one report (Sukhtankar and Ko 2013), a finding that was not repeated in another study (Mishra et al. 2012). Therefore, a role of Nmb in itch sensation is still controversial.

The search for the neurotransmitter used by DRG neurons identified a new itch-specific neuropeptide, natriuretic polypeptide b (Nppb) (Mishra and Hoon 2013). Nppb, also called BNP, is a 32-amino-acid cyclic peptide that was discovered about 25 years ago (Seilhamer et al. 1989) (Fig. 1). Nppb, as the name implies, is better known as a peptide that is released by the heart and controls blood sodium (and blood volume), activating its receptor in blood vessels and in the kidney. Nppb is part of a small gene family that encodes three distinct secreted peptides, Nppa, Nppb, and Nppc. Nppa and Nppb are both produced by heart muscle, are released

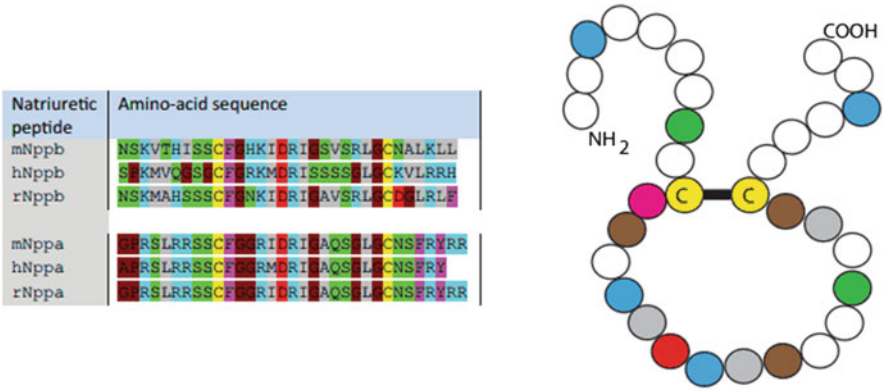

Fig. 1 Structure of natriuretic peptides. A comparison of the primary structure (table) of mouse, human, and rat natriuretic polypeptides reveals that Nppa displays greater sequence conservation than Nppb. Despite these differences, both Nppa and Nppb activate the same receptor; Npr1. Amino acids are color coded to reflect their characteristics. The structure of the natriuretic peptides is schematized (to the *right*) to highlight the core conserved ring that forms the key structural element of these transmitters. Only amino acid residues that have high sequence conservation between species and between Nppa and Nppb are colored. The cysteine residues and their critical disulfide bond are marked in *yellow*. The diagram also illustrates that the N-terminal and C-terminal loops exhibit low sequence identity and are not thought to be important determinants in receptor binding

Table 1 Table of the major itch neurotransmitters and their receptors

Receptor	Gene name	Agonist	Antagonist	Transducer
Natriuretic peptide receptor A	Npr1	Nppa (Atlas et al. 1985) Nppb (Tse et al. 2001)	Anantin (Wyss et al. 1991)	Guanylyl cyclase (Garbers and Lowe 1994)
Gastrin-releasing peptide receptor	Bb2	GRP (Jensen et al. 2008)	BIM 189 (Coy et al. 1990)	Gq/G_{11} family (Hellmich et al. 1997)
Somatostatin receptor 2	Sstr2	Sst (Kardon et al. 2014) Octreotide (Raynor et al. 1993)	CYN-154806 (Bass et al. 1996)	G_i/G_o family (Hou et al. 1994)
Kappa-opioid receptor	Opkr1	Dynorphin (Kardon et al. 2014) Nalfurafine, U-50,488 (Morgan and Christie 2011)	Nor BNI, 5′-GNTI (Morgan and Christie 2011)	G_i/G_o family (Dhawan et al. 1996)

The receptors for the four major itch neurotransmitters are listed together with a partial list of their known agonists and antagonist, as well as their coupling to cellular signaling cascade. Notably, Npr1 and Grpr receptors stimulate excitatory signaling processes which cause elevation of intracellular concentrations of cGMP or PLC metabolites, respectively. In contrast, the Sstr2 and Opkr1 receptors activate downstream components which cause a lowering of intracellular cAMP concentration and produce an inhibition of neuronal firing. Abbreviations: *Nor BNI* norbinaltorphimine, *5′GNTI* 5′-guanidinonaltrindole

into the blood, and activate the same receptor, Npr1 (Fig. 1 and Table 1). The Nppc peptide activates a related receptor Npr2. Nppb was identified in DRG (Fig. 2), by screening for genes that are preferentially expressed in cells that express or have developmentally expressed the capsaicin receptor, TRPV1 (Mishra et al. 2011). This population of neurons was screened because they have been shown to be required for itch responses and because the TRPV1-ion channel is essential for some types of itch behavior (Imamachi et al. 2009). Analysis of the expression pattern of Nppb revealed that it is expressed in a subset of TRPV1 neurons. As expected for an itch neurotransmitter, Nppb is also co-expressed with the itch receptors MrgprA3 and MrgprC11 (Liu et al. 2009, 2011) and is co-expressed with the critical downstream signaling molecule PLCβ3 (Han et al. 2006). The novel role for the natriuretic peptide Nppb was uncovered by analyzing the behavior of Nppb null mice. Animals lacking Nppb exhibit profoundly attenuated itch responses to all pruritogenic compounds tested but reacted normally to other somatosensory stimuli. Nppb has also been reported to serve as a modulator of pain signals (Zhang et al. 2010); however, mice lacking Nppb display no nociceptive deficits contradicting this finding. Importantly intrathecal administration of Nppb recapitulates the same behavior as intradermal injection of pruritogens, namely, it elicits scratching behavior in wild-type and Nppb knockout mice.

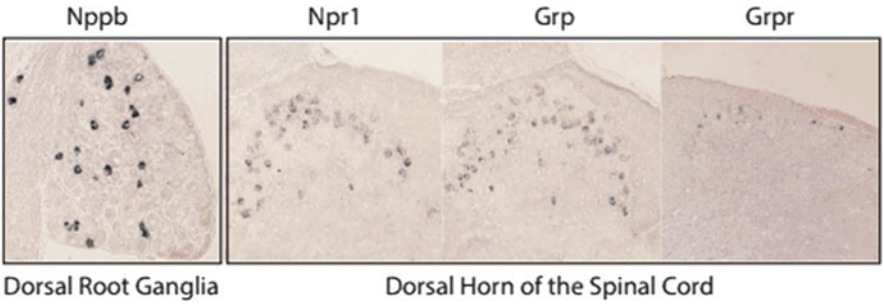

Fig. 2 Expression pattern of itch-specific neurotransmitters and their receptors. In situ hybridization shows the neurons which express the transmitters Nppb and Grp and their respective receptors, Npr1 and Grpr. Nppb is expressed in about 6 % of DRG neurons that have small diameters. The receptor for Nppb, Npr1, is expressed in dorsal horn neurons. Likewise, Grp and Grpr are also expressed in the superficial dorsal lamina of the spinal cord

Therefore, Nppb has many characteristics of the bona fide primary itch transmitter, and it was predicted that the receptor for Nppb, Npr1, should be expressed in interneurons located in the superficial layers of the dorsal horn. Indeed, consistent with Nppb acting as the transmitter for itch, Npr1-expressing neurons are present in the spinal cord (Fig. 2). In addition, elimination of Npr1 neurons by intrathecal administration of Nppb-neurotoxin conjugate (Nppb-saporin) led to loss of itch reception while not effecting other somatosensory responses, including those produced by thermal and mechanical stimuli. Together these data strongly support the proposal that Nppb and glutamate are the principal selective neurotransmitters used by pruritic sensory neurons.

2 Excitatory Neurotransmission in the Spinal Cord

The dorsal horn of the spinal cord is well known to not only act as the conduit of pruritic, painful, and thermal input but is also known to be a site where somatosensory afferent information is integrated. An indication of the complexity of the circuits in the spinal cord is provided by the demonstration of a large number of transmitters that are present in this tissue (Su et al. 2014). Despite this apparent overwhelming complexity, significant headway has been made in unraveling the transmitters and cellular mechanisms involved in itch transmission.

The neurons which express the primary itch transmitter receptor, Npr1, are thought to be the first element in the itch neural circuit in the spinal cord (Mishra and Hoon 2013). It was noticed that ablation of these neurons (using Nppb-saporin neurotoxin) did not eliminate Grp-induced itch. This result is consistent with the transmitter Grp being downstream of Nppb. Furthermore, in mice lacking Nppb, intrathecal administration of Grp elicited normal itch responses. What is more, antagonist blockade of Grpr or elimination of Grpr neurons inhibited equally Grp- and Nppb-elicited itch behavior, concordant with a linear circuit for itch in the

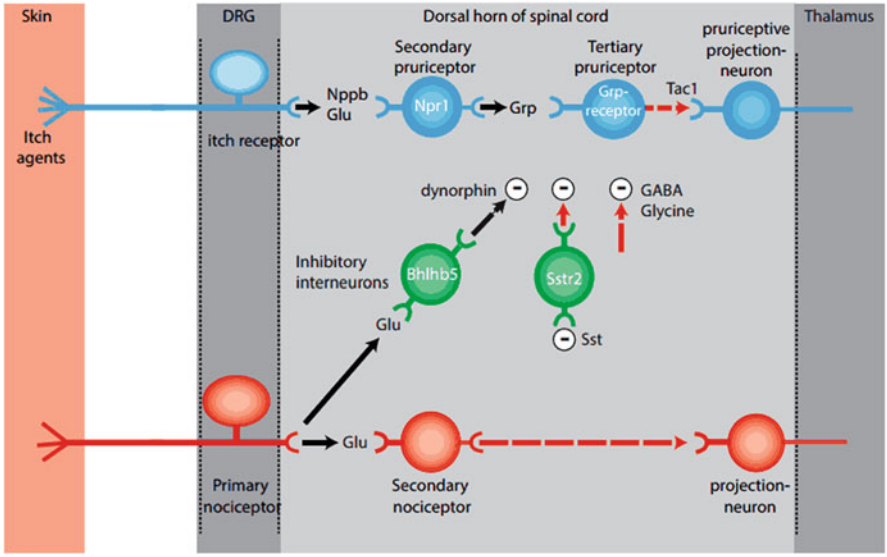

Fig. 3 Schematic of the itch circuit. Schematic representation of the distribution and function of itch-selective transmitters and their neuronal targets illustrates the simplicity of the itch circuit. Excitatory pathway (*blue*-colored cells): (1) glutamatergic and Nppb excitatory input from pruritic neurons activates Npr1-expressing spinal cord neurons. (2) Npr-1-expressing neurons release Grp which in turn activate tertiary pruriceptive neurons that express the Grpr receptor. (3) The transmitter used by Grpr-expressing neurons has not yet been identified; however, substance P (Tac1) is believed to play a role in the activation of the last step in the spinal cord itch circuit and the stimulation of spinothalamic projection interneurons. Inhibitory pathway (*green*-colored neurons): (1) glutamatergic afferents of nociceptors (*red*-colored neurons) stimulate Bhlhb5-inhibitory neurons. (2) Bhlhb5-expressing neurons release dynorphin (Pdyn) which activates Kappa-opioid receptors in postsynaptic membranes of unknown pruritic pathway neurons. (3) Somatostatin (Sst) from dorsal horn or DRG neurons activates and in turn inhibits neurons which express the Sstr2 receptor. Since Sstr2 leads to reduced cell activity, Sst-mediated activation of these neurons causes disinhibition and therefore potentiates itch behavior. The inhibitory neurotransmitters GABA and glycine are also involved in reducing itch responses. The segments of the pathway that are unknown or controversial are marked in *red*

dorsal horn of the spinal cord (Fig. 3). By assaying the expression of Grp, it was revealed that Grp is present at high levels in interneurons in the dorsal horn of the spinal cord (Fig. 2) (Fleming et al. 2012; Li et al. 2006; Mishra and Hoon 2013; Wang et al. 2013; Xu et al. 2013). To better define the neurons that express Grp, mice in which Npr1-cells are ablated were examined to see if the removal of these neurons leads to a concomitant reduction of Grp-expressing cells. As expected for the co-expression of Grp and Npr1, there was a dramatic reduction of Grp-expressing spinal cord neurons after Nppb-saporin treatment. Double-labeling experiments further confirmed that Grp and Npr1 are co-expressed (Mishra and Hoon 2013). The Grp/Npr1 class of neurons in addition to being stimulated by Nppb is likely activated by glutamate (Koga et al. 2011). Therefore, there is strong evidence that Grp is downstream of Nppb and that Npr1-spinal cord interneurons

are activated by the presynaptic release of Nppb and glutamate from DRG afferents (Fig. 2). Nevertheless, the importance of Grp in the itch pathway is undisputed; ablation of Grpr neurons, elimination of the Grpr receptor itself, or administration of Grpr antagonist causes the selective loss of itch responses (Sun and Chen 2007; Sun et al. 2009). A recent pair of papers confirms the crucial roles of Grpr- and Grp-expressing interneurons in itch (Wang et al. 2013; Xu et al. 2013). The conditional deletion of the testicular orphan nuclear receptor 4, TR4, results in the loss of several types of dorsal horn neurons and also causes a dramatic reduction in responsiveness to itch-inducing agents. TR4 mutant mice have normal numbers of primary sensory neurons and projection neurons and only show deficits in the number of excitatory dorsal horn neurons. The neurons lost in TR4 conditional knockouts include those expressing Grp and Grpr. Using a different genetic strategy, another study examined the consequence of the loss of the T-cell leukemia homeobox 3 gene, Tlx3, in dorsal horn neurons (Xu et al. 2013). Tlx3 conditional knockout mice lack a subset of spinal cord excitatory neurons in laminae I and II, including those which express Grp and Grpr. These mutant mice, like TR4 knockout animals, have lost responses to itch-inducing agents. However, as both these mutant mice have reduced responses to other somatosensory stimuli, assessment of the specificity of Grp and Grpr neurons and where they fit in itch-circuit models cannot be directly deduced from these studies.

Another excitatory neuropeptide that may be used by the spinal cord itch pathway is substance P (Stander et al. 2010; Trivedi and Bergasa 2010). Although the gene that encodes substance P, Tac1, is not necessary for itch responses, several studies which used substance P antagonists reported that it can attenuate pruritic behavior (Akiyama et al. 2014; Cuellar et al. 2003). In addition, the ablation of substance P receptor-expressing (NK-1) dorsal horn neurons with substance P-saporin also greatly reduced responses elicited by pruritic compounds (Carstens et al. 2010). Lastly, spinothalamic projection neurons which convey itch signals from the spinal cord to the brain are immunopositive for the substance P receptor, NK-1 (Todd et al. 2000). The ablation of NK-1-expressing neurons causes a deficit in responses to painful stimuli as well as itch, which together with electrophysiological results has been interpreted to show convergence of sensory information in spinal cord projection neurons (Davidson et al. 2009, 2012; Mantyh et al. 1997).

3 Inhibitory Transmission in the Spinal Cord

Thermal, painful, and mechanical stimuli can inhibit itch (Bromm et al. 1995; Yosipovitch et al. 2007). An important clue to the mechanism for the inhibition of itch by counter-stimuli was obtained by examining the effect of silencing glutamate transmission from nociceptive neurons (Lagerström et al. 2010; Liu et al. 2010). By selectively abolishing glutamate neurotransmission (by eliminating VGlut2) in various classes of nociceptors, including those which express the voltage-gated sodium channel Nav1.8, pruritogen-induced itch was markedly increased, while nociceptive responses were greatly reduced. As a note

of caution, a comparable but not identical study in which other somatosensory-specific conditional VGlut2 mice were examined showed no change in itch behavior in mutant animals (Scherrer et al. 2010). At present it is not possible to reconcile these differing results. Nevertheless, an explanation for the novel itch behavior described for Nav1.8 conditional VGlut2 mice was proposed in which these animals lose inhibition of pruritic input (Lagerström et al. 2010; Liu et al. 2010). It was suggested that normally tonic pruritic input is silenced by paired inhibitory nociceptive input. A model to account for this explanation posits that a class of inhibitory neurons is activated by nociceptive input; these cells in turn normally inhibit itch signals (Fig. 3). Indeed, this model is supported by mutant mice that lack a specific class of inhibitory neurons (Bhlhb5 cells) and display a similar spontaneous itch phenotype to that of conditional VGlut2 mutants (Ross et al. 2010). A search for the mechanism by which Bhlhb5 neurons inhibit itch revealed that they likely use the inhibitory transmitter, dynorphin, a kappa-opioid receptor agonist (Table 1). Initially, it was shown that the kappa-opioid receptor agonist nalfurafine inhibits scratching (Inan and Cowan 2006; Kardon et al. 2014), suggesting that kappa-opioid receptors are part of the inhibitory pathways for itch. Recently, it was shown that the endogenous kappa-opioid receptor agonist, dynorphin, is expressed in Bhlhb5 neurons (Kardon et al. 2014). Grp-induced itch is inhibited by nalfurafine suggesting that dynorphin probably inhibits either Grpr neurons or cells further downstream in the itch pathway (Inan et al. 2011; Kardon et al. 2014). These results establish that dynorphin is an important negative modulator of itch signals. However, it must be noted that dynorphin knockout mice exhibit no itch phenotype, suggesting that there may be functional redundancy in the transmitters for inhibition or there is development plasticity which makes the role of dynorphin dispensable (Kardon et al. 2014). In addition to dynorphin, the neuropeptide somatostatin (Sst) has been reported to induce itch-scratching responses (Kardon et al. 2014). A receptor for Sst, Sstr2 (Table 1), is found in many inhibitory neurons in the dorsal horn of the spinal cord (Polgar et al. 2013). Bhlhb5 neurons co-express the Sstr2 receptor and can be hyperpolarized by application of Sst, suggesting that Sst may induce scratching by disinhibiting Bhlhb5 neurons. However, Sstr2 receptors are expressed in other classes of spinal cord interneurons, as well as in DRG cells, and an alternate explanation of the inhibitory effects of Sst is that it triggers responses in these other classes of cells (Polgar et al. 2013; Shi et al. 2014; Takeda et al. 2007; Yasaka et al. 2010). In addition, the effects of Sst on nociception are well characterized, which, given the interplay between itch and nociception input, could account for the induction of itch by Sst. Lastly, other inhibitors of itch are the neurotransmitters GABA and glycine (Akiyama et al. 2011). At present the exact source and cellular substrates for these transmitters are unknown. Cumulatively, we are beginning to see the outline of the inhibitory transmitters and interneurons that are responsible for the cross talk between different sensory modalities which is crucial for the sentient perception of our environment.

4 Concluding Remarks

In this review we have given our view of the itch circuit, which we believe is rather simple and is likely to be highly selective. However, we would like to stress that this model of itch is still a work in progress. As such, there may still be some surprises and there are also parts of the pathway that, while they are correct, lack detailed characterization. For instance, are the neuropeptides Nppb, Grp, dynorphin, and somatostatin neurotransmitters or neuromodulators? What is the precise anatomy of the neurons and how are they connected? Are some types of itch caused by perturbation of components in the itch circuit? In this rapidly moving field, these questions and more should be answered in the next few years and will build our knowledge of itch sensation and will ultimately produce a better understanding of the causes of chronic itch.

Much of the current work on itch has focused on the basic mechanisms by which signals are conveyed from peripheral pruritic neurons to the spinal cord. Having gained these key insights, some of these breakthroughs are starting to be moved into translational studies. For instance, small molecular inhibitors of Grpr have been used to reduce itch in a mouse model (Zhao et al. 2013) and the κ-opioid agonist nalfurafine shows promise in the clinic at ameliorating uremic pruritus (Kumagai et al. 2012; Wikström et al. 2005). Similarly, antagonists of Npr1 and Sstr2 receptors may be tractable alternative strategies for treatment. However, without exception, all the transmitters in the itch circuit perform "double duty" as components in non-itch processes in either other areas of the brain or in other parts of the body. Therefore, drugs which interfere with the receptors for these transmitters might produce unwanted side effects, and while not necessarily precluding their usefulness, these off-target effects may curtail their utility in the clinic.

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Role of Cytokines and Chemokines in Itch

Eoin R. Storan, Susan M. O'Gorman, Ian D. McDonald, and Martin Steinhoff

Contents

Abstract

Cytokines classically are secreted "messenger" proteins that modulate cellular function of immune cells. Chemokines attract immune cells to the site where they exert various functions in inflammation, autoimmunity or cancer. Increasing evidence is emerging that cytokines or chemokines can act as "neuromodulators" by activating high-affinity receptors on peripheral or central neurons, microglia cells or Schwann cells. Very recently, cytokines have been shown to act as pruritogens in rodents and humans, while a role of chemokines in

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itch has thus far been only demonstrated in mice. Upon stimulation, cytokines are released by skin or immune cells and form a "bridge of communication" between the immune and nervous system. For some cytokines such as IL-31 and TSLP, the evidence for this role is strong in rodents. For cytokines such as IL-4, there is some convincing evidence, while for cytokines such as oncostatin M, IL-2, IL-6, IL-8 and IL-13, direct evidence is currently limited. Current clinical trials support the idea that cytokines and chemokines and their receptors or signalling pathways are promising targets for the future therapy of certain subtypes of itch.

Keywords
Cytokines • IL-31 • Oncostatin M • Pruritus • Nerve • Skin • Receptor • Cell signalling • Atopic dermatitis

Abbreviations

AD	Atopic dermatitis
CLA	Cutaneous lymphocyte-associated antigen
CD	Cluster of differentiation
DRG	Dorsal root ganglion
ET-1	Endothelin 1
IFNγ	Interferon gamma
IL	Interleukin
IL-31RA	IL-31 receptor alpha
KO	Knockout
MAPK	Mitogen-activated protein kinase
mRNA	Messenger RNA
mrgpr	Mas-related G protein-coupled receptor
OSM	Oncostatin M
OSMRβ	OSM receptor beta
STAT	Signal transducer and activator of transcription
TH	T helper cells
TRPA1	Transient receptor channel ankyrin subtype 1
TRPV1	Transient receptor potential cation channel subfamily V member 1
TSLP	Thymic stromal lymphopoietin
SEB	Staphylococcal enterotoxin B

1 Introduction

Cytokines are secreted proteins, which modulate a wide spectrum of cellular functions, including immune cell recruitment, activation and differentiation. Cytokines are considered part of the immune system and have long been known

to modulate innate and adaptive immune responses. However, immune cells are not the only source of cytokines and chemokines. For example, keratinocytes have been identified to produce cytokines decades ago (Luger et al. 1981). In the skin, almost all cells can produce cytokines or chemokines and express high-affinity receptors for many of them. So far, 38 cytokines and dozens of chemokines have been identified as immunomodulators. Cytokines and chemokines have also been identified as "messengers" of pain and neurogenic inflammation (Zhang and An 2007) indicating a role in the peripheral and central nervous system as well as for microglia function (Hanisch 2002). Emerging evidence, however, demonstrates that the immune system, through these "messenger molecules", is able to communicate with the nervous system and modulate its function. Considerable proof suggests that cytokines and chemokines play a key role in itch, with much of this evidence relating to the study of the prototypic pruritic skin condition, atopic dermatitis. This chapter summarises our knowledge about the cytokines and chemokines involved in the pathophysiology of itch in rodents and/or men and their receptors. Where applicable, we discuss the recent clinical human studies or trials addressing this topic and strategies to treat recalcitrant or chronic itch.

2 IL-31

Interleukin-31 (IL-31; see Fig. 1), a member of the IL-6 family of cytokines, is the best studied cytokine so far that has been implicated in itch and has the strongest evidence supporting its role in humans. A gene on chromosome 12q24.31 codes for IL-31 in humans (Dillon et al. 2004). Linkage markers for asthma are also found at this locus (Raby et al. 2003).

In murine models, IL-31 is released from activated TH_2 cells and in lower levels from TH_1 cells (Dillon et al. 2004). In humans, IL-31 is predominantly released from CLA^+(skin-homing+) $CD45RO^+$ memory T cells (Bilsborough et al. 2006). An analysis of human T-cell subtypes demonstrated IL-31 to be expressed in TH_2 cells but not TH_0, TH_1 or TH_{17} cells (Cevikbas et al. 2014). In addition, IL-31 mRNA is identifiable in mature dendritic cells, albeit at significantly lower levels than in TH2 cells. This suggests that mature dendritic cells may also be capable of producing IL-31 under certain circumstances. Whether this concentration is physiologically relevant in some diseases remains to be determined. Thus, TH_2 cells are the primary source of IL-31 in human skin diseases so far investigated. IL-31 mRNA is not identifiable at any level in keratinocytes, skin fibroblasts or dermal endothelial cells (Cevikbas et al. 2014). Factors influencing the production of IL-31 in humans are known to include antimicrobial peptides, cathelicidin LL-37 and human beta-defensin (Niyonsaba et al. 2010).

IL-31 acts through a heterodimer receptor complex, related to the glycoprotein-130 receptor family, composed of an IL-31-specific subunit, IL-31 receptor alpha (IL-31Rα) and oncostatin M receptor beta (OSMRβ), a subunit that IL-31 shares with OSM.

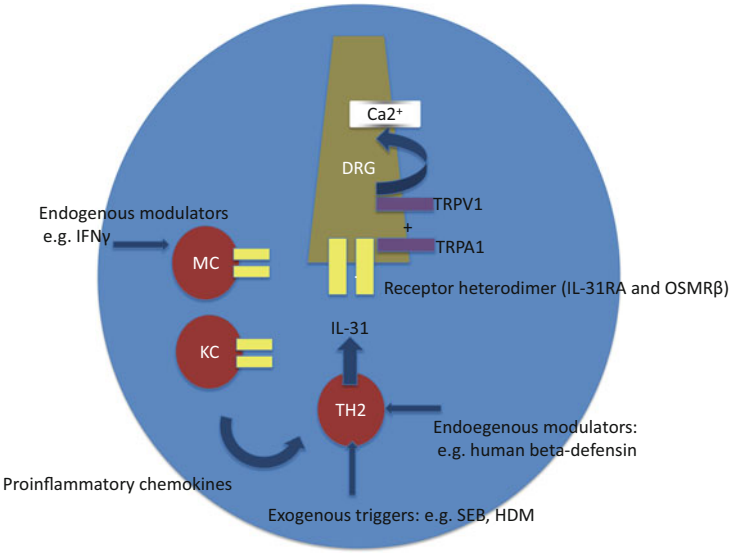

Fig. 1 IL-31 is predominantly produced by TH2 cells in humans. Factors influencing this release include exogenous triggers such as staphylococcal enterotoxin B (SEB), house dust mites (HDM) and endogenous triggers such as beta-defensins. IL-31 acts upon a heterodimer receptor (IL-31R) located on dorsal root ganglion neurons. This receptor is also present on monocytes (MC) and keratinocytes (KC). IFNγ upregulates the expression of IL-31R in human monocytes

IL-31R is expressed by dorsal root ganglion (DRG) neurons, mature dendritic cells and keratinocytes, with the highest level of expression seen on the DRG neurons (Sonkoly et al. 2006). The expression of IL-31R on DRG neurons has been demonstrated at the RNA (Sonkoly et al. 2006; Bando et al. 2006) and protein level (Bando et al. 2006). IFNγ upregulates the expression of IL-31R in human monocytes (Dillon et al. 2004) and in human dermal microvascular endothelial cells (Feld et al. 2010). IL-31R appears to be constitutively expressed in the skin, as no antigenic stimulus is required to induce the atopic dermatitis (AD) phenotype, while the lack of lung pathology in IL-31 transgenic mice suggests that the asthma phenotype may require an allergenic stimulus (Dillon et al. 2004).

2.1 Evidence for the Role of IL-31 in Itch

Atopic dermatitis, a highly pruritic chronic inflammatory disorder, is characterised by a TH_2-mediated immune response. Activated skin-homing (CLA+) TH_2 cells in patients with atopic dermatitis produce higher levels of IL-31 than controls (Bilsborough et al. 2006). IL-31 mRNA is upregulated in murine models of atopic dermatitis, including an ovalbumin (OVA)-induced model and a model induced by topical application of superantigen *Staphylococcus aureus* toxin B (SEB) (Cevikbas et al. 2014). IL-31 overexpression in transgenic mice induces an atopic-like

dermatitis (Dillon et al. 2004; Grimstad et al. 2009). IL 31 injected into mice induces pruritus (Arai et al. 2013) and alopecia (Dillon et al. 2004) but not pain (Cevikbas et al. 2014). Interestingly, IL-31-induced alopecia is dependent on the presence of functioning lymphocytes, while IL-31-induced pruritus is not (Dillon et al. 2004). Cutaneous (Dillon et al. 2004) as well as intrathecal (Cevikbas et al. 2014) injections induce itch. The effect of intrathecal injection, in bypassing the skin, suggests that IL-31 can hypothetically trigger pruritus centrally in a direct fashion. Whether this is relevant in certain pathophysiologies remains to be determined. Cutaneous injection of IL-31 induced pruritus after 3–4 days in mice, and pruritus persisted for 6–9 days after administration had ceased (Dillon et al. 2004). One recent study in humans indicated that IL-31 does not induce an immediate itch following a skin challenge (Hawro et al. 2014). Of note, in a further study, the clinically relevant pruritic effect of injected IL-31 occurred despite only 4 % of DRGs being IL-31RA^{+}(Cevikbas et al. 2014). The delayed onset and sustained nature of IL-31-induced pruritus (Arai et al. 2013) is characteristic of C-fibre activation. A Japanese team of researchers demonstrated both delayed itch in mice injected with intravenous IL-31, and also that intravenously administered IL-31R antibody reduced itch (Kasutani et al. 2014). In that study, the authors used two dermatitis models: firstly, a contact sensitivity reaction model and, secondly, a chronic AD-like model. They found that IL-31R antibody reduced itch both when used in a preventative manner and also after the disease was established. In the chronic AD-like model, IL-31R antibody reduced ear swelling and the dermatitis score. Existing drugs, including glucocorticoids, naloxone, calcineurin inhibitors (tacrolimus and pimecrolimus) and the H1 receptor antagonist, terfenadine, did not impact IL-31-induced itch (Kasutani et al. 2014). This supports the findings of a murine study in which IL-31-induced pruritus was unchanged in mast cell-deficient c-kit mutant mice compared to wild-type controls, indicating that neither histamine nor tryptase, pruritogens released from mast cells, is required for IL-31-mediated itch (Cevikbas et al. 2014). In a separate study, anti-IL-31 antibodies reduced itch in a murine model of atopic dermatitis (Grimstad et al. 2009) suggesting that an anti-IL-31/IL-31 blocking strategy may be beneficial to treat itch in atopic dermatitis.

Patients with AD express higher levels of IL-31 in their skin (Nobbe et al. 2012). IL-31 is upregulated in pruritic skin in AD compared with non-pruritic skin affected by psoriasis (Neis et al. 2006; Sonkoly et al. 2006). In patients with AD, IL-31 levels are higher in lesional compared to non-lesional skin. However, levels of IL-31 RNA are nonetheless fourfold higher in non-lesional skin of AD patients compared to skin of healthy controls (Sonkoly et al. 2006). The highest levels of IL-31 are seen in prurigo nodularis, a condition characterised by severe pruritus (Sonkoly et al. 2006). A haplotype of the IL-31 gene is associated with atopic dermatitis in humans. The high levels of IL-31 associated with $CD3^{+}$ $CD4^{+}$ $CD26^{-}$ T-lymphocytes, the cell type having undergone malignant transformation in cutaneous T-cell lymphoma, may shed light on the mechanism of itch in that disorder (Singer et al. 2013).

2.2 Mechanism of IL-31-Induced Itch

As previously outlined, IL-31 is upregulated in pruritic skin disorders. One trigger identified for this upregulation is the exposure to staphylococcal superantigens, and while upregulation occurs in the peripheral blood monocytes of both AD patients and healthy controls, the increase is greater in AD patients (Sonkoly et al. 2006). IL-31, an immune system-derived cytokine, is capable of "crosstalk" with the nervous system. The highest level of expression of the IL-31 receptor is on DRGs. IL-31-induced pruritus is significantly reduced in TRPV1- and TRPA1-deficient mice, and additionally, IL-31RA is largely expressed on neurons that also express TRPV1 (Cevikbas et al. 2014). Only a subset of neurons expressing TRPV1 express IL-31RA (Cevikbas et al. 2014). The co-localisation of IL-31RA and TRPV1 is of functional significance as following intradermal IL-31 injections scratching behaviour was reduced in TRPV1 knockout mice and following intrathecal injection scratching behaviour was similarly reduced following capsaicin-mediated TRPV1 neuronal ablation (Cevikbas et al. 2014). IL-31 has been demonstrated to trigger intracellular Ca^{2+} influx, which is in someway TRPV1/TRPA1 dependent (Cevikbas et al. 2014). TRPA1 is a channel required for mas-related G protein-coupled receptor (Mrgpr) and endothelin-1 (ET-1)-mediated itch. IL-31-mediated itch is reduced in TRPA1 knockout mice (Cevikbas et al. 2014). In vitro evidence of IL-31 acting via ERK1/2 phosphorylation is supported by in vivo reduction in scratching behaviour in the setting of a specific inhibitor of ERK1/2 activation (Cevikbas et al. 2014).

IL-31-induced pruritus is independent of IgE (Dillon et al. 2004). Similarly, IL-31-mediated itch is independent of the mast cell-derived pruritogens, histamine and tryptase, and is similarly independent of the PAR-2 pathway (Cevikbas et al. 2014). Of IL-31 responsive neurons, 67 % are also responsive to capsaicin, 30 % to allyl isothiocyanate (AITC), 38 % to histamine and 21 % to PAR-2. The fact that 90 % of IL-31-responsive neurons also respond to chloroquine, an exogenous pruritogen which functions through the MrgprA3 receptor, is of unclear significance but is possibly related to the co-localisation of IL-31R and TRPA1, as TRPA1 is a channel required for mas-related G protein-coupled receptor (Mrgpr). MrgprA3 KO mice could be used to assess if this channel functions in IL-31-mediated itch.

IL-31 activates the MAPK, P13K/AKT and JAK/Stat pathway (STAT1, STAT3 and STAT 5) (Dillon et al. 2004; Ghilardi et al. 2002; Diveu et al. 2004; Dreuw et al. 2004).

Genetic variation may predispose some of us to IL-31-mediated itch, with a G allele variant of IL-31 associated with higher rates of non-atopic dermatitis (Schulz et al. 2007).

The delayed itch response after IL-31 injection seems incompatible with direct activation of IL-31R as the sole mechanism behind IL-31-mediated itch. IL-31 receptors are present on keratinocytes, eosinophils (Cheung et al. 2010), mast cells (Yamaoka et al. 2009), activated monocytes and macrophages (Bilsborough et al. 2006; Kasraie et al. 2010) in addition to neuronal cells such as DRGs;

therefore, tissue-specific knockout studies are required to assess the relative contribution of these tissue types to IL-31-mediated itch.

Of particular interest is the role that keratinocytes play in IL-31-mediated itch. Keratinocytes express the IL-31 receptor. Intriguingly, IL-31RA is upregulated in the keratinocytes of patients with AD compared to controls (Bilsborough et al. 2006), suggesting that these patients are primed to respond to IL-31. Whether this is a cause or effect of their condition is unclear. Keratinocytes play an active role in modulating the neuronal experience of pain and pruritus, at least in part, through the release of mediators such as neurotrophins, neuropeptides or proteases (Paus et al. 2006; Steinhoff et al. 2006). IL-31 induces keratinocyte-associated inflammatory chemokines such as CXCL1, CCL17 and CCL22 (Dillon et al. 2004) and thymic stromal lymphopoietin (TSLP). Keratinocytes release IL-1 and IL-6, which may play a role in neuronal communication and pruritus. A study assessing IL-31 administration in the setting of keratinocyte-specific IL-31RA knockout would provide useful insight into the significance of their role.

There are myriad signalling pathways in afferent neurons, activated by a range of pruritogens, and the specificity of the process perhaps depends on the combination of signalling pathways engaged by a given pruritogen.

3 Oncostatin M

Oncostatin M (OSM) is a cytokine that belongs to the IL-6 family. It plays a critical role in inflammation, autoimmunity and in cancer (Silver and Hunter 2010), with a loss of responsiveness to OSM linked to disease progress in cancer patients (Lacreusette et al. 2007). OSM mRNA has been detected in various tissues in the human body, including T cells, neutrophils, eosinophils as well as microglia in the central nervous system (Repovic and Benveniste 2002; Tamura et al. 2002). OSM released from T cells is a potent stimulator of keratinocyte migration, and OSM transcripts are enhanced in the skin of patients with atopic dermatitis, a pruritic skin disease (Boniface et al. 2007). OSM may play an essential role in the development of a subtype of nociceptive neuron in the dorsal root ganglia (Morikawa et al. 2004; Bando et al. 2006), suggesting that OSM may represent a link between nerves and T cells in the skin.

OSM shares the glycoprotein-130 signal-transducing receptor that forms part of the receptor complex for all of the IL-6 family (Silver and Hunter 2010). The OSM receptor (OSMR) is a member of the type 1 cytokine receptor family (Homey et al. 2006). Murine OSM binds to a selective heterodimer of the OSMR beta (OSMRβ) receptor and the glycoprotein-130 receptor family (Tanaka et al. 1999). The type I OSM receptor is identical to the leukaemia inhibitory factor receptor (LIFR beta) and gp130 heterodimer, and the type II OSM receptor consists of gp130 and the OSMRβ subunit (Tanaka et al. 1999). A missense mutation in the OSMRβ gene has been reported to occur in patients with familial primary localised cutaneous amyloidosis (Arita et al. 2008), an autosomal-dominant disorder associated with chronic pruritus. This suggests that OSMRβ plays an important role in human

itch. There is little evidence at present to suggest that OSM has a direct role in the genesis of pruritus.

4 TSLP

Thymic stromal lymphopoietin (TSLP) is an epithelial-derived cytokine that is implicated in the pathogenesis of diseases characterised by TH2 responses, such as atopic dermatitis (Leyva-Castillo et al. 2013). TSLP acts as a potent stimulator of Th2 cytokines including IL 4, 5, and 13 with subsequent IgE production (Brandt and Sivaprasad 2011). Wilson et al. (2013) discovered that TSLP acts as a primary pruritogen, whereby calcium-dependent TSLP release by keratinocytes directly activates primary afferent sensory neurons as well as stimulating immune cells to induce itch and promote inflammatory responses in the skin. The authors identified the ORAI1/NFAT calcium signalling pathway as an essential regulator of TSLP release from keratinocytes in the skin. The TSLP receptor (TSLPR), a heterodimer of an IL-7 receptor alpha chain and a TSLP-specific receptor chain, is expressed in neuronal tissue in human dorsal root ganglia. These TSLP-sensitive neurons appear to represent a novel, distinct subset of neural tissue. TSLP acts directly on transient receptor potential (TRP) TRPA1-positive sensory neurons, which are downstream of TSLPR, to trigger itch. Histamine-dependent itch requires TRPV1 (Imamachi et al. 2009) but Wilson et al. (2013) showed that TRPV1-deficient mice displayed normal TSLP-evoked itch behaviours, indicating that TSLPR activation of primary afferent sensory neurons occurs independently of TRPV1. Wilson and colleagues showed that histamine, and other pruritogens, is not required to generate TSLP-evoked itch and that acute TSLP-evoked itch is independent of lymphocytes.

Cyclosporine is an inhibitor of NFAT-mediated transcription and is used in the management of pruritic inflammatory skin diseases, such as atopic dermatitis (Madan and Griffiths 2007). The authors suggest that the effectiveness of cyclosporine in treating chronic itch may be partially due to its effect on keratinocyte-mediated TSLP release (Wilson et al. 2013).

5 IL-4 and IL-13

IL-4 and IL-13 are both cytokines produced by TH2 cells that play an important role in the development of atopic dermatitis (Hamid et al. 1994). There is evidence that IL-4 is involved in the development of pruritus. Chan et al. (2001) showed that transgenic mice expressing epidermal IL-4 at the basal keratinocytes spontaneously developed a pruritic inflammatory skin disease reproducing all of the key features of human atopic dermatitis. A recent murine model in atopic-like mice showed that IL-4 was significantly upregulated in the skin following the application of the superantigen, *Staphylococcus aureus* toxin B (Cevikbas et al. 2014). These indicate that IL-4 may play an important role in the development of pruritus and inflammation in atopic dermatitis. Levels of IL-4, as well as IL-13, have been shown to

correlate with levels of IL-31, a known pruritogen, in skin biopsies of patients with atopic dermatitis (Neis et al. 2006).

A transgenic murine study showed that the dermal expression of IL-13 in the skin resulted in a chronic pruritic inflammatory skin condition resembling eczema (Zheng et al. 2009). There are no studies demonstrating that IL-13 plays a direct role in the development of pruritus.

IL-4 and IL-13 have been the focus of a recent therapeutic advancement in atopic dermatitis. Dupilumab is a monoclonal antibody that blocks the activity of IL-4 and IL-13. A recent randomised, double-blind, placebo-controlled trial evaluated the safety and efficacy of dupilumab in the treatment of patients with moderate-to-severe atopic dermatitis in four clinical trials (Beck et al. 2014). Four weeks of treatment with dupilumab resulted in a dramatic, dose-dependent improvement in itch and biomarker profiles, and extension of treatment to 12 weeks resulted in a statistically significant reduction of 55.7 % in the mean score on the pruritus numerical-rating scale. Combination therapy with topical glucocorticoids for 4 weeks was associated with a statistically significant, rapid and sustained reduction in the pruritus numerical-rating scale ($P = 0.005$), despite 50 % less topical glucocorticoid use than the control group. In conclusion, there is convincing evidence that IL-4 and, to a lesser degree, IL-13 play a role in the development of pruritus.

6 Interleukin-6

Interleukin-6 (IL-6) and IL-6 receptors are expressed in Schwann cells of peripheral nerves (Grothe et al. 2000). IL-6-like immunoreactivity has been shown to be significantly increased in nerve-like fibres in skin biopsies taken from patients with positive epicutaneous patch test reactions to nickel sulphate and from patients with atopic dermatitis and prurigo nodularis, a chronic, highly pruritic skin disease (Nordlind et al. 1996), implicating IL-6 in the development of pruritus. Prick testing of atopic patients and controls with supernatants of mite or birch pollen antigen, however, showed no increase in levels of IL-6 (Lippert et al. 1998), drawing into question the role IL-6 plays in the development of itch.

7 Interleukin-2

IL-2 is a cytokine produced by activated T-lymphocytes that is implicated in the symptom of itch. The IL-2 receptor (IL-2R) is made up of 3 subunits—alpha (α), beta (β) and gamma (γ). The α and β chains bind IL-2, and the γ chain is involved in signal transduction following interaction with the cytokine (Cantrell and Smith 1984). Epidermal injection of IL-2 has been reported to cause a local, low intensity, pruritogenic effect (Darsow et al. 1997) in healthy individuals and in patients with atopic dermatitis (Wahlgren et al. 1995). Of interest, pruritus and erythema developed earlier in patients with atopic dermatitis than in healthy controls. The cause of

this is unclear and whether IL-2 receptors are upregulated in these patients has not been investigated. Prick testing with supernatants of mitogen-stimulated peripheral blood mononuclear cells containing increased concentrations of IL-2 and IL-6 resulted in pruritus and wheal formation in patients with atopic dermatitis and had no effect on healthy controls (Cremer et al. 1995).

Serum levels of IL-2 have been shown to be significantly higher in patients on haemodialysis with uraemic pruritus, a condition frequently refractory to anti-pruritic therapies, than patients without pruritus on haemodialysis. IL-4 levels were not found to be elevated in these patients (Fallahzadeh et al. 2011).

Aldesleukin is an IL-2 drug used for the treatment of advanced melanoma and renal cell carcinoma. High-dose IL-2 is capable of inducing flushing, vasodilatation and itch in cancer patients; however, there is a latency period of 2–3 days preceding the itch response that suggests an indirect pruritogenic effect of IL-2 via other mediators (Gaspari et al. 1987). It is unclear if the induction of itch is by a direct, receptor-mediated process or whether it is an indirect process, acting via mast cells or endothelial cells (Steinhoff et al. 2006). There may also be synergistic amplification or receptor transactivation; for example, there is evidence that bradykinin augments the effect of IL-2-induced pruritus in sensory nerves (Martin 1996).

Systemic and topical immunosuppressants such as tacrolimus, pimecrolimus and cyclosporine A inhibit the production of IL-2 with subsequent attenuation of pruritus in patients with atopic dermatitis (van Joost et al. 1987; Wahlgren et al. 1990), supporting the theory that IL-2 is involved in the development of pruritus. Further study into the effects of blocking the IL-2R with a targeted antibody would be interesting to evaluate if this is a potential therapeutic target in the management of itch.

8 Interleukin-8

Interleukin 8 (IL-8) is a chemokine that had been postulated to act as a mediator of itch in atopic dermatitis; however, intracutaneous application of IL-8 did not induce pruritus or erythema in human skin (Stander and Steinhoff 2002). A study showed increased levels of IL-8 following prick testing of atopic dermatitis patients with supernatants of mite or birch pollen antigen, but there was no correlation with the induction of itching or whealing (Lippert et al. 1998). These findings suggest that IL-8 does not play a central role in the symptom of itch.

9 TNF Alpha

TNF-α is an adipokine that plays an important role in neuropathic pain. Animal models of neuropathic pain secondary to various types of nerve injury implicate TNF-α in the initiation and propagation of pain (George et al. 1999; Shubayev and Myers 2000). In rats with spinal root injuries, TNF receptor 1 (TNFR1) elicits excitatory responses in the dorsal root ganglia of adjacent uninjured roots, and

TNFR2 excites dorsal root ganglia neurons from injured roots (Schafers et al. 2008). The authors conclude that TNFR1 may be predominantly involved in the excitation of sensory neurons and induction of pain behaviour in the absence of nerve injury (Schafers et al. 2008). In humans, nerve biopsies from patients with centrally mediated mechanical allodynia showed higher levels of TNF-α receptor I especially in myelinating Schwann cells (Empl et al. 2001). Intra-sciatic injection of TNF-α in rats has been shown to cause mechanical allodynia and thermal hyperalgesia (Wagner and Myers 1996). Furthermore, clinical studies have shown that TNF-α used as chemotherapy in isolated limb perfusion leads to the development of peripheral neuropathy in the perfused limb (Drory et al. 1998). There are no reports as yet linking TNF alpha to itch.

10 Other Cytokines

There is no convincing evidence that IL-1, IL-7, ciliary neurotrophic factor and neuropoitin correlate with itching. IL-18 has been shown to contribute to the spontaneous development of atopic dermatitis-like inflammatory skin lesions (Konishi et al. 2002), but there is no convincing evidence that IL-18 acts as a key mediator in pruritus.

11 Conclusions

There is strong evidence that in humans, monkeys and rodents IL-31 and in mice, thymic stromal lymphopoietin (TSLP) acts as a key mediator in the development of itch, with clinical but less mechanistic evidence also existing for IL-4 and/ or IL-13 in humans. Chronic pruritus causes significant morbidity, and effective treatments are limited. Targeted therapy against IL-4 and IL-13 with dupilumab has already demonstrated efficacy in the treatment of human itch. The door is now open for the development of further cytokine-targeted therapies for the treatment of this highly distressing symptom (Steinhoff et al. 2012).

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Interactions Between Keratinocytes and Somatosensory Neurons in Itch

Jamie Schwendinger-Schreck, Sarah R. Wilson, and Diana M. Bautista

Contents

Abstract

Keratinocytes are epithelial cells that make up the stratified epidermis of the skin. Recent studies suggest that keratinocytes promote chronic itch. Changes in skin morphology that accompany a variety of chronic itch disorders and the multitude of inflammatory mediators secreted by keratinocytes that target both sensory neurons and immune cells highlight the importance of investigating the connection between keratinocytes and chronic itch. This chapter addresses some of the most recent data and models for the role keratinocytes play in the development and maintenance of chronic itch.

Keywords

Atopic dermatitis • Chronic itch • Cytokines • DRG • Keratinocytes • Neurogenic inflammation • TSLP

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Abbreviations

AD	Atopic dermatitis
CALCRL	Calcitonin receptor-like receptor
CCL5	Chemokine ligand 5
CGRP	Calcitonin gene-related peptide
DRG	Dorsal root ganglia
ER	Endoplasmic reticulum
ET-1	Endothelin-1
H1R	Histamine 1 receptor
IL	Interleukin
KLK	Kallikrein
NFAT	Nuclear factor of activated T cells
NGF	Nerve growth factor
NK1R	Neurokinin 1 receptor
ORAI1	Calcium release-activated calcium channel protein 1
PAR2	Protease-activated receptor 2
SLIGRL	Ser–Leu–Iso–Gly–Arg–Leu
SP	Substance P
T_H2	T helper type 2
TRPA1	Transient receptor potential cation channel A1
TRPV1	Transient receptor potential cation channel V1
TSLP	Thymic stromal lymphopoietin
VCAM-1	Vascular cellular adhesion molecule 1

1 Introduction

Keratinocytes are the predominant cell type making up the layers of the epidermis in the skin. Their precise role in the development and maintenance of acute and chronic itch remains a major question. Data supporting a role for keratinocytes in chronic itch draw attention to neuron-to-keratinocyte communication and suggest that the activation of itch-sensitive sensory neurons (pruriceptors) itself may lead to observed changes in skin morphology. Amid the emerging data, a model is forming whereby there is multidirectional cross talk between keratinocytes, sensory neuron afferents innervating the skin, and local immune cell populations that occurs under pathophysiological states in chronic itch (Fig. 1). Here, we address some of the current data and models for the role keratinocytes play in itch transmission. We refer readers to reviews that comprehensively cover the role of keratinocytes and sensory neurons in itch (Ansel et al. 1997; Boguniewicz and Leung 2011; Gutowska-Owsiak and Ogg 2012; Kabashima 2013; Bautista et al. 2014).

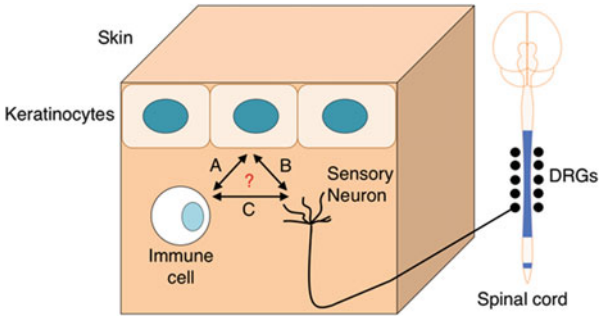

Fig. 1 Keratinocytes, epidermal immune cells, and sensory neuron afferents communicate via cell–cell signaling factors. Keratinocytes release factors that influence immune cell activity (**A**), such as chemokines and cytokines. Immune cells also release chemokines and cytokines (**A**) that modulate keratinocyte activity. Both keratinocytes (**B**) and immune cells (**C**) can also release pruritogens, algogens, and antinociceptive factors that all act on sensory neurons, whereas sensory neurons release neuropeptides that can target both keratinocytes (**B**) and immune cells (**C**)

2 The Skin and the Detection of Itch Stimuli

The skin is a stratified epithelium, home to a variety of epithelial cells, resident immune cells, and innervating somatosensory nerve fibers (Fig. 2). Keratinocytes, the resident epithelial cells making up the epidermis, adopt unique morphologies and differentiation states as they progress from deeper to more superficial layers. Those attached to the basement membrane in the stratum basale remain proliferative, while daughter cells in the stratum spinosum exit the cell cycle. Tight junctions connect the flattened keratinocytes in the stratum granulosum, forming a cornified envelope. Finally, in the outer layer, the stratum corneum, squamous keratinocytes are continually shed as new cells proliferate and replace them from below (Simpson et al. 2011). The stratum corneum is known to play an important role in the prevention of itch disorders such as atopic dermatitis (Hatano et al. 2009).

The skin-resident immune population includes macrophages, dendritic cells, mast cells, and γδ T cells. Macrophages in the skin function much as they do in the body, by orchestrating inflammatory responses and clearing invading pathogens, cellular debris, and apoptotic cells through phagocytosis. Dendritic cells may reside in the epidermis as Langerhans cells or the dermis as dermal dendritic cells (Tay et al. 2013). Both dermal dendritic and Langerhans cells function primarily to recognize and capture antigens present in the skin for processing and presentation in initiation of the adaptive immune response. Mast cells are particularly abundant in tissues that form interfaces with the external environment, like the skin, suggestive of their role as the first line of host defense. Upon activation, mast cells release potent inflammatory mediators that are stored in their secretory vesicles, such as histamine, proteases, cytokines, proteoglycans, and

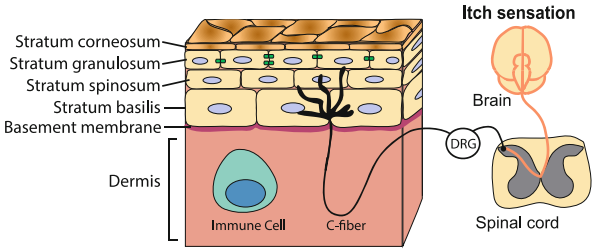

Fig. 2 The skin is a layered epithelium containing sensory nerves and immune cells. The epithelium is made up of several layers of differentiated keratinocytes. In the stratum granulosum, tight junctions (*green*) connect neighboring keratinocytes. Unmyelinated, nociceptive C-fibers detect pain, temperature, and itch. The cell bodies of primary sensory fibers are in the dorsal root ganglia (DRG) and project to the spinal cord. Interactions between keratinocytes, sensory neurons, and resident immune cells play an important role in chronic itch

lipid mediators. γδ T cells are a subset of T cells that are highly enriched in the epithelium. They have been proposed to play roles in monitoring and destroying stressed epithelial cells, promoting wound healing, and regulating inflammatory responses (Girardi 2006).

Somatosensory neuron terminals densely innervate the skin. Itch and other environmental stimuli, such as mechanical force and temperature, are detected by the somatosensory system. The cell bodies of somatosensory neurons are located in the trigeminal ganglia near the base of the skull and dorsal root ganglia (DRG) adjacent to the spinal cord. Sensory neurons in the trigeminal ganglia innervate the skin and organs of the head and face and send efferent projections directly to the brain stem. Sensory neurons in the DRG project peripheral afferent fibers to the skin and organs in the rest of the body and project central fibers to the dorsal horn of the spinal cord, where they synapse onto dorsal horn neurons that ultimately send signals to the brain stem and thalamus (Fig. 1).

A heterogeneous population of primary sensory neurons mediate itch and terminate in the skin as free nerve endings (Dong et al. 2001; Imamachi et al. 2009; Han et al. 2013; Bautista et al. 2014). Approximately 5–20 % of the total population of primary afferent C-fibers are activated by endogenous itch-producing compounds released by nonneuronal cells in the skin (e.g., mast cell-derived histamine), as well as by exogenous pruritogens, such as chloroquine (Ikoma et al. 2006; Imamachi et al. 2009; Liu et al. 2009). Pruriceptors are categorized by the compounds they respond to (e.g., histamine, serotonin, and chloroquine) and the compounds they release (e.g., neuropeptides like calcitonin gene-related polypeptide (CGRP) or neurotransmitters like glutamate). CGRP-expressing neurons have been shown to be required for full expression of chloroquine- and histamine-evoked itch behaviors (McCoy et al. 2013) and terminate in the stratum spinosum (Lumpkin and Caterina 2007). Interestingly, different layers of the epidermis secrete molecules with opposing roles in pain and itch. In the superficial epidermis, stimulation of keratinocytes results in the release of the antinociceptive molecule beta-endorphin, while deeper keratinocytes release pro-nociceptive endothelin-1 (ET-1) when stimulated

(Lumpkin and Caterina 2007). These data suggest that the different epidermal layers may specialize in specific somatosensory modalities, though how these layer-specific differences pertain to the sensation of itch remains unknown.

3 Keratinocyte-Mediated Modulation of Pruriceptors

Keratinocytes interact with neurons to promote itch both indirectly and directly. First, keratinocytes can promote itch indirectly through the release of inflammatory mediators that activate immune cells, which in turn stimulate pruriceptors. Second, keratinocytes are the primary cellular component of the epidermis. Physical disruption of the skin barrier enables entry of exogenous pruritogens into the body, indirectly promoting itch and inflammation. Direct interactions between keratinocytes and neurons occur via the release of pruritogens by activated keratinocytes, as well as the release of growth factors that encourage nerve sprouting and sensitization. Both direct and indirect interactions between neurons and keratinocytes are facilitated by the location of itch-transducing C-fibers as free nerve endings in the skin. Peptidergic itch-transducing C-fibers terminate in the stratum spinosum, while non-peptidergic C-fibers penetrate more superficially to the stratum granulosum (Zylka et al. 2005). Although classical synapses have not been observed between keratinocytes and neurons, electron microscopy of the skin reveals membrane–membrane apposition, where gap junctions or chemical synapses could exist (Hilliges et al. 1995).

4 Indirect Interactions Between Keratinocytes and Sensory Neurons

Upon activation by pruritogens, keratinocytes release a number of inflammatory molecules, which have been proposed to account for enhanced pruriceptor sensitivity under chronic itch conditions. For example, histamine can act on keratinocytes to increase the production of proinflammatory cytokines such as IL-6 and IL-8 (Kohda et al. 2002) and the proinflammatory chemokine CCL5 (Giustizieri et al. 2004). Similarly, keratinocytes treated with substance P or CGRP upregulate the expression of the inflammatory cytokine IL-1α and inflammatory chemokine IL-8 at both the mRNA and protein levels (Dallos et al. 2006). Keratinocytes can also be activated by PAR agonists (e.g., tryptase), endothelin-1 (ET-1), vasoactive intestinal polypeptide (VIP), and galanin (Yohn et al. 1994; Santulli et al. 1995; Dallos et al. 2006). Some chemokines and cytokines released by keratinocytes activate sensory neurons directly, while others recruit immune cell populations such as mast cells and T cells, which can then release sensory neuron-activating pruritogens like histamine and IL-31, respectively (Fig. 3; Tay et al. 2013). IL-31 can directly activate neurons in culture, and injection of IL-31 causes robust scratching that is independent of mast cells (Cevikbas et al. 2014). The

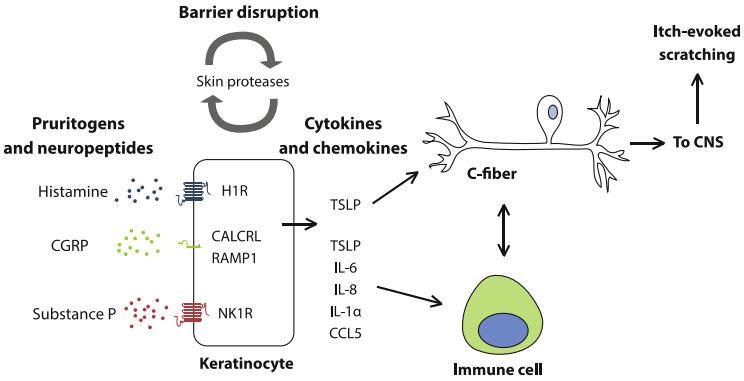

Fig. 3 Keratinocytes indirectly promote itch in sensory nerves. Keratinocytes are activated by pruritogens and neuropeptides such as histamine, CGRP, and SP. Keratinocytes release chemokines and cytokines that activate the immune system and result in release of pruritogens like TSLP and IL-31. TSLP also activates sensory neurons, inducing itch directly. Sensory neurons and immune cells also interact, for example, through the release of IL-31 by T cells and inflammatory mediators by neurons. In addition, chronic itch is associated with disruption of barrier function, which is maintained by keratinocytes. Barrier disruption may result from increased levels of skin proteases, such as kallikreins and tryptase, which normally regulate stratum corneum turnover

dysregulation of these feedback loops among keratinocytes, immune cells, and sensory neuron afferents is common in chronic itch diseases of the skin such as psoriasis (Raychaudhuri and Raychaudhuri 2004; Raap et al. 2011).

Keratinocytes are also critical for maintaining the epidermal barrier, which prevents both entry of exogenous objects and exit of internal components. Thus, the epidermal barrier disruption common to chronic itch disorders may facilitate entry of pruritogens and allergens. For example, in the NC/Nga atopic dermatitis (AD) mouse model, scratching itself exacerbates the itch phenotype (Hirano et al. 2011). Indeed, in AD patients, lesions develop at the site of scratching, and barrier defects in the skin are frequently associated with the development of AD (Elias and Schmuth 2009; Leung 2013). Epidermal serine proteases known as kallikreins (KLK) also mediate a positive feedback loop between keratinocyte activation and barrier disruption. KLKs function at the outer layer of the epidermis, enabling desquamation of the stratum corneum. Activated keratinocytes release KLKs, which in turn break down the corneodesmosomes holding together the outer layer of keratinocytes, thereby enabling shedding of the top layer of the epidermis and its replacement by new cells from the stratum granulosum (Tanaka et al. 2011). Unregulated KLK activity results in excessive loss of outer skin cells, and improper KLK regulation is believed to contribute to the impaired skin barrier seen in AD (Tanaka et al. 2011). As such, transgenic mice with overexpression of KLK5 in the stratum corneum and stratum granulosum have defective skin barrier function and significantly higher transepidermal water loss than wild-type mice. Further, these mice exhibit continuous scratching (Furio et al. 2014).

Physical perturbation of keratinocytes may also lead more directly to itch, as tape stripping results in increased production of the cytokine thymic stromal lymphopoietin (TSLP) (Kabashima 2013), which can activate itch directly through sensory neurons as well as indirectly via immune cells (Ziegler and Artis 2010; Wilson et al. 2013b). KLK5, in addition to physically disrupting the epidermis, also activates PAR2 in keratinocytes, leading to expression of several inflammatory mediators including TSLP (Stefansson et al. 2008; Briot et al. 2009). Another protease that leads to itch is cathepsin S, a cysteine protease that also activates PAR2 (Reddy et al. 2010; Elmariah et al. 2014). Further, overexpression of cathepsin S leads to atopic dermatitis phenotypes in mice, and levels correlate with seborrhoeic dermatitis in humans (Kim et al. 2011; Viodé et al. 2014).

5 Direct Interactions Between Keratinocytes and Sensory Neurons

Keratinocytes release a number of pruritogens that can directly activate itch fibers, including histamine, TSLP, and ET-1 (Fig. 4; Lumpkin and Caterina 2007). Histamine is a pruritogen that is secreted by a variety of cells including mast cells (Chatterjea and Martinov 2014), basophils, and keratinocytes. Cultured keratinocytes release histamine upon radiation by ultraviolet light B (Malaviya et al. 1996), and the release of histamine by keratinocytes was recently confirmed in vivo (Inami et al. 2013). Histamine acts directly on primary sensory neurons to induce itch primarily through the H1 receptor (Jeffry et al. 2011). The ion channel TRPV1 is required for both histamine-evoked calcium signals in DRG neurons and histamine-evoked itch behaviors in mice (Kim et al. 2004; Imamachi et al. 2009).

As mentioned above, TSLP can also be released by keratinocytes upon PAR2 activation. Cleavage of the N terminus of the PAR2 receptor leads to the release of intracellular calcium stores, causing activation of STIM1 and influx of extracellular calcium through the channel ORAI1. Calcium influx in turn activates calcineurin, which dephosphorylates NFAT and enables its translocation to the nucleus. NFAT is a transcriptional activator of TSLP, leading to release of TSLP into the extracellular space (Fig. 5; Wilson et al. 2013b). TSLP directly activates sensory neurons through the TSLP receptor and requires the ion channel TRPA1 (Wilson et al. 2011, 2013a, b). TSLP release by keratinocytes also contributes indirectly to itch. TSLP promotes inflammatory immune responses, leading to increased cytokine production by mast cells and T helper type 2 (T_H2) differentiation of CD4+ T cells (Ziegler and Artis 2010). However, while activation of immune responses by TSLP plays a key role in allergic inflammation and contributes to the development of chronic itch, neither T and B cells nor mast cells are required for acute TSLP-induced itch (Wilson et al. 2013b).

Keratinocytes, along with sensory neurons and immune cells, also secrete ET-1, a pruritogen that directly activates ~3 % of DRG neurons in culture (Kido-Nakahara et al. 2014). Injection of ET-1 has been reported to induce *either* pain or itch or *both* pain and itch in mice (Khodorova et al. 2003; Liang et al. 2010; Gomes et al. 2012;

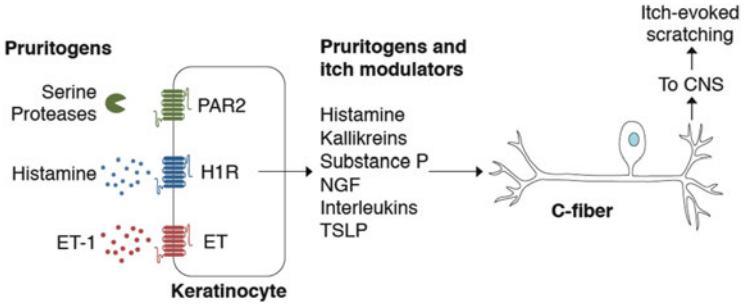

Fig. 4 Keratinocytes act in concert with somatosensory neurons to detect and transduce itch stimuli. Keratinocytes are activated by a number of pruritogens including histamine, SLIGRL, and ET-1 and secrete pruritogens and other itch mediators in response. C-fibers terminate as free nerve endings in the skin, enabling rapid signaling between keratinocytes and itch fibers

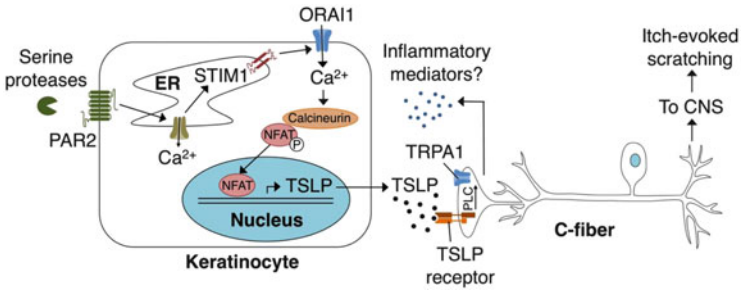

Fig. 5 Schematic diagram depicting the ORAI1 signaling pathway in keratinocytes that links PAR2 to TSLP secretion and activation of itch neurons. Activation of PAR2 triggers release of Ca^{2+} from the ER and activation of STIM1, which opens ORAI1 channels to promote Ca^{2+} influx. Ca^{2+} activates the phosphatase calcineurin, which dephosphorylates NFAT and causes nuclear translocation, thus inducing transcription of TSLP. Secreted TSLP depolarizes a subset of C-fibers to evoke itch, in a TSLPR- and TRPA1-dependent manner. Activation of TRPA1-expressing sensory neurons can then lead to release of neuropeptides in the skin in a process known as neurogenic inflammation

Kido-Nakahara et al. 2014). However, when ET-1 is injected intradermally into the mouse cheek, an assay used to distinguish itch from pain, both wiping (indicative of pain) and scratching (indicative of itch) occur (Gomes et al. 2012). To further complicate the action of ET-1, two ET-1 receptors, ET_A and ET_B, are expressed in the skin and exert opposing effects. ET_A is expressed by neurons and mast cells and activation promotes scratching. In contrast, ET_B is expressed by keratinocytes and appears to inhibit both itch and pain (Gomes et al. 2012). Scratching responses to ET-1 are dependent on the ion channel TRPA1 but not TRPV1. However, loss of TRPA1 does not affect the proportion of DRG neurons that respond to ET-1 in culture (Kido-Nakahara et al. 2014). Further studies are needed to understand the exact roles of ET-1 receptors in somatosensation.

Keratinocytes also mediate itch by the release of the neurotrophin nerve growth factor (NGF). When treated with substance P or CGRP, keratinocytes increase expression of NGF, which sensitizes somatosensory neurons and may contribute to increased innervation in chronic itch conditions (Urashima and Mihara 1998; Dallos et al. 2006). NGF secreted by keratinocytes is able to induce neurite outgrowth in culture (Di Marco et al. 1991), and NGF also interacts with the immune system to degranulate mast cells and activate T lymphocytes (Raychaudhuri and Raychaudhuri 2004).

6 Neuronal Modulation of Keratinocytes

Neurons, in turn, influence keratinocytes by releasing inflammatory mediators that can activate keratinocytes, as well as by promoting epidermal thickening and the proliferation of keratinocytes. As mentioned above, keratinocytes are activated by a number of pruritogens and inflammatory mediators, including histamine, substance P, and CGRP.

Activated peripheral terminals of small-diameter sensory neurons have been shown to release a variety of neuropeptides and proinflammatory molecules. The aforementioned substance P and CGRP have been suggested to be major initiators of this neurogenic (neuronal origin) inflammation. These neuropeptides are located in a subset of small dorsal root ganglion (DRG) cells, which give rise to the lightly myelinated Aδ and unmyelinated C-fibers (Willis and Coggeshall 1991). Substance P and CGRP produce symptoms of neurogenic inflammation by interacting with a variety of cell types, including endothelial and immune cells (Richardson and Vasko 2002). A number of stimuli have been shown to cause or augment the release of these neuropeptides such as ATP, bradykinin, capsaicin, mustard oil, heat, low pH, NGF, trypsin, tryptase, and prostaglandins (Richardson and Vasko 2002). Substance P can then go on to activate keratinocytes and induce release of cytokines such as IL-1, IL-8, and vascular cellular adhesion molecule 1 (VCAM-1) (Ansel et al. 1997). In addition, CGRP has been linked to increased expression of vascular endothelial growth factor (VEGF) in cultured keratinocytes (Yu et al. 2006). Interestingly, increased VEGF expression is common in the skin of psoriatic patients, suggesting that dysregulation of neuron-to-keratinocyte signaling may play a role in this chronic itch condition.

Sensory neurons may also play a role in epidermal homeostasis. In an innervated skin model using coculture of human fibroblasts and keratinocytes with porcine DRG neurons, neurons were found to induce epidermal thickening and keratinocyte proliferation in a CGRP-dependent manner (Roggenkamp et al. 2013). Similar results are also found using in vivo models. In a mouse model of dry skin-evoked itch, TRPA1-expressing sensory neurons drive epidermal thickening along with gene expression changes and scratching behaviors (Wilson et al. 2013a).

7 Keratinocytes and Chronic Itch

Many forms of chronic itch are refractory to available therapies, including antihistamines. Other treatments for chronic itch include topical steroids, immunomodulators, and moisturizers to prevent water loss from the skin. However, these therapies are not always effective and some require extensive treatment regimens, resulting in low compliance rates (Patel and Yosipovitch 2010a, b; Elmariah and Lerner 2011). Therefore, there is intense interest in identifying the underlying molecular mechanisms of chronic itch.

Many of the molecules involved in communication between keratinocytes, sensory neurons, and the immune system are misregulated in humans with chronic itch disorders or mouse models of chronic itch. Clinically, PAR2 and PAR2 agonists such as tryptase and kallikreins are upregulated in the skin of AD patients (Steinhoff et al. 2003; Komatsu et al. 2007). Expression of the pruritogenic cytokine TSLP is increased in keratinocytes from AD patients (Soumelis et al. 2002), and genetic variants of TSLP are associated with AD (Gao et al. 2010). Overexpression of TSLP in keratinocytes, either by an inducible transgene or deletion of the retinoid X receptors α and β, results in AD phenotypes in mice (Li et al. 2005; Yoo et al. 2005). In addition, TSLP has a well-known role in the activation of inflammatory immune responses, leading to allergy and asthma (Ziegler and Artis 2010; Siracusa et al. 2011; Kim et al. 2013; Ziegler et al. 2013). Protein levels of ET-1, a pruritogen that both activates and is secreted by keratinocytes, are higher in patients with prurigo nodularis, a disease characterized by the formation of hard nodules on the skin that itch intensely (Kido-Nakahara et al. 2014). Further, inhibition of the ET-1 receptor ET_A reduces scratching in a mouse model of chronic itch (Kido-Nakahara et al. 2014). IL-31, which links immune cells and sensory neurons, is overexpressed in the skin of atopic dermatitis patients, though levels do not correlate with disease severity (Neis et al. 2006; Siniewicz-Luzeńczyk et al. 2013). In contrast, plasma NGF levels are high in patients with atopic dermatitis and do correlate with disease severity (Toyoda et al. 2002). Further, immunohistochemistry of skin samples from patients with prurigo nodularis shows increased levels of NGF primarily in the upper dermis (Johansson et al. 2002). Finally, epidermal thickening, which is dependent on innervation, is further exacerbated when epidermal cells of AD patients are used for coculture (Roggenkamp et al. 2013).

Here, we have highlighted the importance of the interaction between keratinocytes and neurons in producing itch. Keratinocytes both respond to and release pruritogens. Additionally, mechanical or chemical disruption of the skin results in barrier loss and dry skin, which are both associated with chronic itch. An important goal in the field is to elucidate the signaling pathways responsible for the development and maintenance of chronic itch. In particular, further work is required to better understand the molecular mechanisms that cause keratinocytes to release pruritogens and other neuromodulators in chronic itch conditions and the effects of such molecules on neurons and immune cells. A better understanding of

these mechanisms may lead to itch-selective therapies that improve the quality of life for chronic itch patients.

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Itch and Its Inhibition by Counter Stimuli

Lindsey M. Snyder and Sarah E. Ross

Contents

Abstract

Recent studies have made significant progress in the knowledge of how itch sensation is processed, especially the molecular identity of neurons involved in itch signaling, both in the dorsal root ganglion and spinal cord. Despite these advances, the organization of these neurons in dorsal spinal cord circuits and how they interact with other somatosensory modalities, such as pain or temperature, remain relatively unexplored. Recent work from our lab and others has begun to shed light on these questions and will be the focus of this chapter. Here we describe the discovery of B5-I neurons, a population of inhibitory interneurons that function to inhibit itch, and review the evidence that these

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neurons mediate the inhibition of itch by counter stimuli. These studies are helping to solve the long-standing question of why itch makes us scratch.

Keywords
Pruritus • Itch • Dynorphin • Kappa opioid • KOR • Galanin • nNOS • Bhlhb5 • Bhlhe22

1 Introduction

Itch is a very distinctive sensation—it's irritating but not really painful, and it is associated with a very strong desire to scratch. Moreover, scratching causes at least transient relief from itch. In this regard, itch is unmistakably different than pain, which elicits the urge to protect the affected tissue rather than scratch it. And yet, itch and pain can be triggered by the very same chemical agents. For instance, in the eye or an open wound, capsaicin—a prototypical algogen—invariably causes pain. But when applied to undamaged skin, capsaicin can cause itch as well as pain. Histamine—a prototypical pruritogen—causes a pure itch sensation when it is released from mast cells during a bout of hives. But when histamine is injected subcutaneously, it causes pain rather than itch. Furthermore, this itch/pain dichotomy is true for almost every agent that has been studied—formalin, serotonin, endothelin-1, SLIGRL, acetylcholine, and prostaglandin-E2—all of these agents can cause either pain or itch depending on the manner in which they are applied (Ross 2011). These observations imply that the primary sensory afferents that convey itch and those that convey pain must express overlapping subsets of receptors for these chemical agents. But if the sensory neurons for pain and those for itch express the same receptors, how does the nervous system distinguish itch from pain?

One attractive model that may help explain how itch is distinguished from pain is the idea that lateral interaction, possibly at the level of the spinal cord, is involved in sharpening sensory acuity. In particular, inhibitory interneurons may provide cross-modal inhibition to help the nervous system decode somatosensory input. Thus, just as a center-surround network of excitation and inhibition sharpens spatial acuity in the visual system, a stimulus-counter-stimulus network of excitation and inhibition may sharpen the modality input in the somatosensory system. According to this model [which is an elaboration of the selectivity theory (McMahon and Koltzenburg 1992)], the selective activation of sensory neurons that are tuned to detect itch would result in itch, whereas the coactivation of a larger subset of primary afferents (e.g., ones that are tuned to detect noxious input) would result in the inhibition of itch (Fig. 1). One of the reasons that this model is attractive is because a simple neural circuit of this type could explain the everyday phenomenon that scratching (and other counter stimuli) inhibits itch.

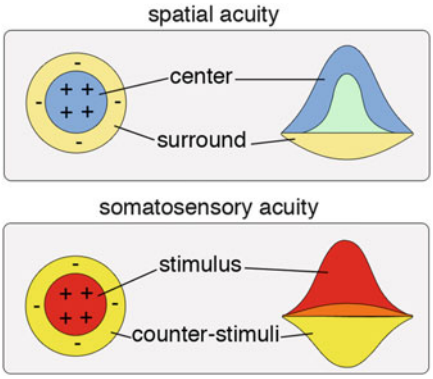

Fig. 1 Sharpening sensory acuity through lateral interaction. In the visual system, a center-surround receptive field sharpens the spatial input for visual acuity. By analogy, a stimulus-counter-stimulus receptive field may sharpen the modality input for somatosensory acuity. In this way, counter stimuli could, for example, inhibit itch

Some indirect evidence for this idea came from two groups that were examining the role of vGLUT2 in somatosensation. These groups made the same fundamental observation, which unexpectedly gave insight into the coding of itch (Lagerstrom et al. 2010; Liu et al. 2010). Glutamate is the fast excitatory neurotransmitter that is released by all primary somatosensory neurons. Although there are three glutamate transporters (vGLUT1–3), many sensory neurons express only vGLUT2. Hence, when vGLUT2 is conditionally removed from primary afferents, the "vGLUT2-only" subset is no longer able to signal via glutamate. Since the vGLUT2-only subset is a large proportion of the C fibers, it was not surprising that the loss of vGLUT2 in primary afferents resulted in blunted pain responses. However, the completely unexpected finding was the vGLUT2-lacking mice scratched all the time and showed elevated itch. These findings imply that glutamate signaling from vGLUT2-only primary afferents normally inhibits itch. However, the neural circuitry underlying this phenomenon remained unclear.

Around this time, strong evidence emerged, suggesting that itch is under inhibitory control at the level of the spinal cord. To study the neural circuits underlying itch, Akiyama et al. (2011) had developed a clever method to record from spinal neurons that are presumably involved in the transmission of itch: they used a model of dry skin itch (to drive ongoing itch) and then recorded from spontaneously active neurons in the spinal cord. Consistent with the idea that counter stimuli inhibit itch, they found that scratching, pinch, and noxious heat inhibited the firing rate of spontaneously active neurons. Moreover, treatment with either strychnine (to block glycinergic inhibition) or bicuculline and saclofen (to block GABAergic inhibition) strongly reduced the scratch-evoked inhibition of these cells. These findings suggested that both GABAergic and glycinergic inhibitory mechanisms in the spinal cord are involved in the inhibition of itch. But the identity of these inhibitory interneurons was not known.

Now, recent work from our lab has revealed that itch is inhibited by a population of spinal interneurons called B5-I neurons, and there is some tantalizing evidence that B5-I may mediate the inhibition of itch by counter stimuli (Kardon et al. 2014;

Ross et al. 2010). Here, we review how these inhibitory interneurons were first discovered and what we know about them.

2 Transcription Factors and the Development of the Dorsal Spinal Cord

Itch, pain, touch, and temperature are first detected in primary sensory afferents that convey this information to the dorsal horn in the spinal cord, particularly laminae I–III. Notably, less than 1 % of the neurons in the dorsal spinal cord are projection neurons that convey somatosensory input to the brain (Todd 2010). The vast majority of neurons in the dorsal horn (~99 %) are local interneurons, a fact which strongly implicates spinal microcircuits in the integration of somatosensory input. Moreover, it is becoming increasingly clear that these neural networks are made of discrete subtypes of spinal interneurons that form stereotyped connections with one another. Importantly, the wiring among these neurons appears to be developmentally programmed by a series of transcription factors. Thus, to understand these local networks, we need to understand the ontogeny of spinal interneurons.

Interneurons within the dorsal horn arise from progenitors that reside in the ventricular zone of the developing spinal cord (Helms and Johnson 2003). These neurons, which are among the last to differentiate, undergo their final round of cell division in between embryonic day 12 and embryonic day 14.5. Two basic types of neurons are born at this time, the so-called dorsal interneurons late A and B (dILA and dILB), which develop into inhibitory and excitatory neurons of the dorsal horn, respectively. Various transcription factors are expressed in neural progenitors and early postmitotic neurons during this time, and these factors are involved in specifying neuronal identity and mediating the differentiation of a neuronal precursor into a specific cell type with stereotyped connectivity.

In particular, the transcription factors of the basic helix-loop-helix (bHLH) and homeodomain factors appear to play key roles in these processes. For instance, inhibitory interneurons in the dorsal horn are not generated in mice lacking the bHLH factor *Ptf1a*, emphasizing the important role of the Ptf1a in mediating inhibitory neuronal fate (Glasgow et al. 2005). Excitatory neurons, in contrast, require the homeodomain factor Tlx3, which is required to suppress the GABAergic fate (Cheng et al. 2004). Both excitatory and inhibitory neurons diversify further during maturation into a large array of distinct neural subtypes (the number of which is not yet known). For instance, various neuropeptides, receptors, and other neuronal markers are expressed by distinct subpopulations of dorsal horn neurons (Polgar et al. 2013b). But, while transcription factors are thought to mediate the terminal differentiation of neurons and their connectivity, the identity of these factors and their specific functions remain poorly understood. Bhlhb5 (also called Bhlhe22) is a bHLH transcription factor that is expressed in the dorsal spinal cord within subsets of dILA and dILB neurons from the time they are postmitotic approximately until P14 (Ross et al. 2010). Thus, based on the

expression pattern of Bhlhb5, we hypothesized that this transcription factor was involved in the terminal differentiation and connectivity of subsets of neurons in the dorsal horn.

3 Mice Lacking the Transcription Factor Bhlhb5 Show Abnormally Elevated Itch

Bhlhb5 is a neural-specific basic helix-loop-helix (bHLH) transcription factor related to the *Drosophila* proneural factor *atonal* (Ross et al. 2003). Bhlhb5 and other closely related family members (namely, Bhlhb4 and Oligs1–3) all function as transcriptional repressors. However, Bhlhb5 is distinct from its family members because Bhlhb5 is selectively expressed in postmitotic neurons rather than in proliferating progenitors. Thus, whereas the Oligs (and likely Bhlhb4) are involved in neuronal fate specification, Bhlhb5 is involved in terminal neuronal differentiation. To investigate the function of Bhlhb5, two independent groups—ours and that of Lin Gan—made *Bhlhb5* knockout mice. These studies revealed an important role for Bhlhb5 in the retina, where it is required for the survival of some amacrine and cone bipolar cells (Feng et al. 2006). In addition, Bhlhb5 is required for the acquisition of area-specific fates in the cortex (Joshi et al. 2008). We found that Bhlhb5 is a transcriptional repressor that uses Prdm8 as an obligate cofactor and that both factors are required for the proper axonal targeting of all cortical projection neurons (Ross et al. 2012). Thus, Bhlhb5 has multiple roles in different regions of the nervous system. However, the most striking phenotype of $Bhlhb5^{-/-}$ mice is that they all develop self-inflicted skin lesions, which prompted us to investigate somatosensation in these mice (Ross et al. 2010).

Initially, it was not clear why $Bhlhb5^{-/-}$ mice, which behave normally until around 4–6 weeks of age, suddenly develop self-inflicted skin lesions. At the time of these studies, only a few animals with skin lesions had been analyzed in detail, and in these cases it was concluded that the mice in question either lacked sensitivity to pain ($Drg11^{-/-}$ mice) or suffered from obsessive-compulsive disorder ($Hoxb8^{-/-}$ mice) (Chen et al. 2001; Greer and Capecchi 2002). Indeed, when we (naively) first analyzed somatosensation in adult $Bhlhb5^{-/-}$ mice (which, importantly, already had skin lesions), we found that these mice showed a very blunted response to noxious input. Based on these findings, we erroneously concluded that the self-injury observed in $Bhlhb5^{-/-}$ mice was due to an absence of pain. Fortunately (and as a testimony to the efficacy of the review process), the reviewers of our manuscript questioned our interpretation and asked us to investigate the possibility of abnormal itch in $Bhlhb5^{-/-}$ mice. When we reevaluated the behavior of $Bhlhb5^{-/-}$ mice, this time analyzing mice before the onset of skin lesions, we realized that our first interpretation was completely wrongheaded. In fact, prior to the onset of skin lesions, $Bhlhb5^{-/-}$ mice show normal responses in most sensory tests including chemical, mechanical, and heat nociception (Ross et al. 2010). However, $Bhlhb5^{-/-}$ mice scratch significantly more than wild-type littermates following the application of all of the itch-inducing agents tested. Therefore, the

lack of Bhlhb5 expression led to an increase in itch sensitivity, but left pain and other somatosensory modalities relatively intact. Furthermore, these findings suggested that the self-inflicted skin lesions in *Bhlhb5*$^{-/-}$ mice were the result of excessive licking and scratching due to elevated itch.

4 Pathological Itch in Bhlhb5$^{-/-}$ Mice Is Due to the Loss of B5-I Neurons

Having determined that *Bhlhb5*$^{-/-}$ mice have abnormally elevated itch, the next step was to identify which cells were responsible for this phenotype. Since Bhlhb5 is expressed in numerous regions of the nervous system, the cellular basis of elevated itch was not completely obvious. Though Bhlhb5 is expressed in subsets of neurons in the dorsal horn (where itch is first integrated), this transcription factor is also expressed in some primary sensory afferents, the brainstem, and many other regions of the brain that might theoretically be involved in the processing of itch. To determine the neurons responsible for the elevated itch in *Bhlhb5*$^{-/-}$ mice, we used a genetic approach to selectively remove *Bhlhb5* from different regions of the nervous system (Ross et al. 2010). Using this conditional ablation strategy, we were able to ask whether deletion of *Bhlhb5* in specific areas of the nervous system was sufficient to recapitulate the phenotype seen in the constitutive *Bhlhb5*$^{-/-}$ mice. Upon loss of *Bhlhb5* from primary afferents, the resulting mice were normal with respect to itch. Likewise, upon loss of *Bhlhb5* from the dorsal telencephalon, the resulting mice had no sensory phenotypes. However, loss of *Bhlhb5* from the spinal cord (using the Hoxb8-cre line) was sufficient for the abnormally elevated itch and the development of skin lesions (unpublished observation). This finding suggested a key role of Bhlhb5 in the spinal cord. Since Bhlhb5 is expressed in both excitatory and inhibitory neurons within the spinal cord, we used cre lines that caused selective removal of *Bhlhb5* in excitatory and inhibitory neurons, respectively, to determine which type of spinal neurons were involved. These experiments revealed that loss of *Bhlhb5* within excitatory neurons of the dorsal horn (using Tlx3-cre) had no effect on itch sensitivity, whereas loss of *Bhlhb5* within inhibitory neurons (using Pax2-cre) was sufficient for abnormally elevated itch. Together, these experiments revealed that Bhlhb5 is required in inhibitory spinal interneurons for normal itch.

In newborn mice, Bhlhb5 is expressed in 7 % of neurons within the dorsal spinal cord. Of these, approximately one quarter of Bhlhb5-expressing neurons are excitatory and three quarters are inhibitory. At the time, there were no other markers for the Bhlhb5-expressing cells, and so there was no way to see what happened to the Bhlhb5-expressing cells in the absence of *Bhlhb5*. To resolve this problem, we used another genetic approach to permanently label all the cells that had ever expressed *Bhlhb5*. Specifically, we generated a *Bhlhb5-cre* knockin allele, which we then crossed with cre-responsive reporters (Ross et al. 2010). Using this approach, we discovered that Bhlhb5 is required for the survival of Bhlhb5-expressing neurons in the spinal cord. Without it, many of the neurons that should have expressed Bhlhb5

were missing. This discovery implied that the loss of a specific population of inhibitory interneurons during development results in abnormal itch, and we called these spinal interneurons B5-I neurons, since they are the ***I***nhibitory subset of Bhlh***b5***-expressing neurons.

5 B5-I Neurons Function to Inhibit Itch

Although we had identified the neurons responsible for abnormal itch, we still didn't know very much about them. To study these cells in greater detail, we began collaborating with Andrew Todd. His previous work had revealed that spinal inhibitory neurons can be divided into subgroups based on the expression of neurochemical markers (Polgar et al. 2013b). In particular, approximately one half of the inhibitory neurons in laminae I and II of the spinal cord express somatostatin receptor sst_{2A}, and B5-I neurons were found to belong to this subset (Kardon et al. 2014). The discovery that all B5-I neurons express sst_{2A} (which inhibits neurons) was important because it provided us with the tools that we needed to address a key question. Specifically, hitherto it was still not clear whether B5-I neurons function in the adult animal to inhibit itch or whether the survival of B5-I neurons was critical for the establishment of proper itch circuits—in other words, we could not distinguish between an adult function and a developmental role for B5-I neurons. Fortuitously, the finding that B5-I neurons express sst_{2A} provided an opportunity to directly test whether B5-I neurons inhibit itch in adult mice. Specifically, we reasoned that if B5-I neurons suppress itch, then inhibition of B5-I neurons through activation of sst_{2A} would result in spontaneous itch. In keeping with this idea, we found that intrathecal injection of the sst_{2A} agonist, octreotide, resulted in spontaneous scratching behavior. Moreover, this effect was lost in $Bhlhb5^{-/-}$ mice that are lacking B5-I neurons (Fig. 2). These data revealed that disinhibition of B5-I neurons causes itch, indicating that B5-I neurons normally function to inhibit itch.

6 B5-I Neurons Release the Kappa Opioid Dynorphin and Kappa Agonists Inhibit Itch

The inhibitory interneurons that express sst_{2A} are not a single population, and previous work by Andrew Todd had shown these interneurons can be further subdivided based on the expression of distinct neurochemical markers (Polgar et al. 2013a; Spike et al. 1998). When we looked at which of these subpopulations made up B5-I neurons, we discovered that B5-I neurons are composed of two mostly nonoverlapping subpopulations, one expressing the neuropeptide galanin and one expressing neuronal nitric oxide synthase (nNOS). Moreover, both galanin-expressing and nNOS-expressing subpopulations were almost completely missing in $Bhlhb5^{-/-}$ mice, whereas other populations of inhibitory neurons were unaltered (Kardon et al. 2014).

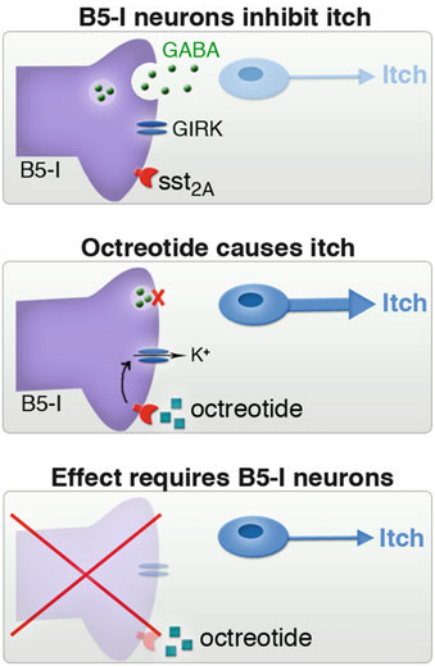

Fig. 2 Evidence that B5-I neurons function to inhibit itch. (**a**) Normally, itch is inhibited by B5-I neurons. (**b**) Activation of the somatostatin receptor sst_{2A} with octreotide results in the inhibition of B5-I neurons and spontaneous itch. (**c**) Octreotide has no effect in $Bhlhb5^{-/-}$ mice, which are lacking B5-I neurons

This discovery raised the important question of whether one or both of these subpopulations are involved in the inhibition of itch. Although we still don't know the answer to this question with certainty, we were particularly interested in the galanin subpopulation because previous work had shown that galanin-expressing interneurons also express the endogenous kappa opioid, dynorphin (Sardella et al. 2011). The idea that the release of dynorphin from B5-I neurons could be a potential mechanism through which they inhibit itch sensation was an attractive idea since there was already some precedent for the idea that kappa opioids inhibit itch (Inan and Cowan 2004; Ko et al. 2003; Togashi et al. 2002). Consistent with these previous studies, we found that pretreatment with kappa opioid agonists significantly decreased scratching in response to both histamine-dependent and histamine-independent pruritogens in wild-type mice (Kardon et al. 2014). Kappa opioid agonists also significantly reduced the elevated scratching response observed in $Bhlhb5^{-/-}$ mice, consistent with the idea that it is the absence of dynorphin in $Bhlhb5^{-/-}$ mice that is partially responsible for their elevated itch.

These findings raised the questions as to whether the GABA/glycine-mediated inhibition provided by B5-I galanin and nNOS cells was responsible for the elevated itch in $Bhlhb5^{-/-}$ mice or whether it was due solely to the decrease of dynorphin signaling. To evaluate this question, we analyzed the preprodynorphin knockout mice ($PPD^{-/-}$), which are missing dynorphin but not spinal dynorphin-expressing neurons. We found that loss of $PPD^{-/-}$ had no effect on itch sensitivity, and $PPD^{-/-}$ mice do not develop self-inflicted skin lesions (Kardon et al. 2014).

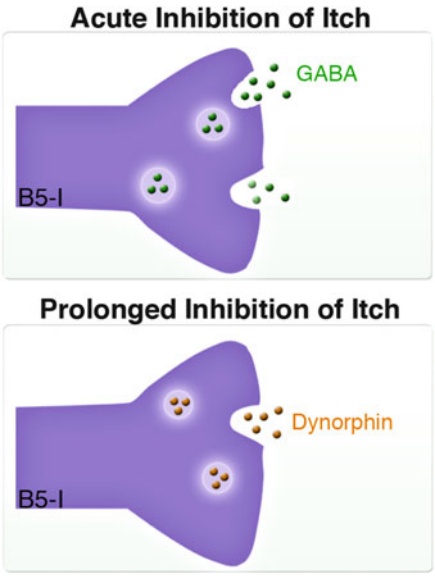

Fig. 3 Model for the acute and prolonged inhibition of itch by B5-I neurons. Fast-acting inhibitory neurotransmitters such as GABA may provide instantaneous relief from itch. The inhibitory neuromodulator dynorphin may provide sustained relief from itch, lasting minutes to hours

These observations point to a key difference between the loss of a neuropeptide and a loss of a neuronal subtype. Thus, whereas compensatory mechanisms may be able to atone for the loss of dynorphin, they cannot fully compensate for the loss of dynorphin-releasing neurons in *Bhlhb5*$^{-/-}$ mice. In conjunction, this evidence points to a role for both GABA/glycine- and kappa opioid-mediated inhibition in the regulation of itch sensation. One possibility is that GABA and glycine, which are fast-acting neurotransmitters, are involved in the immediate relief of itch that is felt upon scratching, whereas dynorphin, which is a neuromodulator that signals via Gi/o-coupled signaling cascades, is involved in the prolonged inhibition of itch (Fig. 3).

The identity of the cells expressing the kappa opioid receptor (KOR) is of particular interest, as they are likely to be the neurons that are inhibited by dynorphin and hence are likely intimately involved in the processing of itch sensation in the dorsal horn. Previous studies have established a key role for gastrin-releasing-peptide (GRP) receptor expressing excitatory interneurons in the transmission of itch signals within the dorsal horn (Sun et al. 2009; Mishra and Hoon 2013), and so we wondered whether these neurons might express KOR. We found that pretreatment with a kappa agonist significantly reduced scratching in response to GRP application (Kardon et al. 2014). This finding suggests that B5-I neurons could possibly target GRPR neurons directly. On the other hand, it is possible that dynorphin targets other interneurons downstream of the GRPR neurons. In either case, identifying the spinal neurons that express KOR is important since they will likely have an important role in the integration of itch.

7 Kappa Agonists as a Therapeutic Agent for Chronic Pruritus

Given the huge number of people worldwide that suffer from chronic itch, it is important to consider the therapeutic potential of kappa opioid agonists. To address this question, we used *Bhlhb5*$^{-/-}$ mice that have skin lesions as a model of neuropathic itch. Importantly, treatment of these mice with kappa opioid agonists significantly decreased time spent scratching, supporting the idea that kappa opioid agonists may serve as potentially effective clinical treatments (Kardon et al. 2014). Indeed, several clinical trials support the feasibility of kappa opioid agonists to treat chronic pruritus (Kumagai et al. 2012; Wikstrom et al. 2005). More recently, the neuropathic itch in *Bhlhb5*$^{-/-}$ mice has also been resolved by transplanting GABAergic neurons into the spinal cord. Thus, it appears that either increased GABAergic tone in the spinal cord or increased KOR signaling can reduce itch.

7.1 Kappa Agonists Are Selective for Itch

The kappa opioid receptor is closely related to the mu and delta opioid receptors. When the kappa opioid receptor was first discovered, there was great enthusiasm in the pharmaceutical industry for the idea that kappa opioids might offer relief from pain without the addiction and potential for abuse, which are observed with mu agonists like morphine. Numerous kappa agonists were developed and many were tested in clinical trials. Unfortunately, kappa agonists turned out to be poor at blocking pain, and although there are hundreds of papers testing the role of kappa agonists in animal models, only one agonist (U50,488) was ever tested in the KOR$^{-/-}$ mouse to confirm specificity of the agonist. Furthermore, even in this case, the dose of U50,488 that results in decreased nociceptive responses is one that appears to have sedative effects in the mouse (Simonin et al. 1998). Thus, the degree to which kappa opioid signaling truly inhibits pain remains an open question. This issue prompted us to analyze the degree to which the kappa agonists used on our study were selective for pain vs. itch. To address this question, we turned to the cheek model of itch developed by Lamotte's group (Shimada and LaMotte 2008). In this model, itch and pain behaviors can be distinguished in the same assay by quantifying scratching vs. wiping responses. Agents that cause pain result in wiping with the forepaw, whereas agents that cause itch result in scratching with the hindpaw. Using this model, we found that the kappa agonist nalfurafine significantly reduced chloroquine-induced scratching but had no effect on capsaicin-induced wiping (Kardon et al. 2014). Thus, at least under some conditions, kappa agonists selectively inhibit itch but not pain.

8 Increasing Evidence That Opioid Receptor Subtypes Show Modality Selectivity

An exciting idea that emerges from these experiments is the possibility that different opioid subtypes are involved in regulating different aspects of somatosensation. Mu opioid agonists, such as morphine, are among the most widely used for the treatment of pain. However, it has been reported that epidural morphine administration in patients often resulted in pruritus (Kjellberg and Tramer 2001). This side effect, however, is reduced by treatment with the drug nalbuphine, which acts as a kappa opioid agonist and mu opioid antagonist (Liao et al. 2011). Thus, nalbuphine may reduce itch, at least in part, through its action at the kappa opioid receptor. Consistent with this idea is the report of kappa opioid agonists reducing morphine-induced itch in monkeys (Ko et al. 2003). Considering these data, it is possible that mu and kappa opioids play distinct, opposing roles in somatosensory modulation in which mu opioids decrease pain and kappa opioids decrease itch. Intriguingly, studies of the delta opioid receptor are now suggesting that delta agonists are likewise specific to certain modalities of somatosensation. In particular, the delta opioid receptor is specifically expressed in mechanically sensitive primary afferents, and delta agonists specifically reduce mechanical pain (Bardoni et al. 2014; Scherrer et al. 2009). Thus, mu agonists may target heat pain, delta agonists may reduce mechanical pain, and kappa agonists may selectively inhibit itch. The idea that distinct opioid subtypes may differentially modulate neural function is in keeping with what is observed in other regions of the nervous system where, for example, mu and kappa opioids have opposing effects on body-temperature regulation in the hypothalamus (Rawls and Benamar 2011) and on emotional state in the limbic system (Schlaepfer et al. 1998).

9 Inhibition of Itch by Counter Stimuli

Several studies in humans have reported that both noxious hot and cold can decrease reported itch sensation following the application of histamine to the skin (Ward et al. 1996; Yosipovitch et al. 2007). Previous work has shown that glycine and GABA activity within the dorsal horn is important for the decrease in pruritogen-evoked activity of neurons within the dorsal horn caused by counter stimuli (Akiyama et al. 2011). This finding suggests that inhibitory interneurons mediate the inhibition of itch by different sensory modalities such as scratching, noxious input, heat, and cool (Ma 2010; Patel and Dong 2010; Ross 2011). Since B5-I neurons function to inhibit itch, we reasoned that these neurons might be the neural substrate that mediates the inhibition of itch by counter stimuli. To test this idea, we performed a couple of different types of experiments to address whether B5-I neurons might be involved in this type of inhibition. Here we describe the electrophysiological and behavioral experiments that suggest that B5-I neurons may mediate the inhibition of itch by chemical counter stimuli.

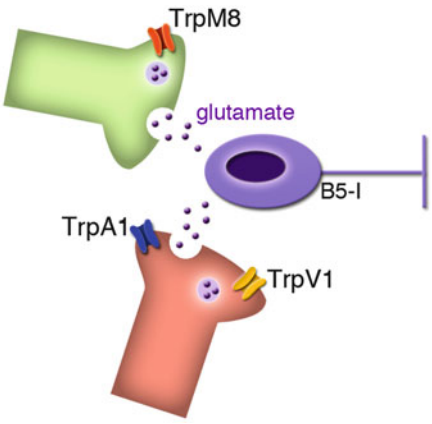

Fig. 4 TRPM8-, TRPA1-, and TRPV1-expressing sensory neurons directly target B5-I neurons. Depolarization of primary afferents by central application of menthol, mustard oil, or capsaicin causes glutamate release onto B5-I neurons

Chemicals such as capsaicin, mustard oil, and menthol are included in creams used to treat itch (Patel and Yosipovitch 2010). These chemicals are agonists for cation channels TRPV1, TRPA1, and TRPM8, respectively, which are expressed on primary afferent nerves. We therefore reasoned that if B5-I neurons mediate the inhibition of itch by these chemical counter stimuli, then B5-I neurons ought to receive input from these primary afferent fibers. To test this idea, we took advantage of the fact that application of these chemicals to an in vitro spinal cord preparation activates the primary afferent terminals, causing the release of glutamate and subsequent activation of postsynaptic neurons in the spinal cord. In order to determine if B5-I neurons receive input from these classes of primary afferents, electrophysiological recordings of B5-I neuron activity were performed during the application of each of these three chemicals. Consistent with our hypothesis, we observed a significant increase in the frequency of excitatory postsynaptic currents (EPSCs) following the application of each chemical (Kardon et al. 2014). Furthermore, this increase in EPSC frequency was maintained in the presence of tetrodotoxin (TTX), which blocks action potential propagation. These results suggest that B5-I neurons receive direct synaptic input from primary afferent terminals expressing TRPV1, TRPA1, and TRPM8 channels (Fig. 4).

To more rigorously test the idea that B5-I neurons mediate the inhibition of itch by counter stimuli, we developed a behavior model of the inhibition of itch by menthol. Prior studies in humans had shown that topical application of menthol reduces itch intensity, and menthol is commonly used in topical antipruritic treatments (Bromm et al. 1995; Patel et al. 2007). We found that, just as is observed in humans, topical application of an 8 % menthol-containing solution significantly reduced pruritogen-induced itch in mice. Next we reasoned that if B5-I neurons mediate the inhibition of itch by menthol, then menthol should not inhibit itch in mice that lack B5-I neurons. Consistent with this idea, we found that although menthol reduces itch in wild-type mice, it did not do so in *Bhlhb5*$^{-/-}$ mice, which lack B5-I neurons. These data suggest that B5-I neurons are necessary for mediating the inhibition of itch by the counter stimulus, menthol (Fig. 5).

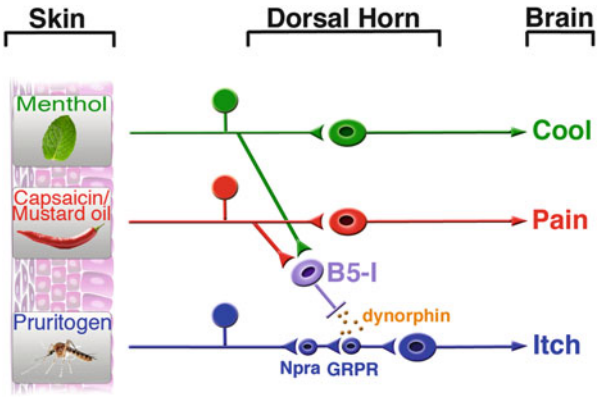

Fig. 5 B5-I neurons may mediate the inhibition of itch by the counter stimuli menthol, capsaicin, and mustard oil. Mice lacking B5-I neurons no longer exhibit the inhibition of itch by counter stimuli

While B5-I neurons seem selective for the inhibition of itch, it is possible that other inhibitory interneuron populations in the spinal cord are involved in modulating other somatosensory modalities. A recent paper suggests that a population of inhibitory interneurons mediates the heat inhibition of cold sensation (McCoy et al. 2013). They propose this model in light of the finding that ablating primary afferents containing CGRPα reduced heat and itch sensitivity but increased cold sensitivity. Although the identity of these interneurons remains to be determined, it is possible that these and other subsets of interneurons within the dorsal horn play similar roles as B5-I neurons but mediate different sensations.

10 Conclusions

Though the specific circuitry underlying the processing of itch sensation in the dorsal spinal cord remains largely uncharacterized, significant advances are being made, describing populations of interneurons and outlining their importance in somatosensory processing (Kardon et al. 2014; Mishra and Hoon 2013; Ross et al. 2010; Sun et al. 2009; Wang et al. 2013; Xu et al. 2013). Discovering the molecular identity of these neurons is important, as it gives us the opportunity to identify and manipulate these populations. Using molecular genetic tools combined with other techniques allows us to probe the circuitry underlying itch sensation. Further understanding of the properties and connections of neurons involved in itch sensation can potentially lead to the development of more effective pharmacological treatments for pruritic conditions.

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Noradrenergic Modulation of Itch Transmission in the Spinal Cord

Yasushi Kuraishi

Contents

Abstract

Inhibition of both itching and scratching is important in the treatment of chronic pruritic diseases, because itching has a negative impact on quality of life and vigorous scratching worsens skin conditions. Pharmacological modulation of itch transmission in the dorsal horn is an effective way to inhibit both itching and scratching in pruritic diseases. Pruriceptive transmission in the spinal dorsal horn undergoes inhibitory modulation by the descending noradrenergic system. The noradrenergic inhibition is mediated by excitatory α_1-adrenoceptors located on inhibitory interneurons and inhibitory α_2-adrenoceptors located on central terminals of primary sensory neurons. The descending noradrenergic system and α-adrenoceptors in the dorsal horn are potential targets for antipruritic drugs.

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Keywords
Alpha-adrenoceptor • Biting • Counterstimulus • Descending noradrenergic system • Dorsal horn • GABAergic interneuron • Glycinergic interneuron • Locus coeruleus • Noradrenaline • Scratching • Tonic inhibition

Abbreviations

GABA γ-Aminobutyric acid
mRNA Messenger ribonucleic acid

1 Pharmacological Significance of Itch Regulation in the Dorsal Horn

Itch is an annoying and impatient sensation that provokes a desire to scratch. Since chronic itching has a negative impact on quality of life and vigorous scratching worsens skin conditions, inhibition of both itching and scratching is important in the treatment of chronic pruritic diseases. Since scratching is not only induced by an itching sensation but also mediated by a spinal reflex (scratch reflex) (Sherrington 1906), inhibition of the production of the itch signal in the periphery is an ideal pharmacological intervention in the treatment of pruriceptive itching (Fig. 1). There are many endogenous itch mediators, and various itch mediators are involved in itching in pruritic diseases (Ständer et al. 2011; Kuraishi 2013). Therefore, selective antagonists and inhibitors that block itch signal production may not effectively relieve different types of pruritic disease. Recent studies show that a few groups of dorsal horn neurons selectively receive pruriceptive information from the skin (Sun et al. 2009; Mishra and Hoon 2013). Thus, it is expected that pharmacological modulation of itch transmission in the dorsal horn will be an effective way to inhibit both itching and scratching in pruritic diseases (Fig. 1). Itch perception is suppressed by various stimuli from the external environment. For example, itching is inhibited by counterstimuli, including noxious stimuli. Similar to nociceptive information, pruriceptive information that reaches the dorsal horn of the spinal cord from the skin is not automatically transferred to the brain. The dorsal horn of the spinal cord is a major site for modulation of nociceptive information. Supraspinal neurons with long descending axons are known to directly and indirectly modulate nociceptive transmission in the spinal dorsal horn via interneurons (Sandkühler 1996). In this chapter, I describe inhibition of itch sensation by exogenous sensory stimuli and noradrenergic inhibition of pruriceptive transmission in the spinal cord.

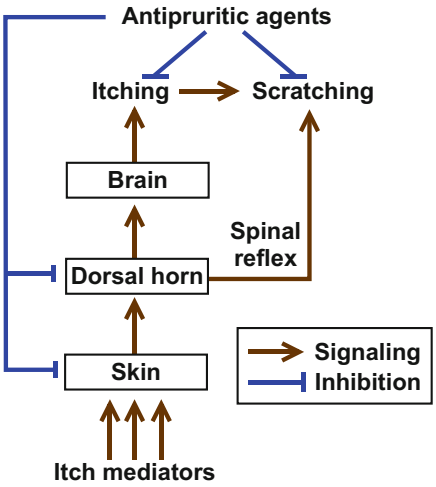

Fig. 1 Schematic representation of desirable sites of action of antipruritic agents. Since vigorous scratching worsens skin conditions, suppression of both itching and scratching is important in the treatment of chronic pruritic diseases. Scratching is not only induced by an itching sensation but also mediated by a spinal reflex. Therefore, in order to suppress itch-associated scratching, antipruritic agents should inhibit the generation of itch signals in the skin or the synaptic transmission of itch signals in the dorsal horn. Various itch mediators are involved in the generation of itch in the skin, whereas only a few groups of dorsal horn neurons selectively receive itch signals from the skin. Therefore, pharmacological modulation of itch transmission in the dorsal horn is an effective way to suppress both itching and scratching in pruritic diseases

2 Central Regulation of Itch Sensation

2.1 Counterirritants

Noxious counterstimuli applied to skin areas adjacent to the itch site suppress itch perception (Ward et al. 1996). The antipruritic state produced by brief noxious stimuli persists for longer than 30 min (Ward et al. 1996). Subpopulations of wide-dynamic-range (responsible for all somatosensory modalities and a wide range of intensity of peripheral stimulation) and nociceptive-specific neurons in the superficial dorsal horn receive itch signals from the skin (Andrew and Craig 2001; Jinks and Carstens 2002; Omori et al. 2009). Primate spinothalamic tract neurons and murine superficial dorsal horn neurons respond to an intradermal injection of histamine, a pruritogen, and these responses are inhibited by scratching of the receptive field, a counterstimulus (Davidson et al. 2009; Akiyama et al. 2012). In contrast, scratching does not inhibit the responses of these neurons to painful stimuli (Davidson et al. 2009; Akiyama et al. 2012). Scratching within an area 5–17 mm distant from the injection site inhibits the pruriceptive response, but

scratching of the injection site does not (Akiyama et al. 2012). Thus, scratching exerts site- and state-dependent inhibition of pruritogen-responsive spinal neurons.

Superficial dorsal horn neurons in mice with pruritic dry skin exhibit high levels of spontaneous firing that is inhibited by scratching, pinching, and noxious heat stimulation (Akiyama et al. 2011). Scratch-induced inhibition is suppressed by spinal administration of strychnine (a glycine receptor antagonist), bicuculline (a $GABA_A$ receptor antagonist), and saclofen (a $GABA_B$ receptor antagonist) (Akiyama et al. 2011). These findings suggest that glycinergic and GABAergic interneurons are involved in the inhibition of the pruriceptive responses of the dorsal horn neurons induced by noxious counterstimuli. In addition, scratch-induced inhibition is attenuated by cold block and transection of the upper spinal cord, suggesting that descending pathways from the brain are also involved in the inhibition of pruriceptive responses (Akiyama et al. 2011).

Lowering skin temperature from 33 °C to 30 °C by innocuous cooling reduces the intensity of histamine-induced itch; moreover, topical application of menthol reduces itch intensity, although skin temperature does not decrease (Bromm et al. 1995). These temporal reductions in itch may be mediated by activating receptor channels of the transient receptor potential family, particularly transient receptor potential M8 channel (Peier et al. 2002; Story et al. 2003). Inhibition of itch-related behaviors by menthol has been shown to be mediated by a subpopulation of inhibitory interneurons (B5-I neurons) in the dorsal horn (Kardon et al. 2014).

2.2 Distraction

Innocuous stimulation, such as vibration and warming, of skin areas adjacent to the itch site reduces histamine-induced itch (Ward et al. 1996). Vibration of the opposite side of the body also reduces itch (Melzack and Schecter 1965). In addition, these innocuous stimuli mildly reduce mustard oil-induced pain (Ward et al. 1996). These inhibitions do not persist for longer than 20 s when the counterstimuli are removed and may be because of distraction, a state in which attention is diverted from the central part of an experience (Ward et al. 1996). Distraction with noncontact stimulation also inhibits itching in humans (Leibovici et al. 2009) and scratching behaviors in mice (Tohda et al. 1997; Yamaguchi et al. 2001).

3 Noradrenergic Inhibition of Itch Transmission

3.1 Descending Noradrenergic System

Depletion of spinal noradrenaline by intrathecal injection of 6-hydroxydopamine, the catecholaminergic neurotoxin, increases itch-related behavior, including biting of the affected area of the hind paw (Hagiwara et al. 1999; Gotoh et al. 2011b;

LaMotte et al. 2011), induced by acute cutaneous allergy and intradermal pruritogen injection in mice (Gotoh et al. 2011b, c). The itch-related response is inversely correlated with noradrenaline content in the spinal cord in mice that are treated intrathecally with 6-hydroxydopamine (Gotoh et al. 2011b). Intrathecal injection of phentolamine, the nonselective α-adrenoceptor antagonist, also increases itch-related behavior induced by acute allergy and pruritogen injection (Gotoh et al. 2011b, c). These findings suggest that noradrenaline is an itch-inhibiting transmitter in the spinal cord and that itch signaling is under tonic inhibitory control of the descending noradrenergic system, which has been long known as a pain-inhibiting system. In contrast, depletion of spinal serotonin, another monoaminergic transmitter of the descending pain-inhibiting system, by intrathecal injection of 5,7-dihydroxytryptamine, the serotonergic neurotoxin, does not increase itch-related behavior induced by acute cutaneous allergy (Gotoh et al. 2011c) and pruritogen injection (unpublished observation).

In rats, wide-dynamic-range neurons in the superficial dorsal horn respond to intradermal histamine injection within the receptive field; however, these neurons respond to algogenic stimuli as well (Jinks and Carstens 2002). Electrical stimulation of the midbrain periaqueductal gray suppresses the responses of these neurons to histamine (Carstens 1997). Electrical stimulation of the midbrain periaqueductal gray also increases the release of noradrenaline in the spinal cord (Cui et al. 1999). Noradrenaline-containing neurons are not present in the midbrain periaqueductal gray, which densely innervates A6 (locus coeruleus) and A7 catecholamine cell groups (Bajic and Proudfit 1999). Electrical stimulation in the locus coeruleus and at sites near the A7 cell group inhibits nociception mediated by descending noradrenergic systems at the spinal cord level (Jones and Gebhart 1986; Yeomans et al. 1992). It is possible that these noradrenergic pain-inhibiting pathways are also involved in inhibiting itch signaling in the spinal cord (Fig. 2). Noradrenergic terminals have hardly any synaptic contacts with central terminals of primary sensory neurons and with dorsal horn neurons, and volume transmission (transmitter diffusion to the target cells in the extracellular space) has been suggested to have an important role in the noradrenergic modulation of neuronal activity (Hagihira et al. 1990; Rajaofetra et al. 1992). Therefore, itch and pain transmission may be modulated by common noradrenergic pathways. Neurons in the locus coeruleus exhibit spontaneous action potential discharge in vivo (Sugiyama et al. 2012), suggesting that noradrenaline is released spontaneously from the axon terminals of noradrenergic neurons in the locus coeruleus. This raises the possibility that noradrenergic neurons in the locus coeruleus play a role in the tonic inhibitory control of itch signaling in the spinal cord.

3.2 α_1-Adrenoceptors

Although pruritogen-induced itch-related behavior increases upon intrathecal injection of phentolamine, it is not affected by an intrathecal injection of the α_1-adrenoceptor antagonist, prazosin, or the α_2-adrenoceptor antagonist, yohimbine

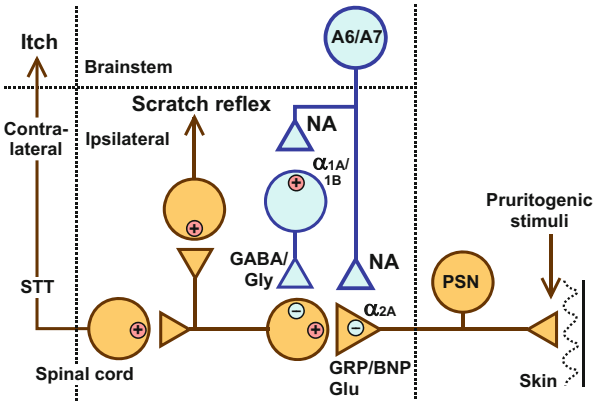

Fig. 2 Schematic illustration of the itch-signaling pathway and its inhibition by the descending noradrenergic system. Itch signals that originate in primary sensory neurons (PSN) are transmitted to interneurons in the dorsal horn by glutamate (Glu) and gastrin-releasing peptide (GRP)/B-type natriuretic peptide (BNP). Itch signals are further conveyed to the contralateral brain by spinothalamic tract (STT) neurons to form an itch sensation. Itch signals conveyed in the ipsilateral white column evoke the scratch reflex. The descending noradrenergic system that inhibits itch transmission in the dorsal horn probably originates from A6 (locus coeruleus) and A7 catecholamine cell groups. The antipruritic activity of noradrenaline (NA) may be mediated by inhibitory α_{2A}-adrenoceptors presynaptically located on the central terminals of primary afferents and excitatory α_{1A}- and α_{1B}-adrenoceptors postsynaptically located on the inhibitory interneurons, which use γ-aminobutyric acid (GABA) or glycine (Gly) as a neurotransmitter

(Gotoh et al. 2011b). Therefore, α_1- and α_2-adrenoceptors may play significant roles in noradrenergic inhibition in the spinal cord. Itch-related behavior induced by intradermal pruritogen injection is inhibited by intrathecal injections of the α_1-adrenoceptor agonist phenylephrine and the α_2-adrenoceptor agonist clonidine. In fact, phenylephrine and clonidine cause almost complete inhibition at doses that do not affect locomotor activity (Gotoh et al. 2011a, b). These findings support the idea that both α_1- and α_2-adrenoceptors are involved in inhibitory regulation of itch signaling in the spinal cord (Fig. 2). The antipruritic action of phenylephrine is antagonized by intrathecal treatment with prazosin, the α_1-adrenoceptor antagonist (Gotoh et al. 2011b), which has similar binding affinities for α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors (Bylund et al. 1994). However, it is not antagonized by intrathecal treatment with the α_1-adrenoceptor subtype-selective antagonists 5-methylurapidil, cyclazosin, and BMY 7378 (Gotoh et al. 2011b), which have relatively high binding affinities for α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors, respectively. α_{1A}- and α_{1B}-adrenoceptor mRNAs are expressed in the spinal dorsal horn and dorsal root ganglion; however, α_{1D}-adrenoceptor mRNA is not expressed in these regions in rodents (Nicholson et al. 2005; Gotoh et al. 2011b). Taken together, these findings suggest that α_{1A}- and α_{1B}-adrenoceptors are involved in the noradrenergic regulation of itch signaling (Fig. 2).

Noradrenaline and phenylephrine directly excite GABAergic and glycinergic inhibitory interneurons in the spinal lamina II (substantia gelatinosa) (Baba

et al. 2000a). Prazosin and the selective α_{1A}-adrenoceptor antagonist WB 4101 antagonize the excitatory action of phenylephrine (Baba et al. 2000b; Gassner et al. 2009). Such a mechanism is likely to be involved in α_1-adrenoceptor-mediated inhibition of itch signaling in the superficial dorsal horn (Fig. 2).

3.3 α_2-Adrenoceptors

There are three types of α_2-adrenoceptors: α_{2A}-, α_{2B}-, and α_{2C}-receptors. The antipruritic action of intrathecally administered clonidine is almost completely antagonized by intrathecal injection of yohimbine, the α_2-adrenoceptor antagonist (Gotoh et al. 2011a), which has similar binding affinities for α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors (Uhlen et al. 1994). However, it is not inhibited by prazosin (Gotoh et al. 2011a), the α_1-adrenoceptor antagonist with substantial binding affinities for α_{2B}- and α_{2C}-adrenoceptors (Bylund et al. 1994). Therefore, α_{2A}-adrenoceptors may play an important role in the antipruritic action of clonidine (Fig. 2). The α_{2A}-adrenoceptors also have a major role in the pain-suppressive effect induced by α_2-adrenergic agents (Pertovaara 2006). α_{2A}-Adrenoceptors are located presynaptically on the central terminals of capsaicin-sensitive sensory neurons (Stone et al. 1998). Neurons expressing the BB_2 bombesin receptor (a receptor for gastrin-releasing peptide) or the natriuretic peptide receptor A, on which B-type natriuretic peptide mainly acts in the dorsal horn, may play key roles in itch signaling in the superficial dorsal horn (Sun et al. 2009; Mishra and Hoon 2013). Primary afferent C-fiber-evoked responses of the superficial dorsal horn neurons that respond to gastrin-releasing peptide are blocked by antagonists of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate glutamate receptors, suggesting that glutamate plays a major role in itch signaling (Koga et al. 2011). Capsaicin-induced glutamate release from the dorsal horn in vitro is suppressed by clonidine, and this effect is antagonized by yohimbine (Ueda et al. 1995). Inhibition of the release of transmitter(s) from the central terminals of primary afferents may be a mechanism underlying α_2-adrenoceptor-mediated noradrenergic inhibition of itch signaling in the dorsal horn (Fig. 2).

4 Systemic Pharmacological Agents

4.1 α-Adrenoceptor Agonists

Intraperitoneal administration of clonidine suppresses pruritogen-induced itch-related behavior: doses of 1–10 μg/kg produce dose-dependent inhibition with nearly complete inhibition at 10 μg/kg (Gotoh et al. 2011a). Intraperitoneal clonidine injection at a dose of 100 μg/kg causes almost complete inhibition of acetic acid-induced pain-related behaviors in mice, whereas 10 μg/kg causes partial inhibition (Bezerra et al. 2008). Thus, antipruritic doses of clonidine are lower than antinociceptive doses. Itch-related behavior is inhibited by intrathecal but not

intraplantar nor intracisternal clonidine injections, and the antipruritic effect of intraperitoneal clonidine is almost completely reversed by intrathecal yohimbine (Gotoh et al. 2011a). It is suggested that the antipruritic effect of clonidine is predominantly mediated by α_2-adrenoceptor stimulation in the dorsal horn.

4.2 Noradrenaline Reuptake Inhibitors

Tonic inhibition of spinal itch processing by the descending noradrenergic system raises the possibility that antidepressants that block noradrenaline reuptake may exert inhibitory effects against cutaneous pruritus. As expected, pruritogen-induced itch-related behavior in mice is suppressed by intrathecal injection of milnacipran, a serotonin–noradrenaline reuptake inhibitor. In contrast, it is not inhibited by intrathecal injection of fluvoxamine, a selective serotonin reuptake inhibitor (Andoh et al. 2013). Intraperitoneal administration of milnacipran also inhibits itch-related behavior, which is almost completely reversed by intrathecal injection of phentolamine (Andoh et al. 2013). Thus, agents that inhibit noradrenaline reuptake represent potential spinal-acting antipruritic drugs.

4.3 Other Potential Drugs

There are some drugs that have been shown to produce analgesic and/or antiallodynic effects mediated by the descending noradrenergic system. These include gabapentin/pregabalin (Tanabe et al. 2005; Takeuchi et al. 2007; Kitamura et al. 2014) and tramadol (Ide et al. 2006; Kimura et al. 2012). Gabapentin acts presynaptically on GABAergic nerve terminals in the locus coeruleus in vitro (Takasu et al. 2008). Since systemic injections of gabapentin and pregabalin decrease the release of GABA in the locus coeruleus but not in the spinal cord (Yoshizumi et al. 2012), local disinhibition of noradrenergic neurons in the locus coeruleus may increase the release of noradrenaline in the dorsal horn. Gabapentin and pregabalin inhibit itch-related behaviors in mice with allergic contact dermatitis (Tsukumo et al. 2011). It has been suggested that in this murine model, gabapentin exerts antipruritic effects through action on the $\alpha_2\delta$-1 subunit of the voltage-dependent Ca^{2+} channel upregulated in primary sensory neurons (Tsukumo et al. 2011). However, it is possible that the antipruritic activity of systemically administered gabapentin also involves the descending noradrenergic system. Tramadol inhibits noradrenaline uptake into brain synaptosomes (Raffa et al. 1992), and its metabolite, mono-*O*-desmethyl-tramadol, exhibits μ-opioid agonistic activity (Gillen et al. 2000). Collaboration between these actions may enhance the function of the descending noradrenergic system (Ide et al. 2006). The descending noradrenergic system and α-adrenoceptors in the dorsal horn are potential targets for antipruritic drugs.

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Protease-Activated Receptors and Itch

Tasuku Akiyama, Ethan A. Lerner, and E. Carstens

Contents

Abstract

Protease-activated receptors (PARs) have been implicated in a variety of physiological functions, as well as somatosensation and particularly itch and pain. Considerable attention has focused on PARs following the finding they are upregulated in the skin of atopic dermatitis patients. The present review focuses on recent studies showing that PARs are critically involved in itch and sensitization of itch. PARs are expressed by diverse cell types including primary sensory neurons, keratinocytes, and immune cells and are activated by proteases that expose a tethered ligand. Endogenous proteases are also released from diverse

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cell types including keratinocytes and immune cells. Exogenous proteases released from certain plants and insects contacting the skin can also induce itch. Increased levels of proteases in the skin contribute to inflammation that is often accompanied by chronic itch which is not predominantly mediated by histamine. The neural pathway signaling itch induced by activation of PARs is distinct from that mediating histamine-induced itch. In addition, there is evidence that PARs play an important role in sensitization of itch signaling under conditions of chronic itch. These recent findings suggest that PARs and other molecules involved in the itch-signaling pathway are good targets to develop novel treatments for most types of chronic itch that are poorly treated with antihistamines.

Keywords
Brain • GPCR • Human • Mouse • Pain • Pruritus • Rat • Scratching • Spinal cord

Abbreviations

ACC	Anterior cingulate cortex
AYPGKF	Ala-Tyr-Pro-Gly-Lys-Phe-NH_2
BAM8-22	Bovine adrenal medulla 8-22
KLK	Kallikrein
Mrgpr	Mas-related G-protein-coupled receptors
NGF	Nerve growth factor
PARs	Protease-activated receptors
PCC	Posterior cingulate cortex
PLC	Phospholipase C
SLIGRL	Ser-Leu-Ile-Gly-Arg-Leu-NH_2
spink5	*serine protease inhibitor Kazal type 5*

1 Protease-Activated Receptors and Itch

Protease-activated receptors (PARs) are unique among G-protein-coupled receptors. This uniqueness stems from the fact that PARs are activated following protease cleavage of part of the extracellular domain. Cleavage exposes a new amino terminus which acts as a ligand to activate PARs. PARs have various physiological and pathophysiological roles in cardiovascular and respiratory systems, nervous systems, the gastrointestinal tract, musculoskeletal systems, renal systems, embryogenesis, and cancer (Adams et al. 2011). In the skin, physiological functions of PARs include skin barrier homeostasis, inflammation, as well as itch and pain (Lee et al. 2010; Vellani et al. 2010; Adams et al. 2011; Akiyama

and Carstens 2013; Kempkes et al. 2014). This review will highlight the role of PARs in itch and sensitization of itch.

PARs consist of four members: PAR-1, PAR-2, PAR-3, and PAR-4. PARs other than PAR-3 are likely involved in acute itch. Hexapeptide agonists derived from the tethered ligand sequences, including TFLLR (PAR-1), SLIGRL (PAR-2), and AYPGKF (PAR-4), are known to elicit scratching in mice, but SFNGGP (PAR-3) failed to elicit scratching (Tsujii et al. 2008; Akiyama et al. 2009a, b, c, 2010b, 2012a). Although proteases efficiently lead to PAR activation, the hexapeptides are weak pruritogens in mice, being active at micromolar levels. This weakness may explain why neither SLIGRL nor AYPGKF elicits scratching in Sprague-Dawley rats (Klein et al. 2011). The activity of PARs is controlled by activating and deactivating proteases (Adams et al. 2011). The proteases originate from endogenous sources including keratinocytes, mast cells, macrophages, dendritic cells, B cells, T cells, and neutrophils, as well as from external sources including mites, fungi, cockroaches, bacteria, and plants (Shpacovitch et al. 2007; Lee et al. 2010; Reddy and Lerner 2010; Meyer-Hoffert 2012; Page 2012) (Fig. 1). As proteases activate PARs and injection of proteases into human skin can cause itch, it is possible that tryptase and chymase, serine proteases released from mast cells, contribute to itch (Hägermark et al. 1972; Kivinen et al. 2001; Steinhoff et al. 2003; Moormann et al. 2006; Sharma et al. 2007; Groschwitz et al. 2013). Intradermal injection of tryptase elicits scratching in mice (Ui et al. 2006). Several kallikreins, also serine proteases, including kallikrein (KLK) 1, 4, 5, 6, 7, 8, 9, 10, 11, 13, and 14, are expressed in the stratum corneum of the epidermis (Komatsu et al. 2003, 2005). KLK5 and KLK14, but not KLK7 or KLK8, act on PAR-2, while KLK1 acts on PAR-1 (Stefansson et al. 2008; Gao et al. 2010). Intradermal injection of KLK elicits itch in humans (Hägermark 1974). Cathepsin S, a cysteine

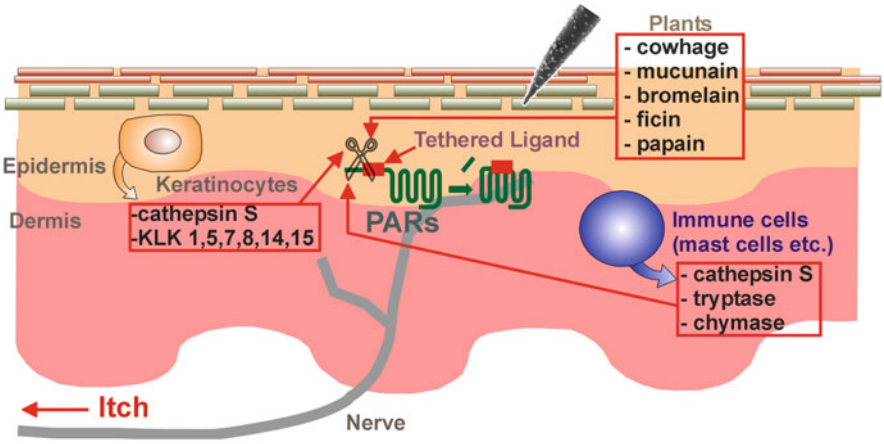

Fig. 1 Schematic diagram of origins of PAR ligands in the skin. Figure shows cross section through the skin. The proteases originate from endogenous sources including keratinocytes and immune cells as well as from external sources, such as plants

protease, is expressed in antigen-presenting cells, including B cells, macrophages, and dendritic cells as well as keratinocytes (Schwarz et al. 2002; Lutzner and Kalbacher 2008). Cathepsin S delivered by inactivated spicules of cowhage elicited itch through PAR-2 and PAR-4 (Reddy et al. 2010). Cowhage spicules, which constitute itch powder, contain the active component mucunain, a cysteine protease that acts at PAR-2 and PAR-4 to produce itch (Reddy et al. 2008). Other plant cysteine proteases activate PAR-2 and PAR-4 to produce itch, such as bromelain (pineapple stem), ficin (fig tree latex), and papain (papaya) (Reddy and Lerner 2010).

Whether the activation of PAR-2 contributes to initiating itch in mice remains controversial. SLIGRL- and trypsin-evoked scratching remained in PAR-2 knockout mice (Liu et al. 2011). In contrast, tryptase-evoked scratching was inhibited by genetic knockout of PAR-2 as well as pharmacological blockade of PAR-2 (Ui et al. 2006). Different types of agonists (e.g., SLIGRL, tryptase, and trypsin) may activate different signaling pathways. Alternatively, roles of PAR-2 may be different between neurons and keratinocytes.

PARs are involved in pathological conditions accompanied by chronic itch. The number of tryptase-containing mast cells in the upper dermis is increased in atopic dermatitis as well as psoriatic skin (Harvima et al. 1990; Jarvikallio et al. 1997). Mutations in the *serine protease inhibitor Kazal type 5* (*spink5*) gene, which encodes the protease inhibitor lymphoepithelial Kazal-type-related inhibitor, result in upregulation of KLK5 activity which is involved in the formation of atopic dermatitis-like skin lesions via PAR-2 (Briot et al. 2009) (Table 1). Consistent with this, transgenic KLK5 overexpressor mice displayed signs of severe inflammation and pruritus (Furio et al. 2014). Cathepsin S is mainly present in the dermis of normal human skin. In contrast, cathepsin S is upregulated and detected in keratinocytes in psoriatic skin (Schonefuss et al. 2010). Overexpression of cathepsin S induces atopic dermatitis-like skin through an increase in PAR-2 expression in dendritic cells (Kim et al. 2012). Transgenic expression of the serine protease channel-activating protease-1 in the skin induces atopic dermatitis-like skin accompanied by increased spontaneous scratching (Frateschi et al. 2011). These phenotypes are completely negated when superimposed on a PAR-2 null background. In the skin of NC mice, an animal model of atopic dermatitis, the activity of

Table 1 Protease-related mutant mice with atopic dermatitis-like skin

Mutations	Upregulated proteases	Subtype of PARs	References
Serine protease inhibitor Kazal type 5	KLK5	PAR-2	Briot et al. (2009)
KLK5	KLK5	Not tested	Furio et al. (2014)
Cathepsin S	Cathepsin S	PAR-2	Kim et al. (2012)
Serine protease channel-activating protease-1	Serine protease channel-activating protease-1	PAR-2	Frateschi et al. (2011)

serine proteases as well as the number of PAR-2-positive keratinocytes is increased (Tsujii et al. 2009). These findings imply that PARs play an important role in chronic itch under pathological conditions.

2 Mechanisms of Protease-Activated Receptor-Mediated Itch

PARs are expressed broadly in neuronal as well as nonneuronal cells (Shpacovitch et al. 2002, 2007; Steinhoff et al. 2003; Zhu et al. 2005; Moormann et al. 2006; Vellani et al. 2010). The activation of PAR-2 on keratinocytes induces the release of LTB4 (Zhu et al. 2009b), which elicits itch through BLT1-expressing neurons (Andoh and Kuraishi 1998, 2005). Activation of PAR-2 in keratinocytes also induces release of thymic stromal lymphopoietin, a pruritogenic cytokine that excites TRPA1-expressing sensory neurons (Wilson et al. 2013). PARs are expressed by a variety of different immune cells, including neutrophils, eosinophils, monocytes, macrophages, and mast cells (Shpacovitch et al. 2007). In particular, PAR-1 is expressed in mast cells. Considering the partial inhibition of the PAR-1 agonist-evoked scratching by an H1 histamine receptor antagonist (Tsujii et al. 2008), PAR-1 agonist-evoked itch can be partially attributed to histamine released from mast cells. Although PAR-2 and PAR-4 are expressed by mast cells, an intradermal injection of PAR-2 or PAR-4 agonist presumably does not activate these receptors expressed by mast cells, since H1 histamine receptor antagonists failed to inhibit scratching evoked by the PAR agonists (Tsujii et al. 2008; Akiyama et al. 2012a). In addition to mast cells, other immune cells may be activated through PARs under pathophysiological conditions to release certain pruritogens. PAR-1, PAR-2, and PAR-4 have been found to be expressed in primary sensory neurons (Steinhoff et al. 2003; Zhu et al. 2005; Vellani et al. 2010). Proteases such as trypsin and thrombin and hexapeptide ligands of PAR-1, PAR-2, and PAR-4 can activate primary sensory neurons (Amadesi et al. 2004; Akiyama et al. 2010a; Vellani et al. 2010). Direct activation of primary sensory neurons through these receptors might contribute to itch. A PAR-2 agonist and either a PAR-1 or PAR-4 agonist apparently activate different subpopulations of primary sensory neurons (Vellani et al. 2010).

The neuronal pathway for PAR-mediated itch has been studied mainly using cowhage and PAR-2 tethered ligands such as SLIGRL. Itch elicited by intradermal insertion of cowhage spicules is considered to be non-histaminergic based on the following observations. (1) In contrast to histamine, cowhage spicules elicit itch without accompanying flare (Johanek et al. 2007; Sikand et al. 2009). (2) While histamine-elicited itch is described as mosquito bite-like, cowhage-elicited itch is described as stinging, sharp, and prickly (Kosteletzky et al. 2009). (3) Desensitization of the skin with topical capsaicin abolished cowhage-induced itch but not histamine-induced itch (Johanek et al. 2007). (4) Cowhage-evoked itch was not inhibited by pretreatment with an H1 histamine receptor antagonist (Johanek et al. 2007). Itch elicited by cowhage spicules is apparently mediated by

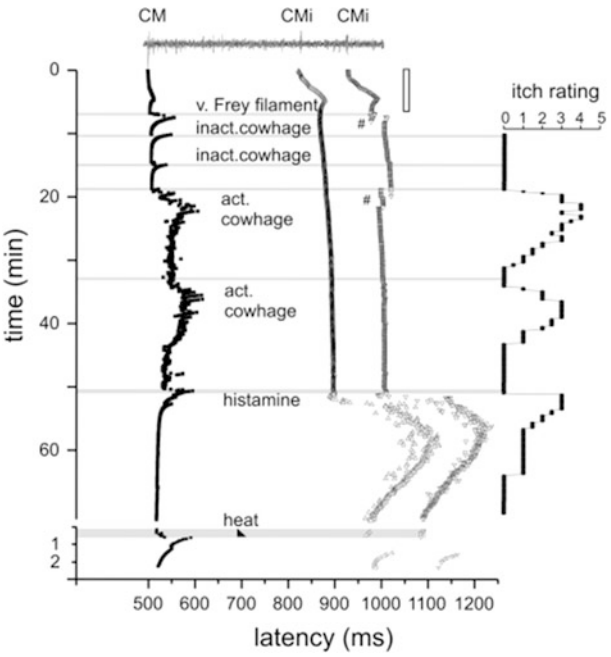

Fig. 2 Specimen of a multifiber recording from 1 mechano-responsive (CM) and 2 mechano-insensitive nociceptors (CMi). A trace of the raw signal containing the C-fiber action potentials is shown on *top*. Conduction latencies of these three marked fibers (*filled square*, *open triangle*) in response to successive electrical stimulation at the receptive field are plotted from top to bottom. *Top traces* were recorded during stimulation with increasing frequencies (see *open square* on *right side*), followed by traces recorded during stimulation with mechanical stimuli (v. Frey filament), inactive (inact.) and active (act.). Adapted from Namer et al. (2008)

populations of C- as well as Aδ-fibers that are distinct from those mediating histamine-elicited itch. Mechano-insensitive C-fibers preferentially respond to histamine but not cowhage (Schmelz et al. 1997; Namer et al. 2008) (Fig. 2). In contrast, mechanosensitive, polymodal C-fibers readily responded to cowhage with lesser or no responses to histamine (Johanek et al. 2008; Namer et al. 2008). Mechanosensitive A-fibers also responded more vigorously to cowhage than to histamine, but some exclusively responded to histamine (Ringkamp et al. 2011). At the level of the spinal cord, cowhage and histamine activated separate subpopulations of primate spinothalamic tract neurons (Davidson et al. 2007, 2012). In the mouse, a majority of spinal neurons were activated by both SLIGRL and histamine (Akiyama et al. 2009a, b). This difference between murine and primate spinal neurons might be due to the agonistic activity of SLIGRL on Mas-related G-protein-coupled receptors C11 (MrgprC11) (Liu et al. 2011). In the brain, cowhage and histamine activated largely overlapping areas including thalamus, primary and secondary somatosensory cortices, posterior parietal cortex, superior and middle temporal cortices, PCC, ACC, precuneus, and cuneus.

However, some areas exhibited more extensive activation by cowhage, including the insular cortex, claustrum, basal ganglia, putamen, thalamic nuclei, and pulvinar (Papoiu et al. 2012).

The downstream signal transduction molecules involved in PAR-mediated itch have not yet been specified. Histaminergic itch requires TRPV1, whereas non-histaminergic itch via MrgprA3 and MrgprC11 agonists requires TRPA1 (Shim et al. 2007; Imamachi et al. 2009; Wilson et al. 2011). PAR-1-mediated itch may require TRPV1, but it is not currently known if itch mediated by PAR-2 and PAR-4 requires TRPV1 or TRPA1. Scratching evoked by trypsin, which acts at PAR-1, PAR-2, and PAR-4, was reduced in knockout mice lacking TRPV1 (Costa et al. 2008). Trypsin-evoked scratching was inhibited by an H1 histamine receptor antagonist as well as depletion of mast cells by repeated treatment with compound 48/80 (Costa et al. 2008), suggesting that mast cells play a major role in itch evoked by trypsin. Knockout mice lacking PAR-2 exhibited greater scratching compared to wild types (Liu et al. 2011), implying that PAR-2 is not involved in trypsin-evoked scratching. Considering that PAR-1 agonist-evoked scratching was partially inhibited by the H1 histamine antagonist, trypsin-evoked scratching is presumably mediated by PAR-1. It would be interesting to know whether scratching evoked by the PAR-2 and PAR-4 agonists requires TRPV1 or TRPA1. Phospholipase C (PLC) plays a key role in intracellular signaling by G-protein-coupled receptors. While PLCβ3 contributes to itch evoked by histamine and 5-HT, PLCβ3 does not appear to be involved in SLIGRL-evoked itch (Imamachi et al. 2009). Pirt (phosphoinositide-interacting protein) binds to phosphatidylinositol (4,5)-bisphosphate, TRPV1, and other ion channels to potentiate them. Knockout mice lacking Pirt exhibited a significant loss of scratching evoked by histamine, 5-HT, endothelin-1, and the MrgprA3 agonist chloroquine (Patel et al. 2011). On the other hand, SLIGRL-evoked scratching was not significantly inhibited in Pirt knockout mice.

3 Itch Sensitization via Protease-Activated Receptors

PARs are involved in sensitization of itch, with the features of spontaneous itch, hyperknesis (enhanced itch to a normally itchy stimulus), and alloknesis (itch elicited by an innocuous touch stimulus). These manifestations of itch sensitization are observed in patients suffering from chronic itch (Schmelz et al. 2003; Ikoma et al. 2004; Hosogi et al. 2006). In mice, hyperknesis has been demonstrated in a model of chronic dry skin itch. We reported that mice treated with drying agents exhibited significantly greater scratching following intradermal injections of 5-HT and SLIGRL delivered in the dry skin treatment area compared to control (water-) treated mice (Akiyama et al. 2010a). In contrast, histamine-evoked scratching was not significantly enhanced in dry skin-treated mice. DRG cells taken from the dry skin-treated mice exhibited significantly greater responses to 5-HT and SLIGRL, but not histamine, consistent with the behavioral results (Fig. 3). Superficial dorsal horn neurons receiving afferent input from a dry skin-treated hind paw exhibited significantly enhanced responses to SLIGRL, but not histamine, compared to units recorded in control animals (Akiyama et al. 2011). These findings suggest that the

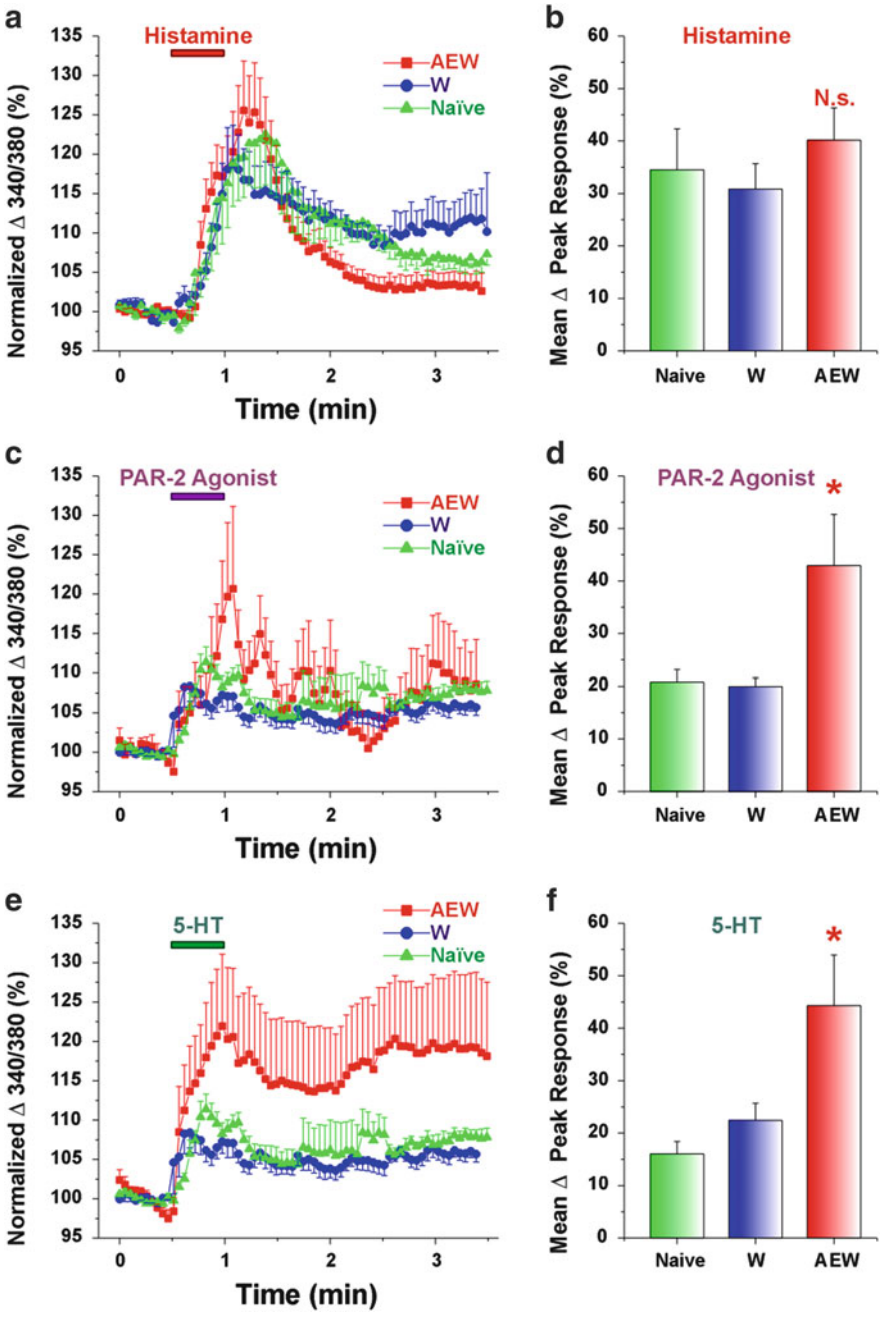

Fig. 3 DRG cells from AEW-treated mice show enhanced responses to PAR-2 agonist and 5-HT but not histamine. (**a**) Histamine. Mean normalized ratiometric responses of DRG cells from each treatment group vs. time relative to histamine perfusion (*red bar*). *Error bars*: SEM. (**b**) Mean

enhanced response to SLIGRL may be attributed to peripheral sensitization of pruriceptors projecting to the recorded dorsal horn neurons. NGF might account for this peripheral sensitization. In dry skin, NGF levels are elevated and might contribute to peripheral sensitization of pruriceptors (Tominaga et al. 2007). Intradermally administered NGF enhanced itch induced by cowhage but not histamine in humans (Rukwied et al. 2013) (Fig. 4). PAR-2 may be sensitized in the skin in which NGF levels are elevated under chronic itch conditions. Moreover, not only may pruriceptors expressing PARs become sensitized, but PARs may also contribute to sensitization of non-histaminergic pruriceptors expressing transduction molecules such as MrgprA3 and MrgprC11. Pretreatment with SLIGRL, but not BAM8-22, resulted in an enhancement of responses of primary sensory neurons to MrgprA3 and MrgprC11 agonists, as well as enhanced scratching evoked by the MrgprA3 and MrgprC11 agonists (Akiyama et al. 2012b). Pretreatment with SLIGRL failed to enhance scratching evoked by histamine, 5-HT, the PAR-4 agonist, or SLIGRL (Akiyama et al. 2009c).

Certain PARs are also involved in alloknesis, the phenomenon in which itch is induced by low-threshold mechanical stimulation of skin surrounding the site of the pruritic stimulus. Cowhage spicule-elicited itch is accompanied by alloknesis (Sikand et al. 2009), implying the involvement of PAR-2 and/or PAR-4 since they are activated by mucunain in cowhage (Reddy et al. 2008). Alloknesis is a common and often distressing symptom for many patients suffering from chronic itch. The neural mechanisms underlying alloknesis are poorly understood, partly due to a lack of animal models for alloknesis. To begin to investigate alloknesis, we recently developed an animal model (Akiyama et al. 2012a). C57BL/6 mice do not normally respond to innocuous mechanical stimulation of the rostral back. However, following intradermal injection of histamine and certain other pruritogens, low-threshold mechanical stimuli delivered to the skin around the injection site reliably elicited discrete hind limb scratch bouts directed to the stimulus. The time course of touch-evoked scratching had a slower onset and longer duration compared to the pruritogen-evoked scratching that usually ceased within 30 min. Touch-evoked scratching was observed following histamine, 5-HT, the PAR-4 agonist, and BAM8-22 but not SLIGRL or chloroquine (Fig. 5). Considering that alloknesis was evoked by the PAR-4 agonist but not SLIGRL, alloknesis evoked by cowhage is presumably mediated by PAR-4.

Fig. 3 (continued) peak response (% change from baseline) of DRG cells to histamine for each treatment group. N.s.: no significant difference compared to W. (**c**, **e**) as in (**a**) for PAR-2 agonist and 5-HT, respectively. (**d**, **f**) as in (**b**) for PAR-2 agonist and 5-HT, respectively. *Asterisk*, significantly different compared to W ($p < 0.05$, unpaired *t*-test). ($n = 14$–23/group). Naïve data from cervical DRG cells ($n = 719$) obtained from untreated mice. Adapted from Akiyama et al. (2010a)

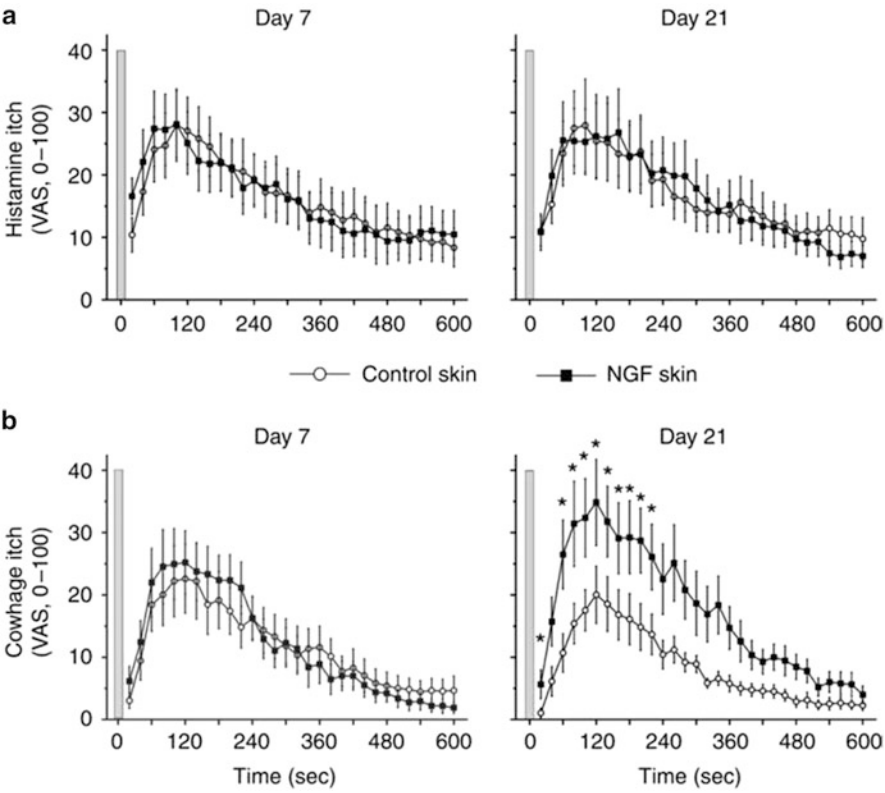

Fig. 4 Nerve growth factor (NGF) sensitizes cowhage- but not histamine-induced itch. Itch sensation recorded upon (**a**) histamine iontophoresis and (**b**) cowhage spicule insertion at day 7 (*left panel*) and day 21 (*right panel*) after administration of 1 μg NGF ($n = 12$). In comparison with control skin, cowhage-induced itch was perceived significantly stronger at the NGF-treated sites at day 21 (marked by *asterisks*, Wilcoxon test, $P < 0.05$). *Gray bars* indicate the time point of histamine iontophoresis and gray cowhage application. *Error bars* indicate SEM. VAS, visual analog scale. Adapted from Rukwied et al. (2013)

4 Protease-Activated Receptors and Pain

PARs have been shown to play a role in modulating nociception. PAR-2 is expressed in nociceptive primary sensory neurons, and its activation leads to hyperalgesia (Amadesi et al. 2004; Dai et al. 2004, 2007; Wang et al. 2012). PAR-2 is involved in joint, visceral, and somatic pain through the sensitization of TRPV1, TRPA1, TRPV4, and P2X3 (Amadesi et al. 2004; Dai et al. 2004, 2007; Sipe et al. 2008; Helyes et al. 2010; Lam et al. 2012; Wang et al. 2012; Poole et al. 2013). In contrast, PAR-1 and PAR-4 agonists exert antinociceptive effects such as increased thermal and mechanical nociceptive withdrawal thresholds

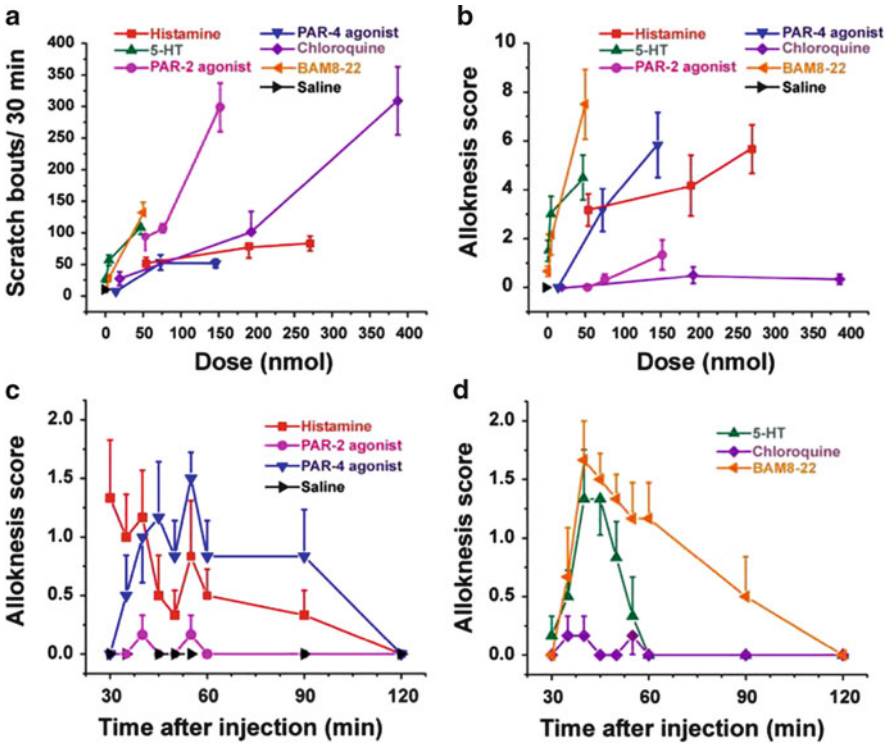

Fig. 5 Scratching and alloknesis elicited by different pruritogens. (**a**) Dose-response curve for scratch bouts (assessed over 30 min) elicited by intradermal injection of pruritogens indicated in each figure. *Error bars*: SEM ($n = 6$/group). (**b**) Dose-response curve for alloknesis score elicited by the same pruritogens. (**c**) Time course of alloknesis for histamine (271 nmol/10 μl), PAR-2 agonist SLIGRL-NH_2 (76 nmol/10 μl), PAR-4 agonist AYPGKF-NH2 (146 nmol/10 μl), and saline vehicle. (**d**) Time course of alloknesis for 5-HT (47 nmol/10 μl), chloroquine (193 nmol/10 μl), and BAM8-22 (50 nmol/10 μl). Adapted from Akiyama et al. (2012a)

(Asfaha et al. 2002, 2007; Auge et al. 2009; Karanjia et al. 2009; Annahazi et al. 2012) with one exception (McDougall et al. 2009). High doses of PAR agonists can induce inflammation, presumably explaining the pro-nociceptive action of PAR-4 agonists in the study of joint pain (McDougall et al. 2009).

5 Protease-Activated Receptors as Target Molecules for Future Treatments of Chronic Itch

The studies discussed above provide strong evidence for the participation of PARs in acute itch as well as itch sensitization. Thus, the clinical treatment of chronic itch is likely to benefit from the development of drugs directed at PARs. There are two strategies to block PAR-mediated itch signaling: (1) inhibit the protease agonists of

PARs and (2) antagonize PARs. Nafamostat mesilate, a serine protease inhibitor, inhibited scratching evoked by tryptase and compound 48/80 as well as spontaneous scratching in NC mice exhibiting atopic dermatitis-like skin lesions (Ui et al. 2006; Tsujii et al. 2009). Leupeptin, a protease inhibitor, inhibited scratching evoked by compound 48/80 as well as passive cutaneous anaphylaxis in mice (Ui et al. 2006; Zhu et al. 2009a). The chymase inhibitor SUN13834 inhibited spontaneous scratching in a mouse dermatitis model induced by repeated treatments of hapten (Terakawa et al. 2008). Similar to the serine protease inhibitors, PAR-2 antagonists have been used successfully to inhibit itch-related behavior in mice. Scratching evoked by tryptase and compound 48/80 was inhibited by the PAR-2 antagonist FSLLRY, as well as by an anti-PAR-2 antibody (Ui et al. 2006). Spontaneous scratching in dry skin-treated mice, as well as in NC mice, was inhibited by an anti-PAR-2 antibody (Tsujii et al. 2009; Akiyama et al. 2010a). FK506 is a drug that provides temporary itch relief in atopic dermatitis. Its antipruritic effect might be due to inhibition of PAR-2-mediated signaling (Nakano et al. 2008). Overall, PARs are attractive targets for the development of treatments for itch. PAR-2 antagonists, such as the novel low molecular weight, non-peptide PAR-2 antagonist GB88, have been developed recently. Their antipruritic effects await future testing (Suen et al. 2012). Further studies will reveal the detailed mechanisms underlying the role of PARs in acute and, especially, chronic itch.

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NK-1 Antagonists and Itch

Sonja Ständer and Thomas A. Luger

Contents

Abstract

Substance P (SP) is an important mediator of pro-inflammatory mechanisms in the skin. It targets multiple cells such as keratinocytes, mast cells, and fibroblasts which are involved in the cutaneous generation of pruritus. This suggests that SP is an interesting target for therapy. In fact, in recent case reports and case series, SP antagonists demonstrated a significant antipruritic effect in acute and chronic pruritus such as drug-induced pruritus, paraneoplastic pruritus, prurigo nodularis, cutaneous T-cell lymphoma, and brachioradial pruritus.

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Abbreviations

ACE	Angiotensin-converting enzyme
AD	Atopic dermatitis
CGRP	Calcitonin gene-related peptide
CMH fibers	Mechano- and heat-sensitive C-nerve fibers
CMi fibers	Mechano-insensitive C-nerve fibers
CNS	Central nervous system
CP	Chronic pruritus
CRH	Corticotropin-releasing hormone
CRHR-1	Corticotropin-releasing hormone receptor-1
CRPS	Complex regional pain syndrome
DNFB	Dinitrofluorobenzene
H1	Histamine-1 receptor
IL	Interleukin
MC	Mast cell
NEP	Neutral endopeptidase
NGF	Nerve growth factor
NK1R	Neurokinin-1 receptor
NK2R	Neurokinin-2 receptor
NK3R	Neurokinin-3 receptor
NKA	Neurokinin A
NKB	Neurokinin B
NKγ	Neuropeptide-γ
NPK	Neuropeptide K
PN	Prurigo nodularis
PPTA	Preprotachykinin-A
SP	Substance P
TNF	Tumor necrosis factor
TRPV1	Transient receptor potential vanilloid subtype 1
VEGF	Vascular endothelial growth factor

Chronic pruritus (>6 weeks of duration) is a frequent symptom of dermatological, systemic and neurological diseases (Ständer et al. 2007). Given that up to 23 % of all adults in different populations suffer from the symptom (Matterne et al. 2011), chronic pruritus (CP) represents a burden on patients worldwide (Weisshaar and Dalgard 2009). Left untreated, CP may lead to severe cutaneous scratch lesions,

impaired quality of life, and psychogenic reactions. Though medical care of patients with CP has improved during the past few years by establishment of specialized itch clinics, a classification system, and publication of guidelines (Ständer et al. 2007, 2012a, b), the currently available therapy modalities are not sufficiently efficacious in the majority of patients with pruritus (Ständer and Weisshaar 2012). The current guidelines suggest the use of antihistamines, pain modulators, opioid antagonists, and antidepressants in treating CP (Ständer et al. 2012b; Weisshaar et al. 2012). A considerable number of patients experience relief from these drugs but mostly no complete resolution is achieved (Siepmann et al. 2011). In addition many of the recommended therapies carry a large number of adverse effects and cannot be used on a long-term basis. For example, gabapentin has a negative impact on the excretory function of the kidney potentially leading to chronic renal insufficiency; the antidepressant amitriptyline may lead to electrocardiogram changes and impair the heart function. Thus, therapy of patients with CP still remains challenging and new therapies are urgently needed. We (Pogatzki-Zahn et al. 2008) and others (Kumagai et al. 2010) have pursued the concept to develop target-specific treatments targeting pathophysiological mechanisms specific for pruritus. Among this, substance P (SP) is a very interesting candidate. SP is an important mediator in the induction and maintenance of pruritus in the central nervous system (CNS) and skin; recent case reports and case series suggest that targeting the SP receptor (neurokinin-1 receptor; NK1R) represents an interesting target for an antipruritic treatment.

1 Substance P and Neurokinin Receptors

1.1 Cutaneous Transmission of Itch

The neuroimmunological peripheral and central mechanisms underlying CP are not yet fully elucidated (Ikoma et al. 2006). In the skin, a complex crosstalk of keratinocytes, mast cells (MC), transient inflammatory cells, fibroblasts, macrophages, and sensory neurons underlies the induction and chronification of the symptom. Concerning the involved sensory nerves, histamine-selective, mechano-insensitive C-fibers (CMi fibers), which are known for their relevance for capsaicin-induced hyperalgesia, were demonstrated to transmit pruritus (Serra et al. 2004; Ikoma et al. 2006). More recent studies clearly demonstrated that, in addition to the CMi fibers, other subtypes of cutaneous Aδ- and C-fibers are also involved in itch in humans. It was clearly shown that the naturally occurring cowhage from the plant *Mucuna pruriens* excites cutaneous, mechano-, and heat-sensitive C-fibers (CMH fibers) (Johanek et al. 2007) and induces itch with nociceptive subqualities such as pricking, stinging, and burning in healthy volunteers. The C-fibers also respond to capsaicin. Capsaicin-mounted spicules applied to the skin produce an itch signal (LaMotte et al. 2009). Interestingly, in experimental studies, cowhage-induced itch could not be blocked by pretreatment with antihistamines but by a 3-day topical application of capsaicin (Johanek et al. 2007). Capsaicin

selectively binds to the ion channel *transient receptor potential V1* (TRPV1), which is expressed on keratinocytes and several cutaneous C-fiber classes including CMH fibers (Benecke et al. 2013a, b). It is well known that histamine requires the function of TRPV1 on CMi fibers for the transmission of an itch signal (Imamachi et al. 2009). The CMi and CMH fibers are not only relevant for their afferent function; they are peptidergic in nature, i.e., they contain neuropeptides, which are released upon neuronal activation into the surrounding tissue. This is an important step for the induction of neurogenic inflammation and a first step in the peripheral sensitization which is defined by increased responses of primary sensory neurons to itch and pain mediators (Liu and Ji 2013).

1.2 The Tachykinin Family

The most prominent neuropeptides released by peptidergic neurons are calcitonin gene-related peptide (CGRP) and substance P (SP). SP is a member of the tachykinin family which comprises also neurokinin A (NKA), neurokinin B (NKB), hemokinin 1, neuropeptide-γ (NKγ), neuropeptide K (NPK), and endokinins. The tachykinins are synthesized in and released from neurons of the CNS, capsaicin-sensitive primary afferent neurons, and capsaicin-insensitive intrinsic neurons of the gastrointestinal tract as well as nonneuronal cells such as immune and inflammatory cells (Patacchini et al. 2004). SP is composed of 11 amino acids (Arg-Pro-Lys-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH2) [(Harmar et al. 1986; Datar et al. 2004); UniProtKB P20366; TKN1_HUMAN; http://www.uniprot.org/uniprot/P20366]. It is encoded by the gene *TAC1* on chromosome 7 (7q21-q22) and derives from the precursor preprotachykinin-A (PPTA), which also encodes for NKA, NKγ, and NPK (Datar et al. 2004). The corresponding G protein-coupled receptors for the tachykinins are the neurokinin-1 receptor (NK1R), the neurokinin-2 receptor (NK2R), and the neurokinin-3 receptor (NK3R) (Datar et al. 2004). The genes for the receptors are located on chromosome 2 (2p12; TACR1, tachykinin receptor 1; Gene ID: 6869; http://www.ncbi.nlm.nih.gov/gene/6869), on chromosome 10 (10q22.1; TACR2, tachykinin receptor 2; Gene ID: 6865; http://www.ncbi.nlm.nih.gov/gene/6865), and on chromosome 12 (12q13-q21; TACR3, tachykinin receptor 3; Gene ID: 6866; http://www.ncbi.nlm.nih.gov/gene/6866). SP is the endogenous ligand with the highest affinity at NK1R which binds also with low-affinity NPγ, NPK, and NKA (Scholzen et al. 1998; Datar et al. 2004). NK2R has a high binding affinity for NKA and low affinity for NKB and SP (Scholzen et al. 1998; Datar et al. 2004). In NK3R, the order of binding affinity is NKB, NKA, and SP (Scholzen et al. 1998).

All NK receptors have seven hydrophobic transmembrane regions with an extracellular amino and cytoplasmatic carboxyl terminus. The C-terminus domain was identified to be essential for SP-induced endosome-mediated internalization of the receptor (Bowden et al. 1994), desensitization, and recycling (Böhm et al. 1997; Scholzen et al. 1998; Orel et al. 2012). Two naturally occurring forms of NK1R exist that differ in the length of their C-terminus but which are indistinguishable in

their agonist binding affinity (Lai et al. 2006; Orel et al. 2012). The first is a full-length receptor (407 amino acids) which mediates most of the functions and the second a truncated receptor (311 amino acids) which results in abnormal resensitization. Recently the C-terminus was characterized in more detail; it was shown to have clearly defined secondary (25 % α-helix, 27 % unordered structure, and 48 % β-sheets and β-turns) and tertiary structures which are tightly related to multiple functions (Orel et al. 2012). After internalization, NK1R returns to the cell surface and rapidly regains the ability to bind SP and undergo endocytosis (Southwell et al. 1998). Interestingly, in an animal study, a sex difference related to estradiol was observed concerning NK1R internalization. Intact female rats had greater internalization of NK1R than intact male rats while ovariectomy (but not castration) reduced the internalization (Nazarian et al. 2014). These data suggest that the sex difference may contribute to a differential development of central sensitization and nociceptive behaviors in males and females (Nazarian et al. 2014).

1.3 Biological Role of SP and NK1R in the Skin

NK1R is expressed in the CNS and peripheral tissues such as intestine, lung, immune cells (T cells, B lymphocytes, macrophages) and in the skin on keratinocytes, epithelial cells of hair follicles, MCs, fibroblasts, epidermal dendritic cells, and endothelial cells (Scholzen et al. 1998). It is debated if NK1 receptors are also present on peripheral sensory neurons (Scholzen et al. 1998); however, they are expressed on the superficial lamina I dorsal horn neurons of the spinal cord (Carstens et al. 2010) where they contribute to pain and itch transmission (Akiyama et al. 2013). For example, it was shown that, following a peripheral nerve lesion, NK1 receptors are upregulated on lamina I projection neurons and contribute by this route to neuropathic pain (Saeed and Ribeiro-da-Silva 2013). NK1 receptors are also present on human melanoma cells and mediate the viability of tumor cells (Muñoz et al. 2010). NK2R is predominantly expressed in the peripheral nervous system and keratinocytes (Scholzen et al. 1998), whereas NK3R is found in the CNS (Scholzen et al. 1998).

The NK1R and SP are involved in multiple physiological and pathophysiological processes, including pain and pruritus, vomiting reflex, depression, anxiety, change in cardiovascular tone, stimulation of salivary secretion, vasodilatation, modulation of cell proliferation, and regulation of immune and inflammatory responses (Orel et al. 2012; DeVane 2001). The list of their functions in various organs including the skin is long and can be presented only in an exemplary fashion in this chapter (Table 1). For example, SP, a neuropeptide and an abundantly present neurotransmitter in the CNS, is involved in sensory neurotransmission and nociception. Together with its role as a pro-inflammatory molecule, it is involved in facilitating nociceptive sensitization in inflammatory pain (Sahbaie et al. 2009). Thus, SP contributes to mechanical allodynia, central sensitization in trigeminal ganglia (Takeda et al. 2012), heat hyperalgesia associated with

Table 1 Functions of substance P and NK1R in the skin (examples)

Cell/ structure	Effects of SP/NK1R	Potential translational importance	References
Epidermal dendritic cells	Increased migration to skin-draining lymph nodes; inhibition of IL-10 synthesis and secretion	NK1R agonists: potential adjuvant for enhancing the efficiency of DC vaccines	Janelsins et al. (2009, 2013)
Fibroblast	Production of IFN-gamma, IL-1beta, and IL-8	NK1R antagonists: suppression of SP-induced inflammation	Liu et al. (2008)
Hair follicle	Stress-induced hair growth termination (catagen induction) accompanied by neurogenic inflammation in connective tissue sheath	NK1R antagonists: interruption of stress-mediated hair loss	Peters et al. (2007)
Keratinocyte	Production of NGF, leukotriene B4, IFN-gamma, IL-1beta, and IL-8	NK1R antagonists: suppression of SP-induced inflammation	Liu et al. (2008), Andoh et al. (2001)
Mast cell (MC)	Release of histamine, leukotriene B4, prostaglandin D2, or tumor necrosis factor-α Increased expression of NK1R on MCs in mastocytosis Degranulation, activation, accumulation in CRPS	NK1R antagonists: suppression of mast cell degranulation in mastocytosis, atopic dermatitis NK1R antagonists: management of CRPS	Li et al. (2012), Maintz et al. (2011)
Melanoma cells	Suppression of melanogenesis, cell growth of melanoma cells	NK1R agonists: management of skin pigmentation NK1R antagonists: cell growth inhibition and apoptosis of melanoma cells	Ping et al. (2012), Muñoz et al. (2010)

CRPS complex regional pain syndrome

inflammation or nerve injury (Teodoro et al. 2013), or complex regional pain syndrome (Li et al. 2012).

Next to its sensory function, SP has abundant **efferent function** after release from sensory nerve fibers upon activation of C- and Aδ-fibers by a pain or itch signal. SP is a mediator of inflammation in the skin which is terminated after release by cutaneous angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP). A major role of SP in the skin is the induction of **neurogenic inflammation,** which is induced by SP binding on MC and blood vessels. After release from nerve fibers, SP induces vasodilatation of short duration (Weidner et al. 2000) and leads to MC degranulation with release of histamine, leukotriene B4, prostaglandin D2, tumor necrosis factor-α, and vascular endothelial growth factor (VEGF) (Hägermark et al. 1978; Andoh et al. 1998; Kawana et al. 2006). Histamine binds to histamine 1 (H1) receptors on CMi fibers and induces pruritus (Ikoma et al. 2006).

Clinically, the neurogenic inflammation results in erythema, whealing, and pruritus. It was shown experimentally several times by intradermal injection of SP (10(-5) and 10(-6) mol/l) in healthy skin of volunteers, in inflamed skin of volunteers pretreated with 1 % sodium lauryl sulfate, and in psoriasis patients. This led to pruritus (in all groups) of the same intensity, flare, and wheal reaction (Thomsen et al. 2002; Amatya et al. 2010). In mice, cutaneous SP injections resulted in a dose-dependent increase in scratching of the injected site (Andoh et al. 1998). **Cutaneous thermal hyperemia** by local heating has an axon reflex-mediated component and a vascular component (production of local vasodilators), too, both of which are based on cutaneous SP effects and, accordingly, can be blocked by NK1R antagonists (Wong and Minson 2011). Interestingly, also in pain such as **complex regional pain syndrome** (CRPS), the efferent function of SP plays a role. In CPRS, release of SP into the tissue induces mast cell degranulation, accumulation, and activation that can be inhibited by NK1R antagonist (Li et al. 2012). In mastocytosis, characterized by increased numbers of MCs in the skin and pruritus, there is increased expression of NK1R on cutaneous MCs; furthermore, increased levels of SP have been shown in the serum of patients (Maintz et al. 2011). Moreover, SP increases **corticotropin-releasing hormone receptor-1** (CRHR-1) expression on MCs as well as release of IL-8, tumor necrosis factor (TNF), and VEGF by mast cells, which can be blocked by NK1R antagonists (Asadi et al. 2012). Corticotropin-releasing hormone (CRH) induces NK-1 gene expression in mast cells. CRH is released, for example, as a **stress response** and can induce mast cell degranulation and augment skin vascular permeability. This suggests a link between SP, CRH, and inflammatory diseases that worsen with stress, such as psoriasis or atopic dermatitis (AD), as well as associated pruritus (Singh et al. 1999; Alysandratos et al. 2012). This was also demonstrated in an AD animal model. Stress primarily exacerbates AD (e.g., increased eosinophil infiltration, vascular cell adhesion molecule-positive blood vessels, and epidermal thickness) via SP-dependent cutaneous neurogenic inflammation. This was inhibited in NK1R knockout mice (Pavlovic et al. 2008). Interestingly, SP shifted the cytokine profile toward TH2 in skin (Pavlovic et al. 2008) and thus may have additional influence on inflammation.

In both **keratinocytes and fibroblasts**, SP induces proliferation and the production of interferon gamma, interleukin (IL)-1beta, and IL-8 and fibroblast migration (Tanaka et al. 1988; Kähler et al. 1993; Liu et al. 2008). Interestingly, cetirizine, the H1 antihistamine, significantly inhibited the expression of NK1R and SP-induced IL-1beta and IL-8 production in HaCaT cells and fibroblasts (Liu et al. 2008). These results suggest a link between histamine and SP-induced inflammation in keratinocytes and fibroblasts. In keratinocytes, SP can directly induce nerve growth factor (NGF) mRNA expression and the secretion of bioactive NGF protein (Burbach et al. 2001). Topical capsaicin application results in significant upregulation of keratinocyte NGF expression in the epidermis (Burbach et al. 2001). SP further leads to an increased expression of leukotriene B4 in keratinocytes (Andoh et al. 2001) that can be blocked by azelastine, the H1 antihistamine (Andoh and Kuraishi 2002). The release of SP-mediated

pro-inflammatory mediators from mast cells and keratinocytes attracts inflammatory cells to invade into the skin. This pro-inflammatory action of SP can be blocked by addressing NK1R as demonstrated in animal models. NK1R knockout mice displayed a significantly reduced allergic contact dermatitis (ACD) in response to dinitrofluorobenzene (DNFB) with histological evidence of less edema and 50 % fewer infiltrating leukocytes compared with the ACD response in wild-type animals (Scholzen et al. 2004). In addition, administration of a NK1R antagonist before sensitization, significantly inhibited the augmented effector phase of ACD in mice with a heterozygous deletion of somatic ACE (Scholzen et al. 2003).

1.4 Role of SP in Pruritus

By promoting the production of NGF in keratinocytes and the release of histamine and other pro-inflammatory mediators from mast cells, SP leads to sensory nerve fiber sprouting and augmentation of skin inflammation. This contributes to the development and maintenance of cutaneous pruritus. In fact, in AD, prurigo nodularis (PN; pruritus with chronic persistent scratch lesions), and normal looking skin of chronic pruritus patients, an increased density of dermal SP-positive nerve fibers was found (Abadía Molina et al. 1992; Järvikallio et al. 2003; Haas et al. 2010). In addition, one of the key pruritogenic factors in dermatoses is the worsening of pruritus by stress as also mediated by SP (Hosokawa et al. 2009).

Accordingly, SP is reported to be involved in the pathogenesis of pruritus in several entities such as pruritus in psoriasis (Nakamura et al. 2003; Chang et al. 2007), AD (Hosokawa et al. 2009), and cholestatic pruritus (Trivedi and Bergasa 2010). In patients with pruritic psoriasis, an increase in SP-positive nerve fibers in perivascular areas, decreased expression of NEP in the epidermal basal layer, and increased expression of epidermal NK1R were observed compared to patients with non-pruritic psoriasis, suggesting a role of SP and epidermal NK1R-positive keratinocytes in the induction of itch (Nakamura et al. 2003; Chang et al. 2007). Another study demonstrated increased expression of SP and NK1R in dermal inflammatory cells (lymphocytes, mast cells) in involved, compared to noninvolved, psoriatic skin that was not related to pruritus but to stress levels (Remröd et al. 2007). SP plasma levels do not seem to be related to psoriasis-associated pruritus as a negative correlation between pruritus intensity and levels of SP has been found (Reich et al. 2007).

Animal models demonstrated a role for SP in AD. Administration of an NK1R antagonist (BIIF 1149 CL) significantly decreased scratching behavior in NC/Nga mice (Ohmura et al. 2004). Induction of a DNFB-mediated allergic contact dermatitis in mice could be inhibited by depleting substance P by tacrolimus with resulting reduced scratching behavior (Inagaki et al. 2010). In the same study, DNFB-induced ear swelling and scratching behavior were significantly inhibited by FK888, a NK1R antagonist (Inagaki et al. 2010). In another study, oral application of aprepitant reduced the serum IgE level, tissue substance P levels, and the

infiltration of Treg cells but not the inflammation as assessed by a total clinical severity score and ear thickness in NC/Nga mice (Lee and Cho 2011).

In human AD skin, dermal contacts between mast cells and sensory nerves were increased in number in both lesional and non-lesional samples of AD when compared to those in normal controls (Järvikallio et al. 2003). SP-positive nerve fibers in the epidermis and in the papillary dermis were markedly increased in lesional AD as compared to non-lesional controls (Järvikallio et al. 2003). In the blood plasma, patients with AD had significant increases in levels of NGF and SP compared with controls (Toyoda et al. 2002; Salomon and Baran 2008; Hosokawa et al. 2009). A significant correlation of plasma NGF and SP levels with disease activity (as measured by the grading system of Rajka and Langeland, objective severity scoring of AD and Eczema Area and Severity Index - EASI) was found (Toyoda et al. 2002). However, another AD study demonstrated that SP plasma levels did not seem to be a sensitive marker for disease severity because the distribution window of actual values of plasma SP levels was relatively small (10 to <500 pg/mL) (Hosokawa et al. 2009). Further, in children, no correlation between plasma SP concentration and the subjective symptoms of pruritus or sleep-loss scores as reported by the parents in the SCORAD (SCORing Atopic Dermatitis) was found (Hon et al. 2007). In sum, cutaneous SP seems to play a role in AD pruritus induction, but it is questionable if SP plasma levels can serve as biomarkers.

Thus, SP is a key mediator of itch induction in the skin. Vice versa, skin scratching may influence SP and NK1R content of the skin. Skin-scratching stimulation in mice resulted in immediate depletion of SP from sensory nerves, while the expression of NK1R was upregulated in basal keratinocytes within days (Yamaoka and Kawana 2007). Interestingly, in some entities such as cholestatic pruritus, increased serum concentrations of SP have been found. This may be related to scratching and thus be a secondary finding, as other mediators such as autotoxin have recently been defined as primary mediators of cholestatic pruritus (Kremer et al. 2012).

2 Neurokinin Receptor Antagonist, Aprepitant

Aprepitant (bis(trifluoromethyl) morpholine, MK-869) is a selective high-affinity, CNS-penetrant, oral NKR1-antagonist with little or no affinity for other neurokinin receptors (Kramer et al. 1998). It was developed for the therapy of pain and depression, but studies failed to demonstrate an effect in a nontoxic dosage range (DeVane 2001). In 2003, aprepitant was approved for the prevention of chemotherapy-induced emesis and is usually administered for 3 days only (Hesketh et al. 2003; Dando and Perry 2004).

2.1 Adverse Events with Aprepitant

Aprepitant is an inducer of cytochrome P450 3A4 isoform (CYP3A4) activity and to a modest extent, activity of CYP2C9 (Shadle et al. 2004; Ruhlmann and

Herrstedt 2011). As a consequence, there is a risk of drug-drug pharmacokinetic interactions. Several drugs should not be applied in association with aprepitant such as rifampicin, phenytoin, carbamazepine, phenobarbital, ketoconazole, itraconazole, cyclosporine, and tacrolimus.

In studies investigating the use of aprepitant in patients with depression, the drug was applied for 6–8 weeks (Table 2). Mild to moderate side effects occurred but there were no serious adverse events. In one of the first studies including 213 patients who received 300 mg aprepitant (MK-869) for 6 weeks, the safety and tolerability of MK-869 were generally similar to placebo, except for mild and typically transient somnolence and asthenia (Kramer et al. 1998). The most common clinical adverse events observed in patients receiving MK-869 were headache (32 %), somnolence (20 %), nausea (18 %), and asthenia/fatigue (14 %). In studies investigating potential antidepressant effects, the percentage of patients discontinuing therapy because of adverse effects was greater with paroxetine (19 %) than with MK-869 (9 %) or placebo (9 %) (Kramer et al. 1998). Subsequent studies using aprepitant in a lower dosage (80–250 mg) but for a long period (2–8 weeks) confirmed the safety of the substance (Table 2). In general, it can be

Table 2 Adverse events of aprepitant as reported in long-term treatment trials

Indication	Number of patients	Duration of treatment	Oral dosage of aprepitant	Adverse events (AE)	Authors
HIV infection	30	2 weeks	125 mg ($n = 9$) 250 mg ($n = 8$)	Aprepitant induced more grade 2–4 AEs than placebo ($p = 0.042$)	Tebas et al. (2011)
Overactive bladder	125	8 weeks	160 mg	Aprepitant induced more AEs than placebo ($p = 0.001$), although AEs were mild and unlikely to be of clinical significance	Green et al. (2006)
Depression	2,526 (summary of five studies)	8 weeks	160 mg ($n = 806$) 80 mg ($n = 479$)	No significant differences versus placebo in any of the five studies. Aprepitant 80 mg showed no significant differences versus placebo on any of the summary safety measures	Keller et al. (2006)
Depression	213	6 weeks	300 mg	No significant differences versus placebo	Kramer et al. (1998)

concluded that aprepitant may have more adverse events (AEs) than placebo (Green et al. 2006; Tebas et al. 2011), but studies including large numbers of patients (Kramer et al. 1998; Keller et al. 2006) concluded that the substance is generally well tolerated over a long period—up to 8 weeks.

3 Antipruritic Effects of Aprepitant

Given the important function of SP in skin inflammation and peripheral and central pruritus transmission, aprepitant was applied in acute and chronic pruritus patients.

3.1 Acute Pruritus (Table 3)

In **pruritic drug reactions** due to the modern class of antineoplastic drugs, in total 48 patients have been reported to respond to aprepitant. A first report described a 44-year-old woman and a 74-year-old man, each with stage IV non-small cell lung cancer, with an erlotinib (epidermal growth factor receptor inhibitor)-induced pruritic acneiform skin rash (duration of pruritus: 1 week) (Vincenzi et al. 2010a). Application of aprepitant (125 mg for 1 day, 80 mg for 2 days) resulted in a decrease of pruritus (visual analogue scale, VAS 8 and 9 to VAS 0 and 1). After this, application of erlotinib together with an altering dosage of 125 mg and 80 mg aprepitant for 2 months prevented recurrence of the symptom though the rash continued to be present. No side effects were mentioned in the report (Vincenzi et al. 2010a).

A 54-year-old patient with lung adenocarcinoma received aprepitant (80 mg per day) for erlotinib-induced pruritic rash. Rash and pruritus regressed during an 8-day therapy (Mir et al. 2011). The authors mention that the patient still receives aprepitant (80 mg per day) and erlotinib (150 mg per day) without recurrence of pruritus, but the precise duration of therapy as well as occurrence of side effects was not mentioned.

In an open-label, uncontrolled, and non-randomized pilot study (Santini et al. 2012), 45 patients with solid tumors were treated with aprepitant for acute pruritus due to the application of an antineoplastic drug (erlotinib, $n = 16$; cetuximab, $n = 23$; sunitinib, $n = 3$; lapatinib, imatinib, gefitinib, each $n = 1$). Patients were grouped into a *refractory group* (for patients with pruritus refractory to standard treatment with prednisone 25 mg/day or fexofenadine 180 mg/day) or a *naive group* (for patients who had not been previously treated for pruritus). Aprepitant (125 mg on day 1; 80 mg on day 3; 80 mg on day 5) was given to patients in the refractory group after at least 1 week of standard systemic treatment. In the naive group, aprepitant was given in the same schedule as the refractory group, after the first onset of severe pruritus (Santini et al. 2012). Forty-one (91 %) patients responded to aprepitant. Median VAS dropped from 8 to 1 (in the refractory group; $p < 0.0001$) and from 8 to 0 (in the naive group; $p < 0.0001$). No side effects related to aprepitant occurred (Santini et al. 2012).

Table 3 Current evidence of an antipruritic effect of aprepitant

Indication	Number of patients	Dosage of aprepitant	References
Acute pruritus (n = 50)			
Drug-induced pruritus (with or without skin rash)			
Erlotinib-induced acneiform pruritic rash in stage IV non-small cell lung cancer	2	125 mg (day 1), 80 (day 2–3); then altering dosage of 125 mg and 80 mg for 2 months	Vincenzi et al. (2010b)
Erlotinib-induced pruritic rash in lung adenocarcinoma	1	80 mg for 1 week (therapy still ongoing)	Mir et al. (2011)
Antineoplastic drug-induced pruritus in metastatic solid tumors	45	125 mg (day 1), 80 mg (day 3; day 5) for one cycle	Santini et al. (2012)
Paraneoplastic pruritus			
Metastatic soft tissue sarcoma, metastatic breast carcinoma	2	125 mg (day 1), 80 mg (day 2–3)	Vincenzi et al. (2010a)

Given that aprepitant is a CYP3A4 inhibitor, there is risk of an increase in erlotinib concentrations and decrease in erlotinib clearance upon continuous administration of aprepitant (Levêque 2010; Mir et al. 2011; Mir and Coriat 2012). An accumulation of mast cells in the lesional skin of patients treated with erlotinib might be responsible for the induction of pruritus; prevention of SP-induced degranulation of mast cells by aprepitant might explain the antipruritic effect of the NK1R antagonist in these patients (Gerber et al. 2011).

In two patients with potential **paraneoplastic pruritus** (a male with metastatic soft tissue sarcoma and a female with metastatic breast carcinoma) receiving systemic chemotherapy, pruritus (duration not provided) occurred without clear correlation to chemotherapy or any other origin (Vincenzi et al. 2010b). These patients received aprepitant during chemotherapy (125 mg for 1 day, 80 mg for 2 days) and both reported a drop of 8 VAS points from initial 8 and 9, respectively. After interruption of aprepitant, pruritus recurred within 3 days. No relevant side effects related to aprepitant administration were observed in the two patients (Vincenzi et al. 2010b).

3.2 Chronic Pruritus (Table 4)

In an open-label proof-of-concept study, it was demonstrated that targeting of NKR1 is of high clinical relevance for CP patients (Ständer et al. 2009, 2010). Twenty patients with chronic, yet therapy-refractory, pruritus of various origins experienced a significant antipruritic effect ($p < 0.001$) upon monotherapy with aprepitant 80 mg within 1 week. Pruritus intensity was assessed by VAS daily. The VAS was reduced from VAS_{pre} 8.4 points (SD ± 1.7) before treatment to VAS_{post}

Table 4 Current evidence of an antipruritic effect of aprepitant in chronic pruritus

Indication	Number of patients	Dosage of aprepitant	References
Chronic pruritus (n = 67)			
Chronic pruritus of various origins			
$n = 5$: chronic kidney disease $n = 7$: multiple causal factors $n = 8$: pruritus of unknown origin Among them: 10/20 atopic predisposition	20	80 mg for 3–13 days (mean, 6.6 days)	Ständer et al. (2009, 2010)
Prurigo nodularis			
Prurigo nodularis of different etiology	13/20 (see above)	80 mg for 3–13 days (mean, 6.6 days)	Ständer et al. (2010)
Prurigo nodularis of different etiology	36	80 mg for 1–4 weeks	Zeidler, Luger, Ständer, unpublished
Cutaneous T-cell lymphoma (CTCL)			
Sézary syndrome	3	80 mg (once?; no duration of therapy provided)	Duval and Dubertret (2009)
Erythrodermic CTCL $n = 3$: Sézary syndrome $n = 2$: erythrodermic mycosis fungoides	5	125 mg (day 1); 80 mg (days 2–3), in 2-week repetitions for a median time of 15 weeks	Booken et al. (2011)
Sézary syndrome	2	80 mg for 15 days; 80 mg every other day for 10 days	Torres et al. (2012)
Neuropathic pruritus			
Brachioradial pruritus	1	80 mg 7 days, after 2 days break, then again for 2 weeks	Ally et al. (2013)

4.9 points (SD ± 3.2; $p < 0.001$; CI 1.913–5.187) after treatment. Among the patients were those with chronic kidney disease-associated pruritus and pruritus of multiple etiologies; the response in this group was not convincing. However, the most significant response rate was observed in patients with **atopic predisposition** (VAS_{pre} 8.2, SD ± 1.8; VAS_{post} 3.8, SD ± 2.8; $p = 0.001$; CI 2.144–6.656) and **prurigo nodularis** (PN) (VAS_{pre} 8.4, SD ± 1.8; VAS_{post} 4.4, SD ± 3.2; $p = 0.001$; CI 1.863–6.137). The latter observation could be confirmed in 36 subsequent patients with PN receiving aprepitant 80 mg for 1–4 weeks (Zeidler, Luger, Ständer, unpublished observation). The antipruritic effect (VAS_{pre} 7.0 ± 2.2; VAS_{post} 4.5 ± 2.8) was stable over 4 weeks and allowed improvement of PN with partial healing of lesions. Side effects remained mild (nausea, vertigo, and drowsiness) and occurred in three patients (Ständer et al. 2010). The mechanism of the antipruritic effect of aprepitant is assumed to be based on the multiple functions of SP in the skin and CNS. The previous findings of increased SP-positive skin nerve

fibers in patients with AD and PN and our observation of a major pruritus reduction in these entities argue for a mainly peripheral effect.

An antipruritic activity of aprepitant in CP patients was confirmed in subsequent case reports. For example, in three patients with pruritus (duration not provided) related to **cutaneous T-cell lymphoma** (CTCL) of the type Sézary syndrome and mycosis fungoides (MF), a VAS reduction of 5–7 points could be noticed 1 day after the start of aprepitant 80 mg therapy (Duval and Dubertret 2009). Interestingly, no effect was seen on erythroderma. No side effect was mentioned in this case report.

A set of five patients (three men, mean age 61) with CP (duration of pruritus: mean, 25 months) in erythrodermic MF ($n = 2$) and Sézary syndrome ($n = 3$) received aprepitant 125 mg on day 1 and 80 mg on days 2 and 3 in 2-week repetitions for a median time of 15 weeks (range 6–24) and a median number of 7 therapy cycles (range 3–12) (Booken et al. 2011). One patient failed to show any response; four showed a decrease of itch intensity. VAS score at the beginning of the study was 9.8 ± 0.4 and after intervention 4.3 ± 3.4 ($P = 0.125$). Improvement of pruritus was already observed after the first cycle of therapy and was stable over 2 weeks. Oral aprepitant was well tolerated and no side effects were observed (Booken et al. 2011).

A further two patients with CP secondary to CTCL type Sézary syndrome were treated with aprepitant 80 mg (Torres et al. 2012). Two days after starting treatment, the patients reported great improvement of pruritus. VAS dropped from 8 and 9 to 2 and 3, respectively, on day 5. Aprepitant was applied daily for 15 days, and then the dosage was tapered to 80 mg every other day for 10 days. After stopping aprepitant, no rebound effects of pruritus occurred. No relevant side effects were observed in the two patients (Torres et al. 2012).

Interestingly, a neuropathic form of CP was reported recently to respond to aprepitant (Ally et al. 2013). A 61-year-old woman with bilateral **brachioradial pruritus** related to bilateral neuroforaminal stenosis between C4 and C6 was treated with 80 mg of aprepitant for 7 days. Within 2 days she reported vast improvement of pruritus and scratch lesions. At day 9, after 2 days without aprepitant, pruritus recurred and aprepitant was started again for 2 weeks but response was weak. No adverse effects were reported. The failure of aprepitant to achieve significant pruritus relief in this case report argues again for a mostly peripheral antipruritic mechanism of action of aprepitant.

In sum, there is strong evidence from recent case reports and case series from multiple observers that SP is indeed a key mediator of pruritus in many diseases and that aprepitant, the NK1R antagonist, exhibits antipruritic activity. Based on these encouraging reports, many health care providers are already using aprepitant in the treatment of patients with pruritus. Controlled trials in different entities such as CTCL, PN, and drug-induced pruritus, however, are lacking and urgently needed.

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Antihistamines and Itch

Robin L. Thurmond, Kayvan Kazerouni, Sandra R. Chaplan, and Andrew J. Greenspan

Contents

Abstract

Histamine is one of the best-characterized pruritogens in humans. It is known to play a role in pruritus associated with urticaria as well as ocular and nasal allergic reactions. Histamine mediates its effect via four receptors. Antihistamines that block the activation of the histamine H_1 receptor, H_1R, have been shown to be effective therapeutics for the treatment of pruritus associated with urticaria, allergic rhinitis, and allergic conjunctivitis. However, their efficacy in other pruritic diseases such as atopic dermatitis and psoriasis is limited. The other histamine receptors may also play a role in pruritus, with the exception of the histamine H_2 receptor, H_2R. Preclinical evidence indicates that local antagonism of the histamine H_3 receptor, H_3R, can induce scratching perhaps via blocking inhibitory neuronal signals. The histamine H_4 receptor, H_4R, has received a significant amount of attention as to its role in mediating pruritic signals. Indeed, it has now been shown that a selective H_4R antagonist

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can inhibit histamine-induced itch in humans. This clinical result, in conjunction with efficacy in various preclinical pruritus models, points to the therapeutic potential of H_4R antagonists for the treatment of pruritus not controlled by antihistamines that target the H_1R.

Keywords
Pruritus • Atopic dermatitis • Urticaria • Allergic rhinitis • Allergic conjunctivitis • H4R antagonists • H1R antagonists

1 Introduction

Histamine is a ubiquitous bioamine that has a variety of physiological functions. First synthesized in 1907, it was recognized as a natural substance in 1910 by Sir Henry Dale and colleagues, who found it in ergot (Windaus and Vogt 1907; Barger and Dale 1910). It was not until 1927 that it was shown to be a natural component of animal tissue and therefore of physiological relevance (Best et al. 1927). It is now known that histamine acts via four receptors, namely, the histamine H_1 receptor (H_1R), the histamine H_2 receptor (H_2R), the histamine H_3 receptor (H_3R), and the histamine H_4 (H_4R) (Table 1). Drugs that target the H_1R have been in use since Bovet and Staub showed that the actions of histamine could be blocked by synthetic compounds (Bovet and Staub 1937; Staub and Bovet 1937). This led to the development of the first clinical antihistamine, 2339 RP/phenbenzamine/Antergan, which was shown to be effective in blocking the effects of intradermal histamine injection and was used to treat urticaria and serum sickness (Celice et al. 1942; Decourt 1942; Halpern 1942; Parrot 1942). The term "antihistamine" is largely used to describe compounds that block the actions of the H_1R, and predates knowledge of the existence of the other receptors. Drugs that target the H_2R are also in clinical use for the treatment of gastric ulcers. The first of these was burimamide, shown to inhibit gastric acid secretion in humans (Black et al. 1972;

Table 1 Histamine receptors

Receptor	Role in itch	Other uses
H_1	Mediates itch in urticaria, allergic rhinitis, and allergic conjunctivitis	The treatment of hives, overall allergic symptoms in rhinitis and conjunctivitis, insomnia
H_2	No known role	The treatment of duodenal and gastric ulcers, gastroesophageal reflux disease
H_3	Local antagonist induces itch in preclinical models	Potential indications include excessive daytime sleepiness and attention-deficit hyperactivity disorder
H_4	Antagonist inhibits itch in several preclinical models	Potential indications include atopic dermatitis, psoriasis, asthma, and rheumatoid arthritis

Wyllie et al. 1972). The field is still awaiting the development of drugs that target the H_3R and H_4R, but clinical studies are ongoing with ligands for both receptors.

The various histamine receptor ligands have been crucial in distinguishing the four receptor subtypes and in defining their physiological functions. Indeed, the identity of the receptor mediating a particular function is defined on a pharmacological basis. For example, a function that is modulated by ligands active at the H_1R is deemed to be mediated by that receptor. Limitations of the use of ligands as pharmacological tools in this manner have appeared as the new histamine receptors have been discovered and the affinity of the available drugs for the various receptor subtypes has been better characterized (Table 2). For example, thioperamide was originally used to characterize the function of the H_3R, but it is now known that the compound has significant H_4R activity. Similarly, efficacy of clinical H_1R and H_2R ligands has been used to define whether histamine is involved in a particular disease. Thus, the itch in both urticaria and allergic rhinitis is effectively blocked by H_1R antihistamines and can be properly considered to be histamine mediated. Conversely, these drugs are not effective against the itch in either atopic dermatitis or psoriasis; therefore, it has been concluded that the itch in these conditions is not histamine mediated. Since only H_1R and H_2R antagonists are available for clinical use in diseases where they fail to control symptoms, the conclusion that histamine is not involved may not be accurate. In addition to the H_1R and H_2R, histamine may have clinically important activity via the H_3R or H_4R. Therefore, the conclusions of some of the literature published prior to the characterization of the H_3R and H_4R should be revisited in light of the newer receptors.

Table 2 Binding affinities for selected histamine receptor ligands

Ligand	Typically ascribed function	hH_1R pK_i[a]	hH_2R pK_i[b]	hH_3R pK_i[c]	hH_4R pK_i[d]
Histamine	HR agonist	4.4	5.1	8.2	8.1
Betahistine	H_1R agonist	4.5[e]		5.2[f]	4.1
Alcaftadine	H_1R antagonist	8.5	7.2	<5	5.4
Cetirizine	H_1R antagonist	8.2	<6	<6	<5
Chlorpheniramine	H_1R antagonist	8.6	5.1	5.5	4.6
Desloratadine	H_1R antagonist	8.4	6.4[e]	<5[g]	5.1[g]
Diphenhydramine	H_1R antagonist	7.9	5.8	4.6	4.4
Fexofenadine	H_1R antagonist	7.5			<5
Ketotifen	H_1R antagonist	9.3	6.0	5.6	4.3
Levocetirizine	H_1R antagonist	8.2	<6	<6	<4
Loratadine	H_1R antagonist	7.2		<5[g]	4.7
Olopatadine	H_1R antagonist	7.5	4.0	4.1	<4[g]
4-Methylhistamine	H_2R/H_4R agonist	<5[g]	5.1	4.7	7.7
Cimetidine	H_2R antagonist	4.8	5.8	4.7	5.0
Famotidine	H_2R antagonist	<5[e]	7.6		<5
Ranitidine	H_2R antagonist	4.5	6.7	4.9	<5
R-α-Methylhistamine	H_3R/H_4R agonist	<5[g]	<4	8.7	6.8

(continued)

Table 2 (continued)

Ligand	Typically ascribed function	hH_1R pK_i[a]	hH_2R pK_i[b]	hH_3R pK_i[c]	hH_4R pK_i[d]
Pitolisant	H_3R antagonist	5.8	5.0	8.1	<4
Thioperamide	H_3R antagonist	3.9	4.3	7.6	7.0
JNJ 7777120	H_4R antagonist	<5	<6	5.3	8.1

[a]Data averaged from Tran et al. (1978), Chang et al. (1979), Arrang et al. (1985), De Backer et al. (1993), Leurs et al. (1994b), Sharif et al. (1996), Kato et al. (1997), Merlos et al. (1997), Bakker et al. (2001), Gillard et al. (2002), Esbenshade et al. (2003), Govoni et al. (2003), Seifert et al. (2003), Thurmond et al. (2004), Bielory et al. (2005), Ligneau et al. (2007), Yu et al. (2010), Rossbach et al. (2011), Appl et al. (2012), Gallois-Bernos and Thurmond (2012)
[b]Data averaged from Harada et al. (1983), Gantz et al. (1991), Eriks et al. (1993), Leurs et al. (1994a), Leurs et al. (1995), Sharif et al. (1996), Kreutner et al. (2000), Saitoh et al. (2002), Esbenshade et al. (2003), Thurmond et al. (2004), Bielory et al. (2005), Lim et al. (2005), Preuss et al. (2007), Yu et al. (2010), Rossbach et al. (2011), Appl et al. (2012), Gallois-Bernos and Thurmond (2012) (Levocetirizine New Drug Application #22-064; Loratadine New Drug Application #21-165)
[c]Data averaged from Arrang et al. (1985), Sharif et al. (1996), Kato et al. (1997), Lovenberg et al. (1999), Coge et al. (2001), Liu et al. (2001a), Wieland et al. (2001), O'Reilly et al. (2002), Wellendorph et al. (2002), Wulff et al. (2002), Esbenshade et al. (2003), Thurmond et al. (2004), Bielory et al. (2005), Lim et al. (2005), Gbahou et al. (2006), Ligneau et al. (2007), Yu et al. (2010), Rossbach et al. (2011), Appl et al. (2012), Gallois-Bernos and Thurmond (2012) (Levocetirizine New Drug Application #22-064)
[d]Data averaged from Liu et al. (2001a, b), Morse et al. (2001), Zhu et al. (2001), O'Reilly et al. (2002), Esbenshade et al. (2003), Thurmond et al. (2004), Lim et al. (2005), Gbahou et al. (2006), Ligneau et al. (2007), Deml et al. (2009), Yu et al. (2010), Rossbach et al. (2011), Appl et al. (2012), Gallois-Bernos and Thurmond (2012)
[e]Data from guinea pig
[f]Data from rat
[g]Unpublished data

2 Histamine and Itch

Early investigators noted that when histamine is delivered to the skin, subjects experience the sensation of itch that starts 20–30 s after application and then dissipates by about 10 min (Eppinger 1913; Sollmann and Pilcher 1917; Bickford 1937). The itch is in addition to the triple response of local vasodilation, local edema, and flare described by Lewis and co-workers and summarized in his book *The Blood Vessels of the Human Skin* (Lewis 1927). Indeed, any kind of injury that produces the triple response will also produce itch due to the release of histamine (Lewis 1942). Early clinical studies showed that the first clinical antihistamine, Antergan, could reduce the itch, wheal, and flare caused by intradermal injection of histamine (Decourt 1942; Parrot 1942), and it was recognized early on that a comparison of the antipruritic activity of antihistamines could be made by measuring the changes in the threshold of histamine concentrations required to induce itch (Cormia and Kuykendall 1954).

Histamine has emerged as the best-characterized human pruritogen; but what are the mechanisms that lead to the sensation of itch? A neuronal basis for histamine-induced itch sensation was suggested by the fact that certain direct stimuli such as scratching or painful stimuli such as a pin prick, heat, or cold can inhibit the itch response (Bickford 1937; Graham et al. 1951). Indeed, this is true even if such stimuli are given distal to the site of itch (Graham et al. 1951; Yosipovitch et al. 2005, 2007). In addition, it appears that the application of local anesthetics can inhibit the itch sensation (Shelley and Melton 1950). These observations suggest that histamine-induced itch is mediated via neuronal activation. This was confirmed by the identification of histamine sensitive C-fibers that transmit the itch response to the spinal cord (Schmelz et al. 1997). These C-fibers can also respond to other nociceptive compounds such as capsaicin and bradykinin in addition to histamine, but do not respond to mechanical stimuli (Schmelz et al. 2003). The possibility that other polymodal C-fibers play a role is not excluded, however (Handwerker et al. 1991; Schmelz et al. 1997; Johanek et al. 2008). The transmission of histamine-induced pruritic signals appears to require TRPV1, since scratching was reduced in TRPV1-deficient mice or those treated with an inhibitor (Shim et al. 2007). Transmission of pruritus to the brain also appears to be mediated via specific spinothalamic tract neurons (Andrew and Craig 2001). Interestingly, these neurons are polymodal and can respond to mechanical stimuli (Schmelz 2001; Simone et al. 2004; Davidson et al. 2007), which may explain why scratching can reduce the histamine-induced activity of these neurons and may provide a mechanism for the inhibition of itch by other stimuli (Davidson et al. 2009). In the brain, peripheral itch stimuli evoke a complex pattern of regional neuronal activation that contains elements of sensation, emotion, and motivation (Hsieh et al. 1994; Darsow et al. 2000; Drzezga et al. 2001; Mochizuki et al. 2003, 2007; Herde et al. 2007; Schneider et al. 2008; Papoiu et al. 2012). However, there is some evidence that different pruritus stimuli result in different patterns of neuronal pathway activation and that the processing of chronic pruritic signals may differ from acute pruritus (Leknes et al. 2007; Schneider et al. 2008; Papoiu et al. 2012). Data have shown that in mice, many itch signals, including those of histamine, are transmitted via MrgprA3-expressing neurons (Han et al. 2013). However, itch-transmitting neurons do not appear to be interchangeable. Roberson et al. (2013) used a method to silence neurons after they have been activated to show that specific afferent fibers mediate itch generated by different pruritogens. In both cases, it appears that these neurons do not process pain sensations (Han et al. 2013; Roberson et al. 2013).

3 The Histamine H_1 Receptor

The H_1R is expressed on many cell types and mediates several important physiological functions that have been exploited from a therapeutic perspective. For example, as above, expression on both peripheral and central neurons mediates pruritic responses. The H_1R is also expressed on neurons in the central nervous system with a role in sleep/wake cycles. Blockade of the H_1R by compounds that

cross the blood–brain barrier results in sedation, which is undesirable in the treatment of allergy but useful in the treatment of insomnia. On smooth muscle cells and endothelial cells, activation of the H_1R drives vascular permeability responses in the skin and other organs.

The currently marketed H_1R antihistamines are divided into two categories. First-generation H_1R antihistamines are characterized by poor selectivity for the H_1R as well as their ability to easily cross the blood–brain barrier. Second-generation antihistamines are more selective for the H_1R, thus reducing the side-effect burden, and are mildly sedating or nonsedating, since they do not penetrate into the brain as readily. Despite these differences, it appears that antipruritic efficacy is similar between first- and second-generation H_1R antihistamines. However, second-generation antihistamines are preferred, due to safety advantages and dosing convenience. In addition, second-generation H_1R antihistamines have been more rigorously studied in the clinic since they were introduced after many of the clinical trial efficacy and safety requirements went into effect.

As discussed earlier, intradermal injection of histamine into the skin of humans produces a sensation of itch as well as a wheal and flare response. This is also true with intradermal injection of an H_1R agonist, 2-methylhistamine, whose effects can be blocked by an H_1R antagonist (Davies and Greaves 1980). Much of histamine activity in the skin is mediated via activation of the H_1R, since antihistamines that target this receptor can inhibit these reactions (Hagermark 1973; Davies et al. 1979; Hagermark et al. 1979; Davies and Greaves 1980; Levander et al. 1985, 1991; Coulie et al. 1991; Lahti and Haapaniemi 1993; Weisshaar et al. 1997; Clough et al. 2001; Furue et al. 2001; Denham et al. 2003; Morita et al. 2005; Kupczyk et al. 2007; Tanizaki et al. 2012). For example, two highly selective H_1R antagonists, cetirizine and its active enantiomer levocetirizine, were able to almost completely reduce histamine-induced itch in humans, as well as significantly inhibit the wheal and flare response (Coulie et al. 1991; Levander et al. 1991; Lahti and Haapaniemi 1993; Clough et al. 2001; Denham et al. 2003). This effect could also be mimicked by another selective H_1R antagonist, fexofenadine (Tanizaki et al. 2012).

When injected into the skin, other agents such as compound 48/80, platelet-activating factor, prostaglandin E2, substance P, and other neuropeptides can cause itch as well as a wheal response that can be blocked by H_1R antagonists (Hagermark et al. 1978; Fjellner and Haegermark 1981, 1985; Rukwied et al. 2000; Hosogi et al. 2006). These agents appear to be producing their effects by causing the release of histamine at the site of injection, although it is also possible that they directly or indirectly stimulate c-afferent fibers and generate an upstream histamine-mediated neuronal response that is H_1R mediated. Some pruritogens appear to work via a different mechanism, though, since the itch generated by intradermal injection of substances such as serotonin or cowhage is not inhibited significantly by H_1R antihistamines (Weisshaar et al. 1997; Hosogi et al. 2006; Johanek et al. 2007).

The role of the H_1R in histamine-induced itch in the skin provides a clear rationale for the use of antihistamines that target this receptor for dermal pruritus. The best example of success in this area is with urticaria. Urticaria is characterized

by highly pruritic skin wheals, which can be either acute or chronic, persisting for 6 weeks or longer. For many patients, the triggers are unknown, with the exception of physical urticarias that are induced by exposure to heat, cold, sun, pressure, vibration, water, exercise, or contact (Greaves 1995; Zuberbier and Maurer 2007). The similarity in symptoms between urticaria and histamine injection into the skin is suggestive for a role of histamine. This is supported by the observations of increased histamine levels and mast cell numbers in the affected skin of urticaria patients (Kaplan et al. 1978; Natbony et al. 1983). One of the first antihistamines, diphenhydramine, was shown to be effective in treating urticaria, with one early study showing that 25 of 35 patients with urticaria experienced immediate and complete relief of symptoms (Curtis and Owens 1945; O'Leary and Farber 1945). Several H_1R antihistamines are approved for the treatment of urticaria, including fexofenadine. In double-blind, placebo-controlled studies, fexofenadine was shown to reduce pruritus by about 1 point on a 0–4 scale and to improve the wheal score as well (Finn et al. 1999; Kaplan et al. 2005). In addition to fexofenadine, clinical studies (Table 3) have shown efficacy with bilastine (Zuberbier and Maurer 2010), loratadine (Monroe 1992; Dubertret et al. 1999), cetirizine (Breneman 1996), desloratadine (Monroe et al. 2003; Ortonne et al. 2007), levocetirizine (Nettis et al. 2006), mizolastine (Brostoff et al. 1996), and rupatadine (Gimenez-Arnau et al. 2007). These clinical studies have resulted in the recommendation of second-generation H_1R antihistamines as the standard of care for patients with urticaria, according to several clinical guidelines and expert opinions (Kaplan 2002; Powell et al. 2007; Khan 2008; Zuberbier et al. 2009; Ortonne 2011). Of note, the clinical data are mainly concerned with chronic idiopathic urticaria and, with the exception of several studies of cold urticaria (Bonadonna et al. 2003; Juhlin 2004; Magerl et al. 2007; Siebenhaar et al. 2009; Krause et al. 2013), the benefits of treatment for other physical urticarias (e.g., heat and solar) have not been well characterized.

However, not all chronic urticaria patients experience complete symptomatic relief with second-generation H_1R antihistamines. Some of these subjects are given sedating first-generation H_1R antihistamines, especially when the symptoms interfere with sleep at night. In clinical guidelines it has been recommended that these refractory patients may benefit from increasing the dose of the second-generation H_1R antihistamine up to four times the standard dose (Zuberbier 2012). Clinical data showing greater efficacy with increasing doses of levocetirizine, desloratadine, and rupatadine have been used to support this claim (Kameyoshi et al. 2007; Gimenez-Arnau et al. 2009; Siebenhaar et al. 2009; Staevska et al. 2010). One study compared the effects of increasing the dose of either levocetirizine or desloratadine (Staevska et al. 2010). Urticaria patients were randomized in a blinded fashion to 5 mg of either levocetirizine or desloratadine. After 1 week, patients who did not respond were instructed to double the dose and then to double the dose again if they did not respond after an additional week. Increasing the dose of either drug was associated with an increase in treatment success rate, suggesting that the higher doses were beneficial in patients who did not respond to lower doses. However, it should be noted that in this study, the subjects were not blinded to the increase in dose. An early study with fexofenadine showed an advantage of doses of

Table 3 Clinical studies for selected H_1R ligands

	Clinical studies		
Antihistamine	Histamine itch	Urticaria	Allergic rhinitis
Cetirizine	Coulie et al. (1991)	Breneman (1996)	Howarth et al. (1999)
	Levander et al. (1991)		Murray et al. (2002)
	Lahti and Haapaniemi (1993)		Noonan et al. (2003)
	Furue et al. (2001)		
	Morita et al. (2005)		
	Weisshaar et al. (1997)		
Mepyramine	Hagermark et al. (1979)		
Chlorpheniramine	Davies and Greaves (1980)		
	Davies et al. (1979)		
Chlorcyclizine	Hagermark (1973)		
Levomepromazine	Hagermark (1973)		
Acrivastine	Lahti and Haapaniemi (1993)		
Levocetirizine	Clough et al. (2001)	Nettis et al. (2006)	Potter (2003)
	Denham et al. (2003)		
Loratadine	Clough et al. (2001)	Dubertret et al. (1999)	Oei (1988)
	Kupczyk et al. (2007)		Storms et al. (1989)
Epinastine	Furue et al. (2001)		
Olopatadine	Morita et al. (2005)		Meltzer et al. (2005)
			Ratner et al. (2005)
Hydroxyzine	Levander et al. (1991)		
Desloratadine	Denham et al. (2003)	Monroe et al. (2003)	Berger et al. (2002)
		Ortonne et al. (2007)	Kim et al. (2006)
Fexofenadine	Tanizaki et al. (2012)	Kaplan et al. (2005)	Bernstein et al. (1997)
			Wilson et al. (2002)
Bepotastine	Tanizaki et al. (2012)		
Bilastine		Zuberbier and Maurer (2010)	Kuna et al. (2009)
Mizolastine		Brostoff et al. (1996)	
Rupatadine		Gimenez-Arnau et al. (2007)	
Azelastine Nasal			LaForce et al. (2004)
			Lumry et al. (2007)

60–240 mg bid over 20 mg bid, but did not detect differences between the higher doses (Finn et al. 1999). However, an open-label study in Japanese subjects with chronic urticaria showed that 120 mg fexofenadine bid was more effective than 60 mg in reducing itch and severity score (Tanizaki et al. 2013a). This study also included a double-blind, placebo-controlled crossover study assessing the effects on histamine-induced wheal, flare, and itch in healthy volunteers. The higher dose of fexofenadine was more effective at reducing itch at early time points, but at 6 and 12 h after dosing, the results were the same for both doses. Similar effects on histamine-induced itch have been reported for increased doses of levocetirizine (Tanizaki et al. 2013b). Inhibition of the itch intensity and the wheal area was significantly greater with 10 mg levocetirizine compared to 5 mg at both 1 and 24 h. The difference at intermediate time points was not statistically significant. Increasing the dose of bilastine has also been reported to improve its effectiveness with respect to the wheal threshold in cold contact urticaria, but not on the pruritus score (Krause et al. 2013). It should be noted that it is currently unclear as to whether increasing the dose is more effective due to more complete inhibition of the H_1R- or to other non-H_1R-mediated anti-inflammatory properties (Wang et al. 2005; Weller and Maurer 2009).

Mosquito bites also cause pruritic wheals that are similar to those seen in urticaria and are mediated in part by the local release of histamine (Horsmanheimo et al. 1996). As with urticaria, H_1R antihistamines have shown efficacy in clinical trials with ebastine, cetirizine, and levocetirizine decreasing bite pruritus by 60–70 % (Reunala et al. 1993; Karppinen et al. 2000, 2006).

The H_1R also mediates histamine-induced nasal itch. Nasal challenge with histamine or the H_1R agonist, betahistine, leads to itching, congestion, rhinorrhea, and sneezing (Mygind 1982; Miadonna et al. 1987; Shelton and Eiser 1994). Antihistamines that target the H_1R completely block all of the histamine-induced symptoms, with the exception of congestion, indicating that these effects are due to activation of the H_1R (Secher et al. 1982; Kirkegaard et al. 1983; Mygind et al. 1983; Naclerio and Togias 1991; Hilberg 1995; Wood-Baker et al. 1996; Wang et al. 2001; Taylor-Clark et al. 2005). For example, cetirizine was shown to almost completely inhibit histamine-induced itch, secretions, and sneezing, but only partial effects were observed for congestion-related parameters (Hilberg 1995).

The efficacy of H_1R antihistamines in blocking histamine-induced nasal symptoms provides a mechanistic rationale for their effects in allergic rhinitis. Allergic rhinitis is a very common allergic disease with symptoms that include nasal and pharyngeal pruritus, as well as sneezing, rhinorrhea, coughing, and congestion: effects that are very similar to those observed with administration of histamine. H_1R antihistamines have been one of the mainstays for the treatment of allergic rhinitis ever since diphenhydramine, one of the first antihistamines, showed effects in patients with hay fever (Koelsche et al. 1945). Since then, several topical and oral H_1R antihistamines have been approved for use in allergic rhinitis based on controlled clinical studies (for a review see Benninger et al. 2010). Many H_1R antihistamines have shown efficacy in reducing nasal pruritus as well as other

symptoms in well-conducted, placebo-controlled studies in allergic rhinitis. These include bilastine (Kuna et al. 2009), cetirizine (Howarth et al. 1999; Murray et al. 2002; Noonan et al. 2003), desloratadine (Berger et al. 2002; Kim et al. 2006), fexofenadine (Bernstein et al. 1997; Wilson et al. 2002), levocetirizine (Potter 2003), and loratadine (Oei 1988; Storms et al. 1989) and the intranasal H_1R antihistamines, including azelastine (LaForce et al. 2004; Lumry et al. 2007) and olopatadine (Meltzer et al. 2005; Ratner et al. 2005). For example, 5 mg of desloratadine reduced nasal itching by about 0.7 points on a four-point scale in patients with seasonal allergic rhinitis compared to placebo, which only had a reduction of 0.4 points (Berger et al. 2002). A similar magnitude of effect was also seen for all of the other symptoms measured (Berger et al. 2002). There is also some limited evidence that the combination of an H_1R antihistamine with intranasal corticosteroids has an additive benefit on nasal itch (Simpson 1994; Anolik 2008; Ratner et al. 2008).

The H_1R also mediates ocular pruritus. H_1R antihistamines completely block the itch induced with ocular administration of histamine in addition to having a partial effect on redness (Abelson et al. 1980; Kirkegaard et al. 1982; Abelson and Smith 1988), supporting their use in allergic conjunctivitis. This ocular allergy is triggered by exposure of the conjunctiva to environmental allergens that initiate an inflammatory cascade. One of the primary symptoms is pruritus, but tearing, lid and conjunctival edema and erythema, and photophobia also occur. In clinical studies, the symptoms of allergic conjunctivitis are induced by the application of allergen in a controlled setting. Antihistamines that target the H_1R such as alcaftadine (Greiner et al. 2011; Torkildsen and Shedden 2011), azelastine (Ciprandi et al. 1997; Horak et al. 1998), bepotastine (Abelson et al. 2009; Macejko et al. 2010), emedastine (Abelson and Kaplan 2002; D'Arienzo et al. 2002; Borazan et al. 2009), epinastine (Lanier et al. 2004; Whitcup et al. 2004), ketotifen (Crampton 2002; Greiner and Minno 2003), levocabastine (Zuber and Pecoud 1988; Abelson et al. 1995), and olopatadine (Abelson 1998; Spangler et al. 2001; Mah et al. 2007) demonstrated superiority over placebo in such studies. For example, both alcaftadine and olopatadine were able to inhibit ocular itching after conjunctival allergen challenge with a mean difference compared to placebo of about 1.7–1.9 points on a 0–4 pruritus scale (Greiner et al. 2011). In a natural allergen exposure setting, an ophthalmic solution of bepotastine gave a mean improvement of 30–40 % greater than placebo (Carr et al. 2013). These drugs are able to reduce many of the symptoms of allergic conjunctivitis including pruritus and are typically used as first-line therapy for the disease. Topical ocular H_1R antihistamines appear to have superior efficacy to oral antihistamines (Abelson and Welch 2000; Spangler et al. 2003), although the combination of ocular and oral H_1R antihistamines is superior to either treatment alone (Abelson and Lanier 1999; Crampton 2003). Finally, head-to-head studies have shown that topical H_1R antihistamines are more effective than mast cell stabilizers, such as cromolyn or nedocromil (Orfeo et al. 2002; Greiner and Minno 2003), or topical NSAIDS such as ketorolac (Discepola et al. 1999; Yaylali et al. 2003).

Despite the successes in treating itch associated with urticaria, allergic rhinitis, and allergic conjunctivitis, not all pruritic diseases are controlled by H_1R antihistamines. One such disease is atopic dermatitis, which is an inflammatory skin disease where pruritus is one of the most common and characteristic symptoms (Williams 2005). Despite evidence of elevated histamine levels and increased numbers of mast cells in pruritic skin of atopic dermatitis patients (Johnson et al. 1960; Juhlin 1967; Ikoma 2009), there is little clinical evidence that H_1R antihistamines have any effect. Several clinical studies have been conducted, and reviews of these studies suggest that any effect seen is due to sedation by blocking the central H_1R or off-target anti-inflammatory or skin barrier effects (Klein and Clark 1999; Akdis et al. 2006; Amano et al. 2007; Tamura et al. 2008; Saeki et al. 2009; Buddenkotte et al. 2010).

Other pruritic diseases where antihistamines that target the H_1R are ineffective are psoriasis, cutaneous T-cell lymphomas (CTCL), and chronic cholestatic liver disease. Psoriasis symptoms include inflamed, dry and scaling skin lesions that are pruritic in many patients (Yosipovitch et al. 2000). Histamine has been associated with the disease; it has been reported that histamine is increased in psoriatic skin and in addition there are increases in the number of mast cells and in particular degranulated mast cells (Brody 1984; Krogstad et al. 1997). However, no controlled clinical trials have been conducted, and it is not thought that H_1R antihistamines are effective in treating the pruritus of psoriasis. Clinical studies have been conducted testing H_1R antihistamines in the itch associated with CTCL, but it was found that they were not effective (Meyer et al. 2010; Ahern et al. 2012). Pruritus is also a frequent symptom in patients with either primary biliary cirrhosis or primary sclerosing cholangitis (Wiesner et al. 1985; Mela et al. 2003), but histamine is not thought to play a major role, although first-generation H_1R antihistamines are prescribed for their sedative effects (Herndon 1975; Bergasa and Jones 1991). Similarly, histamine has been discounted as cause for pruritus in patients with end-stage renal disease. With the exception of a few small studies where doxepin, ketotifen, and terfenadine showed some benefit, trials of oral H_1R antihistamines have not in general demonstrated efficacy in relieving uremic pruritus (Russo et al. 1986; Francos et al. 1991; Pour-Reza-Gholi et al. 2007). Despite the lack of clinical evidence, first-generation H_1R antihistamines are occasionally used for their sedative properties.

4 The Histamine H_2 Receptor

After the discovery of the first antihistamines, it was recognized that they could not block all of the physiological effects of histamine. It was proposed that the receptor blocked by the known antihistamines be called the H_1 receptor and that another histamine receptor mediated the histamine role in functions such as gastric acid secretion and the positive chronotropic response in isolated atria (Ashford et al. 1949; Trendelenburg and Hobbs 1960; Ash and Schild 1966). The receptor that modulates gastric acid secretion was defined as the H_2 receptor (H_2R) after

specific ligands were found that block this effect of histamine (Black et al. 1972; Wyllie et al. 1972). While the H_2R is expressed in many tissues and cell types, its most clinically relevant pharmacological activity is mediated via its expression on parietal cells in the gastric mucosa. Parietal cells are the main acid-secreting cells in the gastrointestinal system. Histamine release from enterochromaffin-like cells enhances this secretion by activating H_2R on the parietal cells. Therefore, antagonists of the H_2R will decrease gastric acid secretion. This has led to the clinical use of such antagonists for lowering gastric acid levels in patients with gastric or duodenal ulcers or with gastroesophageal reflux disease. However, since the development of proton pump inhibitors, H_2R antagonists are no longer considered the frontline therapy. The most commonly used H_2R antagonists are cimetidine, ranitidine, and famotidine.

There has been some work suggesting that the H_2R is involved in dermal responses to histamine. In one study, neither the H_2R antagonist cimetidine nor the H_1R antagonist chlorpheniramine was able to shift the threshold for itch induction in response to increasing concentrations of histamine, although chlorpheniramine exhibited a trend toward increasing this threshold when compared to placebo (Davies et al. 1979). These results may have been complicated by a high placebo response. Nevertheless, the combination of cimetidine and chlorpheniramine did significantly suppress histamine-induced itch (Davies et al. 1979). A second paper indicated that both the H_2R antagonist metiamide and cimetidine given intradermally could inhibit histamine-induced itch, but the inhibition was modest and not dose dependent, leading the authors to conclude that the effects were not due to the H_2R (Hagermark et al. 1979). Another study showed that the H_2R antagonist ranitidine given orally was able to inhibit itch when subjects were simultaneously given skin-prick testing of histamine, codeine, and grass pollens (Kupczyk et al. 2007). The levels of inhibition were statistically significant compared to placebo and similar in magnitude to the H_1R antagonist loratadine, but since all skin tests were given at the same time, it was impossible to tell if there was any specificity to the response. The effects of H_2R antagonists on histamine-induced wheal and flare responses are modest at best (Marks and Greaves 1977; Hagermark et al. 1979; Meyrick-Thomas et al. 1985a, b; Kupczyk et al. 2007). Finally, preclinical data do not show any effect of H_2R antagonists on histamine-induced itch in mice (Bell et al. 2004; Dunford et al. 2007). Therefore, the current data do not support a major role for the H_2R in mediating histamine effects in the skin. This also appears to be true in the nasal mucosa where H_2R antagonists do not reduce symptoms induced by histamine, although there is some indication of an effect in combination with H_1R antagonists (Secher et al. 1982; Havas et al. 1986; Wood-Baker et al. 1996).

Despite this, H_2R antagonists are sometimes prescribed to treat itch in refractory chronic urticaria and psoriasis. Currently, there is little clinical trial evidence supporting this use. Ranitidine failed to show benefit in a large placebo-controlled study in psoriasis patients (Zonneveld et al. 1997). In addition, a Cochrane review concluded that the data on the use of H_2R antagonists did not support their use in urticaria (Fedorowicz et al. 2012). However, one study in subjects with acute

allergic conditions suggested that a combination of ranitidine and diphenhydramine could reduce wheal formation in subjects with urticaria (Lin et al. 2000). Therefore, currently there is no conclusive clinical evidence that H_2R antagonists are effective against itch in either of these two conditions.

5 The Histamine H_3 Receptor

Like the H_2R, the H_3R was identified based on a pharmacological activity of histamine that could not be attributed to the known receptors. It was shown that histamine inhibited its own release in neurons but that neither H_1R nor H_2R ligands could reproduce this effect. Although the H_3R was defined on a pharmacological basis in 1983 (Arrang et al. 1983), it was not cloned until years later (Lovenberg et al. 1999). The H_3R is a presynaptic autoreceptor that is expressed on histamine synthesizing neurons that project throughout all major areas of the brain (Panula and Nuutinen 2013). The receptor also acts as a heteroreceptor on non-histamine-containing neurons in both the central and peripheral nervous systems where it impacts the release of a variety of neurotransmitters (Panula and Nuutinen 2013). Histamine and the other neurotransmitters modulated by the H_3R play a role in a variety of physiological functions such as sleep/wake cycles, cognition, and attention. This, therefore, has attracted a lot of interest in developing H_3R ligands as treatments for a variety of neurological disorders. Antagonists to the H_3R have been shown to reduce daytime sleepiness in patients with narcolepsy or Parkinson's disease (Lin et al. 2008; Arnulf 2009; Iannone et al. 2010; Inocente et al. 2012). Positive results have been reported with one H_3R antagonist in a clinical study in patients with attention-deficit hyperactivity disorder (Schwartz 2009); however, others have not shown a benefit (Kuhne et al. 2011; Herring et al. 2012; Weisler et al. 2012). In addition to these neurological disorders, H_3R antagonists have also been studied in nasal allergen challenge models in humans where reductions in nasal symptoms, including itch, were observed (Stokes et al. 2012; Barchuk et al. 2013).

Expression of the H_3R in the nervous system suggests that it could be involved in the transmission of neuronal itch signals. However, systemic administration of a selective H_3R antagonist could not block histamine-induced itch in mice (Dunford et al. 2007). The story appears to be different when the antagonists are given locally. Scratching could be induced in mice by intradermal injection of iodophenpropit, clobenpropit, or thioperamide, H_3R antagonists that also have H_4R activity (Hossen et al. 2003; Sugimoto et al. 2004). The scratching induced by iodophenpropit and clobenpropit still occurred in H_1R-deficient mice and could not be inhibited by H_1R antagonists (Hossen et al. 2003, 2006). As further evidence for the role of the H_3R in this effect, the H_3R/H_4R agonist R-α-methylhistamine was able inhibit the scratching induced by thioperamide (Sugimoto et al. 2004). These previous studies employed compounds with both H_3R and H_4R activity, but the highly selective H_3R antagonist, pitolisant, has shown the same effect. Intradermal injection of pitolisant induced scratching in mice and this effect was completely

blocked by the H_3R agonist, immethridine (Rossbach et al. 2011). Of particular interest is the fact that the scratching could also be partially inhibited by either the H_1R antagonist, cetirizine, or the H_4R antagonist, JNJ 7777120, with the combination completely inhibiting scratching. All three receptors were found to be expressed on TRPV1-positive dorsal root ganglion neurons, and in these neurons, pitolisant induced a calcium response similar to that of an H_1R or H_4R agonist (Rossbach et al. 2011). The mechanisms of the effects of the H_3R antagonist are not clear, but it is known that activation of the receptor can inhibit neuronal histamine release as well as the release of other neurotransmitters (Panula and Nuutinen 2013). Therefore, the H_3R may serve as an inhibitory receptor in the peripheral or central nervous system that dampens histamine signals. Antagonism of the receptor could lead to enhanced release of histamine or other neurotransmitters that either directly or indirectly activate itch pathways. Ligands for the receptor are still in clinical development and so there are limited tools to address this in humans. Kavanagh et al. (1998) studied intradermal injections of the H_3R agonist, R-α-methylhistamine, in humans and showed that it caused a weak wheal and flare response; however, no assessments of itch were included. One study has shown an effect of an H_3R antagonist on nasal itch in response to allergen challenge. Here the H_3R antagonist, JNJ 39220675, given orally, significantly reduced the itch component of the total nasal symptom score compared to placebo as was the total symptom score itself (Barchuk et al. 2013). Pseudoephedrine was also included in the study, and while it did have a significant reduction in the total nasal symptom score versus placebo, there was no effect on the itch component of the score. For the H_3R antagonist, the other three components of the total nasal symptom score were also reduced so it is difficult to determine whether the effects on itch are direct or indirect. While the hypothesis that the H_3R may be involved in itch in humans is intriguing, more clinical work is needed.

6 The Histamine H_4 Receptor

With the cloning of the H_3R, searches of genomic databases using the H_3R sequence uncovered a novel receptor that bound to histamine (O'Donnell et al. 2006). The discovery of histamine H_4 receptor (H_4R) was almost simultaneously reported by six different groups in 2000–2001 (Nakamura et al. 2000; Oda et al. 2000; Liu et al. 2001a; Morse et al. 2001; Nguyen et al. 2001; Zhu et al. 2001). After the identification of the new receptor, investigators searched the previous literature to find examples of histamine function that were not mediated by the H_1R, H_2R, or H_3R. The first example noted was an increase in calcium induced in eosinophils treated with histamine. Here, the rank order of potency of histamine receptor agonists did not match the pharmacology of the known histamine receptors (H_1R–H_3R), and it was speculated that this represented a novel receptor (Raible et al. 1994). Pharmacological characterization of the H_4R on eosinophils identified it as the receptor that mediated the calcium response (Buckland et al. 2003; Ling et al. 2004).

Much of the attention as to the physiological function of the H_4R has focused on its role in the immune response primarily driven by the original observations that the receptor was most highly expressed on immune cells. Anti-inflammatory activity of antagonists for the receptor has been noted in preclinical models of colitis, asthma, allergic rhinitis, and atopic dermatitis (Varga et al. 2005; Dunford et al. 2006; Seike and Furuya 2007; Deml et al. 2009; Takahashi et al. 2009; Cowden et al. 2010; Suwa et al. 2011; Ohsawa and Hirasawa 2012; Shiraishi et al. 2013). In addition to inflammatory conditions, the H_4R has also been suggested to mediate nervous system disorders, based on expression in the brain, spinal cord, and dorsal root ganglion although this data is controversial (Strakhova et al. 2009; Galeotti et al. 2013).

Preclinical studies suggest a role of the H_4R in itch. Histamine-induced itch in mice was shown to be reduced in mice that lack the H_4R and in ones that are treated with H_4R antagonists (Dunford et al. 2007; Yamaura et al. 2009; Shin et al. 2012). Further support of a role for the H_4R in mediating pruritus is given by the fact that agonists to the H_4R can induce scratching in mice when injected intradermally, although not when administered to H_4R-deficient mice (Bell et al. 2004; Dunford et al. 2007; Yu et al. 2010). These findings have now been validated in humans. A selective H_4R antagonist, JNJ 39758979, was shown to inhibit histamine-induced pruritus in healthy volunteers (Kollmeier et al. 2014). This was clearly attributable to the H_4R as the H_1R-mediated wheal and flare responses were no affected. These are the first clinical results showing the therapeutic utility of H_4R antagonists.

Antagonists to the H_4R have also been shown to inhibit itch induced by other pruritogens such as substance P or H_3R antagonists (Yamaura et al. 2009; Rossbach et al. 2011). The H_4R also appears to mediate itch driven by allergic responses to haptens. Acute application of fluorescein isothiocyanate to sensitized mice led to immediate scratching behavior followed by dermal inflammation. The H_4R antagonist, JNJ 7777120, was able to inhibit the scratching and the inflammation in this model (Cowden et al. 2010). JNJ 7777120 also blocked itch generated by the acute application of either toluene-2,4-disiocyanate or 2,4-dinitrochlorobenzene, but in this case, there was no effect on the inflammation (Rossbach et al. 2009). A role has also been observed in more chronic models. One study used application of 2,4,6-trinitrochlorobenzene three times a week over 99 days (Suwa et al. 2011). Starting on day 63, JNJ 7777120 was given orally once a day, and both the scratching and the clinical score of skin symptoms were inhibited, whereas the H_1R antagonist fexofenadine had no effect on either parameter (Suwa et al. 2011). Effects on inflammation were also noted in a similar study (Matsushita et al. 2012). When NC/Nga mice where sensitized and then challenged once a week for 10 weeks with picryl chloride, JNJ 7777120 reduced the scratching to a similar extent as the H_1R antagonist olopatadine (Ohsawa and Hirasawa 2012). However, this effect may be specific for hapten allergens; when a protein allergen was used to induce dermatitis in NC/Nga mice, the H_4R antagonists, JNJ 7777120 and JNJ 28307474, failed to inhibit scratching or reduce disease scores (Kamo et al. 2014). Similar results on lesion scores were found in a model where dogs were challenged with

Dermatophagoides farinae (house dust mite), but scratching was not assessed (Baeumer et al. 2011).

Similar effects are seen when studies were conducted looking at ocular or nasal itch. Topical JNJ 7777120 was shown to be as effective as the H_1R antagonist, levocabastine, in reducing scratching associated with ocular application of histamine, but it did not impact the symptom score (Nakano et al. 2009). When allergen was used to induce the ocular symptoms, a trend toward inhibition of scratching was seen for both drugs, but did not reach statistical significance. In addition it was found that the H_4R agonist, 4-methylhistamine, could induce scratching and allergic-like symptom when given ocularly and this could be inhibited by JNJ 7777120, but not levocabastine (Nakano et al. 2009). Both JNJ 7777120 and levocabastine inhibited scratching and symptoms induced by the H_1R agonist, HTMT (Nakano et al. 2009). Of potential interest, alcaftadine, a topical antihistamine approved for allergic conjunctivitis, does exhibit some H_4R antagonist activity that may contribute to its efficacy (Namdar and Valdez 2011; Gallois-Bernos and Thurmond 2012). In an allergen-induced allergic rhinitis model in mice, JNJ 7777120 was able to suppress the sneezing and nasal rubbing to a similar extent as ketotifen when given intranasally or orally. Improvement in upper airway function has also been shown in another model of allergic rhinitis with either JNJ 7777120 or H_4R-deficient mice (Shiraishi et al. 2013).

One common finding in the preclinical models is that there may be a benefit of combining an H_4R antagonist with an H_1R antagonist. The combination of JNJ 7777120 and diphenhydramine completely eliminated histamine-induced itch in mice, and the residual scratching to histamine observed in H_4R-deficient mice could be completely eliminated by an H_1R antagonist (Dunford et al. 2007). The combination of an H_4R antagonist and an H_1R antagonist was also more effective in inhibiting itch induced by acute application of toluene-2,4-disiocyanate and fluorescein isothiocyanate, as well as chronic picryl chloride-induced itch (Rossbach et al. 2009; Cowden et al. 2010; Ohsawa and Hirasawa 2012). The combination was also more effective on the skin lesions in the chronic models (Matsushita et al. 2012; Ohsawa and Hirasawa 2012). In the allergen-induced ocular model, neither JNJ 7777120 nor levocabastine had a statistically significant effect on scratching or symptoms, but the combination of the two drugs was effective (Nakano et al. 2009). These data suggest that for both itch and inflammation, the combination of an H_1R antagonist with an H_4R antagonist may be more effective than either on its own.

The mechanism by which the H_4R drives pruritic responses is still unknown. In mice it was shown that H_4R-mediated scratching did not require mast cells and the scratching could not be restored by reconstituting H_4R-deficient mice with wild-type bone marrow (Dunford et al. 2007). These results show that in mice, the effect is not mediated by hematopoietic cells. The H_4R effects are most likely modulated via specific neuronal pathways as has been shown for the H_1R. In support of this hypothesis, there is some evidence, albeit controversial, that the H_4R is expressed in the spinal cord, dorsal root ganglia, and brain. Expression has been detected by RT-PCR in several regions of the human brain including the amygdala, cerebellum,

corpus callosum, cortex frontal cortex, hippocampus, and thalamus (Strakhova et al. 2009). Immunohistochemistry has detected H_4R expression in laminae I–VI of the cerebral cortex with the strongest expression in layer IV (Connelly et al. 2009), although the selectivity of the antibody used has been questioned (Beermann et al. 2012). Expression has also been shown in the hippocampus, granule cell layer of the cerebellum, and the thalamus (Connelly et al. 2009). Of note, in a panel of human tissues including the CNS regions, heart, liver, and spleen, H_4R mRNA expression was highest in the spinal cord (Strakhova et al. 2009). Expression in mouse and rat brains appeared to be similar. In the rat, expression of the H_4R is prominent in dorsal root ganglion neurons of all size classes, with roughly one third of neurons showing H_4R immunoreactivity. H_4R immunoreactivity is also present in the spinal cord, in a pattern consistent with primary afferent terminal staining, in laminae II and IV and more intensely in laminae I–II (Strakhova et al. 2009). In whole cell clamp recordings of layer IV somatosensory cortex cells from mice, activation of the H_4R induced hyperpolarization in the majority of neurons (Connelly et al. 2009). In addition it has been shown that an H_4R antagonist can block the depolarization-induced firing of rat vestibular ganglion neurons (Desmadryl et al. 2012). The receptor also mediates action potential discharge in human submucous neurons (Breunig et al. 2007). In the mouse, skin-specific sensory neurons have been identified that express functional H_4R (Rossbach et al. 2011). Direct stimulation of neurons by compound 48/80 induces scratching in mice that is inhibited by an H_4R antagonist or in H_4R-deficient mice indicating that the H_4R is downstream of sensory fiber activation (Dunford et al. 2007). The scratching induced by compound 48/80 is not due to degranulation of mast cells since it still occurs in mast cell-deficient mice and it is known to activate sensory neurons directly (Eglezos et al. 1992; Dunford et al. 2007). Therefore, the current data suggest that itch stimuli in the skin, whether due to histamine or other pruritogens, lead to the activation of the H_4R on sensory neurons which then transmit the signal to the brain. Whether these pathways are similar to those mediated by the H_1R is currently unknown, but in the work of Rossbach et al. (2011), none of the mouse neurons tested responded to both an H_1R agonist and an H_4R agonist. The possible neuronal activity of the H_4R may also explain the efficacy of H_4R antagonists in both nociceptive and neuropathic pain models (Coruzzi et al. 2007; Altenbach et al. 2008; Cowart et al. 2008; Hsieh et al. 2010).

All of the preclinical data support the exploration of the effectiveness of H_4R antagonists for the treatment of pruritus that is not well controlled by the current antihistamines that target the H_1R or H_2R (Fig. 1). One particular disease of interest is atopic dermatitis since, as discussed above, H_1R antihistamines are generally considered ineffective (Klein and Clark 1999). The role of the H_4R in histamine-induced itch in humans and in numerous preclinical models of itch, including some thought to mimic atopic dermatitis, suggests that the H_4R may play a major role in controlling histamine effects in this disease. In addition, there is other evidence for a role of the receptor in mediating the inflammation in atopic dermatitis. The H_4R is

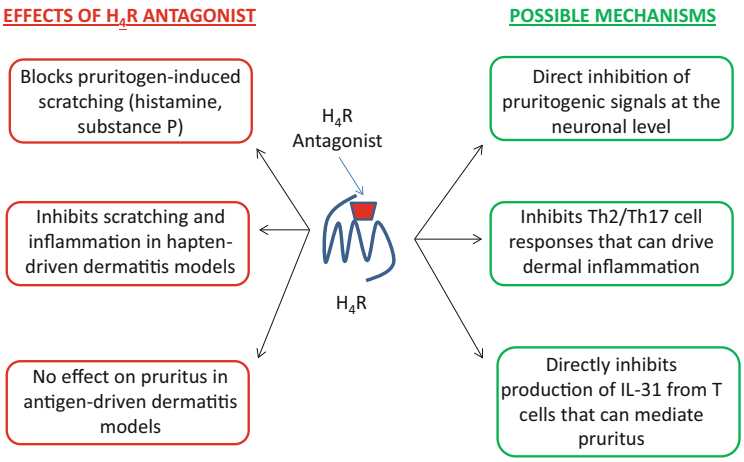

Fig. 1 The preclinical evidence for a role of the H_4R in mediating itch and possible mechanisms involved

expressed on keratinocytes, fibroblasts, and inflammatory dendritic epidermal cells that are associated with atopic dermatitis (Bäumer et al. 2008; Dijkstra et al. 2008; Ikawa et al. 2008; Gschwandtner et al. 2011a, b; Glatzer et al. 2013). Proliferation of keratinocytes from atopic dermatitis has been shown to be increased upon activation of the H_4R (Glatzer et al. 2013). The receptor also mediates the production of IL-31 by Th2 T cells from atopic dermatitis patients (Gutzmer et al. 2009). IL-31 is thought to play a role in mediating both pruritus and inflammation in atopic dermatitis (Sonkoly et al. 2006). In preclinical models, H_4R antagonists reduce the production of inflammatory cytokines and the infiltration of inflammatory cells (Cowden et al. 2010; Seike et al. 2010; Suwa et al. 2011; Matsushita et al. 2012; Ohsawa and Hirasawa 2012). For example, in the mouse fluorescein isothiocyanate-induced dermatitis model, treatment with the H_4R antagonist, JNJ 7777120, reduced the tissue accumulation of eosinophils and mast cells (Cowden et al. 2010). Furthermore, reductions in tissue levels IL-4, IL-6, IL-1β, and TNF were observed (Cowden et al. 2010). Interestingly, as discussed above, the H_4R appears to play a stronger role in models driven by haptens instead of proteins. The difference between hapten and protein models may suggest a relationship to intrinsic versus extrinsic atopic dermatitis. Intrinsic atopic dermatitis is characterized by normal barrier function and low IgE levels compared to extrinsic atopic dermatitis where there is elevated IgE levels and impaired barrier function (Tokura 2010; Kabashima 2013). It has been speculated that extrinsic atopic dermatitis is driven by protein antigens whereas in intrinsic atopic dermatitis the antigens are not proteins (Tokura 2010). Perhaps of interest, it has been shown that intrinsic atopic dermatitis shows increases in Th17-associated cytokines (Suarez-Farinas et al. 2013) and in the fluorescein isothiocyanate dermatitis model an H_4R antagonist was shown to reduce IL-17 levels (Cowden et al. 2010). Expression of

the H_4R has been detected in Th17 cells from psoriasis patients where it modulates cytokine production (Mommert et al. 2012). This, in conjunction with the expression and effects on chemotaxis in plasmacytoid dendritic cells, suggest that the receptor may also play a role in psoriasis (Gschwandtner et al. 2011a).

7 Conclusions and Future Directions

Histamine is one of the best-characterized inducers of itch in humans. Most of its effects are mediated via activation of the H_1R, although there is growing speculation (and for the H_4R clinical data) that the H_4R and H_3R may also be involved in histamine-induced itch. Drugs that target the H_1R are quite effective in blocking acute itch in conditions such as acute urticaria, allergic rhinitis, and allergic conjunctivitis. However, these drugs are not thought to be effective in more chronic pruritic conditions such as atopic dermatitis and psoriasis. The field is anxiously awaiting the development of new drugs that target the H_3R and H_4R in hopes that they will be more effective in the treatment of chronic pruritus.

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Targeting Itch with Ligands Selective for κ Opioid Receptors

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Abstract

Several chemically diverse pruritogens, including bombesin, compound 48/80, norbinaltorphimine, and 5′-GNTI, cause rodents to scratch excessively in a stable, uniform manner and consequently provide convenient animal models of itch against which potential antipruritics may be evaluated, structure–activity relationships established, and the nature of spontaneous, repetitive behavior itself analyzed. Decreasing the number of scratching bouts in these apparently simple models has been the requisite first step in the progress of kappa opioid agonists such as nalbuphine, asimadoline, and CR845 toward clinical testing as antipruritics. Nalfurafine is the prime example of a kappa agonist spanning the developmental divide between scratching mice models and commercialization within 10 years. Patients undergoing hemodialysis and suffering from the itching associated with uremic pruritus, and potentially those inflicted with atopic dermatitis, are the beneficiaries.

Keywords

Arylacetamide • Benzomorphan • Bombesin • Compound 48/80 • CR845 • Gastrin-releasing peptide • GNTI • JDTic • Kappa opioid receptor • Mouse behavior • Nalfurafine • Norbinaltorphimine • Rat behavior • Scratching • Zyklophin

Abbreviations

A_{50} (antiscratch-50)	The dose of test compound that antagonizes pruritogen-induced scratching by 50 % on a scale that ranges from vehicle (control) value to 100 %
ACTH	Adrenocorticotropic hormone
ADL 10–0101	*N*-Methyl-*N*-[(1*S*)-(1-phenyl-2-pyrrolidinyl)ethyl]-2-methanesulfonamidylphenylacetamide methanesulfonate
EKC	Ethylketocyclazocine
GR 94839	4-Acetyl-1-(3,4-dichlorophenyl)acetyl-2-[(3-hydroxy-1-pyrrolidinyl)methyl]piperazine
GRP	Gastrin-releasing peptide
ICI 204,448	(*RS*)-[3-[1-[[3,4-Dichlorophenyl)acetyl]methylamino]-2-(1-pyrrolidinyl)ethyl]phenoxy]acetic acid
icv	Intracerebroventricular
JDTic	(3*R*)-7-Hydroxy-*N*-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl]-1,2,3,4-tetrahydro-3-isoquinoline-carboxamide

JNJ 10191584	1-[(5-Chloro-1*H*-benzimidazol-2-yl)carbonyl]-4-methylpiperazine
JNJ 39758979	(*R*)-4-(3-Amino-pyrrolidin-1-yl)-6-isopropyl-pyrimidin-2-ylamine
KOP	Kappa opioid
McN-A-343	4-(*m*-Chlorophenyl-carbamoyloxy)-2-butynyltrimethylammonium chloride
norBNI	Norbinaltorphimine
ODT8-SS	des-AA^{1,2,4,5,12,13}[D-Trp^{8}]somatostatin
RC-3095	[D-Tpi^{6} Leu^{13}ψ(CH_2NH)Leu^{14}]bombesin (6–14)
U-50,488	*trans*-(±)-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide

1 Introduction

This chapter describes a mainly behavioral analysis of prominent kappa (κ) opioid receptor ligands that either cause repetitive scratching episodes in rodents (kappa antagonists) or reduce the number of such bouts when elicited by various pruritogens in these animals (kappa agonists). Related work on the scratching associated with the spinal administration of mu (μ) opioid analgesics, and the alleviation of this recognized side effect, is reviewed by Dr. Ko in the next chapter (Ko 2015). Our approach has observed long-standing principles of classical in vivo pharmacology. For example, dose–response curves have been obtained in rodents for the kappa opioid ligands under test at behaviorally nondepressant doses and at times of peak effect; stereoselectivity has been examined; various routes of administration have been studied; and tolerance development has been investigated. The key issue being addressed here is as follows: to what extent has preclinical animal research from our laboratory, and elsewhere, contributed to the discovery and evaluation of druggable molecules that hold promise as novel antipruritic agents?

2 Kappa Receptors and Itch/Scratch: Background

Compounds classified as agonists at kappa opioid receptors first entered the pharmacological literature in the late 1970s after the influential report by Martin et al. (1976) introduced ketocyclazocine as the prototype kappa agonist in chronic spinal dogs. Several analgesics that featured in the basic and clinical research literature of that era possess various degrees of selectivity for kappa opioid

receptors (Martin et al. 1976; Yaksh and Wallace 2011). Examples include nalorphine, cyclazocine, ethylketocyclazocine, butorphanol, nalbuphine, pentazocine, and U50,488. These compounds each represent important stepping stones in the history of opioid pharmacology and have endured a proliferation of descriptors over the years, such as narcotic antagonist analgesic, mixed agonist–antagonist, mixed mu/kappa agonist, KOP, partial kappa agonist, kappa partial agonist, and full kappa agonist.

Early associations between kappa-type compounds and excessive self-scratching behavior (and, by implication, the sensation of itch) were noted in "kappa-dependent" patas monkeys (1) during abrupt withdrawal from multiple injections of either nalorphine or cyclazocine and (2) subsequent to challenge with naloxone, the universal opioid antagonist (Cowan 1973). Body scratching was not observed as a component of the *qualitatively different* abstinence syndrome in additional monkeys that received morphine thrice daily for 1 month. These findings were subsequently confirmed in rhesus monkeys (Gmerek et al. 1987) by testing U-50,488, an *N*-(2-aminocyclohexyl)arylacetamide that has become the standard reference kappa receptor agonist in preclinical opioid research (Szmuszkovicz 1999; Russell et al. 2014).

Numerous studies examining the pharmacological bases of peptide-induced overt, ongoing behavior in mice and rats were conducted during the 1980s at a time when kappa analgesics were being enthusiastically developed for eventual clinical testing. Examples of such kappa opioids include bremazocine (Römer et al. 1980), tifluadom (Römer et al. 1982), and spiradoline (Von Voigtlander and Lewis 1988). One common link between the two fields of research was naloxone, which was routinely used to unveil and/or confirm the type of receptor involved in (1) the actions and side effects of prospective kappa agonists and (2) the compulsive body grooming and/or scratching precipitated in rodents by, for example, bombesin (Katz 1980; Gmerek and Cowan 1983a; Van Wimersma Greidanus et al. 1985b), adrenocorticotropic hormone (Van Wimersma Greidanus et al. 1986), and somatostatin (Van Wimersma Greidanus et al. 1987).

Our own interest centered on bombesin, a tetradecapeptide originally isolated from the skin of the frog *Bombina bombina* (Anastasi et al. 1971) that caused excessive scratching in several species and to which tolerance did not develop (Kulkosky et al. 1982; Cowan et al. 1985; Van Wimersma Greidanus et al. 1985a; but see Su and Ko 2011). When given by the intracerebroventricular (icv) route to rats, bombesin precipitated quick onset (1–2 min), reproducible, and dose-related bouts of neck scratching by the hind paws for at least 45 min—excellent experimental conditions against which potential antagonists could be assayed. For example, negative results with mepyramine, diphenhydramine, hydroxyzine, cimetidine, and cyproheptadine indicated that histamine was not necessarily involved in bombesin-induced scratching (Gmerek and Cowan 1983c). Although gastrin-releasing peptide (GRP-27) (Masui et al. 1993; Jensen et al. 2008) and neuromedin B (Wada et al. 1991; Su and Ko 2011), two mammalian peptides that share close

structural homology with bombesin, also elicited scratching in rats, we selected bombesin as the standard/reference pruritogen, based on our prior behavioral data and on potency (bombesin > $GRP_{18–27}$ > neuromedin B) and efficacy (bombesin = $GRP_{18–27}$ > neuromedin B) considerations (Cowan 1988; Van Wimersma Greidanus and Maigret 1991).

This demonstration of a strong link between bombesin-like peptides and repetitive scratching in mice and rats thus provides the historical prelude to eventual reports, more than two decades later, championing the role of GRP as a key spinal neurotransmitter for mediating histamine-independent itch (Sun and Chen 2007; Sun et al. 2009; Liu et al. 2014), along with the associated controversy implicating natriuretic polypeptide B (Mishra and Hoon 2013; Goswami et al. 2014; Solorzano et al. 2015).

Critical points, relevant to this chapter, which emerged from the extensive "scratch" literature of the 1980s are summarized in Table 1. Additionally, early benzomorphan compounds, showing preferential agonism at kappa opioid receptors, antagonized icv bombesin-induced scratching in rats in a pharmacologically meaningful manner (Gmerek and Cowan 1984). Thus, the scratching was antagonized in a stereoselective and dose-related way by systemically administered benzomorphan analgesics such as ethylketocyclazocine (EKC). Of academic (and potentially clinical) significance, tolerance developed to this attenuating action of EKC. The important contribution of kappa receptors was further established through an experiment where mu opioid receptors were occluded by buprenorphine yet EKC was still able to antagonize bombesin-evoked scratching. Subsequent studies revealed chemically diverse kappa agonists (e.g., tifluadom, a benzodiazepine derivative, and U50,488) to be active in this rat scratch test. By way of contrast, morphine and other mu agonists were ineffective against bombesin when tested at behaviorally nondepressant doses (Cowan and Gmerek 1986). These early results provided points of reference and support for continued structure–activity experiments linking kappa agonism with suppression of

Table 1 Characteristics of icv bombesin-induced scratching in rats

Qualitatively different from ACTH-(1–24)-elicited body grooming[a]
Mechanistically different from the scratching associated with ODT8-SS, a somatostatin analogue[b]
Also observed after intraperiaqueductal[b] gray infusion and intrathecal administration[c] but not after intravenous injection
Unaffected by either hypophysectomy or adrenalectomy[b]
Not markedly affected by behaviorally nondepressant doses of haloperidol, morphine, naloxone, haloperidol, or antihistamines[b]
Provides a possible animal model for preclinical screening of potential antipruritic agents[d]
Antagonized in a dose-dependent manner by agonists at kappa opioid receptors[e]

[a]Van Wimersma Greidanus et al. (1985a)
[b,d]Gmerek and Cowan (1983a,b,c)
[c]Gmerek et al. (1983)
[e]Cowan and Gmerek (1986)

scratching and led to the eventual discovery of a clinical antipruritic candidate—nalfurafine (Phan et al. 2012).

In the 1990s, more selective kappa agonists were being evaluated as analgesics, and the question arose at the bench level as to whether such second-generation compounds might also possess antiscratch activity. Enadoline is just one example from the arylacetamide class that was tested in phase II clinical trials but, like so many similar analgesics possessing that chemical scaffold, was never commercialized (Hunter et al. 1990; Reece et al. 1994; Pande et al. 1996). The main reason for such failures is well known to analgesic researchers and can be stated bluntly in the presence of two worrisome side effects that have been intimately linked with the evolution of kappa agonists over the years: dysphoria and psychotomimesis (Kumor et al. 1986; Pfeiffer et al. 1986; Walsh et al. 2001). Despite kappa agonists being perceived as offering greater safety than traditional morphine-like compounds, a view based on the milder form of physical dependence that develops in animals, and limited actions on respiration and gastrointestinal transit, the associated psychotoxicity has dimmed the clinical prospects of these agents as analgesics (and possibly as potential antipruritics). One response to this situation has been to investigate the pharmacological profiles of peripherally directed kappa agonists given that kappa opioid receptors are present on the peripheral terminals of primary afferent neurons. ICI 204,448 (Shaw et al. 1989), GR 94839 (Rogers et al. 1992), and asimadoline (Barber et al. 1994) are examples of kappa analgesics that are believed to pass across the blood barrier with great difficulty, if at all. We compared ICI 204,448, a diarylacetamide derivative, with the centrally penetrating enadoline in the following two experimental models: (1) the rat paw formalin test for persistent pain (Wheeler-Aceto and Cowan 1991) and (2) the rat bombesin test for antiscratch activity. The subcutaneous antinociceptive-50 (A_{50}) value of enadoline against formalin-elicited late phase flinching was 0.19 mg/kg. The corresponding value for ICI 204,448 was 6.9 mg/kg which reflects suppression of inflammatory pain by means of peripheral kappa opioid receptors. The subcutaneous A_{50} value of enadoline against scratching elicited by bombesin was equally impressive (0.012 mg/kg), whereas ICI 204,448 showed no antipruritic potential in this procedure, the A_{50} value being greater than 30 mg/kg.

Results from this particular study reinforced the prevailing view that antagonism of bombesin-evoked scratching requires penetration across the blood–brain barrier and subsequent agonism at central kappa receptors, physicochemical attributes associated with enadoline but not with ICI 204,448. Inactivity against bombesin-induced scratching was nevertheless helpful for screening and characterizing in vivo those kappa agonists with the preferred *peripheral* locus of action. There was an obvious need for additional animal models that generate itch stimuli at local, peripheral sites. One such model made use of the pruritogenic properties of compound 48/80 in mice.

3 Compound 48/80-Induced Scratching in Mice

Pharmacologists and chemists were alerted to the possibility of establishing structure–activity relationships for potential antipruritic agents in mice with the publication of a standardized, apparently straightforward, and efficient methodology by Kuraishi and colleagues in 1995. Their approach did not involve the laborious procedure of cannulating the cerebroventricles of rats but, rather, depended on a single subcutaneous injection of pruritogen behind the neck of awake, ddY mice and the immediate monitoring of spontaneous bouts of scratching by hind paws at the injection site. Importantly, Kuraishi et al. (1995) concluded that the excessive scratching was associated with the sensation of itch (elicited by compound 48/80, a mast cell degranulator and pruritogen in humans) and not with the sensation of pain (provoked by either capsaicin or dilute formalin). The mechanism responsible for this repetitive behavior requires further study particularly since compound 48/80 still causes scratching in mast cell-deficient mice (Dunford et al. 2007) and, additionally, is only weakly active in Sprague Dawley rats (Thomsen et al. 2001).

The compound 48/80 behavioral test has face validity (symptom homology) and enjoyed subsequent popularity as an initial, general screen for detecting compounds with antiscratch activity in much the same way as the mouse abdominal constriction test (e.g., Pearl et al. 1968) has been used historically to obtain dose–response curves for possible antinociceptive agents. In both cases, activity in the screen may be taken as a positive indication of chemical progress, but implicating human itch or pain states would be premature in the absence of more comprehensive testing. That being said, it should be noted that Green et al. (2006) injected eleven inbred strains of mice with a different pruritogen (chloroquine, s.c., in the nape of the neck) and concluded that their results supported the utility of the Kuraishi model in the general area of itch research.

Our initial work was greatly influenced by findings from the Kuraishi group and involved assessing the antiscratch activities of subcutaneously administered opioids in Swiss Webster mice that were subsequently challenged with compound 48/80. Results from these early experiments revealed that neither naloxone (0.10–3 mg/kg) nor the standard kappa receptor antagonist, norbinaltorphimine (norBNI, 20 mg/kg at −15 h), had marked effects on compound 48/80-induced scratching. Notably for this review, both enadoline (0.0025–0.02 mg/kg) and ICI 204,448 (2.5–10 mg/kg) attenuated the scratching in a dose-related manner, giving antiscratch-50 values of 0.004 and 2.82 mg/kg, respectively (Cowan and Kehner 1997). Confidence in the pharmacological underpinnings of the Kuraishi model was reinforced by our observation of stereoselectivity for enadoline. Thus, as just noted, (−)-enadoline was active against compound 48/80-elicited scratching, whereas (+)-enadoline, which has very low affinity for kappa opioid receptors (Halfpenny et al. 1990), had no marked antiscratch effect (Kehner 2002) (Fig. 1 and Table 2).

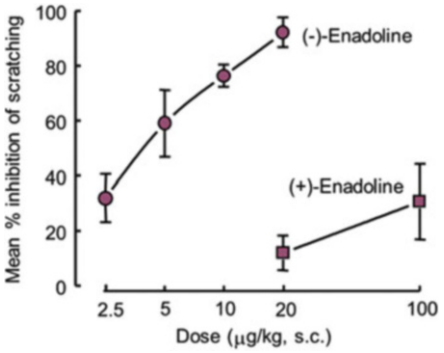

Fig. 1 Effects of (−)- and (+)-enadoline on scratching bouts in mice ($n = 7$–12). (−)-Enadoline (active enantiomer) dose-dependently antagonized scratching induced by compound 48/80 with an A_{50} value of 0.004 (0.002–0.005) mg/kg. (+)-Enadoline (essentially inactive enantiomer) had no marked antiscratch effect ($A_{50} > 0.10$ mg/kg). Both enantiomers were supplied by Parke-Davis, Cambridge, UK)

Table 2 Rank order of potency of opioid receptor ligands in suppressing repetitive scratching caused by compound 48/80 (2 mg/kg, s.c.) in mice[a]

Compound	A_{50} (mg/kg, s.c.)
(−)-Enadoline	0.004 (0.002–0.005)
Nalfurafine	0.007 (0.004–0.009)[b]
CR845	0.077 (0.035–0.24)[c]
Asimadoline	0.51 (0.28–0.76)
U-50,488	1.34 (1.23–4.54)
ICI 204,448	2.82 (1.38–4.26)
ADL 10-0101	13.6 (7.4–19.7)
(+)-Enadoline	>0.10
Naloxone	>3.0
norBNI	>20

[a]Data from Cowan lab
[b]Wang et al. (2005)
[c]CR845 injected i.v.

This result is in line with the comparable finding of stereoselective antagonism of bombesin-elicited scratching in rats by ethylketocyclazocine, the benzomorphan analgesic (*vide supra*).

Kappa receptors seem to be involved in this blunting of 48/80-evoked scratching since the behaviorally neutral dose of norBNI (20 mg/kg at −15 h) antagonized the antiscratch activity of a submaximal dose of either enadoline (0.01 mg/kg) or ICI 204,448 (5 mg/kg). Overall, these results strengthened the proposition that kappa agonists as a class are potential antipruritic agents. Moreover, the activity of ICI

204,448 (in contrast to the inactivity of this compound in the rat bombesin scratch model) established, for the first time, the possibility of developing peripherally directed kappa agonists as therapeutically important antipruritics. The hydrophilic ICI 204,448 has been a valuable pharmacological tool in basic animal studies on itch. For example, the standard dose (5 mg/kg) provides a stable antagonism (50–70 %) of compound 48/80-provoked scratching for as long as 2 h (when given as a pretreatment in different groups of mice) and then gradually fades over the following 3 h. A second example of the usefulness of a kappa agonist with low CNS penetration was our demonstration of antagonism of chloroquine-induced, quick onset scratching in mice by ICI 204,448, this finding clearly implicating peripheral kappa receptors in the compulsive behavior (Inan and Cowan 2004). Parenthetically, it may be of clinical interest to note that nalfurafine (*vide infra*), the centrally active kappa agonist, also suppressed this chloroquine-elicited, histamine-independent, frenzied scratching when administered (either subcutaneously or orally) to additional mice (Inan and Cowan 2004). Next, we conducted experiments with ICI 204,448 to address the following unanswered pharmacological question: does tolerance develop to the antiscratch activity of a peripherally restricted kappa agonist in the compound 48/80 model? Two studies were initiated with the acceptance that some ICI 204,448 would likely cross the blood–brain barrier under the multiple dosing schedules utilized. When ICI 204,448 (5 mg/kg, s.c.) was administered to mice every other day for 5 days (i.e., on days 1, 3, and 5), in parallel with appropriate control groups, there was no behavioral tolerance (Fig. 2, top panel). In contrast, when different groups of mice were similarly injected with 5 mg/kg of ICI 204,448 for 5 consecutive days, tolerance clearly developed (Fig. 2, bottom panel). So, as with all whole animal tolerance experiments, the conclusion is only meaningful when linked and discussed with the specific dosing schedule employed.

In developing the chronology of the kappa antagonist-scratching story, it should be appreciated that parallel experiments with antihistamines also showed suppression of compound 48/80-evoked scratching. Thus, Sugimoto and colleagues (1998) called attention to histamine H-1 receptors by reporting that either chlorpheniramine or diphenhydramine antagonized subsequent scratching elicited by compound 48/80 (50 μg, s.c. in the nape of the neck) in BALB/c mice. More recent reports have stressed the prominent involvement of histamine H-4 receptors in mediating the compound 48/80 itch-associated response (e.g., Dunford et al. 2007). Structure–activity studies in the lowly mouse scratch test have encouraged further experimentation and, ultimately, demonstration of some efficacy for oral JNJ 39758979, a histamine H-4 antagonist, in attenuating histamine-induced pruritus in healthy (nonallergic) humans (Kollmeier et al. 2014).

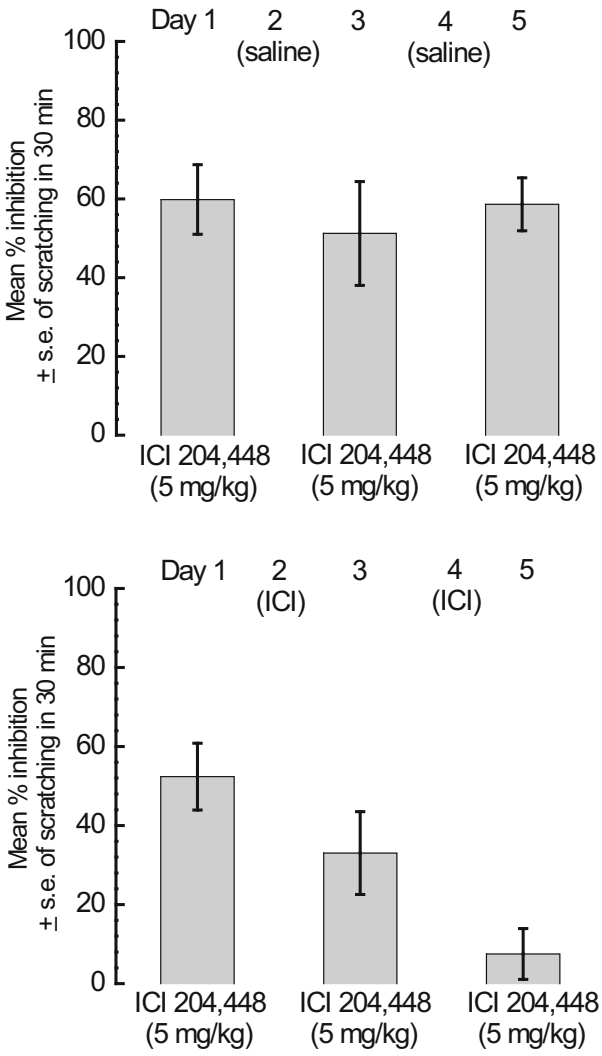

Fig. 2 The antiscratch effects of ICI 204,448 in the compound 48/80 mouse model were maintained after *every-other-day* injection of the kappa agonist for 5 days (*top panel*). In separate groups of mice, injected *every day* for 5 days, partial tolerance occurred on day 3, and full tolerance was demonstrated on day 5 (*bottom panel*). Each group represents the mean percent inhibition of scratching compared to controls ($n = 7$–12) (Kehner 2002)

4 Pruritogenic Activity of norBNI in Mice

Norbinaltorphimine (norBNI) is a bivalent ligand containing two naltrexone-derived pharmacophores (Fig. 3). Despite its description and widespread use as the prototype kappa receptor antagonist for almost three decades (Portoghese et al. 1987; McCurdy et al. 2006; Briggs and Rech 2009), the selectivity of norBNI (especially across the first hour or so after injection in rodents) has been questioned (Birch et al. 1987; Endoh et al. 1992; Spanagel et al. 1994). What is not in doubt is its long duration of action (a matter of weeks) as a kappa antagonist in mouse

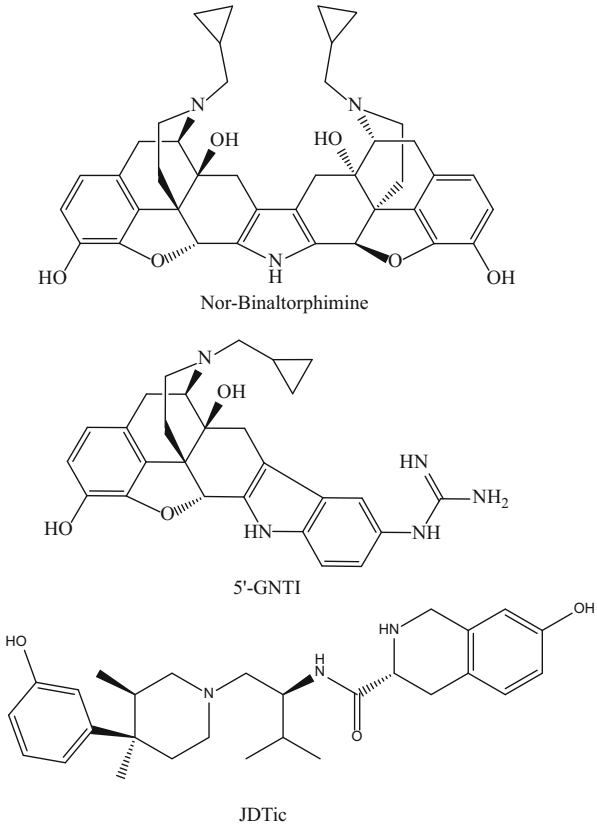

Fig. 3 Chemical structures of norbinaltorphimine, 5′-GNTI, and JDTic

antinociceptive tests (Endoh et al. 1992; Horan et al. 1992; Broadbear et al. 1994). This long duration of action is a property shared by at least two other kappa opioid antagonists/pruritogens, JDTic and 5′-GNTI (*vide infra*) (Carroll et al. 2004; Bruchas et al. 2007). Explanations include activation of the c-Jun N-terminal kinase 1, mitogen-activated protein kinase cascade and, in consequence, inhibition of signaling at the kappa receptor (Bruchas et al. 2007; Melief et al. 2011), and long-term presence of compound (norBNI) in the central nervous system of mice (Kishioka et al. 2013; Patkar et al. 2013).

All three compounds possess slow onsets of action as kappa antagonists (Broadbear et al. 1994; Carroll et al. 2004; Metcalf and Coop 2008). Neither attribute—delayed onset and long duration of action—was reported by Kamei and Nagase (2001) when they described, for the first time, the itch-associated properties of norBNI after subcutaneous injection (behind the neck) in ICR mice. Similarly, JDTic (0.30–5 mg/kg, s.c.), the phenylpiperidine-based kappa antagonist (Carroll and Carlezon 2013), caused quick onset scratching of very short duration in Swiss Webster mice (DiMattio, Liu-Chen, and Cowan, unpublished results).

The demonstration of repetitive scratching in mice by norBNI was notable for introducing a new class of pruritogen (and research tool) to the field of dermatopharmacology. Proposing an overall mechanism of action is no easy matter. norBNI caused no excessive scratching when given either icv to ddY mice (Umeuchi et al. 2003) or subcutaneously to rhesus monkeys (Ko et al. 2003). Kamei and Nagase (2001) reported that oral pretreatment with either chlorpheniramine or U-50,488 antagonized, in a dose-dependent manner, scratching precipitated by norBNI (10 mg/kg). On the basis of their interactional studies, these latter workers offered a conservative interpretation and speculated that the repetitive behavior was caused, in part, by (1) the release of histamine, followed by (2) antagonism of kappa opioid receptors.

5 Pruritogenic Activity of 5′-GNTI in Mice

We selected 5′-guanidinonaltrindole (5′-GNTI) for in-depth study as an alternative pruritogen to norBNI because of its superior potency and receptor selectivity as a kappa antagonist in standard smooth muscle preparations (Jones and Portoghese 2000; Stevens et al. 2000). Such was our thinking—associating kappa antagonism with body scratching—at the beginning of the 2000s (Cowan et al. 2002). Additionally, 5′-GNTI provided interesting structural novelty (Fig. 3), being the 5′-guanidinyl derivative of naltrindole the well-recognized nonpeptidic antagonist at *delta* opioid receptors (Portoghese et al. 1988, 1990).

5′-GNTI (0.03–3 mg/kg), but not naloxone (3 mg/kg), caused vigorous, compulsive scratching bouts within 3–5 min of subcutaneous injection in the nape of the neck of male Swiss Webster mice. 5′-GNTI was more efficacious and more potent (×44) than norBNI (Cowan and Inan 2009). A submaximal dose of 0.30 mg/kg of 5′-GNTI was chosen as standard for all experiments summarized below. Optimal scratching with this dose occurred between 10 and 30 min and faded gradually between 30 and 80 min. So, as with norBNI (Sect. 4), there is a temporal disconnect between the scratching phase and the time of peak effect of 5′-GNTI as a kappa antagonist (Munro et al. 2012). As of 2014, the display of scratching in mice as a consequence of kappa opioid antagonism (or not) represents a conundrum yet to be solved.

Scratching also occurred after intradermal injection of 5′-GNTI into the rostral back of mice but not when delivered by intraperitoneal, spinal (lumbar), or icv routes of administration. Sprague Dawley rats were essentially unaffected when given 5′-GNTI (0.30 or 3 mg/kg, s.c.) behind the neck, highlighting species as a variable in basic itch research. For context, Thomsen et al. (2001) reported dose-related scratching with serotonin in Sprague Dawley rats and characterized histamine, compound 48/80, and substance P as weak/inactive pruritogens in this strain.

The behavioral pharmacology of 5′-GNTI has been extended with the following observations (Inan et al. 2009a; Inan 2010; Cowan and Inan 2013): (1) tolerance to the scratch-inducing action of 5′-GNTI did not develop when mice were injected with the pruritogen once every day for 8 consecutive days, recalling a similar conclusion with bombesin in rats (Sect. 2); (2) the scratching was resistant to

antagonism by naloxone (3 mg/kg, s.c.) or norBNI (20 mg/kg, i.p. at −18 h) but suppressed by intradermal 2 % lidocaine; (3) 5′-GNTI precipitated scratching in mu, kappa, or delta opioid knockout mice (C57BL/6 J background) to the same extent as in respective wild-type littermates; (4) oral pretreatment at −45 min with either a histamine H-1 antagonist (fexofenadine, 20–60 mg/kg) or a H-4 antagonist (JNJ 10191584, 10–60 mg/kg) proved ineffective; and (5) 5′-GNTI was more efficacious and potent (12.5×) than the 6′-regioisomer which, nevertheless, was a very active pruritogen in mice. Both 5′-GNTI and 6′-GNTI therefore exhibit a common overt behavior despite the latter compound being described as a selective *agonist* for the spinal kappa–delta heteromer (Van Rijn et al. 2010) and also as a *biased agonist* at kappa receptors (Rives et al. 2012).

Additionally, we investigated if gastrin-releasing peptide (GRP) might play a role in kappa antagonist-evoked scratching particularly in view of the report by Sun and Chen (2007) claiming that the spinal receptor for GRP mediates scratching in mice receiving chemically diverse pruritogens. We pretreated mice with RC-3095 (10 or 30 mg/kg, s.c.) in the flank (Inan et al. 2011). This pseudopeptide is a GRP (BB_2) antagonist (Jensen et al. 2008; Andoh et al. 2011), which is bioavailable after systemic administration and which had no marked effect on the incidence of scratching associated with our standard dose of 5′-GNTI. We obtained similar negative results with [D-Phe^6]bombesin(6–13)methyl ester (2–100 nmoles intraspinally), the peptide antagonist of GRP (BB_2) receptors that was used previously by Sun and Chen (2007).

We submitted a sample of 5′-GNTI (Tocris Bioscience) to Caliper Life Sciences (now called PerkinElmer) for preliminary binding affinity screening at 1 μM against a panel of neurotransmitters, ion channels, steroids, second messengers, prostaglandins, growth factors, hormones, brain–gut peptides, and enzymes. Modest binding to muscarinic M-1 receptors was detected and subsequently confirmed by Munro et al. (2013). Our studies in mice involving interactions between 5′-GNTI and muscarinic M-1 agonists or antagonists are ongoing. A common interpretational problem in rodent behavioral research was encountered with intraspinal McN-A-343 (1.5–15 μg), the M-1 agonist: demonstration of statistically significant suppression of 5′-GNTI-evoked scratching (e.g., 70 %, $p < 0.01$ with 15 μg) despite the group mean from the 30 min observation session remaining impressively high (190 ± 35 [s.e.m.] scratches) (Inan et al. 2011).

So, as with many contemporary studies in the field of experimental pruritus, the following pesky question needs an answer: What is the pharmacological basis of the residual bouts of scratching often observed after incomplete antagonism of pruritogen-induced scratching, in this case, from pairing McN-A-343 with 5′-GNTI?

6 Behavioral Effects of Zyklophin in Mice

Zyklophin is a metabolically stable dynorphin analogue of 11 amino acids (Patkar et al. 2005). It is a selective kappa receptor antagonist that differs from the small molecule norBNI, 5′-GNTI, and JDTic, respectively, in being a cyclic peptide and in

possessing a much shorter duration of action as a kappa antagonist (<12 h) after subcutaneous administration in the mouse tail withdrawal test (Aldrich et al. 2009). The peptide structure and shorter duration of action notwithstanding, zyklophin (0.10–1 mg/kg, s.c.) caused Swiss Webster mice to scratch their necks excessively within 1 min of injection. The extent of this behavior was dose related with 1 mg/kg of the peptide eliciting around 350 bouts in the 30 min observation period. Most of the scratching occurred within 15 min of injection and was essentially over by +30 min.

Pretreating mice with norBNI (20 mg/kg, i.p. at −18 h) had no marked influence on scratching associated with a submaximal dose of zyklophin (0.30 mg/kg). This result calls to mind the ineffectual antagonism of 5′-GNTI-evoked scratching by norBNI mentioned in Sect. 5. Similarities between zyklophin and 5′-GNTI were also apparent in their respective abilities to provoke scratching in kappa receptor knockout mice (C57BL/6 J background), comparable to that of wild-type C57BL/6 J controls (DiMattio et al. 2014; Sect. 5). This work unveiled an additional finding: male, wild-type C57BL/6 J mice are much less sensitive to a standard dose of zyklophin than are corresponding Swiss Webster animals. Still to be determined is the molecular target(s) at which zyklophin acts to elicit scratching.

7 Antipruritic Profile of Nalfurafine (TRK-820)

Nalfurafine holds pride of place in the history of kappa opioid pharmacology. Although identified initially as a prospective analgesic, this indication was never realized clinically and the compound was recast and developed as an antipruritic. Introduced as a kappa agonist by Nagase and colleagues in 1998, this 4,5-epoxymorphinan represents the only marketed product to emerge from the active field of kappa opioid research. Nalfurafine (as Remitch® capsules) was launched in Japan in 2009 for the treatment of uremic pruritus in subjects with end-stage renal disease receiving hemodialysis and is currently a part of ongoing trials in the United States (uremic pruritus) and Japan (cholestatic itch).

Togashi et al. (2002) described the antiscratch activities of oral nalfurafine in ICR mice. Thus, the scratching invoked by either intradermally injected histamine or substance P was markedly suppressed by nalfurafine (0.10 mg/kg), and this action, in turn, was antagonized by norBNI, given subcutaneously at −24 h. The three main metabolites of nalfurafine were essentially inactive in the substance P itch model (Nakao et al. 2012). In our hands, nalfurafine was a very potent antagonist of either compound 48/80-induced scratching (Wang et al. 2005) or 5′-GNTI-induced scratching (Inan et al. 2009a) in Swiss Webster mice. In the latter study, nalfurafine was active when administered *either* before or after 5′-GNTI with doses that did not affect spontaneous locomotion. At the neuronal level, nalfurafine inhibited c-fos expression elicited by either 5′-GNTI or compound 48/80 in the dorsal horn of mouse spinal cord. This result supports a spinal locus of action for nalfurafine as an antiscratch agent (but not exclusively) against both pruritogens (Inan et al. 2009b).

Tolerance did not develop over 10 daily injections of nalfurafine (0.02 mg/kg, s.c.) to its antiscratch activity against 5′-GNTI. This finding was of translational value

and contrasted with a previous report by Suzuki et al. (2004) stating that tolerance develops after only five administrations (over 3 days) to both the antinociceptive and sedative effects of nalfurafine in mice. As a pertinent footnote to this issue, tolerance did not occur to the well-known side effect—water diuresis—that accompanied daily injections of nalfurafine (0.02 mg/kg, s.c.) to rats for 7 days (Inan et al. 2009c).

The high selectivity of nalfurafine for the kappa opioid receptor over other opioid receptors was described by Wang et al. (2005). In routine screening for binding affinity at nonopioid receptors (quoted by Nakao and Mochizuki 2009), the profile for nalfurafine was unremarkable except for the detection of low binding affinity at muscarinic M-1 receptors, bringing to mind the same result with 5′-GNTI (Sect. 5). Nonetheless, this profile is sufficient to promote nalfurafine as a multitarget ligand with an increasingly wide spectrum of antiscratch activities. Here are two examples. First, in a rat model of scratching behavior secondary to cholestasis induced by chronic injections of ethynylestradiol, nalfurafine (0.005–0.04 mg/kg, s.c.) suppressed the whole-body scratching (suggestive of generalized pruritus) in a dose-dependent manner (Inan and Cowan 2006). And second, more recently, Akiyama et al. (2015) studied a mouse model of chronic dry skin itch (acetone/diethyl ether/water applied to the nape of each animal neck twice daily for 8 days) and found that systemically injected nalfurafine (0.02 mg/kg) abolished the usual spontaneous scratching.

First reports linking the beneficial effects of selective kappa agonists with pruritic states in humans date back to 2001 with a communication from Toray Industries on nalfurafine in uremic pruritus at the first meeting of the International Forum for the Study of Itch in Singapore, and a press release from Adolor Corporation on a successful proof-of-concept trial with ADL10-0101 against experimentally provoked poison ivy itch in adult volunteers. This latter compound was one of a series of peripherally directed arylacetamides that were active in animal models of itch (Table 2) and visceral pain. The itch project was subsequently terminated in favor of the pain project (Eisenach et al. 2003). With nalfurafine, several small, open-label studies led to the much quoted "Kappa opioid system in uremic pruritus: multicenter, randomized, double-blind, placebo-controlled clinical studies" by Wikström et al. (2005) in which belief in the safety and clinical potential of nalfurafine in alleviating pruritus in patients on hemodialysis was reinforced and eventually strengthened (Kumagai et al. 2010, 2012; Ueno et al. 2013). Mitsubishi Tanabe Pharma Corporation has partnered with Toray Industries to introduce MT-9938 (nalfurafine) into North America for treatment, in the first instance, of uremic pruritus in subjects with end-stage renal disease receiving hemodialysis.

8 Butorphanol and Nalbuphine as Antipruritics

Butorphanol is a synthetic analgesic from the 14-hydroxymorphinan series. It was designated originally as a (mu) agonist–(mu) antagonist (Pircio et al. 1976) but nowadays is burdened with a range of terms depending on the level of investigation:

cell line, research animal, or human. "Butorphanol is an opioid analgesic with partial agonist actions at both mu and kappa opioid receptors" (Lee et al. 2007) would seem a reasonable compromise for present purposes.

Shortly after a nasal spray formulation of the highly lipophilic butorphanol (Stadol NS®) was launched in the United States in 1992 for the relief of moderate to severe pain, Dunteman et al. (1996) conducted an uncontrolled, pilot, clinical study and described the positive effect of intranasal butorphanol on opioid-induced pruritus. A decade later, Dawn and Yosipovitch (2006) reported a similar positive effect for intranasal butorphanol in a small, open-label study of patients with intractable pruritus associated with inflammatory skin or systemic diseases.

Whereas butorphanol is not under commercial development specifically as an antipruritic at present, nalbuphine (Schmidt et al. 1985), a second well-known analgesic from the 1970s, will undergo clinical evaluation against uremic pruritus as well as prurigo nodularis by Trevi Therapeutics. Nalbuphine ER is the oral extended release form under development and is described as having a dual mu antagonist/kappa agonist mechanism of action. Nalbuphine (10–30 mg/kg, s.c.) was active as a pretreatment against substance P in the Kuraishi mouse scratch model (Hawi et al. 2013). The extended release tablet was safe and well tolerated in humans with pruritus and undergoing hemodialysis. Itch was reduced in these subjects and so this open-label study provided early promise for nalbuphine ER as an antipruritic (Sciascia et al. 2014).

9 Potential of Peripherally Directed Kappa Agonists

The preclinical pharmacology of asimadoline, an orally active diarylacetamide, was published by Barber and colleagues in 1994. It was characterized as a selective kappa opioid receptor agonist with a limited ability to penetrate the blood–brain barrier. Asimadoline inhibited hyperalgesia pressure nociception in rats. The authors attributed this action to mediation by kappa receptors on the endings of sensory nerve fibers. Hopes were that asimadoline might treat peripheral pain of, for example, arthritis. An early setback occurred with the report that oral asimadoline provided unimpressive pain relief in patients following knee surgery (Machelska et al. 1999).

Tioga Pharmaceuticals obtained worldwide rights to asimadoline from Merck KGaA in 2005 and progressed the compound to Phase III clinical testing in patients suffering from abdominal pain and diarrhea of irritable bowel syndrome. In a change of research direction, this company has now focused on asimadoline as a potential antipruritic, given its good safety profile in humans and encouraging results from preclinical itch models (data on file at Tioga Pharmaceuticals). Our own work with asimadoline shows it to be active against compound 48/80-induced scratching in mice, with an antiscratch-50 value approximately five times lower than that of ICI 204,448, the other peripherally directed diarylacetamide (Table 2). As of 2014, a Phase II clinical trial is planned to assess if asimadoline can alleviate pruritus associated with atopic dermatitis.

CR845 (Cara Therapeutics) is a second peripherally restricted, selective agonist at kappa opioid receptors that may have clinical applicability against itch. It is a chemically interesting all D-amino acid tetrapeptide (D-Phe-D-Phe-D-Leu-D-Lys-[γ-(4-*N*-piperidinyl)amino carboxylic acid] acetate salt) (Chalmers 2011) that represents the culmination of previous studies on this novel class of tetrapeptide as antinociceptive agents (e.g., Vanderah et al. 2008). In our hands, CR845 antagonized scratching in a dose-related manner in both compound 48/80 (Table 2) and 5′-GNTI mouse models of itch. A Phase II clinical trial has been initiated by Cara Therapeutics to evaluate an intravenous formulation of CR845 against the itching of hemodialysis patients with uremic pruritus.

10 Perspectives

Selective kappa opioid receptor antagonists have been used widely as pharmacological tools in opioid research. Recently, these compounds are being evaluated as potential treatments for mood disorders and cocaine addiction. There is therefore a need to study the preclinical pharmacology of this class of opioid ligand in detail and establish whether or not a key attribute—excessive body scratching in mice—is a factor in their role as psychiatric drugs. This robust behavior, an interesting phenomenon in its own right, provides an animal model of human compulsive/perseverative behavior and, in an entirely different context, an animal model suitable for the investigation of dermatological agents, specifically antipruritics.

In the latter case, the scratching behavior does not seem to be contingent upon the kappa antagonist properties of the opioid ligand, given its resilience in kappa receptor knockout mice and also the chemical diversity and pharmacological nature of the pruritogens examined. Nonetheless, there is a kappa link inasmuch as kappa agonists as a class decrease the number of scratching bouts across the standard rodent models of itch. For example, nalfurafine suppresses scratching in bombesin, compound 48/80, 5′-GNTI, and chloroquine animal itch models and is the first-in-class drug against itch in chronic renal failure in humans. So, nalfurafine may claim "universal antipruritic" status and reinforce the belief that activation of kappa opioid receptors will dampen scratching in animals and humans, but just how a single agent is efficacious across the whole itch spectrum poses an interesting question. Might dynorphin and spinal B5-I interneurons (Kardon et al. 2014) be the universal answer?

Additional questions to be addressed in the unveiling of any "new generation" kappa antipruritic include the following: (1) Is the incidence of scratching eliminated, rather than just reduced? (2) Does tolerance develop to the antipruritic action across time? (3) To what extent is peripheral selectivity retained after multiple doses of a peripherally directed kappa agonist? (4) Is *some* central nervous system activity beneficial despite off-target issues? (5) How detrimental is a schedule II designation?

5′-GNTI, as a standard antagonist of kappa opioid receptors, is of particular interest to opioid researchers. Surely the combined talents of the field can

characterize the molecular processes behind such a prominent and measurable behavior. For a start, are Mas-related G protein-coupled receptors or protease-activated receptors involved in the 5′-GNTI scratching signal? How about toll-like receptors? One update is our recent finding that mouse repetitive scratching, evoked by either 5′-GNTI or compound 48/80, is accompanied by activation of microglial cells in the spinal cord (Zhang et al. 2015). Every little helps in coming to terms with the "shivering titillations of itch" (Connor 2004).

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Neuraxial Opioid-Induced Itch and Its Pharmacological Antagonism

Mei-Chuan Ko

Contents

Abstract

Given its profound analgesic nature, neuraxial opioids are frequently used for pain management. Unfortunately, the high incident rate of itch/pruritus after spinal administration of opioid analgesics reported in postoperative and obstetric patients greatly diminishes patient satisfaction and thus the value of the analgesics. Many endeavors to solve the mystery behind neuraxial opioid-induced itch had not been successful, as the pharmacological antagonism other

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than the blockade of mu opioid receptors remains elusive. Nevertheless, as the characteristics of all opioid receptor subtypes have become more understood, more studies have shed light on the potential effective treatments. This review discusses the mechanisms underlying neuraxial opioid-induced itch and compares pharmacological evidence in nonhuman primates with clinical findings across diverse drugs. Both nonhuman primate and human studies corroborate that mixed mu/kappa opioid partial agonists seem to be the most effective drugs in ameliorating neuraxial opioid-induced itch while retaining neuraxial opioid-induced analgesia.

Keywords
Agonist • Analgesics • Antagonist • Antipruritics • Epidural • Intrathecal • Itch • Monkey • Mouse • Opioid receptor • Pain • Pruritus • Rat • Spinal cord

Abbreviations

DOP	Delta opioid receptor
GRPR	Gastrin-releasing peptide receptor
KOP	Kappa opioid receptor
MOP	Mu opioid receptor
NOP	Nociceptin/orphanin FQ peptide receptor

1 Neuraxial Opioids

1.1 Clinical Applications of Neuraxial Opioids

Neuraxial administration of drugs offers the techniques that deliver drugs in close proximity to the spinal cord, i.e., intrathecally into the cerebrospinal fluid or epidurally into the fatty tissues surrounding the dura. Neuraxial administration of opioids provides effective analgesia before and after a surgical procedure. The modern era of spinal opioid administration began with a report by Yaksh and Rudy in 1976, demonstrating analgesia in rats by intrathecal administration of morphine (Yaksh and Rudy 1976). In 1979, Wang et al. published the first controlled clinical study of intrathecally administered opioid in humans conducted in a double-blind placebo setting. Eight cancer patients were selected based on severe pain in the back and legs and failure to respond to systemic analgesics when given at reasonable dose levels and frequencies. Each patient received both morphine at either

0.5 mg or 1.0 mg dosage and the placebo saline. Injections were administered at the second or third lumbar interspace at various intervals ranging from 4 to 48 h, depending on each patient's response to the treatment. In the end, 17 injections of morphine and 12 injections of saline were administered in total. Three quarters of the patients reported long-lasting pain relief after being treated with intrathecal morphine, suggesting that there is a clear distinction between the analgesic effects of morphine and placebo saline (Wang et al. 1979). No sign of central nervous system depression was reported in this study; hence, it was concluded that intrathecally administered opioids were advantageous for relieving pain and free from compromising sensory and motor functions (Wang et al. 1979).

These findings encouraged further studies of intrathecally administered opioids to explore the possibilities in obstetrics and postoperative pain treatment. Later studies concluded that because of its selective blockade in pain conduction, i.e., an absence of sympathetic blockade, spinal opioid allows patients' motor functions to remain intact upon receiving treatment, rendering spinal opioid therapeutically advantageous over local anesthetics (Cousins and Mather 1984).

1.2 Side Effects of Neuraxial Opioids

The use of neuraxial opioids to relieve pain is not without its side effects, however. Itch/pruritus, nausea, vomiting, urinary retention, and respiratory depression are the prominent side effects. This review focuses on the discussion of itch/pruritus because it can sometimes become a more irritating problem than pain itself. Spinal opioid-induced pruritus is an unwanted itch sensation often seen in obstetric and postoperative patients, with an incidence of 20–100 % (Ganesh and Maxwell 2007; Krajnik and Zylicz 2001; Szarvas et al. 2003). The onset of pruritus begins shortly after analgesia, differing in severity and duration depending on different classes of opioids and the dosage used. This unpleasant sensation, causing a reflex or desire to scratch, may warrant the use of antipruritic drugs, which may in turn create hormonal changes in obstetric patients (Ganesh and Maxwell 2007; Szarvas et al. 2003).

The itch sensation caused by neuraxial opioid is not only disturbing to and inconvenient for patients but also a self-limiting factor as it reduces the efficacy of neuraxial opioids for pain relief (Ballantyne et al. 1988; Cousins and Mather 1984; Ganesh and Maxwell 2007; Szarvas et al. 2003). This long-standing troublesome problem associated with neuraxial opioid-induced itch has prompted many scientists and physicians to target studies on potential treatment options (Dominguez and Habib 2013; Ganesh and Maxwell 2007; Kumar and Singh 2013; Waxler et al. 2005). In an attempt to prevent or treat neuraxial opioid-induced itch, a wide variety of pharmacological agents have been evaluated in both animals and humans. However, there is not yet a widely accepted non-opioid drug for treating neuraxial opioid-induced itch. From the perspective of receptor mechanisms underlying opioid-induced itch, the purpose of this review is to discuss the treatment of neuraxial opioid-induced itch and its pharmacological antagonistic

mechanisms through examining the evidence provided by preclinical and clinical studies from available literature to date.

2 Mechanisms of Neuraxial Opioid-Induced Itch

2.1 The Molecular Basis

The molecular mechanism of neuraxial opioid-induced itch has been somewhat unveiled by a recent study (Liu et al. 2011). Liu et al. conducted a series of elegant experiments, demonstrating the uncoupling of morphine-induced itch and morphine-induced analgesia in the mouse spinal cord. The mu opioid receptor (MOP) isoform MOP1D is required for intrathecal morphine-induced itch. MOP1D heterodimerizes with gastrin-releasing peptide receptor (GRPR) in the spinal cord, together relaying itch neurotransmission. In particular, MOP agonist-induced scratching responses were nearly abolished in GRPR knockout mice and intrathecal morphine-induced scratching was inhibited by coadministration with a GRPR antagonist (Liu et al. 2011). However, the presence of MOP1D in the rat spinal cord has been questioned (Oldfield et al. 2008). A recent study in monkeys showed that the GRPR antagonist could not attenuate scratching responses elicited by intrathecal administration of an MOP-preferring ligand, β-endorphin, but the same GRPR antagonist significantly attenuated intrathecal gastrin-releasing peptide-induced scratching (Ko 2013). Although the identification of MOP1D in mice implicates that there may be a possible groundbreaking analgesic treatment without causing the pruritic side effect (Liu et al. 2011), future studies are warranted to investigate whether these exciting findings can be translated to other species and advanced to the clinical setting.

As promising as the separation of morphine-induced itch and morphine-induced analgesia implies, rodents subjected to intrathecal morphine did not display profound scratching activities. It is worth noting the dramatic differences in the scratching activities elicited by intrathecal morphine between rodents and primates. Both the magnitude and duration of intrathecal morphine-induced scratching in mice are very mild, i.e., 15 scratching bouts as peak activity 10 min after injection and the increased scratching activity only lasted for 10–15 min (Liu et al. 2011). As compared to scratching responses elicited by intrathecal vehicle or saline, mice display a similar profile of scratching activity which made other researchers conclude that intrathecal morphine failed to elicit scratching responses in mice (Sukhtankar and Ko 2013). Intrathecal morphine over a wide dose range also failed to elicit scratching responses in rats (Lee et al. 2003). Nevertheless, intrathecal morphine elicited profound scratching responses in nonhuman primates, i.e., approximately 600 scratches within a 15-min bin/time sampling and such profound scratching lasted for several hours (Ko and Naughton 2000; Ko et al. 2004). Such dramatic species differences in intrathecal morphine-induced scratching may affect the interpretations of the pharmacological and neurobiological findings.

2.2 The Cellular Basis

The cellular mechanisms of neuraxial opioid-induced itch have been elucidated in depth by pharmacological studies in nonhuman primates. First, microinjection of a kappa opioid receptor (KOP) agonist, U-50488H, or a delta opioid receptor (DOP) agonist, DPDPE, into the medullary dorsal horn did not evoke facial scratching in monkeys (Thomas et al. 1992). Second, intrathecal administration of U-50488H and a DOP agonist, SNC80, produced moderate antinociception, but both ligands did not produce scratching in monkeys (Ko et al. 2003a). Third, intrathecal administration of a nociceptin/orphanin FQ peptide receptor (NOP) agonist produced full antinociceptive effects without eliciting scratching (Ko et al. 2006). Fourth, the antagonist potency of nalmefene, an MOP-preferring antagonist, validates that MOP mainly mediates intrathecal morphine-induced itch scratching (Ko and Naughton 2000). Last, pretreatment with an MOP antagonist (clocinnamox), rather than a DOP antagonist (naltrindole) or a KOP antagonist (nor-binaltorphimine), blocked intrathecal morphine-induced scratching (Ko et al. 2004). Taken together, these findings clearly demonstrate that the MOP, but not other opioid receptor subtypes, mainly mediates neuraxial opioid-induced itch in primates.

There is a well-known theory for opioid-induced itch. As pain inhibits itch, opioid analgesics elicit itch sensation by providing pain relief, i.e., removal of pain unmasks itch sensation (Ikoma et al. 2006; McMahon and Koltzenburg 1992). However, functional evidence from pharmacological studies does not support this notion. By using receptor-selective ligands, pharmacological approaches allow researchers to elucidate the function of each opioid receptor subtype in modulating pain and itch sensations. The DOP, KOP, and NOP agonists produce analgesic properties across diverse pain modalities following intrathecal and systemic administration (Brandt et al. 2001; Butelman and Kreek 2013; Butelman et al. 1993; Hu et al. 2010; Ko et al. 2009; Sukhtankar et al. 2014). Interestingly, these three types of opioid receptor agonists do not elicit scratching responses over a wide antinociceptive dose range (Ko et al. 2004, 2009; Sukhtankar et al. 2014). These findings clearly demonstrate that only MOP agonists produce analgesic effects accompanied by itch scratching responses. Other opioid receptor subtypes, DOP, KOP, and NOP, do not mediate neuraxial opioid-induced itch. It is important to further investigate physiological properties of sensory neurons expressing MOP and/or other opioid receptor subtypes in the spinal cord. More importantly, opioid-induced analgesia and itch can be distinguished at the receptor level. From the perspective of developing novel neuraxial opioids, it is promising to reveal that spinal administration of NOP agonists produces morphine-comparable analgesic effects without evoking itch in nonhuman primates (Hu et al. 2010; Ko and Naughton 2009; Ko et al. 2006). Such important findings will facilitate future advances of spinal analgesics (Lin and Ko 2013; Molinari et al. 2013; Schröder et al. 2014).

2.3 Animal Models with Translational Values

By using intrathecal administration, animal studies have shown that intrathecal morphine elicited scratching responses of different magnitudes and temporal patterns between rodents and nonhuman primates (Ko and Naughton 2000; Kuraishi et al. 2000; Lee et al. 2003; Liu et al. 2011; Sukhtankar and Ko 2013). Perhaps, the most important characteristic of intrathecal morphine is that it simultaneously provides pain relief and elicits itch sensation in patients. To the best of our knowledge, the nonhuman primate model can simulate this therapeutic profile very well. Intrathecal morphine over a wide dose range (10–320 μg) produced antinociceptive effects and it also produced profound scratching responses for several hours in rhesus monkeys (Ko and Naughton 2000; Ko et al. 2004). These observations closely parallel the behavioral effects and physiological relevance of spinal morphine in humans (Bailey et al. 1993; Palmer et al. 1999; Waxler et al. 2005). Accordingly, nonhuman primates can serve as a translational bridge to explore and validate potential drugs that may be effective in treating neuraxial opioid-induced itch in humans.

Recently, several studies using in vivo electrophysiology have shed light on regulation of pain and itch sensations by sensory neurons. Moser and Giesler (2013) have identified trigeminothalamic tract (VTT) neurons in anesthetized rats that are differentially affected by morphine. Briefly, intrathecal morphine increased the ongoing activity of pruriceptive VTT neurons but inhibited the ongoing activity and responses to noxious stimuli in nociceptive VTT neurons. In addition, the spinothalamic tract (STT) responds to pruritogens with activation that reflects the time course of histamine-induced itch sensation in humans (Andrew and Craig 2001; Davidson et al. 2007; Simone et al. 2004). More interestingly, the responses of STT neurons to histamine were inhibited by scratching the skin, indicating a neural correlate of scratching-induced relief and the importance of spinal processing in controlling itch neurotransmission (Davidson et al. 2009). These observations pinpoint pruriceptive STT neurons being positioned within a plastic circuitry that can provide a locus for pharmacological management of itch (Davidson et al. 2009, 2012). Future research integrating both pharmacological and electrophysiological approaches in both rodents and nonhuman primates will advance our understanding of how the spinal neural circuits regulate MOP-mediated itch and analgesia. Based on the current literature, several pharmacological studies in nonhuman primates have been conducted to evaluate the effectiveness of diverse ligands in treating intrathecal morphine-induced scratching in adult rhesus monkeys (Table 1). These summarized findings in nonhuman primates are discussed and compared in the sections below (pharmacological antagonism) in terms of effectiveness of opioid- and non-opioid-related ligands in treating neuraxial opioid-induced itch in adult patients.

Table 1 Summary of preclinical studies evaluating the effectiveness of diverse ligands in managing neuraxial opioid-induced scratching in adult rhesus monkeys

Neuraxial opioids	Treatment drug and doses	Outcomes and conclusion	References
Intrathecal morphine 10–320 μg	Intravenous nalmefene (MOP antagonist) 10–32 μg/kg	Effective Scratching responses: ↓	Ko and Naughton (2000)
Intrathecal morphine 32 μg	Intramuscular clocinnamox (MOP antagonist) 0.1 mg/kg	Effective Scratching responses: ↓	Ko et al. (2004)
Intrathecal morphine 32 μg	Subcutaneous butorphanol (mixed KOP/MOP partial agonist) 10–32 μg/kg	Effective Scratching responses: ↓	Lee et al. (2007)
Intrathecal morphine 10–32 μg	Subcutaneous U-50488H (KOP agonist) 0.1–0.32 mg/kg	Effective Scratching responses: ↓	Ko et al. (2003b)
Intrathecal morphine 32 μg	Intramuscular nalfurafine (KOP agonist) 0.3–1 μg/kg	Effective Scratching responses: ↓	Ko and Husbands (2009)
Intrathecal morphine 32 μg	Intramuscular naltrindole (DOP antagonist) 1 mg/kg	Ineffective No change in scratching responses	Ko et al. (2004)
Intrathecal morphine 50 nmol	Intrathecal N/OFQ (NOP agonist) 10–100 nmol	Ineffective No change in scratching responses	Ko and Naughton (2009)
Intrathecal morphine 32 μg	Intravenous ondansetron (5HT3 antagonist) 0.1–3.2 mg/kg	Ineffective No change in scratching responses	Fig. 1
Intrathecal morphine 32 μg	Intramuscular diphenhydramine (antihistamine) 0.32–3.2 mg/kg	Ineffective No change in scratching responses	Ko et al. (2004)
Intrathecal morphine 32 μg	Intravenous ketorolac (NSAID) 1–10 mg/kg	Ineffective No change in scratching responses	Fig. 2

Note: ↓= decrease/ inhibition, *MOP* mu opioid receptor, *KOP* kappa opioid receptor, *NOP* nociceptin/orphanin FQ peptide receptor, *DOP* delta opioid receptor, *NSAID* non-steroidal anti-inflammatory drug

3 Pharmacological Antagonism by Opioid-Related Ligands

3.1 Mu Opioid Receptor Antagonists

As most opioid analgesics used in the clinics are MOP agonists, it is expected that MOP antagonists are effective in treating neuraxial opioid-induced itch in patients (Dominguez and Habib 2013; Ganesh and Maxwell 2007; Kumar and Singh 2013; Waxler et al. 2005). A systematic review of randomized trials involving obstetric

patients indicated that intravenous naloxone (0.25–2.4 μg/kg/h) was effective in managing opioid-induced itch (Kjellberg and Tramer 2001). However, MOP antagonists are not widely useful in patients receiving neuraxial opioids for pain relief because MOP antagonists reverse or shorten neuraxial opioid-induced analgesia (Abboud et al. 1990; Cohen et al. 1992; Rawal et al. 1986; Wang et al. 1998).

Antagonist studies in nonhuman primates demonstrate that pretreatment with a single dose of nalmefene (32 μg/kg) was equally potent to block intrathecal morphine-induced itch scratching and antinociception (Ko and Naughton 2000). In this study, the in vivo pK_B analysis was used to verify functional receptor populations underlying the actions of intrathecal morphine. The same dose of nalmefene produced approximately tenfold rightward shifts in each subject's dose–response curves of intrathecal morphine for scratching and antinociception. Accordingly, nalmefene pK_B values were similar for both endpoints, indicating that intrathecal morphine-induced scratching and antinociception are mediated by the same MOP population in primates (Ko and Naughton 2000). These findings indicate a narrow window between reversal of itch and analgesia by MOP antagonists and support the clinical findings that MOP antagonists such as naloxone and nalmefene may not be ideal drugs for treating pruritus in obstetric patients. Nevertheless, the MOP antagonist is one of the treatment options for ameliorating cholestatic pruritus, which may be caused by elevated levels of endogenous opioid peptides (Bergasa 2008; Jones and Bergasa 1992).

3.2 Opioid Receptor Partial Agonists

Both nalbuphine and butorphanol are opioid receptor partial agonists that have been used clinically as analgesics with limited abuse liability (Preston and Jasinski 1991). The radioligand binding assay suggests that both drugs have reasonable binding affinity for both MOP and KOP sites in monkey brain membranes, although nalbuphine has a higher selectivity for MOP over KOP (Butelman et al. 1998). In the cell lines expressing MOP or KOP, both drugs displayed low-mid efficacy as measured by the stimulation of [^{35}S]GTPγS binding, i.e., low-mid intrinsic activity (Emmerson et al. 1996; Remmers et al. 1999; Zhu et al. 1997). Interestingly, due to its low efficacy, nalbuphine displays partial MOP agonist actions with its context-dependent agonist/antagonist effects in nonhuman primate behavioral assays (Gerak et al. 1994; Gerak and France 1996). By contrast, butorphanol is characterized as a partial agonist acting at both KOP and MOP sites by diverse in vivo assays in nonhuman primates (Butelman et al. 1995; Lee et al. 2007; Vivian et al. 1999).

Both nalbuphine and butorphanol are effective in alleviating neuraxial opioid-induced itch (Table 2). In particular, systemic nalbuphine between 3 and 10 mg seems effective in decreasing the incidence of pruritus in most of the clinical studies. However, with a high dose of nalbuphine (20 mg), Morgan et al. (1991) did not find pruritus relief by nalbuphine. Butorphanol seems less popular than nalbuphine for treating opioid-induced itch probably due to potential drowsiness

Table 2 Summary of clinical studies evaluating the effectiveness of opioid receptor partial agonists in managing neuraxial opioid-induced pruritus in adult patients

Neuraxial opioids	Treatment drugs and doses	Outcomes and conclusion	References
Epidural morphine 0.1 mg/kg	Intravenous nalbuphine 0.1 mg/kg	Effective Pruritus score: ↓	Penning et al. (1988)
Epidural morphine 5 mg	Intravenous nalbuphine 20 mg	Ineffective No change in the degree of pruritus	Morgan et al. (1991)
Epidural morphine 5 mg	Intravenous nalbuphine 5 mg	Effective Severity of pruritus: ↓	Cohen et al. (1992)
Epidural morphine 5 mg	Intravenous nalbuphine 2.5 mg/h	Effective Pruritus score: ↓	Kendrick et al. (1996)
Epidural morphine 3 mg/12 h	Intravenous nalbuphine 60 μg/kg/h	Effective Incidence of pruritus (13 %): ↓	Wang et al. (1998)
Epidural morphine 1.5 mg/12 h	Intramuscular nalbuphine 10 mg	Effective Incidence of pruritus (44 %): ↓ Severity of pruritus: ↓	Liao et al. (2011)
Intrathecal morphine 200 μg	Nalbuphine (no specified delivery route) 5–10–10 mg, stepwise	Effective VAS score of zero (83 %)	Alhashemi et al. (1997)
Intrathecal morphine 200 μg	Intravenous nalbuphine 3 mg	Effective Treatment success rate (83 %): ↑	Charuluxananan et al. (2001)
Intrathecal morphine 200 μg	Intravenous nalbuphine 4 mg	Effective Pruritus score: ↓ Request for pruritus treatment: ↓	Charuluxananan et al. (2003)
Intrathecal morphine 150 μg	Intravenous nalbuphine 2–3 mg	Effective % of successful treatment (87–97 %): ↑	Somrat et al. (1999)
Intrathecal fentanyl 50 μg	Intravenous nalbuphine 4 mg	Partially effective Incidence of pruritus (61 %)	Ben-David et al. (2002)
Epidural morphine 4 mg	Epidural butorphanol 3 mg	Effective % patients treated for pruritus (0 %): ↓	Lawhorn et al. (1991)
Epidural morphine 4 mg	Epidural butorphanol 3 mg	Effective Incidence of pruritus (20 %): ↓	Wittels et al. (1993)
Epidural morphine 3 mg	Epidural butorphanol 3 mg	Ineffective No change in VAS for pruritus	Gambling et al. (1994)

(continued)

Table 2 (continued)

Neuraxial opioids	Treatment drugs and doses	Outcomes and conclusion	References
Epidural morphine 60 μg/kg	Epidural butorphanol 30 μg/kg	Effective Severity of pruritus: ↓	Bailey et al. (1994)
Intrathecal morphine 150 μg	Intravenous butorphanol 2 mg	Ineffective No change in the intensity of pruritus	Sakai et al. (2001)
Intrathecal morphine 100 μg	Intravenous butorphanol Bolus 1 mg with 0.2 mg/h	Effective Incidence of pruritus (13 %): ↓	Wu et al. (2012)

Note: *VAS* visual analog scale, ↓ = decrease/inhibition, ↑ = increase

following systemic administration. Nevertheless, several studies have shown a decreased incidence of pruritus without other side effects when butorphanol was administered with morphine epidurally in pediatric patients (Bailey et al. 1994; Gunter et al. 2000; Lawhorn et al. 1995; Lawhorn and Brown 1994). A recent systematic review also indicates the potential benefits of using butorphanol to prevent neuraxial morphine-induced itch and decrease pain intensity and postoperative nausea and vomiting without increasing other side effects (Du et al. 2013). Importantly, a pharmacological study demonstrates that butorphanol's partial agonist actions at both MOP and KOP sites contribute to its antipruritic actions, i.e., low-efficacy ligands antagonize high-efficacy ligand's action in producing itch sensation (Lee et al. 2007). Compared with MOP antagonists, opioid receptor partial agonists seem to have an advantage for ameliorating itch while retaining analgesia (Dominguez and Habib 2013; Ganesh and Maxwell 2007; Kumar and Singh 2013; Waxler et al. 2005). These observations are in line with preclinical studies demonstrating that butorphanol is effective in alleviating MOP agonist-induced itch without reversing analgesia in nonhuman primates (Lee et al. 2007). Due to butorphanol's unique pharmacological profile, i.e., partial agonist actions at both MOP and KOP sites, dermatologists are very interested in developing a transdermal formulation of butorphanol for the treatment of chronic itch (Dawn and Yosipovitch 2006; Lim et al. 2008).

3.3 Kappa Opioid Receptor Agonists

Numerous preclinical and clinical studies have indicated that KOP is a viable therapeutic target for potential antipruritics (Cowan and Gmerek 1986; Ko et al. 2003b; Kumagai et al. 2010, 2012). Original studies in rodents showed that systemic administration of KOP agonists inhibited scratching activity evoked by pruritogens such as bombesin-related peptides (Gmerek and Cowan 1983, 1984). In particular, KOP agonists inhibited scratching behavior without interfering with

locomotor activity in rodents (Inan et al. 2009; Togashi et al. 2002; Wang et al. 2005). Recent studies have identified a subset of inhibitory interneurons regulating itch in the dorsal horn of mouse spinal cord (Ross et al. 2010). It will be important to investigate the role of KOP modulating these inhibitory interneurons. Furthermore, pharmacological studies in nonhuman primates have demonstrated that KOP agonists, at nonsedating doses, can attenuate intrathecal morphine-induced scratching without affecting antinociception (Ko and Husbands 2009; Ko et al. 2003b). These findings facilitated the development of a KOP agonist, nalfurafine, as an antipruritic. To date, two clinical trials have reported that nalfurafine is a safe and effective antipruritic in hemodialysis patients suffering from uremic pruritus (Kumagai et al. 2010, 2012).

KOP agonists produce several effects opposite to those of MOP agonists in primates. For example, MOP agonists produce euphoria, whereas KOP agonists produce dysphoria (Kumor et al. 1986; Walsh et al. 2001); MOP agonists produce antidiuretic effects, while KOP agonists produce diuresis (Peters et al. 1987; Weiskopf et al. 1987). Although there is no selective KOP agonist approved for treating neuraxial opioid-induced itch, it seems promising to develop KOP-related ligands, especially mixed KOP/MOP agonists for this purpose or as spinal analgesics. Clinically used mixed KOP/MOP agonists such as butorphanol and pentazocine have a low incidence of pruritus and are effective in treating spinal morphine-induced itch (Abboud et al. 1989; Ackerman et al. 1989; Lawhorn et al. 1991; Tamdee et al. 2009). In addition, butorphanol produces neither euphoria nor dysphoria in humans and it does not cause diuresis (Butelman et al. 1995; Dershwitz et al. 1991). These findings strengthen the notion that mixed KOP/MOP agonists may have a therapeutic advantage over selective MOP agonists. It will be important to further develop novel opioid agonists with dual actions at both KOP and MOP sites with different degrees of intrinsic efficacy and advance the medicine of neuraxial opioids.

4 Pharmacological Antagonism by Non-Opioid Ligands

4.1 Serotonin 5-HT3 Receptor Antagonists

The effectiveness of a 5-HT3 receptor antagonist, ondansetron, in treating neuraxial opioid-induced itch varies across different clinical studies (Table 3). Several studies showed that intravenous ondansetron (4–8 mg) was effective in decreasing the incidence of pruritus in patients receiving either epidural or intrathecal morphine, fentanyl, or combination of MOP agonists. However, several other studies concluded that ondansetron was ineffective in treating itch in most of the patients receiving intrathecal fentanyl or combination of MOP agonists (Bonnet et al. 2008). It will be important to investigate whether fentanyl, sufentanil, or a combination of MOP agonists elicits a higher intensity of itch as both fentanyl and sufentanil have been characterized in the agonist stimulation of [^{35}S]GTPγS binding as full MOP

Table 3 Summary of clinical studies evaluating the effectiveness of a 5-HT3 receptor antagonist, ondansetron, in managing neuraxial opioid-induced pruritus in adult patients

Neuraxial opioids	Treatment drug and doses	Outcomes and conclusion	References
Epidural morphine, 2 mg Intrathecal morphine, 0.2 mg	Intravenous ondansetron 8 mg	Effective Success rate (70 %): ↑	Borgeat and Stirnemann (1999)
Epidural morphine 3 mg	Intravenous ondansetron 4 mg	Effective Incidence of pruritus (28 %): ↓	Tzeng et al. (2003)
Intrathecal sufentanil 2.5 μg and morphine 100 μg	Intravenous ondansetron 8 mg	Ineffective No change in the frequency and severity of pruritus	Yazigi et al. (2002)
Intrathecal morphine 160 μg and fentanyl 15 μg	Intravenous ondansetron 8 mg	Ineffective No change in the incidence of pruritus	Sarvela et al. (2006)
Intrathecal morphine 250 μg	Intravenous ondansetron 4 mg	Effective Incidence of pruritus (34 %): ↓	Iatrou et al. (2005)
Intrathecal morphine 200 μg	Intravenous ondansetron 4–8 mg	Effective Request for pruritus treatment: ↓	Charuluxananan et al. (2003)
Intrathecal morphine 200 μg	Intravenous ondansetron 4 mg	Effective Treatment success rate (80 %): ↑	Charuluxananan et al. (2000)
Intrathecal morphine 200 μg	Intravenous ondansetron 4 mg Orally disintegrating tablets 8 mg	Effective Incidence of pruritus (56–66 %): ↓	Pirat et al. (2005)
Intrathecal morphine 150 μg	Intravenous ondansetron 0.1 mg/kg	Effective Incidence of pruritus (25 %): ↓	Yeh et al. (2000)
Intrathecal fentanyl 25 μg	Intravenous ondansetron 4–8 mg	Ineffective No change in the incidence and severity of pruritus	Wells et al. (2004)
Intrathecal fentanyl 25 μg	Intravenous ondansetron 8 mg	Effective Incidence of pruritus (39 %): ↓	Gurkan and Toker (2002)
Intrathecal fentanyl 25 μg	Intravenous ondansetron 8 mg	Effective Incidence of pruritus (6 %): ↓	Gulhas et al. (2007)
Intrathecal fentanyl 15 μg	Intravenous ondansetron 8 mg	Ineffective No change in the incidence of pruritus	Browning et al. (2013)

(continued)

Table 3 (continued)

Neuraxial opioids	Treatment drug and doses	Outcomes and conclusion	References
Intrathecal fentanyl 10 μg	Intravenous ondansetron 4–8 mg	Ineffective No change in the incidence and severity of pruritus	Korhonen et al. (2003)
Intrathecal sufentanil 10 μg	Intravenous ondansetron 8 mg	Ineffective No change in the incidence and severity of pruritus	Waxler et al. (2004)

Note: *VAS* visual analog scale, ↓ = decrease/inhibition, ↑ = increase

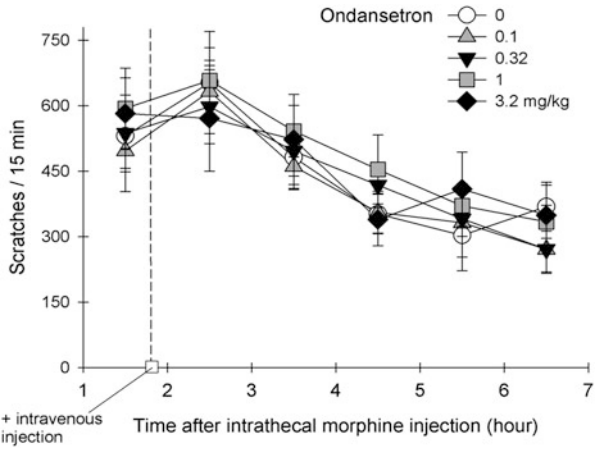

Fig. 1 Effects of intravenous ondansetron on intrathecal morphine-induced itch scratching responses in rhesus monkeys. Each value represents means +/− S.E.M. ($n = 8$)

agonists with higher intrinsic activity as compared to morphine (Emmerson et al. 1996).

The exact mechanism for ondansetron to alleviate itch is unknown. Although the 5-HT3 receptors can be identified in the spinal cord of rodents and primates (Laporte et al. 1996; Waeber et al. 1988), there is no anatomical evidence for the co-localization of the 5-HT3 receptor with MOP in the spinal cord or functional evidence for the interaction between the 5-HT3 receptor and MOP in any animal models. Since patients with cholestatic pruritus have elevated levels of endogenous opioids, there were several randomized controlled trials exploring the effects of ondansetron (Jones et al. 2007). It was concluded that ondansetron has negligible effect on cholestatic or uremic pruritus based on a recent systematic review (To et al. 2012). Figure 1 illustrates the effects of ondansetron on intrathecal morphine-induced scratching in monkeys. Intrathecal administration of morphine (32 μg) elicited profound scratching responses (i.e., ~600 scratches within a 15-min bin/time sampling) in rhesus monkeys ($n = 8$) (unpublished data from the Ko lab). Intravenous ondansetron (0.1–3.2 mg/kg) was given approximately 2 h after

subjects received intrathecal morphine. Within these doses tested herein, ondansetron was ineffective in attenuating intrathecal morphine-induced scratching. A higher dose of ondansetron (10 mg/kg) caused extrapyramidal reactions in monkeys (i.e., involuntary head jerking, both legs were rigid and were in extensor spasm) which led to the termination of experiments.

4.2 Histamine H1 Receptor Antagonists

Although morphine can trigger the release of histamine from mast cells, clinical studies have indicated that antihistamines are not effective in relieving neuraxial opioid-induced itch (Dunteman et al. 1996; Horta et al. 2006). Pharmacological studies in nonhuman primates also found that an antihistamine, diphenhydramine, over a wide dose range could not attenuate intrathecal morphine-induced scratching (Ko et al. 2004). Moreover, other MOP agonists such as fentanyl and alfentanil do not stimulate histamine release (Hermens et al. 1985; Rosow et al. 1982), whereas they evoke itch/scratching in humans and nonhuman primates (Ellis et al. 1990; Ko et al. 2004). As tachyphylaxis develops quickly in response to histamine-induced itch, the role of histamine is minimal in both neuraxial opioid-induced itch and chronic itch. Nevertheless, the sedative effects of antihistamines may be helpful by providing needed sleep and interrupting the itch–scratch cycle while being barely effective in decreasing the severity of itch (Krajnik and Zylicz 2001; Szarvas et al. 2003).

4.3 Nonsteroidal Anti-Inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) attenuate inflammatory pain by inhibiting cyclooxygenases and decreasing prostaglandin levels. Intravenous tenoxicam and rectal diclofenac have been reported to attenuate neuraxial opioid-induced itch (Colbert et al. 1999a, b). However, other studies using celecoxib and lornoxicam found no changes in the severity of pruritus (Gulhas et al. 2007; Lee et al. 2004). In the nonhuman primate inflammatory pain model, systemic administration of ketorolac (0.3–10 mg/kg) dose-dependently attenuated carrageenan-induced thermal allodynia/hyperalgesia (Sukhtankar et al. 2014). However, the same dose range of intravenous ketorolac did not attenuate scratching responses elicited by intrathecal morphine (32 μg) (unpublished data from the Ko lab). Figure 2 compares the effects of ketorolac and nalmefene on intrathecal morphine-induced scratching in the same rhesus monkeys ($n = 5$). Either ketorolac (10 mg/kg) or nalmefene (32 μg/kg) was administered intravenously approximately 2 h after subjects received intrathecal morphine (32 μg). In this experimental setting, intravenous nalmefene, but not ketorolac, significantly attenuated scratching responses. Based on these results, NSAIDs may not be useful therapeutic agents to treat neuraxial opioid-induced itch. It seems unlikely that prostaglandins play a significant role as itch mediators associated with neuraxial opioids.

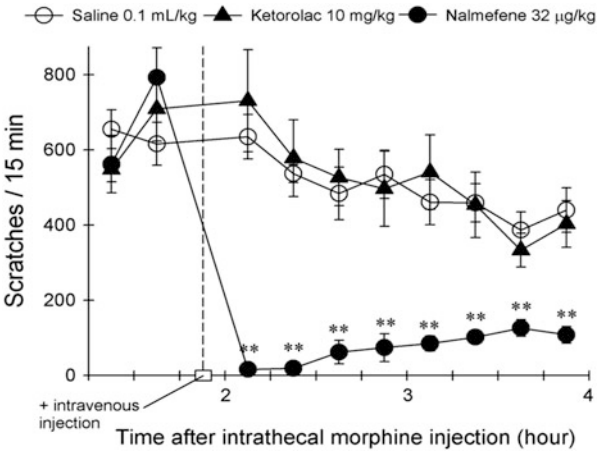

Fig. 2 Effects of intravenous ketorolac and nalmefene on intrathecal morphine-induced itch scratching responses in rhesus monkeys. Each value represents mean +/− S.E.M. ($n = 5$). The *asterisks* represent significant differences from the saline condition

5 Conclusion

A variety of drugs have been evaluated in treating neuraxial opioid-induced itch. These diverse drugs, including gabapentin, dopamine D2 receptor antagonists, propofol, mirtazapine, and dexamethasone, have been discussed in recent review articles, but all have mixed results from a very limited number of clinical studies (Dominguez and Habib 2013; Ganesh and Maxwell 2007; Kumar and Singh 2013). As these drugs have not been extensively studied in nonhuman primates, there is no further discussion on the potential pharmacological antagonism of these drugs on neuraxial opioid-induced itch. Most importantly, accumulated pharmacological evidence in nonhuman primates (Table 1, Figs. 1 and 2) supports that (1) MOP antagonists and mixed KOP/MOP partial agonists are the most effective treatment options for managing neuraxial opioid-induced itch (Table 2) and (2) non-opioid ligands, including the 5-HT3 antagonist ondansetron, antihistamines, and NSAIDs, are not effective in treating neuraxial opioid-induced itch (Table 3). Collectively, these pharmacological studies indicate that rhesus monkeys may serve as a surrogate species for humans in preclinical studies to identify effective treatments for neuraxial opioid-induced itch.

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Current Topical and Systemic Therapies for Itch

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Abstract

Itch is a common distressing symptom which may be caused by multifactorial aetiologies including inflammatory skin diseases, systemic diseases, neuropathic conditions and psychogenic disorders. Itch is a term used synonymously with pruritus and is defined as acute if it lasts less than 6 weeks or chronic if it persists for more than 6 weeks. It can have the same impact on the quality of life as chronic pain and shares many of the same pathophysiological pathways. Depending on the aetiology of the itch, different pathogenic mechanisms have been postulated with a number of mediators identified. These include histamine, leukotrienes, proteases, neuropeptides, cytokines and opioids, which may activate peripheral itch-mediating C-fibres via receptors on the nerve terminals and central neuronal pathways. *Therefore, there is no single universally effective anti-itch treatment available*. First-line treatments for itch include topical therapies, such as emollients, mild cleansers (low pH), topical anaesthetics, steroids, calcineurin inhibitors and coolants (menthol). Treatment with systemic therapies can vary according to the aetiology of the chronic itch. Non-sedating antihistamines are helpful in conditions such as urticaria where the itch is primarily histamine mediated. Although the itch of eczema is not mediated by histamine, sedating antihistamines at night are helpful to break the itch-scratch cycle. Chronic itch may also be treated with other systemic therapies, such as anticonvulsants, antidepressants as well as mu-opioid antagonists, kappa-opioid agonists and phototherapy, depending on the cause of the itch. This article summarises the topical and systemic therapies available with our current understanding of the pathophysiology of itch.

Keywords

Itch • Pruritus • Pain • Topical • Systemic • Antihistamines • Anticonvulsants • Capsaicin • Glucocorticoids • Antidepressants • Opioids • Phototherapy • Emollients • Menthol • C-fibres • Central neuronal pathways • Peripheral neuronal pathways • Chronic • Acute

1 Introduction

Itch (pruritus) is a common distressing symptom that has multifactorial aetiologies and therefore may be the result of various mechanisms. The pathophysiology of itch is poorly understood, and to date there is still a significant lack of randomised controlled trials, due to the diversity and complexity of this symptom, multiple aetiologies and lack of well-defined outcome measures (Weisshaar et al. 2012). However, recent studies are making a major impact on our understanding of this symptom. Itch is defined as acute if it lasts less than 6 weeks and chronic if lasting more than 6 weeks. It is more common in women than in men, with its incidence increasing with age. Itch shares similar pathophysiological pathways and mechanisms with pain, and itch can be as debilitating as chronic pain with the same impact on quality of life (Yosipovitch and Bernhard 2013). The patient may have localised or widespread itch, depending on its aetiology. There are four major causes of chronic itch. These are *dermatological* (the most common being atopic eczema, urticaria, psoriasis and skin dryness), *systemic* (end-stage chronic kidney disease, cholestasis, myeloproliferative disorders and hyperthyroidism), *neuropathic* (notalgia paresthetica, brachioradial pruritus and postherpetic neuralgia itch) and *psychogenic* (obsessive-compulsive disorders, depression and fibromyalgia) (Yosipovitch and Bernhard 2013).

The treatment of acute itch is usually straightforward, but the management of chronic itch can be much more challenging. In chronic generalised itch, in the absence of skin disease, the cutaneous changes may be non-specific and secondary to scratching, rubbing and picking (lichenification, prurigo nodules, patches of dermatitis and excoriations). Therefore, systemic causes cannot be ruled out by skin examination alone. Therapies should aim to influence cutaneous and central mechanisms, and if an underlying disease can be identified, then this should be treated. However, in many cases the cause may be multifactorial or of unknown origin. So there is no standard recommendation for the treatment of itch or an effective, universal treatment for itch (Leslie 2013).

The primary step in the management of itch is to determine whether it is due to a dermatological disease or whether an underlying non-cutaneous cause is present as the treatments may vary based on the underlying aetiology.

First-line treatments for itch are topical therapies, which include emollients, coolants such as menthol, as well as topical anaesthetics, capsaicin, antihistamines, corticosteroids and calcineurin inhibitors. Recent studies into the pathogenic mechanisms of itch have identified agonists, including proteases, neuropeptides, cytokines and opioids, as well as their receptors. These in turn may activate itch-mediating unmyelinated C-fibres via receptors on the nerve terminals, which may also lead to itch sensitisation (Raap et al. 2011). Systemic treatment with oral antihistamines has traditionally been thought to be the mainstay of itch management, especially sedative antihistamines, which help the patient sleep at night and so break the itch-scratch cycle. Chronic itch may also be treated systemically with phototherapy, anticonvulsants, antidepressants and mu-opioid antagonists and kappa-opioid agonists.

It must be remembered that many therapies for itch are not licensed and may only be prescribed ‘off-label’. It is therefore important to establish individual

therapy regimens according to the patient's age, pre-existing diseases, medications and quality and intensity of itch. Most importantly, elderly patients, pregnant women and children need special attention. The patient should be fully involved with their treatment to increase compliance, leading to more successful outcomes in the management of their itch (Weisshaar et al. 2012).

2 Topical Treatments

Topical therapies are still the first-line treatment for mild itch and for xerosis. They are the mainstay of dermatological management of localised or acute itch, especially where systemic therapies are contraindicated (Table 1).

2.1 Emollients

Emollients, moisturisers and barrier repair creams are important antipruritics which soften the outermost layer of dry skin (stratum corneum) and improve skin barrier function. This is often impaired in inflammatory skin diseases and can be exacerbated by repetitive scratching, facilitating the entry of irritants, allergens and infectious pathogens that can precipitate itch (Elmariah and Lerner 2011). Transepidermal water loss (TEWL) can reflect impaired epidermal barrier function, leading to greater itch intensity, especially in patients with atopic dermatitis and at night, when TEWL is increased (Patel and Yosipovitch 2010). The choice of emollient should be based on the nature of the underlying condition, its severity

Table 1 Common topical treatments for pruritus

Agent	Indications	Major adverse effects
Coolants: menthol, camphor, phenol	Most pruritic conditions	Skin irritation
Capsaicin 0.025–0.1 %	• Neuropathic itch • Prurigo nodularis • Aquagenic pruritus • Uremic pruritus	Initial burning sensation
Anaesthetics	Neuropathic itch	Numbness
Calcineurin inhibitors	Eczema (various types) and anogenital pruritus	Transient burning sensation
N-palmitoylethanolamine	Atopic dermatitis, dry skin	Skin irritation
Doxepin	• Atopic dermatitis • Localised pruritus	• Drowsiness in 25 % of patients • Allergic contact dermatitis
Aspirin and salicylates	Lichen simplex chronicus	Transient burning sensation

Adapted from Tey HL, Yosipovitch G, Bernhard J (2014) Scientific American Medicine, 3rd edn

and patient preference. Emollients are best applied after a bath or shower. They may contain preservative or other additives and can occasionally cause sensitisation.

Patients should avoid alkaline soaps, which increase the secretion of serine proteases, such as mast cell tryptase (an endogenous PAR2 agonist), that play an important role in mediating pruritus, via activation of protease-activating receptor 2. Such soaps should be avoided in favour of moisturisers and cleansers with a low pH, which are useful in improving skin barrier function by maintaining the natural acidic pH of the skin surface. If secondary infection is present, then it should be treated.

2.2 Oatmeal Moisturisers

Colloidal oatmeal has been used for centuries as a soothing agent to relieve itch and irritation associated with eczema and various dry skin dermatoses. Avenanthramides are phenolic compounds present in oats at approximately 300 parts per million (ppm). Avenanthramides showed a significant inhibition of tumour necrosis factor-alpha (TNF-alpha) and reduction of interleukin-8 (IL-8) release. Additionally, topical application of 1–3 ppm avenanthramides countered neurogenic inflammation and reduced pruritogen-induced scratching in a murine itch model (Sur et al. 2008).

2.3 Menthol

Menthol and aqueous cream combinations can be applied for short-term reduction of itch. Menthol (a naturally occurring terpene alcohol, $C_{10}H_{20}O$) has been used in dermatology for its cooling, antipruritic, analgesic and antiseptic properties. The cooling sensation appears to reduce itch and is effective in low concentrations (1–5 %) as higher concentrations can cause irritation (Yosipovitch and Bernhard 2013).

Itch is known to be aggravated by warmth and reduced by cooling the skin. Menthol induces a subjective feeling of cold lasting up to 70 min and thereby can reduce the sensation of itch. This may be via the transient receptor channels (TRP), which are a family of sensory receptors activated by both chemical and physical factors. TRPA1 and TRPM8, expressed in the subpopulation of C-fibres, with possible downstream release of substance P, appear to be highly sensitive menthol receptors and may play a part in the pathogenesis of itch. Of note, there is a subset of patients where menthol actually aggravates itch, and studies in primates suggest that A delta fibres that transmit cold may have some role in itch (Ringkamp et al. 2011).

Camphor activates the TRPV3 channel and has been used for centuries as an antipruritic. Although there is little literature on its effects and its antipruritic efficacy has not been evaluated in double-blind studies, it is used in conjunction with other topical and systemic therapies (Weisshaar et al. 2012).

2.4 Calamine

Calamine contains zinc oxide, ferric oxide and phenol. Its antipruritic effect is attributed to phenol with its cooling and mild local anaesthetic action. It is used to temporarily relieve itch in poison ivy and dermatitis.

2.5 Topical Anaesthetics

Topical local anaesthetic preparations include pramoxine 1 % and mixtures of lidocaine 2.5–5 % and prilocaine 2.5 % cream. They can treat itch due to neuropathic causes and facial or anogenital itch. Although the data is insufficient, there has been a report of pramoxine 1 % cream being useful in treating the itch of chronic kidney disease (Young et al. 2009). More data on the safety of long-term use over a large area of skin is required. Polidocanol has both local anaesthetic and moisturising properties. Polidocanol 3 % combined with urea 5 % reduced pruritus in patients in a number of skin diseases (Freitag and Hoppner 1997). Short-term application of topical local anaesthetics can be recommended with low risk of sensitisation in some situations (Weisshaar et al. 2012).

2.6 Capsaicin

Topical capsaicin is derived from chilli peppers and is an exogenous vanilloid used as a pain-relieving medication (Szolcsányi 2004). It exerts its antipruritic effect via TRPV1, which has been associated with the pathogenesis of itch (Imamachi et al. 2009). Unmyelinated peripheral C-nerve fibres at the site of itch can be desensitised by depletion of substance P (Jessell et al. 1978). Localised itch disorders, particularly those of neuropathic origin, such as notalgia paresthetica, brachioradial pruritus and postherpetic neuralgia, can be treated with capsaicin. It has also been used for psoriatic itch and uremic and aquagenic pruritus (Papoiu and Yosipovitch 2010; Tey and Yosipovitch 2011; Yosipovitch and Bernhard 2013). Concentrations of capsaicin ranging from 0.025 % up to 0.1 % are available as OTC and are commonly used.

The main adverse effect is an initial burning sensation which usually lasts several days. This may lessen compliance. Patients can be advised to apply a topical anaesthetic, such as lidocaine or eutectic mixture of local anaesthetic (EMLA), before application of capsaicin in the first 2 weeks of treatment in order to reduce the discomfort. The topical anaesthetic, in addition, serves as an antipruritic treatment in itself. Pretreatment with a topical anaesthetic gives effective reduction of burning sensation and attenuates heat hyperalgesia during capsaicin treatment.

A new, high-potency transdermal formulation containing capsaicin at 8 % has recently been approved in Europe and the USA. For the treatment of postherpetic neuralgia, a single 30- or 60-min application may provide up to 3 months of

localised pain relief with minimal adverse effects. Although it is not indicated for neuropathic itch, this formulation has been reported to be effective in case series for neuropathic itch (Papoiu and Yosipovitch 2010; Metz et al. 2011).

2.7 Topical Glucocorticoids

Topical glucocorticoids are frequently used to treat patients with itch. They have an anti-inflammatory effect, rather than acting as a direct antipruritic, and range in potency. Inflammatory skin conditions have been effectively treated with both high- and moderate-potency preparations in randomised controlled trials. Hydrocortisone (2.5 %) has been shown to be significantly more effective than placebo in the reduction of itch that was induced experimentally (Zhai et al. 2000a). Clinical experience has shown that secondary manifestations of chronic itch (e.g. prurigo nodularis, lichen simplex chronicus) can be treated successfully with potent glucocorticoids (Weisshaar et al. 2012; Yosipovitch and Bernhard 2013).

The precise antipruritic mechanism of topical corticosteroids is not known. It is suggested that glucocorticoid receptors are activated, which in turn inhibit the action of cytokines and reduce the local inflammation; therefore, itch is controlled indirectly (Elmariah and Lerner 2011). Patients with noninflammatory itch usually do not benefit from topical corticosteroids.

The use of topical steroids in cases of generalised cutaneous disease, in the absence of a primary rash or in prolonged daily treatment should be limited to short periods and avoided long term (Weisshaar et al. 2012). Local adverse effects may include atrophy, striae, pigment alteration, acne, petechiae and telangiectasia, as well as potential systemic absorption, with hypothalamus-pituitary axis suppression. There may be a reduction in response after the administration of several doses of topical steroids (tachyphylaxis), which has been shown in experimental settings for itching disorders, including atopic dermatitis and psoriasis (Elmariah and Lerner 2011).

2.8 Topical Calcineurin Inhibitors

Tacrolimus and pimecrolimus are topical calcineurin inhibitors (TCIs) that have been shown to reduce itch in inflammatory skin conditions such as eczema (Kaufmann et al. 2006). Studies have also shown that TCIs can be successful in treating graft-versus-host disease, lichen planus, lichen sclerosis and prurigo nodularis (Ständer et al. 2006b). The mechanism of action of these agents in reducing itch is unclear and possibly multifactorial. TCIs regulate T-cell activation and inhibit the release of inflammatory cytokines. However, rather than acting solely through anti-inflammatory properties, these agents may also have an antipruritic effect that is mediated by the activation and subsequent desensitisation of TRPV1, located on unmyelinated peripheral C-nerve fibres. The efficacy of TCIs has been demonstrated in randomised trials, improving itch within 48 h of the first application and maintaining antipruritic effects throughout their continued use (Kaufmann et al. 2006).

Topical calcineurin inhibitors can cause an initial burning sensation, which may be due to activation of TRPV1. This may serve as a biomarker for antipruritic effect in those patients. The burning sensation generally reduces after repeated application over several days. TCIs do not cause skin atrophy with prolonged use and are therefore suitable for treating facial, genital and intertriginous areas of the skin. Patients with atopic dermatitis have been shown to have no significant risk of systemic immunosuppression or increase in the rate of serious infections (McCollum et al. 2010). In 2006, the FDA issued a black box warning of risk of lymphoma in paediatric or adult atopic populations using TCIs. This risk seems extremely rare (Siegfried et al. 2013). Long-term safety studies are required to investigate the risk of lymphomas (Elmariah and Lerner 2011).

2.9 Topical Antihistamines

Topical antihistamines are frequently used to treat itch; however, their value as a therapy for itching conditions is limited. Studies on topical antihistamines have been generally inconsistent and of inadequate design (Eschler and Klein 2010). Doxepin is an oral tricyclic antidepressant, which has also been manufactured as a topical 5 % cream and has been used successfully in patients with atopic dermatitis. It has anti-H_1 and anti-H_2 properties and is also thought to have some antimuscarinic effects (Drake et al. 1994). Doxepin is beneficial in patients with lichen simplex chronicus, nummular dermatitis and contact dermatitis (Patel and Yosipovitch 2010) but is ineffective in other conditions that cause itching (Yosipovitch and Bernhard 2013).

Adverse effects of topical doxepin, especially when applied to large areas, can include drowsiness caused by absorption through the skin, localised burning and allergic contact dermatitis. It is usually avoided in children and the elderly. Due to an increased risk of contact allergy, especially when the treatment exceeds 8 days, topical doxepin is not recommended by the latest chronic pruritus guidelines (Weisshaar et al. 2012).

2.10 Topical Cannabinoids

The efficacy of cannabinoids in pruritus has been demonstrated in a few studies. In a large industry-sponsored open-label trial in more than 3,000 patients, a cream containing *N*-palmitoylethanolamine (PEA) significantly reduced pruritus and improved disease severity in AD. PEA exhibits little affinity for cannabinoid receptors but may act by enhancing the effect of anandamide, an endocannabinoid, through inhibition of the enzyme fatty acid amide hydrolase (FAAH). Topical cannabinoids have been reported anecdotally to be effective for lichen chronicus, prurigo nodularis and uremic pruritus, with few to no side effects (Ständer et al. 2006a).

2.11 Topical Prostanoid Inhibitors

Topical acetylsalicylic acid has been described as having antipruritic effects, most probably by its effect on prostaglandin E2, a known itch enhancer, in double-blind study against lichen simplex chronicus—a form of localised severe itch (Yosipovitch et al. 2001; Patel and Yosipovitch 2010).

2.12 Miscellaneous Ion Channel Blockers

Topical strontium gel, a calcimimetic that blocks ion channels in nerves, has been shown in a double-blind study to inhibit itch induced by cowhage and histamine and has been used against different types of chronic itch (Zhai et al. 2000b; Papoiu et al. 2013).

Topical ketamine up to 5 %, combined with either amitriptyline or lidocaine, has shown antipruritic effects in recalcitrant cases of chronic itch and neuropathic itch in noncontrolled studies (Poterucha et al. 2013a, b).

3 Systemic Treatments

3.1 Systemic Antihistamines

Systemic antihistamines are the most frequently used drugs to relieve the symptoms of chronic itch due to dermatological and non-dermatological causes. First-generation antihistamines (chlorpheniramine, diphenhydramine, hydroxyzine and promethazine) are known to bind to H1 receptors as well as muscarinic, alpha-adrenergic, dopamine and serotonin receptors, which lead to a central sedative effect. They are therefore most useful at night for reducing itch and the itch-scratch cycle in a number of dermatoses, especially eczema and urticaria. Second-generation antihistamines (cetirizine, levocetirizine, desloratadine, ebastine, fexofenadine and loratadine) have little activity on non-histamine receptors—so are less sedative—and have longer durations of action (Weisshaar et al. 2012). Non-sedating H1-receptor antagonists are effective in the treatment of urticaria (Zuberbier et al. 2014; Maurer et al. 2013). However, histamine is not usually a major factor in conditions other than urticaria or mastocytosis, and so, while antihistamines such as loratadine, desloratadine, cetirizine and levocetirizine have commonly been used for daytime relief, the non-sedating H_1-receptor and H_2-receptor antagonists generally have limited use in treating itch (O'Donaghue and Tharp 2005). When choosing an antihistamine the disease, age and likelihood of other medical conditions or drug interactions should be considered. There is therefore not a single preferred antihistamine for the management of itch. Certain antihistamines (e.g. loratadine) are thought to be safer during pregnancy and lactation and in young children (Zuberbier et al. 2014; Lawlor 2014) (Table 2).

Table 2 Current systemic therapies for pruritus

Medication class	Medication and dosages	Main indication	Major side effect
Antihistamine	*1st generation:* usually only given at night due to their sedative effect Hydroxyzine Adults: 30–100 mg/day in 3 divided doses Children 30 months–15 years: 1 mg/kg/day in divided doses Diphenhydramine Adults: 25–50 mg bd Children > 2 years old: 1–2 mg/kg 6–8H Chlorpheniramine maleate Adults: 4 mg 6–8H Children: 0.1 mg/kg 6–8H	Nocturnal itch	Sedation
	2nd generation		
	Loratadine Adults and children ≥12 years: 10 mg qd Children 2–12 years: >30 kg:10 mg qd; ≤30 kg:5 mg qd	Urticaria Mastocytosis Insect bite reactions	Infrequent Drowsiness Dry mouth
	Cetirizine Adults and children ≥ 6 years: 10 mg qd or 5 mg bd Children 2–5 years: 2.5 mg bd or 5 mg qd Renal or hepatic insufficiency: reduce dosages by half		
	Fexofenadine Adults and children ≥12 years: 60 mg bd or 180 mg qd Children 6–11 years: 30 mg bd Renal impairment: consider lower dose of 60 mg qd		
Anticonvulsants	Gabapentin 300–3,600 mg/day in 3 divided doses Reduced dose in renal impairment	Neuropathic itch Uremic pruritus Prurigo nodularis Postburn pruritus	Drowsiness Leg swelling Blurred vision Constipation Ataxia

(continued)

Table 2 (continued)

Medication class	Medication and dosages	Main indication	Major side effect
	In dialysis patients, 100–300 mg after each dialysis		
	Pregabalin 150–450 mg/day in 2–3 divided doses Dose reduction in renal impairment		
Mu-opioid receptor antagonists	Naltrexone 25–50 mg om	Pruritus associated with Cholestasis Atopic dermatitis Chronic urticaria	Nausea and vomiting Insomnia Reversal of opioid analgesia Hepatotoxicity rarely
Kappa-opioid receptor agonists	Butorphanol 1–4 mg intranasally on		Butorphanol Drowsiness Nausea and vomiting
	Nalfurafine 2.5–5 μg om	Uremic pruritus (nalfurafine)	Nalfurafine Insomnia
Antidepressants	Mirtazapine 7.5–15 mg on initially, up to 45 mg on	Malignancy-associated pruritus Nocturnal pruritus in atopic dermatitis	Mirtazapine Drowsiness Weight gain
	SSRIs Paroxetine 10–40 mg qd Sertraline 75–100 mg qd Fluvoxamine 25 mg for 3 days, then 50–150 mg qd	Consider in pruritus associated with depression and/or anxiety Pruritus associated with haematological malignancies and solid tumours (paroxetine) Cholestatic pruritus (sertraline)	SSRIs Drowsiness Insomnia Sexual dysfunction
	Tricyclic antidepressants Doxepin 10–100 mg on Amitriptyline 25–75 mg on	Chronic idiopathic urticaria (doxepin) Neuropathic itch (amitriptyline)	Anticholinergic effects Drowsiness Dry eyes and mouth Blurred vision Urinary retention

(continued)

Table 2 (continued)

Medication class	Medication and dosages	Main indication	Major side effect
			Cardiovascular effects Orthostatic hypotension Conduction disturbances
Thalidomide	100–200 mg qd	Prurigo nodularis Uremic pruritus Actinic prurigo	Teratogenicity Peripheral neuropathy Drowsiness
Neurokinin-1 receptor antagonist	Aprepitant 80 mg qd	Itch associated with Haematological malignancies Solid tumours Biological cancer drugs Prurigo nodularis	Nausea Dizziness
Phototherapy	UVB, broad- and narrowband UVA Combined UVA and UVB PUVA, oral and topical	Atopic dermatitis Psoriasis Uremic pruritus Cholestatic pruritus	Tanning Increased itch Skin malignancies

Adapted from Tey HL, Yosipovitch G, Bernhard J (2014) Scientific American Medicine, 3rd edn

3.2 Glucocorticosteroids

Glucocorticosteroids have no direct antipruritic actions but have been effective in patients with inflammatory dermatoses, such as urticaria, dermatitis and bullous pemphigoid, reducing itch and controlling these diseases due to their anti-inflammatory effects. Recent data suggest they decrease the secretion of interleukin-31, an 'itchy' cytokine involved in the itch of eczema and cutaneous T-cell lymphoma (Singer et al. 2013).

Prednisolone is the most commonly selected systemic corticosteroid and should only be used as a short-term treatment (not longer than 2 weeks) due to potential side effects (Streit et al. 2002). Prednisolone may be used for a longer time in the treatment of inflammatory dermatoses, usually at a tapering dose to avoid long-term side effects.

4 Drugs That Work on Central Mechanisms of Itch

There is increasing evidence to support the phenomenon of central sensitisation in chronic pruritus, a process analogous to central sensitisation in chronic pain (Yosipovitch et al. 2007; Davidson and Giesler 2010; Akiyama and Carstens

2014). This process is thought to occur in the spinal cord and brain. In addition, the perception and interpretation of pruritus are very much influenced by cognitive and affective processes (Mochizuki et al. 2014). The use of antidepressants (selective serotonin reuptake inhibitors), mirtazapine and GABAergic drugs (gabapentin and pregabalin) may reduce this sensitisation and some of the cognitive aspects of chronic itch. Their exact mechanisms of action are unknown, but they most probably modulate itch perception.

4.1 Gabapentin

Antiepileptic drugs, such as gabapentin and pregabalin, are useful in neuropathic disorders causing itch or pain. These structural analogues of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), have an unclear mechanism but may be associated with the prevention of nociceptive sensations to the spinal cord and brain (Patel and Yosipovitch 2010). There have been case reports where their use has reduced neuropathic itch (Ehrchen and Ständer 2008) and gabapentin, in particular, has been successfully used in itch associated with nerve entrapment, such as notalgia paresthetica and postherpetic neuralgia (Loosemore et al. 2007). Recommended doses range between 300 mg and 1,200 mg three times a day. In controlled trials, low doses of gabapentin (100–300 mg three times per week) improved the itch associated with chronic kidney disease with significantly more efficacy than placebo (Yosipovitch and Bernhard 2013). Itch related to cutaneous lymphoma has also been treated with gabapentin. However, in patients with itch related to cholestasis, gabapentin increases the perception of itch and scratching and is therefore not recommended in these cases.

Pregabalin is a newer compound, similar in structure and function to gabapentin, and is considered to have fewer adverse effects. It is effective in patients with chronic itch, in particular neuropathic itch, in doses ranging from 25 mg up to 200 mg, twice a day; however, as with gabapentin, there is a risk of withdrawal symptoms if treatment is stopped suddenly (Ehrchen and Ständer 2008). Other common adverse effects of gabapentin and pregabalin include constipation, weight gain, drowsiness, ataxia and blurred vision (Yosipovitch and Bernhard 2013). The dosages of both these drugs require adjustment in patients with renal impairment.

4.2 Antidepressants

Mirtazapine, a selective norepinephrine reuptake inhibitor, reduces itch in patients with advanced cancer, leukaemia, lymphoma, chronic kidney disease, cholestasis and atopic dermatitis (Hundey and Yosipovitch 2004). A combination of low-dose mirtazapine and either gabapentin or pregabalin reduces intractable itch in patients with cutaneous T-cell lymphoma (Yosipovitch and Bernhard 2013). Its H_1-antihistamine properties also impart a sedative effect (Patel and Yosipovitch 2010). There are no serious adverse effects associated with mirtazapine, and it can

be particularly helpful in treating night-time itching. Oral mirtazapine (15 mg daily) reduces various kinds of nocturnal itch, particularly itching related to cancer (Hundey and Yosipovitch 2004).

Selective serotonin reuptake inhibitors may be useful when treating psychogenic itch, as well as other types of generalised itch. Paroxetine and fluvoxamine have a reasonable antipruritic effect (improving itch in 68 % of patients with chronic itch) especially when associated with atopic dermatitis, systemic lymphoma and solid carcinoma (Ständer et al. 2009). Sertraline is well tolerated and effectively treats cholestatic itch. Neuropathic or psychogenic forms of itch may also be treated with tricyclic antidepressants (e.g. amitriptyline) although there are no randomised trials that have studied these agents as yet (Yosipovitch and Bernhard 2013).

4.3 Mu-Opioid Antagonists

Mu-opioid receptor antagonists reverse the side effect, itch, caused by analgesics acting as mu-opioid receptor agonists. The antipruritic effects of naltrexone, nalmefene and naloxone have been reported in patients with cholestatic pruritus, chronic urticaria, atopic eczema and end-stage renal disease in randomised controlled trials (Phan et al. 2010). Endogenous activation of the mu-opioid receptor is presumed to be involved in mediating chronic itch, particularly in systemic diseases such as chronic kidney disease and cholestasis. However, findings from studies in the treatment of itch relating to chronic kidney disease using mu-opioid antagonists are inconsistent, and adverse effects such as diarrhoea, abdominal cramps, loss of appetite and nausea have restricted their use (Yosipovitch and Bernhard 2013).

4.4 Kappa-Opioid Agonists

Nalfurafine, a kappa opioid launched in Japan in 2009, has proved successful in end-stage renal disease patients on hemodialysis with pruritus (Kumagai et al. 2010). This drug has not been approved as yet in other countries. Butorphanol, a kappa-opioid agonist/partial mu antagonist and antimigraine agent, is also effective against intractable nocturnal itch of different types (Dawn and Yosipovitch 2006). It is administered intranasally (1 mg dose up to 4 mg a day).

4.5 Neurokinin-1 Inhibitors

Aprepitant, an oral antiemetic drug that antagonises the action of substance P on neurokinin type 1 receptors, is effective against pruritus associated with prurigo nodularis and lymphoproliferative diseases, in particular, intractable itch of Sézary syndrome (Ständer et al. 2010; Raap et al. 2011). A major drawback for its current use in the USA is that it is extremely expensive.

4.6 Thalidomide

Thalidomide has been used successfully in some cases of chronic pruritus, probably due to its central depressant effect (Daly and Shuster 2000). It is useful in prurigo nodularis and uremic pruritus (Maurer et al. 2004). Thalidomide is teratogenic with a risk of neuropathy; therefore, it is not recommended routinely for the treatment of chronic pruritus and is used for limited durations of up to 1 year.

5 Immunosuppressants for Chronic Itch

The oral immunosuppressants, cyclosporine and azathioprine, have demonstrable antipruritic effects in patients with atopic dermatitis. Cyclosporine has also shown antipruritic effects in lichen planus and urticaria (Madan and Griffiths 2007). Widespread use of this agent has been limited due to the risk of significant hypertension, elevated creatinine and blood urea nitrogen, immunosuppression and renal toxicity. Monotherapy with oral azathioprine in a double-blind, placebo-controlled study resulted in significant improvements in itch score, disease activity and quality of life in patients with atopic dermatitis. Caution is advised when prescribing this medication due to the risk of dose-dependent myelotoxicity. The susceptibility of individuals to myelosuppression induced by azathioprine relates to the activity of thiopurine methyltransferase (TPMT), a key enzyme in azathioprine metabolism. Thus, measurement of erythrocyte TPMT activity before initiation of therapy helps identify those patients at high risk of this serious side effect. African Americans display lower enzyme activity, and therefore extreme caution should be taken when using this drug in these patients with intermediate levels of TPMT (Patel and Yosipovitch 2010). It is recommended that oral cyclosporine and azathioprine only be used in the short term for patients with atopic dermatitis who have failed conventional therapy and with appropriate monitoring.

Mycophenolate mofetil (MMF), an ester prodrug of mycophenolate acid, acts by selectively inhibiting lymphocyte proliferation and the production of antibodies, to exert an immunosuppressive effect. Although not licensed for use in dermatology, treatment with oral MMF (2 g daily) is safe, well tolerated and effective in severe atopic eczema in adults (Neuber et al. 2000). It has also been used successfully as a second-line therapy to treat patients with chronic autoimmune and idiopathic urticaria, with some achieving complete control of their symptoms. Gastrointestinal complaints are a common concern (Zimmermann et al. 2012). Caution must be taken in the elderly because of increased risk of infection and gastrointestinal haemorrhage.

Methotrexate, a derivative of folic acid, inhibits cell division by suppressing keratinocyte proliferation and inhibiting DNA synthesis in immunologically active cells. It also exhibits anti-inflammatory effects on lymphocytes and neutrophils (Smith and Barker 2002). Methotrexate is licensed for use in severe psoriasis, but is also useful in other dermatological diseases. A randomised controlled trial concluded that methotrexate achieved clinical improvement in patients with severe

atopic eczema. It is safe in the short term and therefore an appropriate treatment option for adults (Patel et al. 2012). Treatment of generalised lichen planus is often associated with relapse. Low-dose oral methotrexate (15 mg/week for adults and 0.25 mg/kg/week for children) improved the condition in a small study, with complete remission of the disease in 14 of 24 patients and no relapses during posttreatment follow-up of 3 months (Kanwar and De 2013).

Dapsone (25–50 mg/day) is effective in different types of chronic urticaria and angioedema, including spontaneous chronic urticaria. The addition of dapsone to desloratadine in a randomised study was associated with a higher rate of complete remission compared with desloratadine alone, although urticaria activity scores were not reduced (Engin and Ozdemir 2008). A combination of dapsone, systemic corticosteroids and phototherapy has been shown to produce a good response in cases of hypereosinophilic dermatitis (Kemmler et al. 2005). Dapsone therapy also alleviates the characteristic intense pruritus that accompanies dermatitis herpetiformis, as well as other highly variable cutaneous symptoms and signs (Pfeiffer 2006). While dapsone is usually well tolerated, there are rare adverse effects, which include anaemia (related to the dose), as well as skin rash, peripheral neuropathy, gastrointestinal problems, hepatotoxicity, blood dyscrasias and methaemoglobinaemia. It can also cause drug reaction with eosinophilia and systemic symptoms and is contraindicated in G6PD-deficient patients due to increased risk of haemolysis (Engin and Ozdemir 2008).

More recently, the newer biologic agents have been shown to reduce itch in a number of dermatoses. These include biologics such as omalizumab, anti-IgE, licensed for use in patients with chronic urticaria. Omalizumab is a recombinant humanised monoclonal IgG antibody, which binds free IgE and is thought to downregulate mast cell function. It has emerged as an effective treatment for antihistamine-unresponsive chronic urticaria, demonstrated by numerous case reports and studies (Asero et al. 2013). A randomised, double-blind, placebo-controlled trial has shown subcutaneous omalizumab (150–300 mg) to be effective and well tolerated in chronic urticaria patients aged 12–75 years (Maurer et al. 2013). However, symptoms gradually reappear 4–10 weeks after drug discontinuation, and the use of the drug is also limited by high cost (Kaplan et al. 2013). Anti-TNF-alpha agents, such as etanercept and adalimumab, have been used in patients with itch secondary to psoriasis (Krueger et al. 2005). Both have shown antipruritic activity in psoriatics (Gottlieb et al. 2010; Sola-Ortigosa et al. 2012).

Apremilast, a specific inhibitor of phosphodiesterase 4, modulates pro-inflammatory and anti-inflammatory cytokine production. It is licensed for use in psoriatic arthritis, with potential benefits for a range of other inflammatory conditions. A randomised controlled study of patients with moderate to severe plaque psoriasis resulted in an improved pruritus visual analogue scale after 16 weeks of treatment with apremilast. A pilot study evaluating doses of apremilast for atopic dermatitis in adults found that 20 mg twice daily significantly reduced pruritus at 3 months (Samrao et al. 2012).

6 Phototherapy

The positive immunomodulatory effects of UV therapy for the treatment of chronic itch of various types are well documented (Steinhoff et al. 2011). Broad- or narrowband ultraviolet B (UVB) radiation, used alone, or in combination with ultraviolet A (UVA) radiation, has been found by observational studies to improve the itch associated with chronic kidney disease and also in skin diseases including psoriasis, atopic eczema and cutaneous T-cell lymphoma (Rivard and Lim 2005). A single-blind, randomised trial for uremic itch showed no significant difference in the efficacy of UVA radiation compared with UVB radiation (Ko et al. 2011). Phototherapy may be given in combination with topical and/or systemic therapy, except with calcineurin inhibitors or immunosuppressive drugs, where its use may lead to increased risk of malignancy.

7 Conclusion

Current available topical and systemic therapies have been discussed; there is no universal treatment regimen that is satisfactorily effective in all cases of chronic itch. This is due to the fact that itch has multifactorial aetiologies and can involve different mechanisms. An understanding of the pathophysiology of chronic itch is only partially complete at present. Chronic itch management is challenging and should be instituted early and address the underlying pathology and central hypersensitisation. With a better understanding of the pathological mechanisms of itch processing, the targeting of specific mediators and neuronal pathways will offer a promising approach.

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Atopic Itch in Dogs: Pharmacology and Modeling

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Abstract

Itch is the most common clinical problem seen in dogs with skin diseases. Although an etiological classification of canine pruritus does not yet exist, most causes would likely fall into the IFSI class I (dermatological) itch. One of the most common causes of canine itch is that associated with atopic dermatitis, and there is randomized controlled trial grade evidence of the efficacy of several antipruritic interventions. At this time, the mainstay of treatment of canine atopic itch relies principally on the use of topical and/or oral glucocorticoids and oral cyclosporine. Type 1 receptor antihistamines are notorious in their inconsistency in reducing pruritus in atopic dogs. A new Janus kinase (JAK)-1 inhibitor has recently been approved for treatment of allergic itch in dogs, and its onset of efficacy is remarkably fast. Modeling itch in dogs can be achieved by allergen sensitization (fleas, house dust mites), and challenges that elicit pruritic manifestation can be used for mechanistic studies as well as for testing of novel anti-itch modalities.

Keywords

Animal model • Atopic dermatitis • Canine • Dog • Pruritus

Abbreviations

AD	Atopic dermatitis
JAK	Janus kinase
NFAT	Nuclear factor of activated T cells
NT	Not tested
PAR	Proteinase-activated receptor
RCT	Randomized controlled trial
VAS	Visual analog scale

1 Etiology of Itch in Dogs

In a recent epidemiological study, pruritus (itch) was found to represent the most common clinical sign (>30 % of cases) exhibited by dogs presented to veterinarians for skin diseases, the latter being the number one cause (>20 % of cases), motivating owners to bring their dogs to veterinarians outside of preventive health care (Hill et al. 2006). At the time of this writing, an etiological classification of itch in dogs has not been established. We propose herein to use the 2007 nosology

Table 1 Proposal for a classification of itch in dogs

Category	Diseases in dogs
I. Dermatological	*Arising from "diseases of the skin"*: e.g., ectoparasites (e.g., scabies, fleas, etc.), allergies (e.g., atopic dermatitis, urticaria, flea allergy dermatitis, etc.), infections (staphylococcal folliculitis, *Malassezia* dermatitis, etc.), and neoplastic (e.g., epitheliotropic T-cell lymphoma, mast cell tumor, etc.)
II. Systemic	*Arising from "diseases of organs other than the skin"*: none recognized so far in dogs
III. Neurological	*Arising from "diseases or disorders of the central or peripheral nervous system"*: e.g., syringomyelia (Chiari-like malformation), acral mutilation syndrome, etc.
IV. Psychogenic-psychosomatic	For example, acral lick dermatitis, tail chasing, etc.
V. Mixed	*Overlapping and coexistence of several diseases*: e.g., atopic dermatitis with staphylococcal folliculitis, epitheliotropic T-cell lymphoma with secondary Malassezia surface proliferation, etc.
VI. Others	*Undetermined origin*

proposed by members of the International Forum for the Study of Itch (IFSI) (Stander et al. 2007). In dogs, like in humans, the etiology of itch can be logically subdivided in one of six categories (Table 1). Currently recognized forms of canine itch are likely to belong to the first category (i.e., "dermatological itch"), meaning that pruritus occurs in association with, or secondary to, cutaneous inflammation. Using a 10-point visual analog scale validated for use in dogs, the pruritus associated with atopic dermatitis (AD) was found to range in values between 2.0 and 9.5 (median: ~6.0) (Rybnicek et al. 2009). In dogs, erythema and pruritus scores are normally correlated, but there are animals with high pruritus VAS and low erythema values and vice versa (Hill et al. 2010).

A notable difference between dogs and humans is the lack of pruritus recognized to be associated with chronic liver or kidney disease in animals. There is no logical explanation for this discrepancy between species.

2 Pharmacology of Itch in Dogs

There are only two canine pruritic skin diseases in which there is randomized controlled trial (RCT)-level evidence of efficacy of interventions to treat itch and skin lesions: atopic dermatitis (AD) and flea allergy dermatitis. As the use of an insecticide remains the treatment of choice for the latter, this chapter will be limited to the discussion of interventions shown in RCTs to be consistently effective to reduce pruritus in dogs with AD.

2.1 Glucocorticoids for Canine Atopic Itch

The systemic administration of glucocorticoids has been the first line of treatment for pruritus of allergic origin in dogs for decades. Their efficacy has been demonstrated in numerous RCTs [reviewed in Olivry et al. (2010) and Olivry and Bizikova (2013)].

2.1.1 Mechanism of Action of Glucocorticoids in Canine Atopic Itch

Glucocorticoids show a broad anti-inflammatory effect. Interestingly, in all RCTs where both lesions and pruritus are evaluated, a concurrent reduction of pruritus and skin lesions is normally seen, thereby indicating that glucocorticoids likely act via the inhibition of pro-inflammatory and pruritogenic cytokines, as well as nerve hypersensitivity secondary to inflammation. However, laboratory animal data suggest that a direct effect on sensory neurons also exists, as scratching induced by histamine, substance P, serotonin, and a PAR-2 agonist can be attenuated by topical administration of the strong dermocorticoid clobetasol propionate, even in mast cell-deficient mice (Sekine et al. 2012). There are few canine studies on the change in skin inflammatory mediators after glucocorticoid administration. In an experimental dog model of cutaneous late-phase reactions induced by anti-canine IgE antiserum, the mRNA expression of the cytokines IL-13, CCL2, CCL5, and CCL17 was suppressed by prednisolone (Pucheu-Haston et al. 2006). Recently, recombinant canine IL-31 was found to induce pruritus in dogs, and it is elevated in more than 50 % of dogs suffering from AD (Gonzales et al. 2013b). Prednisolone given 1 h before IL-31 injection to dogs does not reduce its induced pruritus, but it does so when given 2–10 h beforehand (Fleck et al. 2012).

2.1.2 Efficacy of Glucocorticoids for Canine Atopic Itch

Oral Glucocorticoids

There are at least seven RCTs demonstrating the efficacy of prednisone, prednisolone, or methylprednisolone for treatment of pruritus associated with AD in dogs. Typical dosages range between 0.5 and 1.0 mg/kg per day, and the doses are normally decreased after 1 or 2 weeks to reduce side effects. The overall reduction of pruritus varies from study to study and ranges from 40 % to 85 % (maximum assessment: 16 weeks) (Olivry et al. 2010; Olivry and Bizikova 2013).

Topical Glucocorticoids

As the long-term use of oral glucocorticoids is associated with side effects that include polydipsia, polyuria, and urinary infections, the topical administration of glucocorticoids offers a valid substitute with fewer systemic side effects.

One RCT reported the use of 0.015 % triamcinolone acetonide solution (Genesis, Virbac) in dogs with pruritus of suspected allergic origin (DeBoer et al. 2002). Nearly 70 % of the dogs treated with the active intervention exhibited greater than 50 % reduction in pruritus scores compared to only 24 % of dogs receiving placebo (DeBoer et al. 2002).

In another RCT, a 0.0584 % hydrocortisone aceponate spray (Cortavance, Virbac) was tested over 84 days: 67 % of the atopic dogs exhibited a reduction of pruritus of at least 50 % and 21 % of at least 90 % (Nuttall et al. 2012). Again, the decrease of pruritus showed a very similar time response as the reduction of lesions indicating that the antipruritic effect might be primarily secondary to the reduction of inflammation. Importantly, the long-term use of topical glucocorticoids leads to skin atrophy, the severity of this problem depending upon the strength of the product and the duration of treatment.

2.2 Cyclosporine for Canine Atopic Itch

2.2.1 Mechanism of Action of Cyclosporine for Canine Atopic Itch

Calcineurin inhibitors act mainly (but not exclusively) as T-cell inhibitors. By blocking the nuclear translocation of the nuclear factor of activated T-cell (NFAT) transcription factor, the secretion of cytokines such as IL-2 and interferon gamma is reduced in T cells. A direct effect of cyclosporine on canine T cells reveals that therapeutic doses of cyclosporine (5–10 mg/kg/day) reduce the expression of IL-2 and interferon gamma in in vitro stimulated T cells purified from peripheral blood mononuclear cells of orally treated dogs (Archer et al. 2011).

Specific studies on the influence of cyclosporine on mediators of itch have not yet been performed in dogs. As cyclosporine treatment in atopic humans is accompanied by a decrease of serum levels of IL-31 (Otsuka et al. 2011), the effect of cyclosporine A on canine serum IL-31 or in activated canine T cells and mast cells should be investigated.

The action of cyclosporine in atopic dermatitis (and itch) is most likely not restricted to that on T cells: the NFAT transcription factor is also found in other cells such as dendritic cells, eosinophils, mast cells, and keratinocytes, and cyclosporine is known to also affect the function of these cells (Fric et al. 2012). For instance, protease-induced secretion of GM-CSF was reduced in a canine keratinocyte progenitor cell line by cyclosporine (Kimura et al. 2013). Also the lipopolysaccharide-induced PGE_2 synthesis was significantly reduced by cyclosporine in primary canine keratinocytes (Baumer and Kietzmann 2007). On the contrary, a recently published study revealed that cyclosporine might enhance the response to Toll-Like receptor agonists Pam3CSK4 and staphylococcal peptidoglycan, as mRNA expression of TNFα and IL-8 was enhanced in canine keratinocytes (Hendricks et al. 2012). Future studies should focus on the recently published influence of cyclosporine and PAR-2-activated TSLP secretion in canine keratinocytes, as this could be a crucial inducer of itch in dogs, as shown recently in mice (Wilson et al. 2013).

2.2.2 Efficacy of Cyclosporine for Canine Atopic Itch

There are several RCTs showing a significant reduction of pruritus in dogs suffering from AD after treatment with oral cyclosporine (Atopica, Novartis Animal Health) starting at 5 mg/kg once daily. Depending on the study, approximately 50–70 % of

dogs exhibit a reduction of pruritus score equal or greater than 50 %. The maximal antipruritic effect is generally seen after 4–6 weeks, and the efficacy appears to remain stable even over longer treatment periods (Olivry et al. 2010; Olivry and Bizikova 2013; Steffan et al. 2006). Similar to that of glucocorticoids, the antipruritic effect of cyclosporine seems to be linked to the anti-inflammatory action of this drug, as the evolution of pruritus reduction appears to most often parallel that of the reduction in skin lesions. The concurrent use of oral prednisolone increases the speed of antipruritic action of cyclosporine (Dip et al. 2013).

A topical nanoencapsulated cyclosporine formulation was reported recently to be capable of reducing pruritus in atopic dogs to an extent similar to that of orally administered cyclosporine (Puigdemont et al. 2013).

2.3 Type 1 Antihistamines for Canine Atopic Itch

2.3.1 Mechanism of Action of Type 1 Antihistamines for Canine Atopic Itch

The classic H1 antihistamines are competitive inhibitors at the H1 receptor (Simons and Simons 2011). Histamine binds to four different G protein-coupled receptors. These are widely distributed in the body: to simplify, the H1R is found on smooth muscle, endothelial, as well as immune cells, and it plays a role in the genesis of immediate-type hypersensitivity reactions; the H2R plays a role in gastric acid production, whereas the H3R is mainly found in the central nervous system and on peripheral neurons. Interestingly, the H4R is mainly expressed on hematopoietic cells (neutrophils, eosinophils, monocytes, dendritic cells, Langerhans cells, T lymphocytes, basophils, mast cells), fibroblasts, endocrine cells, and neurons, and this leads to a suspected role in allergy and inflammation (Zampeli and Tiligada 2009). Importantly, histamine-induced pruritus in mice seems to be mediated via the histamine H1 and H4 receptors, while the H3R appears to have a negative regulatory role (Rossbach et al. 2011). Typical H1 antihistamines like diphenhydramine, cetirizine, loratadine, and hydroxyzine have only low binding affinity to the H2R, H3R, or H4R (Lim et al. 2005). With regard to pruritus, it might be important to distinguish centrally acting antihistamines (first-generation antihistamines like diphenhydramine) from those that lack (or have a vastly reduced) central action, as these hardly cross the blood-brain barrier (loratadine).

2.3.2 Efficacy of Type 1 Antihistamines for Canine Atopic Itch

There are several clinical trials that tested the efficacy of both first- and second-generation H1R antihistamines in dogs with AD [reviewed in Olivry et al. (2010), Olivry and Bizikova (2013) and Olivry and Mueller (2003)]. In general, the quality of these trials was found to often be poor, and most RCTs did not document a clinically relevant efficacy of this class of drugs (Olivry et al. 2010; Olivry and Bizikova 2013; Olivry and Mueller 2003; Eichenseer et al. 2013). The main limitations of these studies are a short duration of action and, possibly, inappropriate dosages, as these are often extrapolated from those used in humans without

further dog-specific pharmacokinetic/pharmacodynamic justification. In spite of the lack of consistently supportive RCT results, many clinicians anecdotally report a low to medium efficacy of H1R antihistamines to control pruritus in dogs with AD (Dell et al. 2012). To date, there has been no trial-based evaluation of the possible benefit of H4R (or combined H1R and H4R) antihistamines to control AD-associated itch in dogs.

2.4 Janus Kinase (JAK) Inhibitors

2.4.1 Mechanism of Action of JAK Inhibitors for Canine Atopic Itch

The Janus kinase (JAK) family encompasses four intracellular tyrosine kinases (JAK1, JAK2, JAK3, and TYK2) that transduce signals of numerous cytokine and chemokine receptors via the STAT signaling pathway (O'Shea and Plenge 2012). Oclacitinib (Apoquel, Zoetis) is a novel JAK inhibitor shown to principally inhibit the function of JAK1-dependent cytokines involved in allergic inflammation (IL-2, IL-4, IL-6, IL-13); it appears to have minimal activity against JAK2-dependent cytokines involved in hematopoiesis or those associated with the innate immune response (Gonzales et al. 2013a). Oclacitinib reduces IL-31-induced pruritus in dogs, likely because of its interference with the IL-31 receptor signal transduction.

2.4.2 Efficacy of JAK Inhibitors for Canine Atopic Itch

A recent RCT documented the rapid efficacy of oclacitinib (0.4–0.6 mg/kg twice daily) to decrease itch, as well as skin lesions, in dogs with allergic skin diseases (Cosgrove et al. 2013). Oclacitinib significantly reduced pruritus compared to placebo as early as the first day, and approximately 60 % of treated dogs had at least a 50 % reduction in pruritus as early as 4 days of treatment (placebo 23 %) (Cosgrove et al. 2013).

3 Modeling Canine Atopic Itch

3.1 Pruritus Induction by Single Substances

There have been several attempts to experimentally induce pruritus in dogs. Several agents, which are known to be pruritogenic in humans and/or rodents, have been investigated for a similar effect in dogs. Unfortunately, the intradermal administration of mast cell-degranulating substances like compound 48/80, anti-canine IgE antibodies, as well as histamine, serotonin, tryptase, and substance P all failed to induce a reliable itch-scratching reaction (see Table 2 as an overview) (Hill et al. 2001; Carr et al. 2009; Rossbach et al. 2009). The administration of cowhage, known to activate PAR-2, was recently shown to inconsistently induce pruritus manifestations in dogs (Olivry et al. 2013).

Table 2 Effect of selected substances on their pruritogenic behavior induction (+) in mice, dogs, and humans

Substance	Mice	Humans	Dogs	Comment
Histamine	+	+	–	Dogs develop wheals and erythema
Serotonin	+	+	–	Dogs develop wheals and erythema
Substance P	+	±	–	Dogs develop small wheals
Compound 48/80	+	+	±	Dogs develop wheals and erythema
Leukotriene B4	+	NT	–	
Tryptase	+	NT	–	
Cowhage/ mucunain	+	+	±	Dogs show inconsistent itch manifestations
IL-2	NT	+	–	Dogs develop wheal and erythema
IL-31	+	+	+	Most of the dogs tested show itch behavior

Data from Carr et al. (2009), Olivry et al. (2013), Akiyama and Carstens (2013), and our own unpublished observations
NT not tested

3.2 Flea Allergic Pruritus Dog Model

A model used to test the efficacy of new antipruritic compounds in dogs is the flea allergy model. Flea allergy dermatitis is a highly pruritic skin disease with itch VAS scores comparable to those of canine AD (Rybnicek et al. 2009). An experimental flea allergy model was used to test the efficacy of topical hydrocortisone aceponate on the time and frequency of pruritic events (Bonneau et al. 2009). Similarly, the recently FDA-approved JAK inhibitor oclacitinib was also found to significantly reduce flea allergy-associated pruritus and skin lesions (Wheeler et al. 2012).

Laboratory beagles are often used for this model. Flea allergy dermatitis is induced by controlled infestations with cat fleas (*Ctenocephalides felis*). Experimental protocols do vary, but the principle is a sensitization by frequent infestations with about 100 fleas per dog followed by a rest period of several months (Bonneau et al. 2009). The challenge step involves several successive infestations with cat fleas (e.g., every 4 days). The infestation of dogs with 5 to 30 fleas induces moderate levels of pruritus in most dogs. Pruritic manifestations, such as chewing, scratching, biting, licking, rolling, and rubbing, can be monitored. With this model, the antipruritic effect of pharmacological agents can be compared to that of placebo or active drugs. In a recent study using this model, the efficacy of a topical hydrocortisone aceponate spray (Cortavance, Virbac) was monitored over 7 days, and, although dogs with placebo also exhibited a reduction in pruritus, the effect on itch was significantly higher in hydrocortisone aceponate-treated dogs.

There are several limitations with the use of this flea allergic pruritus dog model. Until now, this model has been insufficiently characterized, especially in regard to the development of flea allergen-specific IgE serum levels; cytokine profiles and inflammatory infiltrates before and after flea allergen challenge have shown conflicting results (Wuersch et al. 2006). Furthermore, there are no data available for possible alterations of skin barrier function and skin lipid composition, which

are among the main focuses on AD-associated inflammation and possibly itch (Marsella et al. 2011; Olivry 2011).

3.3 House Dust Mite Allergic Pruritus Dog Model

In the last decade, Marsella and colleagues from the University of Florida pioneered the use of house dust mite (HDM)-sensitized beagles exposed to environmental, epicutaneous, inhalation, or oral allergen challenges (Marsella et al. 2006a, b). Sensitized beagles reliably develop skin lesions, but pruritus is not consistently reported, nor is it typically taken into consideration in data analyses.

A second model developed at NC State University involves dogs that spontaneously developed food hypersensitivity at a young age. These Maltese-beagle atopic (MBA) dogs exhibited pruritus and AD-like lesions following food allergen ingestion, as reported initially by Jackson and colleagues (Jackson and Hammerberg 2002). These dogs are easily sensitized to aeroallergens including HDM. In sensitized dogs, but not in controls, the epicutaneous application of HDM induces skin lesions that clinically and histologically resemble those of AD (Fig. 1) (Pucheu-Haston et al. 2008; Olivry et al. 2011). In the original challenge design, HDM allergens are applied to a small area in one of the dog's axillae in order to have non-challenged control sites on other parts of the body as well as to limit discomfort to the animal.

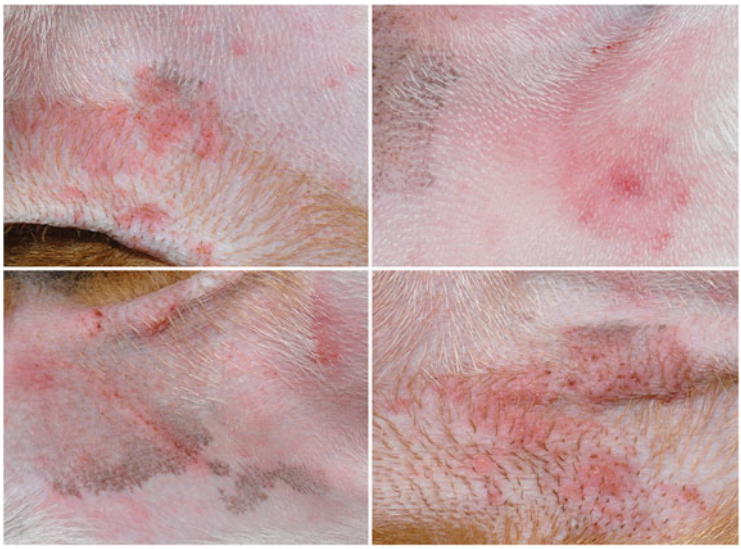

Fig. 1 Macular, papular, or patchy erythema, with or without excoriations 24 h after HDM application to the axillary skin of Maltese-beagle atopic dogs sensitized to this allergen; these lesions are reminiscent of those of acute AD in humans and dogs

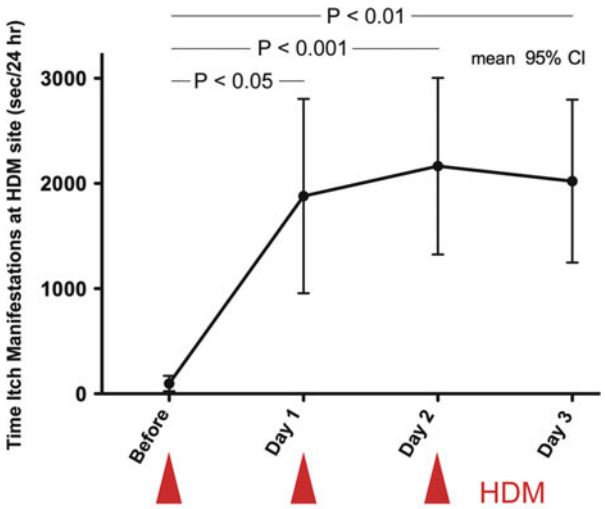

Fig. 2 House dust mite allergens were applied once daily for 3 consecutive days (*arrowhead*) to the abdominal skin of four Maltese-beagle atopic dogs sensitized to these allergens. Dogs were monitored by 24 h video surveillance. The time spent scratching, licking, biting, and chewing the allergen application sites was counted. In these dogs, HDM allergen application led to a significant increase in itch manifestations at the challenge site; applying the allergen on days 2 and 3 did not lead to a marked increase in itch manifestations compared to those seen on day 1

To increase pruritogenic stimuli, investigators now apply higher doses of HDM allergens to a wider surface on the abdomen, and the dog's pruritic manifestations are recorded via video and accelerometers. With this stronger challenge, a reliable pruritus induction is obtained (Fig. 2), with itch starting within minutes of allergen application. Itch induction is usually, but not always, associated with skin lesions at the site of application (Olivry and Paps unpublished data).

The models discussed above provide a strong advantage for studying the pathogenesis of canine AD and its itch as (i) within each colony, there is homogeneity of genetic heritage, environment, diet, and hygiene, (ii) skin lesions and itch can be timed after allergen challenge, and (iii) comparisons can be made between dogs sensitized or not to a particular allergen or between skin areas in which the allergen is applied or not.

As a result of these models, notable advances have been made in the understanding of canine AD, including the latest impetus on the likely importance of the skin barrier in the genesis of AD skin lesions (Olivry et al. 2011; Marsella et al. 2010; Hightower et al. 2010; Stahl et al. 2012). Additionally, these models have proven useful to test the efficacy of pharmaco- or immunotherapeutic interventions, for example, our recent demonstration that the administration of selected type 1 or type 4 antihistamines does not prevent AD acute lesion development (Baumer et al. 2011).

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