EFFECT OF ATP AND ADP ON THE ZETA POTENTIAL OF PLATELETS

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The effect of ATP and ADP, in doses of 5-50 μg/ml, on the zeta potential of platelets was studied by determination of electrophoretic mobility in a chamber for microelectrophoresis and by phase-contrast microscopy. An increase in the ADP concentration causes a decrease in the zeta potential of intact and washed platelets. Small doses of ATP increase the zeta potential of platelets, but higher doses decrease it. The value of the platelet zeta potential depends not only on the concentration of ATP and ADP, but also on the duration of their incubation with platelets.

The functional state of platelets is determined by the plasma proteins, vasoactive substances, and in particular, adenyl nucleotides (ATP and ADP) adsorbed on them [3-6, 8, 10-12]. It has been shown recently that the most accurate index of functional activity of the platelets is the magnitude of their zeta potential, formed at the boundary between the adsorbed and diffuse layers surrounding the platelet [7, 9, 13].

In this investigation the effect of ATP and ADP on the platelet zeta potential was studied.

EXPERIMENTAL METHOD

Experiments were carried out with a suspension of platelets from citrated rabbit's blood. The zeta potential of the platelets was determined from their electrophoretic mobility in the chamber of Abramson and Moyer [2]. The flat part of the chamber was connected by means of three-way cocks with nonpolarizing Ag–AgCl electrodes. The electrophoretic mobility was investigated by a phase-contrast microscope, ocular-micrometer, and seconds counter. The velocity of movement of the platelets was determined at 0.211 and 0.79 depths of the chamber. During the investigation recordings were made of the voltage applied to the electrodes, the current strength, and the viscosity and pH of the medium in which the zeta potential was determined. This was calculated from the formula

$$E = \frac{4n\eta sl}{Dvt},$$

where F represents the zeta potential of the platelets in mV, η the viscosity (0.026 for blood, 0.01 for physiological saline), D the dielectric constant (120 for blood, 89 for physiological saline), v the voltage between electrodes in the experiments, l the length of the chamber in cm, S the distance traveled by the platelet in unit time in cm, and t the time in sec. ATP and ADP (Reanal) were added to the suspension of intact and washed platelets in concentrations of 5, 12.5, 25, and 50 μg/ml. The platelets were incubated with ATP and ADP for 1, 3, and 10 min in a water bath at 37°. To wash the platelets to remove the layer of plasma proteins adsorbed on them, they were centrifuged 15 times with physiological saline [1]. The zeta potential of intact platelets and of platelets washed in physiological saline was determined. The plasma and physiological saline were titrated to pH 7.46-7.48. Experiments were carried out on 34 rabbits weighing 2.5-3 kg.

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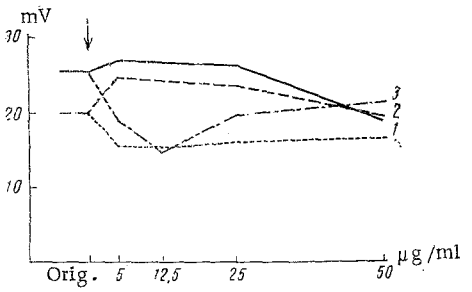

Fig. 1

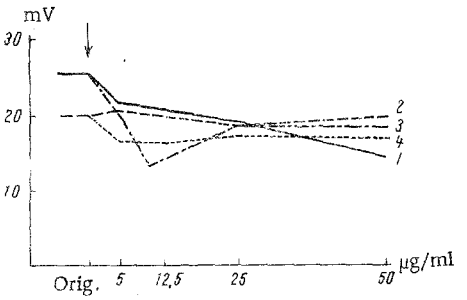

Fig. 2

Fig. 1. Zeta potential of intact and washed platelets after incubation with ATP and ADP for 1 min. Here and in other figures: 1) effect of ATP on zeta potential of intact platelets; 2) effect of ATP on zeta potential of washed platelets; 3) effect of ADP on zeta potential of intact platelets; 4) effect of ADP on zeta potential of washed platelets.

Fig. 2. Zeta potential of intact and washed platelets after incubation with ATP and ADP for 3 min.

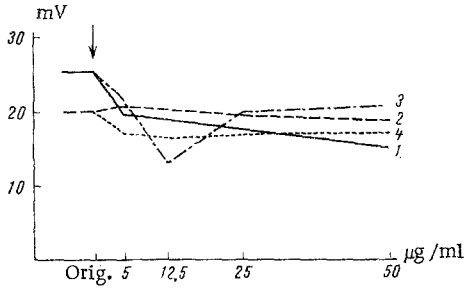

Fig. 3. Zeta potential of intact and washed platelets after incubation with ATP and ADP for 10 min.

EXPERIMENTAL RESULTS

The zeta potential of the intact platelets was 25.6±0.45 mV. After washing of the platelets 15 times with physiological saline, their zeta potential fell to 20.2±0.6 mV ($P < 0.001$). ATP, in a concentration of 5 and 25 μg/ml, increased the zeta potential of the platelets by 2 mV ($P < 0.02$) and 0.3 mV ($P > 0.5$), respectively. An increase in the amount of ATP added to the plasma to 50 μg/ml was accompanied by a decrease in the zeta potential of the platelets by 6.6 mV, ($P < 0.001$). The addition of ATP to a suspension of washed platelets in a concentration of 5 and 25 μg/ml increased their zeta potential by 4.5 mV ($P < 0.01$) and 3.6 mV ($P < 0.05$), respectively. The zeta potential of washed platelets fell very slightly after treatment with ATP in a dose of 50 μg/ml. By contrast with ATP, ADP reduced the zeta potential of the platelets. A more marked decrease was observed when ATP was added to intact platelets. A sharp decrease in the zeta potential of intact platelets (by 10.8 mV, $P < 0.001$) was observed when ADP was added to the plasma in a dose of 12.5 μg/ml. A further increase in the amount of ADP added to the plasma was accompanied by restoration of the normal zeta potential of the intact platelets. The addition of ADP to washed platelets in doses of 5 and 12.5 μg/ml likewise reduced the zeta potential of the platelets by 3.4 mV ($P < 0.001$) and 3.6 mV ($P < 0.001$), respectively. An increase in the dose of added ADP was accompanied by a very slight increase in the zeta potential of the washed platelets. Incubation of intact and washed platelets with ATP and ADP for 3 min caused somewhat different changes in the zeta potential (Fig. 2). Addition of ATP in concentrations of 5, 25, and 50 μg/ml to a suspension of intact platelets caused a well marked decrease in the zeta potential, by 3.2, 6.0, and 10.1 mV, respectively ($P < 0.001$). When ATP was added to a suspension of washed platelets, no significant changes were found in their zeta potential. Addition of ADP to intact and washed platelets was accompanied by a decrease in their zeta potential. The greatest decrease was observed by the action of ADP in a concentration of 12.5 μg/ml on intact platelets (by 12.8 mV, $P < 0.001$).

Incubation of intact and washed platelets with ATP and ADP for 10 min caused similar changes in the zeta potential (Fig. 3). The greatest decrease in zeta potential of the intact platelets was observed after addition of ATP in a concentration of 50 μg/ml, and in this dose ATP had practically no effect on the zeta potential of the washed platelets. ADP likewise reduced the zeta potential of the intact and washed platelets. The most marked decrease was observed after addition of ADP in a concentration of 12.5 μg/ml of intact platelets (by 11.7 mV; $P < 0.001$).

ATP and ADP thus produce marked changes in electrical activity of platelets. These changes are dependent not only on the structure on the nucleotides, but also on their dose and the duration of incubation

with the platelets. Low concentrations of ATP, with short periods of incubation, increase, while large doses decrease the zeta potential of intact and washed platelets. With an increase in the time of incubation of ATP with the platelets, their zeta potential is reduced. By contrast with ATP, ADP reduces the zeta potential of platelets regardless of the dose added or the time of incubation with the platelets. The greatest decrease is observed by the action of ADP in a concentration of 12.5 μg/ml on intact platelets. This dose of ADP is the threshold at which aggregation of the platelets takes place [8]. No increase was observed in the electrophoretic mobility of the platelets during the action of small doses of ADP on them, as some workers have described [9, 13]. Since ATP and ADP have a greater effect on the zeta potential of intact than of washed platelets, this suggests that the nucleotides interact with the components of the blood clotting system and with other substances adsorbed on the surface of the platelets. Changes in the zeta potential of washed platelets during the action of ATP and ADP may be due to their effect on ion transport through the cell membrane. The more marked effect of ADP is probably associated with the negative charge of its molecule and its greater adsorptive capacity than that of ATP. The increase in the blood concentration of ATP and ADP, resulting in lowering of the zeta potential of the platelets, can be presumed to facilitate their adhesion and aggregation and, consequently, to promote intravascular thrombosis.

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