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Involvement of Thrombin in Activation of Heparin Secretion by Mast Cells in Immobilization Stress in Rats

B. A. Umarova, F. B. Shapiro, A. E. Kogan, S. V. Kulieva, and S. M. Strukova

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Activation of heparin secretion by connective tissue mast cells under conditions of immobilization stress is determined by activation of the sympathoadrenal system, secretion of adrenocorticotropic hormone, and possible generation of thrombin. Generation of thrombin in the blood under these conditions is confirmed by a significant drop in the proenzyme protein C concentration by 23%, a decrease in the activity of factor V (substrate of protein C) by 36%, and prolongation of activated partial thromboplastin time by 40%. It is shown that 30-min immobilization leads to a 3-fold depletion of the heparin pool in mast cells. Intravenous injection of hirudin, a specific thrombin inhibitor, before immobilization slightly diminishes the stimulating effect of stress on heparin secretion. These data suggest that apart from catecholamines and adrenocorticotropic hormone, thrombin generated in the bloodstream during stress also markedly contributes to activation of heparin secretion by mast cells.

Key Words: *thrombin; protein C; hirudin; mast cells; stress*

Modern concepts of the development of stress reactions do not exclude generation of thrombin in the bloodstream. We have previously demonstrated a sharp increase in the release of heparin, an immediate anticoagulant, from the connective tissue mast cells (MC) [4]. A question arises, is the thrombin generated in the bloodstream involved into stimulation of heparin secretion, i.e., in adaptive reactions aimed at maintenancing homeostasis. The validity of such a question is strengthened by previous findings that intravenous injection of α-thrombin leads to activation of heparin secretion by MC [2].

The aim of the present study is to reveal generation of thrombin in a certain type of stress, to prevent its effect using a specific thrombin inhibitor,

M. V. Lomonosov Moscow State University

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and to evaluate the intensity of heparin secretion by MC.

MATERIALS AND METHODS

Hirudin (Sigma), specific activator of protein C, protease from *Agkistrodon blomhoffi* venom, obtained as described elsewhere [11], factor V-deficient plasma, and APTT reagent (Sigma) were used.

Experiments were carried out on Wistar male rats weighing 180-200 g. Immobilization (fixation to a table) of varying duration served as stress factor. Blood samples (0.5 ml) were taken from *v. jugularis* before and during immobilization (on minutes 15 and 30) and mixed with 3.8% sodium citrate (9:1, v/v). In order to block thrombin activity the animals were intravenously injected with 50 units hirudin in a volume of 1 ml. Control rats received the same volume of physiological saline. The activity of factor V was measured according to the protocol recommended by Sigma, and the concentration of protein C was evaluated by its amidase activity after activation of the proenzyme with specific snake venom protease [11]. Platelets were counted in a 220 LA aggregometer. Physiological status of the subcutaneous and mesenterial populations of MC after a 30-min immobilization was assessed morphometrically. The indexes of heparin saturation and degranulation served as the main criteria of secretory activity of MC [1]. The data were analyzed statistically using the Student test.

RESULTS

In the first experimental series we studied generation of thrombin in the bloodstream of immobilized rats. Protein C is known to be extremely sensitive to blood, because thrombin with high affinity binds to endothelial thrombomodulin and rapidly activates protein C [7,8]. Active protein C (C_a) cleaves coagulation factors V/V_a and $VIII/VIII_a$. Therefore, the appearance of thrombin in the blood was judged from the drop in the protein C content due to its activation and, consequently, utilization, and from the decrease in the factor V, a substrate of protein C. Additional information on thrombin generation was obtained from platelet count, since the decreased platelet count implies their aggregation, which may be induced by thrombin. Intravascular platelet activation in repeated cold stress has been previously described in rats and mice [9,10].

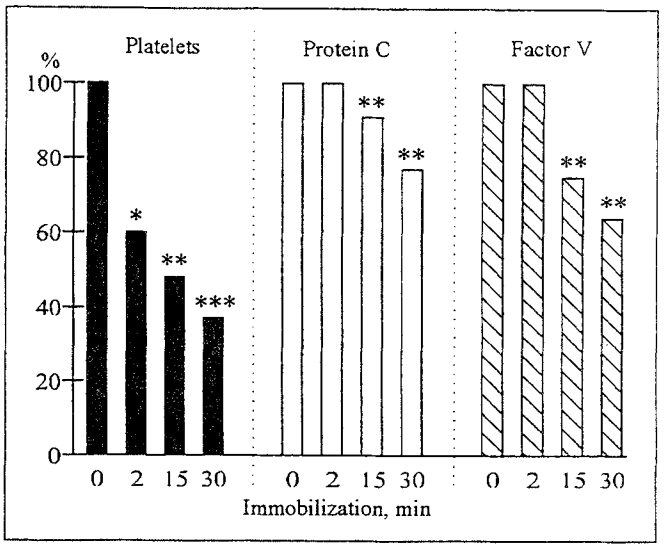

Fig. 1. Activity of platelets, protein C, and factor V in rats during a 30-min immobilization. Values before immobilization are taken as 100%. *$p<0.05$, **$p<0.02$, ***$p<0.001$ compared with the initial level.

As seen from Fig. 1, under conditions of immobilization stress blood concentration of protein C dropped to 91 and 77% on the 15th and 30th min, respectively. In parallel, the content of factor V decreased to 75 and 64%, respectively. Activated partial thromboplastin time was prolonged to the end of immobilization by 40% ($p<0.001$), which also attested to activation of the protein C system [13]. Platelet count decreased to 60% as soon as after 2 min of immobilization and to 48 and 37% after 15 and 30 min, respectively. This attests to intense platelet aggregation in stress induced under these conditions apparently by both thrombin and epinephrine. Activation of platelet aggregation during

TABLE 1. Effect of Immobilization Stress on Functional Status of Mast Cells ($M \pm m$)

Group	Mesentery		Subcutaneous fat	
	index of heparin saturation	index of degranulation	index of heparin saturation	index of degranulation
Intact (n=9)	1.93±0.12	0.01±0.003	1.83±0.1	0.03±0.004
Immobilization 30 min+0.85% NaCl (n=9)	0.70±0.03	0.05±0.003	0.67±0.04	0.05±0.004
Immobilization 30 min+hirudin (n=18)	0.98±0.06*	0.03±0.005	0.75±0.04	0.04±0.003

Note. *$p<0.001$ compared with group 2.

the first few minutes of immobilization may be caused by increased epinephrine secretion, while further activation is more likely to be governed by thrombin. It should be noted that thrombin acts sinergically with catecholamines and stimulates aggregation [12].

Thus, these findings suggest that thrombin generation occurs in this type of stress.

In the second experimental series we used hirudin, a specific inhibitor of thrombin, to assess possible participation of thrombin, which appears in the blood under conditions of immobilization stress, in activation of heparin release from MC (Table 1). In the control animals injected with physiological saline and subjected to 30-min immobilization, heparin secretion by mesenterial and subcutaneous MC was markedly enhanced due to activation of degranulation. As a result, heparin pool in these MC population to the end of immobilization constituted about 1/3 of the initial pool (intact animals). If rats were injected with hirudin before immobilization, the stimulating effect of stress was noted only for subcutaneous MC. The level of heparin secretion in mesenterial MC was significantly lower compared with that in the control animals, and the heparin pool in these cells by the end of immobilization was decreased by 50% ($p<0.001$). The intensity of stress-induced degranulation of mesenterial MC in control rats increased 4.5-fold, while in hirudin-treated rats it increased 3-fold in comparison with intact animals. Thus, inhibition of thrombin appearing in the bloodstream during stress prevents the development of appropriate adaptive reaction directed toward the maintenance of adequate anticoagulant potential of the blood, since not all MC populations respond to stress by intense heparin production.

From these data the following conclusions on the mechanisms involved into the stress-induced activation of secretory activity of MC were made. Catecholamines and adrenocorticotropic hormone play an important role in this activation [3,6]. Now, the contribution of thrombin arising in the bloodstream during stress to the stimulation of heparin secretion by MC also should be taken into account. The stimulating effect of thrombin on heparin secretion was noted only for mesenterial, but not for subcutaneous MC. No exhaustive explanation of this phenomenon can be given without appropriate investigations; however, it can be hypothesized that subcutaneous population of MC is more sensitive to the stress factor than mesenterial MC and, therefore, immobilization led to a more rapid depletion of heparin stores in subcutaneous MC than in mesenterial MC. For instance, after a 7-min immobilization heparin pool of subcutaneous MC is depleted by 60% and then slightly decreases, whereas heparin pool in the mesenterial MC population still remains unchanged [5]. Moreover, under physiological conditions the depletion of MC is apparently limited. This follows from the fact that about 25-30% of the initial heparin content is retained in MC in any type of stimulation [4]. Presumably, thrombin generated under stress conditions has no time to activate subcutaneous MC, which are already stimulated by neuroendocrine factors. Unlike subcutaneous MC, the less reactive mesenterial MC have an opportunity to accept the stimulating signal of thrombin and, therefore, hirudin injected into the bloodstream significantly suppresses their activation in immobilization stress.

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