BIOPHYSICS AND BIOCHEMISTRY

Gadolinium Blocks Proton-Activated Currents in Isolated Purkinje Cells

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Patch-clamp and concentration jump studies showed that gadolinium ions dose-dependently reduced the amplitude of proton-activated currents (pH 6.5) in isolated Purkinje cells (gadolinium concentration producing a half-maximum effect was 69 μM for the peak and 8 μM for the stationary component of the current). The magnitude of the block did not depend on membrane potential at negative clamping potentials. Gadolinium blockade of activated receptor was more rapid and intensive in comparison with blocking of silent receptor.

Key Words: *proton-activated channels; gadolinium; cerebellum; potential fixation method*

Acid-sensing ion channels (ASIC; H^+-activated channels) are ligand-operated receptors, which are activated upon the increase in extracellular proton concentration (pH<7.0) [7,12]. Four genes encoding 6 ASIC subunits were identified: ASIC1a, -1b, -2a, -2b, -3, and -4. The ASIC isoforms are differently presented in the central and peripheral nervous systems [1,12,14], which determines the difference in the biophysical and pharmacological characteristics of these receptors in neurons of different types [6,14].

The functional role of ASIC in central neurons is little studied. They were reported to be involved in learning and plastic restructuring of the CNS [13], as well as in ischemic injuries to neurons [15]. The characteristics of ASIC in the CNS are little studied, which impedes the understanding of their functional role and limits the possibilities of their pharmacological modulation.

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Lanthanide gadolinium (Gd^{3+}) is most often used in experimental pharmacology and biophysics as a blocker of mechanosensitive channels [5]. Since ASIC belongs to the same family of degenerin-sensitive channels as mechanosensitive channels, this drug is also used as a physiological tool in studies of ASIC characteristics. Experiments on recombinant receptors expressed in *Xenopus* oocytes showed that Gd^{3+} sensitivity depended on the subunit composition of the receptors [1].

We studied characteristics and mechanisms of Gd^{3+} interactions with ASIC in Purkinje cells.

MATERIALS AND METHODS

Experiments were carried out on 32 neurons isolated from the cerebellar sections of young (14-20 days) rats by vibrodissociation [10]. Brain slices were incubated at ambient temperature in saline containing (in mM): 124 NaCl, 5 KCl, 1.3 $CaCl_2$, 1.5 $MgCl_2$, 1.3 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose. The solution was saturated with carbogen (95% O_2, 5% CO_2). Cell isolation and registration

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of their activity were carried out in solution of the following composition (mM): 150 NaCl, 5 KCl, 2.5 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES. Micropipettes with 2-3 MΩ resistance made from borosilicate glass were filled with a solution of the following composition (mM): 40 CsF, 100 CsCl, 2 Na_2-ATP, 4 $MgCl_2$, 5 EGTA, and 10 HEPES-Na. The experiments were carried out at 20-23°C. H^+-activated currents were recorded using whole-cell patch-clamp technique. The substances were delivered using a rapid application system [11]. The rate of solution replacement near the recorded neuron was about 20 msec. Activation of ASIC was induced by rapid application of solution with pH<7.0 within 1-3 sec.

A series of experiments with isolated application of Gd^{3+} was carried out in order to detect possible interactions between this lanthanide and the membrane. Paired application of H^+, 500 msec each, at 100-1000 msec intervals between paired applications, was used in these experiments. Control solution or solution containing 100 μM Gd^{3+} (pH 7.5) was applied during the intervals between the pairs.

The concentration dependence for H^+-activated currents was described by a logistic equation: $A=1/(1+(pH_{0.5}/[H^+])^n)$, where $A=I_A/I_{A(pH\ 5)}$ is the response caused by H^+; $pH_{0.5}$ is H^+ concentration, inducing the half-maximum response; and n is Hill's coefficient. The modulating effect of Gd^{3+} on currents activated by permanent concentration of H^+ was quantitatively evaluated by the equation: $B=1/(1+([Gd^{3+}]/IC^{Gd}_{50})^n)$, where $B=I_{pH+Gd}/I_{pH}$ was the extent of H^+-activated currents blocking under the effect of Gd^{3+} standardized to current amplitude without Gd^{3+}; IC^{Gd}_{50} is Gd^{3+} concentration causing the half-maximum effect; and n is Hill's coefficient.

The data are presented as the mean±standard error in the mean.

RESULTS

Rapid acidification of the medium from the neutral value of 7.5 to 7.0 and lower (i.e. with higher concentration of H^+) at clamping potential of -70 mV induced inward current with an amplitude increasing with the increase in H^+ concentration. The $pH_{0.5}$ was 6.32±0.05, Hill's coefficient 1.27± 0.19 (n=7) for solutions with pH 7.5-5.0 and external Ca^{2+} concentration of 2.5 mM. H^+-activated currents were desensitized during application, the desensitization rate increasing with pH decrease. Current decrease at $pH_{0.5}$ was observed at a time constant of 1.1-1.7 sec. The H^+-activated currents were blocked with amiloride with IC_{50} 13.06±0.78 μM (Hill's coefficient 1.00±0.06; n=8), which suggests referring the receptors mediating these currents to ASIC [13]. Comparison of the described characteristics of the native H^+-activated currents with the characteristics of recombinant receptors [6] and with published data on the distribution of ASIC subunit subtypes [4,6,9] suggests that ASIC in the studied neurons are most likely multiheterooligomers consisting of ASIC1a and ASIC2b or ASIC1a and ASIC2a subunits.

Combined application of low pH solution and Gd^{3+} reduced the amplitude of H^+-activated currents in all studied neurons (Fig. 1, *a*). The degree

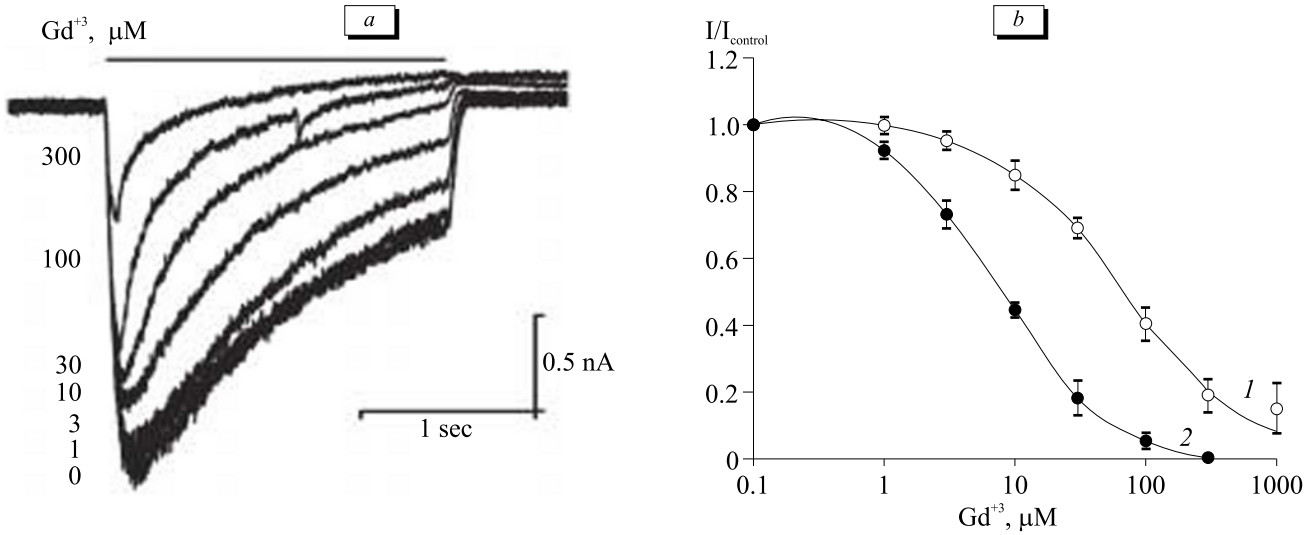

Fig. 1. Blocking of H^+-activated currents in Purkinje cells with Gd^{3+}. *a*) changes in currents caused by application of solution with pH 6.5 during parallel application of H^+ and Gd^{3+} ions in ascending concentrations. Here and in Figs. 2, 3: periods of application are shown with a horizontal line. Clamping potential -70 mV. *b*) dose—response relationship for the blocking effect of Gd^{3+} on the peak value of current activated by solution with pH 6.5 (*1*) and on the stationary component of response (*2*).

of blockade depended on Gd^{3+} concentration and exposure. Quantitative evaluation of Gd^{3+} effects was carried out under conditions of ASIC activation with solutions with pH 6.5, which was close to $pH_{0.5}$ value. The relationship between Gd^{3+} effects and concentration was approximated by Hill's equation (Fig. 1, *b*). In our application protocol, the intensity of ASIC current blockade increased during application, and hence, the extent of response suppression was evaluated at the start and at the end of 2-sec application. IC_{50} for Gd^{3+} estimated for the response peak and plateau were 68.59±6.68 and 7.95±0.47 μM, respectively, Hill's coefficient 0.92± 0.08 and 1.10±0.05, respectively (n=3-6). In contrast to Purkinje cells, the peak and stationary components of responses of ASIC2a+3 recombinant receptors were blocked with Gd^{3+} equally effectively [1]. The Gd^{3+} IC_{50} for response peak suppression in our experiments was similar to that detected for ASIC2a+3 recombinant receptors (about 50 μM) [1], the stationary component of the response being suppressed much more effectively. The IC_{50} for suppression of the stationary component was similar to that described for mechanosensitive channels (about 5 μM) [3,5]. The differences in the type of Gd^{3+} effect on H^+-activated currents in Purkinje cells and oocytes [1] seemed to be due to different types of ASIC mediating them. The precise subunit composition of ASIC in Purkinje cells is unknown, but the characteristics of H^+-activated currents in these neurons suggest their relation to activation of heterooligomers consisting of ASIC1a and ASIC2b or ASIC1a and ASIC2a subunits. Though ASIC2a+3 were detected in rat cerebellum, they were located mainly in Golgi's cells [1], and hence, it seems that the effects of Gd^{3+} in Purkinje cells reflect the absence of ASIC2a+3 in these cells. In addition, high Gd^{3+} sensitivity of Purkinje cells indicates that these neurons have no homomeric ASIC2a, whose Gd^{3+} sensitivity surpasses 1 mM [1].

In order to detect the mechanisms of Gd^{3+} blockade of ASIC currents, we studied the relationship between the block and activation of receptor and membrane potential. The effects of Gd^{3+} developed rapidly after application following ASIC activation: the block development time constant for 100 μM Gd^{3+} was about 120 msec (Fig. 1, *a*), which presumably reflected the direct interaction of metal ions with the receptor molecule. Study of the relationship between the block and membrane potential showed that Gd^{3+}-induced block virtually did not depend on clamping potential if its value varied from -110 to -10 mV (Fig. 2, *a*, *b*). However, at positive values of clamping potential, the block was replaced by response potentiation (Fig. 2, *a*, *b*). These characteristics of the block preclude an unambiguous assumption that the mechanism of Gd^{3+} blocking effect consists in blockade of open ionic channel, as is the case with Gd^{3+} blockade of mechanosensitive channels [3]. Replacement of the blocking effect with a potentiating one presumably results from Gd^{3+} interaction with selective filter of the channel.

Recovery of the response after Gd^{3+} washout was slow in experiments with Gd^{3+} application after

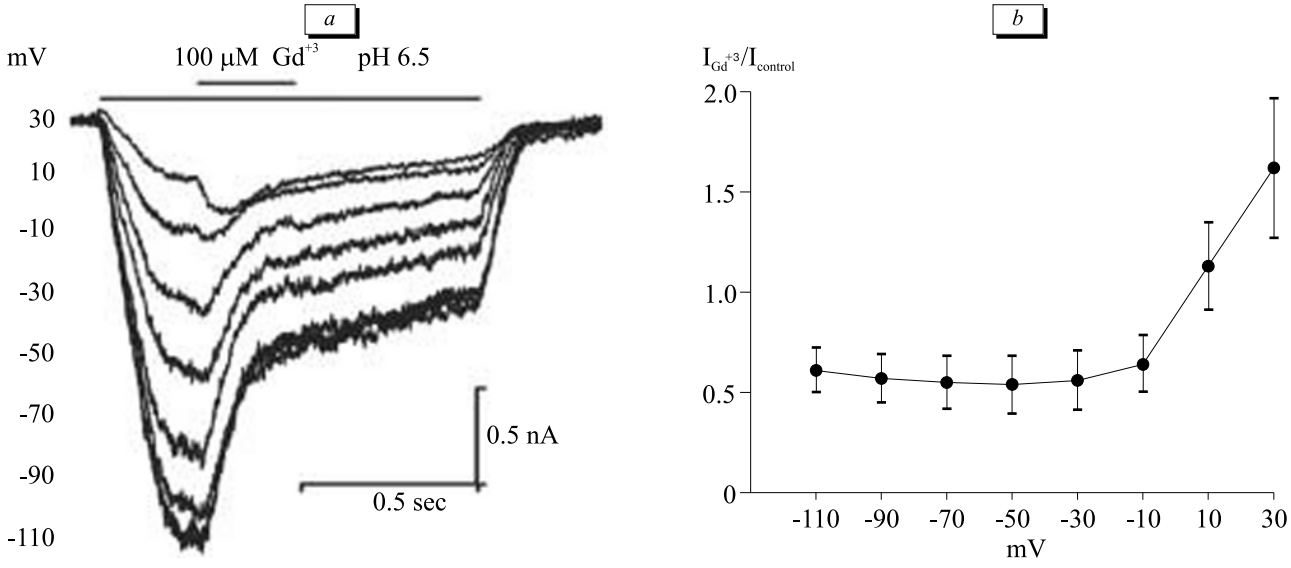

Fig. 2. Relationship between Gd^{3+} effects and membrane potential. *a*) currents recorded in a Purkinje cell clamped at different potentials in response to application of solution with pH 6.5. Gd^{3+} (100 μM) was applied after receptor activation for 500 msec; *b*) relationship between Gd^{3+} effects (100 μM) and membrane potential.

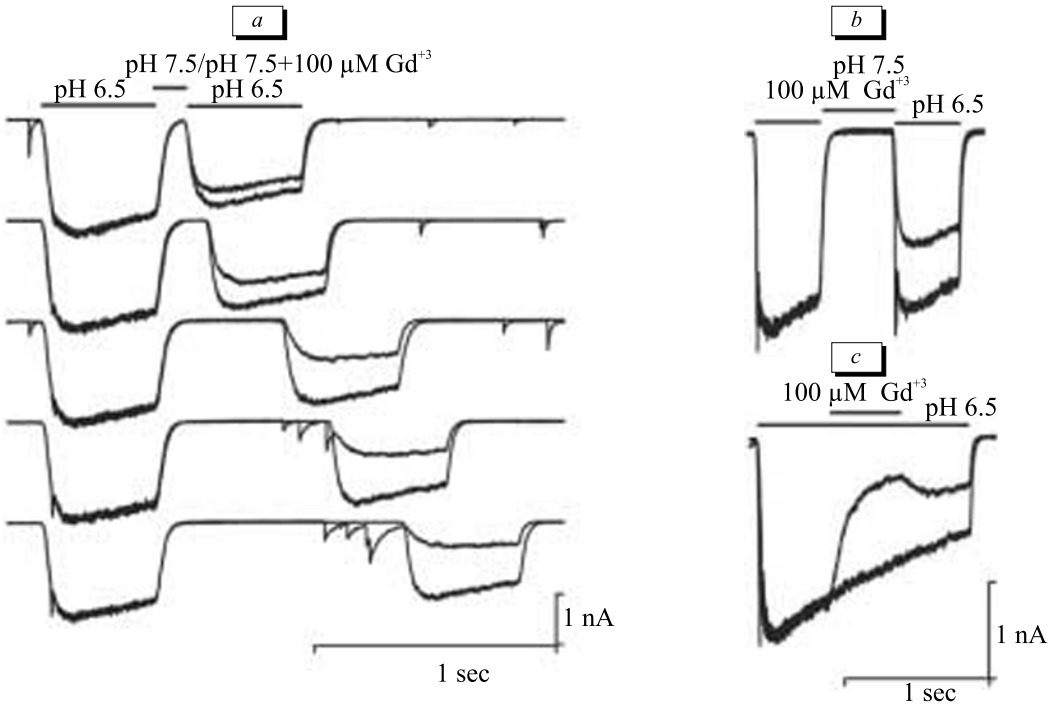

Fig. 3. Effect of Gd^{3+} on silent and activated receptors. *a*) paired application of solution with pH 6.5 at 100-1000 msec intervals between applications. Two control responses and two responses between which an isolated application of Gd^{3+} (100 μM) was made are superimposed for each record. The second response in the pair, having a lower amplitude, is the response after Gd^{3+} application. The blocking effect of Gd^{3+} on silent (*b*) and activated receptor (*c*).

receptor activation; this suggests other mechanisms of Gd^{3+} effect, in addition to interaction with the receptor protein. Lanthanides are characterized by high affinity to cell membrane phospholipids and cause structural changes in the lipid bilayer, which can serve as a method for regulation of the mechanosensitive channel open/closed status [2]. Application of Gd^{3+} without ASIC activation caused blockade of H^+-activated current, the magnitude of this block increased with prolongation of Gd^{3+} application (Fig. 3, *a*). However, the efficiency of Gd^{3+} blockade in this application mode was lower than its effect on the open ionic channel. Application of Gd^{3+} for 200 msec without ASIC activation caused just a 20% reduction of the response (Fig. 3, *a*), while 200-msec parallel application of H^+ and Gd^{3+} produced pronounced blockade (Fig. 3, *c*). Prolongation of isolated application of Gd^{3+} led to a more pronounced blocking effect, its value approaching that observed for open ionic channel (Fig. 3, *a*). These results indicate that Gd^{3+} can interact with closed channel, but preclude unambiguous interpretation of blockade of silent receptor as a result of Gd^{3+} effect on the lipid bilayer. At the same time, Gd^{3+} modifies the electric and elastic characteristics of lipid membranes even in micromolar concentrations [2], and therefore the relationship between the extent of the blocking effect without ASIC activation and duration of Gd^{3+} exposure can be regarded as an argument supporting the hypothesis that these effects are caused by Gd^{3+} interaction with the neuronal membrane, which results in modification of its mechanical characteristics. Higher efficiency of the block after receptor activation in comparison with blockade of the closed channel can be due to the fact that receptor has a binding site for Gd^{3+} available only after receptor activation, and also to the fact that the increase in H^+ concentration leads to increase in membrane and/or receptor protein affinity for Gd^{3+} ions.

Thus, specific blocker of mechanosensitive channels Gd^{3+} in micromolar concentrations blocks native ASIC expressed in the Purkinje cells. Gd^{3+} interacts with activated and silent receptors, but blockade of activated receptor is more rapid and effective.

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