STATE OF PLATELET–VASCULAR HOMEOSTASIS IN RABBITS WITH PARATHYROPRIVAL HYPOCALCEMIA

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A key role in the maintenance of functional unity of the vessel – blood system is played by metabolites of arachidonic acid (AA), turnover of which supplies the main types of regulators of this system: prostaglandins (PG), prostacycline (PGI_2), and thromboxanes (TX). As activators of phospholipases C and A_2, Ca^{++} ions play a triggering role in the metabolic cascade of AA transformations both in platelets and in the vessel wall. Reliable proof has now been obtained of the modulating role of changes in the Ca^{++} concentration in the cytosol in the regulation both of platelet aggregation [11] and of the antiaggregation activity (AAA) of the vessel wall [7]. Furthermore, Ca^{++} ions also play a leading role in the release of the very powerful proaggregant known as platelet activating factor (PAF) [12]. The present writers discovered hyperaggregability of platelets in hypoparathyroid animals [2].

The aim of this investigation was to study AA levels in the aortic wall in animals with specific hypocalcemia caused by parathyroidectomy.

EXPERIMENTAL METHOD

Experiments were carried out on 40 rabbits weighing 2.8-3 kg. The animals were used in the experiments on the 5th-6th day after surgical removal of the parthyroid glands under pentobarbital anesthesia (40 mg/kg, intraperitoneally), when the clinical picture of neuromotor disorders had developed and the serum Ca^{++} concentration, determined by De Waard's method, had fallen. Animals of the control group underwent a mock operation. Platelet-rich (PRP) and platelet-deprived (PDP) plasma were obtained by differential centrifugation of blood taken from the heart and treated with 3.8% sodium citrate (9:1). Platelets were counted in PRP samples on a "Picoscale" automatic blood cell counter (Hungary). Vascular AA was tested by the method in [9] with perfusion with PRP (200,000-250,000 cells in 1 µl) through an isolated segment of aorta. To assess the degree of inhibition of platelet aggregation, the degree of aggregation of PRP perfused through the vessel was compared with aggregation of the same PRP, passing under the same conditions through a pump, bypassing the vessel (nonperfused control). Platelet aggregation was studied on induction by ADP and collagen (Dade, $2 \cdot 10^{-5}$ M and $2 \cdot 10^{-4}$ g/ml, respectively) on a "Payton" two-channel aggregometer (USA), by the method in [6]. Using this technique four series of experiments were undertaken: 1) PRP of control animals was perfused through a vessel taken from a control animal; II) PRP from rabbits with parathyroprival hypocalcemia (PHC) was passed through a vessel from a rabbit with PHC; III) PRP from animals with PHC was perfused through a vessel from a control animal; IV) PRP of control rabbits was perfused through a vessel taken from a rabbit with PHC. The vascular segment was resected from the thoracic aorta and chosen so that the area of endothelium in contact with plasma was the same in all experiments. The numerical data were subjected to statistical analysis by the Wilcoxon–Mann–Whitney nonparametric test and Student's parametric test.

EXPERIMENTAL RESULTS

When the data were analyzed it was found that the ability of the vessels from animals with PHC to inhibit aggregation, whether induced by ADP or by collagen, was considerably increased. For instance, in the series with perfusion of the vessels of rabbits with PHC with their own PRP, the increase in AAA of the vessels was 165.8% ($P < 0.001$) for ADP and 32.6%

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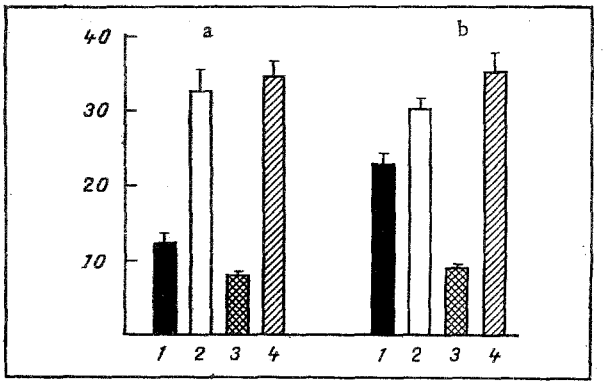

Fig. 1. AAA of aortic wall (in %) of rabbits with PHC: 1) Perfusion of control vessel with control PRP; 2) perfusion of experimental vessel with experimental PRP; 3) perfusion of control vessel with experimental PRP; 4) perfusion of experimental vessel with control PRP. a) ADP; b) collagen.

($P < 0.01$) for collagen-induced aggregation compared with the control (Fig. 1: 1, 2). Similar changes in AAA of the vessels of animals with PHC compared with the control also were obtained on perfusion of a vessel from one animal with PRP from another (to discover any intraspecific influences), whether in the control or in the experiment.

If vessels of animals with PHC were perfused with the control PRP, an even greater increase in vascular AAA was observed: by 180.3% for ADP- and 54.3% for collagen-induced aggregation compared with the control ($P < 0.001$; Fig. 1: 1, 4).

It will be clear from Fig. 1 that AAA of the vessels from animals with PHC, perfused with PRP from control animals, was much greater still than that in experiments in which the control vessel was perfused with experimental PRP, namely 327.2% for ADP- and 293% for collagen-induced aggregation ($P < 0.001$; Fig. 1: 3, 4).

The least AAA of the vascular wall was observed when the control vessel was perfused with PRP from rabbits with PHC. Thus the weakening of this effect compared with the control was 34.4% ($P < 0.05$) for induction of aggregation by ADP and 60.9% ($P < 0.01$) for induction of aggregation by collagen (Fig. 1: 1, 3).

The study of the serum calcium concentration of animals with PHC showed a decrease compared with the control (from 3.65 ± 0.14 to 2.2 ± 0.12 mM; $P < 0.001$).

As the writers showed previously, platelets from animals with PHC possess hyperaggregating activity, the mechanism of which, it can be tentatively suggested, is through intensification of AA metabolism and PAF release.

The complexity of the functional organization of the vessel–blood system requires the presence of equally complex regulatory systems, among which the PG undoubtedly play a leading role. Large quantities of PG and other AA metabolites, affecting the functional state of the vessel wall and of the blood cells, and combining them into a single system, responding synchronously to various influences, are produced in platelets and in the vessel wall. The results now obtained show that under conditions of PHC the AAA of the vessel wall is enhanced. This enhancement is independent of whether the vessel of the animal with PHC is perfused with control PRP or with PRP obtained from animals with PHC (Fig. 1). This fact is evidence that the dominant role in AAA of the vascular wall is played by substances secreted by the vessel. We know that a complex of highly active effectors, with anti- and proaggregant activity, is secreted by a vascular segment and may affect vascular–platelet homeostasis significantly. Analysis of the results of this investigation, showing enhancement of vascular AAA of animals with PHC suggests that in this condition, among other as yet unknown changes, biosynthesis of PGI_2 is intensified in the vessels. This is all the more likely because the effect of many agents on PGI_2 synthesis is based on lowering of the cAMP level and elevation of the intracellular Ca^{++} concentration [7], and under conditions of parathyroid deficiency, both a fall in the cAMP level in the target cells [5] and a rise of the intramitochondrial Ca^{++} level [3] are observed. In the vessels of control animals, as the results show, no such change takes place, because the AAA of these vessels is substantially below that of the experimental animals, regardless of with which PRP they were perfused (Fig. 1). In other words, mechanisms

capable of reducing the hyperaggregability of platelets under conditions of PHC are not triggered in the vessels of the control animals.

However, as the results in Fig. 1 show, the plasma-platelet factor also plays a definite role in the mechanism of the antiaggregation effect of the vessel. For instance, on perfusion of blood vessels of control animals with PRP from animals with PHC, weakening of the vascular AAA of the control animals was observed (Fig. 1: 1, 3). The same effect of the experimental PRP, but this time on AAA of parathyroprival vessels, was observed mainly when collagen was used and vessels of animals with PHC were perfused with control and experimental PRP (Fig. 1: 2, 4). Consequently, an increase in the concentration not only of proaggregants (PAF, TXA_2), as the writers demonstrated previously, but also of certain other factors reducing vascular AAA, is possible in the PRP of animals with PHC. Here, in particular, the possible weakening of the ability of plasma proteins to stabilize PGI_2 secreted by the vessel wall may be mentioned [1]. It has also been shown that the number of receptors on the surface of platelets is not a constant value but may change under the influence of various factors, and in particular, of Ca^{++} [10]. Thus the appearance of new thrombin receptors on the surface of platelets when activated by Ca^{++} has been described [4]. In this connection some very interesting results have been obtained in an investigation which showed various types of sensitivity of platelets to PGI_2 in patients with pathology of the vascular system, and this effect is regarded as the result of changes in the state of their receptor apparatus [1]. The possibility therefore cannot be ruled out that when calcium metabolism is disturbed in hypoparathyroidism there may be not only an increase in the number of receptors interacting with aggregating agents, but also some decrease in platelet sensitivity to the antiaggregation effect of both PGI_2 and other antiaggregants, secreted by the vascular wall. These effects are most marked when aggregation is induced by collagen, not by ADP (Fig. 1), and this can evidently be explained on the grounds of differences in the mechanisms of their aggregation-inducing action. Unlike ADP, for instance, collagen is an activator of PAF in platelets [12], and it releases TXA_2, which acts primarily on their dense granules. This more marked ability of PRP from animals with PHC to inhibit vascular AAA under conditions of collagen-induced compared with ADP-induced aggregation is further evidence of the hypothesis put forward previously, for we know that PG-aggregants can block the rise of the cAMP level in platelets under the influence of PG-antiaggregants, by acting directly on Ca^{++} release from depots within the platelets [8].

Consequently, the leading role of products of the AA metabolite cascade in changes in vascular–platelet homeostasis is not in dispute. Analysis of the data indicates the complex character of the effect of parathyroid deficiency on the level of vascular–platelet homeostasis. Both vascular (stimulation of biosynthesis of PGI_2 and other antiaggregation agents) and plasma–platelet factors (increased PAF and TXA_2 production, depression of platelet sensitivity to the action of PGI_2, reduced ability of plasma proteins to stabilize PGI_2) may participate in the mechanisms of the changes discovered in PHC.

Considering that in hypoparathyroidism platelet aggregability is increased it can be postulated that the enhancement of AAA of the vascular wall found in this pathology serves to compensate hyperaggregation of platelets and to facilitate the restoration of physiological equilibrium in the vessel–blood system.

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