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THE SIGNIFICANCE OF ACTH FOR THE PROCESS OF FORMATION OF COMPLEX HEPARIN COMPOUNDS IN THE BLOOD DURING IMMOBILIZATION STRESS /244*

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Total nonenzymatic fibrinolytic activity increases four times and more than 1.5 times -- its proportion in the general fibrinolytic activity of the blood plasma in the rat in a stress state caused by 30-minute immobilization. The same stress effect against the background of exogenic ACTH leads to a still more pronounced increase in nonenzymatic fibrinolysis (2.5 times higher than without the administration of ACTH). ACTH causes a significant increase in the formation of heparin complexes even in the absence of the stress factor. When ACTH secretion is blocked by the administration of DOCA, immobilization stress is not accompanied by an increase in the process of complex formation. The effect of ACTH on the formation of heparin complexes is mediated through its stimulation of the adrenal cortex. This is indicated by the results of experiments when ACTH was administered to rats at different times following adrenalectomy. 96 hours following adrenalectomy, the additional cortical tissue already reacts to the administration of ACTH, and correspondingly, the stress factor against the ACTH background causes a signficantly greater increase in non-enzymatic fibrinolysis than the same factor without ACTH.

One can now consider it firmly established that the hypothysary - adrenal cortex system participates in the complex physiological processes which ensure preservation of the liquid state of the circulating blood. Injury of any of the elements of this system fundamentally results in the adaptive possibilities of the

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anticoagulating system being sharply reduced in a stress state that requires mobilization of all protective mechanisms of the organism. We have shown that the various indices which characterize the functional condition of the anticoagulating system differ from the corresponding indices in intact animals and indicate disruption of its function in adrenalectomized animals, as in animals with ACTH secretion blockage by the hypophysis under conditions of stress. These indices normalize when necessary replacement hormonal therapy is carried out [9,11]. Such an important index of the functional activity of the anti-coagulating system, which sensitively reacts to stress factors, as the process of formation of complex heparin compounds with specific blood proteins and biogenic amines [8] is also disrupted following adrenalectomy. These complex heparin compounds have anticoagulation activity, as investigations conducted by B. A. Kudryashov et al. [2,4,5] show, and are capable of dissolving the unstabilized fibrin clots. Inasmuch as the adrenalectomized animals cannot respond to the stress factor caused by precisely the same increase in heparin complex formation as the intact animals, the anticoagulating potential of their blood is sharply reduced. One can normalize the process of formation of the heparin complexes under stressful conditions by replacement administration of corti- /245
costeroids. These data indicate that heparin complex formation requires a certain level of corticosteroids for its realization. The question naturally arises whether the process of heparin complex formation is increased in animals with hyperfunctioning of the adrenal cortex, in which case an excess of adrenal cortical hormones would exist in the organism. The explanation of this question was also a goal of this investigation.

The Method

Mongrel male rats weighing 170-200 grams were used in the work. Hyperfunctioning of the adrenal cortex was caused by the intraabdominal administration of ACTH (the S. M. Kirov meat combination)

in physiological solution (1.0 ml). The one-time dose was 5 units of ACTH administered triply with an interval of 24 hours, or singly. Blood with sodium nitrate (9:1) was taken from the jugular vein 24 hours or 30 minutes after administering ACTH. Animals that were administered physiological solution and intact animals were the control. The stress state was caused by 30 minute immobilization (the animals were bound to a table). Total fibrinolytic blood activity was determined according to the Astrup and Mullertz method [12]. Nonenzymatic fibrinolysis by complex heparin compounds was determined by the B. A. Kudryashov and L. A. Lyapina method with epsilon aminocaproic acid (EACA), which suppresses enzymatic fibrinolysis [6]. Fractional extraction of the following complex heparin compounds was carried out: with fibrinogen (FH) -- according to the method of B. A. Kudryshov, T. M. Kalishevskaya and L. A. Lyapina [3]; Adrenaline (AH) -- according to the method of B. A. Kudryashov and L. A. Lyapina [7], plasminogen (PGH) and Plasmin (PH) -- according to a method we described [8]. The activity of FH, AH, and PGH complexes was determined on factor XIII - unstabilized fibrin platelets. The activity of the PH complex, unlike that of the other complexes, was determined on stabilized fibrin platelets. The activity of PGH and PH complexes was determined on fibrin platelets heated for 30 minutes at 85° [13]. 0.05 ml of plasma or a complex was added to the platelets during the determination of lytic activity and the platelets were then incubated for 24 hours at 37°. The value of fibrinolictic activity, nonenzymatic fibrinolysis, and activity of FH, AH, PGH and PH complexes were judged according to the magnitude of lysis zones on the fibrin platelets (in mm^2).

Results of the Investigation and their Discussion

Before investigating the lytic activity of complex heparin compounds under conditions of immobilization stress in animals with hyperfunctioning of the adrenal cortex caused by the admin-

istration of ACTH, it was necessary to obtain "background data", i.e., data on how complex formation changes in intact animals with the given kind of stress. In accordance with what we had earlier shown [8], immobilization led to a significant increase in both total nonenzymatic fibrinolytic activity and the activity of separate complex heparin compounds (tables 1 and 2, groups 1 and 2; figures 1 and 2). The value of nonenzymatic fibrinolytic activity increased nearly four times (35.8 $\pm$ 4.4 mm^2 instead of 9.7 $\pm$ 1.5 mm^2), and by more than 1.5 times of its proportion in total blood fibrinolytic activity (48.1 $\pm$ 3.7% instead of 28.7 $\pm$ 3.8%), while the activity of the FH, AH, and PGH complexes increased 5-9 times and activity of the PH complex even increased 13 times.

As one can see from the data presented in table 1, the stress factor led to a much greater increase in both the total fibrinolytic activity of the blood, and particularly, the nonenzymatic fibrinolytic activity in all groups of animals which received ACTH (groups 4,6 and 8), regardless of the total dose and time of administration, than was the case in the intact animals. Total and nonenzymatic fibrinolytic blood activity increased within practically the same limits during stress as in the intact animals and control animals (groups 3, 5, and 7), which received physiological solution instead of ACTH.

Thus, an increase in nonenzymatic fibrinolytic activity in excess of the control figures (by an average of 2.5 times) was detected both in animals with clearly pronounced adrenal hypertrophy which developed as the result of 3 time ACTH administration (the weight of the adrenals increased by approximately 30%, group 4), and in those animals which received ACTH singly, and not only over 24 hours (group 6), but also simultaneously with the onset of the stress factor (group 8), and in which adrenal hypertrophy was not detected.

Table 1. Total nonenzymatic fibrinolytic activity, size of the lysis zone (mm^2). /246

Group of animals	Number of animals	Lysis zone (A)	Lysis zone with EACC(B)	BX100/A
1. Intact, without immobilization	20	35.3±2.7	9.7±1.5	[illegible]
2. Intact, with immobilization	26	65.2±3.6	35.8±4.4	[illegible]
3. Physiological solution intra-abdominally, 3 days, with immobilization	31	81.0±7.1	37.0±3.3	[illegible]
4. ACTH intraabdominally, 3 days, with immobilization.	34	122.0±7.2	71.2±5.1	[illegible]
5. Physiological solution singly, intraabdominally, over 24 hrs. with immobilization.	10	60.6±10.1	23.5±4.4	[illegible]
6. ACTH singly in 24 hours, intraabdominally, with immobiliztion.	12	150.0±10.5	90.7±6.5	[illegible]
7. Physiological solution singly, in 30 minutes, intraabdominally, with immobilization.	24	77.5±4.3	37.1±1.9	[illegible]
8. ACTH intraabdominally, singly, in 30 minutes, with immobilization.	36	136.7±4.2	80.3±2.3	[illegible]
9. Physiological solution singly, intraabdominally, in 24 hours without immobilization.	11	37.9±10.7	10.9±2.3	[illegible]
10. ACTH singly, in 24 hours, intraabdominally, without immobilization.	12	92.2±4.2	56.6±2.3	[illegible]
11. DOCA intraabdominally, in 24 hours with immobilization.	16	31.4±7.6	9.3±0.7	[illegible]
12. Solvent intraabdominally, over 24 hours, with immobilization.	16	59.2±2.2	27.4±1.6	[illegible]

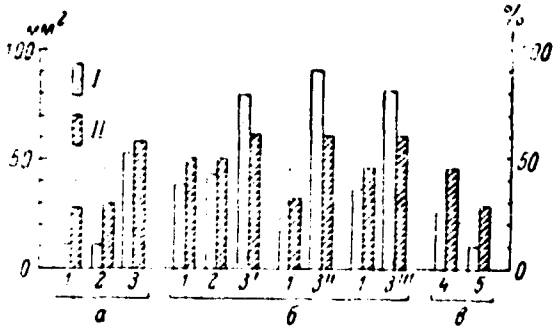

Figure 1. Effect of intra-abdominally administered ACTH on total nonenzymatic fibrinolytic activity. I - absolute value; II - percentage of total fibrinolytic activity. a-without immobilization; b,c - immobilization. 1 - Intact rats. Rats which received the following intraabdominally: 2-physiological solution; 3 - ACTH, 5 units, in 24 hours, 3'- ACTH, 15 units in 24 hours, 3''- ACTH, 5 units in 24 hours, 3''' ACTH, 5 units, in 30 minutes; 4 - solution 5 - DOCA.

Table 2. Lytic activity of complex heparin compounds, size of lysis zones (mm^2).

Group of animals	Number of animals	FH	A	PGH	PH
1. Intact, without immobilization	20	14.2±2.5	4.4±1.1	4.8±1.2	1.0±0.2
2. Intact, with immobilization	26	71.7±17.3	35.0±0.3	31.7±3.8	13.6±2.1
3. Intraabdominal physiological solution, 3 d., with immobilization.	24	69.8±3.9	39.0±3.9	38.7±3.9	14.6±3.9
4. ACTH intraabdominally, 3 d., w/immobilization	26	99.6±5.3	64.2±3.9	49.6±5.4	20.4±4.9
5. Physiological solution intrabdominally, singly, in 24 hrs, with immobilization.	10	18.5±3.4	24.4±3.2	17.3±3.4	15.8±4.8
6. ACTH, intraabdominally singly, in 24 hrs., with immobilization	12	74.0±6.3	56.3±5.8	55.5±5.7	37.4±4.7
7. Physiological solution intraabdominally, singly in 30 minutes, with immobilization.	24	62.1±4.5	36.2±1.2	7.8±1.8	8.2±1.4
8. ACTH intraabdominally, singly in 30 minutes, with immobilization.	36	91.3±3.2	50.4±2.0	39.6±3.4	24.7±2.3
9. Physiological solution intraabdominally, singly in 24 hrs, without immobilization.	11	18.5±3.4	24.4±3.2	17.3±3.4	15.8±4.8
10. ACTH intrabdominally, singly, in 24 hrs. without immobilization.	12	55.2±3.1	24.8±2.3	16.8±5.8	10.7±2.5
11. DOCA intraabdominally, in 24 hrs. with immobilization.	16	10.1±3.0	2.9±1.0	1.2±0.6	1.5±1.4
12. Solution intraabdominally, in 24 hrs., with immobilization	16	63.7±3.0	20.9±2.2	14.2±2.8	8.6±2.2

The increase in total and nonenzymatic fibrinolytic activity in animals which received ACTH was statistically reliable in all cases, in comparison with the intact and control animals which received

physiological solution.

An increase in the proportion of ACTH in the total fibrinolytic activity of the blood to 57.7-62.7%, observed in all cases, indicates that nonenzymatic fibrinolysis specifically increases in the stress state which developed against the background of the effect of exogenic ACTH, while at the same time as the proportion of ACTH is 43.7 - 46.7% in the control groups. This difference is reliable for groups 3 and 4 ($t = 4$) and for groups 7 and 8 ($t = 6$); obviously, reliability is somewhat lower ($t = 2$) for groups 5 and 6 because of the relatively small number of animals.

The activity of individual complexes was greater in all groups of animals which received ACTH in the stress state than in the control animals (Table 2). The increase in the activity of FH and AH complexes in these groups, on the average, exceeded their increase in the control groups by 65-70%, while the PGH and PH complexes even exceeded them by 130-167%.

The fact that administering ACTH even in the absence of the stress factor causes an equally significant increase in the formation of heparin complexes is of particular interest. One can see from the data presented in tables 1 and 2 (groups 9 and 10) that nonenzymatic fibrinolytic activity increased in animals which received 5 units of ACTH 24 hours before blood taking if they were exposed to the stress factor, and namely -- the absolute value of fibrinolytic activity exceeded the control Figures 2 times. In /248
this case, only the FH complex activity increased (3 times). The activity of the AH, PGH, and PH was unchanged.

Hence, one can state that ACTH apparently evokes a "stress state" in the organism and has a quite prolonged effect in this regard, inasmuch as the effect of its administration can be detected a day later.

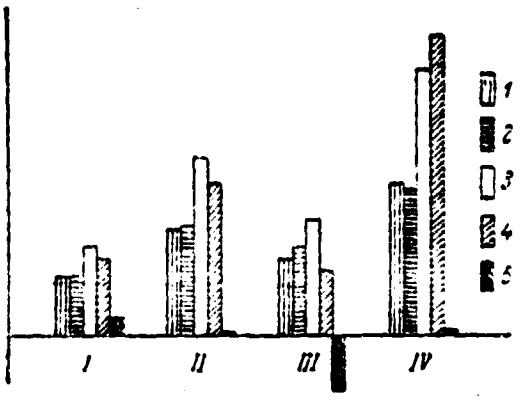

Figure 2. The effect of intraabdominally administered ACTH on the activity of complex heparin compounds. I -- fibrinogen -- heparin complex (FH), II -- adrenaline -- heparin complex (A), III -- Plasminogen-heparin complex (PGH); IV -- plasmin-heparin complex (PH). 1 - Intact rats. Rats which received the following intraabdominally: 2 -- NaCl solution; 3 --15 units of ACTH in 24 hours; 4 - ACTH, 5 units, in 30 minutes; 6 -- DOCA, Horizontal line -- level of activity of complexes in intact rats without immobilization.

The ACTH value for realizing the complex forming process is also confirmed by the results of a specially conducted series of experiments with ACTH secretion blockage. For this purpose, one group of animals was intraabdominally administered DOCA (20 mg/100g), while the other, for a control, was administered an equal volume of solvent and a conventional experiment with immobilization stress was performed with them 24 hours later (Tables 1 and 2, groups 11 and 12; Figures 1 and 2). As should have been expected, the stress factor did not cause the conventional increase in com- /249
plex formation against the background of secretion blockage of ACTH -- it was the same as in the intact animals without stress.

If the hypothesis that ACTH has its effect in mediated fashion on complex formations via stimulation of the adrenal cortex is correct, then the effect of administering ACTH should be absent in animals deprived of their adrenals. In order to test this hypothesis, the authors administered ACTH to adrenalectomized animals in the

Table 3. Total enzymatic fibrinolytic activity, size of the lysis zones (mm^2).

Group of animals	Number of animals	Lysis zone (A)	Lysis zone with EACC (B)	BX100/A
1. 48 hours after adrenolectomy, intravenous physiological solution singly, in 30 minutes, with immobilization.	10	49.7±2.4	12.8±1.7	25.7±3.4
2. 9-10 days following adrenolectomy, with immobilization	23	54.0±5.5	15.0±3.4	25.8±6.6
3. 48 hours after adrenolectomy, intraabdominal ACTH singly, in 30 minutes, with immobilization.	10	53.7±4.3	14.8±1.2	27.5±1.7
4. 96 hours after adrenolectomy, intraabdominal ACTH singly, in 30 minutes, with immobilization.	8	67.5±4.5	28.1±2.6	41.6±1.0
5. 9-10 days following adrenolectomy, intraabdominal ACTH in 30 minutes	14	90.5±4.6	52.4±4.8	57.9±2.6
6. 9-10 days after adrenolectomy, intraabdominal ACTH singly, in 24 hours, without immobilization.	19	52.9±3.0	20.2±1.7	38.3±2.2
7. 9-10 days following adrenolectomy, intraabdominal physiological solution singly, in 24 hours, without immobilization.	15	20.6±2.1	4.7±0.9	20.0±4.0

subsequent series of experiments. Table 3 and Figure 3 give the results of this series. For brevity, we shall cite only data relative to total nonenzymatic fibrinolytic activity.

As is known, one quite frequently encounters additional adrenal tissue in albino rats which can be activated following adrenalectomy and can secrete corticosteroids even after four days [1]. The results of an experiment conducted by the authors with the administration of ACTH to adrenalectomized animals at different times following the operation are in agreement with these data and indicate the mediated character of its effect on the process of heparin complex formation.

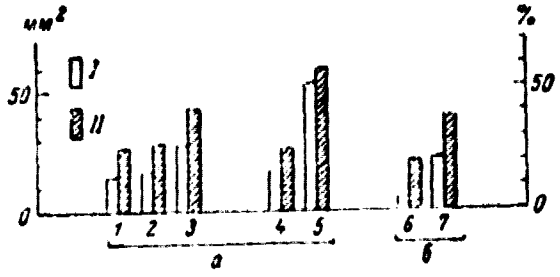

Figure 3. The effect of intraabdominally administered ACTH on total nonenzymatic fibrinolytic activity in adrenalectomized rats. I -- absolute value, II -- percentage of total fibrinolytic activity. a -- Immobilzation; b -- without immobilization. 1 - 48 hours after adrenolectomy, physiological solution; 2 - 48 hours after adrenolectomy, ACTH; 3 - 96 hours after adrenolectomy; ACTH; 4 - 9-10 days after adrenolectomy; 5 - 9-10 days after adrenolectomy, ACTH; 6 - 9-10 days after adrenolectomy, physiological solution; 7. 9-10 days after adrenolectomy, ACTH.

When examining the data in Table 3, one should primarily note that immobilization does not cause complex formation to occur more intensively in adrenalectomized animals than in intact animals without stress. The same low level of complex formation was detected both 48 hours and 9-10 days following removal of the adrenals (Table 3, groups 1 and 2). Obviously, if activation of the additional cortical tissues occurred 9-10 days following adrenolectomy, then its secretion of corticosteroids was insufficient for the complete development of the stress reaction[10]. The administration of ACTH 48 hours following adrenolectomy produced no effect--complex formation remained at precisely the same low level during stress (group 3). Evidently, this period of time is still insufficient for the additional cortical tissue to begin to react to the ACTH administered to the organism.

Administering ACTH 96 hours after adrenalectomy already caused an increase in complex formation, and total nonenzymatic fibrinolytic activity reliably increased during stress in comparison with

nonenzymatic fibrinolytic activity in animals which received ACTH 48 hours after adrenalectomy (t = 8.3). A still greater increase in nonenzymatic fibrinolytic activity during stress was evoked by administering ACTH to adrenalectomized animals 9-10 days following the operation (group 5). This increase is reliably higher than the increase which occurred upon administering ACTH 96 hours after adrenalectomy (t = 5.8). Moreover, administering ACTH 9-10 days after adrenolectomy causes a reliable increase in complex formation even without the stress factor, as occurs in intact animals (groups 6 and 7), although it occurs at a significantly lower level. Thus, we see that the additional cortical tissue already reacts to exogenic ACTH 96 hours following adrenalectomy /250 and its functional possibilities increase with the passage of time, although, of course, they do not fully compensate for the deficit of corticosteroids which exists in the organism following removal of the adrenals. Inasmuch as the administration of ACTH in the early terms following adrenolectomy stimulates the process of formation of heparin complexes, the mediated character of its effects in the later periods is certain.

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