PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY ROLE

OF CYCLIC AMP IN REGULATION OF PLATELET SHAPE

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Recent years have seen a steady increase in the evidence for an important role of cyclic nucleotides in platelet function [1]. Compounds leading to an increase in the intracellular cyclic AMP content have been shown to inhibit platelet aggregation [3, 4]. A fall in the cyclic AMP level in these cells has the opposite action. Meanwhile the importance of changes in platelet shape for the aggregation process is well known [2, 4].

In the investigation described below the effect of a change in the cyclic AMP level on the shape of platelets was studied.

EXPERIMENTAL METHOD

Blood was taken from the abdominal aorta of rats. A 3.8% solution of sodium citrate (blood—anticoagulant ratio 9:1) was used as the anticoagulant. Platelet-rich plasma was obtained from citrated blood by centrifugation at 250g for 6 min at room temperature. Platelet-enriched plasma diluted 1:6 with magnesium-free Tyrode solution, pH 7.4, was used in the experiments.

Chlortetracycline (final concentration in the cuvette 10^{-5} M) was used as the Ca-sensitive fluorescent probe. Fluorescence and transmittance of the platelet suspension were measured on a Hitachi MPF-4 fluorescence spectrophotometer. Fluorescence was recorded at an angle of 90° with the front surface of a square quartz cuvette (length of side of square 1 cm). Fluorescence was excited by light with a wavelength of 400 nm (width of slit 4 nm) and light with a wavelength of 530 nm was recorded (width of slit 10 nm). Transmittance was recorded at a wavelength of 620 nm. The temperature in the cuvette was kept constant (35°C) by means of a special cuvette holder and a UT-15 water ultrathermostat. The contents of the cuvette were constantly stirred by means of a vibromixer.

The ATP used in the investigation was from Reanal, Hungary, the chlortetracycline from Calbiochem, USA, the dibutyryl-cyclic AMP (dbc-AMP) from Serva, USA. Prostaglandin (PG) E_2 was generously provided by Professor Bergstrom (Sweden). The remaining preparations were of Soviet origin and of the optically pure and chemically pure grades.

EXPERIMENTAL RESULTS

Addition of dbc-AMP to the platelet suspension did not cause any significant change in its transmittance, evidence that there were no changes in shape of the cells. However, preincubation of platelets with this agent for 2 min inhibited ADP-induced changes of shape (Fig. 1A). An increase in the cAMP content in the cell due to papaverine, which inhibits phosphodiesterase, also blocked changes in shape caused both by ADP (Fig. 1B) and by PGE_2 (Fig. 1C). Elevation of the cAMP level in the cell thus prevents changes in shape of the platelets. Incubation of platelets at pH 9.0 or the addition of PGE_2 to them caused a lasting change of shape. If dbc-AMP or papaverine was added to the modified platelets, it stimu-

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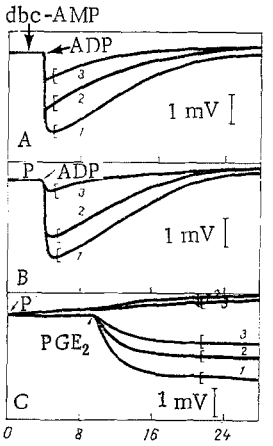

Fig. 1

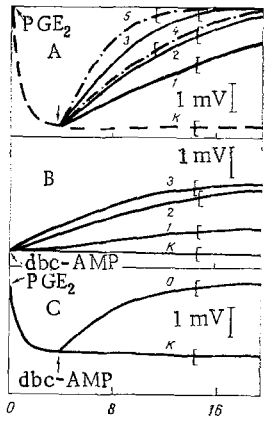

Fig. 2

Fig. 1. Inhibition of changes in platelet shape by agents raising intracellular cAMP level. A) Effect of different concentrations of dbc-AMP on ADP-induced change in transmittance: 1) Control, 2) 2mM, 3) 6 mM, B) effect of papaverine (P) on ADP-induced change in platelet shape: 1) control, 2) 0.01 mM, 3) 0.1 mM: C) inhibition by papaverine of changes in platelet shape induced by different concentrations of PGE_2: 1, 1') 1.4×10^{-5} M; 2, 2') 0.6×10^{-5} M; 3, 3') 0.35×10^{-5} M. 1, 2, 3) Without addition of papaverine, 1', 2', 3') after preliminary incubation with papaverine (0.1 mM). Abscissa, time (in min); ordinate, intensity of transmittance.

Fig. 2. Restoration of shape of modified platelets by means of papaverine and dbc-AMP. A) Normalization of shape of platelets modified by PGE_2 (1.4×10^{-5} M) by means of dbc-AMP [1) 1 mM, 2) 2 mM, 3) 4 mM] and papaverine [4) 0.01 mM, 5) 0.1 mM, K) control], B) increase in transmittance of platelets incubated at pH 9.0 after addition of dbc-AMP: K) control, 1) 0.5 mM, 2) 1 mM, 3) 4 mM, C) restoration of fluorescence of chlortetracycline bound with platelets after PGE_2-induced changes of shape under the influence of dbc-AMP (1 mM). Abscissa, time (in min), ordinate: A, B) intensity of transmittance, C) intensity of fluorescence.

lated a return to the native shape of the cell (Fig. 2A, B). Restoration of the initial level of membrane-bound calcium, modified by PGE_2, also was observed (Fig. 2C).

It can thus be postulated that the intracellular cAMP level regulates activity of the system which restores the native shape of platelets.

LITERATURE CITED

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