CHANGE IN ACTIVITY OF FACTOR XIII IN THE PLASMA DURING PLATELET AGGREGATION

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During platelet aggregation induced by ADP or thrombin, a reduction in the activity of factor XIII is observed in platelet-enriched rat plasma. On the addition of active factor XIII (XIIIa) to this plasma, besides the increased ADP-induced aggregation, activity of factor III in the plasma is reduced. In the case of thrombin-induced platelet aggregation, addition of factor XIIIa is accompanied by a marked decrease in its activity in the plasma. The degree of aggregation under these circumstances is lower than in control samples. The observed differences in the character of aggregation taking place in the presence of factor XIIIa, when different aggregants (ADP and thrombin) are used, are evidently due to interaction between active factor XIII and thrombin added to the plasma as the aggregant.

KEY WORDS: factor XIII; platelets; aggregation.

Besides its principal function – the stabilization of fibrin – factor XIII also affects the aggregation of platelets, as experimental evidence [1, 3] shows.

In a previous investigation [3] the writers showed that active factor XIII increases platelet aggregation in platelet-enriched plasma on the addition of the aggregating agent ADP. Intravenous injection of a preparation of factor XIII into rats also leads to increased aggregation of platelets induced by ADP.

In this investigation the action of factor XIII was studied on platelet aggregation induced by various aggregants, especially ADP and thrombin, and the changes in its activity in the plasma during the aggregation process were examined.

EXPERIMENTAL METHOD

Aggregation of platelets was determined by Born's turbidimetric method [4] in platelet-enriched rat plasma and expressed as indices at the time of its greatest change. The aggregating agents used were the sodium salt of ADP, in a final concentration of $5 \cdot 10^{-5}$ M, and a preparation of plasminogen-free thrombin manufactured by the Kaunas Bacterial Preparations Factory with an activity of 0.4 NIH unit/ml plasma. The activity of factor XIII in the plasma was determined by Buluk and Januszko's method [5] and expressed in units/ml plasma. The preparation of factor XIII was obtained by Loewy's method [7] from bovine blood and partially purified on a column with DEAE-cellulose.

EXPERIMENTAL RESULTS

As previous experiments [3] showed, addition of active factor XIII (XIIIa) in vitro to platelet-enriched plasma led to an increase in ADP-induced aggregation. Similar results were obtained in the present experiments also on the addition of 200 units factor XIIIa/ml plasma (aggregation index in control samples 1.9, in experimental 2.6; $P < 0.01$). Inactive factor XIII caused no significant changes in aggregation. The activity of factor XIII was determined before the beginning of aggregation and again 1, 3, 5, 8, and 10 min after the beginning of aggregation, simultaneously in the same samples as had been used for recording the optical

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TABLE 1. Activity of Factor XIII in Plasma during Platelet Aggregation in vitro on Addition of Active Factor XIII (aggregant ADP)

Sample of plasma	Statistical index	Activity of factor XIII (in %)		
		before aggregation	after 3-5 min (maximum of aggregation)	after 10 min (de-aggregation)
Control	n	7	7	7
	M	100	80,2	89
	$\pm m$	—	7,8	4,9
	P	—	$<0,05$	$\leqslant 0,05$
With addition of inactive factor XIII	n	7	7	7
	M	100	80,0	82
	$\pm m$	—	6,5	8
	P	—	$<0,01$	$\leqslant 0,05$
With addition of active factor XIII	n	7	7	7
	M	100	52	66
	$\pm m$	—	5,8	4,7
	P	—	$<0,001$	$<0,001$

TABLE 2. Degree of Aggregation of Platelets in vitro in Presence of Factor (XIIIa (aggregant thrombin)

Sample of plasma	Statistical index				
	n	M	$\pm m$	t	P
Control	10	100	—	—	—
With addition of inactive factor XIII	9	51,5	7,0	6,2	$<0,001$
With addition of active factor XIII	7	70,0	8,2	3,4	$<0,01$

Legend. Aggregation expressed as percentage of control.

density. The results in Table 1 show that during the maximum of aggregation (an interval of 3-5 min) in the control samples, a decrease in the activity of factor XIII to 80.2% of the initial level was observed, followed by recovery to 89% at the end of the determination (interval 10 min).

Addition of inactive factor XIII to the plasma affected neither the degree of platelet aggregation nor the character of the change in activity of factor XIII in the plasma. The addition of active factor XIII to platelet-enriched plasma increased aggregation of the platelets and led to a more marked decrease in its activity (to 52%) during the period of maximal aggregation, followed by very slight recovery of activity during the period of deaggregation.

The decrease in activity of factor XIII in the blood plasma can evidently be explained by its utilization during the stabilization of fibrin conformed in the process of aggregation as the result of the ensuing activation of thrombinogenesis [2, 6, 9]. The fact will also be noted that the greater decrease in activity of factor XIII in the experimental samples of plasma (Table 1) was connected with the discovery of a greater degree of aggregation.

In the next series of experiments platelet aggregation induced by thrombin was studied in the presence of factor XIIIa (200 units/ml plasma), and the change in its activity in the plasma during aggregation was recorded. It will be clear from Table 2 that the addition of thrombin alone to platelet-enriched plasma caused marked aggregation (control). The activity of factor XIII in the plasma fell under these circumstances to 67% of its initial level in the period of maximal aggregation (3-5 min), followed by a return to normal in the period of de-aggregation (Table 3); in other words, changes similar to the changes in activity of factor XIII in the plasma during ADP-induced aggregation were observed.

Under the same conditions but after the addition of inactive factor XIII a marked increase in the activity of factor XIII in the plasma took place compared with initially, but this was evidently the result of activation of the inactive factor XIII by thrombin added to the plasma as the aggregant. The degree of aggregation in this case was lower than in the control.

TABLE 3. Activity of Factor XIII in Plasma during Platelet Aggregation in vitro on Addition of Active Factor XIII (aggregant thrombin)

Sample of plasma	Statistical index	Activity of factor XIII (in %)		
		before aggregation	after 3-5 min (maximum of aggregation)	after 10 min (de-aggregation)
Control	n	7	7	7
	M	100	67	103
	$\pm m$	—	7,5	10,5
	P	—	<0,01	>0,5
With addition of inactive factor XIII	n	7	7	7
	M	100	122	94
	$\pm m$	—	7	7,5
	P	—	<0,01	>0,5
With addition of active factor XIII	n	7	7	7
	M	100	60	63
	$\pm m$	—	10,2	9
	P	—	<0,01	<0,01

Addition of active factor XIII to platelet-enriched plasma in the presence of thrombin (0.4 unit/ml) led, just as during ADP-induced aggregation, to a marked decrease in the activity of factor XIII in the plasma during the period of maximal aggregation. However, the degree of aggregation was not increased in this case and remained at a lower level than in the control.

The observed differences in the character of aggregation taking place in the presence of factor XIIIa when different aggregants (ADP and thrombin) were used can evidently be explained by interaction between this factor and the thrombin added to the plasma as aggregant.

Data in the literature [8, 10] show that incubation of factor XIIIa with thrombin leads to a decrease in its activity. The presence of factor XIIIa and of thrombin added to induce aggregation in the experimental samples of plasma may have led to a similar phenomenon, as a result of which the activity of factor XIII in the plasma fell and aggregation took place to a lesser degree than after the addition of thrombin alone to platelet-enriched plasma.

The present experiments in fact showed that on the addition of a small dose of thrombin (0.4 unit/ml) to plasma containing factor XIIIa (200 units/ml), no increase in its activity took place; on the contrary, a more than threefold decrease in the activity of facotor XIII in the plasma was observed by the end of the 10th minute of incubation at 37° C.

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