Cannabinoids and Anxiety

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Abstract The term cannabinoids encompasses compounds produced by the plant *Cannabis sativa*, such as Δ^9-tetrahydrocannabinol, and synthetic counterparts. Their actions occur mainly through activation of cannabinoid type 1 (CB1) receptors. Arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG) serve as major endogenous ligands (endocannabinoids) of CB1 receptors. Hence, the cannabinoid receptors, the endocannabinoids, and their metabolizing enzymes comprise the endocannabinoid system.

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Cannabinoids induce diverse responses on anxiety- and fear-related behaviors. Generally, low doses tend to induce anxiolytic-like effects, whereas high doses often cause the opposite. Inhibition of endocannabinoid degradation seems to circumvent these biphasic effects by enhancing CB1 receptor signaling in a temporarily and spatially restricted manner, thus reducing anxiety-like behaviors. Pharmacological blockade or genetic deletion of CB1 receptors, in turn, primarily exerts anxiogenic-like effects and impairments in extinction of aversive memories. Interestingly, pharmacological blockade of Transient Receptor Potential Vanilloid Type-1 (TRPV1) channel, which can be activated by anandamide as well, has diametrically opposite consequences. This book chapter summarizes and conceptualizes our current knowledge about the role of (endo)cannabinoids in fear and anxiety and outlines implications for an exploitation of the endocannabinoid system as a target for new anxiolytic drugs.

Keywords Cannabinoids · Endocannabinoids · Fear · Anxiety · Stress

Abbreviations

2-AG	2-Arachidonoyl glycerol (endocannabinoid; i.e., activates CB1 receptors)
AA-5HT	Arachidonoyl serotonin (synthetic inhibitor of FAAH-mediated endocannabinoid degradation and TRPV1 antagonist)
AEA	Anandamide or arachidonoyl ethanolamide (endocannabinoid and endovanilloid; i.e., activates CB1 receptors and TRPV1)
AM251	(Synthetic CB1 receptor antagonist)
AM404	(Synthetic inhibitor of endocannabinoid uptake and FAAH-mediated degradation)
CB1	Cannabinoid type 1 receptor
CB2	Cannabinoid type 2 receptor
CBD	Cannabidiol (phytocannabinoid)
CP-55940	(Synthetic CB1 receptor agonist)
Cre/loxP	(Recombinatory system used in conditional mouse mutagenesis)
FAAH	Fatty acid amide hydrolase (major degrading enzyme of AEA)
GABA	Gama-aminobutyric acid (major inhibitory transmitter of the brain)
GPR55	(G-protein-coupled cannabinoid receptor)
HU210	(Synthetic CB1 receptor agonist)
JZL184	(Synthetic inhibitor of MGL-mediated endocannabinoid degradation)
MGL	Monoacylglycerol lipase (major degrading enzyme of AEA)
PAG	Periaqueductal grey (matter brain structure orchestrating fear responses)

PPARα	(cannabinoid receptor)
SB366971	(Synthetic TRPV1 antagonist)
SR141716A	Rimonabant/acomplia^{TM} (synthetic CB1 receptor antagonist)
SR144528	(Synthetic CB2 receptor antagonist)
Δ^9-THC	Δ^9-Tetrahydrocannabinol (phytocannabinoid; major psychoactive ingredient of *Cannabis sativa*)
TRPV1	Transient receptor potential vanilloid type-1 channel
UCM707	(Synthetic inhibitor of endocannabinoid uptake)
URB597	(Synthetic inhibitor of FAAH-mediated endocannabinoid degradation)
VDM11	(Synthetic inhibitor of endocannabinoid uptake)
WIN-55212-2	(Synthetic CB1 receptor agonist)

> "Lets us hope that through better understanding of Cannabis chemistry in the brain we may also approach the chemistry of emotions."
> Mechoulam et al. (1991)

1 Introduction

The plant *Cannabis sativa* has been used for medical and recreational purposes for more than 5000 years. It has been known since these early times that smoking of Cannabis (marijuana) may induce a state of euphoria, relaxation, sense of delight, and increase in sociability. Occasionally, however, it may cause anxiety, panic, delusions or psychotic-like symptoms. The psychoactive ingredient of Cannabis could be identified and characterized only in 1964. Raphael Mechoulam and his coworker (1964) isolated it as a lipid compound, an aromatic terpenoid named Δ^9-tetrahydrocannabinol (Δ^9-THC; Fig. 1). Today we know that Cannabis extracts contain more than 70 biologically active compounds, including the nonpsychoactive cannabidiol (Fig. 1) and cannabinol.

The identification of these natural cannabinoids, or phytocannabinoids, led to the synthesis of several analogues, termed synthetic cannabinoids, which were able to mimic the effects of Δ^9-THC with higher potency and which contributed to elucidating the pharmacology of this group of substances. Among the main synthetic cannabinoids are nabilone, CP-55940, and WIN-55212-2 (Fig. 1). In addition, the refinement of animal models allowed a better characterization of their effects. For instance, with the Tetrad test a pharmacological assay was developed in which cannabinoids could be identified by their capacity to induce analgesia, hypothermia, hypolocomotion, and catalepsy. Nonetheless, it took several decades until their mechanisms of action started to be unraveled.

For a long time, Δ^9-THC and other cannabinoids were thought to exert their biological effects by changing biophysical characteristics of cell membranes. Only at the end of the 1980s a cannabinoid receptor could be identified, followed

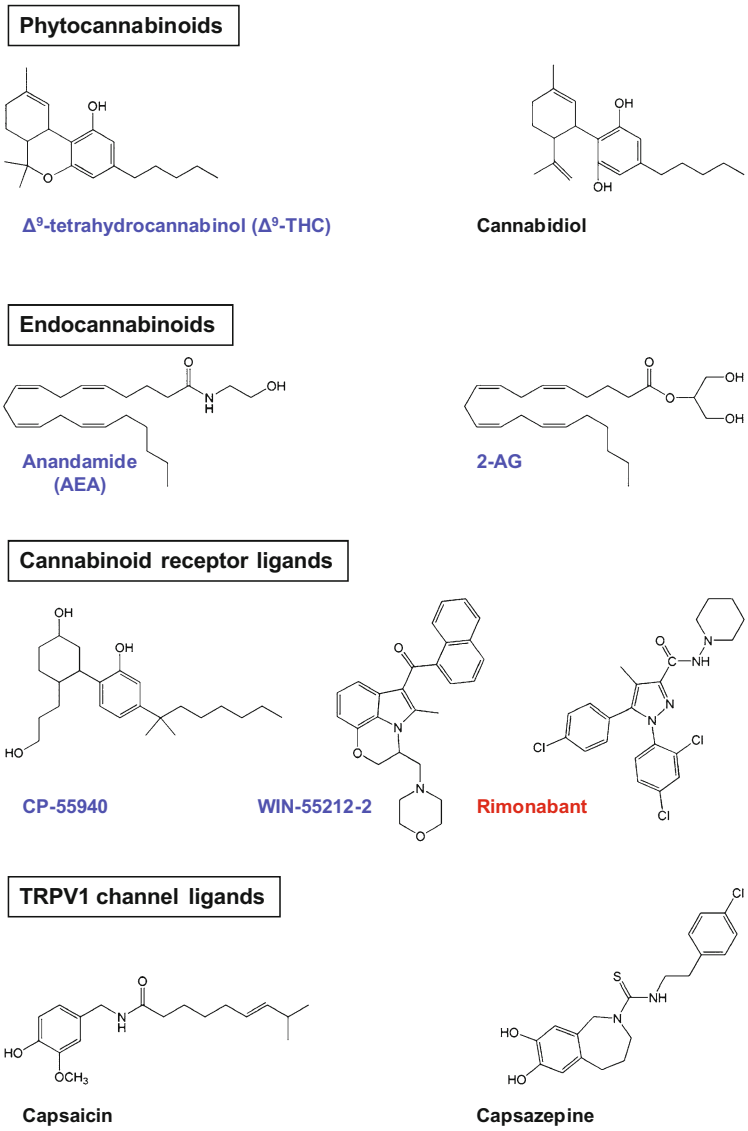

Fig. 1 Chemical structures of selected compounds that act on the endocannabinoid/endovanilloid system. Phytocannabinoids (originating from *C. sativa*), endocannabinoids and synthetic ligands activate (blue), inhibit (red) or bypass (black) cannabinoid type 1 (CB1) receptor signaling. Anandamide may activate Transient Receptor Potential Vanilloid Type-1 (TRPV1) channels in addition to CB1, thus acting as an endovanilloid and endocannabinoid at the same time (see text for further details)

by the discovery of endogenous ligands, referred to as endocannabinoids. The endocannabinoids along with their receptors and enzymes for synthesis and catabolism constitute the endocannabinoid system. Thus, a clear distinction should be

made between the terms cannabinoids (i.e., phytocannabinoids or synthetic analogues) and endocannabinoids synthesized by vertebrates.

Today we know that the endocannabinoid system is involved in a plethora of biological functions ranging from brain development and organogenesis to control of energy expenditure, food intake, pain perception, and inflammation to regulation of cognition, stress responses, and positive as well as negative affects. This chapter will summarize current evidence concerning the effects of cannabinoids and the role of endocannabinoids in fear and anxiety, ending with conceptualizing principles of endocannabinoid action and recommendations for a pharmacological exploitation of the endocannabinoid system for the treatment of human anxiety disorders.

2 The Endocannabinoid System of the Brain

The first cannabinoid receptor was identified in the rodent brain after studies with radioactively labeled synthetic cannabinoids in 1988 (Devane et al. 1988), followed by its cloning (Matsuda et al. 1990). The biochemical characterization unveiled a 7-transmembrane, G-protein-coupled receptor. Later on, another metabotropic receptor was identified. The International Union of Basic and Clinical Pharmacology (IUPHAR) named these receptors Cannabinoid receptor type 1 (CB1) and Cannabinoid receptor type 2 (CB2), respectively (Howlett et al. 2002). Most of the psychoactive actions of Cannabis seem to be mediated via CB1 receptors. Its strong expression within the brain fits well with these neuropharmacological effects. For instance, CB1 receptors are densely expressed throughout the cerebral cortex and the hippocampal formation, possibly explaining why Cannabis smoke induces impairments in learning and memory. Furthermore, CB1 receptor density is also extremely high in the basal ganglia and cerebellum, which may account for the motor impairment induced by marijuana. Furthermore, the expression in amygdala and periaqueductal gray may account for the emotional effects and analgesia. The absence in midbrain regions responsible for the control of breathing rhythms may explain why cannabinoids, contrary to opioids, do not induce respiratory depression (Howlett et al. 2002).

The story of cannabinoids recapitulates the discovery of the mechanisms of opioid action. Morphine was isolated in the nineteenth century and opioid receptors were identified in the 1970s, followed by the characterization of endogenous ligands (endorphins) shortly thereafter. Accordingly, the race for the identification of endogenous binding partners was opened as soon as the first cannabinoid receptor had been discovered. The first endocannabinoid was identified in the porcine brain in 1992. In line with the chemistry of natural and synthetic cannabinoids, this mammalian cannabinoid proved to be also a lipid, the arachidonic acid derivate arachidonoyl ethanolamide (Fig. 1), also termed anandamide (from the Sanskrit word *ananda* for “bliss”) (Devane et al. 1992). A second endocannabinoid could be identified as 2-arachidonoyl glycerol (2-AG; Fig. 1) shortly thereafter (Mechoulam et al. 1995; Sugiura et al. 1995). Other putative

endocannabinoids are *N*-arachidonoyl dopamine, virodhamine, and noladin ether, all amide or ester analogues of arachidonic acid (Di Marzo et al. 2004; Piomelli 2003).

Endocannabinoids have several characteristics that distinguish them from classical neurotransmitters. According to the traditional view, neurotransmitters are synthesized in presynaptic neurons and stored in vesicles to be released after neural activation and subsequent calcium influx. On the contrary, endocannabinoids are synthesized from membrane lipids in postsynaptic neurons after calcium influx that follows neural activation. Anandamide synthesis primarily depends on the activity of phospholipase D, whereas 2-AG synthesis involves phospholipase C. Also diverging from the classical concept of neurotransmission, endocannabinoids immediately diffuse to the synaptic cleft, instead of being stored in vesicles (Piomelli 2003). Complementing this picture, CB1 receptors are mainly located in presynaptic terminals. Activation of CB1 receptors leads to a decrease in synaptic transmission via a complex set of intracellular signaling cascades. Thus, endocannabinoids act as retrograde messengers, which are synthesized and released on demand following depolarization of the postsynapse to reach presynaptically localized CB1 receptors, where they restrain the release of neurotransmitters (Fig. 2).

Not only the release of endocannabinoids, but also their range of action are tightly controlled in temporal and spatial terms. Endocannabinoids are internalized and hydrolyzed inside neurons. The exact uptake mechanisms responsible for these processes are still unknown. Two – mutually not exclusive – possibilities have been proposed in this context: First, endocannabinoids might cross the cell membrane along a concentration gradient in a passive process not governed by any enzyme or transport protein. Second, a protein transporter might exist, which favors the movement of endocannabinoids through the cell membrane. The later view is supported by experiments showing that the uptake process is saturable, temperature-dependent, and can be inhibited by specific compounds (Piomelli 2003). Nonetheless, an "endocannabinoid transporter" could not be identified and cloned yet.

Once inside neurons, endocannabinoids are catabolized by different enzymes. Anandamide undergoes hydrolysis by the enzyme fatty acid amide hydrolase (FAAH), an integral membrane protein identified primarily in postsynaptic sites in rodents and primates (Fig. 2). FAAH breaks down anandamide into arachidonic acid and ethanolamine. 2-AG, in contrast, is hydrolyzed mainly by presynaptically localized monoacylglycerol lipase (MGL; Piomelli 2003; Fig. 2).

In the past decade it became evident that endocannabinoid-controlled neuronal activity represents a fundamental principle of the central nervous system with large implications on brain development (Harkany et al. 2007), synaptic plasticity (Chevaleyre et al. 2006), and behavior (Moreira and Lutz 2008). This might be partially explained by the observation that CB1 receptors belong to the most abundantly expressed G-protein-coupled receptors of the brain. Nevertheless, CB1 receptors are not ubiquitously distributed throughout the entire brain, but show different levels of expression depending on the neuronal subtype. For instance, CB1 receptors are densely expressed in cholecystokinin-positive basket

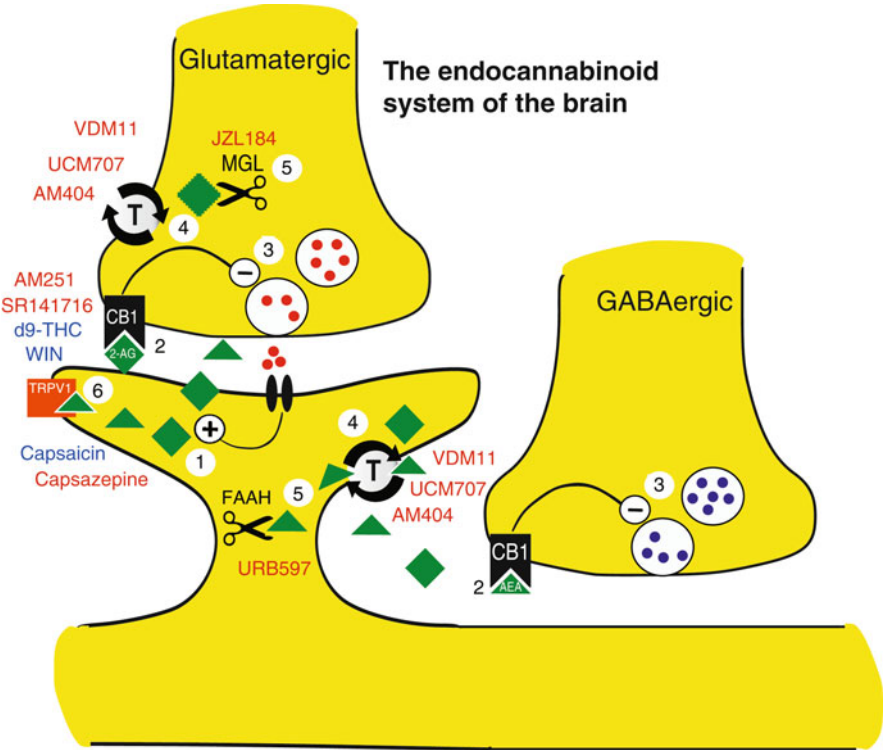

Fig. 2 The endocannabinoid system of the brain. Endocannabinoids (anandamide, AEA, and 2-AG) are synthesized on demand following depolarization of the postsynaptic membrane (1) and/or activation of postsynaptic metabotropic receptors (not shown). They diffuse into the synaptic cleft to bind to presynaptically localized cannabinoid type 1 receptors (CB1) (2). Activation of CB1 receptors leads to an inhibition of transmitter release (3). Endocannabinoid signaling is terminated by cellular uptake processes, which likely involve transporter proteins (4), followed by intracellular hydrolysis of 2-AG by presynaptic monoacylglycerol lipase (MGL) (5) and of AEA by postsynaptic fatty acid amide hydrolase (FAAH) (5). It remains to be shown that the same processes (1–5) apply to all of the different neuronal populations expressing CB1 receptors. Pharmacological compounds indicated in blue promote and those in red inhibit the respective processes (for chemical structures see Fig. 1). Noteworthy, AEA may additionally bind to cytosolic domains of postsynaptically localized Transient Receptor Potential Vanilloid Type-1 (TRPV1) channels (6), thereby promoting activation of postsynaptic terminals

neurons that release gamma-aminobutyric acid (GABA). The density seems to be lower, though not less functionally significant, in glutamate-releasing neurons. Indeed, pharmacological stimulation of CB1 receptors may inhibit either GABA- or glutamate-mediated neural transmission (Marsicano and Lutz 2006).

In summary, the endocannabinoid system comprises the cannabinoid receptors, of which CB1 seems to be the most relevant in the brain, the endogenous ligands (endocannabinoids), such as anandamide and 2-AG, as well as the enzymes for synthesis and catabolism. Interestingly, anandamide seems to bind not only to

CB1, but also to the Transient Receptor Potential Vanilloid Type-1 (TRPV1) channel, the receptor for capsaicin, the pungent ingredient of red hot chilli pepper (Fig. 1; Ross 2003). Within the central nervous system, TRPV1 might be expressed in postsynaptic terminals (Fig. 2). Activation of TRPV1 by intracellular binding of anandamide leads to an increase in postsynaptic activity, which is in striking contrast to the consequences of extracellular binding of anandamide to presynaptic CB1 receptors, thus suggesting opposing functions of the two receptor types (Starowicz et al. 2007). Other potential cannabinoid receptors are the GPR55, a G-protein-coupled receptor, and the peroxisome-proliferator-activated receptor α (PPARα), a nuclear receptor (Brown 2007; O'Sullivan, 2007). Finally, an allosteric site has been identified at the CB1 receptor, which remains to be further investigated (Ross 2007).

3 How to Study the Role of the Endocannabinoid System

Experiments with natural or synthetic cannabinoids may provide important insights into their pharmacological characteristics as a prerequisite for their future pharmacological exploitation. However, ubiquitous activation of CB1 receptors by exogenous agonists provide little – if any – information about the role of endogenous binding partners, keeping in mind the highly abundant expression of CB1 receptors and the diversification of the endocannabinoid system in terms of the endocannabinoids (which differ in their synthesis, range/duration of action, and degradation), the neuronal subpopulations affected, and the differential role of CB1 vs. TRPV1. Consequently, the biological functions of the endocannabinoid system in general and its implication in fear and anxiety in particular can only be achieved by monitoring the activity status of the endocannabinoid system and/or by inhibiting, respectively, increasing endocannabinoid signaling.

3.1 Monitoring of Endocannabinoid Signaling

As for many other transmitter systems, indirect conclusions about a potential involvement of the endocannabinoid system in distinct biological functions can be drawn by monitoring changes in its activity status, ranging from alterations in surface expression of CB1 receptors to changes in the activity of anabolic and catabolic enzymes to changes in endocannabinoid levels within the brain. Quantification of endocannabinoid levels turned out to be technically challenging due to the physicochemical characteristics and relatively low concentrations of the endocannabinoids. Other confounding factors are that both anandamide and 2-AG may result from rapid membrane degradation, thus requiring immediate processing of biological specimens, and that 2-AG is an intracellular intermediate product of multiple metabolic processes unrelated to endocannabinoid signaling. To deal with

this problem, recent studies tried to monitor the dynamics of 2-AG (and anandamide) release by means of in vivo microdialysis, thereby focusing on the amount of endocannabinoids, which reach the extracellular space, and thus become biologically active at CB1 receptors.

3.2 Attenuation of Endocannabinoid Signaling

Rimonabant (SR 141716A; Fig. 1; Rinaldi-Carmona et al. 1994) was the first CB1 receptor antagonist synthesized. This drug has provided invaluable insights into the physiology of the endocannabinoid system as well as in the pharmacology of cannabinoids. It blocks the action of cannabinoids in animals and of marijuana in humans and had been approved for the treatment of obesity in humans in Europe. In the meantime, several other CB1 antagonists have been developed, including AM251 (Gatley et al. 1996). In vitro experiments have suggested that both rimonabant and AM251 might act as inverse agonists, rather than silent antagonists (Pertwee 2008). As for the other cannabinoid receptors, selective CB2 antagonists have also been developed, such as SR144528 (Pertwee 2008). In addition, there are also antagonists for TRPV1 receptors, such as capsazepine (Fig. 1), 6-iodonordihydrocapsaicine, iodo-resiniferatoxin, and SB366971 (Di Marzo et al. 2008).

By specific blockade of CB1 receptors (or TRPV1 channels) it is possible to measure cellular, physiological or behavioral consequences of attenuated endocannabinoid signaling. However, a certain lack of specificity of the anatagonists, in particular at higher doses, hampers the unequivocal interpretation of the findings. To circumvent these problems, mutant mice have been generated, which lack expression of CB1 (CB1-KO) or TRPV1 (TRPV1-KO). As expected, CB1-KO does not respond to cannabinoid injection (Fig. 3). Further refinements of mouse genetics led to the development of conditional CB1 receptor knockout mice, whose CB1 receptor is deleted in neuronal subpopulations, such as GABAergic or cortical glutamatergic neurons by means of the Cre/loxP system (Fig. 3). The power of this approach has been recently demonstrated in context with the dissection of the transmitter systems implicated in tetrad effects of Δ^9-THC (Monory et al. 2007; Fig. 4).

3.3 Facilitation of Endocannabinoid Signaling

Two main strategies have emerged for increasing endocannabinoid signaling at the level of their receptors: First, chemical compounds, such as AM404, VDM11 or UCM707, may interfere with the uptake of the endocannabinoids into pre- and/or postsynaptic terminals, thereby increasing the availability of endocannabinoids at CB1 receptors. However, their exact mechanisms of action still remain to be characterized. Moreover, these compounds lack specificity, as they may also interfere with endocannabinoid degradation and TRPV1 channels (Piomelli 2003).

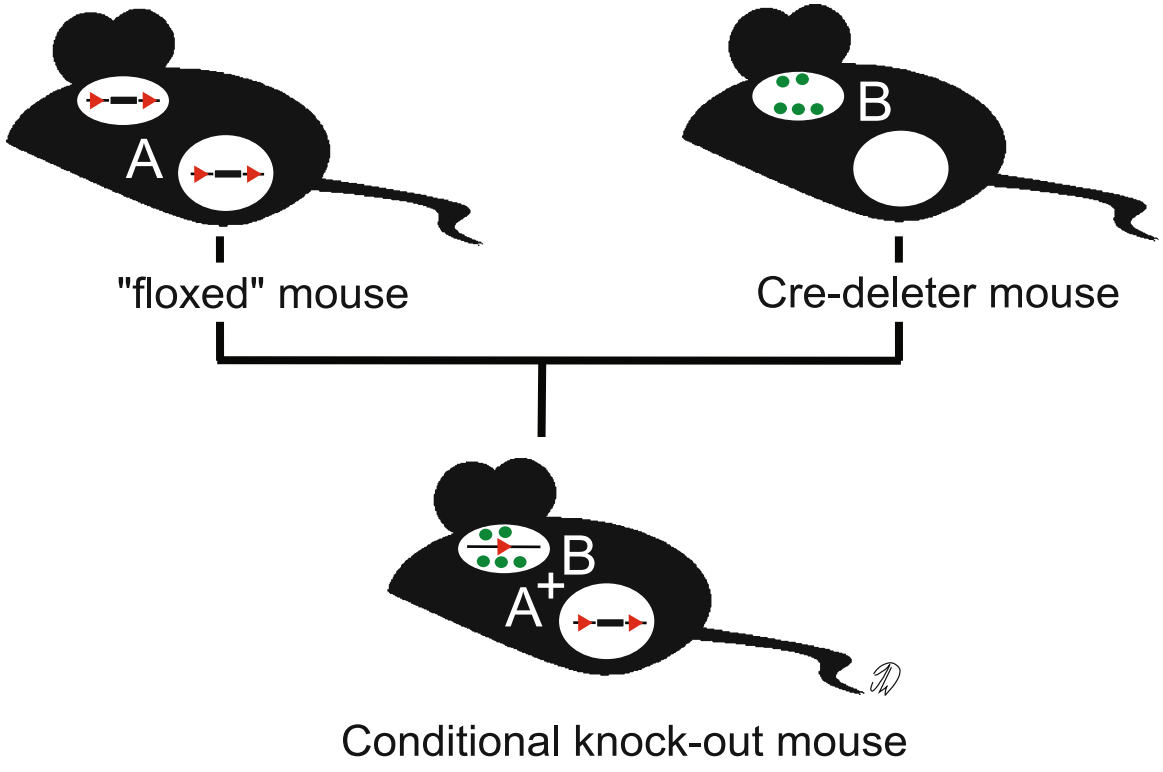

Fig. 3 Conditional mutagenesis. Schematic description of the basic principles of conditional mutagenesis based on the Cre/loxP system, illustrating that conditional mutants originate from two different mouse lines. In one line (A), the gene of interest contains two short DNA fragments (loxP sites), which are inserted into the genome by homologue recombination. These animals, in which the gene of interest is flanked by loxP (i.e., "floxed"), show normal expression of the gene product as long as they are not mated with mice of another line (B), which expresses Cre recombinase under control of a specific promoter. Expression of Cre leads to excision of DNA localized between two loxP sites. The promoter of the Cre transgene determines in which cell type and at which time point of development the recombinase is expressed and, thus, the gene of interest "knocked out" (A+B)

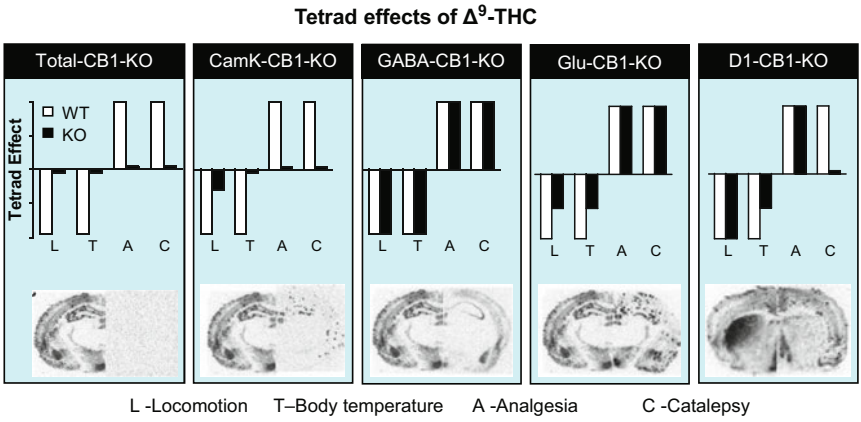

Fig. 4 Dissection of the neurochemical signature of tetrad effects of D9-THC. In pharmacological studies, the efficiency of cannabinoids is assessed by four criteria, which include reduction in locomotion, decrease in body temperature, increase in analgesia and increase in catalepsy (i.e., tetrad effects). Wild-type mice (white bars) respond to D9-THC (10 mg/kg) in the expected manner, whereas mice with complete absence of CB1 (Total CB1-KO) fail to respond at all, thus indicating the dependency of the tetrad effects on CB1. To further dissect the neurochemical signature of the tetrad effects, a variety of different conditional knockout mice that lack expression of CB1 in projection neurons of the forebrain (CamK-CB1-KO), in all GABAergic neurons of the brain (GABA-CB1-KO), in cortical glutamatergic neurons (Glu-CB1-KO) or in neurons expressing the dopamine D1 receptor (D1-CB1-KO) were treated with D9-THC. The insets depict representative in situ hybridizations of CB1 mRNA in wild-type mice (left half) and conditional mutants (right half). By means of this approach it became evident that distinct neuronal subpopulations are responsible for distinct tetrad effects (e.g., D1 receptor-expressing neurons seem to mediate the effects of D9-THC on catalepsy) (modified from Monory et al. 2007)

Second, a very promising strategy for enhancing endocannabinoid signaling is based on the inhibition of their hydrolysis. Specific inhibitors of FAAH and MGL have been developed, which are able to increase the brain levels of anandamide and 2-AG, respectively. The most prominent inhibitor of FAAH is URB597, which causes a 5-fold increase in the brain levels of anandamide (Kathuria et al. 2003), thus corroborating findings obtained with genetic deletion of FAAH (Cravatt et al. 2001). MGL, in turn, is inhibited, among others, by JZL184, which causes an 8-fold increase in 2-AG tissue content. Contrary to blockade of FAAH, MGL blockade induces tetrad effects in mice (Long et al. 2009).

4 Animal Models of Fear and Anxiety

As described in other chapters of this book in more detail, fear and anxiety responses can be assessed in an unconditioned or a conditioned manner. Animal models of unconditioned fear and anxiety are based on the conflict between two opposing innate motivations: the drive to explore a novel environment (presumably

in order to obtain food, shelter, to escape, or to find mating partners) on the one hand and avoidance of potentially dangerous places on the other hand. Behavioral paradigms most widely used in this context are the elevated plus maze (EPM), the light–dark avoidance test and the open field test, all of which measure avoidance of aversive compartments, such as of elevated open spaces (EPM), brightly lit compartments (light-dark avoidance), or the centre of an open field. Other models are the social interaction test (i.e., confrontation with a conspecific in a neutral test environment) and the Vogel conflict test (water consumption with the risk of receiving a mild electric shock; Sousa et al. 2006). Anxiolytic drugs shift the balance between approach and avoidance toward approach responses. The emotional load of the test situations can be modified, for instance, by changing the light conditions.

In the scientific literature, the terms "fear" and "anxiety" are often synonymously used, despite fundamental differences between the two emotional states. In their two-dimensional system of defense that is largely based on the conceptual work of Gray (1982), Blanchard and Blanchard (1988) and Deakin and Graeff (1991), McNaughton and Corr (2004) combined the concepts of defensive approach/avoidance and defensive distance, whereby defensive approach refers to anxiety states and defensive avoidance to fear (Fig. 5). Fear typically arises in situations with explicit (i.e., physical) confrontation with a threat, and fear responses (e.g., fight/flight, freezing, tachycardia) are often phasic and of reflexive nature. This might be explained by the fact that major relay stations and integrative centers of fear responses (e.g., periaqueductal gray, PAG) are located in the mid-/hindbrain. The

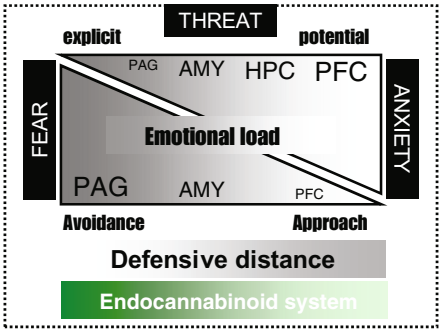

Fig. 5 The two-dimensional defense system. The two-dimensional defense system predicts that defensive distance to an explicit (fear) or a potential (anxiety) threat determines both fear (defensive avoidance) and anxiety (defensive approach) responses (modified from McNaughton and Corr 2004). A decrease in defensive distance coincides with an increase in negative emotional load. There is accumulating evidence that the endocannabinoid system becomes activated primarily in highly aversive situations with strong emotional load, thus suggesting its particular involvement in the control of fear reactions (see also Fig. 8). Fear and anxiety responses differ in the underlying neuronal circuitries (AMY – amygdala complex, HPC – hippocampus formation, PAG – periaqueductal grey, PFC – prefrontal cortex; the letter size indicates the strength of activation)

more tonic and diffuse anxiety responses, in contrast, crucially depend on the prefrontal cortex, the hippocampus and the amygdala (Fig. 5).

Animal models of conditioned fear are based on Pavlovian conditioning, whereby the animals associate an a priori neutral stimulus (i.e., cued fear conditioning) or environment (i.e., contextual fear conditioning) with a punishment (e.g., air puff or electric foot shock). On subsequent confrontation with that stimulus or environment, animals show a number of characteristic fear responses, including freezing (i.e., immobility) and potentiated startle responses. Interestingly, these fear responses are diminished following repeated exposure to the fear-eliciting stimuli in a process termed fear extinction (Myers and Davis 2007). Since conditioned fear is typically acquired in a single conditioning session, conditioning paradigms are predisposed for dissecting the role of cannabinoids and endocannabinoids in acquisition, consolidation, expression, and extinction of fear responses.

5 Role of Cannabinoids and Endocannabinoids in Fear and Anxiety – Animal Studies

Cannabinoids and drugs that interfere with the endocannabinoid system have been extensively studied in animal models of fear and anxiety. Considering that users self-administer marijuana to achieve a state of relaxation and bliss, one could infer that cannabinoids would induce anxiolytic-like effects in rodents. Yet, their effects remain controversial and a diversity of results has been reported. Actually, cannabis itself may also induce anxiety and panic attacks. Furthermore, its effects may depend on previous experiences and the context of use.

5.1 Unconditioned Fear/Anxiety

5.1.1 Natural and Synthetic Cannabinoids

Several cannabinoids have been investigated in animal models of anxiety-like behavior. Their effects tend to be complex and influenced by diverse factors. However, some common patterns start to emerge: as the main psychoactive ingredient of cannabis, Δ^9-THC has attracted considerable attention and has been investigated, among others, in the elevated plus maze and the light–dark box. Its effects tend to be biphasic – doses below 1 mg/kg generally induce anxiolytic-like effects, whereas the opposite occurs with higher doses up to 10 mg/kg. Interestingly, Δ^9-THC causes a strong activation of the hormonal stress responses at similarly high doses (Steiner and Wotjak 2008), thus indicating that it might be perceived as particularly stressful. Several synthetic cannabinoids have been investigated as well, including HU210, WIN-55212-2, and CP-55940, yielding similar results as those seen with Δ^9-THC. Though complex, their pattern of action actually

mimics the effects of cannabis in humans, which may depend on dose, environmental influences and previous stress experiences (Viveros et al. 2005).

Apart from Δ^9-THC, the only other phytocannabinoid that has been investigated in such models is cannabidiol (CBD), which also induces anxiolytic-like effects in the elevated plus maze and in the Vogel conflict test (Guimarães et al. 1990; Moreira et al. 2006). The effects of Δ^9-THC and synthetic cannabinoids are blocked by the CB1 antagonist rimonabant, though the mechanisms of action of CBD remain unknown.

Some potential explanations for the diversity of cannabinoid effects are the interference with diverse neurotransmitter systems and the action in various brain regions. Depending on the dose administered, cannabinoids could inhibit GABA or glutamate activity in the brain, thus modulating neurotransmitters with opposite functions on fear and anxiety. Alternatively, the extensive distribution of CB1 receptors in the brain suggests that cannabinoids could differentially affect negative emotions at the different brain sites of the fear and the anxiety matrix, respectively (cf. Fig. 5; Moreira and Lutz 2008).

5.1.2 Enhancing the Levels of Endocannabinoids

The experiments described above are relevant for clarifying the pharmacology of cannabinoids, though they do not provide information about the role of the endocannabinoid system. Since endocannabinoids are produced on demand followed by rapid uptake and degradation, drugs that inhibit their hydrolysis lead to an activation of CB1 signaling in a temporally and spatially restricted manner. The anandamide-transporter inhibitor AM404, for instance, induces anxiolytic-like effects in the elevated plus maze, an effect blocked by rimonabant (Bortolato et al. 2006). This drug causes an increase in the brain levels of anandamide, but not of other endocannabinoids (Bortolato et al. 2006). However, a more extensively explored strategy is the inhibition of FAAH. The prototypical compound is URB597, which induces anxiolytic-like effects, in parallel with an increase in anandamide, but not 2-AG (Kathuria et al. 2003). Contrary to CB1 agonists, no bell-shaped curves have been reported for drugs that inhibit anandamide transport or hydrolysis. However, prevention of AEA degradation not necessarily exerts anxiolytic-like effects, which seem to critically depend on the test situation (Moreira and Lutz 2008). In addition, it remains to be shown how blocking of 2-AG degradation affects fear and anxiety.

5.1.3 Inactivation of CB1 Receptors

Inhibition of CB1 signaling by pharmacological or genetic means provides the most reasonable approach for studying the involvement of the endocannabinoid system in fear and anxiety. In the majority of studies, systemic treatment with CB1 receptor antagonists induces anxiogenic-like effects (Patel and Hillard

2006), in particular if the animals are tested under highly aversive conditions, such as in brightly lit environments (Haller et al. 2004). The same holds true for CB1 knockouts, which show an increase in anxiety-like behavior only in situations with high emotional load. The mechanisms underlying these anxiogenic-like effects are still unknown. They may involve an imbalance between GABAergic and glutamatergic transmission (Moreira and Lutz 2008). Certainly, behavioral analysis of conditional mutants with cell type-specific deletion of CB1 will help to resolve this issue.

Noteworthy, a shift of anandamide actions from CB1 towards TRPV1 receptors, which exert opposite effects on neurotransmission (Di Marzo et al. 2008), may lead to an opposite behavioral phenotype, in particular since attenuation of TRPV1 signaling has anxiolytic-like consequences (Marsch et al. 2007; Terzian et al. 2009).

5.1.4 Intracerebral Injections

The diversity of the responses obtained after systemic cannabinoid injections might be due to the differential role of CB1 receptors in specific brain regions. Therefore, intracerebral injections may unveil the function of this receptor and help to clarify the effects of cannabinoids (Moreira et al. 2009). For instance, anandamide induces anxiolytic-like effects when injected into the dorsolateral PAG, an effect which is blocked by the CB1 antagonist AM251 and mimicked by the selective CB1 agonist arachidonoyl chloroethylamide (Moreira et al. 2007). Anxiolytic-like effects were also observed after injection of Δ^9-THC into the ventral hippocampus or the prefrontal cortex (Rubino et al. 2008). Therefore, these structures could be part of the brain circuitry responsible for the anxiolytic-like effects of cannabinoids (Fig. 5).

5.2 Conditioned Fear

In classical or Pavlovian conditioning paradigms, animals learn to associate a priori neutral stimuli or situations with a punishment. Subsequent encounter of these stimuli leads to pronounced fear responses, which are largely reflexive by nature (e.g., potentiated startle or freezing responses). In the absence of the predicted punishment, however, the fear responses decline both during the exposure and between exposures (i.e., within and between-session extinction). Fear extinction can result from habituation-like processes within the fear matrix as well as from relearning processes, during which the animals form a new inhibitory memory (stimulus–no-punishment association) that suppresses the original stimulus–punishment memory trace (Fig. 6). CB1 receptor signaling turned out to play an important role, not in acquisition of fear memories, but in fear extinction, since both

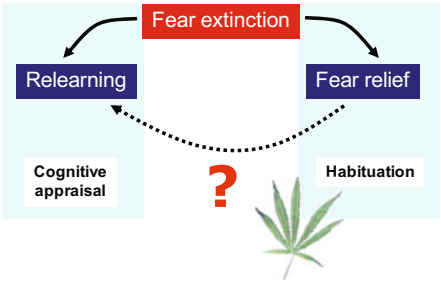

Fig. 6 Principles of fear extinction. In classical conditioning paradigms, animals and humans learn to associate a priori neutral stimuli or situations with a punishment. Re-exposure to those stimuli/situations triggers fear responses, which vanish in the absence of additional punishments. This process is termed fear extinction. It may result from relearning processes ("this stimulus/situation does not predict a punishment anymore"), which inhibit expression of the original memory trace ("this stimulus/situation predicts a punishment"). In addition, fear response may wane in a nonassociative manner via habituation-like processes (fear relief). Evidence exists that endocannabinoids are primarily involved in fear relief, which, however, might be permissive for relearning processes

genetic and pharmacological inactivation of CB1 were accompanied by sustained, nondecaying fear responses following fear conditioning (Marsicano et al. 2002). Interestingly, endocannabinoids appear to be specifically involved in extinction of *aversive* memories, since extinction of *appetitive* memories was intact in CB1-deficient mice (Hölter et al. 2005). Endocannabinoid-controlled fear extinction seems to critically depend on the amygdala, where the endocannabinoid system becomes activated during recall of aversive memories (Marsicano et al. 2002). Furthermore, systemic treatment with inhibitors of endocannabinoid uptake and/or degradation facilitated extinction of conditioned fear in a CB1-dependent manner (Chhatwal et al. 2005). Recent evidence suggests that endocannabinoids primarily affect habituation-like processes, which are thought to underlie acute fear relief rather than relearning processes per se (Kamprath et al. 2006; Fig. 6). In this context, it became evident that the averseness of a stimulus or test situation has to exceed a certain threshold, before the endocannabinoid system becomes activated and exerts its fear alleviating effects, most likely by influencing cortical glutamatergic neurons (Kamprath et al. 2009). Consequently, the endocannabinoid system seems to serve as a protective system which prevents the occurrence of exaggerated fear responses, e.g., by controlling glutamatergic transmission within the fear matrix (Fig. 7).

It is of note that extinction training resembles repeated exposures to homotypic stressors, which are associated with a priming of the endocannabinoid system (in particular of 2-AG signaling) in cortical brain structures that actively mediates long-term habituation of the stress responses during subsequent encounters (Patel and Hillard 2008). It is tempting to assume that similar processes account, at least in part, for endocannabinoid-controlled fear extinction.

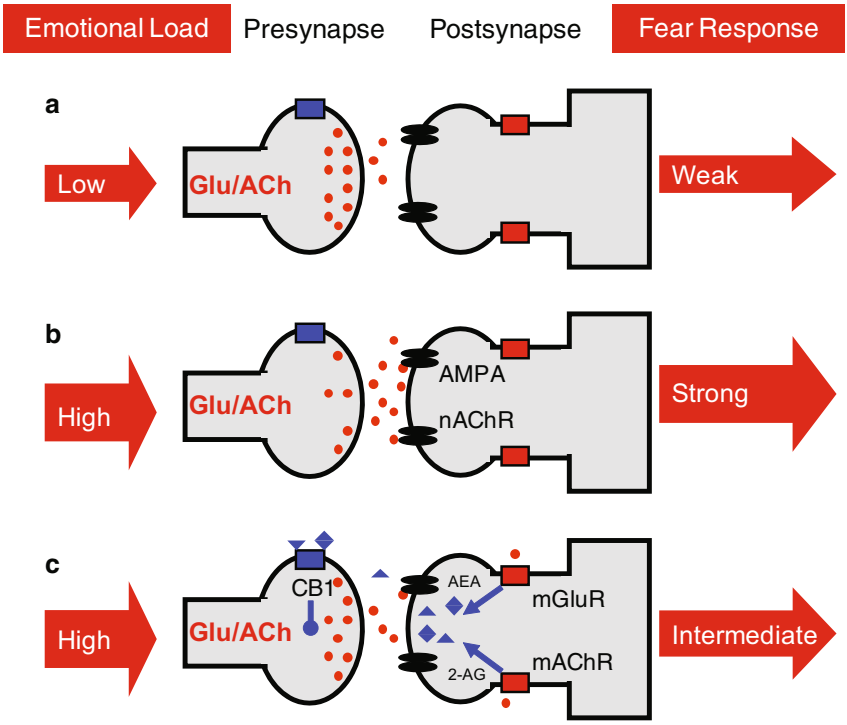

Fig. 7 A cellular model of endocannabinoid-controlled fear relief. (**a**) In situations with low negative emotional load, animals show negligible levels of fear. (**b**) In situations with high negative emotional load, in contrast, the fear matrix becomes strongly activated (see Fig. 5). (**c**) As a consequence of the intense activation of interneuronal communication, neurotransmitters [e.g., glutamate (Glu) or acetylcholine (Ach)] may reach extrasynaptically localized metabotropic receptors by synaptic spill-over, thus triggering endocannabinoid [i.e., anandamide (AEA) and 2-AG] synthesis and release. The endocannabinoids, in turn, downregulate transmitter release via presynaptically localized CB1 receptors, thereby mediating fear relief

Similar to unconditioned fear, the role of TRPV1 in fear conditioning appears to be opposite to that of CB1, since TRPV1 knockout mice show reduced levels of conditioned fear (Marsch et al. 2007).

6 Role of (Endo)Cannabinoids in Fear and Anxiety – Situation in Humans

Information concerning the role of endocannabinoids in the modulation of anxiety in humans is still quite limited. Cannabis consumption may induce a diversity of effects, ranging from relaxation and a “high” to anxiety and panic attacks (Hall and

Solowij 1998), implying a complex role of CB1 signaling on the modulation of emotional states. Pure Δ^9-THC injection may not entirely mimic the effects of Cannabis, which has been reported to induce "bliss," though also psychotic states and anxiety (Ranganathan and D'Souza 2006). In addition, CB1 receptors rapidly desensitize following their activation by Δ^9-THC, which renders it likely that part of the phenotype caused by Δ^9-THC or Cannabis intoxication relates to attenuated rather than promoted CB1 signaling.

Interestingly, clinical trials investigating the therapeutic potential of the CB1 receptor antagonist rimonabant for the treatment of obesity and cardiovascular disorders, such as the Rimonabant in Obesity (RIO) or the Strategy to Reduce Arteriosclerosis Development Involving Administration of Rimonabant—The Intravascular Ultrasound Study (STRADIVARIUS) studies, revealed the importance of endocannabinoids in the modulation of emotionality in humans, since a significant percentage of patients taking rimonabant reported feelings of anxiety and depression and an increase in suicidal thoughts (Christensen et al. 2007; Nissen et al. 2008). Rimonabant eventually reached the market in Europe as Acomplia™, only to be withdrawn shortly thereafter due to major concerns about psychiatric side-effects. Because of the same reasons, the Federal Drug Administration failed to approve the prescription of rimonabant in North America. In any case, the rimonabant saga provided evidence that endocannabinoids may tonically modulate mood and anxiety not only in lab animals, but also in humans, thus illustrating the power of translational research.

7 The Endocannabinoid System in Fear and Anxiety – Theoretical and Practical Considerations

The majority of preclinical and clinical studies report a distinctive role of the endocannabinoid system in dampening negative affects associated with fear, anxiety, and stress. However, even in normal healthy subjects, the endocannabinoid system shows a limited range of action with a lower and upper threshold (Fig. 8). The lower threshold depends on the averseness of a test situation, since the negative emotional load of a test situation has to exceed a certain threshold in order to activate endocannabinoid synthesis and release. Because of the close relationship between decrease in defensive distance, lack of controllability, and increase in emotional load (McNaughton and Corr 2004; cf. Fig. 5), this applies in particular to highly aversive situations, which cannot be avoided by the animals. It is conceivable that the spill-over of transmitters out of the synaptic cleft to extra-synaptic metabotropic receptors triggers the activation of endocannabinoid signaling, which in turn downregulates transmitter release from presynaptic butons in the vicinity of the synapse (Fig. 7).

Fear responses may depend on the defensive distance not only in quantitative, but also in qualitative terms. Rats and mice, for instance, switch from arousal and

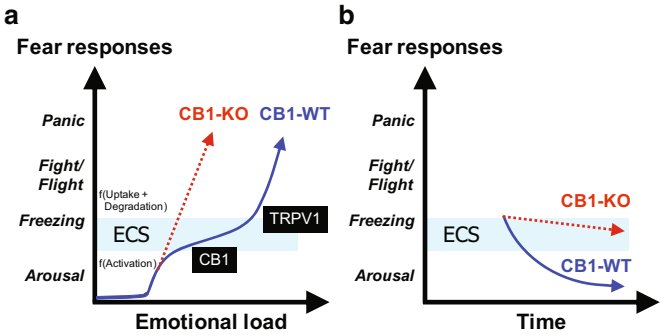

Fig. 8 The critical range hypothesis of endocannabinoid action. (**a**) The negative emotional load (cf. Fig. 5) has to exceed a certain threshold before the endocannabinoid system (ECS) becomes activated (cf. Fig. 7). After binding to CB1 receptors, the endocannabinoids delay the further increase in fear responses despite steadily increasing emotional load. However, the capacity of the endocannabinoid system is limited by efficient uptake and degradation processes. In addition, intracellularly accumulated anandamide may bind to postsynaptic TRPV1 channels. Together, these processes unleash fear responses from regulatory constraints of CB1 signaling. Consequently, in organisms with intact CB1 receptors (e.g., wild-type mice, CB1-WT), the critical range of endocannabinoid action serves as a high- and low-pass filter, which ensures moderate fear responses over a broad range of aversive conditions, without precluding exaggerated fear responses to life-threatening stimuli. This situation may become maladaptive in case of organisms with impaired CB1 signaling (e.g., CB1-deficient mice, CB1-KO), which may show inadequately strong fear responses already to situations of moderate averseness. (**b**) The same model explains dynamic changes of fear responses to stimuli/situations of a given emotional load, whereby intact CB1 signaling (e.g., in CB1-WT) mediates fear relief, whereas blocked CB1 signaling (e.g., in CB1-KO) leads to sustained fear responses

exploratory behavior (risk assessment) to behavioral immobility (freezing) to fight/flight in response to an approaching threat (e.g., a cat; Blanchard and Blanchard 1988). The adaptive value of fear reduction may become maladaptive, if the animals continue to show freezing or arousal in response to the approaching predator, when fight or flight responses would be a more adequate strategy for escaping from the dangerous situation. The endocannabinoid system seems to permit such switches in behavioral strategies despite its fear-alleviating effects, because its range of action appears to be limited by an upper threshold. This threshold is defined by highly efficient uptake and degradation processes, which omit the endogenous binding partners of the CB1 receptors. The resulting increase in anandamide in postsynaptic terminals may lead to an activation of TRPV1 receptor channels, which show a 10-times lower affinity for anandamide than CB1 (Pertwee 2008; Ross 2003) and which promote fear responses (Marsch et al. 2007; Terzian et al. 2009). We, therefore, propose that the endocannabinoid system acts as a kind of high- and low-pass filter which ensures moderate fear responses in situations with intermediate to high emotional load without precluding exaggerated fear responses in life-threatening situations (Fig. 8a). At the same time, the endocannabinoid system mediates fear relief via habituation-like processes to enduring

threatening stimuli (Fig. 8b), thus enabling the animals to return to housekeeping functions (e.g., feeding or nursing) and/or to extinguish aversive memories (Fig. 6).

In translational terms, the theoretical considerations arising from animal studies suggest that imbalances in the endocannabinoid system may contribute to human psychiatric disorders, which are associated with exaggerated fear responses, such as phobias and panic disorder, or the perseverance of aversive memories, such as posttraumatic stress disorder. Accordingly, broadening of the range of endocannabinoid action by pharmacological means might represent a novel therapeutic strategy for the treatment of these disorders. The use of cannabinoids to increase CB1 receptor signaling appears to be less promising, taking into consideration the variety of side effects (Fig. 4) and the possibility of rapid receptor desensitization. Much more promising would be to inhibit endocannabinoid uptake and degradation. In fact, inhibition of 2-AG and/or anandamide hydrolysis would enhance CB1 signaling in a temporally and spatially restricted manner. A particularly innovative approach would be to combine blockade of anandamide hydrolysis with antagonism of TRPV1, thereby promoting fear-alleviating/anxiolytic effects caused by activation of CB1 and preventing fear-promoting/anxiogenic effects mediated by TRPV1 at the same time. The success of this strategy was recently shown for arachidonoyl serotonin (AA-5HT), which exerted prominent anxiolytic effects (Micale et al. 2009).

Despite the tremendous progress in our knowledge about the physiology and pathology of the endocannabinoid system and potential consequences for health and disease in the recent years, this system still keeps a plethora of secrets waiting to be uncovered. Its pharmacological exploitation, which has started with the first use of Cannabis extracts more than 5000 years ago, has not yet come to an end, but, hopefully, will enter a new avenue away from fundamentalist concerns for the benefit of the patients.

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