RELATIONS OF CHANGES IN PLATELET SHAPE WITH CALCIUM BALANCE AND UNCOUPLING EFFECT OF CYTOCHALASIN B

I. M. Antonikov, O. V. Alekseev, and A. M. Chernukh*

UDC 612.117.7.014.2:612.111.7.015.31:546.41].014.46:547.869.2

KEY WORDS: ADP activation; fluorescent probe; cytochalasin B; platelet shape; calcium.

The writers have shown in relation to the endothelium of the microcirculatory system that cell shape is an important functional parameter, which is under strict control [3]. The mechanisms of intercellular interaction, especially the mechanisms of contact and union of cells, which in the case of the endothelium largely determines the state of vascular permeability, are closely connected with this parameter. It has also been shown that regulation of shape of nonmuscle cells, which includes endothelial cells, is based on changes in the structural state and functional activity of the cytoplasmic microfibrillary apparatus, which is independent of the intracellular calcium balance and is blocked by cytochalasin B [1, 2, 6]. Data in the literature indicate that the principles revealed in experiments with the endothelium may be common also to other types of nonmuscle cells and, in particular, platelets. The question of the mechanisms and role of changes in the shape of platelets during a disturbance of their aggregate state is particularly interesting in this connection. The study of this problem was the aim of the investigation described below, in which chlortetracycline, widely used nowadays to study the dynamics of calcium binding by membranes [7, 9], was used as fluorescent probe. Platelet shape was studied with the scanning electron microscope.

EXPERIMENTAL METHOD

Platelet-enriched rat blood plasma, diluted 1:6, was used. The platelet-enriched plasma and platelet-free plasma were obtained by Aoki's method [10]. A 3.8% solution of sodium citrate was used as the anticoagulant (blood:anticoagulant = 9:1 by volume). The plasma thus obtained was diluted with freshly prepared Tyrode solution (without magnesium), pH 7.4. Fluorescence and light transmittance of the platelet suspension were measured on a Hitachi MPF-4 spectrofluorometer (Japan). Chlortetracycline (CT), in a final concentration of 10^{-5} M, was used as the fluorescent probe. The wavelength of the exciting light was 400 nm (slit width 4 nm) and light was recorded from the front edge of the cuvette at an angle of 90° at 530 nm (slit width 10 nm). The temperature in the cuvette was kept constant at −35°C. The suspension was continuously shaken, using a vibromixer. Transmittance was recorded at 620 nm.

The platelets were activated with ADP (10^{-9}-10^{-7} M). All reagents were diluted on addition to the object by 100 times. The ADP used in the work was from Reanal (Hungary), the CT and cytochalasin B from Serva (West Germany). The cytochalasin B was added as a solution in 96% ethanol up to a final concentration of $2.5 \cdot 10^{-5}$ g/ml.

The platelet preparations for scanning electron microscopy were obtained by the method of Nachmias [10] and were studied in the Hitachi S-500 microscope (Japan).

EXPERIMENTAL RESULTS

Addition of ADP in a concentration of 10^{-5} M or more to the platelet suspension caused an initial fall in transmittance, followed by a sharp rise. According to Born's investigations [5], the decrease in transmittance of the platelet suspension reflects changes of shape whereas the subsequent considerable increase reflects aggregation. Addition of a smaller quantity of ADP (10^{-7}-10^{-9} M) caused only the first phase of a decrease in transmittance (Fig. 1A). This indicates that in these ADP concentrations only the shape of the platelets is changed, without any significant change in their state of aggregation. Restoration of the intensity of light transmittance with these doses of ADP shows that the platelet transformations are reversible in character.

*Academician of the Academy of Medical Sciences of the USSR.

Laboratory of General Pathology and Experimental Therapy, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 8, pp. 87-89, August, 1981. Original article submitted January 26, 1981.

 0007-4888/81/9208-1102$07.50

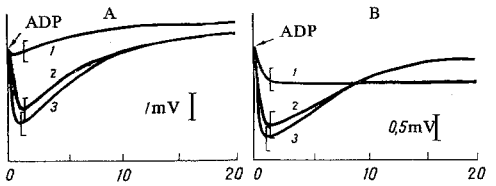

Fig. 1. Effect of cytochalasin B on time course of transmittance of a platelet suspension (A) and of intensity of chlortetracycline fluorescence (B), induced by addition of ADP (10^{-7} M). 1, 2, 3) Preincubation for 40 min with cytochalasin B, with ethanol, and without either agent. Abscissa, time (in min); ordinate, transmittance (A) and intensity of fluorescence (B).

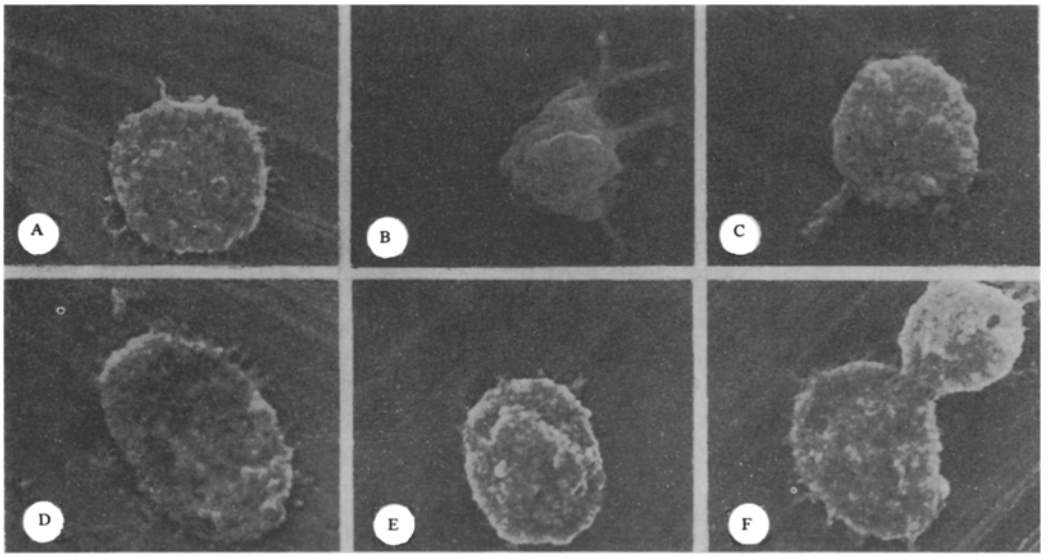

Fig. 2. Effect of cytochalasin B on changes in shape of platelets after their activation ADP (10^{-7} M). A, B, C) Control (preincubation with ethanol), D, E, F) preincubation with cytochalasin B. A, D) Before addition of ADP; B, E) 1 min after addition of ADP; C, F) 20 min after addition of ADP. Calibration (not shown) 5 μ.

Preparations of the platelet suspension were examined in the scanning electron microscope. The discoid shape of the platelets (Fig. 2A) was found to be considerably changed 1 min after addition of 10^{-7} M ADP (Fig. 2B). The original shape of the platelets was restored after 20 min (Fig. 2C).

The results show that under the above-mentioned conditions platelet activation is manifested purely as a reversible change of shape, which is most sensitive during the activation process. It can be concluded from these data that a change in platelet shape itself is not an adequate condition for the development of aggregation.

In the modern view an important role in the initiation of activation and aggregation of platelets is played by calcium. With this in mind, observations were made on the time course of calcium binding by the membrane during a reversible change in platelet shape. The intensity of fluorescence of CT bound with the cells was recorded during the first 20 min after their activation by ADP (Fig. 3). The intensity of fluorescence at first fell, and this was followed by a rise almost to the initial level. The intensity of CT fluorescence is known to reflect the quantity of membrane-bound calcium [9]. It can accordingly be concluded that in the ADP concentrations used, leading to reversible changes in platelet shape, there was a reversible decrease in the quantity of calcium bound by the platelet membrane; the time course of the change in calcium binding agreed closely with that of platelet shape. This suggests that receovery of the shape of platelets after their activation is the result of recovery of the free calcium level in the cytoplasm.

An important role in the mechanism of platelet activation is nowadays ascribed to contractile proteins. It is suggested that there is close interaction between calcium ions and contractile processes in this process. An attempt was

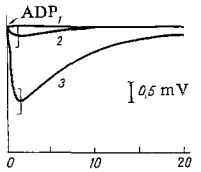

Fig. 3. Effect of various ADP concentrations on time course of intensity of fluorescence of platelet-bound chlortetracycline. Abscissa, time (in min); ordinate, intensity of fluorescence (in mV); 1, 2, 3) ADP concentration: 10^{-9}, 10^{-8}, and 10^{-7} M, respectively.

made to uncouple the change in platelet shape from the membrane-bound calcium concentration during activation of platelets by ADP. This effect was obtained by the action of cytochalasin B on the platelets (Fig. 1). It will be seen that preincubation for 40 min with cytochalasin B blocked changes in platelet shape. This was confirmed by the results of scanning electron microscopy (Fig. 2). Meanwhile changes in the membrane-bound calcium concentration still remained, although they were rather different in form (Fig. 1B). Hence it follows that the change in the calcium level was not itself a sufficient condition for a change in platelet shape. Cytochalasin B is known to prevent the formation of actin threads and to break up an actin gel, whereas it has no significant effect on formed threads. The use of this agent previously led to the discovery of reversible operative structuralization of the microfibrillary apparatus, and the elucidation of its role during changes in the shape of endotheliocytes [2]. Inhibition of the process of ADP-induced change in platelet shape by cytochalasin B is evidence of the existence and importance of the phenomenon of operative structuralization of the microfibrillary apparatus in platelet activation also.

LITERATURE CITED

1. O. V. Alekseev and A. M. Chernukh, in: Problems in the Microcirculation (Function and Structure) [in Russian], Moscow (1977), p. 132.
2. O. V. Alekseev, "Regulatory-effector mechanisms of disturbance of vascular permeability," Author's Abstract of Doctoral Dissertation, Moscow (1979).
3. A. M. Chernukh, P. N. Aleksandrov, and O. V. Alekseev, The Microcirculation [in Russian], Moscow (1975).
4. N. G. Ardlie and P. Han, Br. J. Haematol., 26, 331 (1974).
5. G. V. R. Born, J. Physiol. (London), 209, 487 (1970).
6. A. M. Chernukh and O. V. Alekseev (Alexeyev), in: Abstracts of the 9th World Conference of the European Society for Microcirculation, Antwerp (1976), p. 175.
7. M. B. Feinstein, Thrombos. Haemostas., 42, 432 (1979).
8. N. Aoki, Blood, 52, 1 (1978).
9. V. Nachmias, Blood, 50, 39 (1977).