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MODULATING ACTION OF ADENOSINE ON HUMAN PLATELET ACTIVATION

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KEY WORDS: human platelets; platelet activating factor; ADP; serotonin; adenosine

The study of the functional state of human platelets is of great practical importance for the elucidation of the pattern of development of pathological processes taking place with the participation of these cells. These are, primarily, changes in the blood clotting system connected with hyper- and hyposensitivity of the platelets to the action of activating factors. The study of these cells in connection with their role in the formation of morphological and functional changes in the bronchopulmonary system also remains an urgent problem [7]. In addition, the use of platelets as a model with which to study the state of receptor systems can also help to shed light on the receptor-mediated processes that take place in other cell systems. In the light of these facts it is very interesting to study the action on the cell of physiological effector agents, with specific receptors on the platelet cell surface, such as platelet activating factor (PAF), adenosine diphosphate (ADP), and serotonin (5-HT) [2, 5, 8], and also the ability of adenosine to modulate the formation of the cell response [9]. The reason for the urgency of the study of the action of adenosine is the important role played by this endogenous compound in the regulation of the different systems of the body under both normal and pathological conditions.

The aim of this investigation was to compare changes in the cell response of platelets during modulation of the stimulating effect of PAF, ADP, and 5-HT by adenosine.

EXPERIMENTAL METHOD

Venous blood was obtained from healthy donors and mixed in the ratio 9:1 by volume with anticoagulant of the following composition (in %): sodium citrate 2.5, citric acid 1.37, glucose 20 (pH 6.5). The blood was centrifuged for 15 min at room temperature and at 200*g* to obtain platelet-enriched plasma (PEP). After removal the PEP was subjected to further centrifugation for 10 min at 2000*g* in order to obtain platelet-deprived plasma, which was used in order to adjust the cell count in PEP to $10^5/\mu l$. The aggregating power of the platelets under the influence of agonists was assessed with the aid of an "Elvi-840" two-channel aggregometer (Italy), with simultaneous recording of

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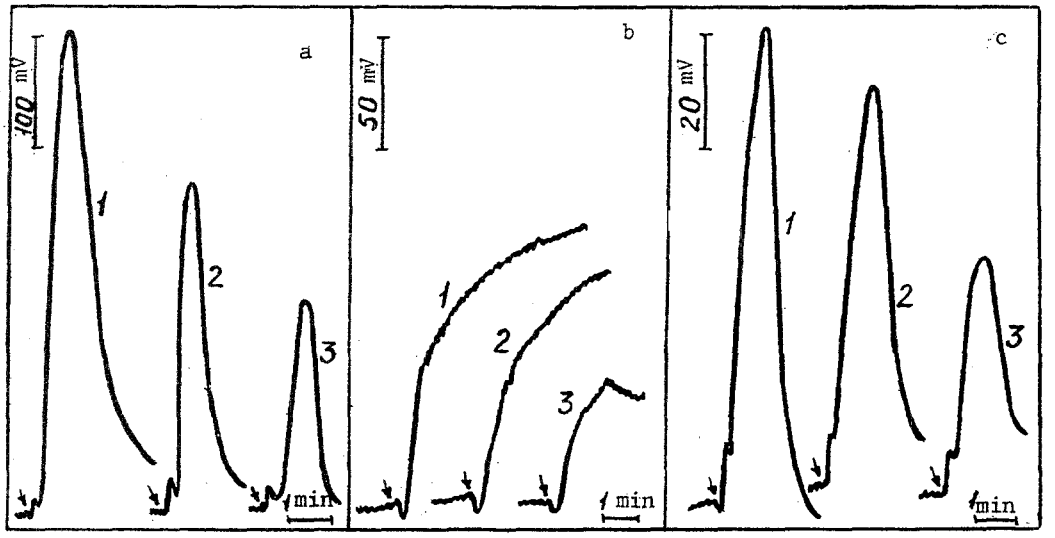

Fig. 1

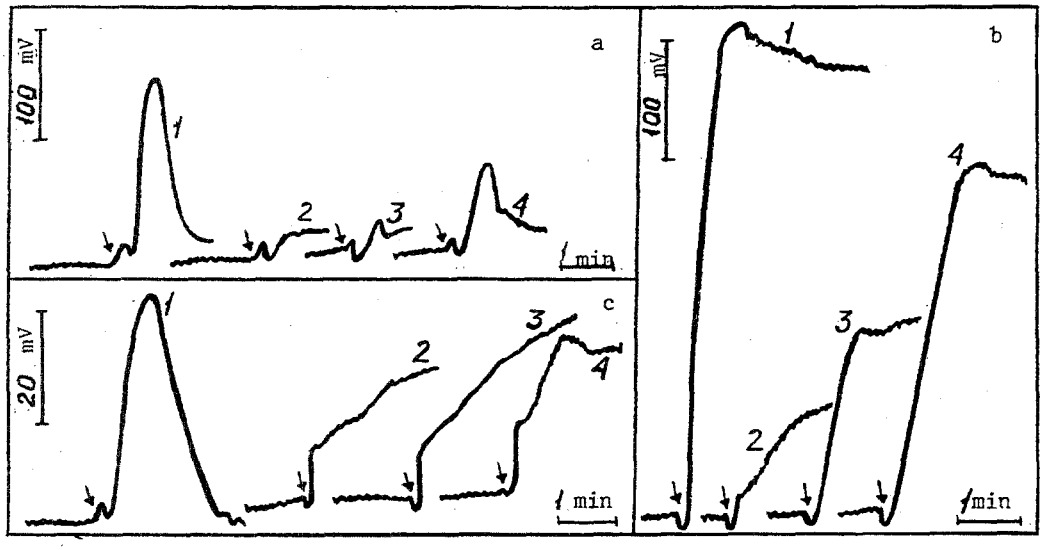

Fig. 2

Fig. 1. Transmittance of human platelet suspension under activation by agonists: a) aggregation curves obtained under the influence of PAF in concentrations of: 1) 0.2 μM, 2) 0.1 μM, 3) 0.05 μM; b) aggregation curves obtained under the influence of ADP in concentrations of: 1) 20 μM, 2) 10 μM, 3) 4 μM; c) aggregation curves obtained under the influence of 5-HT in concentrations of: 1) 5 μM, 2) 1 μM, 3) 0.5 μ. Arrow indicates time of addition of agonist.

Fig. 2. Effect of adenosine on aggregating power of human platelets: a) activation of platelets by 0.04 μM PAF after preliminary incubation of cells with adenosine in concentrations of: 1) 0 μM, 2) 10 μM, 3) 5 μM, 4) 1 μM; b) activation of platelets by 4 μM ADP in presence of adenosine in concentrations of: 1) 0 μM, 2) 10 μM, 3) 5 μM, 4) 0.8 μM. Time of addition of agonist indicated by arrow.

the signal on a two-channel automatic writer (from "LKB," Sweden). After incubation of the cell suspension at 37°C and mixing at a speed of 1000 rpm in the course of 1 min, the test ligands were added in the following concentration range: PAF 0.01-0.20 μM, ADP 1-20 μM, 5-HT 0.1-5 μM. To study the modulating action of adenosine on the cellular response, before activation of the platelets the cells were incubated for 2 min with 0.1-10 μM adenosine.

EXPERIMENTAL RESULTS

Typical aggregation curves reflecting changes in transmittance of light by PEP in response to activation of platelets by PAF, ADP, and 5-HT are shown in Fig. 1. Values of effective concentrations (EC_{50}, in μM, M ± m), were obtained for all compounds, and their values were: for PAF 0.04 ± 0.01, for ADP 4.23 ± 0.70, and for 5-HT 0.61 ± 0.22. The study of changes in the cellular response of the platelets in the presence of adenosine was undertaken during their activation by agonists in concentrations equal to the mean values of EC_{50} for each ligand. In the presence of adenosine, the aggregating power of the platelets in response to stimulation by these same effectors was diminished. Values of effective inhibitory concentrations of adenosine (IC_{50}, μM, M ± m) were: 0.63 ± 0.11 for PAF, 1.47 ± 0.31 for ADP, and 0.64 ± 0.18 for 5-HT. As the results show, IC_{50} of adenosine with respect to its modulation of ADP-induced aggregation was roughly half compared with values of effective inhibitory concentrations of this compound when acting on PAF and 5-HT-platelet activation. The inhibitory effect of the nucleoside was most marked in the case of PAF-induced platelet aggregation. With adenosine in a concentration of about 10 μM virtually the complete absence of changes in transmittance of PEP was recorded in response to the action of this agonist (Fig. 2a). By contrast, complete adenosine-dependent inhibition of the aggregating power of the platelets was not observed under the influence of ADP and 5-HT. Furthermore, in 5-HT-induced platelet aggregation the inhibitory effect of adenosine led not only to a quantitative reduction of the aggregation parameters, but also to a qualitative change in the aggregating power of the cells. Curves showing the aggregating power of the platelets during stimulation by serotonin in the presence of adenosine show that, with a marked decrease in the rate of the change of transmittance of PEP, platelet aggregation became irreversible in character (Fig. 2c).

The action of adenosine may perhaps be mediated through specific receptors located on the outer surface of the cytoplasmic membrane of cells belonging to two subclasses: A_1 and A_2. Depending on activation of a particular type of receptors, the adenylate cyclase activity in the cell decreases or increases [6]. Human platelets have only the A_2-type of receptor sites on their surface, mediating activation of adenylate cyclase and, consequently, increasing the cAMP concentration in the cell [4]. To confirm the receptor-mediated mechanism of inhibition of the cell response under the influence of adenosine and exclusion of any possible involvement of intracellular mechanisms, its effect was studied in the presence of 5 μM dipyridamole, which effectively blocks nucleoside transport through the cytoplasmic cell membrane in this concentration [10]. It was shown that dipyridamole does not reduce the effect of inhibition of platelet aggregation by adenosine in response to the action of PAF, ADP, and 5-HT. The results confirm that the action of adenosine on the aggregating power of the cells is effected through interaction with surface adenosine receptors of the platelets.

During activation of platelets by agonists the initial stages of formation of the cellular response are accompanied, as a rule, by changes in the adenylate cyclase system. For instance, receptor-mediated effects of PAF and ADP reduce adenylate cyclase activity [3, 5]. In seroton-independent platelet activation, no direct change of enzyme activity takes place, but hydrolysis of cAMP is inhibited [1]. Existing differences between the intracellular mechanisms of modulation of the adenylate cyclase system during activation of platelets by PAF, ADP, and 5-HT evidently are responsible for some characteristic features of the inhibitory action of adenosine on aggregation of platelets when stimulated by these ligands.

The experiments thus showed that adenosine is a physiological inhibitor of the aggregating power of human platelets when they are activated by endogenous compounds such as PAF, ADP, and 5-HT.

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INDUCTION OF THE MONOOXYGENASE SYSTEM AND INCORPORATION OF RADIOACTIVITY FROM 2-^{14}C-LYSINE INTO HEPATIC MICROSOMES OF PHENOBARBITAL-TREATED RATS FED A DIET DEFICIENT IN LYSINE, METHIONINE, THREONINE, AND VITAMINS A, C, AND E

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Induction of the cytochrome P-450-dependent microsomal monooxygenase system of the mammalian liver by phenobarbital is known to take place through enhancement of protein synthesis de novo [1]. In this connection, it is logical to suggest on nutritional grounds that under conditions of deficiency of essential components of the diet, the induction of this enzyme system as an adaptive response to those xenobiotics that are inducers of phenobarbital type, may lead not only to redistribution of the flow of nutrients entering with the diet, but also to the outflow of deficient essential components into the liver from other organs and tissues, which could lead to damage to the other functions of the body and could thereby aggravate the manifestations of multiple nutrient deficiency.

To test this hypothesis experimentally we studied induction of the monooxygenase system and incorporation of 2-^{14}C-lysine into microsomal proteins and certain other fractions of liver homogenate under the influence of phenobarbital and in animals with a balanced diet and with a diet deficient in three essential amino acids, namely lysine, methionine, and threonine, and also of vitamins A, C, and E.

EXPERIMENTAL METHOD

Experiments were carried out on 16 growing male WAG rats weighing initially 40-60 g, rising to 160-200 g at the time of sacrifice. The animals, divided into two groups with eight rats in each group, were kept initially for 2 months on a balanced diet (group 1) or on a diet deficient in lysine, methionine, threonine, and vitamins A, C, and E (group 2). The composition of the diets was given previously [4]. In each group the animals were divided into two subgroups: the experimental rats were given phenobarbital (PB, from "Merck," Germany) intraperitoneally for 3 days in a dose of 80 mg/kg, in the form of a sterile solution in 0.9% NaCl, whereas the control rats were given the solvent

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