# Chapter 5
# Study of TRP Channels by Automated Patch Clamp Systems

**Morten Sunesen and Rasmus B. Jacobsen**

**Abstract** Ion channels are responsible for the permeation of ions across the membrane and their central role in cellular physiology is well established. Historically, the direct study of ion channels has been considered technically challenging. As such, a significant barrier to drug discovery for ion channels has been the low throughput of high quality electrophysiological data. The emergence of automated high throughput platforms for studying ion channel kinetics and pharmacology has lowered this barrier. Ion channels are now recognized as increasingly important drug targets and a diverse range of ion channels are implicated in a variety of drug discovery and cardiac safety assessment programs. The TRP (Transient Receptor Potential) superfamily of ion channels play a crucial role in a broad range of sensory functions including vision, taste, olfaction, hearing, touch, pain and thermosensation. Many of the TRP channels are polymodal in their activation and deactivation mechanisms and even with conventional patch clamp electrophysiology, the TRP channels are considered to be a very complex target class. Here we present an update on the significant progress made on the TRP receptor assays with the available automated patch clamp systems.

## 5.1 Introduction

The field of patch clamping has advanced significantly since its introduction by Neher and Sakmann in the 1970s [1]. The discovery of giga-ohm patch clamp recordings, micromanipulated pipettes, and stable specialized amplifiers lead to the successful establishment of the manual patch clamp setups in both academia and industry labs. Since then a plethora of ion channels have been studied functionally and a wide range of ion channel compounds have been described pharmacologically using manual patch clamp. The most commonly used configuration, whole-cell

M. Sunesen (✉)
Sophion Bioscience AS, 2750 Ballerup, Denmark
e-mail: msu@sophion.com

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patch clamp, is a direct method for measuring ion channel activity across the membrane of an entire cell. Although highly informative, the manual (conventional) whole-cell patch clamp technique is slow and requires highly skilled operators. The throughput of manual whole-cell patch clamp experiments is most often limited to examinations of one compound at any one time, and it is extremely labor intensive. As such manual patch clamp represents a major bottleneck in ion channel drug discovery and development.

Recent technological advances have led to the emergence of several automated patch clamp devices in which the classical glass microelectrode is replaced by a planar substrate, which holds the patch clamp hole. The composition of the planar substrate varies from system to system and the tightness of the seal between the cell and the planar substrate may vary according to the composition of the substrate (i.e. glass vs. plastic). However, the fundamental principles remain the same: a cell is drawn to the hole by suction, the cell membrane is ruptured (or perforated) and recordings are obtained in the whole-cell configuration. The level of automation varies a bit from platform to platform. Some systems offer full automation with onboard handling of cells, compounds and consumables, while other platforms are built as simple reader stations, where user interaction is mandatory [2].

With the current availability of devices, a broad range of ion channel currents can be measured. The automated platforms have obtained profound success in areas of electrophysiology where high quality data are needed at a low to medium high throughput. These machines are typically implemented in areas of the drug discovery cascade such as assay optimization, secondary screening, lead optimization and cardiac liability.

Automated patch clamp systems are now routinely deployed in programs with ion channels that can present significant biological and technological challenges. A number of recent publications describe the successful implementation of automated patch clamping in studies of voltage-gated sodium channels [3], voltage-gated calcium channels [4], calcium-activated potassium channels [3, 5], various fast-desensitizing ligand-gated ion channels [4, 6], two pore channels [7], hyperpolarisation activated cyclic nucleotide-gated channels [8] and calcium-release activated calcium channels [9]. This chapter describes the recent studies of a family of technically challenging ion channels, the transient receptor potential (TRP) channels on the currently available automated patch clamp devices: IonWorks (MDC), Patchliner (Nanion), PatchXpress (MDC), and QPatch (Sophion).

The TRP family of ion channels was originally named after the ion channel mutant of the *Drosophila melanogaster* fly that displayed a transient response to light rather than a sustained response [10]. TRP ion channels have six transmembrane domains and all TRP channels are permeable to cations. However, each subtype has its own cationic selectivity and activation mechanism [11]. TRP ion channels are encoded by 28 genes in mammals [12] and they are essential in a broad range of sensory physiology including thermosensation, olfaction, vision, mechanosensation, touch, taste, pain sensation and osmosensation [13]. Since their discovery TRP channels have caught profound attention as an interesting but challenging target class in both academia and industry.

The use of automated patch clamp for studying TRP channels is far from trivial and a number of biological and technological challenges must be addressed: control of intracellular calcium concentration, control of temperature, sensitivity to mechanical stimuli, and differentiation of leak current versus TRP channel permeated current.

Direct control of intracellular calcium concentrations is difficult in most automated patch clamp systems. Some devices use poreformers (e.g., amphotericin) to make holes in the membrane through which only monovalent cations can be exchanged. Manipulation of intracellular calcium levels is therefore poorly controlled on patch clamp systems using poreformers. Other automated patch clamp devices use traditional suction to establish the whole-cell configuration and a more precise control of intracellular calcium can be achieved by using well-known calcium chelator's such as BAPTA and EGTA. Only the Patchliner systems from Nanion allow intracellular solution exchange to directly study the effect of changing intracellular calcium concentrations.

All available automated patch clamp systems suffer from the same disadvantage compared to manual patch clamp setups: the lack of continuous perfusion of the liquid in the recording chamber. The technology that has been developed for automated patch clamp today falls into two categories – either an open-well system, where liquid is added/removed from the top or a laminar flow system, where liquid is drawn through flow channels in discrete bursts upon liquid applications to an inlet well (for review see [2]). Even though the exact mechanism of mechano-activation of the mechano-sensitive TRP channels remains unknown [8, 14, 15], the stop-and-go application of salines and compounds to the recording site of automated patch clamp systems could potentially affect the recordings measured. On the other hand, the stop-and-go feature may be an opportunity to address mechano-sensitivity of TRP channels. In a recent paper, the QPatch was deployed in order to discern the effects of cell swelling from those of mechanical membrane stretch of the potassium channels BK and KCNQ4 [16].

The rapid increase in temperature beyond a TRP-subtype specific temperature will activate the channel and result in an inward current [17]. As none of the available automated patch clamp devices have the capability to change the experimental temperature within seconds without a simultaneous liquid exchange around the cells, the effect of the elevated temperature might result in the activation of the channel caused by mechano-activation resulting from the stop-and-go liquid application. As illustrated in the examples below, the preferred choice when working with TRP channels on automated patch clamp devices has therefore been to either perform the experiments on the TRP channels directly as ligand-gated receptors or to perform the experiments as ligand-gated receptors with some voltage control (i.e., activation by agonist and execution of voltage ramps to detect TRP-specific current changes).

Traditionally, patch clamping has been performed with one cell at a time, however, in 2005 Molecular Devices launched the first automated patch clamp system that recorded from multiple cells residing in the same recording chamber, the IonWorks Quattro. Since then the QPatch HTX multi-hole system has been launched. The multi-hole systems have prominent advantages: the recording

reliability is greatly increased, in some reports run down seems to be minimized, and the success rate of the patch clamp assay is approaching 100% [18–19]. While offering higher and more reliable throughput, the multi-hole systems have their shortcomings. The multi-hole systems lack series resistance compensation and voltage control of the individual cell. Therefore, the same high fidelity of the ion channel recordings you find in single-hole measurements cannot be expected when working with the multi-hole systems. The single-hole systems, therefore, still serve as a very useful platform for functional and biophysical characterization, assay optimization, assay validation and for assays that demands more stringent voltage control.

Automated patch clamp technologies have provided a significantly increased throughput with the same high quality as manual patch clamp recordings. This increased throughput of high quality measurements has lead to a growing number of drug discovery programs targeted towards ion channels including members of the TRP superfamily of ion channels. This review focuses on the various TRP assays that have been established on automated patch clamp systems over the last few years. Illustrated with figures from QPatch experiments from our laboratory, the respective TRPs are reviewed and discussed with special attention to the various assay conditions for automated patch clamp established by the research groups.

## 5.2 Results

### *5.2.1 TRPA1*

The non-selective cation channel TRPA1 acts as a sensor for reactive chemicals in the body [20] and the active ingredients of mustard oil, wasabi and garlic have been shown to activate this channel [8, 15, 21–23]. In addition to activation by reactive chemicals, TRPA1 can also be activated by noxious cold temperatures [21, 24], and by calcium, which seems to desensitize the channel [25, 26].

In order to identify TRPA1 antagonists on PatchXpress, a ligand-gated approach was established in which the channel was activated by mustard oil (AITC) [24]. With a holding potential at –70 mV, the AITC-elicited control response was compared to the AITC signal after preincubation of the specific compounds and the concentration-dependent effect of four compounds was determined.

In a different set of experiments, TRPA1 currents were recorded on QPatch using another approach. Voltage ramps were executed during the course of the experiment and after whole-cell break-in, TRPA1 was activated by application of an agonist. In Fig. 5.1a, the same concentration of supercinnamaldehyde was applied three times and the level of the TRPA1-permeated current was measured using a blocking concentration of Ruthenium Red. Alternatively, the effective $IC_{50}$ of TRPA1 antagonist could be determined by the cumulative addition of increasing concentrations of the antagonist (Fig. 5.1b). To evaluate the stability of the assay, the

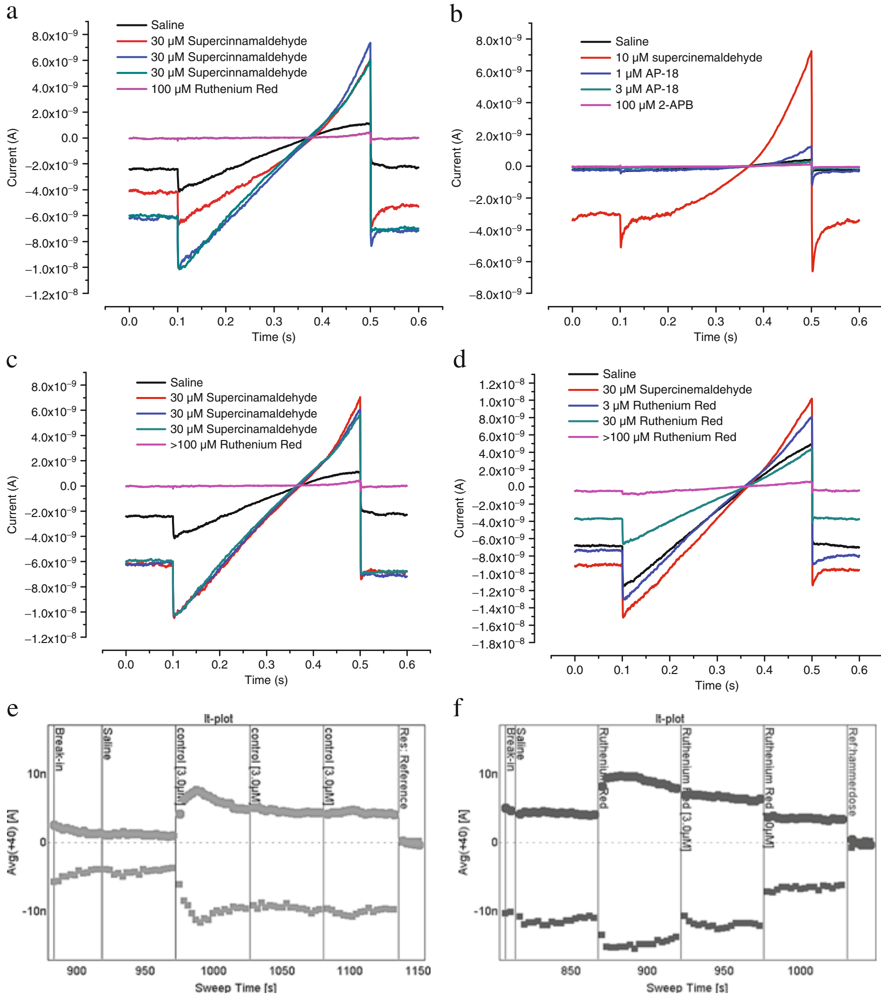


**Fig. 5.1** HEK293-TRPA1 cells tested on QPatch in single- or multi-hole mode. TRPA1 currents were stimulated by a voltage ramp (–100 to +100 mV) upon supercinnamaldehyde activation. (**a**) Single-hole recording of TRPA1 current activated by 30 μM supercinnamaldehyde and blocked by 100 μM Ruthenium Red. (**b**) Single-hole recordings of TRPA1 currents activated by supercinnamaldehyde and subsequently blocked by AP-18 and 2-APB. (**c**) Multi-hole recordings of TRPA1 currents activated by 30 μM supercinnamaldehyde and blocked by Ruthenium Red. (**d**) Multi-hole recordings of TRPA1 currents activated with supercinnamaldehyde and blocked by increasing concentrations of Ruthenium Red. (**e**) Current vs. time plot demonstrating stability of the supercinnamaldehyde elicited current over time. 100 μM Ruthenium Red was used as reference to demonstrate TRPA1 specificity of the measured current. *Dots* are outward current at 40 mV, *squares* are inward current at –90 mV. (**f**) Current vs. time plot of dose response experiment, where TRPA1 currents were activated by supercinnamaldehyde and blocked by increasing concentrations of Ruthenium Red. Again, 100 μM Ruthenium Red was used as reference blocker. *Dots* are outward current at 40 mV, *squares* are inward current at –90 mV

same conditions were tested in a similar set-up on the multi-hole platform QPatch HTX with repeated application of supercinnamaldehyde (Fig. 5.1c) or increasing concentrations of Ruthenium Red (Fig. 5.1d). As illustrated in the current vs. time plots (see Fig. 5.1e, f), the TRPA1 signals remain very stable over time in the multi-hole experiments.

## 5.2.2 TRPCs

TRPCs were the first mammalian homolog of the *Drosophila melanogaster* TRPs to be identified [27]. Like TRPA1, TRPCs are non-selective cationic channels conducting sodium, potassium as well as calcium ions [28]. All TRPCs are activated through downstream pathways of phospholipase C (PLC) stimulation [29]. It has been suggested that certain TRPCs are functionally regulated by interactions with STIM1 [30].

### 5.2.2.1 TRPC1

To evaluate the functional interaction between TRPC1 and STIM1, vascular smooth muscle cells were used in electrophysiological studies on the Patchliner [31]. In whole-cell configuration the cells were clamped at a holding potential of 0 mV and during thapsigargin activation, voltage ramps were applied. In this assay, currents were evoked by applying thapsigargin extracellularly with or without an antibody directed against STIM1 or TRPC1 respectively. These experiments led the authors to conclude that TRPC1 and STIM1 interact and their activation is indeed stimulated by store-depletion but a fraction of the TRPC1 channels remain independent of STIM1 and of calcium store depletion [31].

## 5.2.3 TRPMs

The melastatin subfamily of TRP channels (TRPMs) forms a very diverse group of sensory ion channels that are activated by various subtype-specific mechanisms, which include sensitivity to mechanical or oxidative stress, hormones, temperature, membranous lipid composition and calcium store-depletion. TRPM ion channels are expressed in brain and in many peripheral sensory organs as well as in the immune system [32]. Several of the TRPM ion channels have been successfully tested on automated patch clamp instruments (see below).

### 5.2.3.1 TRPM2

A unique feature of some of the TRPM subfamilies is the presence of an enzymatic domain in the C-terminus, and hence the term *chanzyme* (Channel – Enzyme) was coined for these TRPs. TRPM2 is a chanzyme with an ADP-ribose pyrophatase domain in its C-terminus. It is a non-selective cationic channel that is activated

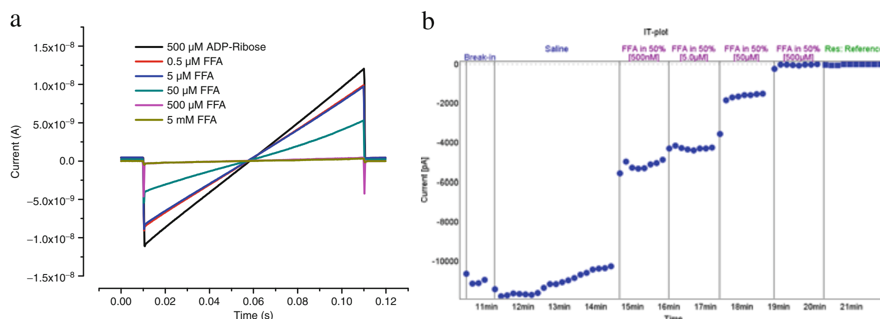


**Fig. 5.2** Current activated by 500 μM ADP-Ribose and blocked by increasing concentrations of flufenamic acid (FFA) in a CHO-TRPM2 cell line. (**a**) Raw traces elicited by a 120 ms ramp from –60 to +60 mV. (**b**) Corresponding current vs. time plot. Using the QPatch assay software the average $IC_{50}$ for FFA was found to be 38 μM, which compared well with literature values 3–30 μM [38]

by ADP-ribose, pyrimidine, nucleotides, NAD, and by oxidative stress [33–35]. TRPM2 channels are important redox sensors in the immune response [32], are involved in insulin secretion [36], and are involved in melanin synthesis and oxidative stress-induced cell death [37].

To study the effect of potential antagonists of TRPM2 on the QPatch, whole-cell currents were obtained by ADP-ribose activation of the channel. As shown in Fig. 5.2a, activation of TRPM2 results in a so-called leak current for which the leak component can be hard to discriminate from the channel permeated current. Therefore standard P/n leak subtraction can be quite problematic for these currents. Instead a known blocker of TRPM2 (5 mM flufenamic acid (FFA)), was used to measure non-TRPM2 mediated currents, which was then used as leak subtraction [18]. In the example in Fig. 5.2, FFA was also used as the test compound in a cumulative four point dose response experiment, which demonstrates the required stability of the TRPM2 current over time (See Fig. 5.2b) as well as an expected $IC_{50}$ of FFA [38, 39].

### 5.2.3.2 TRPM3

TRPM3 seems to be regulated by the lipid structure of the cellular membrane. This TRP channel becomes activated by several membrane components such as sphingosine and the naturally occurring steroid pregnenolone sulphate [12, 40, 41] while being blocked by cholesterol [15]. When heterologously expressed in cultured cells the channel is constitutively open and sensitive to osmolarity of the extracellular solution [40].

TRPM3 is a calcium permeable, non-selective cationic channel but its precise function is still not well described. In hepatocytes, TRPM3 activation causes calcium influx, which results in insulin release and it has therefore been suggested that TRPM3 is involved in glucose homeostasis [41]. However, due to the lack of specific blocking agents the role of TRPM3 remains unclear.

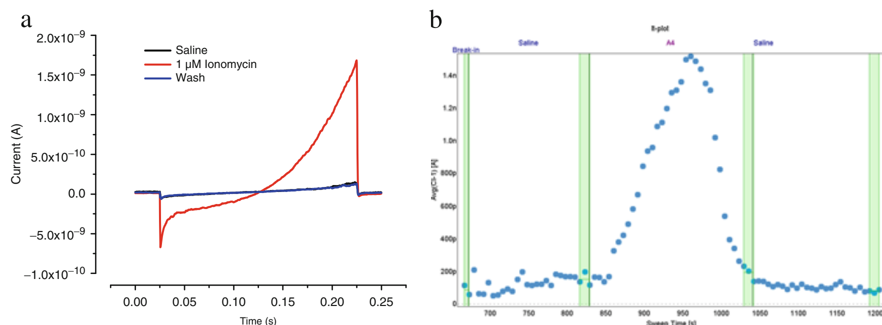


**Fig. 5.3** TRPM5 current activated by application of ionomycin on CHO-TRPM5 cells. (**a**) Raw current traces obtained from a 200 ms ramp (–80 to +80 mV) before and during ionomycin (1 μM) activation and upon a wash step. (**b**) Corresponding current vs. time plot

With the use of automated patch clamp (Patchliner) Dr. Beech and coworkers recently developed specific blocking antibodies to one of the extracellular loops of TRPM3 [42]. On the Patchliner platform the HEK293-TRPM3 cells were clamped at 0 mV and by using voltage ramps from –100 to +100 mV, it was found that 50% of the pregnenolone sulphate response could be blocked by a specific polyclonal antibody directed to the E3 extracellular domain [42]. Approaches like these may in the future be extremely valuable in developing new medicines with an early preclinical prediction of drug action.

#### 5.2.3.3 TRPM5

The TRPM5 channels are unusual to the TRP superfamily in at least three ways: the channels are activated by high concentrations of intracellular calcium, they exhibit some voltage-dependent activation, and they are together with the TRPM4 channels the only TRP channels that selectively permeate monovalent cations [43].

The poreformer ionomycin was used on the QPatch to demonstrate direct activation of TRPM5 by increasing the concentration of intracellular calcium (see Fig. 5.3a). As can be seen on the current versus time plot, a significant activation of TRPM5 is obtained with ionomycin, however, steady state of the signal is very hard to achieve (see Fig. 5.3b). While the TRPM5 assay may be a valuable tool for identifying novel TRPM5 agonists using automated patch clamp, future work will need to establish a reliable automated patch clamp assay for the identification of TRPM5 antagonists and blockers.

#### 5.2.3.4 TRPM8

As demonstrated by its specific expression in cold sensing neurons and by several mice TRPM8 null mutants, the TRPM8 receptor is the primary sensor to innocuous cold temperatures [17, 44]. The TRPM8 channel is activated by cold temperatures and by compounds such as menthol, eucalyptol and icilin – compounds that evoke

the *"cold"* sensation. Quite interestingly, it has been found that irrespective of the sensation of cold – pain or pleasant –TRPM8 plays a central role in the various cold responses [45].

The applicability of automated patch clamping on TRPM8 has been demonstrated in several studies using QPatch systems [4, 46]. In these investigations, the activity of TRPM8 was studied by voltage ramp protocols upon activation by an agonist (menthol) (Fig. 5.4a) plus or minus an antagonist (capsazepine) (Fig. 5.4b). Applications of multiple concentrations of the agonist or antagonist allowed the generation of $IC_{50}/EC_{50}$ data from a single cell or as an average from several cells (see Fig. 5.4c, d). The results were compared to manual patch clamp data and found to be highly similar both with respect to biophysical characteristics and pharmacology [4, 46]. When setting up TRPM8 assays with automated patch clamp, remember the following: none of the existing systems have constant perfusion rather the liquid exchange around the cells happen in discrete bursts designed by the experimenter. For this ion channel the lack of constant perfusion has the consequence that two independent liquid applications are needed to reach steady state. Using this approach a reasonable match of manual and automated patch clamp data was obtained and a relatively high throughput patch clamp assay for secondary screening of TRPM8 antagonists was developed [46]. More specifically, it has been found that an average success rate beyond 60% can be achieved indicating a throughput of more than 30 individual $EC_{50}$s per hour (see Fig. 5.4e).

### *5.2.4 TRPVs*

The TRPV (Vanilliod) family of receptors is like the other TRPs gating cations in general but these channels have higher a preference for permeating calcium and magnesium over sodium and potassium. Also, like other members of the TRP superfamily the TRPVs are activated by multiple apparently disparate mechanisms such as noxious heat, acidic pH, "hot taste" caused by the pungent substances in chili (capsaicin) and black pepper, garlic and camphor, the oils of clove thyme and oregano as well as mechanical stimuli [11, 13].

#### 5.2.4.1 TRPV1

TRPV1 was originally identified and cloned based on the ability of a dorsal root ganglion cDNA fragment to confer capsaicin-evoked changes in intracellular calcium levels [47, 48]. TRPV1 channels are expressed in afferent nociceptor, pain sensing neurons, where they play a central role in transducing chemical and mechanical stimuli. As a result, TRPV1 channels have received a lot of attention in the search of blockers or antagonists for the potential prevention or treatment of pain [49].

Considering automated patch clamp, the TRPV1 channel is probably the most widely tested TRP channel. Data have appeared from nearly all machines including PatchXpress, Patchliner, QPatch, IonWorks and IonFlux. The TRPV1 assay seems to be straight forward: the TRPV1 mediated current is reliably activated by capsaicin

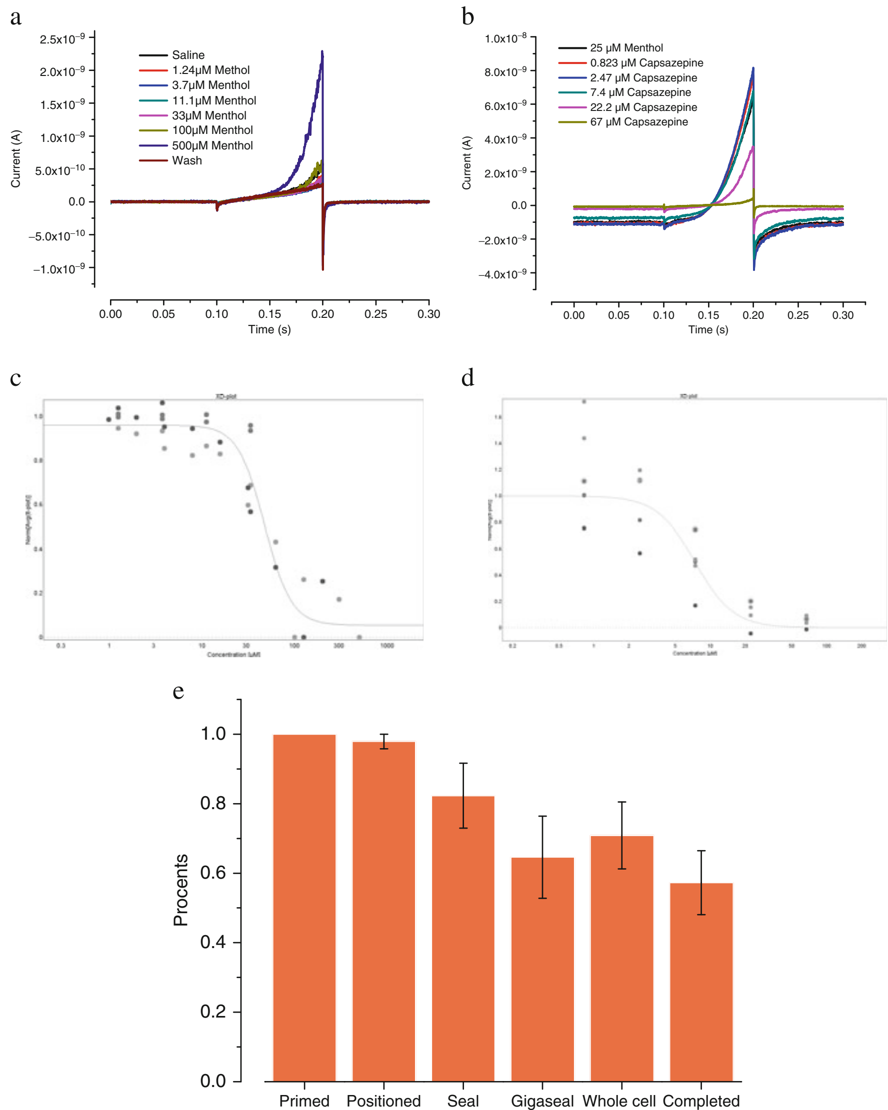


**Fig. 5.4** Characterization of block of the menthol activated current of TRPM8 channels expressed in HEK293. (**a**) Raw traces were obtained from a 300 ms ramp (–80 to +80 mV) with increasing concentration of menthol. (**b**) TRPM8 currents activated with 25 μM menthol and blocked with increasing concentrations of capsazepine. (**c**) Hill fit of normalized data from 10 individual cells. Inward current measured at –60 mV. $EC_{50}$ of menthol was estimated to be 53.30 ± 7.01 μM ($n = 11$) (reference value 101 ± 13 μM [54]). (**d**) Likewise the $IC_{50}$ of capsazepine was estimated to be 5.06 ± 1.26 μM ($n = 7$) (reference value 18 μM [55]). (**e**) Success rates from six QPlates. Shown are percentages of sites primed, cells positioned, cells generating seals above 100 MΩ, cells generating seals above 1 GΩ, cells going whole-cell and cells completing the six point dose response experiment. Results are given as average ± SD

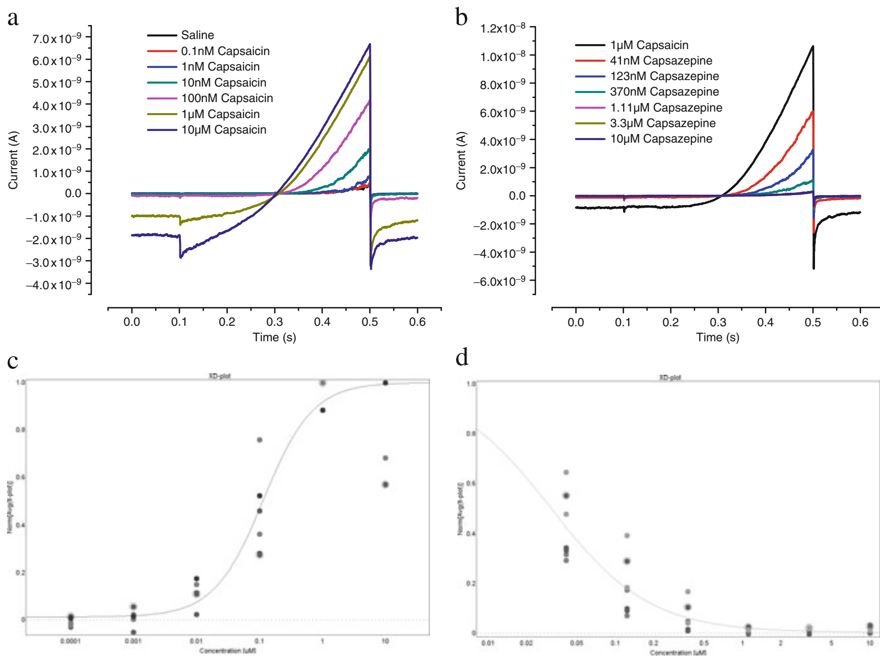


**Fig. 5.5** HEK293 cells stably transfected with TRPV1. A 60 ms ramp from –100 to +100 mV was applied every 5 s. (**a**) Raw current response to increasing concentrations of capsaicin. (**b**) Raw current response to increasing concentrations of capsazepine. (**c**) Normalized group Hill fit of six individual cells. $EC_{50}$ of capsaicin = 110.42 ± 17.55 nM. Same as previously reported [50]. (**d**) Normalized group Hill fit of seven individual cells. $IC_{50}$ of capsazepine was 43.66 ± 7.70 nM. Which correspond well with previous found values [51]

and the capsaicin-activated current can easily be blocked with a reference compound like capsazepine. Also, there are many high quality cell lines available all exhibiting currents in the nA range.

Figure 5.5a shows some typical raw traces from a HEK cell line expressing TRPV1. A –100 to +100 mV voltage ramp protocol was used to monitor TRPV1 activation by increasing concentrations of capsaicin. In Fig. 5.5b, the same voltage protocol was used to determine the dose dependent effect of capsazepine on the capsaicin-induced current. As illustrated in Fig. 5.5c, d, the resultant dose response curves demonstrate that the obtained pharmacology is in range with previously reported values [50, 51].

#### 5.2.4.2 TRPV4

Like many of the other TRPV subfamilies the TRPV4 channel can be activated polymodally and integrate the stimuli from a variety of sources. In addition to temperature TRPV4 is activated by various lipid messengers like endocannabinoids

and some phorbol ester derivatives like 4α-PDD. Furthermore, TRPV4 is activated by hypotonicity (cell swelling) and it has been suggested that TRPV4 is involved in regulation of systemic osmotic pressure [11].

In a study of TRPV4 using PatchXpress and IonWorks, the effect of phorbol ester activation and mechanical stress was measured respectively or in combination [52]. In line with current thinking, it could be clearly demonstrated that the response to the combination of the two stimuli exceeded the effect of either stimuli on its own underscoring the polymodality of TRPV4 activation and the broad applicability of automated patch clamp in addressing polymodality of TRPs.

## 5.3 Conclusions

Driven by the appreciation of whole-cell patch clamp data in ion channel drug discovery and with the desire to characterize compound effects with a faster turn-around time in both drug discovery as well as in safety assessment campaigns, significant efforts have been made to develop automated patch systems [2, 53]. A few years back the automated patch clamp systems had made rather limited impact on the ion channel drug discovery programs but during the last couple of years the flexibility, versatility and reliability of automated patch clamp technologies have made a significant fingerprint on applied workflows in this arena for particular biotech and pharmaceutical companies. The number of high throughput platforms that have been fully integrated into drug discovery programs spanning hit identification, lead optimization, and liability screening has increased dramatically. With the number of programs increasing, researchers are beginning to routinely configure assays where the complexity of the assays and targets can present significant challenges to the automated patch clamp system. Most of the TRP ion channels belong to this category.

Beside the obvious increase in daily data production, a number of experimental advantages can be attributed to automated patch clamp electrophysiology compared with conventional patch clamp. As a positive side effect of automation, the limited user intervention reduces the risk of human errors and thereby increases the likelihood of obtaining reliable, objective, and reproducible data. Furthermore, in conventional patch clamp drugs are routinely applied in a constant perfusion system with flow rates around 1 ml/min. The automated systems by contrast use very small volumes (in the low μl range) thereby reducing waste of highly valuable compounds or even enabling tests at an earlier stage in the drug discovery cascade. With the relative large surface to volume ratio in the flow channels of some of the automated systems, the use of small compound volumes poses a potential problem. As illustrated above, certain sticky compounds need to be added more than once to exert its full effect on the ion channel: a fact that needs to be taking into consideration when designing experiments on automated patch clamp devices.

Being automated and with minimal user intervention, a few issues have shown to be prerequisites for optimal performance of the patch clamp systems. Most notably

the cells have proven to be the key to success. Since the cell to be used in the experiment is selected by random the cell population has to be very homogeneous in particular with respect to expression as non-expressers are extremely costly. As such strict growth and harvest conditions seem to be critical in order to maintain high expression levels, high viability and to avoid clumping of the cell culture. To select the right clone with the right balance of compatibility on the system, current expression, minimal run down during the course of an experiment, and robustness over many cell culture passages, some of the higher throughput platforms like QPatch and IonWorks offer the feature of clone selection.

Generating quality cell lines can be quite cumbersome. Additionally, some TRP channels can be quite difficult to express in the traditional cell lines and expression systems. The result may be either a rather low expression profile or an uneven expression in different cells. With the introduction of the multi-hole systems the sensitivity to such factors are minimized and more stable and reliable recordings are obtainable as demonstrated on several ion channels ([19]). Furthermore, the multi-hole systems allow the usage of various transient transfection methods where the transfection efficiency does not reach 100%. Not only is the assay development time drastically reduced but novel approaches to ion channel research can be applied quickly.

The full potential of automated patch clamp in TRP research still has to be seen. The implementation of automated systems into academic institutions has been somewhat slowed down due to the capital needed to acquire and run an instrument. However, with the aid from various national stimulation packages like the US-based TARP (Troubled Asset Relief Program) there are clear signs that this will change in the next few years. With an often limited freedom and tradition to publish obtained results by groups within the pharmaceutical sector, the number of TRP channels that actually have been tested on the automated platform most probably exceed by far what has been published and reviewed in this chapter.

The availability of high quality stably expressing cell lines has also prevented the full usage of automated patch clamp in certain areas of TRP-directed drug discovery. However, in recent years a number of cell line vendors have generated a very wide panel of TRP expressing cell lines with great promise for the future.

In a meeting report from the 2009 Stockholm TRP meeting it was clearly described how substantial progress has been made in understanding the nature of the various TRP channels [15] but it was also emphasized that a lot of essential questions remain unanswered. For example by which molecular mechanisms are thermosensation and thermoactivation regulated? How is mechano-sensitivity in TRPs triggered? What is the functional consequence of TRP heteromerization or is homomerization only caused by induced fit in highly expressing heterologous expression systems? And what is the role of TRP channels in stem cells?

In the coming years, the automated patch clamp systems will most likely be instrumental in elucidating some of these questions. With the incorporation of temperature control on some of the platforms multiple mutant clones can be characterized efficiently in details that previously would have required a significant effort. Also, work is being done to allow efficient electrophysiological recordings on stem

cells and primary cells on the automated platforms, which should accelerate a better understanding of TRP channels in stem cell development and differentiation. Probably most noteworthy for the contribution of automated patch clamp to the field of TRP channels is the recognition that TRP functions are essential to normal life and that the identification of new compounds that can activate or inhibit a specific TRP channel activity is of paramount importance. The increased throughput of automated patch clamp devices should enable a quicker and more competent search for candidate compounds – be it small molecules, peptides or antibodies.

Although the throughput range of automated patch clamp has not reached the need in primary screening campaigns, the diverse experimental applications, the elevated throughput and the ease of use has found significant foothold in the field of ion channel drug discovery. In the years to come, automated patch clamp is likely to remain an invaluable tool for ion channel drug discovery in general and hopefully for the development of drugs targeting the fascinating TRP ion channels in particular.

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