PLATELET AGGREGATION IN PLASMA AND WHOLE BLOOD AND ATP SECRETION IN HYPOXIC CATS

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An important role in the formation of the vascular responses of the brain and heart to hypoxia has been shown to be played by arachidonic acid (AA) metabolites [2, 10]. In the modern view the blood vessels and the blood flowing along them combine to form a single system, the general regulatory function of which is effected by the AA cascade. Metabolites of the latter, synthesized in the platelets and vessel walls, are in close interaction and a disturbance of the functional unity of this system can lead to increased aggregability of the platelets and to intravascular thrombosis. Under hypoxic conditions prostaglandin production (PGI_2) has been shown to be increased and this plays an important role in the mechanism of hypoxic vasodilatation [12, 14]. Since PGI_2, besides possessing powerful vasodilator activity, also has marked antiaggregation properties, it is interesting to study platelet aggregation during hypoxia, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Acute experiments were carried out on 22 cats weighing 2.5-4 kg, anesthetized with pentobarbital (30 mg/kg, intraperitoneally), with artificial ventilation of the lungs. Hypoxia was induced by the addition of 5% oxygen with nitrogen to the inspired mixture, and monitored by recording the blood gas composition with a "Radiometer" microanalyzer, with maintenance of pO_2 in the arterial blood at 40-45 mm Hg, while pH and pCO_2 were maintained with physiological limits. Platelet aggregation and ATP secretion were studied before (control) and after hypoxia for 30 min. Platelet-enriched and platelet-deprived plasma were prepared by differential centrifugation of citrated blood (9:1). The investigations were synchronized: optical aggregation in the plasma was studied on a Payton aggregometer, and impedance aggregation in whole blood and ATP secretion on a Chrono-Log aggregometer. Born's method [5] was used in the first case, that of Cardinal and Flower [6] in the second case. ATP secretion was modified by recording luminescence in a luciferase system, by means of a photomultiplier, by the method of Feinman et al. [7]. Aggregation was induced by ADP (in a concentration of $2 \cdot 10^{-4}$ M for plasma and $5 \cdot 10^{-6}$ M for blood) and collagen ($2 \cdot 10^{-3}$ and $2 \cdot 10^{-6}$ g/ml, respectively). ADP and Dade collagen were used to investigate aggregation in plasma, and the Chrono-Log method in whole blood. Samples of platelet-rich plasma contained 450 µl, and aggregants accounted for another 50 µl. Aggregation in blood together with ATP secretion were recorded in 900 µl of a mixture of citrated blood with isotonic sodium chloride solution (1:1), and the volume of aggregation inducers was 5 µl for ADP and 2 µl for collagen. The calibration solution of ATP (from Sigma) in a concentration of $3.5 \cdot 10^{-8}$ M was added in a volume of 2 µl, and the luciferin–luciferase reagent (Chrono-Log) in a concentration of 40 mg/ml, in a volume of 100 µl. ATP was calibrated in a separate blood sample. The numerical data were subjected to statistical analysis on the Élektronika computer.

EXPERIMENTAL RESULTS

In hypoxia a decrease in ADP- and collagen-induced aggregation was observed in the plasma, by 18.4 and 42.3%, respectively, compared with the control (Table 1). Meanwhile inhibition of

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TABLE 1. Platelet Aggregation in Platelet-Deprived Plasma (PDP) and Whole Blood, ATP Secretion in the Blood, and Acid-Base Balance of Arterial Blood in Hypoxic Cats (M ± m)

Group of animals	Aggregation inducer	Optical aggregation in PDP, percent	Impedance aggregation in blood, Ω	ATP secretion, 10^{-8} M	pH	pCO_2, mm Hg	pO_2, mm Hg
Control	ADF	61,25±3,40	17,40±1,31	19,88±1,84	7,38±0,01	28,73±1,80	101,20±3,51
Hypoxic		50,00±2,81	17,30±1,50	12,46±0,95	7,40±0,01	26,75±2,11	41,31±2,05
P		<0,05	>0,05	<0,05	>0,05	>0,05	<0,001
Control	Collagen	66,25±5,60	31,20±3,11	27,51±2,33			
Hypoxic		38,25±3,10	22,21±2,31	12,63±1,92			
P		<0,001	<0,05	<0,001			

collagen-induced aggregation took place in whole blood (by 28.8% compared with the control), in the absence of any changes in ADP aggregation. The data in Table 1 indicate that during hypoxia ATP secretion from granules of the platelets is very considerably inhibited during the release reaction induced by collagen and ADP, amounting to 54.1% ($P < 0.001$) in the first and 37.3% in the second case. It can be postulated that the antiaggregation effect of hypoxia is due to activation of the AA cascade, with intensification predominantly of biosynthesis of prostacyclin (PG) of type E (PGE) and PGI_2 in the body or with reduced production of thromboxane A_2 (TxA_2). The basis for this hypothesis was the following data: a) activation of PGE and PGI_2 synthesis during hypoxia, leading to preservation of the cells and stimulation of compensatory mechanisms during hypoxia [2-3, 8, 10, 12]; b) a decrease in TxA_2 synthesis during hypoxia [9].

The level of ADP-induced aggregation falls during hypoxia only in plasma, and is unchanged in whole blood, whereas collagen-induced aggregation is inhibited both in plasma and in blood. It was shown that TxA_2, formed from PG endoperoxides under the influence of TxA_2 synthetase, is an endogenous inducer of aggregation. The key role in the mechanism of the proaggregating effect of TxA_2 is played by endogenous ADP, which is secreted under its influence from the dense granules of platelets during the release reaction [13]. As many of 29,400 receptor binding sites for ADP have been discovered on the surface of the platelet membrane [11]. By interacting with the above receptors, ADP injected as an aggregation inducer triggers a cycle of reactions leading to aggregation. The first of these is a rapid fall of the cAMP level [13]. The results of this investigation show that during hypoxia the sensitivity of the ADP receptors is preserved, for the degree of ADP-induced aggregation in whole blood is not reduced compared with the control. Inhibition of ADP-induced aggregation in plasma, however, is evidently due to the experimental conditions. During hypoxia the platelet count rises, and giant forms predominate [1, 4], whereas during the production of platelet-enriched plasma by the centrifugation method, these are separated first. It may lead to a decrease in the degree of aggregation during hypoxia. On the other hand, the important circumstance must not be forgotten that many biologically active substances secreted by other blood cells and, in particular, by leukocytes and erythrocytes, have a significant effect on the behavior of platelets in whole blood.

Considering the ability of collagen to induce aggregation by a specific mechanism effected through TxA_2, as well as our own data showing inhibition of the aggregability of platelets under hypoxic conditions if collagen is used, the following hypotheses can be put forward: hypoxia either inhibits TxA_2 formation, leading to inhibition of ADP secretion from dense granules, or it reduces sensitivity of the TxA_2 receptors, located in the zone of these granules.

One of the most important stages in platelet aggregation is the release reaction, during which substances such as ADP, ATP, serotonin, calcium, etc., are secreted. Simultaneous investigation of aggregation with the release reaction in platelets also gives some idea about the synthesis of biologically active AA metabolites during aggregation. The results are evidence of considerable inhibition of the release reaction during hypoxia, as reflected in the changes discovered in ATP secretion in the blood. Inhibition of secretion during ADP-induced aggregation naturally does not produce the corresponding effect (inhibition) on aggregation, for ADP-induced aggregation is a primary process, independent of the release reaction.

Under hypoxic conditions the aggregability of the platelets which, as the results of this investigation suggest, is due mainly to the thromboxane component of the AA cascade is thus inhibited. Under these circumstances it is possible that certain other mechanisms of nonthrombocytic genesis (PGI_2, platelet aggregation factor, etc) may participate.

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