The Effect of Myokinase on the Aggregation and Disaggregation of Thrombocytes

Although the aggregation of thrombocytes has been known for more than 5 years[1] its metabolic base has not yet been cleared up completely. It is presumed that an important role can be ascribed to the interference of aggregation factors with the metabolism of macroergic phosphates[2,3].

For this reason, the relation between the aggregation effects of adrenaline and ADP is of great interest. It is presumed that adrenaline induces aggregation by liberating ADP from the interior of thrombocytes and that ADP is the proper aggregation factor[1,2,4]. Nevertheless, evidence has been given that there are differences between the effects of adrenaline and ADP. Lately O'BRIEN[5] described a different effect of ADP and adrenaline on the shape of thrombocytes.

Platelet aggregation due to ADP is reversible and shows a tendency to a spontaneous disaggregation of the formed clumps[6]. When using high concentrations of ADP ($5 \times 10^{-4} M$) we established in our experiments an almost complete disaggregation following 20 min agitating. Contrary to this, the aggregation due to adrenaline in a concentration of $5 \times 10^{-5} M$ under the same conditions showed not the least tendency to spontaneous disaggregation (Table II). In adrenaline-induced thrombocyte aggregation we may suppose a more durable and deeper intervention into thrombocyte metabolism. This possibility has already been pointed out by O'BRIEN.

An attempt to disintegrate adrenaline aggregated thrombocytes therefore seemed not to be without interest.

Thrombocyte disaggregation is explained by the decomposition of ADP into AMP or adenosine and phosphorus, i.e. into factors inhibiting aggregation[3].

As both aggregation and disaggregation depend on the conditions of ATP, ADP and AMP[3] we resolved to study how they would be influenced by myokinase. Myokinase is an enzyme decomposing 2 molecules of ADP into 1 molecule ATP and 1 molecule AMP. Thus we may suppose that it would inhibit not only the aggregation due to ADP, but also that induced by adrenaline, which is mediated by ADP as well. And finally we may expect its disaggregating effect on adrenaline produced aggregates.

In our experiments we used platelet-rich human plasma prepared according to BORN and CROSS[6]. We measured aggregation according to our own modification of O'BRIEN's method[7]. For investigating the inhibition of aggregation, we mixed together myokinase (C. F. Böhringer und Söhne) with platelet-rich plasma in a ratio of 100 µg and 50 µg/ml, respectively. Following a 5-min incubation we added adrenaline or ADP and measured the degree of aggregation. The degree of aggregation was expressed in %. As 100% aggregation we took the drop in extinction following the addition of adrenaline or ADP.

The inhibition was evaluated from the reduction of this drop in extinction and its degree expressed in %.

For measuring disaggregation we agitated the aggregated thrombocytes for 20 min with myokinase or ammonium sulphate. Samples without myokinase or ammonium sulphate were treated in the same way. Disaggregation was evaluated from the increase in extinction due to the presence of dispersed thrombocytes. As 100% disaggregation we took the re-increase in extinction to the original value found prior to addition of the aggregating substance.

We studied the effect of ammonium sulphate as it is part of Myokinase Böhringer. Its concentrations were chosen to correspond to those contained in the myokinase preparations. The aim was to verify the effect of myokinase itself.

Table I shows that ammonium sulphate blocked platelet aggregation induced both by adrenaline and ADP, in keeping with the finding of HASLAM[8]. The presence of myokinase reinforced the inhibitory effect of ammonium sulphate and we may say that myokinase by itself blocked platelet aggregation due to adrenaline and ADP.

More interesting was the finding that myokinase brought about a disaggregation of thrombocytes aggregated by adrenaline (Table II).

Table I. Inhibition of thrombocyte aggregation

Myokinase	$(NH_4)_2SO_4$	Degree of aggregation (%)	% of inhibition[a]	S.D.	No. of experiments
ADP $5 \times 10^{-6} M$					
0	0	+ 100.00	0	7.75	11
0	$6.4 \times 10^{-2} M$	+ 43.21	56.79	9.23	10
0	$3.2 \times 10^{-2} M$	+ 52.12	47.88	11.28	10
100 µg/ml	$6.4 \times 10^{-2} M$	+ 3.23	96.77	12.30	7
50 µg/ml	$3.2 \times 10^{-2} M$	+ 24.31	75.69	6.82	6
Adrenaline $5 \times 10^{-5} M$					
0	0	+ 100.00	0	11.51	25
0	$6.4 \times 10^{-2} M$	+ 68.13	31.87	14.06	10
0	$3.2 \times 10^{-2} M$	+ 75.07	24.93	13.95	10
100 µg/ml	$6.4 \times 10^{-2} M$	− 1.45	101.45	10.83	6
50 µg/ml	$3.2 \times 10^{-2} M$	+ 36.10	63.90	11.29	6

[a] Statistically significant inhibition. For statistical evaluation *t*-test was used.

Table II. Disaggregation of aggregated thrombocytes

Myokinase	$(NH_4)_2SO_4$	% of disaggregation	S.D.	No. of experiments
ADP $5 \times 10^{-6} M$				
0	0	− 88.88[a]	3.78	11
Adrenaline $5 \times 10^{-5} M$				
0	$6.4 \times 10^{-2} M$	− 1.96	8.09	15
0	0	− 3.77	5.33	13
100 µg/ml	$6.4 \times 10^{-2} M$	− 121.69[a]	14.99	15

[a] Statistically significant disaggregation.

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The inhibitory effect of myokinase on adrenaline induced platelet aggregation indicates that this aggregation is caused by an interference of adrenaline with the metabolism of ATP and ADP.

The disaggregating effect of myokinase supports the view that disaggregation is also an active metabolic process in which high energy phosphates play an important role.

The clinical meaning of this finding is that it shows the possibility of reduced platelet aggregability when myokinase is released, for instance from myocardial infarct. Released myokinase could enhance platelet disaggregation and thus reduce the danger of microthrombi formation.

Zusammenfassung. Myokinase, welche die Umwandlung von 2 Molekülen ADP in je 1 Molekül AMP und ATP katalysiert, hemmt die durch ADP sowie durch Adrenalin hervorgerufene Thrombozytenaggregation beträchtlich. Myokinase unterstützt die Desaggregation der durch Adrenalin aggregierten Thrombozyten. Diese Befunde weisen darauf hin, dass die makroergen Phosphate nicht nur für die Aggregation, sondern auch für die Desaggregation eine bedeutende Rolle spielen.

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The Development of the Amylase Activity in Blood and its Behaviour in the Amniotic Fluid in Rats

It is well known that the amylase activity rate in blood, according to its behaviour in the duodenal juice, is slow in the new-born and reaches the normal adult level within the first 2 years of life[1-4]. Employing our own micromethod we examined this course of development in Wistar rats during the first half year of life. To determine amylase activity, 0.02 ml of capillary blood (or amniotic fluid) were suspended in 0.5 ml of 0.9% NaCl-solution, centrifuged, and the supernatant dilution examined in the same way as described for human serum[5].

The results are shown in the Figure. The ferment activity rate is expressed in terms of g% amylase activity (= hydrolysis of 1 g of starch in a 0.12% solution at pH 6.8 and 37 °C within 30 min by 100 ml of blood).

The examination of the amylase activity rate in blood and in the amniotic fluid of the rat foetuses as well as in the blood of the respective pregnant animals yielded the values compiled in the Table.

Amylase activity is observed in the blood of rat foetuses weighing 1 g and more. The mean values increase from birth up until about the twelfth day of life, reaching the normal adult level.

It must be noted that this increase coincides with the period during which the animals ingest exclusively by lactation. Thus, contrary to the assumption with regard to humans[6], it is not necessary to expose the organism to the physiological substrate of the amylase (starch) for the full development of this ferment.

No amylase activity is observed in the amniotic fluid during the early period of foetal development either. Above a foetal weight of 0.9 g the amylase activity rate increases rapidly to values which can amount to about the 20-fold of those of the foetal blood, or the 5-fold of the mother's blood. The amylase activity rate drops considerably at the end of the gravidity with the decrease of the amount of amniotic fluid.

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Amylase activity of the amniotic fluid and in the blood of foetuses and the respective pregnant rats

Weight of foetus (g)	Amniotic fluid				Blood of foetus			Pregnant rat
	No.	Vol. (ml)	Amylase activity $\bar{x}$ (g%)	range (g%)	Amylase activity No.	$\bar{x}$ (g%)	range (g%)	Amylase activity (g%)
0.166	9	~0.2	0	–	–	–	–	11.3
0.320	6	~0.2	0	–	4	0	–	16.3
0.5	2	~0.2	0	–	–	–	–	15.3
0.9	8	~0.3	0	–	8	0	–	22.5
0.9	2	~0.3	2.5	(2.5–2.5)	–	–	–	12.8
1.9	2	~0.5	24.1	(23.7–24.4)	5	4.3	(3.4–6.0)	12.2
2.9	4	~0.5	54.3	(46.0–62.7)	6	6.5	(5.1–7.5)	20.0
4.0	3	< 0.1	17.7	(14.4–19.6)	3	6.7	(6.5–7.0)	21.3
4.5	3	~0.5	91.5	(72.0–114.1)	3	4.7	(2.4–6.3)	22.3
4.5	4	< 0.1	13.9	(10.8–16.1)	6	4.8	(4.1–5.6)	13.7
5.0	2	< 0.1	16.8	(14.4–18.6)	2	5.6	(4.4–7.0)	22.2
New-born 5.0	–	–	–	–	33	6.4	(4.4–9.3)	–