



## CHARACTERIZATION OF ACID-SENSITIVE ION CHANNELS IN FRESHLY ISOLATED RAT BRAIN NEURONS

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**Abstract**—Transient proton-activated currents induced by rapid shifts of the extracellular pH from 7.4 to  $\leq 6.8$  were recorded in different neurons freshly isolated from rat brain (hypoglossal motoneurons, cerebellar Purkinje cells, striatal giant cholinergic interneurons, hippocampal interneurons, CA1 pyramidal neurons and cortical pyramidal neurons) using whole-cell patch clamp technique. Responses of hippocampal CA1 pyramidal neurons were weak (100–300 pA) in contrast to other types of neurons (1–3 nA). Sensitivity of neurons to rapid acidification varied from  $pH_{50}$  6.4 in hypoglossal motoneurons to 4.9 in hippocampal interneurons. Proton-activated currents were blocked by amiloride ( $IC_{50}$  varied from 3.6 to 9.5  $\mu M$ ). Reversal potential of the currents was close to  $E_{Na}$ , indicating that the currents are carried by sodium ions. The data obtained suggest that the proton-activated currents in the neurons studied are mediated by acid-sensitive ion channels. Strong acidification ( $pH < 4$ ) induced biphasic responses in all neuron types: the transient current was followed by a pronounced sustained one. Sustained current was not blocked by amiloride and exhibited low selectivity for sodium and cesium ions. Slow acidification from pH 7.4 to 6.5 did not induce detectable whole-cell currents. At pH 6.5, most of the channels are desensitized and responses to fast pH shifts from this initial level are decreased at least 10 times. This suggests that slow acidification which is well known to accompany some pathological states should rather desensitize than activate acid-sensitive ion channels and depress their function.

Our results provide evidence for a widespread and neuron-specific distribution of acid-sensitive ion channels in the brain. The large amplitudes and transient character of currents mediated by these channels suggest that they could contribute to fast neuronal signaling processes. © 2002 IBRO. Published by Elsevier Science Ltd. All rights reserved.

**Key words:** acid-sensitive ion channel, amiloride, desensitization, pH shifts, proton-activated currents.

Proton-induced cationic currents were found in neurons two decades ago (Krishtal and Pidoplichko, 1980). It was supposed that the currents are mediated by proton-gated ion channels. Later the detailed analysis of their biophysical and functional properties has been done, but the absence of specific antagonists of these channels and their sensitivity to inhibitory action of some typical blockers of  $Ca^{2+}$  channels led to the conclusion that protons may transiently transform  $Ca^{2+}$  channels to proton-gated ones (Konnerth et al., 1987; Davies et al., 1988). The cloning of several subunits forming acid-sensitive ion channels (ASICs) demonstrated clearly that they represent a separate family of  $Na^+$  selective channels (Waldmann et al., 1997a,b; Garcia-Anoveros et al., 1997). To date six members of the ASIC family are known, ASIC1a (BNAc2) and its splice variant ASIC1b, ASIC2a (BNAc1, MDEG1) and splice variant

ASIC2b (MDEG2), ASIC3 (DRASIC) and ASIC4 (SPASIC) (Akopian et al., 2000; Reeh and Kress, 2001). They can form homo- and heteromeric channels. All ASICs are selectively permeable for sodium ions and can be blocked by micromolar concentrations of amiloride. However, the different subunit combinations produce channels with various pH sensitivity, kinetics and permeation properties (McCleskey and Gold, 1999; Reeh and Kress, 2001). Activation of the channels induces transient currents with the exception of ASIC3-containing channels that generate biphasic currents: the transient component is followed by a pronounced sustained one. The distribution of different ASIC subunits in the nervous system is non-uniform. Messenger RNA encoding ASIC1b and ASIC3 subunits were found predominantly in sensory neurons (Chen et al., 1998). In contrast, ASIC2a is abundant in brain but absent in sensory neurons. Other subunits show a widespread distribution pattern (Waldmann and Lazdunski, 1998).

Proton-gated ion channels are abundant in the small primary sensory neurons of dorsal root ganglia and trigeminal ganglia, which participate in pain sensation (Akaike et al., 1990). Therefore, proton-gated channels are thought to take part in the nociception that is accompanied by pH drop (Reeh and Kress, 2001). ASIC3 channels mediate significant sustained currents that may be

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Abbreviations: ASIC, acid-sensitive ion channel; EGTA, ethyleneglycol-bis(2-aminoethyl-ether)- $N,N,N',N'$ -tetraacetic acid; HEPES,  $N$ -(2-hydroxyethyl)piperazine- $N'$ -(2-ethanesulfonic acid); P, postnatal day.

important for the prolonged sensation of pain caused by acids. The specific localization of ASIC3 subunits in sensory neurons supports this hypothesis.

Proton-gated channels were found also in several brain structures, which are not related to nociception. It has been shown that acid-sensitive channels are the earliest  $\text{Na}^+$  channels expressed in undifferentiated neuron precursor and their participation in the neuronal development was discussed (Grantyn et al., 1989). Brain proton-gated channels deeply desensitize within several seconds and can hardly participate in processes which are accompanied by slow and prolonged acidification such as ischemia, inflammation etc., since these channels can be activated only by rapid pH drops. In particular, this might take place during release of synaptic vesicles that have acidic content (Krishtal et al., 1987). But the hypothesis about direct participation of ASICs in synaptic transmission is not confirmed yet by experiments.

Thus, the physiological role of proton-gated ion channels in brain is still not clear. The knowledge of both distribution of ASICs in brain and their properties may help to solve this problem. To date, proton-gated ion channels were found in neurons of the hypothalamic ventromedial nucleus (Ueno et al., 1992), cerebellar granule neurons (Escoubas et al., 2000), tectal neurons (Boonnstra et al., 1983; Grantyn and Lux, 1988) and neocortical pyramidal neurons (Varming, 1999). Unfortunately, proton-gated channels in brain neurons were not studied systematically and the data are still incomplete. The aim of the present work was to carry out a detailed study of proton-activated currents in brain neurons with different functions and localization; hypoglossal motoneurons, Purkinje cells, striatal giant cholinergic interneurons, hippocampal pyramidal neurons and interneurons and cortical pyramidal neurons.

#### EXPERIMENTAL PROCEDURES

Wistar rats (aged 3–18 days, Animal Resources Unit, Sechenov Institute of Evolutionary Physiology, St. Petersburg, Russia) of both sexes were decapitated under urethane anesthesia following a procedure in accordance with the European Communities Council Directive (24th November 1986; 86/609/EEC). All efforts were made to minimize the number of animals used and their suffering. We used postnatal day P3–P6 animals for brain stem slices preparation, and P12–P18 animals for other preparations. The brains were removed rapidly and immediately cooled at 2–4°C in an ice bath. Transverse brain slices (200–300  $\mu\text{m}$  thick) were cut using a Vibroslice-752M (Campden Instruments, Leicester, UK) and stored in a solution containing (in mM): NaCl 124, KCl 5,  $\text{CaCl}_2$  1.3,  $\text{MgCl}_2$  2.0,  $\text{NaHCO}_3$  26,  $\text{NaH}_2\text{PO}_4$  1.24, D-glucose 10, bubbled with 95%  $\text{O}_2$ /5%  $\text{CO}_2$  (pH 7.4–7.5) at 24–26°C. Neurons were isolated from slices by vibrodissociation (Vorobjev, 1991). The experiments were done in hypoglossal motoneurons, Purkinje cells, hippocampal pyramidal neurons and interneurons (isolated from CA1 and CA3 regions), striatal giant cholinergic interneurons and cortical pyramidal neurons. Neurons were identified mainly by their morphology (for details see Samoilova et al., 1999; Fukunishi et al., 1999). In cerebellum, an additional criterium to distinguish between Purkinje cells and Golgi cells was the absence of any responses to 30  $\mu\text{M}$  *N*-methyl-D-aspartate in Purkinje cells. The whole-cell configuration of patch clamp recording techniques was used. The currents were induced by rapid solution exchange

from control pH (7.4) to different values, amplified by an Axopatch 200A (Axon Instruments, Union City, CA, USA), filtered at 5 kHz, sampled and stored on a personal computer for 'on-line' and 'off-line' analysis. The holding potential was  $-80$  mV unless otherwise stated. In most experiments, the interval between applications was 45 s (time of full recovery from desensitization). The extracellular solution contained (in mM): NaCl 143, KCl 5,  $\text{CaCl}_2$  2.5, D-glucose 10, HEPES 10 (pH was adjusted with HCl before each experiment). The pipette solution contained (in mM): CsF 100, CsCl 40, NaCl 5,  $\text{CaCl}_2$  0.5, EGTA 5, HEPES 10 (pH was adjusted to 7.2 with CsOH). Drugs were applied using a fast perfusion technique (Vorobjev et al., 1996). The exposures (5–20 s) were done under computer control.

Concentration dependence of the effects was analyzed using classical equation:

$$I = I_{\max} * (1 / (1 + (\text{EC}_{50} / [C])^s)), \quad (1)$$

where  $I_{\max}$  is current in the presence of a saturating concentration of ligand,  $I$  is current in the presence of ligand concentration  $[C]$ ,  $\text{EC}_{50}$  (or  $\text{IC}_{50}$  in the case of blockade) is the concentration which produces a half maximal effect and  $s$  is the Hill coefficient. In the text,  $\text{EC}_{50}$  values for current activation are expressed in pH units ( $\text{pH}_{50}$ ).

Data were fitted for each neuron separately and then the mean values  $\pm$  S.E.M. for  $n$  neurons were estimated. Significance of the effects was tested by one-way analysis of variance with  $P = 0.05$ . Amiloride was purchased from Sigma (St. Louis, MO, USA).

#### RESULTS

Proton-activated currents were studied in neurons isolated from different brain structures: brain stem, cerebellum, striatum, hippocampus and neocortex. Fast shifts of extracellular pH from 7.4 to  $4 < \text{pH} \leq 6.8$  (mentioned below as pH drops) induced inward currents that decayed to undetectable levels within a few seconds in all investigated types of neurons (Fig. 1). It is noteworthy that proton-activated currents in CA1 hippocampal pyramidal neurons had 10 times smaller amplitudes than in all other types of neurons, including hippocampal interneurons. Pyramidal neurons from the CA3 region (five cells tested) exhibited approximately two times larger responses to pH drops than CA1 pyramidal neurons. The nature of proton-activated currents found was analyzed by the comparison of their properties with characteristic properties of ASICs.

##### *Current activation and desensitization*

In most of the neurons proton-activated currents appeared at the pH drop to 6.8. These currents demonstrated dependence on the proton concentration applied. Mean  $\text{pH}_{50}$  varied from 6.4 for hypoglossal motoneurons to 4.9 for hippocampal interneurons (Table 1). The difference between high sensitivity neurons (hypoglossal motoneurons and Purkinje cells) and low sensitivity neurons (striatal and hippocampal interneurons, cortical pyramidal neurons) was significant. Representative proton-activated currents in high and low sensitivity neurons are shown in Fig. 2. Typically, decay of the proton-activated current was well fitted by a single-exponential function. In some neurons, however, double-exponential approximation provided a better fitting. To make quantitative comparison in the latter case the weighted decay

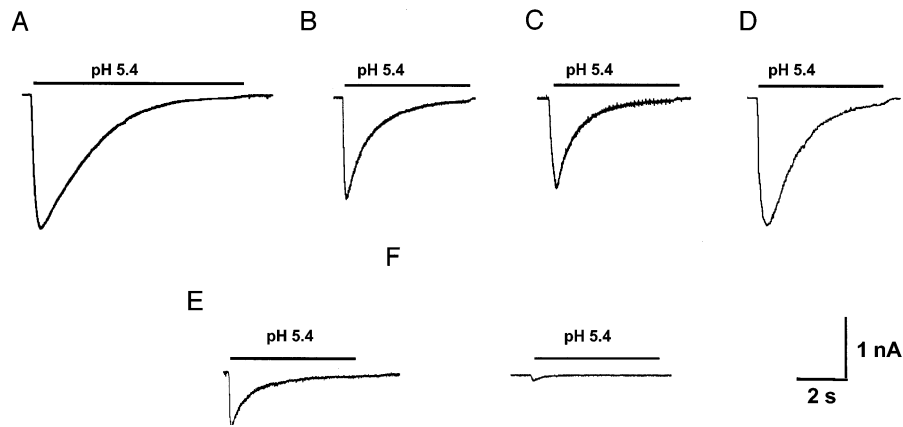


Fig. 1. Representative currents evoked by pH drops to 5.4 in different types of neurons. (A) Hypoglossal motoneuron, (B) Purkinje cell, (C) cortical pyramidal cell, (D) giant aspiny interneuron of striatum, (E) CA1 interneuron, (F) CA1 pyramidal cell. The response of the hippocampal pyramidal neuron is low in contrast to the response of the hippocampal interneuron.

time constant was calculated. At low proton concentrations, decay of the currents was very slow due to competition between asynchronous activation and desensitization (Fig. 2A). Application of higher proton concentrations ( $\text{pH} < \text{pH}_{50}$ ) induced fast activation and the decay time constant became independent of pH. The mean values of the decay time constant shown in Table 1 were measured in this pH range. According to our data, proton-activated currents in hypoglossal motoneurons possess the slowest decay kinetics (see Table 1 and Fig. 1). Other neuron types did not significantly differ in this property.

To characterize the recovery from desensitization, the protocol of double pulse stimulation was used. This protocol included two successive 5-s pH drops to 4.3 separated by a time interval varying from 0.5 to 30 s. Recovery from desensitization was measured as the ratio of the second and first response amplitudes. Full recovery of currents occurred at time intervals greater than 20 s. The example of such an experiment is shown at Fig. 3. Mean values of recovery time constants did not differ in the types of neurons studied (Table 1). However, some hypoglossal motoneurons and Purkinje cells exhibited very large (10–15 s) time constants of recovery from desensitization. Such slow kinetics were not observed in other types of neurons.

#### *Ion selectivity and pharmacological properties*

Selective permeability for sodium ions and sensitivity to the blocking action of amiloride are intrinsic properties of ASICs. Current–voltage relationships of proton-activated currents were obtained for all types of neurons studied. The representative currents (striatal interneuron) are shown in Fig. 4. The current remained inward at potentials up to +60 mV that suggests the reversal at voltages close to  $E_{\text{Na}}$ . For other types of neurons, current–voltage relationships were similar. Consequently, proton-activated currents were mediated mainly by sodium ions. Cesium ions (the internal ion in our experiments) were much less permeant.

Proton-activated currents were effectively blocked by amiloride (Fig. 5).  $\text{IC}_{50}$  values calculated from concentration dependence of block ranged from 3.6  $\mu\text{M}$  in Purkinje cells to 9.5  $\mu\text{M}$  in hypoglossal motoneurons. The currents were also inhibited by an increase of external  $\text{Ca}^{2+}$  ions from 2.5 mM to 10 mM. This increase induced a 34–57% fall of currents elicited by pH drops to 4.3 (Table 1).

The quantitative analysis of proton-activated currents in hippocampal pyramidal neurons was difficult because of their small amplitudes, but the main characteristics (transient character, range of  $\text{pH}_{50}$  values, sodium selec-

Table 1. Characteristics of proton-activated currents in rat brain neurons

| Type of neuron             | Maximal amplitude (nA) | $\text{pH}_{50}$ ( $\text{EC}_{50}$ , $\mu\text{M}$ ) | Hill coefficient   | Time constant desensitization (s) | Time constant recovery (s) | $\text{IC}_{50}$ , amiloride ( $\mu\text{M}$ ) | $I_{\text{Ca10}}/I_{\text{Ca2.5}}$ |
|----------------------------|------------------------|-------------------------------------------------------|--------------------|-----------------------------------|----------------------------|------------------------------------------------|------------------------------------|
| Hypoglossal motoneurons    | $1.5 \pm 0.6$ (8)      | $6.4$ ( $0.40 \pm 0.11$ ) (8)                         | $2.1 \pm 0.3$ (8)  | $1.3 \pm 0.2$ (8)                 | $2.5 \pm 0.5$ (9)          | $9 \pm 3$ (5)                                  | $0.7 \pm 0.1$ (3)                  |
| Purkinje cells             | $3.2 \pm 0.5$ (8)      | $6.3$ ( $0.5 \pm 0.2$ ) (8)                           | $1.7 \pm 0.4$ (8)  | $0.6 \pm 0.3$ (8)                 | $2.9 \pm 0.7$ (8)          | $3.6 \pm 1.2$ (7)                              | $0.4 \pm 0.1$ (3)                  |
| Cortical pyramidal neurons | $1.6 \pm 0.3$ (13)     | $5.5$ ( $3.2 \pm 1.2$ ) (13)                          | $1.0 \pm 0.2$ (13) | $0.7 \pm 0.3$ (10)                | $1.5 \pm 0.2$ (12)         | $3.7 \pm 1.1$ (10)                             | 0.51, 0.50                         |
| Striatal interneurons      | $2.9 \pm 0.8$ (4)      | $5.4$ ( $4.0 \pm 1.6$ ) (4)                           | $1.3 \pm 0.6$ (4)  | $0.6 \pm 0.4$ (7)                 | $1.2 \pm 0.2$ (4)          | $5.0 \pm 0.1$ (6)                              | 0.63, 0.60                         |
| Hippocampal interneurons   | $1.1 \pm 0.4$ (5)      | $4.9$ ( $13 \pm 3$ ) (5)                              | $1.2 \pm 0.3$ (5)  | $0.9 \pm 0.1$ (5)                 | $1.9 \pm 1.0$ (5)          | $6 \pm 2$ (6)                                  | 0.44, 0.34                         |
| CA1 pyramidal neurons      | $0.2 \pm 0.1$ (15)     | from 5 to 6.5 (3)                                     | nd                 | nd                                | nd                         | < 10                                           | $\sim 0.5$                         |

$I_{\text{Ca10}}/I_{\text{Ca2.5}}$  is the ratio of peak amplitudes in solutions containing 10 and 2.5 mM  $\text{Ca}^{2+}$ , respectively. Number of neurons tested is indicated in parentheses. nd, not detected.

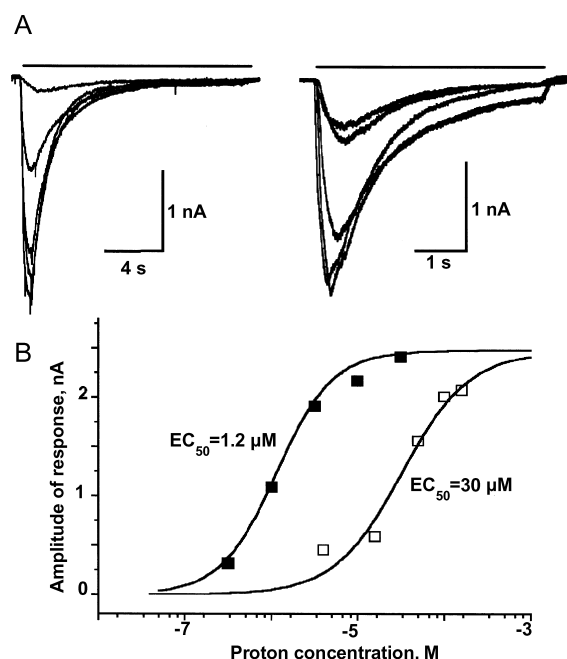


Fig. 2. Sensitivity of hypoglossal motoneuron and hippocampal interneuron to pH drops. (A) Representative responses of hypoglossal motoneuron (left) and hippocampal interneuron (right). (B) Concentration dependence of peak amplitude for the same hypoglossal motoneuron (filled squares) and hippocampal interneuron (open squares) fitted by Eq. 1.

tivity, sensitivity to the inhibitory actions of amiloride and  $Ca^{2+}$ ) were similar to those of other types of neurons.

#### *Sustained component*

Strong acidification (to pH 3.5) produced biphasic currents in all types of neurons: a fast peak component was followed by a slow sustained one. This slow component did not reach the steady state during prolonged applications (up to 20 s). It should be noted that long treatment by very low pH ( $< 4$ ) usually led to progressive decrease of peak response, increase of leak current and finally to cell death. Typically neurons survived two to four 20-s applications of saline with pH 3.5. This made it difficult to quantitatively study the sustained component.

Examples of biphasic currents in cortical pyramidal neurons and hypoglossal motoneurons are shown in Fig. 6. The amplitude of the sustained component was two to four times smaller than that of the peak. Strikingly, the similar ratio of amplitudes of peak and sustained components was observed in hippocampal pyramidal neurons in which proton-activated currents were about 10 times smaller as compared with other types of neurons. The sustained component was not blocked by a high amiloride concentration ( $100 \mu M$ ), while the peak component was completely blocked by this concentration (Fig. 6A). At a holding potential of  $+30$  mV, the sustained component became outward while the peak component was still inward (Fig. 6B). Thus, the basic properties of peak and sustained components were markedly different.

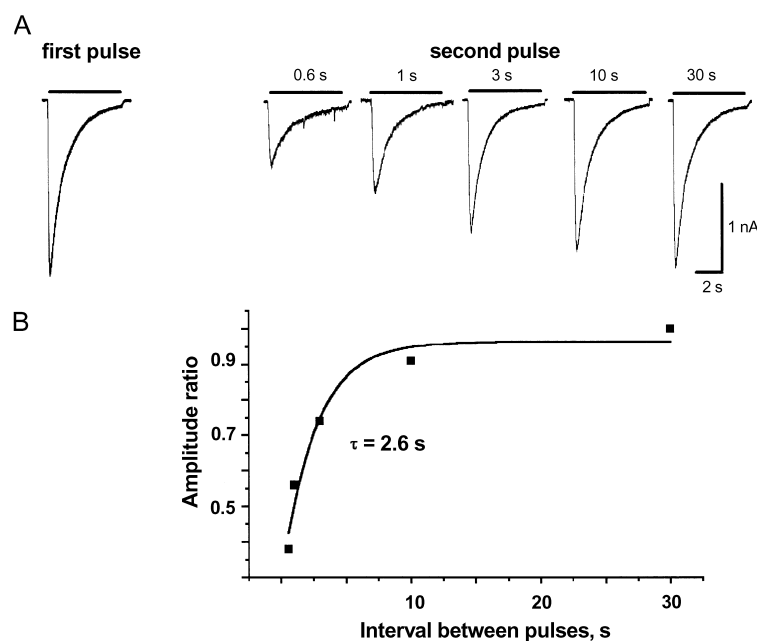


Fig. 3. Recovery from desensitization of proton-activated currents. (A) Representative currents evoked by the double-pulse protocol in Purkinje cells. Successive pH drops to 4.3 are separated by a time interval varied from 0.6 to 30 s (shown above bars). The first response is shown for one pair only. (B) The ratio of amplitudes of second and first responses (from A) is plotted against the time interval. The data were fitted by a single-exponential function.

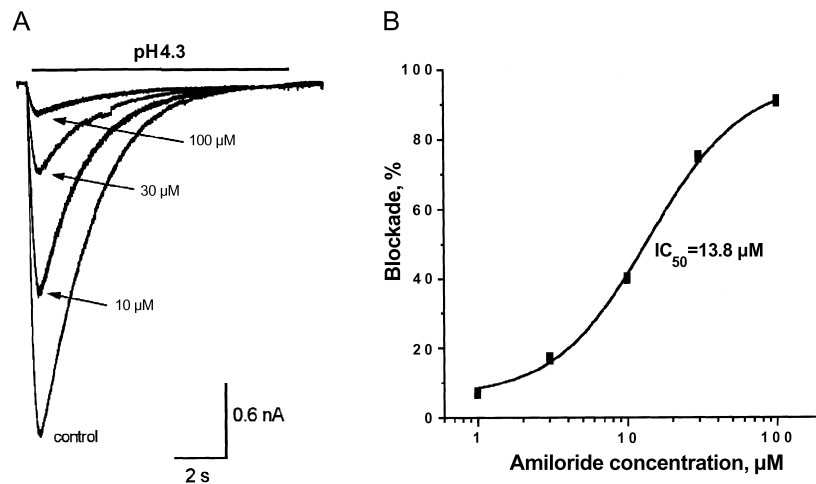


Fig. 4. Blockade of proton-activated currents by amiloride. (A) Representative responses of a hypoglossal motoneuron evoked by pH drops to 4.3 in the absence and presence of different amiloride concentrations. (B) Concentration dependence of amiloride blockade for the same cell fitted by Eq. 1.

#### Effect of slow acidification

The role of proton-gated ion channels in pathological states such as ischemia, inflammation and epilepsy that may be accompanied by significant (about one pH unit) extracellular acidification is widely discussed (Tombaugh and Sapolsky, 1993; Siesjö et al., 1993; Sutherland et al., 2000). To simulate such effects, slow exchange (during 30–50 s) of pH from 7.4 to 6.5 was used. Slow acidification did not induce any detectable currents. This effect can be explained by the kinetics of receptor activation and desensitization. In contrast to pH drop, slow acidification induces asynchronous activation and successive desensitization of proton-gated channels, and no macroscopic currents could be detected. Lowering of the basic pH level to 6.5 led to an about 10 times decrease of response to pH drops (Fig. 7). Since most of proton-gated channels are desensitized at pH 6.5, further acidification can activate only a small fraction of channels, which remain in the resting state. Thus, during ischemia,

inflammation and epilepsy proton-activated channels should be rather depressed than activated.

#### DISCUSSION

The characteristics of proton-activated currents in rat brain neurons match properties of recombinant ASICs. Proton sensitivity varied from  $pH_{50}$  4.9 in hippocampal interneurons to 6.4 in hypoglossal motoneurons. The range of proton sensitivity of homomeric ASICs is similar, ASIC2a and ASIC1a channels have  $pH_{50}$  values 4.3 and 6.4, respectively (Escoubas et al., 2000; Champigny et al., 1998). Transient responses without significant steady-state currents induced by moderate pH drops were reported for all recombinant ASICs (Reeh and Kress, 2001) and were also observed in our experiments. The obtained values for time constants of macroscopic current desensitization and recovery (see Table 1) are also in the range typical for ASICs. Homomeric

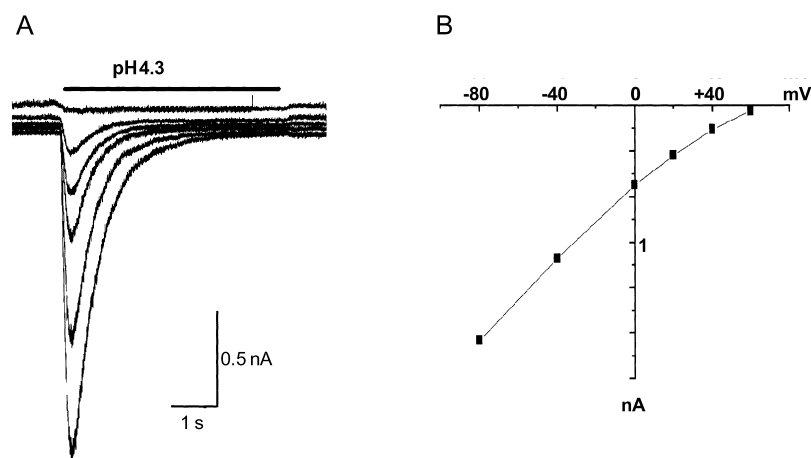


Fig. 5. Voltage dependence of proton-activated currents. (A) Representative responses of a giant interneuron of striatum evoked by pH drops to 4.3 at different holding potentials. (B) Current-voltage relationship of peak amplitude for the same cell. Current remains inward at +60 mV indicating sodium selectivity.



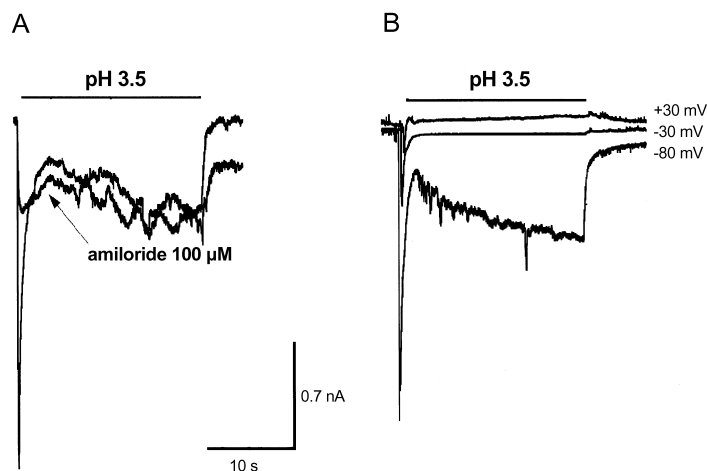


Fig. 6. Properties of the sustained component of proton-activated currents. (A) Representative responses of a cortical pyramidal cell evoked by pH drop to 3.5 in the absence and presence of amiloride. Amiloride completely abolished the peak component while the sustained component of the response was unaffected. After the second application, leak current is increased. (B) Representative responses of a hypoglossal motoneuron evoked by pH drop to 3.5 at different holding potentials. At +30 mV, the peak component remains inward whereas the sustained component is reversed.

ASIC1a channels possess the slowest kinetics with time constants of macroscopic desensitization and recovery of 3.5 and 13 s, respectively. ASIC3 homomeric channels demonstrate fast kinetics; time constants of desensitization and recovery are 0.32 and 0.58 s, respectively (Sutherland et al., 2000). It is also known that ASICs are inhibited by external  $\text{Ca}^{2+}$  ions (Waldmann et al., 1997b) and this property was reproduced herein in experiments on proton-activated currents in rat brain neurons. ASICs belong to the family of amiloride-sensitive sodium channels (Horisberger, 1998). Proton-activated currents in neurons studied were effectively blocked by amiloride and exhibited positive reversal potentials close to  $E_{\text{Na}}$ . Taken together, these data strongly suggest that the proton-activated currents in brain neurons are mediated by ASICs. Sensitivity to extracellular pH and kinetics of desensitization varied

in different types of neurons suggesting a different subunit composition of their ASICs. Most likely, native ASICs studied are heteromeric, because their properties do not match exactly the properties of any known homomeric ASICs. Indeed, properties of heteromeric ASICs with different subunit composition may vary significantly and do not coincide with those of homomeric receptors (Basilana et al., 1997; Reeh and Kress, 2001). Therefore, a conclusion about subunit composition of native ASICs in investigated types of neurons would be premature.

Strong extracellular acidification produced biphasic proton-activated currents in all types of investigated neurons; the peak component was followed by a sustained one. These two components of proton-activated currents differed in their properties. The peak component was sensitive to the blocking action of amiloride and was

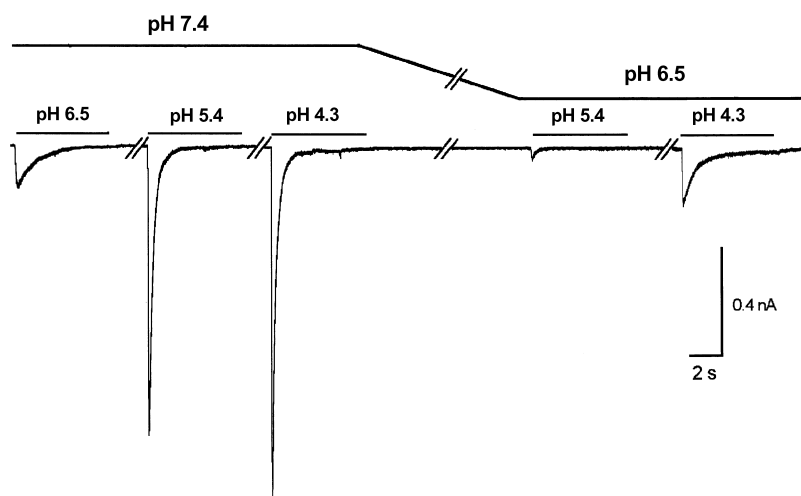


Fig. 7. Effect of slow acidification on proton-activated currents. Representative responses of a giant cholinergic interneuron of striatum evoked by pH drops from 7.4 and 6.5. Breaks correspond to 45-s interval. Slow acidification from 7.4 to 6.5 does not induce detectable current. Response to pH drop depends greatly upon the initial pH level. At pH 6.5, most of proton-gated channels are desensitized and cannot respond to further pH drops.

mediated by sodium ions, while the sustained component was not blocked by a high concentration of amiloride (100  $\mu$ M) and its ion selectivity was weaker. Earlier such biphasic currents were attributed to ASIC3-containing channels (Waldmann et al., 1997a). But mRNA for ASIC3 subunit has not been revealed in most structures of rat brain including hippocampus, striatum and cortex (Babinski et al., 2000). Furthermore, the biphasic proton-activated currents have not been described previously in rat brain. In our experiments, sustained currents appeared at  $\text{pH} < 4$  in contrast to native proton-gated currents in dorsal root ganglion neurons where the pronounced sustained currents were observed at  $\text{pH}$  6.0 (Bevan and Yeats, 1991). The high threshold ( $\text{pH} < 4$ ) of sustained current activation was obtained in recombinant ASIC3-containing channels (Waldmann et al., 1997a). Thus, the nature of sustained proton-activated currents remains unclear. It is possible that the ASIC3 subunit has a wider distribution throughout brain structures than was suggested earlier. It can also be proposed that brain neurons express an ASIC subunit (not cloned yet) that is responsible for sustained currents. We cannot rule out that the sustained component is not related to ASICs and could be a non-specific effect of extremely low  $\text{pH}$  which usually leads to cell death.

Proton-activated currents were found in all types of neurons. In hippocampal neurons, striatal interneurons, Purkinje cells and hypoglossal motoneurons, these currents were described for the first time. It is worthy to note that the proton-activated currents in hippocampal pyramidal neurons are much less pronounced than in hippocampal interneurons and neurons of other structures. This provides evidence of the low abundance of proton-gated channels in the membrane of hippocampal pyramidal neurons. At the same time, mRNA of ASICs was found by *in situ* hybridization in stratum pyramidale

of hippocampus where pyramidal neurons are concentrated (Lingueglia et al., 1997). This disagreement between molecular biology data and the present data needs further investigation. Electrophysiological results obtained on freshly isolated neurons allow characterization of functional ASICs in neuronal membranes whereas other approaches are indirect and their results may be affected by numerous factors.

Moderate slow acidification in some pathological states such as ischemia, inflammation and epilepsy, should induce desensitization of ASICs and inhibit their physiological function. Due to the diversity of properties and expression in functionally distinct neurons, depression of ASICs can lead, for instance, to unbalance of excitatory and inhibitory networks in hippocampus. Evidently, in brain neurons only transient responses of ASICs that appear during rapid acidic shifts may be of physiological significance. ASIC-mediated currents are cell-specific and can have amplitudes comparable to responses of glutamate, which is the major excitatory neurotransmitter in CNS. Therefore their contribution in physiological processes should be important. Unfortunately, fast  $\text{pH}$  drops, which activate brain ASICs, are still not found. Such processes may be revealed by studies of normal synaptic transmission, pathologies and neuronal development. New specific physiological and pharmacological approaches are required to discover the functional role of ASICs in brain.

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