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PARTICIPATION OF THE HYPOPHYSEAL-ADRENAL CORTEX SYSTEM IN THROMBIN CLEARANCE DURING IMMOBILIZATION STRESS

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16. Abstract The examination carried out with thrombin marked with I-131 resulted in a considerable increase of the thrombine clearance rate in healthy male rats during the stress (caused by an immobilization lasting 30 minutes) and in an increase of thrombin deposits in the liver. A further increase of thrombin clearance occurred by a combination of immobilization and administration of ACTH. Contrary to ACTH, the thrombin clearance is not stimulated in healthy animals by hydrocortisone.

Materials and methods used are discussed. Results obtained are presented.

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Participation of the hypophyseal-adrenal cortex system in thrombin clearance during immobilization stress.

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SUMMARY

The examination carried out with thrombin marked with I-131 resulted in a considerable increase of the thrombine clearance rate in healthy male rats during the stress (caused by an immobilization lasting 30 minutes) and in an increase of thrombin deposits in the liver. A further increase of thrombin clearance occurred by a combination of immobilization and administration of ACTH. Contrary to ACTH the thrombin clearance is not stimulated in healthy animals by hydrocortisone. Thrombin clearance and thrombin deposits in the liver are lowered in adrenalectomized rats. In these animals the administration of ACTH does not result in an increase of thrombin clearance. The rate of thrombin clearance is normalized in adrenalectomized animals after administering hydrocortisone without as well as under conditions of stress. In adrenalectomized animals having received hydrocortisone, as well as in healthy animals, the administration of ACTH will result in an increase of thrombin clearance.

It has been proven in several investigations that the hormones /244 * of the hypophyseal-adrenal cortex system (HNNR system = Hypophysen

*Numbers in margin indicate pagination in original foreign text

Nebennierenrinden System) participate in the physiological and biochemical processes that serve to maintain the circulating blood in the liquid state. This is demonstrated particularly by the fact that during stress effects on the organism, ACTH and glucocorticoids increase the blood's anticoagulating power[6,9,13]. As is known from papers by Kudryashov et. al., the anticoagulatory power of blood under stress conditions is determined to a significant extent by the non-enzymatic fibrinolytic activity of the heparin-protein complexes in the blood plasma, and by biogenic amines[3,11,12]. ACTH stimulates the formation of complex heparin combinations and in this manner enhances non-enzymatic fibrinolysis.This stimulation is conditioned by the fact that under the influence of ACTH, heparin enters the blood stream. In contrast, glucocorticoids play a permissive, complex-formation promoting role[7].

It has furthermore been shown that during stress the clearance of the endogenously formed or externally introduced thrombin is accelerated[8], a process also very important for normal activities. This process is causally related to an activation of of the anticoagulating system and an increase in the heparin content of blood, since during clearance from the liver thrombin is enriched with heparin in the form of a complex[5].

It can be assumed that the enhanced thrombin clearance during stress is also hormone-conditioned and dependent on the activation of the HNNR system. The current paper deals with this particular question. /245

Materials and methods

The tests were performed on male, white rats in the weight range 180-220 g. In certain of the tests, animals were used 6 to 7 days as well as 48 hours after adrenalectomy. Stress conditions were generated by immobilization (the animals were tied for 30 minutes to a small table). The blood was removed from the jugular

vein with sodium citrate (9:1).

The thrombin preparation (Manufacturer: Kaunas plant for bacteriological preparations) was labeled with I^{131} following Clement and McNicol's method[10]. In the thrombin iodization, no more than 0.5 g-atoms of I^{131} were used per g-mol of protein. From 95-97% of the radioactivity in the labeled protein was bound in the fraction that is precipitated by means of a 10% solution of trichloro-acetic acid. The specific activity of the I^{131}-labeled thrombin was 0.02-0.06 µci/ml. Thrombin-I^{131} retained its natural properties even many hours after labeling.

ACTH and hydrocortisone were applied intraperitoneally in the rats, in a physiological saline solution immediately prior to immobilization, in doses of 5 to 7 units/200 g and 2 mg/200 g. The volume of the solution applied was 1 ml. In a test with adrenalectomized animals that were not immobilized, the hydrocortisone was applied 30 minutes prior to taking the blood samples. Thrombin-I^{131} (5-7 Wroclaw units/200 g) was applied intravenously. The radioactivity in the blood sample, the liver, the spleen and the lungs was measured 1 to 10 minutes after the application of I^{131}, in Sahli tubes, in the scintillation counter "Gamma Guard 150" of the ICN Co. in Belgium; the specific activity of the organs mentioned was expressed as percentage of the specific activity of the blood.

Results and Discussion

In conformance to the problem posed, we investigated, in the first place, the rate of clearance of thrombin-I^{131} from the blood stream and its concentration by the liver, lungs and spleen, during stress conditions, with normal and adrepalectomized rats, with and without ACTH application.

Table 1 and Figure 1 below show the changes in specific activity of the blood during immobilization stress, during the first 10

TABLE 1. Specific activity in blood, 5-10 minutes after application of thrombin-I^{131}, in relation to the specific activity in blood after 1 minute (100%)

/246

Group	Pretreatment of test animals	Number of animals	Time after application of thrombin-I^{131}	
			5 minutes	10 minutes
1	- (Controls)	6	80,0 ± 1,8	64,8 ± 2,4
2	Immobilization	6	65,0 ± 2,5	50,7 ± 3,0
3	Adrenalectomy	5	90,3 ± 4,1	82,0 ± 3,2
4	Adrenalectomy + immobilization	6	81,2 ± 3,0	68,0 ± 2,7
5	Adrenalectomy + immobilization; physiol.saline sol.	6	82,3 ± 4,3	70,0 ± 3,9
6	Adrenalectomy + immobilization; hydrocortisone	7	67,4 ± 4,2	50,8 ± 2,8
7	Adrenalectomy; hydrocortisone prophyl.	6	79,6 ± 4,0	66,6 ± 3,5
8	Immobilization;ACTH	5	40,0 ± 6,3	25,8 ± 3,3
9	Adrenalectomy + immobilization;ACTH	4	77,7 ± 3,2	64,7 ± 4,4
10	Adrenalectomy + immobilization;ACTH + hydrocortisone	5	51,2 ± 6,7	31,0 ± 6,0

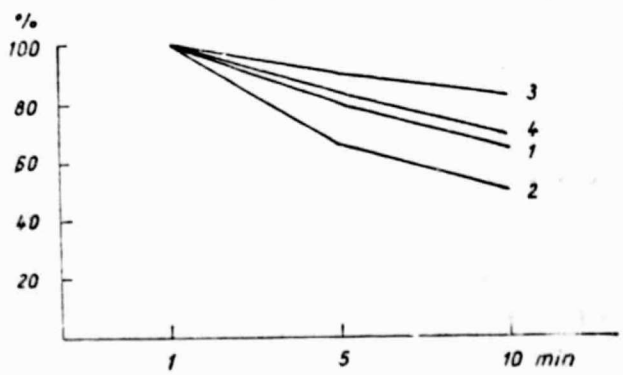

Figure 1. Specific activity in blood during immobilization of rats with unimpaired adrenal gland functioning, and of adrenalectomized animals

1. Non-immobilized animals with unimpaired adrenal gland functions 2: immobilized animals with unimpaired adrenal gland functions; 3: Adrenalectomized animals 4: Adrenalectomized and immobilized animals

minutes after administration of the labeled dose of thrombin-I^{131} to animals with unimpaired adrenal gland functions (groups 1 and 2) and animals with their adrenal gland removed, 6 to 7 days after operation (groups 3 and 4). As can be seen from these data, in agreement with our earlier results[8] the specific activity in blood decreases rapidly, while the rate of clearance increases significantly under the influence of stress conditions. When, for instance, for the case of rats without stress influence the specific activity in blood decreases 20% after 5 minutes, and 35% after 10 minutes, then for immobilized rats these decreases are, for the same times, 35% ($p < 0.01$) and 49% ($p < 0.05$), respectively.

The picture is quite different for adrenalectomized animals. With them, thrombin clearance is considerably lowered than in normal animals. In non-stress situations the specific activity in blood is, 10 minutes after thrombin-I^{131} application, reduced by only 18% ($p < 0.01$). If, instead, adrenalectomized animals are subjected to stress, then their thrombin clearance increases, occurring at the same rate as in animals with unimpaired adrenal gland functions not subjected to stress.

/247

The delayed thrombin clearance in adrenalectomized animals of course leads to a reduced enrichment in thrombin by the liver and lungs, in these animals (see Figure 2, below). If for non-stressed animals with unimpaired adrenal glad functions the specific activity in the liver and lungs, 5 minutes after thrombin administration is approximately 60% of the specific activity in blood, then for the case of adrenalectomized animals this value was closer to 40% ($0.025 > p > 0.01$). During stress the quantity of thrombin enriched in the liver increased, in the case of animals with unimpaired adrenal gland functions up to 98.8%; in this case, accumulation by the lungs was slightly reduced. In the case of adrenalectomized, immobilized rats, the increase in thrombin clearance was paralleled by thrombin enrichment in the liver, to values characteristic of intact, unstressed animals. The quantity of thrombin

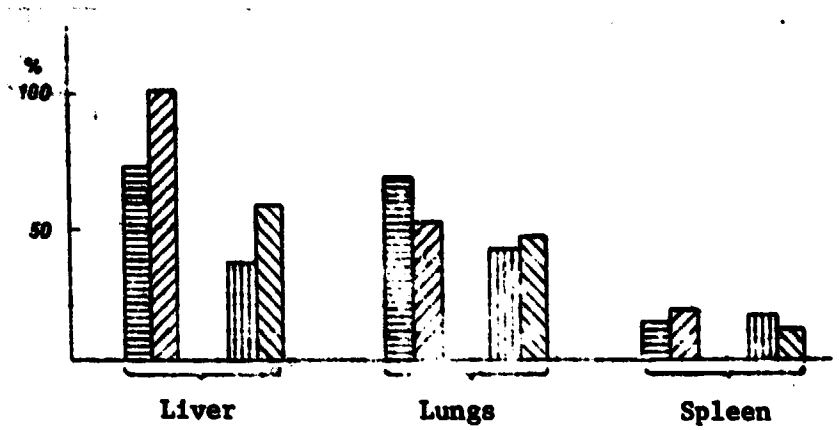

Figure 2. Specific activity in organs 5 min after administration of thrombin-I^{131}

Animals with intact adrenal functions, non-immobilized
Animals with intact adrenal functions, immobilized
Adrenalectomized animals, non-immobilized
Adrenalectomized animals, immobilized

in the lungs of adrenalectomized animals was not reduced by stress. The accumulation of thrombin-I^{131} by the spleen was completely negligible, both in intact and adrenalectomized animals and did not change with stress, either.

As further experimentation showed, the administration of hydrocortisone to adrenalectomized animals can normalize thrombin clearance (Figure 3, below and Table 1, groups 6 and 7; Table 2 groups 4 and 5); both in the cases of stress and non-stress conditions, the clearance of thrombin-I^{131} from the blood and its enrichment in the organs investigated reached the values characteristic of animals with intact cortical functions. A control administration of physiological saline solution to adrenalectomized animals showed no stimulating influence on thrombin clearance (Table 1, group 5 and Table 2 group 3). /249

If the activation of the HNNR system is causally related to increased thrombin clearance during stress, then it must be expected that an additional administration of ACTH to rats with unimpaired adrenal gland functions will further increase thrombin

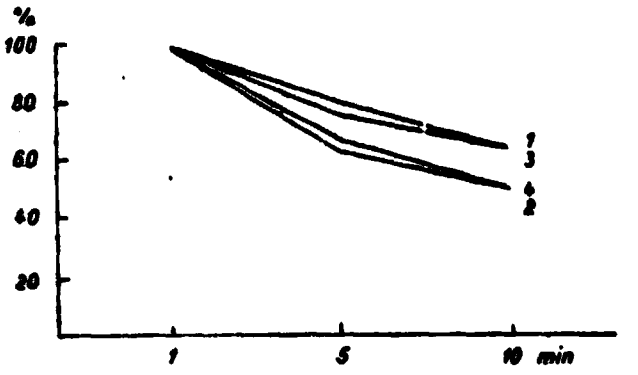

Figure 3. Specific activity in blood after immobilization of rats with intact adrenal functions and of adrenalectomized rats that received hydrocortisone

1 - Non-immobilized rats with intact adrenal functions
2 - Immobilized rats with intact adrenal functions
3 - Adrenalectomized animals; hydrocortisone administered
4 - Adrenalectomized and immobilized animals; hydrocortisone given

TABLE 2. Specific activity in organs of adrenalectomized rats and in rats with unimpaired adrenal gland functioning that received ACTH and hydrocortisone 10 minutes after thrombin-I^{131} administration

Group	Pretreatment of test animals	Number of animals	Liver	Lungs	Spleen
1	- Controls	6	78,7 ± 3,2	79,3 ± 2,7	24,5 ± 4,2
2	Immobilization	6	118,0 ± 5,6	67,2 ± 9,1	32,5 ± 4,2
3	Adrenalectomy + immobilization; phys.saline sol.	6	64,6 ± 3,9	94,3 ± 6,0	20,1 ± 1,8
4	Adrenalectomy + immobilization; hydrocortisone	7	114,2 ± 5,1	78,1 ± 3,2	32,0 ± 2,5
5	Adrenalectomy; hydrocortisone prophyl.	6	79,8 ± 4,0	78,6 ± 2,9	26,4 ± 2,1
6	Immobilization;ACTH	5	181,4 ± 10,0	39,2 ± 4,5	22,2 ± 4,9
7	Adrenalectomy + immobilization;ACTH	4	65,2 ± 4,3	90,8 ± 5,9	25,2 ± 1,6
	Adrenalectomy + immobilization;ACTH + hydrocortisone	5	140,8 ± 6,7	44,0 ± 4,9	23,8 ± 4,4

clearance. To prove this, tests were performed with administration of ACTH to immobilized animals with unimpaired adrenal gland functioning. The results of these tests showed that the administration of ACTH did, in fact, cause an enhancement in thrombin

clearance and an increase in thrombin accumulation by the liver, in comparison to animals that had not received ACTH (Table 1 group 8 and Table 2 group 6).

In contrast, the administration of ACTH to adrenalectomized animals did not prove effective. As is known, white rats often have additional adrenal gland tissue that are activated already 96 hs after adrenalectomy and can secrete corticoids[2]. To completely eliminate the possibility of an enhanced endogenous glucocorticoid level, in the present test we administered ACTH to the rats 48 hs after removal of the cortical gland.

As the results show (Table 1 group 9; Table 2 group 7; Figure 4, below), following ACTH administration the thrombin-I^{131} clearance was the same for adrenalectomized, stressed animals, as that without additional ACTH administration. The quantity of thrombin in the liver did also not change, while that in the lungs increased.

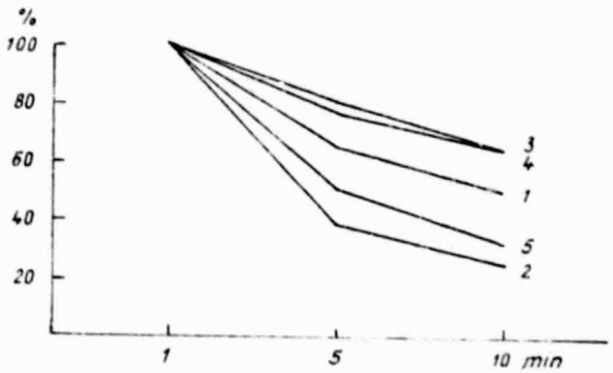

Figure 4. Specific activity in blood following immobilization of rats with intact adrenal functions and in adrenalectomized rats that received ACTH and hydrocortisone

1. Animals with intact adrenal functions, immobilized
2. Animals with intact adrenal functions, immobilized; ACTH
3. Adrenalectomized and immobilized animals; ACTH
4. Adrenalectomized and immobilized animals
5. Adrenalectomized and immobilized animals; hydrocortisone + ACTH

In contrast, when the lack of the adrenal gland was compensated by administration of hydrocortisone, addition of ACTH to these animals caused practically the same increase in thrombin clearance, during stress, as that of animals with intact adrenal functions in corresponding situations, i.e., a combination of stress and ACTH administration (Table 1 group 10 and Table 2 group 8).

Since, as we saw, the stimulating effect of ACTH on thrombin clearance occurs only if the adrenal glands are present or if the corticoid deficit in adrenalectomized animals is compensated by hydrocortisone administration, one could think that the ACTH effect is of a facilitating nature, i.e., that the glucocorticoid function of the adrenal gland is realized due to ACTH activation. It is more likely, however, that in this case - as in the enhancement of non-enzymatic fibrinolysis under the influence of ACTH[7] - we are dealing with the permissive effect of corticoid steroids. This is pointed out directly by the following fact: if the effect of ACTH is facilitated by an increased glucocorticoid secretion, then the administration of glucocorticoids to animals with intact adrenal gland function must cause an ACTH effect. In fact, however, the administration of hydrocortisone to immobilized rats with intact adrenal functions does not increase thrombin clearance; it remains the same as it was without hydrocortisone administration (Table 3).

TABLE 3. Specific activity in organs of animals with unimpaired adrenal gland functions that received hydrocortisone 5 min after administration of thrombin-I^{131}.

Group	Pretreatment of test animals	Number of animals	Liver	Lungs	Spleen
1	Immobilization; phys. saline sol.	6	79.8 ± 4.5	54.3 ± 1.6	21.2 ± 2.1
2	Immobilization; hydrocortisone	6	78.6 ± 5.6	52.5 ± 3.6	21.0 ± 1.7

The dependence of thrombin clearance on the functional state of the anticoagulating system is also demonstrated by results obtained with animals following a longer, atherogenic diet (over 2.5 months)[1,3,4]. In them, a depression of the anticoagulating system developed.

As can be seen in Table 4, the specific activity in blood of animals not subjected to stress and in those immobilized, decreases with a rate characteristic of adrenalectomized animals. The ACTH administration during stress stimulated thrombin clearance in animals with a depressed anticoagulating system. That is, when stress is combined with ACTH administration, there is no difference in thrombin clearance with that of the control animals with intact adrenal functions that have not been subjected to stress (cf. Table 1, groups 1, 3 and 4).

/251

In closing we must repeat once more that the stimulating effects of ACTH on thrombin clearance and on non-enzymatic fibrinolysis are mutually related, since thrombin is accumulated in the liver together with heparin, in a type of complex combination. This

TABLE 4. Specific activity in blood 5 to 10 min after thrombin-I^{131} administration to rats that received an atherogenic diet for over 2.5 months, in relation to the specific activity in blood after 1 min (100%).

Group	Pretreatment of test animals	Number of animals	Time after thrombin administration 5 min	10 min
1	- (Controls)	17	86.2 ± 1.0	77.0 ± 1.5
2	Immobilization	16	80.5 ± 0.9	70.1 ± 1.4
3	Immobilization:ACTH	17	72.8 ± 2.7	64.7 ± 2.0

stimulating effect is based on the ability of ACTH to promote the admission of heparin into the blood stream[7]. However, as has been shown earlier in regard to the formation of complex compounds by

eparin, which accomplish the non-enzymatic fibrinolysis, for the implementation of this process - in the present case, thrombin clearance - a certain physiological concentration of cortical steroids is required. These relationships are particularly apparent in stress situations, since they require the full mobilization of all adaptive possibilities at the organism's command.

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