Reversible Slow Desensitization of the Acid-Sensing Ion Channel (ASIC) 1a

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Research Article

Keywords: tachyphylaxis, gating modulation, voltage-dependence, calcium-dependence, pH-dependence

Posted Date: October 21st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-985337/v1>

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Abstract

Among the proton-activated channels of the ASIC family, ASIC1a exhibits a specific tachyphylaxis phenomenon in the form of a progressive decrease in the response amplitude during a series of activations. This process is well known, but its mechanism is poorly understood. Here, we demonstrated a partial reversibility of this effect by long-term whole-cell recording of CHO cells transfected with rASIC1a cDNA. Long but infrequent acidifications provided the same recovery time course as short acidifications of the same frequency. Steady-state desensitization is not related to the slow desensitization and attenuates the development of the slow desensitization. Consequently, we found that drugs, which facilitate ASIC1a activation (e.g., amitriptyline), cause an enhancement of slow desensitization, while inhibition of ASIC1a by 9-aminoacridine attenuates the slow desensitization. In summary, for influences of vastly different origin, including increase of calcium concentration, different pH conditions, and action of modulating drugs, we found a correlation between the effect on response amplitude and on development of slow desensitization. Thus, our results prove that a slow desensitization requires the open ion-permeable state.

Introduction

Proton-gated channels of the ASIC family are widely distributed in the central and peripheral nervous system. Besides their role in chemosensation and nociception, ASICs participate in synaptic transmission and synaptic plasticity due to the acidic content of synaptic vesicles (see (Uchitel et al. 2019) for a review). Not surprisingly, ASICs are involved in various CNS pathologies and are considered promising pharmacological targets (see (Storozhuk et al. 2021; Baron and Lingueglia 2015) for review). The ASIC subunits ASIC1a, ASIC2a, and ASIC2b are primarily expressed in the CNS and form homo- and heterotrimeric channels. Among the ASICs with different subunit compositions, homotrimeric ASIC1a demonstrates several specific characteristics, including a greater sensitivity to pH changes than is seen for ASIC2a and ASIC2a-containing heteromers (Hesselager et al. 2004). The threshold of activation of homotrimeric ASIC1a is about pH 7.0, and half-maximal activation occurs between pH 5.8 (Hesselager et al. 2004) and pH 6.3 (Shteinikov et al. 2019). ASIC1a channels are permeable not only to sodium but also to calcium ions (Waldmann et al. 1997; Bassler et al. 2001; Yermolaieva et al. 2004).

Acute desensitization terminates the response after activation by acidic solutions, but ASIC1a also demonstrates so-called steady-state desensitization (Babini et al. 2002). This effect is caused by subtle acidifications (pH from 7.2 to 6.9) that do not produce a significant response, but instead render the channels insensitive to further acidifications by pH changes that normally would produce the current. Furthermore, ASIC1a demonstrates an interesting phenomenon usually called tachyphylaxis, which is a progressive decrease in the response amplitude during a series of repetitive stimulations (Gitterman et al. 2005; Neaga et al. 2005; Paukert et al. 2004). Interestingly, the chicken ASIC1a does not exhibit tachyphylaxis (Rook et al. 2020). Systematic analysis of the mechanisms and determinants of the tachyphylaxis effect was undertaken by Chen and Gründer (Chen and Grunder 2007), who suggested that permeating protons contribute to tachyphylaxis. Their work also provided evidence that tachyphylaxis is

due to a long-lived desensitized state of ASIC1a, rather than endocytosis of the channels. A relationship between tachyphylaxis and Ca^{2+} permeability was also supported by the finding that treatment of ASIC1a with trypsin reduces both tachyphylaxis and Ca^{2+} permeability (Neaga et al. 2005).

Kusama et al. (Kusama et al. 2010) have demonstrated that replacement of extracellular Cl^- with the impermeable and inert anion methanesulfonate attenuates tachyphylaxis. Mutation of the residues that form the Cl^- binding site in ASIC1a also abolished the effect. Similarly, their data showed a strong correlation between the rate of desensitization and the degree of tachyphylaxis. ASIC1a currents generated at high Cl^- concentrations displayed the slowest desensitization and the greatest tachyphylaxis (Kusama et al. 2010).

Li et al. (Li et al. 2012) used brief (<100 ms) activation to demonstrate that desensitization of hASIC1a occurs with a short- and a long-lasting state, with time constants of about 0.5 and 229 s, respectively. By contrast, the chicken ASIC1a shows a similar time constant of 4.5 s for both desensitized states. Furthermore, recovery of the chicken ASIC1a from desensitization was unrelated to cytosolic variations in pH, ATP, PIP₂, or redox state, but it was dependent on the hydrophobicity of key residues in the first transmembrane pore-forming segment (Li et al. 2012). The authors also described a kinetic model of ASIC1a desensitization.

These studies have shown that tachyphylaxis depends on the pH, stimulus duration, and recovery period, and that tachyphylaxis is probably linked to the Ca^{2+} permeability of ASIC1a. However, consistent evidence for recovery from tachyphylaxis has still not been obtained, and the determinants of this effect require further analysis. Particularly, many factors, which modulate tachyphylaxis, also affect response amplitude. But these characteristics were not studied together and possible correlations between them are unknown. The aim of the present study was to shed more light on these issues.

Materials And Methods

Chinese hamster ovary (CHO) cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 and maintained under standard culture conditions (Dulbecco's modified Eagle's medium [DMEM/F12] + 10% fetal bovine serum + 50 µg/mL gentamicin). A plasmid encoding the rat ASIC1a subunit was transfected using Lipofectamine 2000 (Invitrogen, CA, USA) following the manufacturer's transfection protocol. Expression vectors encoding rat ASIC1a, as characterized by Hesselager et al. (Hesselager et al. 2004), were a kind gift from Dr. A. Staruschenko (Staruschenko et al. 2007). CHO cells were obtained from Evrogen (<http://evrogen.ru>). Cells were transfected with 0.5 µg rASIC1a cDNA per 35 mm diameter dish + 0.5 µg green fluorescent protein. Experiments were performed 2–3 days after transfection. Transfected cells were identified by their green fluorescence when viewed with a Leica DMIL microscope.

Recordings were made by the whole cell patch clamp technique using an EPC-10 amplifier (HEKA Electronics, Lambrecht, Germany). Currents were filtered at the 0-5 kHz band, digitized with a sampling rate of 1 kHz using the PatchMaster software (HEKA Electronics, Lambrecht, Germany), and stored in a

personal computer for further analysis. The extracellular solution contained (in mM): NaCl 143, KCl 5, MgCl₂ 2, CaCl₂ 2.5, D-glucose 18, HEPES 10, and MES 10 (the pH was adjusted to 7.4 with NaOH). The pipette solution contained (in mM): CsF 100, CsCl 40, NaCl 5, CaCl₂ 0.5, EGTA 5, and HEPES 10 (the pH was adjusted to 7.2 with CsOH). Patch pipettes (2–5 MΩ) were made with a P-97 micropipette puller (Sutter Instruments, CA, USA). Drugs were dissolved in the extracellular solution, and the pH values of the drug-containing solutions were adjusted to the required value immediately before the experiments. A series resistance of about 10 MΩ was compensated by 70–80% and monitored during the experiments. Only recordings showing series resistance and capacitance changes of less than 10% during the experiment were used. All experiments were performed at room temperature (23–25°C). The holding potential was –80mV, unless otherwise noted. Solutions were applied using an ALA-VM8 valve control system (ALA Scientific Instruments, USA) under computer control. The chemicals used in the study were purchased from Sigma-Aldrich and Tocris Bioscience.

All values are presented as the mean ± standard deviation (SD) from at least five experiments. The significance of the effects was tested with a paired t-test (drug versus control). The effects were considered significant at $P < 0.05$, based on at least 5 experiments.

Results

Tachyphylaxis is partially reversible

To induce tachyphylaxis, ASIC1a were activated by extracellular pH drops from 7.4 to 6.5 with a duration of 20 s. The interval between activations was 30 s. In this protocol, ASIC1a-mediated currents progressively decreased during the series of activations (Fig. 1A), so that 10 activations resulted in a $59 \pm 11\%$ ($n = 21$) reduction in the initial amplitude (Fig. 1B).

Previous studies have suggested that tachyphylaxis represents a slow desensitization of ASIC1a (see Introduction). This means that the effect should be reversible. However, recovery was not demonstrated for whole-cell currents. To eliminate this uncertainty and obtain proof of the reversibility, we devised a recovery protocol in which ASIC1a were activated by 5 s acidifications at 300 s intervals. After induction of tachyphylaxis, this protocol showed a clear tendency toward a response recovery (Fig. 1A, C). Note that this experiment required a stable recording lasting more than 30 min. The first 300 s interval did not cause prominent response increase indicating that the 30 s interval between activations is enough for complete recovery from acute desensitization and the entire response decrease is due to tachyphylaxis.

The development of tachyphylaxis and its recovery demonstrated large cell-to-cell variations, and no recovery was observed in some cases. One point to take into account in this respect is that testing acidifications in the recovery protocol also contribute to tachyphylaxis, and this could mask the recovery. To minimize this effect, we used short 1 s testing acidifications in the recovery protocol. Fig. 1D demonstrates that significant recovery was observed in all four experiments. In contrast, 1 s acidification every 30 s did not provide a recovery (data not shown). We also tested effect of long acidifications in the

recovery protocol by 55 s exposure to pH = 6.5 at 250 s intervals to maintain the total interval between acidifications at 305 s. We reduced the uncertainty caused by cell-to-cell variations by applying the 55 s acidifications three times, followed by further application of an acidic solution for 5 s. The results are presented in Fig. 1E. Some cells demonstrated partial recovery, as in the case of 5 s acidifications. The overall time course of recovery was unchanged. If a recovery was not observed with 55 s acidifications, it did not develop even after a switch to 5 s acidifications. Thus, we concluded that slow desensitization did require the open state and that acute desensitization prevents channel transition to the slow-desensitized state. Pooling the data obtained in three recovery protocols together (Fig. 1F), we obtained statistically significant recovery from tachyphylaxis ($n = 22$, $P = 0.011$), thereby confirming its slow desensitization nature.

Modulation of ASIC1a activation affects tachyphylaxis

Previous studies did not analyze possible relationships between the effects of different factors on the channel activation and on the tachyphylaxis. We developed a protocol in which the series of activations under control conditions were alternated with the series of activations under modified conditions, and we then compared the resulting time courses of the experiments with the averaged control curve (Fig. 1B). We tested this protocol by analyzing the slow desensitization caused by strong acidifications (pH = 5.5), which are known to facilitate tachyphylaxis (Chen and Grunder 2007). The results are presented in Fig. 2 A-C. The initial activations caused responses that matched the control curve. Since the initial series of activation was performed under control conditions, similarity of the time course with the average control curve served as independent control. Experiments, in which the relative amplitudes in the initial series deviated from the averaged control curve more than 20%, were not considered. Activations by pH = 5.5 caused an increase in the responses (above the control curve), but responses to subsequent pH = 6.5 activations were significantly below the control curve, suggesting a facilitation of slow desensitization. Thus, the protocol allows the simultaneous estimation of the effects on the response amplitude and on the development of slow desensitization.

Besides the acute desensitization, which terminates the response after its activation by acidic solutions, ASIC1a also demonstrates so-called steady-state desensitization (Babini et al. 2002). This effect is caused by subtle acidifications (pH from 7.2 to 6.9) that do not produce significant response, but instead render the channels insensitive to further acidifications by pH changes that normally would produce the current. To test the relationship between steady-state and slow desensitization, we used our protocol of alternated series of activations with the conditioning pH reduced from 7.4 to 7.1 (Fig. 2D-F). This reduction in the conditioning pH caused a strong decrease in the amplitude due to the steady-state desensitization. However, a return to the control conditions immediately produced the responses with increased amplitude (above the control curve). This result strongly suggests that steady-state desensitization and slow desensitization (tachyphylaxis) are independent processes. Moreover, the increased response after the return to the control conditions suggests that steady-state desensitization prevents channel transition to the slow-desensitized state.

The data in Fig. 2 show that amplitude increase (activating pH=5.5) results in enhancement of tachyphylaxis, while amplitude decrease (conditioning pH=7.1) is accompanied by tachyphylaxis attenuation. We further tested this apparent correlation using two compounds that shift the pH dependence of ASIC1a activation toward more acidic values (9-aminoacridine) (Tikhonova et al. 2015; Shteinikov et al. 2019) and toward less acidic values (amitriptyline) (Nikolaev et al. 2019; Marin et al. 2008). These compounds produce inhibition and potentiation of the responses to pH = 6.5, respectively. Taking into account the previously estimated activities and kinetics of 9-aminoacridine and amitriptyline action, 9-aminoacridine was applied at 500 μ M, simultaneously with acidifications, whereas amitriptyline was applied at 300 μ M during and between the acidifications. Our analysis demonstrated that inhibition by 9-aminoacridine reduced the slow desensitization whereas potentiation by amitriptyline enhanced it (Fig. 3A-F), in agreement with the relation between ASIC1a activation and slow desensitization.

The observation that 9-aminoacridine attenuated the development of slow desensitization prompted us to test whether it facilitates the recovery. The results shown in Fig. 3G confirm this suggestion. Application of 9-aminoacridine during the three first activations in the recovery protocol caused an inhibition of the responses, but strong recovery (18, 48, 8 and 36% of the initial responses in individual experiments) was observed after 9-aminoacridine washout.

ASIC1a also shows complex interactions with calcium ions. These ions serve as permeant ions for this ASIC subtype, but they also block the ion pore and compete with protons (de Weille and Bassilana 2001; Paukert et al. 2004). Previously, Chen and Gründer (Chen and Grunder 2007) demonstrated that slow desensitization is attenuated by increases in extracellular calcium concentration. Conversely, the same work reported a correlation between the calcium permeability of chimeric channels and the development of slow desensitization. A relationship between tachyphylaxis and Ca^{2+} permeability was also supported by Neaga et al. (Neaga et al. 2005). We tested the effect of increase in external calcium concentration in both conditioning and activations solutions from 2.5 to 10 mM (Fig. 4). Indeed, an elevation of external calcium caused an attenuation of slow inactivation, and this effect was accompanied by a strong decrease in the response amplitude. Thus, the increase in calcium attenuated the slow desensitization in the same manner as was observed with 9-aminoacridine and conditioning acidification to pH = 7.1. Taken together, our data suggest that influences of different nature that affect ASIC1a activation cause corresponding effect on the slow desensitization.

Competition between tachyphylaxis and potentiation

Amitriptyline causes a shift of pH-dependence of ASIC1a activation towards less acidic values and thus, potentiates the response. In turn, the amplitude potentiation enhances slow desensitization that results in faster response decrease in a series of activations. The same effect is caused by activation with strong acidifications, responses became larger, but enhanced tachyphylaxis results in a progressive response decrease. To reveal this phenomenon, we analyzed how potentiating effect of amitriptyline (300 μ M amitriptyline was added in both conditioning and activating solutions) depends on the level of slow desensitization. For comparison, we studied how strong acidification (pH=5.5) increases the response

under control conditions (pH=6.5) at different levels of slow desensitization. Thus, in both cases the response to pH=6.5 served as a control. To account the tachyphylaxis the potentiating effect was calculated as $2*I/(I_{\text{control}}+I_{\text{wash}})$, where I is the current amplitude in the presence of amitriptyline or at pH=5.5, I_{control} and I_{wash} are the current amplitudes of preceding control response and response after washout, correspondingly. Figure 5A shows that both potentiating effect of amitriptyline and effect of strong acidification are more pronounced if desensitization is deep.

The formal estimations of the effect values may not be quite correct for the complex experiment. Therefore, we considered the time-course of experiment in details (Fig. 5B and C). We measured the amitriptyline effect on the response amplitude by finding the scaling coefficient that places the amplitudes in the presence of the drug on the control time-course (Fig. 5B). However, finding a single value for the entire experiment was impossible. In this example, scaling the amplitudes in the presence of amitriptyline by 1.35 provided a good fit at the beginning of experiment, but the scale factor of 1.8 was required for the later series with the high level of slow desensitization (Fig. 5B). Fig. 5C shows the correlation field between the amitriptyline effect estimated by this procedure and the level of steady-state desensitization. In all cases the apparent potentiation was smaller in the beginning of experiment, when the level of slow desensitization is modest. The average effect of 300 μM amitriptyline was significantly different for the conditions of high and low levels of slow desensitization (Fig. 5C, $n = 6$, $p = 0.01$). Thus, intrinsic amitriptyline-induced potentiation action is partially screened by an enhancement of slow desensitization and results in the underestimation of potentiating effect. This screening effect is maximal if only a minor fraction of the channels is desensitized. As the slow desensitization approaches equilibrium, the value of the screening effect decreases, and potentiation is estimated more correctly.

The relationships between tachyphylaxis and permeation characteristics

Classical experiments by Chen and Gründer (Chen and Grunder 2007) addressed the impact of ion permeation on slow desensitization. In particular, membrane depolarization that reduced the ion driving force was shown to cause an attenuation of slow desensitization, whereas partial replacement of sodium ions by the impermeant NMDG had no effect. We reproduced these results in our experimental protocol and obtained the same results (Fig. 6). Both depolarization from -80 to -10 mV and partial replacement of sodium by NMDG (10 mM Na^+ and 130 mM NMDG) caused strong reduction of the response amplitudes. Amplitude reduction caused by depolarization was accompanied by attenuation of the slow desensitization in the manner that resembles the effect of 9-aminoacridine, increased calcium and conditioning pH 7.1. However, in the case of sodium replacement with NMDG, the time course of slow desensitization was not affected. Notably, the latter result provided the only example of the drastic reduction in the response amplitude without a significant effect on slow desensitization.

Computer simulations of slow desensitization

Earlier, the kinetic model was proposed to account ASIC1a tachyphylaxis (Chen and Grunder 2007; Li et al. 2012). According to this model channel transition to the slow desensitized state is possible only if the

channel is open. To check if our findings are consistent with this model, we performed simulations by numerical solution of the system of differential equations corresponding to the model (Fig. 7A). Of course, this model shown in Fig. 7A is simplified, particularly it does not reflect binding of multiple protons for activation. Therefore, we did not attempt to find realistic parameters in the kinetic scheme, but simply tried to reproduce the key experimental result, enhancement of slow desensitization by excessive activation. The results are presented in Fig. 7. Control simulations (Fig. 7B) show progressive decrease of the amplitude response in a series of activation and slow recovery as the activation frequency is ten times decreased (red line). Higher agonist concentration (blue line) causes faster and deeper tachyphylaxis (curves are scaled to match the first response amplitude). The green line in the Fig. 7C shows the time course of simulation, in which after the series of control activations the agonist (H^+) concentration was set ten times higher. This results in the prominent increase of the amplitude. However, after returning to the control conditions the green line is below the red one, reflecting the amplitude decrease due to facilitated slow desensitization. Thus, the simple scheme provides reasonable qualitative explanation for relationship between response amplitude and slow desensitization.

Discussion

In the present study, we characterized ASIC1a tachyphylaxis and the modulation of this effect by different factors. We demonstrated that tachyphylaxis is at least partially reversible in whole-cell experiments. This finding unambiguously suggests that tachyphylaxis represents a slow desensitization of ASIC1a. Because the time course of this recovery was very slow, it could be suspected that the increase in current amplitude was due to trafficking and plasma membrane insertion of new channels. However, such effect is hardly possible in long whole-cell experiments with the intracellular content dialysis.

Long-lasting acidifications, which cause fast acute desensitization, do not prevent the recovery. We also found that steady-state desensitization is not related to the slow desensitization. Moreover, acute and steady-state desensitization attenuate the development of the slow desensitization. Thus, our results prove that the slow desensitized state requires the open state further supporting earlier suggestions (Chen and Grunder 2007; Li et al. 2012).

Using the experimental protocol, which allows simultaneous estimation of effects on response amplitude and on tachyphylaxis, we found a relationship between the influences on these characteristics. Reduction of the amplitude by increased calcium attenuates tachyphylaxis while increase of proton concentration causes amplitude increase and enhances tachyphylaxis. To test this relationship, we used the drugs that facilitate (amitriptyline) or inhibit (9-aminoacridine) ASIC1a activation. We found that potentiation by amitriptyline causes enhancement of slow desensitization, while attenuation of ASIC1a activation by 9-aminoacridine attenuates the slow desensitization and speeds up the recovery. Thus, our experiments with potentiating and inhibiting drugs suggest that these influences on slow desensitization are mediated by the corresponding influence on the amplitude, which reflects the open-state probability (Fig. 8). Lowering of conditioning pH to 7.1 that promotes the steady-state desensitization and thus reduces the channel opening, also resulted in attenuated tachyphylaxis. Taken together, our data suggest that

influences of drastically different nature that affect ASIC1a activation cause corresponding effect on the slow desensitization. Indeed, if slow desensitization requires the open state, the large fraction of the open channels, the faster tachyphylaxis development. This finding is well reproduced in computer simulations using simple kinetic model that allows slow desensitization only from the open state.

The next important question is whether slow desensitization depends on the functionally open state *per se* or on the ion transport. Experiments involving changes in the holding voltage favored the second possibility. Under depolarized conditions the open-state probability is not changed, but the currents were significantly smaller and slow desensitization was attenuated. This matches the results of other series (Fig. 8). However, replacement of sodium by NMDG demonstrated that the rate of sodium transport is not essential for the process. Although the current became smaller, the slow desensitization was unaffected. This is the only exception to the correlation between the influences on the current amplitude and on the development of slow desensitization (Fig. 8).

The existence of several distinct mechanisms of desensitization, analogous to the inactivation of voltage-gated channels, is not a unique feature of ASIC1a. Voltage-gated calcium channels and calcium-permeable NMDA receptor channels show the phenomenon of calcium-dependent desensitization (inactivation) (Abderemane-Ali et al. 2019; Sibarov and Antonov 2018; Budde et al. 2002). Another classic example is the fast (or N-type) and slow (or C-type) desensitization of voltage-gated potassium and sodium channels; these types of desensitization are essentially different and are controlled by different parts of the channel proteins (Catterall et al. 2020; Ulbricht 2005). Particularly, the C-type (slow) desensitization is related to ion permeation and the structural stability of the selectivity filter, which strongly interacts with permeant ions (Tikhonov and Zhorov 2007). The difference of the effects caused by membrane depolarization and by partial sodium replacement with NMDG allows to propose that in the case of ASIC1a slow desensitization is also related with ion occupation of the selectivity filter in open channels.

The structural mechanisms underlying the ASIC1a slow desensitization are unknown. Several ASIC1a structures are available (Baconguis et al. 2014; Jasti et al. 2007; Sun et al. 2020; Yoder and Gouaux 2018), but at least three distinct structures are needed, corresponding to acute desensitization, steady-state desensitization, and slow desensitization. Deciphering these structures remains a challenge. In conclusion, the interesting phenomenon of ASIC1a slow desensitization remains incompletely understood and its mechanisms, structural determinants, and possible physiological significance deserve attention in further studies.

Declarations

Statements and Declarations

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

MSK†, ARB† and NNP have performed the experiments and data analysis, DBT designed the experiments and prepared the manuscript draft. All the authors contributed to the manuscript and figure editing.

†These authors have contributed equally to this work and share first authorship.

Funding

The work is supported RSF grant 21-14-00280 for Denis Tikhonov.

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Figures

Figure 1

Reversible tachyphylaxis of ASIC1a responses. A, representative recording of progressive decrease in the response for a series of 10 activations with 30 s intervals and partial recovery by 5 s activations with 300 s intervals. B, averaged curve for tachyphylaxis fitted by a monoexponential curve. Amplitude of the 1st response is assigned 100%. Error bars represent SD from 21 experiments. C, cell-to-cell variations of tachyphylaxis development and recovery in 7 representative experiments. D, results of recovery protocol

with 1 s testing activation. E, recovery takes place even if long (55s) acidifications are used in recovery phase. 8 representative experiments are shown. F, pooled data from the recovery protocols from panels C, D and E demonstrate the significance of the recovery effect between 10th and 15th activations. Individual experiments from panels C, D, and E are shown blue, red and black, correspondingly.

Figure 2

pH-dependence of slow desensitization. A, series of activations under control conditions (pH = 6.5) alternated with activations by pH = 5.5. B, relative response amplitudes from four independent experiments. The average control curve (Fig. 1C) is shown for comparison. Activations by pH = 5.5 produce responses with increased amplitude (above the control curve). Return to the control conditions causes a small-amplitude response (below the control curve), suggesting the desensitization enhancement. C, statistics of each application series demonstrates the significance of the effects ($p < 0.05$, marked by an asterisk) relative to the control. D, responses in a representative experiment in which the standard conditioning pH = 7.4 was alternated with pH=7.1. Application numbers are given above the currents. E, relative response amplitudes for five independent experiments. Although conditioning at pH = 7.1 causes a response reduction due to steady-state desensitization, the return to control conditions produces responses that are above the control curve. F, statistics of each application series demonstrates the significance of the effects ($p < 0.05$, marked by an asterisk) relative to the control.

Figure 3

Modulation of ASIC1a activation affects slow desensitization. A, responses in the representative experiment in which control conditions were alternated with activations in the presence of 300 μ M amitriptyline (Ami). B, relative response amplitudes for four independent experiments. After washout of the potentiating amitriptyline effect, the responses are below the control curve, suggesting an enhancement of slow desensitization. C, statistics of each application series demonstrates the

significance of the effects ($p < 0.05$, marked by an asterisk) relative to the control. D, series of activations under control conditions alternated with activations in the presence of 500 μM 9-aminoacridine (9AA). Application numbers are given above the currents. E, relative response amplitudes from three independent experiments. The average control curve is shown for comparison. 9-aminoacridine causes a strong response inhibition. After drug removal, the responses are above the control curve, suggesting attenuation of the slow desensitization. F, statistics of each application series demonstrate the significance of the effects ($p < 0.05$, marked by an asterisk) relative to the control. G, relative response amplitudes in experiments in which 9AA was applied during the first three activations of the recovery protocol. Strong recovery is observed.

Figure 4

Effect of high extracellular calcium on the ASIC1a response amplitude and on the development of slow desensitization. A, series of activations under control conditions (2,5 mM Ca²⁺) alternated with activations in the presence of 10 mM Ca²⁺. Application numbers are given above the currents. B, relative response amplitudes from three independent experiments. The average control curve is shown for comparison. Increase in calcium concentration causes strong response inhibition and attenuation of the

slow desensitization. C, statistics of each application series demonstrate the significance of the effects ($p < 0.05$, marked by an asterisk) relative to the control.

Figure 5

The apparent potentiating action depends on the level of slow desensitization. A, correlation field between the level of slow desensitization and potentiating effect of 300 μ M amitriptyline (black) and activating pH-5.5 (red). See text for details. In both cases the responses to pH=6.5 are used as controls.

Black and red lines represent linear approximation of the corresponding data sets. B, relative response amplitudes in a representative experiment in which control conditions were alternated by activation in the presence of 300 μ M amitriptyline (Ami). The apparent effect of amitriptyline is determined as a scaling factor that fits the responses in the presence of the drug to those of the control curve. The value 1.35 provides a good fit at the beginning of experiment, with modest slow desensitization. By contrast, a value of 1.8 is required to fit the amplitudes as the slow desensitization deepens. C, correlation field between the level of slow desensitization and the amitriptyline effect calculated as a scaling factor. Averaged values for the amitriptyline effect at low and high levels of slow desensitization (bars) are significantly different ($p=0.002$, $n=6$).

Figure 6

Effects of ion permeation on the development of slow desensitization. A, series of activations under control conditions (-80 mV) alternated with activations at the holding voltage (-10 mV). Application numbers are given above the currents. B, relative response amplitudes from five independent experiments. The average control curve is shown for comparison. Depolarization to -10 mV causes a response reduction. After restoring the holding voltage, the -80 mV responses are above the control curve,

suggesting attenuation of the slow desensitization. C, statistics of each application series demonstrate the significance of the effect ($p < 0.05$, marked by an asterisk) relative to the control. D, responses in a representative experiment in which the control conditions (143 mM Na⁺) were alternated by activation in the presence of 130 mM NMDG and 10 mM Na⁺. Application numbers are given above the currents. E, relative response amplitudes for five independent experiments. Despite the response reduction in the presence of NMDG, the slow desensitization is not changed upon restoration of the control conditions. F, statistics of each application series demonstrate the significance of the effect ($p < 0.05$, marked by an asterisk) relative to the control.

Figure 7

Computer simulation of ASIC1a slow desensitization. A, kinetic scheme used. Symbols C, O, Df and Ds specify closed, open, fast (acute) and slow desensitized states, correspondingly. H^+ marks the only reaction involving the proton binding. B, simulation of tachyphylaxis and recovery in the cases of low (red) and high (blue) proton concentrations. The curves are scaled to match the first response amplitude. In the case of high proton concentration the tachyphylaxis is deeper and faster. C, simulation of the

experiment with alternated activations by low and high proton concentration (green). The red curve is duplicated from panel B for comparison. Activation by high proton concentration provides increased responses, but after returning to the low proton concentration, the responses are smaller than in control (the green line is below the red one) due to enhanced slow desensitization.

Figure 8

Correlation field between changes in the response amplitude (abscissa) and changes in slow desensitization (ordinate) evoked by different factors. Except for partial replacement of sodium by NMDG, other influences induce correlating effects on amplitude and slow desensitization.

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