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Complex action of tyramine, tryptamine and histamine on native and recombinant ASICs

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ABSTRACT

Proton-gated channels of the ASIC family are widely distributed in the mammalian brain, and, according to the recent data, participate in synaptic transmission. However, ASIC-mediated currents are small, and special efforts are required to detect them. This prompts the search for endogenous ASIC ligands, which can activate or potentiate these channels. A recent finding of the potentiating action of histamine on recombinant homomeric ASIC1a has directed attention to amine-containing compounds. In the present study, we have analyzed the action of histamine, tyramine, and tryptamine on native and recombinant ASICs. None of the compounds caused potentiation of native ASICs in hippocampal interneurons. Furthermore, when applied simultaneously with channel activation, they produced voltage-dependent inhibition. Experiments on recombinant ASIC1a and ASIC2a allowed for an interpretation of these findings. Histamine and tyramine were found to be inactive on the ASIC2a, while tryptamine demonstrated weak inhibition. However, they induce both voltage-dependent inhibition of open channels and voltage-independent potentiation of closed/desensitized channels on the ASIC1a. We suggest that the presence of an ASIC2a subunit in heteromeric native ASICs prevents potentiation but not inhibition. As a result, the inhibitory action of histamine, which is masked by a strong potentiating effect on the ASIC1a homomers, becomes pronounced in experiments with native ASICs.

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Introduction

Trimeric proton-gated ion channels of the ASIC family participate in various brain functions and contribute to different pathologies.^{1–3} According to an increasing amount of evidence, they are involved in the postsynaptic response.^{4–7} Since the content of synaptic vesicles is acidic, the release of a neurotransmitter results in synaptic cleft acidification. Protons released together with a neurotransmitter can reach postsynaptic ASICs and activate them, thus producing sodium influx, which additionally depolarizes the postsynaptic membrane. However, available electrophysiological data show very small ASIC-mediated currents, which can hardly play a significant role. Relatively large currents were registered in the Calyx of Held.⁷ This result clearly demonstrates the synaptic localization and function of the ASICs, but the contribution of these channels to the function of classical CNS synapses remains unclear.

A possible solution to the apparent disagreement between the well-established role of ASICs in the CNS and the subtle currents recorded in synaptic preparations is that some presently unknown endogenous ligand(s) potentiate ASICs responses. Many endogenous modulators of the ASICs are known.⁸ Extracellular Ca^{2+} ions inhibit ASICs through two distinct mechanisms. They can bind to the acidic pocket and thus reduce pH sensitivity⁹; another binding site exists at the entrance to the pore.¹⁰ Other divalent cations also inhibit ASICs.^{11,12} Endogenous polyamines agmatine and arcaine cause activation of ASIC3-containing channels.¹³ Spermine potentiates ASICs of different subunit compositions, including native ASICs in hippocampal neurons.^{14,15} The proposed mechanism of spermine action is a shift of pH-dependence of inactivation. Another class of ASIC potentiators includes neuropeptides like FMRF-amide and dynorphin.^{16–19} Arachidonic acid and anandamide potentiate ASICs by shifting pH-dependence of activation.^{20–22} Nitric

oxide also should be mentioned as an ASIC potentiator.²³ Thus, the list of endogenous ASIC modulators is rather long, possible mechanisms are diverse, and it remains unclear how they affect ASIC activity in physiological and pathological conditions. Drug action depends strongly on subunit composition, which greatly complicates the development of ASIC pharmacology. ASIC subunits readily form various functional heteromers and, as a result, native ASICs can show very complex pharmacological profile.^{24,25}

We have found recently that various amine-containing compounds potentiate or inhibit ASICs depending on the structural peculiarities and subunit composition of the target.^{26,27} In particular, the phenylcyclohexyl derivative IEM-2044 in which the hydrophobic moiety is connected to the amino group by two methylene spacers was found to potentiate ASIC1a homomers.²⁸ A similar structural organization occurs naturally in histamine. Subsequent experiments revealed significant potentiation of ASIC1a by histamine.²⁹ It demonstrates the most prominent potentiation at weak acidification conditions, which cause a minor response, and is virtually ineffective at saturating acidifications. Histamine produces a parallel shift of the concentration dependence of ASIC1a activation. This effect may be due to an allosteric increase of proton sensitivity. Alternatively, the amino group of histamine may directly interact with one of the proton binding sites, thus providing the co-agonistic effect. Therefore, histamine can play the role of a positive ASIC1a modulator in the CNS.

A number of synthetic amines were found to modulate ASICs.²⁸ Consequently, endogenous amines besides histamine should be considered as putative ASIC ligands. In the present work, we studied the action of naturally occurring amines tyramine and tryptamine on native and recombinant ASICs and compared them with histamine. The aim was to extend the list of endogenous ASIC modulators and to understand their mechanisms of action.

Different rat brain neurons respond to external acidification by ASIC-mediated currents with varying characteristics.³⁰ The ASICs in the hippocampal CA1 interneurons from the oriens lacunosum-moleculare layer are likely to be composed of ASIC1a and ASIC2 subunits, and they produce rather large currents in response to acidification.³¹ In contrast, the pyramidal neurons from the same area produce only small currents in response to acidification.^{30,31} In our previous

study, synthetic monoamines were shown to significantly affect native ASICs in hippocampal interneurons: IEM-1921 and IEM-2117 potentiate them, whereas memantine and 9-aminoacridine cause inhibition.²⁶ These characteristics do not match the data obtained on homomeric recombinant receptors, suggesting a heteromeric organization of ASICs in hippocampal interneurons. Potentiation caused by IEM-1921 and IEM-2117 is likely to be due to the presence of an ASIC2a in the heteromeric receptors; memantine and 9-aminoacridine work as inhibitors for an ASIC1a or an ASIC1b. Thus, our data agree with earlier results demonstrating ASIC1a and ASIC2 subunits contribution to native ASICs in the hippocampal interneurons. In the present work, we used this type of neurons to study the action of endogenous amines on native ASICs. Taking into account putative subunit composition of ASICs in hippocampal interneurons, we compared the data on these native ASICs with recombinant ASIC1a and ASIC2a.

Results

Action of endogenous amines on native ASICs in hippocampal interneurons

Channels were activated by 7sec pH drops from 7.4 to 6.5 with 30 sec intervals that provide complete recovery from desensitization. Experiments were performed at -80mV holding voltage, unless otherwise stated. Structures of the amines studied are presented in Fig. 1A.

Drug action on ion channels, in particular on ASICs, can be mediated by different molecular mechanisms. To fully understand their effects *in vivo* and to design new potent compounds, we need to uncover the mechanisms of drug action. To that end, after recording the control response (response I in Fig. 1B), we have used three protocols of drug application. The continuous presence of a compound (see response II in Fig. 1B) may produce an environment which is similar to the physiological conditions of action. However, this type of drug application does not allow us to determine if a drug interacts with an open channel, a closed channel, or its binding is state-independent. To reveal an interaction with an open channel, the drugs were applied simultaneously with the activating acidification (see response III in Fig. 1B). When the compounds were applied between activations (see response IV in Fig. 1B), they interacted mainly with

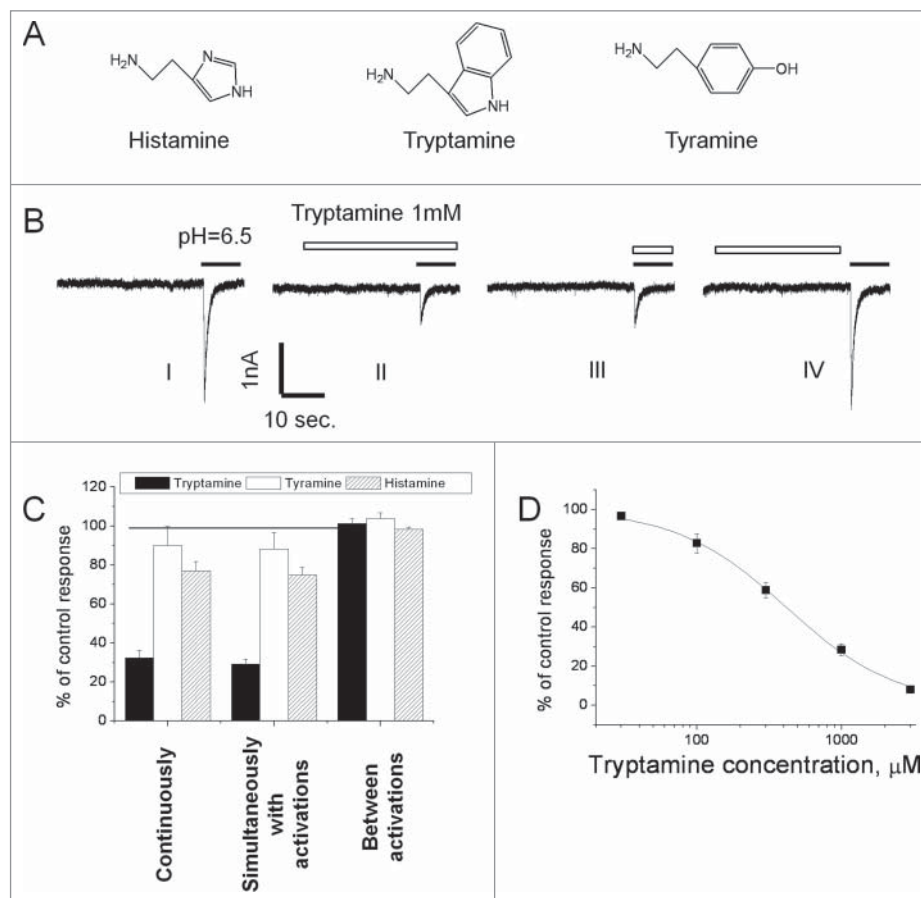


Figure 1. Action of histamine, tyramine and tryptamine on native ASICs. A, chemical structures of the compounds. B, representative recording demonstrates dependence of tryptamine action on the application protocol. After control recording (response I) tryptamine was applied in both neutral- and low-pH solution and caused inhibition (response II). Analogous effect was observed if tryptamine was applied simultaneously with activation (response III). In case of application of tryptamine between activations, no significant effect was detected (response IV). C, summary of effects of tryptamine, tyramine and histamine (1 mM) in three application protocols. D, concentration-dependence of inhibition evoked by tryptamine application simultaneously with activation.

the closed channels. After each type of drug application, we recorded test responses in the absence of a drug, in order to take into account response rundown during the experiment and to ensure reversibility of the effects (not shown).

1 mM tryptamine demonstrated drastically different action in different application protocols (Fig. 1B). Application between activations did not cause any effect, but continuous application and application simultaneous with activation evoked $72 \pm 15\%$ ($n = 6$) and $68 \pm 18\%$ ($n = 6$) inhibition (Fig. 1B and C). According to paired t-test, these effects were not statistically different ($P > 0.1$, $n = 6$). Thus, tryptamine does not affect ASICs in the closed state and inhibits them in the open state. Analysis of the concentration-dependence of tryptamine action was performed for the simultaneous drug application. 3 mM evoked almost complete inhibition, the IC_{50} value was

estimated as $420 \pm 80 \mu\text{M}$ and Hill coefficient was 1.1 ± 0.2 (Fig. 1D).

Our previous study has demonstrated that histamine selectively potentiates recombinant ASIC1a.²⁹ In the present work, we did not observe the potentiating effect of histamine on native ASICs in the hippocampal interneurons (Fig. 1C), thus confirming our previous suggestion that the response evoked in hippocampal interneurons by pH drops is not mediated by a homomeric ASIC1a.

Moreover, we found a subtle, but significant (paired t-test, $n = 6$, $P < 0.05$) difference of the effect in different application protocols. If 1 mM histamine was applied continuously or during activations it caused $25 \pm 6\%$ ($n = 6$) inhibition. Such effect was absent if histamine was applied between ASIC activations. Similar minor effect ($11 \pm 8\%$, $n = 6$) was observed for 1 mM tyramine applied continuously and during

activations (Fig. 1C). This dependence of action on the application protocol suggested an effect of the drugs on the open but not on the closed channels.

The open-channel inhibition effect often occurs if the drug molecule enters the open pore and sterically blocks ion permeation. In such cases, the charged blocking molecule binds in the area of the membrane electric field and the binding depends on the membrane voltage. Analysis of the tryptamine block revealed significant voltage dependence. 1mM produced only about 20% inhibition at 0 mV, but the effect reached 80% at -120 mV (Fig. 2A). Quantitative analysis of voltage dependence was performed using Woodhull model.³² According to this model, the fraction of the membrane field that the tryptamine molecule crosses from external surface to the binding site (δ value) is 0.63 ± 0.17 , $n = 7$ (Fig. 2B). The finding of the voltage dependence of tryptamine action allowed us to readdress the nature of weak effects of histamine and tyramine. In this series of experiments,

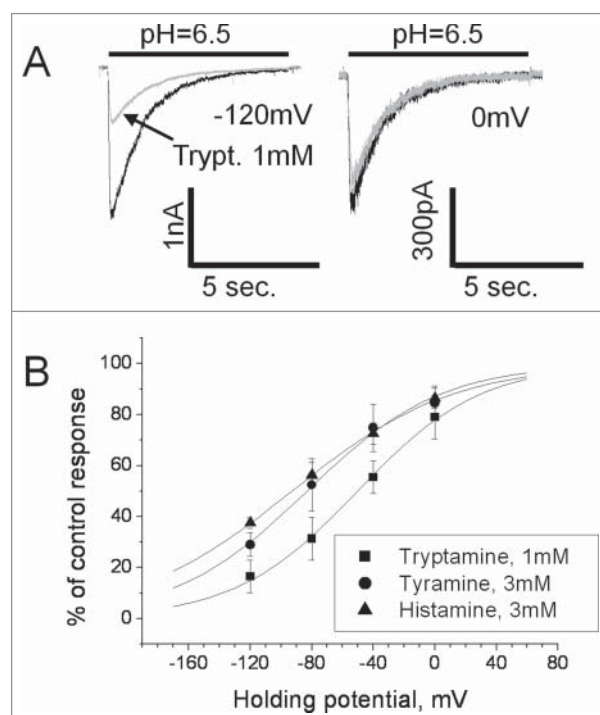


Figure 2. Action of endogenous amines on native ASICs is voltage-dependent. A, control responses (black) are superimposed with responses in the presence of 1 mM tryptamine applied simultaneously with activation (gray). Tryptamine caused strong inhibition at -120 mV but was virtually inactive at 0 mV. B, voltage dependence of action of tyramine, tryptamine and histamine. Although tryptamine is more potent than other amines, all three compounds demonstrated similar voltage dependence of inhibition.

the concentration of tyramine and histamine was increased to 3 mM. They were applied simultaneously with activation since this protocol favors the inhibitory effect. For both drugs significant voltage dependence was detected; δ values for tyramine and histamine were 0.58 ± 0.14 , $n = 5$ and 0.48 ± 0.16 , $n = 6$, respectively. Thus, all three amines cause a voltage-dependent open-channel block of native ASICs in the hippocampal interneurons. Despite certain differences between the δ values obtained for tyramine, tryptamine, and histamine, the data suggest a common binding region in the ion pore of ASICs.

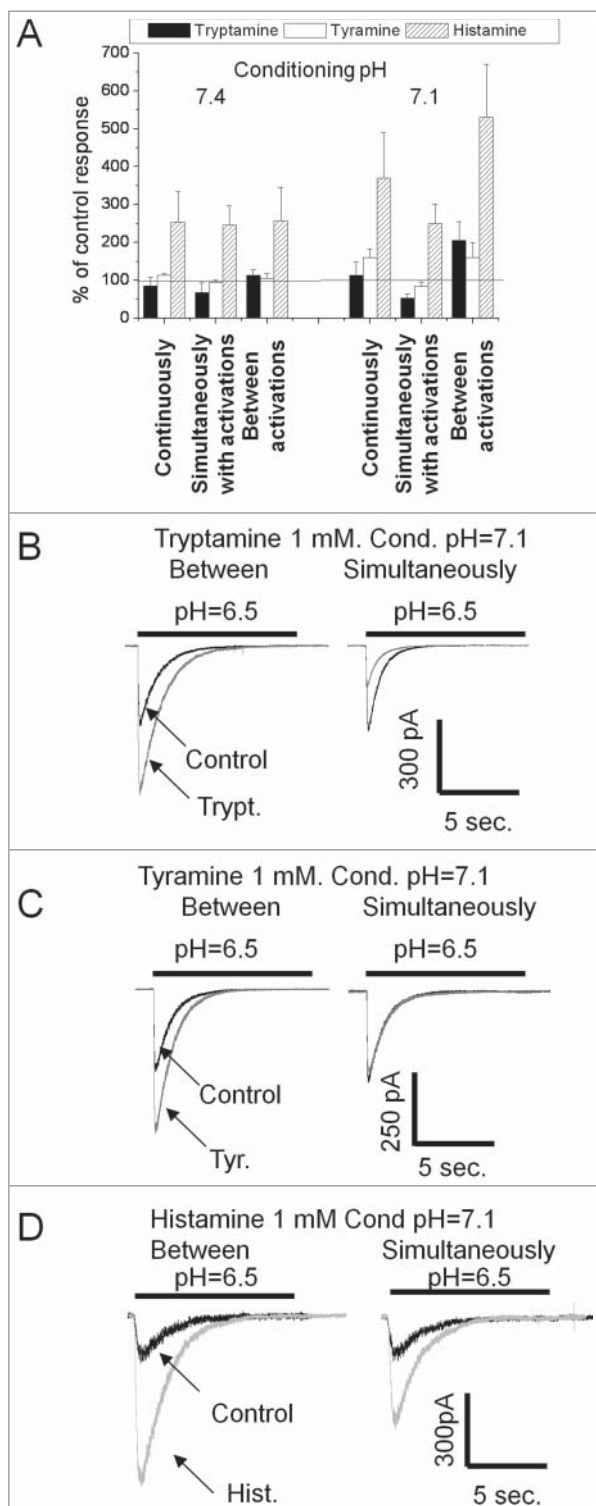
Action of endogenous amines on homomeric ASIC1a

Heteromeric nature of ASIC response in hippocampal interneurons requires analysis of drug action on homomeric recombinant ASICs to reveal subunit-specificity. ASIC1a homomers were activated by 10 sec pH drops from 7.4 to 6.5 at -80 mV holding voltage. The data are presented in Fig. 3.

Histamine caused strong potentiation in all three protocols (Fig. 3A). In contrast, 1 mM tyramine was virtually inactive ($n = 7$, paired t-test drug vs control, $P > 0.1$). When 1 mM tryptamine was applied simultaneously with activations, it caused $31 \pm 18\%$ ($n = 7$ paired t-test drug vs control, $P < 0.05$) inhibition. Surprisingly, application of 1 mM tryptamine between channel activations induced some potentiation of the response ($13 \pm 14\%$, $n = 7$). Although the effect was not statistically significant (paired t-test drug vs control, $P > 0.1$) the result suggested that tryptamine may cause different effects on ASIC1a depending on the application protocol.

If a drug applied between activations significantly interacts with the channels in the resting state, its action can depend on the initial pH value, and in particular on the steady-state desensitization. To test this possibility we performed experiments in which initial pH was reduced from 7.4 to 7.1 (Fig. 3A). Such modest acidification causes 50 – 70% desensitization of the ASIC1a (data not shown). Analysis of tryptamine action on responses evoked by pH drops from 7.1 to 6.5 (Fig. 3B) revealed a strong ($100 \pm 40\%$, $n = 5$) potentiating effect of the drug applied between activations. Fig. 3C shows that we were also able to reveal potentiation by tyramine applied between activations ($62 \pm 19\%$, $n = 5$) at conditions of partially inactivated channels. We also found a significant difference

of histamine action at different application protocols in these experiments. Application simultaneous with activation caused much smaller potentiation than application between activations (Fig. 3D). The continuous presence of the drug resulted in an intermediate effect on the ASIC1a in all cases.



It has been shown in our previous work²⁹ that the potentiating action of histamine strongly depends on the activating pH, and at saturating acidification potentiation becomes negligible. Therefore, we tested the dependence of tryptamine-induced potentiation on the activating pH. Because significant potentiation was observed only for the application between activations at conditioning pH = 7.1, we used this protocol to compare the effect tryptamine had on ASIC1a responses when activated by a pH drop to 6.5 and to 5.0. Direct comparison of the effects in paired experiments did not reveal statistical differences ($n = 6$, paired t-test, $P > 0.1$).

Next, we analyzed voltage- and concentration-dependencies of the potentiating and inhibitory action of tryptamine at conditioning pH = 7.1. Inhibition was studied for the drug application simultaneous with activation. The IC_{50} value for inhibitory action was estimated as 1.3 ± 0.2 mM ($n = 6$). Application of 3mM tryptamine (highest concentration used) between activations resulted in $410 \pm 110\%$ ($n = 5$) potentiation but did not demonstrate saturation, and it was impossible to estimate the parameters of the curve. Qualitatively similar data were obtained for tyramine, but the effects were much weaker in all cases (data not shown). Comparison of the effects at -40, -80 and -120 mV revealed a drastic difference between inhibition and potentiation of the ASIC1a by tryptamine. Inhibition, measured in the protocol of drug application simultaneous with

Figure 3. Action of endogenous amines on ASIC1a. A, summary of effects of tryptamine, tyramine and histamine (1 mM) in three application protocols at two conditioning pH values. Histamine strongly potentiates the response in all conditions. Tryptamine caused inhibition being applied simultaneously with activation. At conditioning pH = 7.1 application of tryptamine between activations potentiates the response. Action of tyramine is weak. Only at conditioning pH = 7.1 continuous application and application between activations caused significant potentiation. B, representative recordings demonstrate opposite effects of tryptamine applied simultaneously with activation (left) and between activations (right). Control responses (black) are superimposed with responses affected by 1 mM tryptamine (gray). C, representative recordings demonstrate that at conditioning pH = 7.1 tyramine causes potentiation (left), whereas application simultaneously with activation does not produce any effect (right). D, at conditioning pH = 7.1 histamine causes strong potentiation being applied between activation (left); application simultaneously with activation produces significantly smaller potentiation.

activation, demonstrated strong voltage dependence (Fig. 4A and B), whereas potentiation caused by application between activations did not demonstrate significant dependence on the holding voltage (Fig. 4C and D).

Although histamine demonstrated potentiating effect in all protocols, it remains possible that a

modest inhibition is masked by the strong potentiation. To test for this possibility we studied the voltage dependence of its action at conditioning pH = 7.1, and found that the potentiation by histamine applied simultaneously with activation is significantly more pronounced at 0 mV than at -140 mV (Fig. 4E and F). The finding of the voltage

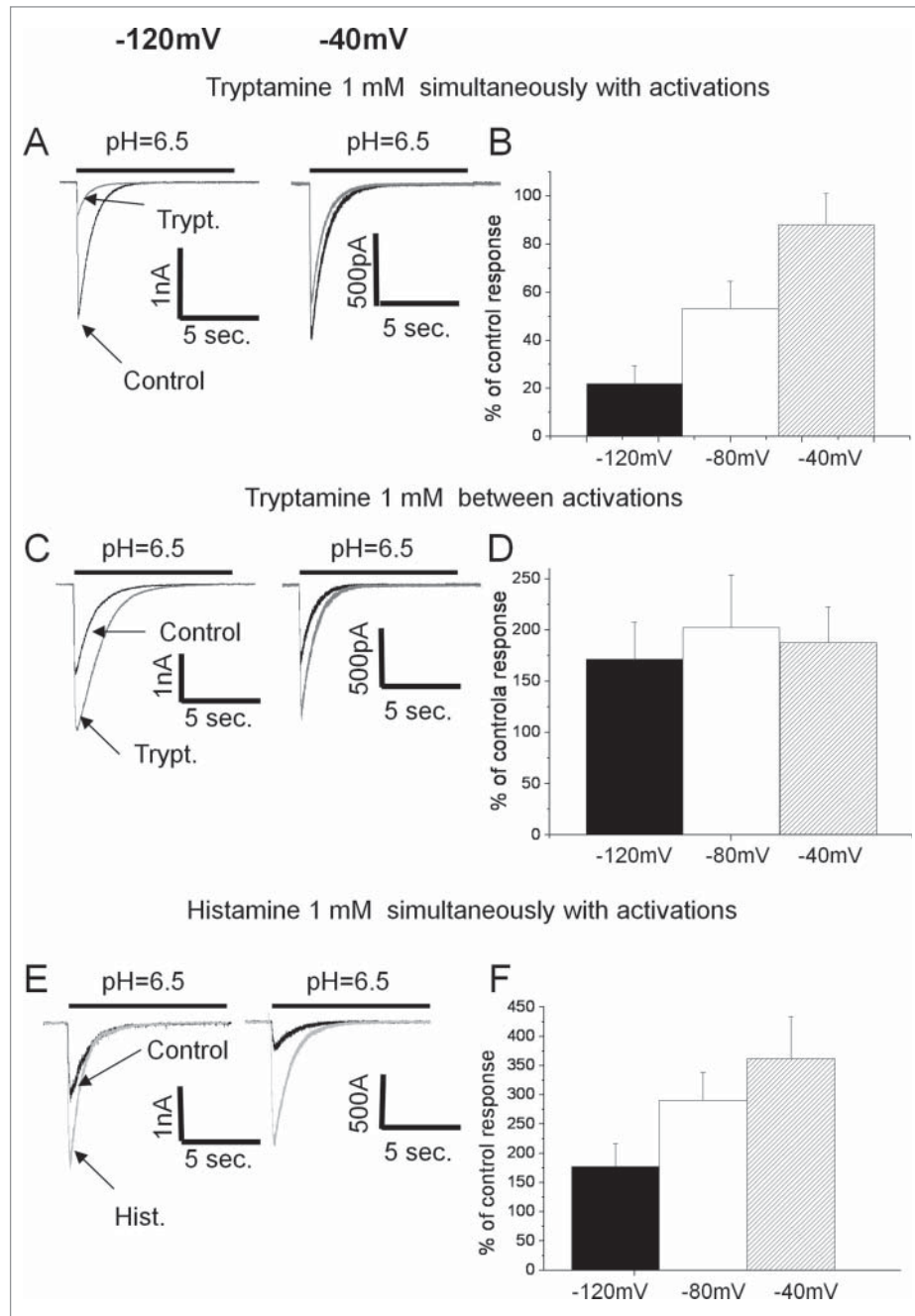


Figure 4. Voltage dependence of action on ASIC1a. A, C and E representative recordings of ASIC1a currents in control (black) superimposed with responses in the presence of endogenous amines (gray) at conditioning pH = 7.1. B, D and F summary data. A and B, inhibitory action of tryptamine applied simultaneously with activation increases with hyperpolarization. C and D, potentiating action of tryptamine applied between activations is voltage-independent. E and F, Potentiating action of histamine applied simultaneously with activation decreases with hyperpolarization suggesting that total effect includes voltage-dependent inhibitory component.

dependence of histamine effect agrees with the data demonstrating a difference between application protocols. Taken together, these results suggest that in addition to a strong potentiation effect described by us previously,²⁹ histamine can block open ASIC1a channels.

Action of endogenous amines on homomeric ASIC2a

Taking into account different sensitivity of ASIC1a and ASIC2a to protons, as well as different activation and desensitization kinetics, ASIC2a homomers were activated by 40sec pH drops from 7.4 to 5.0. Holding voltage was -80mV. To test the possible effect of the steady-state desensitization we also used conditioning pH = 6.5 (Fig. 5A). Tyramine and histamine in a 1 mM concentration were inactive (paired t-test, drug vs control, $P > 0.1$, $n = 5$ for all series) in all six series

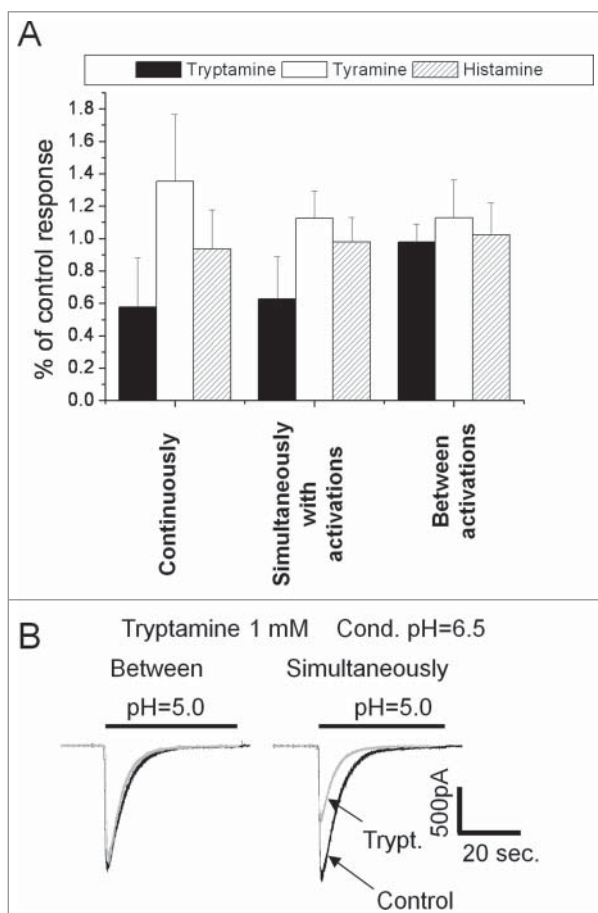


Figure 5. Action of endogenous amines on ASIC2a. A, summary of effects of tryptamine, tyramine and histamine (1 mM) in three application protocols at conditioning pH = 6.5. B, at conditioning pH = 6.5 1 mM tryptamine applied between activations does not produce an effect (left), whereas application simultaneously with activation causes inhibition (right).

of experiments. Tryptamine was ineffective in the protocol of application between activations independently of the value of conditioning pH (data not shown). However, statistically significant inhibition (Fig. 5B) was found in the protocol of application simultaneous with activation ($25 \pm 12\%$, $n = 12$, paired t-test drug vs control, $P < 0.05$). Continuous application also caused statistically significant inhibition ($22 \pm 18\%$, $n = 10$, paired t-test drug vs control, $P < 0.05$). The values of inhibition did not depend on conditioning pH ($n = 5$, paired t-tests, $P > 0.1$). Application of 3 mM of tryptamine caused a $64 \pm 22\%$, $n = 5$ inhibition in the protocol of application simultaneous with activation. The IC_{50} value calculated from the concentration dependence of tryptamine action was 1.5 ± 0.4 mM, $n = 6$. Continuous application produced the same value of inhibition (data not shown).

Thus, there was a drastic difference between the action of amines tested on ASIC1a and ASIC2a. No ASIC2a potentiation was detected even at the most favorable conditions, i.e. in the case of an application between activations at conditioning pH causing partial steady-state inactivation. Significant inhibition was found only for tryptamine, whereas histamine and tyramine were completely ineffective.

Discussion

In the present work, we demonstrated that ASICs are modulated not only by histamine but also by tyramine and tryptamine. The action of these monoamines is relatively weak and it is hardly possible that they significantly contribute to the physiological ASIC modulation. However, the fact that different endogenous amines are able to interact with ASICs should prompt further search for more potent native modulators. Another finding is that histamine, which was previously described as a selective potentiator of the ASIC1a homomers,²⁹ does not potentiate heterogenic ASIC response in the hippocampal interneurons. Moreover, in the absence of potentiation, we can reveal the inhibitory action of histamine.

Various factors could determine the differences of drug action in native and recombinant systems. In the case of neurons, we cannot rule out the possibility of involvement of the histamine receptors (HR1-HR4). However, the obvious similarity of action of all three endogenous amines is evidence against such possibility. Other cell-specific modulation mechanisms,

which can exist in neurons but not in CHO cells, may contribute to the observed differences. However, the most plausible explanation is heteromeric subunit composition of native ASICs in the hippocampal interneurons. Different ASIC subunits readily form functional heteromeric channels and characteristics of heteromeric and homomeric channels are markedly different.³³ Single-cell RT-PCR analysis reveals coexpression of the ASIC1a and the ASIC2 subunit transcripts in hippocampal CA1 interneurons from the oriens lacunosum-moleculare layer.³¹ Notably, the same study demonstrated that tarantula venom psalmitoxin1, a specific blocker for ASIC1a homomers, did not inhibit ASIC currents in these neurons. Therefore, we suggest that the lack of potentiating effect of histamine on hippocampal interneurons is due to the presence of an ASIC2 subunit, which, according to our data, forms histamine-insensitive channels. Inhibitory effects are seen in both the ASIC1a and native ASICs. Thus, the presence of ASIC2a, which is almost insensitive to the amines tested, seems to destroy potentiation but not inhibition. As a result, the inhibitory action of histamine, masked by a strong potentiating effect on the ASIC1a homomers, becomes pronounced in experiments with native ASICs. Systematic testing of this suggestion would require a comparison of action of a representative series of compounds on both heteromeric recombinant and native ASICs from different brain structures.

Comparison of different application protocols allowed us to reveal important determinants of the mechanism of action of endogenous amines on native and recombinant ASICs. The total effect, which is observed in the continuous presence of a drug in solution, is a sum of two independent types of action. If the drugs are applied simultaneously with activation they interact mainly with the open channels. In this case, we observe voltage-dependent channel block. If the drugs are applied only between activations and interact with resting or desensitized channels, they can cause voltage-independent potentiation. The existence of two binding sites and two independent mechanisms of ASIC modulation by amine-containing compounds was proposed by us previously to explain complex structure-activity relationships.²⁸ In the present work, we separated these mechanisms experimentally.

Dualistic ligand action on ASICs is also known for amiloride, which is usually considered to be an ASIC

inhibitor but can induce a potentiating effect.^{34,35}

Although the sites of action are still not exactly known, the X-ray data³⁶ shows that amiloride can bind in the channel pore and cause blocking effect. Another putative site of amiloride is near the “acidic pocket”. Binding to this site can be responsible for the potentiating effect³⁶ since a positively charged group of the amiloride molecule can directly interact with the negatively charged groups in the acidic pocket. Thus, the charged group of an organic ligand can substitute one of the free protons required for activation. Another X-ray structure demonstrates that psalmitoxin also binds in the vicinity of the acidic pocket and that the positively charged groups of this peptide do interact with the negatively charged groups in this site.³⁷ Thus, it appears that the specific pattern of drug interactions with components of the acidic pocket can determine the mode of drug action on ASIC activation and desensitization properties. We have previously shown that synthetic monoamines IEM-1921 and IEM-2117 can potentiate currents both through the native hippocampal ASICs and the homomeric ASIC2a channels.²⁶ The most plausible structural explanation of the difference in the action of IEM-1921 and IEM-2117 and the compounds studied in this work lies in the different distance between the hydrophobic moiety and the amino group. In contrast to IEM-1921 and IEM-2117, in tryptamine, tyramine, and histamine the amino group is connected to the hydrophobic moiety with two methylene spacers. This difference can determine a different pattern of interactions with the receptor. For instance, the amino groups of short (IEM-1921 and IEM-2117) and long (histamine and IEM-2044) amines can interact with different acidic residues in the acidic pocket. It should be noted, however, that there are structural³⁶ and mutational³⁸ data suggesting an involvement of the other sites in ASIC activation and the binding of modulating drugs to these sites. A mutational approach is required to uncover the sites and the structural determinants of action of synthetic and endogenous amines.

Despite the fact that solid conclusion about the binding site requires mutagenesis studies, some suggestions can be made based on the available data. Sequences of rat ASICs differ in the region of acidic pocket. Particularly, ASIC1a contains His residue in position 173, whereas ASIC1b, ASIC2a and ASIC3 contain Ser, Lys and Arg in this position,

correspondingly. It seems likely that ASIC1a-specific ligands interact with the ASIC1a-specific residues. Modeling calculations were performed for the rat ASIC1a using the ZMM program package.³⁹ We found that aromatic moiety of histamine and related amines can bind to histidine in position 173 via the stacking mechanism. Homologous phenylalanine (Phe174 in chicken ASIC1a) strongly interacts with psalmotoxin and amiloride in available X-ray structures (Fig. 6A and B). From this position the amino group connected to the aromatic moiety by two spacers can reach several acidic residues (Fig. 6C and D) including ASIC1a-specific Asp349 (ASIC2a contains Gly residue in this position). Thus, available data on amino acid sequences and spatial structure support the hypothesis that amine-containing ASIC ligands can bind at the acidic pocket region.

We have demonstrated in our previous studies that synthetic amines are a new class of ASIC ligands, which produce various effects depending on the

chemical structure and the subunit composition of the target. Here we found that different endogenous amines also target these channels. Assuming a large diversity of endogenous amines we can suspect that some members of this class can control ASIC activity in physiologically relevant concentrations. It is also important that a number of medically used drugs belong to the same class of amine-containing compounds. Many antidepressants and antihistamine drugs, in particular, should be considered as putative ASIC ligands.

Materials and methods

Native receptors

Experiments were conducted in agreement with the Rules of Animal Care and Use Committee of the Sechenov Institute of Evolutionary Physiology and Biochemistry (IEPHB) of the Russian Academy of Sciences, which is fully compatible with European

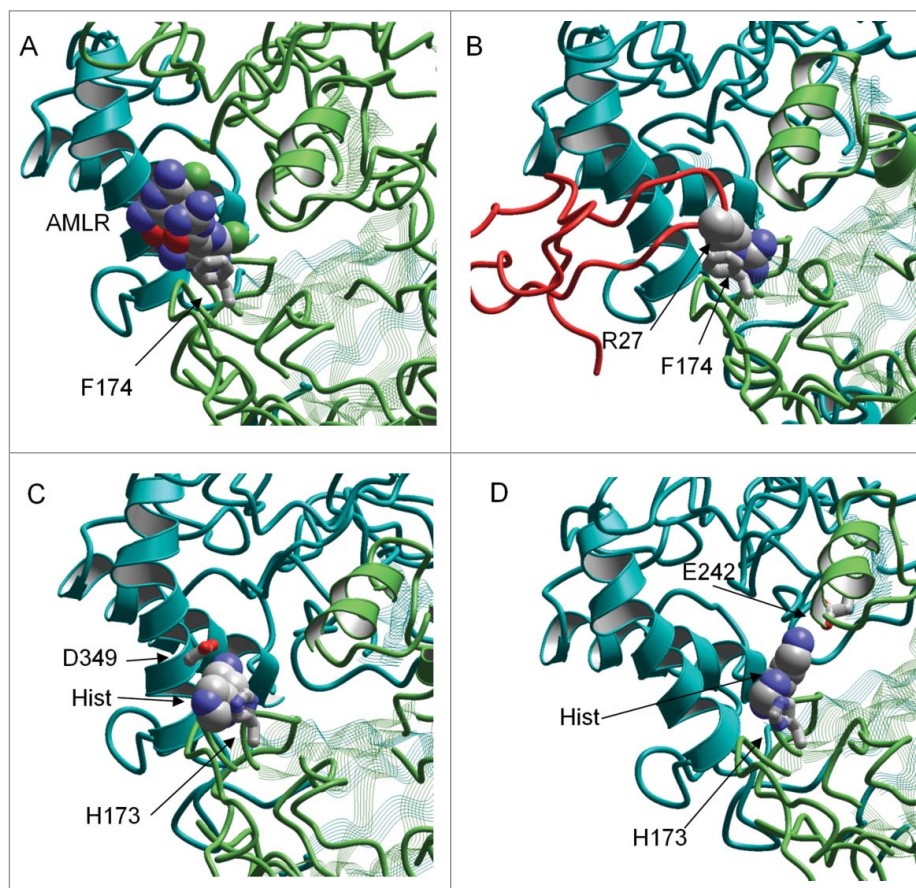


Figure 6. Possible binding of amines at the acidic pocket of ASIC1a. A and B, X-ray structures show interactions of Phe174 with psalmotoxin (A, PDB code 4fz0) and amiloride (B, PDB code 4ntx). C and D, putative binding modes of histamine. Aromatic moiety binds to ASIC1a-specific His173, whereas aminogroup can reach Asp349 (C) or Glu242 (D).

Community Council directives 86/609/EEC. Outbred male Wistar rats 12–17 days old and 25–35g were obtained from a local (IEPHB) animal facility. Rats were anesthetized with urethane and then decapitated. Maximum efforts were made to minimize the number of animals used and to minimize discomfort.

Brains were removed quickly and cooled to 2–4 °C in an ice bath. Transverse hippocampal slices (250 μm thick) were prepared using a vibratome (Campden Instruments Ltd., Loughborough, UK) and stored in a solution containing (in mM): NaCl 124, KCl 5, CaCl_2 1.3, MgCl_2 2.0, NaHCO_3 26, NaH_2PO_4 1.24 and D-glucose 10, aerated with carbogen (95% O_2 , 5% CO_2). Typically, 6–7 slices were obtained from 1 rat brain.

The neurons were isolated from slices with vibrodissociation.⁴⁰ The method allows a cell to be isolated from a local part of the slice under visual control using an inverted microscope. The experiments were carried out on hippocampal interneurons isolated from the lacunosum moleculare and radiatum layers of the CA1 region. Interneurons were identified by morphological and electrical characteristics. The neurons were discriminated from glial cells by the appearance of action potentials in response to depolarizing pulses under the current clamp. Pyramidal neurons usually have pyramidal-like somata and preserved apical and basal (in some neurons) dendrites, the interneurons varied in size and shape; however, most were round to oval. Extracellular acidification at -80mV holding voltage caused larger (up to 2–3 nA) response in interneurons, whereas response of pyramidal neurons was about 0.2 nA only.

The whole-cell patch clamp technique was used to record the membrane currents generated in response to fast acidification. Series resistance of about 20 M Ω was compensated by 70–80% and monitored during the experiments. Currents were recorded using an EPC-8 amplifier (HEKA Electronics, Lambrecht, Germany), filtered at 5 kHz, sampled and stored on a personal computer. Drugs were applied using the RSC-200 perfusion system (BioLogic Science Instruments, Claix, France) under computer control. The extracellular solution contained the following (in mM): NaCl 143, KCl 5, MgCl_2 2.0, CaCl_2 2.5, D-glucose 18, HEPES 10 and MES 10 (pH was adjusted to 7.35 with HCl). The pipette solution contained the following (in mM): CsF 100, CsCl 40, NaCl

5, CaCl_2 0.5, EGTA 5 and HEPES 10 (pH was adjusted to 7.35 with CsOH). All experiments were performed at room temperature (22–24 °C).

Recombinant receptors

CHO cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 . Cells were maintained with standard culture conditions (Dulbecco's modified Eagle's medium DMEM + 10% fetal bovine serum + 5% streptomycin/penicillin). Plasmids encoding ASIC subunits were transfected using Lipofectamine 2000 (Invitrogen, USA) following the manufacturer's protocol. Expression vectors encoding rat ASIC1a and ASIC2a were a kind gift from Dr. A. Staruschenko. For the expression of the homomeric channels, cells were transfected with 0.5 μg rASIC1a or rASIC2a cDNA per 35 mm^2 dish together with 0.5 μg eGFP. Electrophysiological experiments were performed 48–72 h after transfection. Transfected cells were identified with green fluorescence using the Leica DMIL microscope.

Whole-cell currents were recorded in the voltage-clamp mode. The pipette solution contained the following (in mM): CsF 100, CsCl 40, NaCl 5, CaCl_2 0.5, EGTA 5 and HEPES 10 (pH was adjusted to 7.35 with CsOH). Cells were continuously superfused with an extracellular solution containing the following (in mM): NaCl 150, CaCl_2 1, MgCl_2 2, HEPES 10 and MES 10, adjusted to pH 7.35. Current recordings were acquired with an EPC10-USB (HEKA Elektronik, Lambrecht, Germany) patch clamp amplifier connected to a computer running Patchmaster software (HEKA Elektronik, Lambrecht, Germany). Only recordings where access resistance and capacitance changed <10% over the course of the experiment were used.

Data analysis and statistics

All values are presented as mean \pm standard deviation (SD) from at least five experiments. Significance of the effects was tested with paired t-test with $p = 0.05$ (drug versus control).

Disclosure of potential conflicts of interest

The authors declare that they have no conflict of interest.

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