Modulation of GABA-Activated Currents in Rat Isolated Cerebellar Neurons by Lanthanum Ions

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Lanthanum ions (10-1000 μM) potentiated GABA-activated currents and increased the sensitivity of $GABA_A$ receptors in isolated Purkinje cells from rat cerebellum. GABA receptors in Purkinje cells are comparable to receptors on other cells by their sensitivity lanthanum ions.

Key Words: *$GABA_A$-receptor*; *lanthanum ions*; *isolated neurons*; *patch-clamp*

GABA is the main inhibitory neurotransmitter in the central nervous system of mammals. Rapid synaptic inhibition caused by GABA is mediated via $GABA_A$ receptors.

It was previously established that lanthanum in submillimolar concentrations modulates GABA-activated currents [2,9,15]. Similar effects were described for other lanthanides [6].

The effect of lanthanum depends on subunit composition of $GABA_A$ receptors. Experiments with recombinant receptors expressed in heterologous systems [5,12] showed that the modulatory effects of lanthanum and the sensitivity of $GABA_A$ receptors to La^{3+} depended on the type of α-subunit, the most prominent differences were found for receptors containing α1- and α6-subunits. However, the effects of lanthanum on native GABA receptors are little studied.

There are no published data on the modulatory effect of lanthanum on GABA-activated currents in Purkinje cells. Possible effects of lanthanum in these cells can be predicted on the basis on putative subunit composition of $GABA_A$ receptors and on the data obtained on recombinant receptors [5,12]. However, there is now growing evidence that not only the subunit composition but also their interaction with intercellular components affect the properties of GABA receptors [4]. Quantitative characteristics of the effects produced by lanthanum on $GABA_A$ receptors in Purkinje cells allow comparison of these receptors with native receptors on other neurons and recombinant receptors by their sensitivity to lanthanum ions.

MATERIALS AND METHODS

Experiments were carried out on freshly isolated Purkinje cells from cerebellar slices from 14-20-day old rats. Cerebellar slices passed conventional incubation [5], and Purkinje cells were isolated by vibrodissociation method [13]. Borosilicate glass micropipettes for recording electrodes were filled with a solution containing (in mM): 140 CsCl, 0.5 $CaCl_2$, 4 $MgCl_2$, 5 EGTA, 10 HEPES, and 4 ATP-Na_2 (micropipette resistance 2-4 MΩ). Series resistance compensation during recording was >50%. Soluble lanthanum salt ($LaCl_3$) was added with or without GABA to recording solution. Working with high concentrations $LaCl_3$ we took into account recommendations available in literature [11]. Substances were introduced via a rapid perfusion system [14]. The concentration-response curve for GABA-activated currents was described by an equation:

$$R = \frac{1}{1+(EC_{50}/[GABA])^h}, \quad (1)$$

where $R=I_{GABA}/I_{MAX}$ is the relative response to GABA; I_{GABA} is the amplitude of current induced by a given concentration of GABA; I_{MAX} is the saturation current amplitude induced by GABA (100 μM); EC_{50} is the half-maximal effective concentration for

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GABA; and h is Hill coefficient. Similar equation [5,7,12] was used to describe concentration-response curve for lanthanum-induced potentiation:

$$R_{la}=1+\frac{(\text{A-1})}{1+EC^{La}{}_{50}/[\text{La}])^{h}}\times 100\%, \qquad \textbf{(2)}$$

where $R_{La}=I_{GABA+La}/I_{GABA}$ is the potentiation of GABA-activated current by lanthanum, expressed in percent; A×100% is maximum potentiation; $EC^{La}{}_{50}$ is half-maximal effective concentration for lanthanum. The responses were normalized to the amplitude of current induced by GABA alone.

The results of several experiments are reported as mean±standard error. The data were statistically analyzed by ANOVA methods and by two-tail Student's t test at significance level $p<0.05$.

RESULTS

In all neurons (n=30) voltage-clamped at -70 mV, application of GABA (1 to 100 μM) induced an inward current. The amplitude of this current increased with increasing GABA concentration. Repetitive application of GABA (2 μM during 1 sec, every 30 sec) induced currents with stable amplitude (30 min or longer).

In the absence of GABA $LaCl_3$ alone (<1 mM) did not induce transmembrane currents. Combined application of GABA and $LaCl_3$ (10-1000 μM) potentiated GABA-induced responses in a dose-dependent manner (Fig. 1, *a*).

The currents induced by GABA alone or in the presence of $LaCl_3$ were inhibited by 10 μM bicuculline. Reversal potentials for GABA-induced currents were 1.0±0.7 mV in the absence (Fig. 2, *a, c*) and 1.5±0.8 mV in the presence of $LaCl_3$ (Fig. 2, *b, c*). Both reversal potentials differed insignificantly (n=5; p=0.21 and p=0.23, respectively) from the reversal potential for Cl^- ions (0 mV, under these conditions). These data confirm specific effect of La^{3+} on type A GABA-activated chloride channels ($GABA_A$ receptors).

In order to quantify the effects of La^{3+} on $GABA_A$ receptors, 2 μM GABA was applied in combination with La^{3+} in different doses. Current amplitudes were measured in the presence and absence of La^{3+}, and the ratios of these currents were calculated and plotted (Fig. 1, *b*). Experimental curve was approximated by equation **(2)**. The best fit parameters were $EC^{La}{}_{50}$= 88.0±7.7 μM and Hill coefficient 1.40±0.14. The maximum potentiation effect was 355±8% (n=5). Combined application of GABA with La^{3+} not only increased the current amplitude, but also changed the kinetics of the response (Fig. 1, *a*), which reflected the complex of transient processes induced by co-application.

Pretreatment with lanthanum (10-100 μM) potentiated the currents induced by subsequent ap-

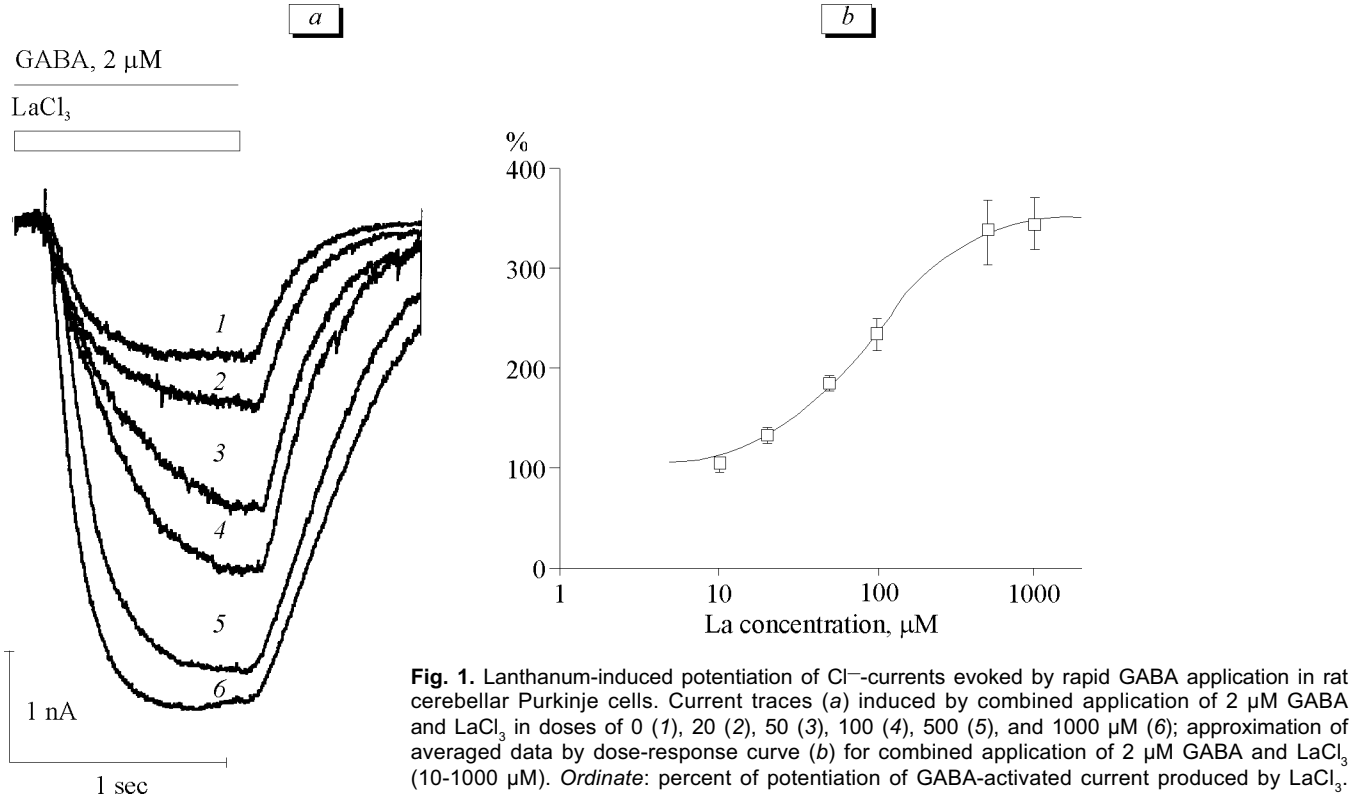

Fig. 1. Lanthanum-induced potentiation of Cl^--currents evoked by rapid GABA application in rat cerebellar Purkinje cells. Current traces (*a*) induced by combined application of 2 μM GABA and $LaCl_3$ in doses of 0 (*1*), 20 (*2*), 50 (*3*), 100 (*4*), 500 (*5*), and 1000 μM (*6*); approximation of averaged data by dose-response curve (*b*) for combined application of 2 μM GABA and $LaCl_3$ (10-1000 μM). *Ordinate*: percent of potentiation of GABA-activated current produced by $LaCl_3$.

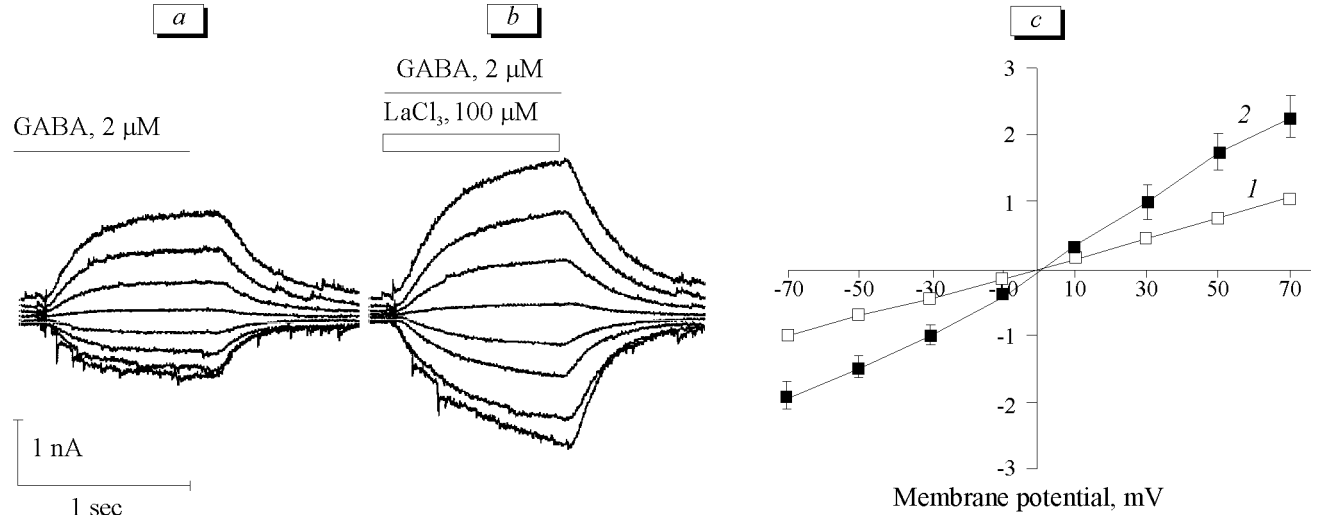

Fig. 2. Effects of lanthanum receptor sensitivity to GABA in Purkinje cells. Current traces induced by application of GABA (*a*) in doses of 2 (*1*), 5 (*2*), 10 (*3*), and 100 μM (*4*), and combined application of GABA and $LaCl_3$ (*b*); dose-response curves (*c*) for application of GABA in the absence (*1*) and presence of 100 μM $LaCl_3$ (*2*). *Ordinate*: normalized response to GABA.

plication of GABA, these effects being dose- and time-dependent (1-30 sec). The higher was the concentration of $LaCl_3$, the shorter duration was needed. It was important to determine, which duration of pretreatment is required to achieve saturation effects for a given concentration of $LaCl_3$. Our results showed that 10-sec pretreatment with 10 μM $LaCl_3$ was sufficient for attaining the maximum effects.

The potentiation effect of La^{3+} decreased with increasing GABA concentration. Thus, for combined application of 2 μM GABA and 100 μM La^{3+} the potentiation effect was 220±20% (n=5), while for 5 μM GABA it constituted 125±11% (n=5), and only 102±2% for 100 μM GABA (n=6).

For evaluation of the mechanisms underlying lanthanum-induced potentiation of GABA-activated currents, we compared the sensitivity of Purkinje cells to GABA before and after $LaCl_3$ treatment. To this end, the currents activated by GABA (2-100 μM) alone or in the presence of 100 μM $LaCl_3$ were recorded (Fig. 3). Taking into account previous results, pretreatment with La^{3+} before application of GABA lasted for 30 sec or longer. Both experimental curves were approximated by equation **(1)**. In the control series, best-fit parameters were EC_{50}= 3.2±0.4 μM and h=2.0±0.2. Lanthanum significantly reduced EC_{50} for GABA to 1.7±0.2 μM (Fig 3, *c*), and Hill coefficient to 1.40± 0.14 (n=6). The amplitude of saturation response induced by 100 μM GABA in the presence of lanthanum remained unchanged (p=0.76; Fig. 3, *a*, *b*). The dose-response curve modified by La^{3+} was left-shifted and had a smoother slope (Fig. 3, *c*).

To study the dependence of lanthanum-induced potentiation on the membrane potential, the percent of potentiation induced by 100 μM La^{3+} was calculated at different holding potential (from -70 to 70 mV) by the formula:

$$P(V)=(I(V)^{GABA+La}/I(V)^{GABA})\times 100\%.$$

We found no voltage dependency of lanthanum-induced effects (p=0.8, n=5).

Our findings are consistent with other data obtained both for native [9] and recombinant receptors with a subtype composition similar to $GABA_A$ receptors of cerebellar Purkinje cells [3,12]. In summary, it was established that extracellular lanthanum ions do not activate transmembrane currents, but potentiate GABA-induced currents. This lanthanum-induced potentiation is associated with interaction between La^{3+} and $GABA_A$ receptors in Purkinje cells. The absence of marked dependency on transmembrane voltage and direct induction of currents by La^{3+} suggest that it modulate GABA-induced currents via binding to extracellular domains of the receptors. Experiments with lanthanum pretreatment indicate that La^{3+} can interact with the receptors in the absence of GABA also. For attaining saturation, La^{3+} should be applied for minimum 10 sec (at concentration of 10 μM). The nature of the lanthanum-induced shift of the dose-response curve and its inability to increase the maximum response suggest that the modulatory effect of La^{3+} is realized predominantly via enhancement of the receptor affinity to GABA. However, the modulatory effect of lanthanum can not be explained only by its effect on receptor affinity, because it changes also the slope of the dose-response curve, *i.e.* lanthanum ions can also affect receptor desensitization processes [15].

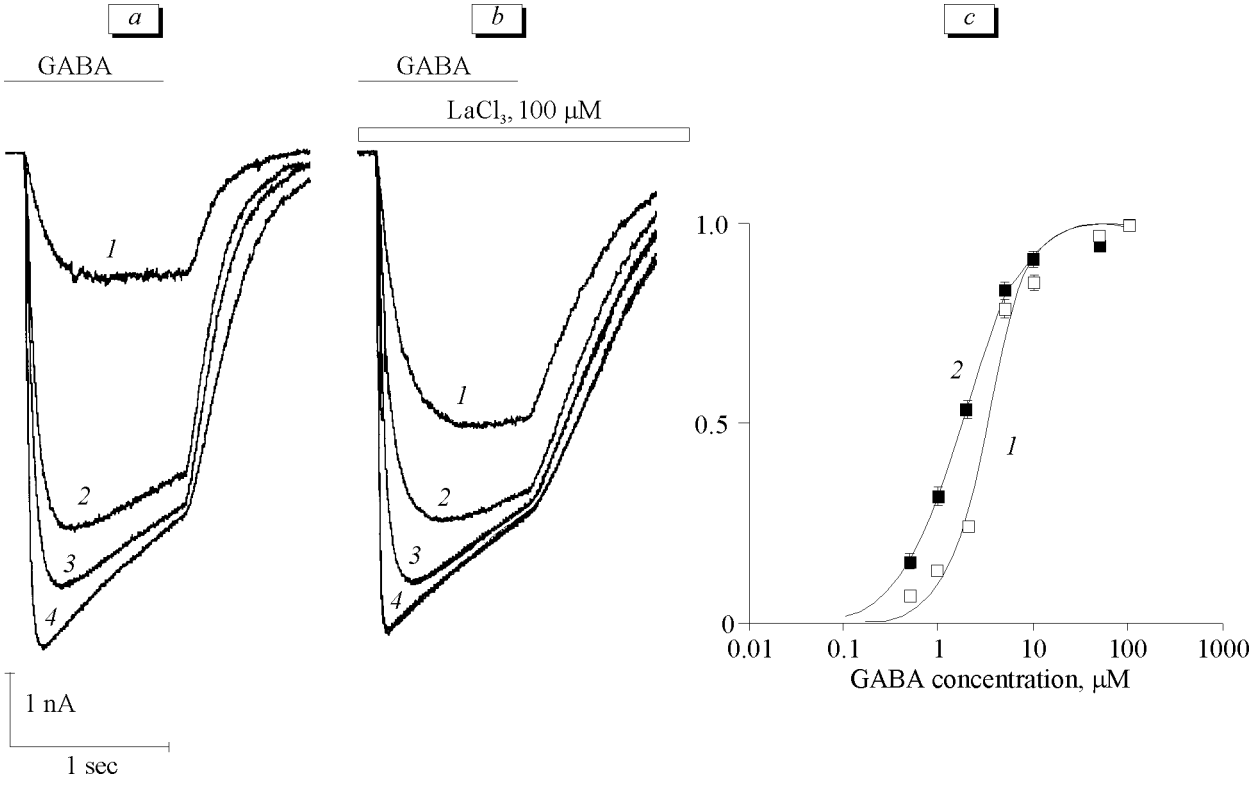

Fig. 3. Independence of modulatory effects of lanthanum on transmembrane voltage. Current traces at different holding voltage steps (from -70 to 70 mV) induced by application of 2 μM GABA (*a*) and combined application of 2 μM GABA and 100 μM $LaCl_3$ (*b*); current-voltage relations (*c*) for currents induced by application of 2 μM GABA (*1*) and combined application of 2 μM GABA and 100 μM $LaCl_3$ (*2*). Current amplitudes were normalized in both series to the current amplitude induced by 2 μM GABA, at clamped voltage -70 mV.

Based on our findings and published data, we compared the modulatory effects of lanthanum ions on $GABA_A$ receptors in various types of neurons. These effects were quantified by the dose-response parameters EC^{LA}_{50} and h and percent of maximum potentiation. Comparison revealed a higher sensitivity $GABA_A$ receptors to lanthanum in Purkinje cells compared with the receptors of neurons in rat dorsal root ganglion, for which the potentiation effect is described by the following parameters: EC^{La}_{50}= 231±32 μM, h= 0.94±0.11, and A×100%= 293±37 [9]. This can be attributed to different subtype compositions of GABA receptors in Purkinje cells (α1β2/3γ2 [2]) and dorsal root ganglion neurones (α2/3/5β2/3γ2 [8]). It is worth to note that the modulatory effect of lanthanum must be compared at equal concentrations of GABA. Thus, the sensitivity of recombinant receptors (α1β3γ2) to lanthanum determined at effective concentration of GABA (EC_{50}) was considerably lower (EC^{La}_{50}=210± 61 μM, h=1.5± 0.11, and A×100%=164±11 [10]), compared to that of GABA receptors in Purkinje cells, although both groups of receptors had similar subunit compositions.

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