BIOPHYSICS AND BIOCHEMISTRY

Lanthanum Potentiates GABA-Activated Currents in Rat Pyramidal Neurons of CA1 Hippocampal Field

A. A. Boldyreva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 10, pp. 398-401, October, 2005
Original article submitted February 2, 2005

In CA1 hippocampal pyramidal neurons, lanthanum ions increased the amplitude of GABA-activated currents and shifted the dose-dependence curve to the left, which attests to increased affinity of $GABA_A$-receptors to GABA. The data made it possible to compare the sensitivity $GABA_A$-receptors of pyramidal neurons and similar receptors of other cells to GABA and lanthanum.

Key Words: *GABA; pyramidal neurons; lanthanum; patch clamp*

GABA is an important inhibitory neurotransmitter in CNS of mammals. GABA-induced rapid synaptic inhibition results from the action of this transmitter on $GABA_A$ receptors. These receptors are presumably pentamers consisting of two α-, two β- and one γ- or δ-subunit [3].

In submillimolar concentrations, lanthanum ions ($LaCl_3$) modulate the GABA-activated transmembrane currents [1,2,6,11]. Similar effects were reported for other rare-earth lanthanide metal ions [5].

The action of $LaCl_3$ on GABA-activated currents is important in view of selectivity of its effects depending on the subunit composition of $GABA_A$-receptors. Experiments with recombinant receptors expressed in heterogeneous systems [4,7] showed that the modulation and sensitivity of $GABA_A$-receptors to $LaCl_3$ is mainly determined by the nature of α-subunit, and the most salient peculiarities were described for receptors with α_1- and α_6-subunits. However, the action of $LaCl_3$ on native receptors is little studied.

This paper continues the study of the lanthanum effects on GABA-activated currents in cerebellar Purkinje cells [1]. Our present aim was to examine the effect of $LaCl_3$ on GABA-activated currents in CA1 hippocampal neurons.

Brain Research Institute, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Experiments were carried out on CA1 hippocampal pyramidal neurons. The cells were isolated using vibrodissociation technique from hippocampal sections from 14-17-day-old rats. The sections were incubated as described elsewhere [9]. Micropipettes made of borosilicate glass were filled with intracellular solution containing (in mM): 2.0 $MgCl_2$, 2.0 Na_2ATP, 100 CsF, 40 CsCl, 10 TEA-Cl, 10 HEPES, 5 EGTA. The resistance of the pipettes was 2-4 MΩ. To study modulation of GABA-activated currents with $LaCl_3$, this soluble salt was added to the bathing solution in combination with GABA. The chemicals were applied using a rapid microperfusion system [10]. The concentration dependence for GABA-activated currents is described by EC_{50} (GABA concentration inducing half-maximum response) and Hill coefficient *n*. To describe the dose-dependence of $LaCl_3$ potentiating effect, we used parameter of EC_{50}^{La}, corresponding to concentration inducing half-maximum potentiation. The amplitudes of currents induced by combined application were divided by amplitudes of currents induced by GABA alone. The data are presented as mean±SEM.

RESULTS

Three experimental series were carried out to study: 1) sensitivity of $GABA_A$-receptors in CA1 hippocam-

0007-4888/05/1404-0403

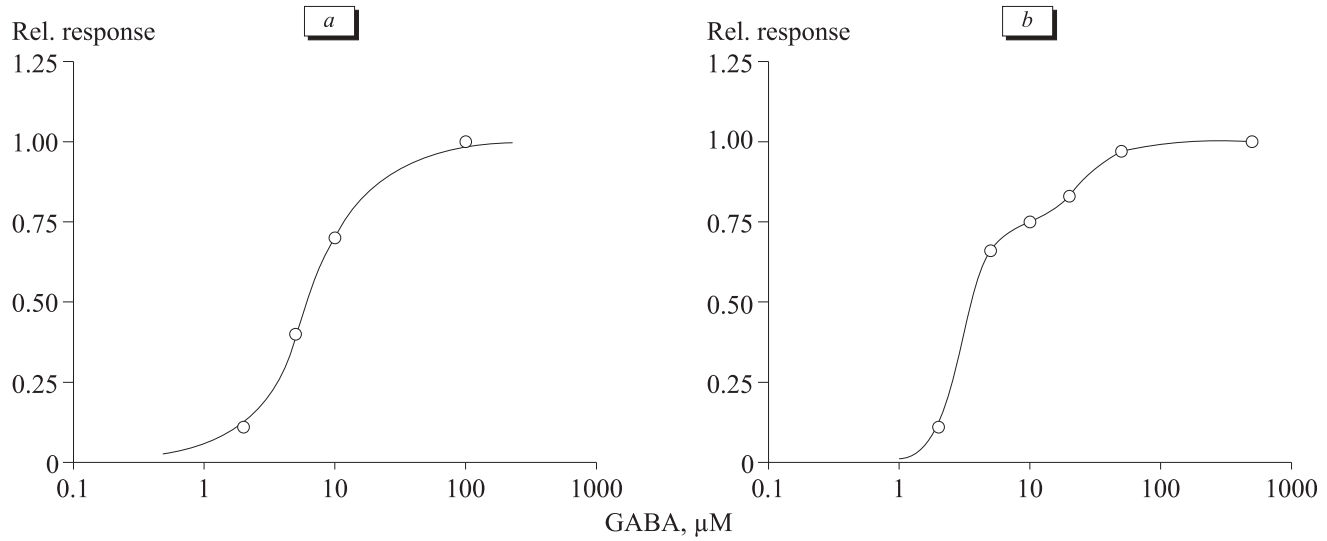

Fig. 1. Sensitivity of pyramidal neurons in CA1 hippocampal field to GABA. An example of GABA dose-dependence plots described by equation with one (*a*) and two (*b*) binding sites.

pal pyramidal neurons to GABA; 2) effect of $LaCl_3$ on GABA-activated currents; and 3) effect of $LaCl_3$ on sensitivity of GABA receptors. In each series, the presented data are the mean values obtained on 3-8 cells.

GABA applied in concentration of 2–500 μM at holding potential -70 mV induced inward current, whose amplitude increased with increasing GABA concentration. In this series, two groups of neurons with different GABA dose-response dependences were revealed (Fig. 1). In group 1, this dependence was described by Hill equation with a single binding site (Fig. 1, *a*). The values of EC_{50} and Hill coefficient were 5.60± 0.19 μM and 1.59±0.08, respectively (n=6). In group 2 (n=2), the dose-response plot was fitted by an equation with two binding sites. The EC_{50} values of the high- and low-affinity sites were 2.3 and 30 μM, respectively (Fig. 1, *b*). The biphasic GABA dose-response curve indicates the existence of two populations of $GABA_A$-receptors in these neurons. Similar data are described elsewhere [8]. In the study of total ionic currents, the existence of receptors with different affinity does not allow unequivocal interpretation of the modulating effects, so we analyzed only the data for neurons with monophasic GABA dose-response dependence.

Application of $LaCl_3$ (3-100 μM) in the absence of GABA did not activate transmembrane currents.

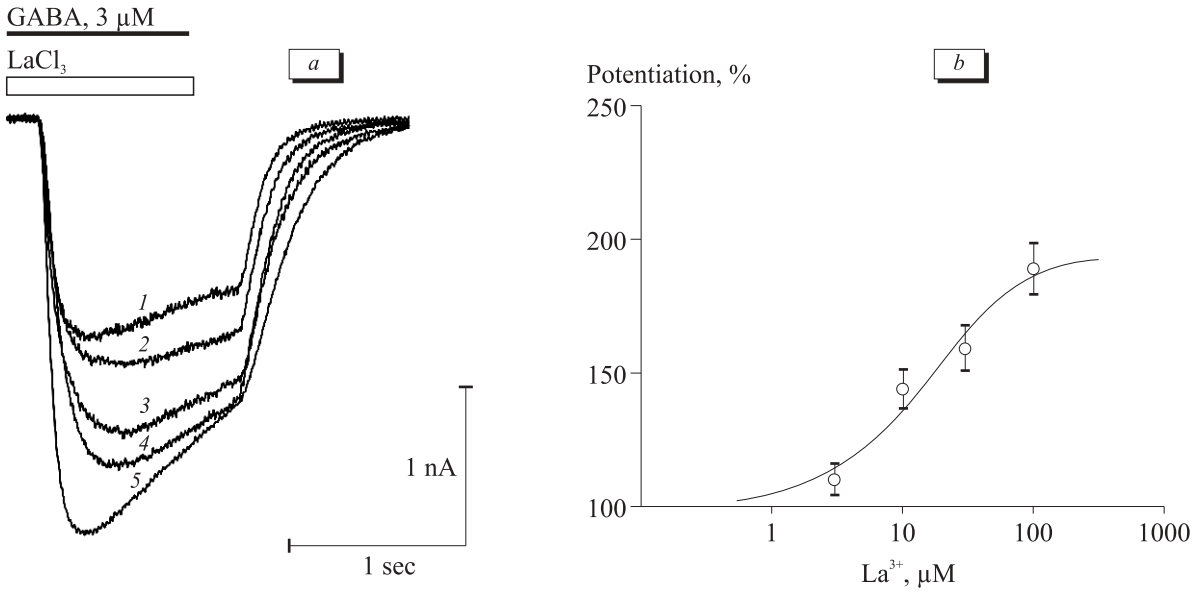

Fig. 2. Potentiating effect of $LaCl_3$ on chloride current induced by rapid application of GABA in pyramidal neurons from CA1 hippocampal field. *a*) ionic currents induced by the combined application of 3 μM GABA and $LaCl_3$ in various concentrations (in μM): 0 (*1*), 3 (*2*), 10 (*3*), 30 (*4*), 100 (*5*); *b*) the corresponding lanthanum dose-response curve fitted by Hill plot.

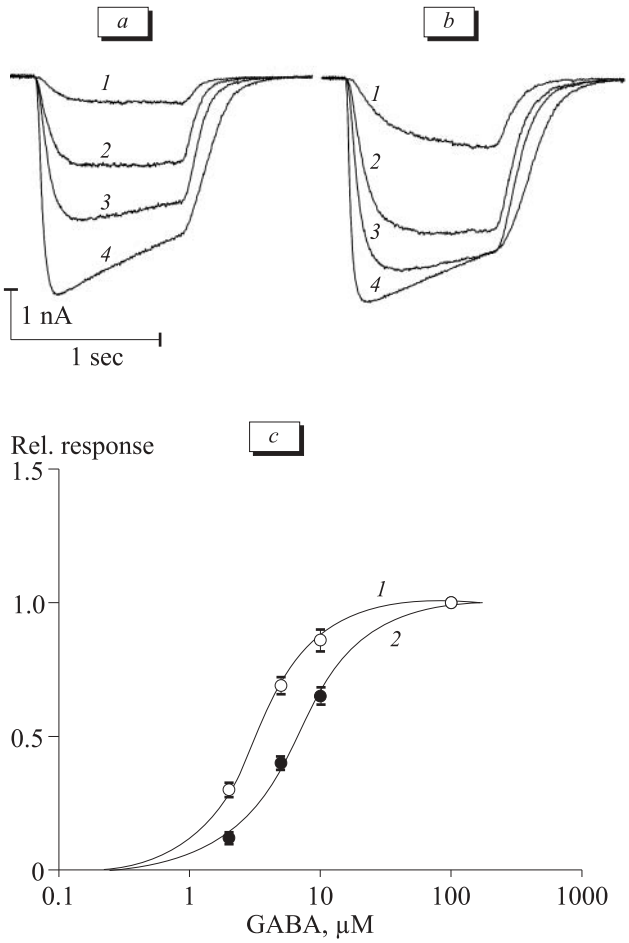

Fig. 3. Effect of $LaCl_3$ on sensitivity of pyramidal neurons isolated from CA1 hippocampal field to GABA. *a*) ionic currents induced by individual GABA application; *b*) the currents induced by the combined application of 100 μM $LaCl_3$ and GABA in various concentrations (in μM): 2 (*1*), 5 (*2*), 10 (*3*), 100 (*4*); *c*) GABA dose-response curves in the absence (*1*) and presence (*2*) of lanthanum ions.

When applied in combination with GABA, this salt potentiated the GABA responses in a dose-dependent manner (Fig. 2, *a*).

To compare our data with [1] for experiments with combined application of a modulator and the agonist, the latter (GABA) was used in a concentration of 3 μM, which induced 20% maximum response (EC_{20}). The resulting dose-dependence plot was fitted with EC_{50}^{La}=19.3±9.1 μM and n=0.92±0.14. The maximum potentiating effect was 208±29% (Fig. 2, *b*).

To analyze the mechanisms underlying potentiating affect of $LaCl_3$ on GABA-activated currents, we examined the dependence of the amplitude of these currents on GABA concentration (2-100 μM) with and without 100 μM $LaCl_3$ (Fig. 3). In these experiments, lanthanum decreased GABA EC_{50} from 5.60±0.19 to 3.20±0.06 μM, did not change the amplitude of the maximum response induced by 100 μM GABA (EC_{98}), and shifted the dose-response curve to the left (Fig. 3, *c*).

Extracellular lanthanum did not activate transmembrane currents, but potentiated GABA-activated ionic currents. The lanthanum-induced a leftward shift of the GABA dose-response curve and inability to change the maximum GABA-induced response by these ions indicate an increase in affinity of $GABA_A$-receptor to this agonist.

The parameters of dose-response curve obtained in this study make it possible to compare the $GABA_A$-receptors in various types of neurons. For example, the receptors of Purkinje cells were less sensitive to $LaCl_3$ than those of pyramidal cells in CA1 hippocampal field. In addition, Purkinje cells are characterized by higher percentage of potentiation. For these cells, the potentiation effect is described by EC_{50}^{La}=88.0±7.7 μM and Hill coefficient 1.40±0.14, the maximum percentage of potentiation being 355±8% [1]. The differences in the sensitivity of GABA receptors in various neurons can be explained by specificity of subunit composition: presumably, the composition of GABA receptors in pyramidal cells of hippocampal CA1 field is $\alpha_{2/5}\beta_{1/3}\gamma_2$ [8], while similar receptors of cerebellar Purkinje cells and giant striatal interneurons are composed of $\alpha_1\beta_{2/3}\gamma_2$ [1] and $\alpha_{2/3}\beta_1\gamma_2$.

This work was supported by the Russian Foundation for Basic Research (grant No. 02-04-48295) and by Leading Scientific Schools Grant of President of Russia, grant No. NSh-1341.2003.4.

REFERENCES

1. S. N. Kolbaev, *Byull. Eksp. Biol. Med.*, **133**, No. 1, 55-59 (2002).
2. B. Barila, A. Cupello, and M. Robello, *Neurosci. Lett.*, **298**, No. 1, 13-16 (2001).
3. E. Costa, *Annu. Rev. Pharmacol. Toxicol.*, **38**, 321-350 (1998).
4. J. L. Fisher, J. Zhang, and R. L. Macdonald, *Mol. Pharmacol.*, **52**, No. 4, 714-724 (1997).
5. J. Y. Ma and T. Narahashi, *J. Neurosci.*, **13**, No. 11, 4872-4879 (1993).
6. T. Narahashi, J. Y. Ma, O. Arakawa, *et al.*, *Cell. Mol. Neurobiol.*, **14**, No. 6, 599-621 (1994).
7. N. C. Saxena, T. R. Neelands, and R. L. Macdonald, *Mol. Pharmacol.*, **51**, No. 2, 328-335 (1997).
8. E. I. Tietz, J. Kapur, and R. L. Macdonald, *J. Neurophysiol.*, **81**, 1575-1586 (1999).
9. V. S. Vorobjev, *J. Neurosci. Methods*, **38**, Nos. 2-3, 145-150 (1991).
10. V. S. Vorobjev, I. N. Sharonova, and H. L. Haas, *Ibid.*, No. 2, 303-307 (1996).
11. W. J. Zhu, H. Wang, L. Corsi, and S. Vicini, *J. Physiol.*, **511**, No. 3, 647-661 (1998).