EFFECT OF CARBON DIOXIDE ON PLATELET AGGREGATION IN CATS

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Products of the arachidonic acid cascade have been shown to play an important role in regulation of the blood supply to several organs, including the brain [1, 8]. Cyclic derivatives of arachidonic acid play a definite role in the mechanisms of action of many known cardiovascular drugs [2].

Platelets have an important functional role in the hemodynamics and in regulation of the state of aggregation of the blood [10] as a result of intensive formation of highly active products of the cyclo-oxygenase and lipoxygenase cascade of arachidonic acid conversions in them. Because of the facts given above, the aim of the present investigation was to study changes in platelet aggregation in the presence of an increased partial pressure of CO_2, one of the most powerful dilators of the cerebral vessels.

EXPERIMENTAL METHOD

Acute experiments were carried out on 45 cats weighing 3-4 kg, anesthetized with pentobarbital (50 mg/kg), during artificial ventilation of the lungs with a mixture of nitrous oxide and oxygen (4:1). Hypercapnia was induced by adding 5% CO_2 to the inspired mixture, and *in vitro* by measured saturation of the blood or plasma with carbogen. The partial pressure of CO_2 was maintained at 90-100 mm Hg. Inhalation of CO_2 lasted 10 min in experiments *in vivo*, and *in vitro* until the necessary pCO_2 level was reached, as verified with the BMS-3

TABLE 1. Changes in Platelet Aggregation in Cats (n = 32) under the Influence of Hypercapnia before and after Administration of Indomethacin (1 mg/ml/min intravenously and $1 \cdot 10^{-5}$ g/ml *in vitro*), on Induction of Aggregation by ADP ($2 \cdot 10^{-5}$ g/ml) and Collagen ($2 \cdot 10^{-3}$ g/ml)

Experimental conditions	Inducer of aggregation	Aggregation, %	V_{max}, mm/min	Latent period, sec
Experiments in vivo				
Control	ADP	72,52±7,3	54,50±4,5	
CO_2		47,48±5,6*	38,80±3,6*	
Control	Collagen	78,41±8,7	73,94±6,8	147,50±40,7
CO_2		27,30±6,5**	47,17±7,5*	183,50±37,0
IM	ADP	62,05±5,3	46,10±6,2	
IM + CO_2		50,85±4,8	34,04±3,7	
IM	Collagen	59,23±8,3	41,44±7,8	141,81±18,5
IM + CO_2		45,60±6,5	24,88±5,3	144,54±21,3
Experiments in vitro				
Control	ADP	66,26±4,5	70,16±15,3	
CO_2		26,25±3,2**	13,00±3,8*	
Control	Collagen	72,90±4,5	60,33±13,5	100,00±20,3
CO_2		30,35±2,3**	11,83±3,7*	216,61±25,8*
IM	ADP	63,30±2,8	78,50±1,3	
IM + CO_2		16,60±1,3	64,51±1,2**	
IM	Collagen	50,00±2,3	31,50±2,3	60,00±13,5
IM + CO_2		20,00±2,1**	11,50±1,2**	150,00±16,3**

Legend. IM) Indomethacin; V) rate of reaching peak of aggregation. *P < 0.05; **P < 0.001.

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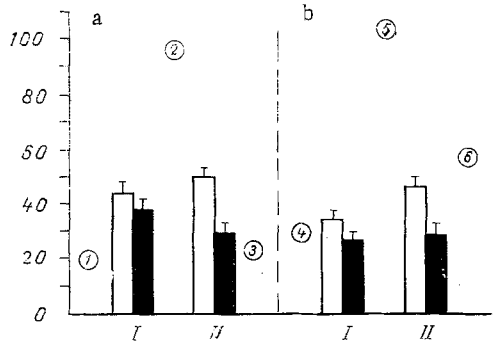

Fig. 1. Changes in platelet aggregation in cats (n = 13) under conditions of hypercapnia, in whole blood (b) and in PRP after normalization of its pCO_2 (a). I) ADP; II) collagen; unshaded columns — control, black columns — hypercapnia; 1, 4) initial pCO_2 level in PRP and blood; 2, 5) pCO_2 during hypercapnia in PRP and blood; 3, 6) pCO_2 in PRP at time of induction of aggregation; *P < 0.05; **P < 0.001. [Asterisks missing in Russian original.] Ordinate, aggregation (in %) and pCO_2 (in mm Hg).

Mark 2 microanalyzer (from Radiometer, Denmark). Platelet aggregation was studied by Born's method [3] on a two-channel "Paiton" aggregometer. Platelet-rich plasma (PRP) and platelet-deprived plasma were obtained by differential centrifugation of blood taken from the carotid artery and treated with sodium citrate (3.8% in the ratio 1:9). Some experiments were carried out when cyclo-oxygenase activity was inhibited by indomethacin (from Sigma Chemicals), injected intravenously (1 mg/ml/min), and also incubated with PRP ($1 \cdot 10^{-5}$ g/ml). ADP and collagen (from Dade), in doses of $2 \cdot 10^{-5}$ and $2 \cdot 10^{-3}$ g/ml, respectively, were used as aggregation inducers. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Experiments *in vivo* (Table 1) showed that, in hypercapnia, ability of the platelets to aggregate is appreciably reduced. With an increase in pCO_2 from 28.82 ± 2.3 to 77.55 ± 1.8 mm Hg (P < 0.05) and with a decrease in pH from 7.32 ± 0.01 to 6.94 ± 0.01 (P < 0.05), inhibition of the ADP-induced and collagen-induced aggregation was observed by 34.5 and 65.2%, respectively. It must be pointed out that parameters of the acid–base balance (ABB) in PRP at the time of induction of aggregation were within the limits of normocapnia (pCO_2 32.12 ± 1.8 mm Hg, pH 7.30 ± 0.01), for, as a result of centrifugation of the blood, CO_2 molecules diffused into the surrounding medium. The ability of CO_2 to depress platelet aggregation *in vivo* was inhibited to a considerable degree by indomethacin (Table 1). The present writers and others [1, 7] showed previously that indomethacin can also depress the vasodilator action of CO_2 in the cerebral vessels.

To determine the degree of participation of platelets in the antiaggregant effect of CO_2 some experiments were carried out at a high pCO_2 level actually in PRP (raised from 20.18 ± 3.5 to 94.48 ± 4.8 mm Hg). Under these conditions, the antiaggregant action of CO_2 was still present, to the extent of 60.4% for ADP-induced and 58.4% for collagen-induced aggregation for normocapnic conditions (Table 1). Meanwhile, a considerable decrease was observed in the maximal rate of aggregation and lengthening of the latent period of collagen-induced aggregation. The fact will be noted that, in experiments *in vitro*, indomethacin did not abolish the antiaggregant effect of CO_2. It must also be pointed out that, unlike in the previous series, induction of aggregation took place under conditions of high pCO_2 and acidosis in PRP (pH 6.80 ± 0.01). The latter could have affected the final results of the experiments because of the direct action of CO_2 and pH on platelets.

To clarify this state of affairs, in the next series of experiments platelet aggregation was studied after normalization of pCO_2 and pH in PRP. In this case (Fig. 1a), the antiaggregant effect of CO_2 was preserved only in the case of collagen-induced aggregation, and it amounted to 40.7% of the control.

In experiments with raised pCO_2 in whole blood *in vitro* (Fig. 1b) depression of ADP- and collagen-induced aggregation also took place under the influence of hypercapnia, and this effect, moreover, was preserved when the degree of hypercapnia was reduced in PRP. Here also it was noted that CO_2 affected mainly collagen-induced aggregation.

Continued resistance of platelets to ADP and collagen after normalization of parameters of ABB in PRP after hypercapnia is an interesting fact and indicates that the mechanism of inhibition of aggregation cannot be entirely attributed to acidification of the medium by CO_2 [5]. Hypercapnia evidently triggers some other mechanism, by means of which its antiaggregant effect continues despite normalization of pCO_2. The factors concerned in the antiaggregant ef-

fect of CO_2 may be increased production of prostacyclin (PGI_2) in the vessel walls, lungs, and leukocytes, or depression of biosynthesis of thromboxane A_2 in the platelets, or a change in the cAMP level in them. Considering that in collagen-induced aggregation PGE_2, $PGF_{2\alpha}$ and, more especially, thromboxane A_2 are formed in the platelets, and that the last of these exerts its proaggregant action through liberation of ADP from dense granules [4, 9, 10], it can be tentatively suggested that during hypercapnia the stage responsible for interaction of thromboxane with ADP suffers the most. This suggestion is confirmed by our data which showed that, after normalization of pH and pO_2 in PRP, ADP-induced aggregation was almost completely restored, but collagen-induced activity remained depressed. Under conditions of a high pCO_2 level in PRP at the moment of aggregation as a result of acidosis and involvement of certain other factors (inhibition of Ca^{++} transport [6]), the antiaggregant effect of CO_2 was more universal in character, i.e., both ADP-induced and collagen-induced aggregation were inhibited.

Another fact which deserves attention is that indomethacin inhibits the antiaggregant activity and vasodilator effect of CO_2 *in vivo* in the same dose [1].

This is evidence both of involvement of products of the cyclo-oxygenase cascade of arachidonic acid conversions in both processes and of the likelihood that their mechanisms are the same. The results suggest that the effect of CO_2 on the cerebral vessels and on platelet aggregation is a manifestation of two different aspects of a common mechanism of action, aimed at maintaining circulatory homeostasis within the bounds of metabolic control of the cerebral circulation.

Hence it follows that when the role of CO_2 as one of the chief components of metabolic control of vascular tone in the brain is assessed, its ability to become involved in mechanisms of regulation of the state of aggregation of the blood must also be taken into account.

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