PHARMACOLOGY

Experimental Approach to Differentiation of the Effects Mediated by Imidazole Receptors and α_2-Adrenoceptors on Platelets

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We studied parameters of specific binding for various ligands of imidazole receptors and α_2-adrenoceptors on human platelets. Pharmacological activity of compounds was evaluated by their effects on platelet aggregation induced by ADP in low concentrations (0.125-1.5 μM). In contrast to α_2-adrenoceptor agonist norepinephrine inducing reversible aggregation of cells, selective stimulation of imidazole receptors with moxonidine produced a disaggregation effect. The data suggest that human platelets can be used as an experimental test system for screening and study of molecular mechanisms underlying the influence of new compounds.

Key Words: *α_2-adrenoceptors; imidazole receptors; human platelets; aggregation*

The hypotensive effect of preparations with central mechanism of action is related to activation of imidazole receptors in the lateral reticular nuclei (*nucleus reticularis lateralis*) of the rostroventrolateral brain region [3,6]. First-generation preparations methyldopa, clonidine, and guanfacine similarly stimulate central imidazole receptors (mainly I_1 receptors) and α_2-adrenoceptors of the locus ceruleus in the pons. These peculiarities explain high incidence of various side effects, including sedation, respiratory depression, orthostatic hypotension, and rebound syndrome (blood pressure rise) after treatment with these drugs [4]. Second-generation preparations moxonidine and rilmenidine selectively bind to I_1 central imidazole receptors and do not cause side effects [5,9].

Radioligand assay allows evaluation of receptor selectivity for new compounds. However, this approach has several limitations. Labeled receptor agonists differently bind to free receptors and receptor-G protein complexes, which makes interpretation of the results difficult [10]. Moreover, specific binding of test substances reflects only their potential activity. Further selection of compounds requires studying of their receptor-mediated intracellular effects. The existence of peripheral imidazole receptors and α_2-adrenoceptors helps to solve this problem, because the response of target cells can serve as a marker of efficiency of new compounds. Human platelets hold much promise in this respect. Platelet membrane contains imidazole receptors and α_2-adrenoceptors. The response of these cells (aggregation) is well studied and easily reproducible [7,8].

Here we developed the method for differential evaluation of the effects mediated by imidazole receptors and/or α_2-adrenoceptors on platelets.

MATERIALS AND METHODS

The method for obtaining suspension of washed platelets was described elsewhere [1]. The receptor agonist

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^{3}H-clonidine (specific activity 70-110 Ci/mmol) and receptor antagonist ^{3}H-idazoxan (65-80 Ci/mol) were used to study receptor binding. Unlabeled ligands (500-fold excess) were presented by selective agonist and antagonists of α_2-adrenoceptors clonidine, yohimbine, and atipamezole and selective agonist of imidazole receptors moxonidine. The suspension of washed cells (10^9/ml) was incubated with labeled compounds (2-50 nM) in the presence or absence of unlabeled substances at 4°C for 2 h. Unbound radiolabeled ligand was removed. Aliquots of platelet suspension (0.1 ml) were placed on GF/C Whatman filters and washed 2 times with 5 ml HEPES buffer at 4°C. Filters were dried and placed in scintillation vials for radiometry.

Parameters of specific binding were determined by Scatchard analysis. Platelet aggregation was studied by the method of Born O'Braine [2]. The results were analyzed by Student's *t* test.

RESULTS

In series I we determined parameters of specific binding for imidazole receptors and α_2-adrenoceptors on platelets. Competitive radioligand assay showed that platelets contain 2 types of specific binding sites differing in their relative affinity for various ligands. Type I receptors displayed affinity for selective α_2-adrenoceptor ligands. The equilibrium dissociation constant (K_d) and maximum binding capacity (B_{max}) for type I receptors were 4.8±1.5 nM and 120±36 fmol/mg protein, respectively. Relative binding activity decreased in the following order: idazoxan → norepinephrine → yohimbine → clonidine → moxonidine.

The K_d and the B_{max} for type II specific imidazole receptors were 11.0±2.1 nM and 274±30 fmol/mg protein, respectively. Affinity of compounds decreased in the following order: moxonidine → clonidine → idazoxan → norepinephrine. By these parameters, binding sites correspond to α_2-adrenoceptors and I_1 imidazole receptors on platelets [10].

In series II we evaluated directionality and degree of the effects produced by test compounds on spontaneous and induced aggregation of platelets.

The influence of preparations on spontaneous platelet aggregation was characterized by considerable intra- and interindividual differences. Therefore, this parameter could not be used to differentiate the effects of substances.

Experiments with platelet-stimulating agent ADP in subthreshold (0.125 μM) and minimal concentrations (0.25 and 0.5 μM) revealed considerable differences in the effect of compounds on induced platelet aggregation (Table 1). The maximum rate of platelet aggregation (*V*) served as a criterion for the degree of aggregation. In control samples not containing test compounds *V* decreased proportionally to the increase in ADP concentration. Aggregation was reversible. The addition of norepinephrine in concentrations of 1 and 2 μM (most significantly) to the cell suspension stimulated platelet aggregation. This effect was especially pronounced after the addition of ADP in low doses.

Clonidine caused no considerable changes in induced platelets aggregation. Significant differences were observed only after treatment with 2 μM clonidine.

The selective agonist of imidazole receptors moxonidine produced an opposite effect on platelet aggregation. Increasing the concentration of moxonidine from 1 to 5 μM led to a decrease in the platelet aggregation rate. Moreover, moxonidine sometimes produced a disaggregation effect.

α_2-Adrenoceptor antagonists atipamezole and yohimbine had no effect on ADP-induced aggregation, reduced cell response to α_2-receptor agonists, and did not modulate the effect of moxonidine.

The differences in K_d (about 10^{-8} M) and acting concentrations of preparations (micromolar range) observed in studying platelet aggregation are related to the formation of complexes with plasma proteins and intracellular accumulation of compounds. It should be emphasized that series I and II were performed on washed platelets and platelet-rich plasma, respectively.

Our results indicate that the response of platelets to various compounds is determined by activation/inhibition of imidazole receptors and α_2-adrenoceptors. Thus, human platelets can be used as an experimental test system for screening and studying of the

TABLE 1. Effects of Test Compounds on the Maximum Rate of Human Platelet Aggregation Induced by ADP (rel. units, *M*±*m*)

Experimental conditions	ADP, μM		
	0.125	0.25	0.5
Control	105±9	144±15	183±19
Norepinephrine			
1 μM	129±10	190±16*	206±16
2 μM	143±11*	221±19*	230±17*
Clonidine			
1 μM	110±9	171±18	199±17
2 μM	125±9*	193±14*	210±14
Moxonidine			
1 μM	107±10	140±15	188±16
2 μM	110±11	135±12	172±16
5 μM	102±9	122±10*	153±15

Note. Results of 3-5 independent experiments. *p<005 compared to the control.

molecular mechanisms underlying the influence of compounds that bind to imidazole receptors and α_2-adrenoceptors.

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