MECHANISMS OF PLATELET AGGREGATION IN EXPERIMENTAL HYPOPARATHYROIDISM

Yu. Ya. Chursina, E. A. Amroyan, and D. N. Khudaverdyan

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An important place in the functioning of the complex multicomponent system regulating the state of aggregation of the blood is ascribed to platelets, which are universal cellular regulators on account of the intensive synthesis of various biologically active substances in them, including highly active products of transformation of arachidonic acid (AA). Calcium plays an essential role in the system regulating the state of aggregation of the blood. It has been suggested that the parathyroid glands, regulating calcium homeostasis in the body, besides their specific action on metabolism of target cells, may also affect calcium-dependent intracellular processes [3].

The specific lowering of the blood Ca^{++} level in hypoparathyroidism and the widespread distribution of hypo- and hypercalcemic states make it imperative to study the mechanisms of platelet aggregation (PA) in experimental parathyroprival hypocalcemia, and the present investigation was undertaken for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 30 rabbits. Parathyroid insufficiency was induced by surgical removal of the glands under pentobarbital anesthesia (40 mg/kg, intraperitoneally). The development of hypoparathyroidism was judged from the severity of clinical manifestations and the fall in the serum Ca^{++} level, determined by De Waard's method. A group of animals undergoing a mock operation served as the control. PA, induced by ADP and collagen ($2 \cdot 10^{-5}$ M and $2 \cdot 10^{-4}$ g/ml, respectively) was studied in a two-channel aggregometer (Payton, USA) by the method in [8]. Platelet enriched (PEP) and platelet deprived (PDP) plasma were obtained by differential centrifugation of blood taken from the heart on the 5th-6th day after the operation and treated with 3.8% sodium citrate (9:1). Platelets in samples of PEP were counted in a "Picoscale" blood cell counter (Hungary). PA was investigated before and during incubation (10 min) of PEP (200,000-250,000 cells in 1 µl) with imidazole (Sigma), an inhibitor of thromboxane A_2 (TxA_2) biosynthesis, with indomethacin (Sigma), an inhibitor of cyclooxygenase, and with mepacrine (Sigma), an inhibitor of phosphorylase A_2, in a final concentration of 10^{-8} M, $3 \cdot 10^{-9}$ M, and $2 \cdot 10^{-9}$ M, respectively.

EXPERIMENTAL RESULTS

The development of hypoparathyroidism did not affect ADP-induced PA (Fig. 1). Imidazole inhibited PA in the control animals by 44% ($P < 0.001$) but did not affect this process in hypoparathyroidism.

Indomethacin inhibited PA both in the control (65%, $P < 0.001$) and in hypoparathyroidism (39%, $P < 0.001$); in the first case, moreover, indomethacin had a much stronger action. Mepacrine inhibited PA equally in intact rabbits and animals with hypoparathyroidism by 72% and 77%, respectively ($P < 0.001$).

In collagen-induced PA this process was intensified in the parathyroidectomized animals, up to 16% ($P < 0.01$). In collagen-induced PA all the compounds studied inhibited aggregation by a greater degree than in the case of ADP-induced PA; imidazole by 65% in the control and by 26% in the experiment, indomethacin by 87% in the control and by 76% in the experiment. These changes were statistically significant ($P < 0.001$).

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In collagen-induced aggregation mepacrine completely inhibited PA both in the control and in hypoparathyroidism (Fig. 1).

A study of the serum calcium concentration of the parathyroidectomized animals revealed a decrease compared with the control (from 3.88 ± 0.13 to 2.35 ± 0.13 mM, $P < 0.001$).

Compared with the control, under conditions of parathormone deficiency intensification of collagen-induced aggregation and weakening of the antiaggregation activity of cyclooxygenase and TxA_2-synthetase inhibitors were thus observed.

The action of parathormone has been shown to be exerted at the plasma membrane level [6, 14], where it activates adenylate cyclase, and thereby stimulates intracellular cAMP formation [7]. According to Rasmussen's hypothesis [12] the action of cAMP on cell metabolism is effected through Ca^{++} redistribution processes. Parathormone deficiency in the animal leads to reduced cAMP formation in target cells [7]. Lowering the cAMP level in platelets is known to disturb phosphorylation of endogenous Ca^{++} binding receptors, as a result of which Ca^{++} is transformed from the bound into the free state and enters the platelets, where it is utilized by them in the mechanism of aggregation, and it also modifies the kinetics of certain calcium-dependent enzymic reactions [1]. In view of the facts described above it can be tentatively suggested that similar changes also are observed in hypoparathyroidism. In that case an increase in the intracellular concentration of Ca^{++} leads to its active transport into mitochondria, which are a depot for intracellular Ca^{++}, and an increase in the Ca^{++}-accumulating capacity of the mitochondria in hypoparathyroidism has indeed been confirmed [5].

The facts described above are evidence that various stimuli (ADP, collagen, etc.) may cause calcium to leave the mitochondria and enter the cytosol, where it participates in the activation of intracellular processes, including the secretion and activation of membrane phospholipase [2, 4].

In the modern view, AA, whose metabolites are very powerful pro- and antiaggregants (endoperoxide, prostacycline, TxA_2) is formed in the platelets at least as a result of activation of phospholipases A_2 and C [11, 13]. Both enzymes are Ca^{++}-dependent. It can be postulated that the intensification of PA in hypoparathyroidism is the result of reduced cAMP formation in the platelets; first, this stimulates aggregation by itself and, second, it promotes activation of phospholipase A_2 and C under the influence of Ca^{++}, with subsequent intensification of AA metabolism. Our results are evidence that in parathormone deficiency, inhibition of TxA_2 biosynthesis by imidazole is not significantly reflected in ADP-induced aggregation. Blockade of AA metabolism at the cyclooxygenase level by indomethacin causes more significant inhibition of aggregation in hypoparathyroidism. This last fact can evidently be explained by the broader spectrum of action of indomethacin. Thus its ability to inhibit phosphodiesterase and lipooxygenase also cannot be ruled out [15].

A membrane phospholipid, released from different kinds of cells during immune and nonimmune processes, and which is a very powerful proaggregant, has been discovered in recent years: platelet activating factor (PAF) [10]. It has been shown that rabbit platelets are most sensitive to it. The mechanism of PAF release from platelets is not clear, but its release is stimulated most actively by Ca^{++}. Collagen, the trigger for TxA_2 secretion in platelets, also releases PAF very actively. Collagen, however, exhibits ionophore properties relative to Ca^{++} [15]. It is also known that PA induced by PAF is not affected by cyclooxygenase inhibitors or ADP antagonists, but it is inhibited by inhibitors of phospholipase A_2 [9]. Thus collagen-induced PA is mainly due to release of PAF and TxA_2 whereas ADP has no PAF-activating action. When collagen is used the increase in PA in hypoparathyroidism compared with the control, and also in experiments without the use of inhibitors, and during incubation with imidazole, is more marked than in the case of induction of aggregation by ADP (Fig. 1), evidence of the intervention of an accessory aggregation factor besides AA metabolites, i.e., evidence of PAF. The antiaggregation effect of indomethacin in collagen-induced aggregation is more marked both in the control and in hypoparathyroidism than ADP-aggregation, and the difference between PA in the control and experimental groups is very considerable. Considering the absence of an inhibitory effect of indomethacin on PAF, it can be tentatively suggested that the effects of indomethacin can be explained by its action on AA metabolism.

Mepacrine is the only inhibitor of those which were used to inhibit PAF-induced PA. Complete suppression of collagen aggregation on incubation with mepacrine, both in the control and in hypoparathyroidism, is evidence in support of the view that activation by PAF is found in rabbits under the influence of collagen. The very low level of aggregation observed as a

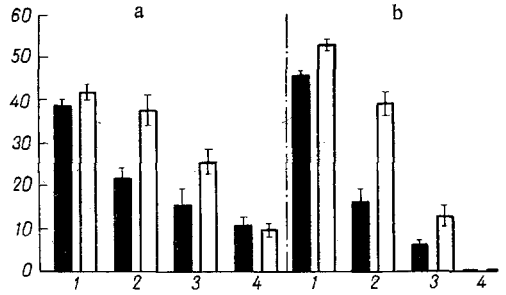

Fig. 1. Effect of imidazole (2), indomethacin (3), and mepacrine (4) on ADP- (a) and collagen-induced (b) PA in intact rabbits (black columns) and rabbits with hypoparathyroidism (white columns). 1) Initial PA. Ordinate, optical density (%).

result of induction with ADP may perhaps be due, besides to the effect of ADP, to activation of AA metabolism through phospholipase C, on which mepacrine does not act [11]. It is important to note that only under the influence of mepacrine was increased aggregating ability of the platelets not observed in hypoparathyroidism, direct evidence of the participation of PAF in this effect.

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