Effect of Metabotropic Receptor Agonists on Ionic Current of Snail Neurons

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The dose-dependent and reversible changes of sodium (I_{Na}), calcium (I_{ca}), slow potassium (I_{Ks}), and fast potassium (I_{Kf}) currents were recorded in isolated snail neurons under the action of κ-opioid agonist butorphanol and chemical agent RU-1203 applied in a concentration range of 1-1000 μM.

Key Words: κ-*receptors; ionic currents; snail neurons;* butorphanol; benzimidazoles

κ-Opioid receptors belong to superfamily of metabotropic G-protein-coupled receptors expressed both in CNS and at the periphery; they are controlled by specific ligands and trigger various signal transduction cascades resulting in unique effects relating to their individual receptor type [4]. Stimulation of κ-receptor structures inhibits adenylate cyclase activity and modulates the calcium as well as potassium permeability [7-9]. Due to negative coupling with adenylate cyclase signaling and cAMP down-regulation, the agents exhibiting the properties of selective κ-agonists will also efficiently block sodium channels resulting in moderation of pacemaker activity, decrease in the amplitude of action potentials, and finally restriction of the flow of nociceptive signals to CNS [5,11].

However, hypothetical possibility of involvement of supplementary non-opioid mechanisms in realization of various pharmacological effects of κ-receptor ligands based on their direct inhibitory action on voltage-operated ionic channels cannot be excluded [6,10,12].

This work was designed to study the changes of membrane ionic currents in isolated snail neurons induced by extracellular application of κ-opioid agonist butorphanol and a synthesized agent RU-1203 with similar properties [2] within the concentration range of 1 to 1000 μM.

MATERIALS AND METHODS

Experiments were carried out with intracellular dialysis and voltage clamp [1,3]. An isolated cell was attached to a polyethylene pipette (in most cases at the holding potential of -90 mV) where the negative pressure pulses were generated to destruct the membrane within the orifice of pipette thereby establishing electric contact between the intracellular medium and a nonpolarizable electrode coupled to a voltage clamp apparatus.

After recording the total ionic currents, the internal and external solutions were replaced which specific salines to measure individual ionic current components [1]. The net calcium or sodium currents with stable parameters considered thereafter as "initial" (control) values were recorded in 3-5 min after complete replacement of solutions; thereafter the test solutions were applied starting from the smallest concentration.

The examined agents were *1*) partial agonist/antagonist μ-opioid receptors and agonist of κ-opioid receptors butorphanol (17-(cyclobutylmethyl)-morphinan-3,14-diol, Moscow Pharmaceutical Factory) and *2*) an agent under a laboratory name of RU-1203

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TABLE 1. Dose-Dependent Effect of RU-1203 (RU) and Butorphanol (BP) on Ionic Currents in Snail Neurons (%; *M±tm*, *n*=5-11)

Ionic current normalized amplitude	Concentration (μM)							
	1		10		100		1000	
	RU	BP	RU	BP	RU	BP	RU	BP
I_{Na}	100.1±2.6	97.5±6.4	95.4±2.8	77.8±8.5	81.8±5.2	29.7±7.2	7.4±6.2	0
I_{Ca}	107.0±3.3	97.2±3.0	102.3±2.5	71.4±6.8	81.1±8.5	44.7±7.2	18.5±5.9	0
I_{Ks}	104.7±3.3	94.2±4.3	101.1±2.0	85.1±8.9	91.5±3.0	54.5±9.6	22.3±5.8	27.1±5.9
I_{Kf}	106.5±5.2	100	100.6±2.4	99.2±1.4	80.7±9.5	84.8±5.4	31.4±6.1	0

Note. Here and in Tables 2: I_{Na}, I_{Ca}, I_{Ks}, and I_{Kf} are the normalized amplitudes of sodium, calcium, slow potassium, and fast potassium ionic currents, respectively. *tm* is the confidence interval for *p*=95%.

(2-fluorophenyl-9-pyrrolidinoethyl-imidazo[1,2-a]benzimidazole). They were tested in concentrations of 1, 10, 100, and 1000 μM. In 2-3 minutes needed to stabilize the ionic currents perturbed by the tested agents, they were recorded to assess the pharmacological effect. Then other concentrations were tested in increasing order. Finally, the initial (agent-free) solution was applied to wash the membrane from the tested agents and to observe restoration of the ionic currents.

The recorded currents were used to plot I-E relations and the dose-response curves. The currents were measured in percentage to their initial amplitude. The data were analyzed statistically using Student's *t* test.

RESULTS

Table 1 summarizes the data on the effect of RU-1203 and butorphanol in various concentrations on the ionic currents in snail neurons. Both substances reversibly inhibited all examined ionic currents in a dose-dependent manner. All the currents restored after a 5-7 washing period virtually to 100% their initial value attesting to a "moderate" degree of binding of the tested agents with the receptive sites of the channels or the nearby membranous structures.

When applied in concentrations of 10 and 100 μM, butorphanol significantly decreased the amplitudes of sodium current to 77.8±8.5% and 29.70±7.23% initial value, respectively. At the greater concentration of 1000 μM, it virtually eliminated the sodium current. Butorphanol produced no changes in kinetics of sodium currents neither a voltage shift in the maximum of their I-E curve. Washing the neurons from this agent for 5-7 min restored sodium currents to 81.60±8.36% initial value. At 1 and 10 μM, butorphanol produced no effect on non-specific leakage current, although the greater concentrations of this agent (100 and 1000 μM) increase it somewhat by 0.5-4.0 nA.

TABLE 2. Half-Maximal Effective Blocking Concentrations (EC_{50}, μM) of RU-1203 and Butorphanol

Substance	I_{Na}	I_{Ca}	I_{Ks}	I_{Kf}
Butorphanol	68.5	70.8	77.9	93.5
Ru-1203	91.4	96.1	98.1	95.4

RU-1203 inhibited the inward sodium current in a dose-dependent manner, produced no effect on kinetics of these currents, and reversibly shifted the maximum of I-E relations to the right (*i.e.*, to depolarizing potentials, Fig. 1, *a*) indicating alteration of the membrane surface potential generated by the fixed charges.

Moreover, butorphanol significantly decreased the calcium currents. At this, it slowed down inactivation but produced no shift in I-E plot. It is noteworthy that butorphanol (100 and 1000 μM) pronouncedly increased the leakage current by 2-7 nA thereby destabilizing the neuron membrane.

The changes of calcium currents induced by RU-1203 in various concentrations are shown in Table 1. This agent accelerated calcium inactivation and slightly shifted the maximum of I-E plot to the right (Fig. 1, *b*). It is noteworthy, that washing the neurons from RU-1203 (1000 μM) for 5-7 min did not completely restore the amplitude of calcium currents.

Butorphanol decreased slow potassium currents (I_{Ks}) to 94.20±4.28% when applied in the smallest tested concentration of 1 μM. At greater concentrations of 10, 100, and 1000 μM, it decreased the amplitude of I_{Ks} to 85.10±8.91%, 54.5±9.6%, and 27.10±5.92%, respectively. In addition, butorphanol produced a certain deceleration of I_{Ks} activation. Washing of the

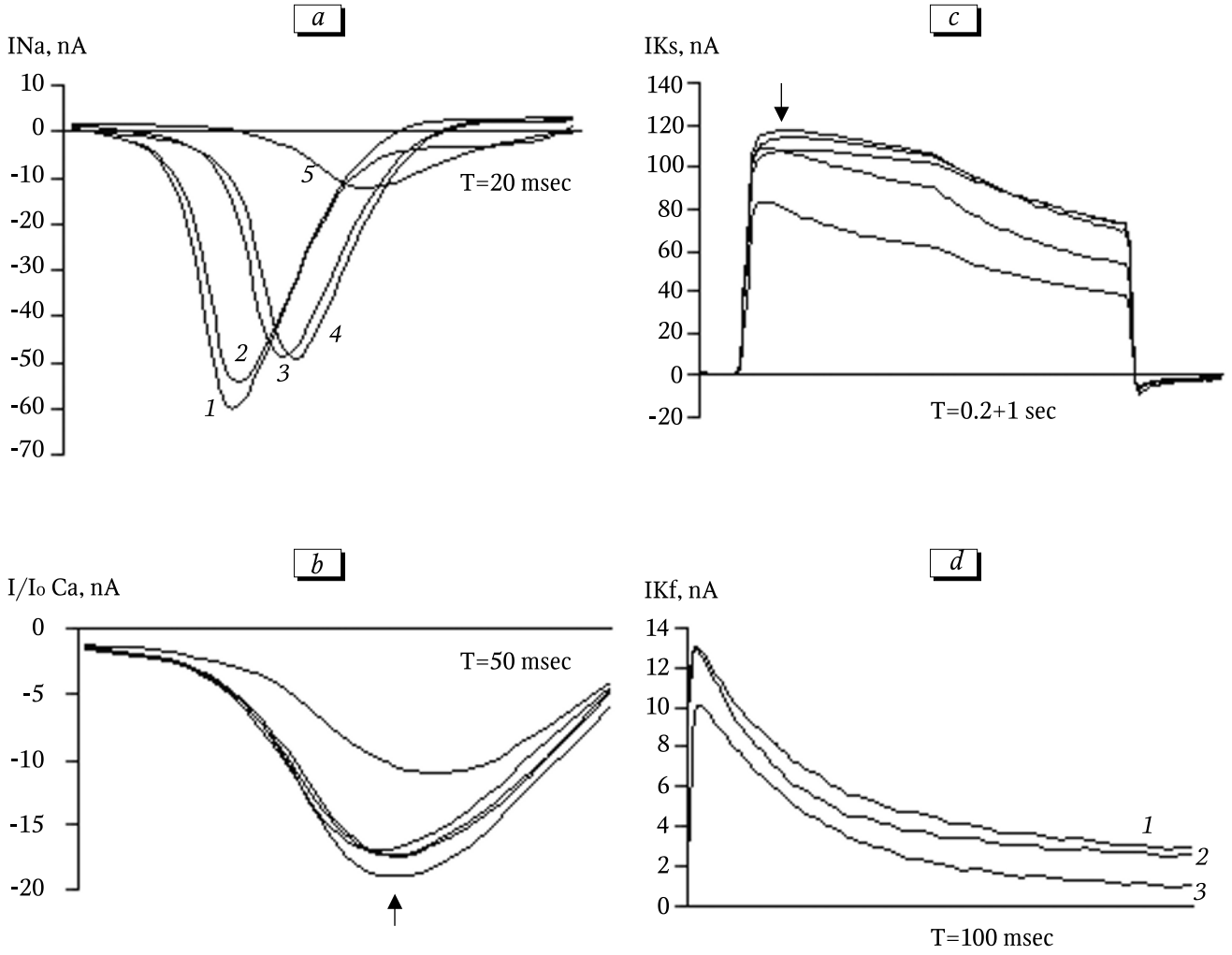

Fig. 1. Dose-dependent effect of RU-1203 on I_{Na}, I_{Ca}, I_{Ks}, and I_{Kf} in snail neurons. *a*) I-E curves of sodium channels. Total abscissa length: duration (20 msec) of voltage ramp from -40 to +10 mV. *1*) control; *2*) washing; *3*) 10 μM; *4*) 100 μM; *5*) 1000 μM. Note the shift of I-E maximum to the right (curves 3-5), *i.e.*, to more positive potentials by 10-20 mV. *b*) I-E curves of calcium channels. Total abscissa length: duration (50 msec) of voltage ramp from -40 to +30 mV; the curves from bottom to top above the arrow: control, 10 μM, washing (the latter curves almost fused), 100 μM, and 1000 μM (note the shift of I-V maximum to the right in latter case). *c*) the changes of slow potassium currents evoked by rectangular voltage pulses from holding potential -90 mV to +40 mV. The first and second halves of the current traces are shown at different sweeps comprising the time segments of 0.2 and 1 sec, respectively. The traces from top to bottom below the arrow: 1 μM, washing, control, 10 μM, and 1000 μM. *d*) the changes of fast potassium currents evoked by voltage ramp pulse. Total abscissa length: duration (100 msec) of voltage ramp. *1*) control; *2*) washing; *3*) 100 μM.

neurons from butorphanol for 5-7 min restored I_{Ks} to 86.70±9.62% initial value. Butorphanol (1000 μM) increased the non-specific leakage current by 0.3-0.5 nA.

Similar to butorphanol, RY-1203 also decreased the amplitude of slow potassium currents in a dose-dependent manner (Fig. 1, *c*). This effect was the most pronounced at 1000 μM RU-1203 as indicated by the decrease in the slope of I-E plots.

Inhibitory action of butorphanol on fast potassium currents (I_{Kf}) was observed only at a large concentration of 100 μM, which decreased I_{Kf} amplitude to 84.8±5.4%, but did not modify its kinetics. The greatest tested concentration (1000 μM) virtually eliminated I_{Kf}. Washing the neurons for 5-7 min restored I_{Kf} amplitude to 95.20±6.67% relatively to initial value. When applied at concentrations of 100 or 1000 μM, butorphanol pronouncedly increased the leakage current by 5-7 nA.

Generally, the mode of action of RU-1203 on fast potassium currents was similar to its action of slow potassium currents, but the effect was somewhat smaller. Washing the neurons resulted in almost complete restoration of I_{Kf} to initial values. RU-1203 produced no effect on I_{Kf} kinetics (Fig. 1, *d*).

It should be stressed that in contrast to butorphanol, RU-1203 decreased the leakage current during recording of any type of ionic currents virtually in the entire range of the tested concentrations, which probably attests to its membrane stabilizing potency.

Effective concentrations (EC_{50}) of the tested agents producing a 50% decrease of the examined

ionic currents showed that butorphanol was more efficient blocker than RU-1203 (Table 2). However, EC50 values calculated for various types of ionic currents inhibited by any of the examined substance were rather similar attesting to a non-selective blocking action of these agents.

Overall, this study demonstrated that butorphanol and RU-1203 are highly efficient membranotropic agents with comparable blocking potency to that of local anesthetics or cardiac antiarrhythmic drugs [1]. It is also important that interaction of butorphanol and RU-1203 with membranous structures of snail neurons was dose-dependent, reversible, and not especially slow. In contrast to butorphanol, RU-1203 decreased the non-specific leakage current in all tested concentrations.

REFERENCES

1. A. I. Vislobokov, Yu. D. Ignatov, P. A. Galenko-Yaroshevsky, P. D. Shabanov, *Membranotropic Effects of Pharmacological Agents* [in Russian], St. Petersburg, Krasnodar (2010).
2. A. A. Spasov, O. Yu. Grechko, N. V. Eliseeva, and V. A. Anisimova, in: *Eksp. Klin. Farmakol., Suppl. 5. International Conference "Biological Fundamentals of Individual Sensitivity to Psychotropic Agents"*, (2010), p. 38.
3. J. V. Aldrich and J. P. McLaughlin, *AAPS J.*, **11**, No. 2, 312-322 (2009).
4. M. R. Bruchas and C. Chavkin, *Psychopharmacology* (Berl.), **210**, No. 2, 137-147 (2010).
5. A. Hudmon, J. S. Choi, L. Tyrrell, *et al*., *J. Neurosci*., **28**, No. 12, 3190-3201 (2008).
6. S. K. Joshi, K. Lamb, K. Bielefeildt, G. F. Gebhart, *J. Pharmacol. Exp. Ther*., **307**, No. 1, 367-372 (2003).
7. M. J. Marinissen and J. S. Gutkind, *Trends Pharmacol. Sci*., **22**, No. 7, 368-376 (2001).
8. G. Piceyro, *Cell. Signal*., **21**, No. 2, 179-185 (2009).
9. R. Sadja, N. Alagem, and E. Reuveny, *Neuron*, **39**, No. l, 9-12 (2003).
10. X. Su, S. K. Joshi, S. Kardos, and G. F. Gebhart, *J. Neurophysiol*., **87**, No. 3, 1271-1279 (2002).
11. A. M. Trescot, S. Datta, M. Lee, and H. Hansen, *Pain Physician*, **11**, No. 2, Suppl. S133-S153 (2008).
12. V. Yarov, J. Brown, E. M. Sharp, *et al*., *J. Biol. Chem*., **5**, No. 1, 20-27 (2001).