PREVENTION OF INTRAVASCULAR BLOOD CLOTTING IN RATS BY DIP-α-THROMBIN

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In the healthy organism neurohumoral mechanisms of the anticlotting system prevent intravascular blood clotting during provocation of thromboinogenesis by releasing into the blood stream humoral agents of the anticlotting system: heparin and tissue plasminogen activator. Injection of heparin can prevent thrombus formation experimentally [1]. The use of heparin in clinical practice to correct hypercoagulation leads to reduction of postoperative thrombosis, although the rise of development of hemorrhagic complications remains high [14]. The potential capacity of the anticlotting system of the body can be estimated by activating it with the natural agent α-thrombin. However, α-thrombin is a polyfunctional enzyme, which has both clotting and procoagulant activity [3, 11]. A form of α-thrombin (DIP-α-thrombin), modified by di-isopropyl fluorophosphate (DFP), has no manifest proteolytic activity but can excite the response of the anticlotting system whether injected intravenously or given by perfusion of the carotid sinus region in the rabbit [4]. The effector act of the anticlotting system on excitation by DIP-α-thrombin includes increased secretion of heparin by mast cells [5]. The ability of DIP-α-thrombin to activate the anticlotting system is due to preservation of a recognition site for high-molecular-weight compounds in its structure, responsible for high-affinity binding of α-thrombin with receptors of blood cells and vessel walls [2, 6] and with specific substrates [9].

The aim of this investigation was to study the possibility of preventing intravascular blood clotting provoked by intravenous injection of lethal doses of thromboplastin, by means of DIP-α-thrombin.

EXPERIMENTAL METHODS

Experiments were carried out on 78 male albino rats weighing 180-200 g. The substances for testing were injected into the jugular vein, from which blood samples (1 ml) also were taken to determine the clotting time of recalcified plasma, the plasma thrombin time, fibrinolytic enzyme activity, plasminogen activator activity and plasmin activity [8], total fibrinolytic activity, and nonenzymic fibrinolysis [2]. The concentration of soluble fibrin was determined by the method in [12]. The results were subjected to statistical analysis. Not more than 2 ml of blood was taken from each animal. Control animals were given an injection of 0.85% NaCl soltuion. Thromboplasin with activity of 17 sec, obtained from rat brain, was used in the experiments. DIP-α-thrombin was obtained by blockade of α-thrombin by DFP (final concentration of 1 μM) for 1 h at pH 7.6. The residual clotting activity did not exceed 0.01 NIH unit/ml. Highly purified α-thrombin, homogenous on polyacrylamide gel electrophoresis was obtained as described in [7]. Preparations of α-thrombin had clotting activity of 2000 NIH units/mg protein. Animals of the experimental groups were given an injection of DIP-α-thrombin in a concentration of 1 μM (1 ml), 1 ml of thromboplastin was injected 5 min later, and blood was taken (1 ml) for analysis after a further 1 min.

EXPERIMENTAL RESULTS

In the experiments of series I the experimental animals (n = 19) were injected with a lethal dose of thromboplastin 5 min after a control injection of 0.85% NaCl solution (Table

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TABLE 1. Changes in State of Clotting and Anticlotting Systems 1 min after Injection of Lethal Doses of Thromboplastin into Rats after Preliminary Injection of DIP-α-Thrombin or of 0.85% NaCl Solution

Parameter of state of clotting and anticlotting systems	Control: 0.85% NaCl solution	Experiment 1: 0.85% NaCl solution + thromboplastin	Experiment 2: DIP-α-thrombin + thromboplastin
Soluble fibrin concentration, μg/ml	177,6±12,7 (10)	372,1±35,7 (10)*	206,1±15,9 (10)
Plasma recalcification time, sec	127,1±2,0 (10)	129,0±3,4 (10)	148,1±2,4 (10)*
Thrombin time, sec	25,0±0,2 (13)	25,3±0,2 (15)	27,8±0,4 (13)*
Nonenzymic fibrinolysis, mm^2	31,5±1,3 (14)	33,2±1,0 (14)	34,9±1,3 (13)
Fibrinolytic enzyme activity, mm^2	64,4±2,7 (14)	0	69,9±3,4 (15)
Plasminogen activator activity, mm^2	53,6±2,5 (14)	0	57,1±2,5 (15)
Plasmin activity, mm^2	10,7±1,6 (14)	0	14,0±1,4 (15)

Legend. Number of animals given in parentheses. *P < 0.001.

1). A sharp increase in the soluble fibrin concentration (up to 372.1 μg, or 209%) was recorded in the blood, and within certain limits this level was directly dependent on the quantity of thrombin appearing in the bloodstream. Massive endogenous thrombin formation as a result of activation of the blood prothrombin by thromboplasin leads to rapid removal of fibrinopeptides from fibrinogen, to the appearance of large quantities of soluble fibrin, and subsequently to intravascular blood clotting. In this series of experiments death of the animals from thrombosis was observed in 90% of cases (n = 17). As a result of binding of plasminogen and its activator with the fibrin thus formed, for which these proteins have high affinity [10], complete disappearance of fibrinolytic enzyme activity in the blood was observed (Table 1). Plasmin formed on the surface of the clots was not accessible for analysis, because its active center remains bound with the fibrin [10]. The natural defensive mechanisms of the anticlotting system were unable to exert any inhibitory action on the reaction of thrombin with fibrinogen, for high concentrations of thrombin block the chemoreceptors of the anticlotting system in the vascular bed.

In the experiments of series II injection of lethal doses of thromboplastin 5 min after injection of DIP-α-thrombin (n = 15) caused death of the animals from thrombosis in only 13% of cases (n = 2). No deaths were observed among animals of the control group, receiving only 0.85% NaCl solution. It can be tentatively suggeted the DIP-α-thrombin, which activates the anticlotting system, mobilizes the anticoagulant fibrinolytic potential of the animal, which is responsible for natural prevention of thrombosis in the vascular bed. Evidence in support of this view is given by the survival of 87% of the animals in this series of experments. We found (Table 1) that injection of thromboplastin after preliminary injection of DIP-α-thrombin causes no sharp change within fibrinolytic enzyme activity. Activity of plasmin and of plasminogen activator was actually a little higher than the values recorded in the control. Evidence of maintenance of the increased anticoagulant potential in the blood was given by a small but statistically significant increase in the plasma recalcification time and thrombin time after injection of a lethal dose of thromboplastin preceded by injection of DIP-α-thrombin. The absence of any significant change in the soluble fibrin concentration will be noted.

Thus DIP-α-thrombin, which possesses no proteolytic activity (but with its recognition site for high-molecular-weight compounds preserved), thus prevents intravascular clotting caused by lethal doses of thromboplstin and protects the experimental animals against thrombus formation, by mobilizing the reserve capacity of the neurohumoral anticlotting system.

The reaction of activation of the anticlotting system can also evidently explain the protective effect of preliminary injection of thrombin against the action of endotoxin and intravascular clotting observed in [13].

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ACTION OF TYRAMINE ON THE SPINAL AFFERENT LINK OF PRESSOR REFLEXES

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Pressor responses of the blood pressure (BP) to electrical stimulation of somatic nerves can be largely suppressed by noradrenalin (NA), applied directly to the region of entry of the stimulated afferents into the spinal cord [2]. Pressor reflexes developing in response to synchronized volleys in somatic Aδ- and C-afferents are the circulatory component of nociceptive responses [3], and this effect of NA can therefore be connected with inhibition both of responses of dorsal horn neurons to nociceptive stimulation when applied iontophoretically close to these neurons [4, 5] and of motor components of nociceptive responses if injected intrathecally [6, 7, 10]. These effects are explained by the action of NA on those adrenergic dorsal horn neurons of the spinal cord on which axons of noradrenergic brainstem neurons, inhibiting transmission of nociceptive impulses at the segmental level, terminate. Thus inhibition of pressor reflexes by NA can be regarded conjecturally as the result of imitation of enhancement of activity of noradrenergic antinociceptive systems. The validity of this hypotheses is increased if it can be shown that this same effect is induced by liberation of NA from axon terminals of noradrenergic neurons. Tyramine has the ability to release NA from these terminals [1].

The aim of this investigation was to determine changes taking place in pressor reflexes to volleys of spinal afferents on application of tyramine to the region of entry of these afferents into the spinal cord, i.e., as a result of release of endogenous NA close to adrenergic neurons composing the sensory systems of the spinal cord. Just as previously [2], the name pressor and depressor components of responses (PCR and DCR respectively) is given not only to the corresponding components of mixed, depressor-pressor responses, but also to responses in one direction, i.e., purely pressor and purely depressor responses.

EXPERIMENTAL METHODS

Experiments were carried out on cats weighing not less than 2 kg, anesthetized (intravenously) with chloralose (20-30 mg/kg) and urethane (330-500 mg/kg). After tracheotomy,

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