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ROLE OF INTERMEDIATE PRODUCTS OF PROTHROMBIN PROTEOLYSIS BY THROMBIN IN STIMULATION OF THE ANTICLOTTING SYSTEM

B. A. Kudryashov, S. M. Strukova, A. S. Orlova, and L. A. Lyapina

UDC 612.115.3:612.111

Two components were isolated from the products of proteolysis of prothrombin by immobilized thrombin: intermediate product 1 (P1) which, under suitable conditions, can be transformed into thrombin, and product 2 (P2), which does not have this property. After intravenous injection of P1 into rats the total clotting time and the plasma recalcification time were lengthened. The total fibrinolytic activity (TFA) and the level of fibrinogen degradation products were raised, the fibrinogen concentration was lowered, and the degree of nonenzymic fibrinolysis and its contribution to TFA rose sharply. Mobilization of the anticoagulant and fibrinolytic potential of the body was due to the response of the second anticlotting system. Intravenous injection of P2 or prothrombin into rats did not stimulate this system.

KEY WORDS: prothrombin; intermediate products 1 and 2; second anticlotting system.

The principal agent stimulating the function of the second anticlotting system (SAS) is thrombin [3]. Thrombin preparations, which differ in their degree of purification but possess equal clotting activity by their direct action on chemoreceptor zones or after intravenous injection, evoke a response from the body which may vary in degree [1, 2]. The strongest effect is observed after injection of unpurified, crude thrombin preparations. In this connection the question arises of the role of intermediate products of prothrombin activation in stimulating the function of SAS. Products of proteolysis of prothrombin by immobilized thrombin were obtained in a previous investigation [5]: intermediate product 1 (P1) and product 2 (P2). Intermediate product 1 (mol. wt. 50,000) was slowly converted into thrombin in vitro under the influence of active factor X or trypsin, but the rate of thrombin generation increased when a relative excess of factor Y, thromboplastin, and Ca ions was added. Before P1 can exhibit clotting activity it had to shed a fragment with molecular weight of 13,000 and one additional peptide bond (Arg–Ile) in the molecule had to be ruptured. Unlike the P1 fragment, P2 is not a thrombin precursor [5, 9].

In this investigation the role of these products of prothrombin proteolysis in activation of the SAS was studied.

Laboratory of Physiology and Biochemistry of Blood Clotting, M. V. Lomonosov Moscow State University.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1172-1174, October, 1976. Original article submitted January 14, 1975.

TABLE 1. Changes in TFA and NF after Intravenous Injection of Intermediate P1 (M ± m)

Preparation injected	Plasma								Euglobulin fraction of plasma					
	TFA				NF				TFA			NF		
	before injection	after 5 min	after 15 min	after 60 min	before injection	after 5 min	after 15 min	after 60 min	before injection	after 5 min	after 15 min	before injection	after 5 min	after 15 min
Physiological saline	30±7,1 (12)	31±9,2 (10)	47±6,2 (15)		8±2,8 (12)	12±5,1 (10)	30±4,6 (16)	11±1,8 (9)	34±1,7 (10)	35±5,7 (10)	39±7,3 (10)	11±1,2 (10)	13±2,1 (6)	19±0,7 (6)
P		>0,5	>0,1			>0,5	<0,001	>0,6		>0,5	>0,5		>0,2	<0,001
P1 (1 mg/kg)	45±8,3 (10)	139±11,1 (16)	100±0,2 (10)		25±1,4 (10)	94±13 (10)	63±9,2 (10)		36±4,7 (6)	91±17,4 (6)	91±18,2 (6)	12±2,9 (6)	56±12,3 (6)	48±11,7 (6)
P		<0,001	<0,001			<0,001	<0,001			<0,02	<0,02		<0,01	<0,02
P1 (2 mg/kg)	43±4,5 (10)	129±9,1 (10)		48±4,5 (10)	12±1,6 (10)	79±5,9 (10)		14±1,3 (8)						
P		<0,001		>0,5		<0,001		>0,5						
P1 (2,5 mg/kg)	45±5 (20)	118±7,8 (16)	89±8,6 (16)		25±1,4 (17)	67±6,2 (16)	51±7 (16)		37±3,0 (16)	78±5,9 (14)	90±7,4 (15)	13±1,8 (16)	42±2,6 (16)	59±8,5 (16)
P		<0,001	<0,001			<0,001	<0,001			<0,001	<0,001		<0,001	<0,001

Legend. Here and in Table 2 number of animals shown in parentheses.

TABLE 2. Changes in Some Indices of Clotting and Anticlotting Systems of the Blood after Intravenous Injection of Prothrombin and P2 (M ± m)

Preparation injection	Plasma recalcification time		Fibrinogen concentration		Fibrinolytic activity [6]		TFA			NF		
	before injection	after 5 min	before injection	after 5 min	before injection	after 5 min	before injection	after 5 min	after 60 min	before injection	after 5 min	after 60 min
Physiological saline	95±12,5 (7)	96±12,0 (7)	541±37 (7)	486±47 (7)	84±30 (7)	80±34 (7)	43±9,0 (7)	45±7,3 (7)		12±2,8 (7)	14±3,1 (7)	
P		>0,5		>0,5		>0,5		>0,5			>0,5	
Prothrombin (2,5 mg/kg)	89±4,5 (15)	88±5,5 (14)	537±29 (15)	501±30 (14)	77±9,5 (12)	82±7,6 (14)	45±6,0 (15)	50±6,3 (14)		18±1,9 (15)	17±2,3 (14)	
P		>0,5		>0,2		>0,5		>0,5			>0,5	
P2 (1,5 mg/kg)	90±6,0 (8)	96±10,0 (8)	612±37 (8)	568±48 (8)			35±2,7 (9)	46±4,5 (10)	41±3,7 (7)	11±2,2 (9)	17±2,6 (10)	12±2,8 (7)
P		>0,5		>0,2				>0,2	0,2		>0,1	>0,5

EXPERIMENTAL METHOD

Prothrombin obtained by the method described previously [5] was homogeneous on electrophoresis in polyacrylamide gel and had an activity of 1500 NIH units/mg protein. Prothrombin was activated by thrombin immobilized on Sepharose 4B, and P1 and P2 were isolated from the proteolysis products by chromatography on DEAE-cellulose [5]. Neither P1 nor P2 possessed clotting or esterase activity and they were homogeneous on electrophoresis in polyacrylamide gel in the presence of sodium dodecylsulfate. The preparations for testing were injected into the jugular vein of male albino rats weighing 180-200 g. Before, and at various time intervals after injection, blood samples were taken and the total clotting time, plasma recalcification time, and thrombin time were measured by the usual methods, the fibrinogen concentration determined as in [7], the level of fibrinogen degradation products as in [8], fibrinolytic activity as in [6], and total fibrinolytic activity (TFA) and nonenzymic fibrinolysis (NF) as in [4]. Physiological saline was injected into the control animals.

EXPERIMENTAL RESULTS

Addition of P1 to plasma in a final concentration of $1.4 \cdot 10^{-6}$-$8.0 \cdot 10^{-6}$ M in vitro did not cause it to clot. No change took place either in the plasma TFA and NF.

Five minutes after intravenous injection of P1 in a dose of 2.5 mg/kg body weight (final concentration $1.4 \cdot 10^{-6}$ M) the total clotting time was lengthened from 69 to 136 sec ($P < 0.001$) and the thrombin time from 40 to 75 sec ($P < 0.001$); the plasma recalcification time increased from 98 to 155 sec ($P < 0.001$). In addition, there was a small (by 20%) but statistically significant ($P < 0.001$) decrease in the fibrinogen concentration 5 and 15 min after injection of P1, which disappeared after 60 min. The concentration of fibrinogen degradation products rose from 156 to 278 mg % ($P < 0.001$) 5 min after injection of P1, and it still remained high after 15 min (234 mg %; $P < 0.001$). The appearance of P1 in the blood stream led to a rapid and considerable increase in TFA (Table 1), which reflects the activity of plasmin, plasminogen activators, and complexes of heparin with proteins in the blood and with biogenic amines. Nonenzymic fibrinolysis also was increased by three to five times. Whereas the contribution of NF to TFA in the control was about 30%, in the experimental series it was increased to 60-70%. This effect is evidence of considerable activation of the SAS. A high level of fibrinolytic activity still persisted 15 min after injection of P1, but it returned to normal after 60 min.

Intravenous injection of prothrombin in a dose of 2.5 mg/kg body weight or of P2 in a dose of 1.5 mg/kg body weight caused no significant change in the state of the clotting and anticlotting systems (Table 2).

Since the prothrombin P1 fragment in vitro is not a fibrinolytic agent and cannot cause clot formation because of the absence of active factor X in the plasma, mobilization of the anticoagulant and fibrinolytic potential of the body following intravenous injection of P1 is due to activation of the SAS. Whether this effect takes place in response to the direct action of P1 on the chemoreceptor zones or indirectly through the rapid generation of thrombin only further investigations will show. The first hypothesis seems the more likely.

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