Potentiation of GABA-Activated Currents by Imidazobenzoimidazole Derivative RU-353 in Isolated Cerebellum Purkinje Cells

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Voltage clamp and concentration jump experiments performed on Purkinje cells isolated from rat cerebellum showed that novel imidazobenzoimidazole derivative RU-353 increased the amplitude of GABA-activated chlorine current in a dose-dependent manner (EC_{50}=15 μM for the currents activated by 1 μM GABA). RU-353 shifted the GABA dose-response curve to the left, but produced no effect on the maximum response (EC_{50} in control and in the presence of 30 μM RU-353 were 6.9 and 2.0 μM, respectively).

Key Words: *imidazobenzoimidazole derivative; RU-353; $GABA_A$-receptor; cerebellum; voltage clamp; concentration jump*

The content of GABA in CNS affects cardiac activity and vascular tone [2,7]. Clinical and experimental data indicate antiarrhythmogenic potency of GABA and agents with GABA-positive activity [6]. We previously showed that imidazobenzoimidazole derivative RU-353 (laboratory code) exhibits pronounced antiarrhythmic and local anesthetic activity [5] and modulates the content of biogenic amines and acetylcholine and anticholinesterase activity in the myocardium and various regions of rat brain [1]. To assess possible interaction of RU-353 with $GABA_A$-receptors of central neurons, we studied modulation of GABA-activated currents by this substance in neurons isolated from rat brain.

MATERIALS AND METHODS

Experiments were carried out on neurons isolated by vibrodissociation technique [10] from cerebellum slices of young rats (14-20 days). The slices were incubated at room temperature in physiological 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 oxygenated with 95% O_2 and 5% CO_2. The medium for isolation and recording contained (in mM): 150 NaCl, 5 KCl, 2.7 $CaCl_2$, 2.0 $MgCl_2$, 10 HEPES. Micropipettes made of borosilicate glass were filled with a solution containing (in mM): 100 CsF, 40 CsCl, 2 ATP, 5 EGTA, 10 HEPES-Na. Micropipettes with tip resistance of 2-3 MΩ were used. The experiments were carried out at room temperature (20-23°C). GABA-activated currents were recorded by patch clamp method in whole cell configuration. Chemical agents were applied by rapid microperfusion system [11]. The solution replacement period was about 20 msec.

The concentration dependence for GABA-activated currents was described by the equation: $A=1/(1+(EC_{50}/[GABA])^n)$, where $A=I_A/I_{A(300\ \mu M)}$ is a relative GABA-induced response; EC_{50} is concentration of the agonist inducing 50% response; *n* is Hill coefficient. To evaluate the potentiating effect of RU-353 on the currents activated by standard GABA con-

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centration, we used the following equation: $B=1+A/(1+(EC_{50}RU/[RU\text{-}353])^n)$, where $B=I_{GABA+RU\text{-}353}/I_{GABA}$ is potentiation of GABA-activated currents caused by RU-353; $EC_{50}RU$ is concentration of RU-353 producing 50% effect; *n* is Hill coefficient. The responses were standardized to current amplitude induced by GABA in various concentrations in the absence of RU-353. The data are presented as mean±SEM.

RESULTS

GABA was applied at a holding potential of -70 mV in concentrations of 1 to 300 μM induced inward currents in all neurons (*n*=29), their amplitude increased with increasing agonist concentration.

In all neurons, combined application of GABA and RU-353 (0.1-100.0 μM) induced currents, which were greater than those induced by individual application of the agonist (Fig. 1, *a*). The degree of this potentiation depended on the dose of RU-353. For evaluation of the effect of RU-353, GABA was applied at a constant concentration of 1 μM (EC ~5%) in combination with modulator RU-353 in various concentrations. In this series of experiments, GABA was applied against the background of RU-353, which was applied 3-5 sec before the agonist (Fig. 1, *a*). In addition, this protocol made it possible to measure the amplitude of ionic current induced by RU-353 alone. A ratio of amplitudes after combined application of GABA and RU-353 and individual application of 1 μM GABA was calculated. The dose-dependence of RU-353 potentiation was fitted by the Hill plot (Fig. 1, *b*), which resulted EC_{50}=15.0±1.8 μM with Hill coefficient of 1.9±0.4 (*n*=7). The maximum potentiation observed at 100 μM RU-353 was 1500±87%. The minimum concentration of RU-353 producing significant potentiation was 3 μM. Preliminary individual application of RU-353 in a concentration of 1-30 μM induced no ionic currents, but the highest concentration tested (100 μM) induced inward current of about 150 pA (Fig. 1, *a*). In contrast to GABA-induced currents, RU-induced current was not blocked by gabazine, a competitive GABA antagonist [12]. This peculiarity showed that RU-induced current was not caused by direct action of RU-353 on the GABA recognition site on the $GABA_A$ receptor. The amplitude of RU-induced current was about 7% of the maximum current induced by GABA.

RU-353 not only potentiated GABA-induced currents, but also markedly modulated their kinetics (Fig. 1, *a*). In the presence of modulator, the rate of ionic current deactivation decreased by several times, and this effect increased with increasing modulator concentration. At the maximum RU-353 concentration (100 μM), the rate of deactivation decreased 25-fold (600 *vs*. 25 msec in the control).

The relative value of potentiation caused by RU-353 depended on GABA concentration. This dependence was examined in a special experimental series, where we measured the amplitude of currents evoked by different GABA concentrations alone or in combination with 30 μM RU-353 (Fig. 2). The relative potentiation of the GABA-induced currents decreased with increasing agonist concentration. Analysis of the dose-response dependence in the control or under the action of RU-353 showed that the modulator shifted

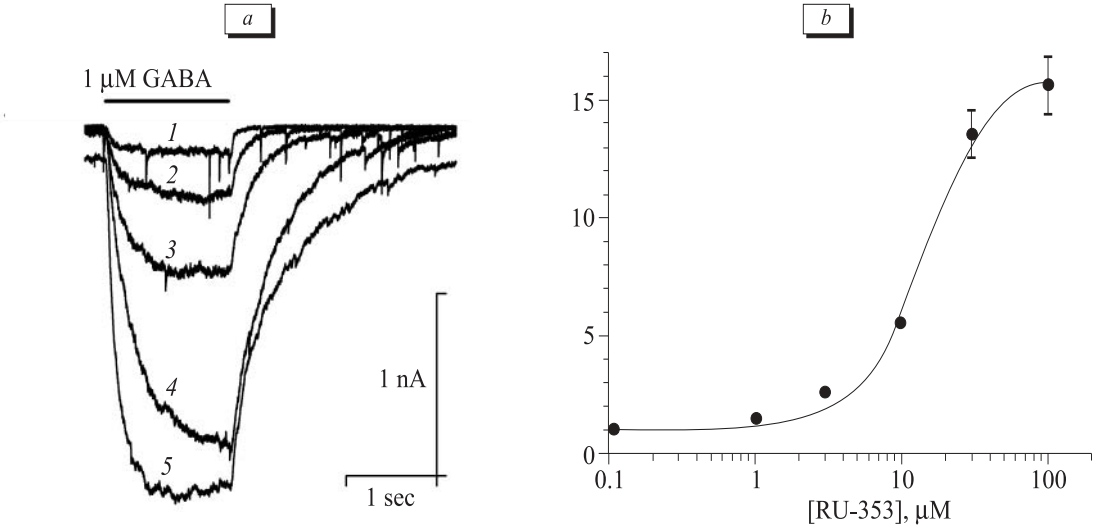

Fig. 1. Potentiation of GABA-activated currents in cerebellar Purkinje cells in the presence of RU-353. *a*: ionic currents induced by 1 μM GABA (horizontal bar) alone (*1*) and in the presence of increasing concentration of RU-353: *2*) 3 μM, *3*) 10 μM, *4*) 30 μM, *5*) 100 μM. Holding potential of -70 mV. *b*: dose-dependence of potentiating effect of RU-353 for currents induced by 1 μM GABA. Ordinate: amplitude of GABA-activated currents relative to the current induced by 1 μM GABA alone. EC_{50} RU-353 and Hill coefficient are 15 μM and 1.9, respectively.

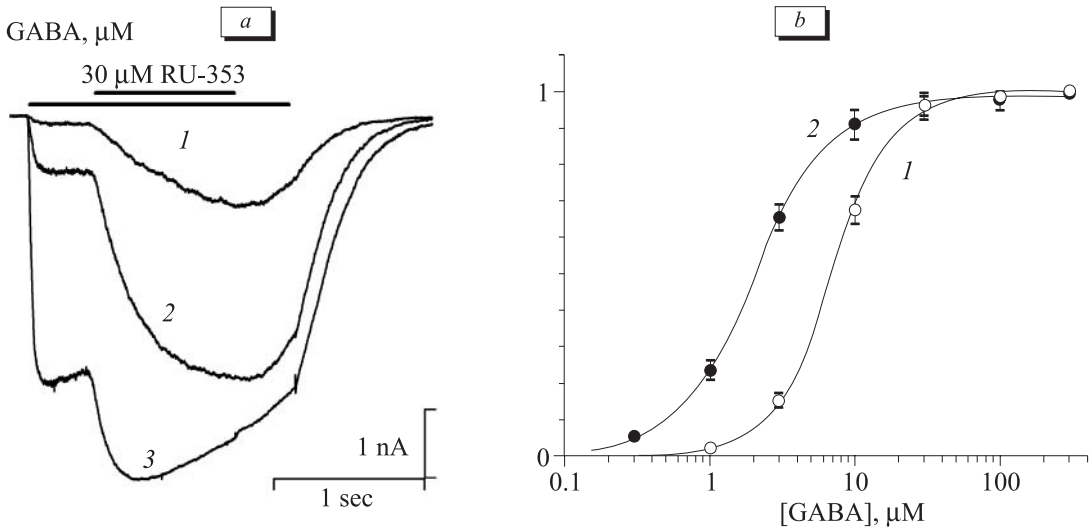

Fig. 2. Potentiating effect of RU-353 at different GABA concentrations. *a*: ionic currents recorded in a Purkinje cell during combined application of 30 μM RU-353 (the horizontal bar) and GABA in increasing concentrations: (*1*) 1 μM, *2*) 3 μM, *3*) 10 μM. *b*: dose—dependence of GABA in the control (*1*) and during combined application of GABA with 30 μM RU-353 (*2*). RU-353 shifted the GABA dose-dependence plot to the left. In the control, EC_{50} and Hill coefficient are 6.9 μM and 2.0, respectively. In the presence of 30 μM RU-353 they became 2.0 μM and 1.6, respectively. The currents were rated against the amplitude of the current induced by 300 μM GABA in the control.

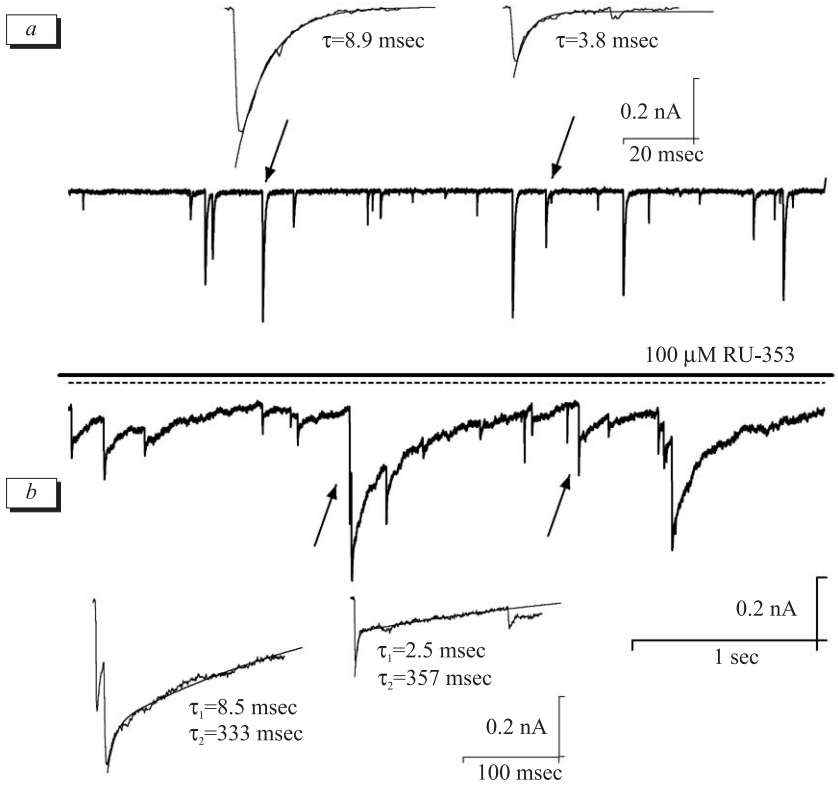

Fig. 3. Effect of RU-353 on spontaneous miniature inhibitory postsynaptic currents (mIPSCs) in isolated Purkinje cell. *a*: spontaneous activity in the control. The inserts shows individual mIPSCs (arrows) at more rapid sweep. The numbers are time constants of the tails fitted by one-exponent plot. *b*: ionic currents in the same cell in the presence of 100 μM RU-353. The inserts show individual mIPSCs (arrows) at greater sweep fitted by two exponents. The dashed line shows membrane potential.

the dose-response curve to the left, but did not change the maximum amplitude of the response (Fig. 2, *b*). In the control, the half-maximum effective GABA concentration was 6.9±0.1 μM with Hill coefficient of 2.00±0.05. In the presence of 30 μM RU-353, EC_{50} and Hill coefficient decreased to 2.0±0.1 μM and 1.6±0.1, respectively. At saturating GABA concentrations (≤100 μM), RU-353 produced no effect on the amplitude of the response. The left shift of the dose-response curve produced by the modulator and inability of this agent to change the amplitude of the maximum response showed that the effect of RU-353 is a result of its allosteric interaction with $GABA_A$ receptor, which increased GABA affinity to the receptor. Similar mechanism is characteristic of the action of some other modulators of $GABA_A$ receptors (diazepam, zolpidem, etomidate, *etc*.) [9].

Apart from currents induced by exogenous GABA, the isolated neurons demonstrated spontaneous synaptic currents. Mechanical isolation of neurons used in our study preserved synaptic boutons, which spontaneously release transmitters activating postsynaptic receptors. The voltage clamp technique makes it possible to resolve spontaneous miniature inhibitory postsynaptic currents (mIPSCs), which were observed in isolated neurons of the basolateral amygdala [8] and in some other preparations. We observed such mIPSCs in most part of isolated cerebellar Purkinje cells, although their frequency and amplitude varied. It was found that the observed mIPSCs were mostly related to the release of the transmitter from inhibitory terminals, which mediated their effect via $GABA_A$ receptor, because virtually all mIPSCs were blocked with bicuculline and gabazine. The external RU-353 markedly increased the duration of mIPSCs (Fig. 3, *b*), which led to summation of frequently generated mIPSCs into the total inward current lasting for up to 1 sec. Under control conditions, the tail of mIPSCs was monoexponential with time constant τ=3-9 msec (the inserts in Fig. 3, *a*). RU-353 (100 μM) significantly increased the tail duration. Moreover, the tail became bi-exponential. The first exponent (τ_1) was similar to the control, but the second exponent (τ_2) was tens times greater (300-400 msec, Fig. 3, *b*). In the presence of gabazine, mIPSCs were almost completely blocked, and no effects of RU-353 were observed. Pronounced deceleration of mIPSCs in the presence of RU-353 shows that this modulator can potentiate the inhibitory effects of GABA during in synaptic transmission *in vivo*.

Therefore, in central neurons RU-353 acts as a positive allosteric modulator of $GABA_A$ receptors. It can be hypothesized that the antiarrhythmic effects revealed during the direct application of this agent [5] into the sympathetic activation center in the medulla oblongata relate mostly to its ability to potentiate the inhibition mediated by $GABA_A$ receptors. It cannot be excluded that the antiarrhythmic effects of RU-353 observed for the arrhythmias of peripheral origin [4] could be partially explained by its central effects.

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