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Mechanisms Underlying Combined Action of Clonidine and Local Anesthetics

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We studied the mechanisms of combined action of clonidine and local anesthetics amethocaine and imidazo-benzimidazole derivative RU-1117. In contrast to amethocaine, RU-1117 in therapeutic concentrations binds to imidazoline receptors and, to a lesser extent, to α_{2A}-adrenoceptors on human platelets. Clonidine and RU-1117 produce opposite effects on platelet aggregation induced by ADP in low concentrations. Our results suggest that the pharmacodynamic interaction of clonidine and RU-1117 is associated with their ability to activate the imidazolinergic system.

Key Words: *local anesthetics; clonidine; α_2-adrenoceptors; imidazoline receptors; human platelets*

α_2-Sympathomimetics are promising analgesic compounds possessing spinal antinociceptive, anxiolytic, and sedative properties. They improve blood hemodynamics and produce a positive effect during perioperative ischemia [5]. Clonidine, most frequently prescribed drug, stimulates not only α-adrenoceptors, but also imidazoline receptors. Published data show that arrhythmogenic and hypotensive changes during surgeries are associated with activation of these receptors [3,6].

Our recent studies revealed a synergetic effect of amethocaine and new anesthetic RU-1117 administered in combination with clonidine [1]. The analgesic effect of local anesthetics was potentiated and prolonged. However, combination treatment with RU-1117 and clonidine was accompanied by an increase in toxicity.

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Here we evaluated trigger mechanisms underlying combined action of anesthetics and clonidine. The effects mediated by imidazoline receptors and adrenoceptors were differentiated by the method consisting in evaluation of affinity and intrinsic activity of compounds in relation to imidazoline receptors and α_2-adrenoceptors on platelets [2].

MATERIALS AND METHODS

The blood (10 ml) was taken from the cubital vein and placed in a plastic tube containing 1.5 ml conserving solution (5 mM glucose, 85 mM sodium citrate, and 65 mM citric acid; pH 6.55). Platelet-rich plasma (PRP) was obtained by centrifugation at 200*g* for 10 min, the supernatant (2.5 ml) was carefully harvested. Cell count was estimated in a Goryaev chamber. PRP was diluted with platelet-depleted plasma to a concentration of $250\text{-}350\times10^9$ platelets/liter. The remaining supernatant was centrifuged at 600*g* for 20 min. The method for isolation of washed platelets included consecutive washing of PRP samples in 3 modified Tyrode's solu-

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tions. T_1 contained 2.8 mM KCl, 2 mM $MgCl_2$, 0.36 mM NaH_2PO_4, 138 mM NaCl, 0.2 mM ethylene glycol-bis(β-aminoethyl ether) N,N,N,N'-tetraacetic acid (EGTA), and 10 mM Hepes. T_2 had a similar composition except for EGTA. T_3 contained 2.8 mM KCl, 0.5 mM $MgCl_2$, 0.36 mM NaH_2PO_4, 138 mM NaCl, 10 mM Hepes, and 1 mM $CaCl_2$ (pH 7.4). Glucose (5 mM) was *ex tempore* added to solutions T_1 and T_2, and pH was brought to 6.55.

Platelet aggregation was recorded on a Biolan device. For standardization we used PRP containing $250\text{-}350\times10^9$ platelets/liter. The measurements were performed on a stir plate at 800 rpm and 37°C.

The effects of the test compounds on induced changes in light transmission were evaluated by the following aggregation parameters: maximum amplitude (percentage of the increase in light transmission after ADP administration); period between induction of aggregation and maximum light transmission; and maximum rate of aggregation. The results were analyzed by Student's *t* test.

RESULTS

For verification of the hypothesis on possible interaction of the test compounds at the level of adrenoceptors and imidazoline receptors we evaluated the ability of clonidine, amethocaine (dicaine, tricaine), and RU-1117 to bind and activate the corresponding receptors on platelets. We chose platelets as the object of investigation since these cells carry membrane imidazoline receptors and α_{2A}-adrenoceptors. These receptors are responsible for the antinociceptive effect of sympathomimetic compounds [8]. This was confirmed in previous experiments on mice with low number of α_{2A}-adrenoceptors (encoding gene knockdown, suppression of synthesis of receptor molecules in the antisense nucleotide sequence during translation) or lacking these receptors (knockout mice, blockade of the gene responsible for the synthesis of receptors in embryogenesis) [4].

We combined data on relative affinity of test compounds for imidazoline receptors and α_{2A}-adrenoceptors on platelets (concentration of inhibitor, IC_{50}; Table 1). Affinity of the test substances for imidazoline binding sites was evaluated by their ability to compete with H^3-idazoxan in the presence of 500-fold excess of norepinephrine displacing the labeled ligand from adrenoceptors [7]. Affinity of substances for α_{2A}-adrenoceptors was determined by competition with H^3-idazoxan for binding to platelets in the presence of 500-fold excess of moxonidine (selective ligand of imidazoline receptors). As differentiated from clonidine, amethocaine in concentrations of 0.1-100 μM did not bind to receptors. This concentration range included variations in therapeutic concentration in the blood (Table 1). RU-1117 efficiently competed for imidazoline binding sites. Relative affinity of this anesthetic for α_{2A}-adrenoceptors was much lower.

For evaluation of pharmacodynamic properties of the test compounds we studied their effects on ADP-induced platelet aggregation. This test system differentiates chemical substances by their relative ability to activate/inhibit imidazoline receptors and α_{2A}-ad-

TABLE 1. Competitive Binding of Test Compounds to Platelets (IC_{50})

Conditions	Clonidine	RU-1117	Amethocaine
^{3}H-idazoxan and norepinephrine	175 nM	330 nM	$>10^{-4}$ M
^{3}H-idazoxan and moxonidine	68 nM	4 μM	$>10^{-4}$ M

Note. Final concentration of labeled idazoxan: 40 nM. Incubation: 4°C, 2 h.

TABLE 2. Effects of Test Compounds on Human Platelet Aggregation Induced by 0.25 μM ADP (*M*±*m*)

Experimental conditions		Maximum aggregation amplitude, %	Maximum rate, rel. units	Aggregation time, min
Control (ADP, 0.25 μM)		23.5±2.0	144±15	3.8±0.2
RU-1117	2 μM	20.9±1.7	120±13	3.7±0.2
	5 μM	16.8±2.2*	101±14*	3.5±0.1
Clonidine	2 μM	31.1±2.7*	193±14*	3.3±0.1
	5 2 μM	33.7±2.6*	209±14*	3.1±0.1
Amethocaine	2 μM	22.4±2.3	141±15	3.7±0.1
	5 μM	25.3±2.0	145±12	3.9±0.2

Note. Results of 3-5 independent experiments. *$p<0.05$ compared to the control.

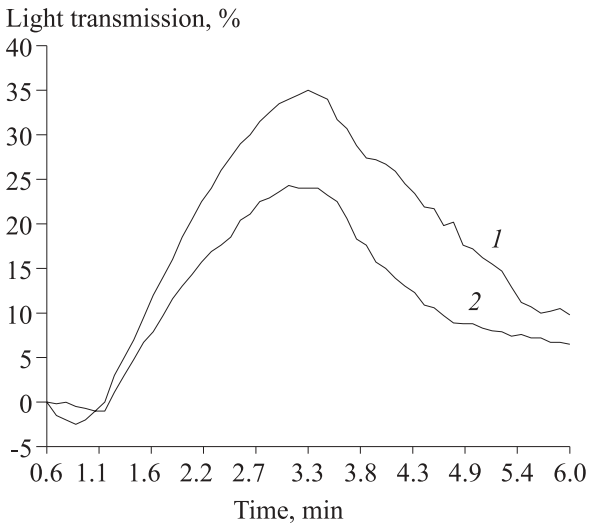

Fig. 1. Effect of RU-1117 on ADP-induced aggregation: ADP (0.5 μM, *1*); ADP (0.5 μM) and RU-1117 (5 μM, *2*).

renoceptors [2]. Parameters of ADP-induced platelet aggregation were determined in the presence of various compounds (Table 2). RU-1117 inhibited platelet aggregation (Fig. 1), which can be explained by predominant activation imidazoline receptors, but not adrenoceptors on platelets. Published data show that the ratio between binding affinities of clonidine for adrenoceptors and imidazoline receptors is 16 [5]. Preferential binding of clonidine to α_{2A}-adrenoceptors explains its potentiating effect on ADP-induced aggregation. In our experiments amethocaine had no effect on ADP-induced platelet aggregation. Therefore, a new anesthetic RU-1117 can bind to and activate imidazoline receptors. There is little likelihood that the effects of RU-1117 in therapeutic concentrations are mediated by α_{2A}-adrenoceptors.

Our results suggest that the pharmacodynamic interaction of clonidine and RU-1117 is associated with their ability to activate imidazoline receptors.

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