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Translation of "Vliyaniye adrenalektomii i vvedeniya kortikosteroidov na fibrinoliticheskuyu aktivnost' kompleksnykh soyedineniy geparina v krovi pri immobilizatsii," Fiziologicheskiy zhurnal SSSR im. I. N. Sechenova, Vol. 59, No. 7, 1973, pp. 1108-1113.

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16. Abstract

Total non-enzymatic fibrinolytic activity in the blood of rats increased three times in response to stress caused by 30 minute immobilization, and the activity of epinephrine-heparin complex increased 9 times. In adrenalectomized animals, which showed a weak response to the same stress, intraperitoneal injection of hydrocortisone 30 minutes prior to immobilization normalized the response. Obtained results indicate that adrenalectomy leads to sharp reduction of heparin complexing with thrombogenic proteins and epinephrine, while substitution therapy with hydrocortisone restores anticoagulation system function.

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THE EFFECTS OF ADRENALECTOMY AND CORTICOSTEROID INJECTION ON THE FIBRINOLYTIC ACTIVITY OF COMPLEX HEPARIN COMPOUNDS IN THE BLOOD DURING IMMOBILIZATION

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Total non-enzymatic fibrinolytic activity of blood plasma in rats /1108* tripled during stress conditioned by 30-minute immobilization. Epinephrine-heparin complex activity concomitantly increased 9 times. A weak response, characterized by a total non-enzymatic fibrinolytic activity approximating that found in intact animals which had not been subjected to stress, was observed in adrenalectomized animals. Following intraperitoneal injection of adrenalectomized rats with hydrocortisone 30 minutes prior to immobilization, stress led to an increase in non-enzymatic fibrinolytic activity to levels present in intact animals which had undergone similar stress (immobilization and intraperitoneal injection of a physiologic salt solution). The obtained results demonstrated that adrenalectomy leads to a sharp reduction in heparin complexing with thrombogenic proteins and epinephrine, while substitution therapy with hydrocortisone ensured complete restoration of the stated system functions.

Numerous works have demonstrated the unquestioned participation of of adrenal cortex hormones in physiological processes ensuring the fluidity of circulating blood. It has been shown that both coagulation and anticoagulation systems are disturbed during stress following adrenalectomy or blocking of hypophysial ACTH functions, when the body's defense mechanisms must be mobilized [7, 8].

B. A. Kudryashov and his associates established that complex heparin compounding with specific blood proteins (fibrinogen, thrombin, plasminogen, plasmin) and some hormones (thyroxin, epinephrine, norepinephrine) occurs in the blood during stimulation of the anticoagulation system, particularly in stressful situations [1, 3, 4].

*Numbers in the margin indicate pagination in the foreign text.

These complex compounds have all of the properties of anticoagulation system agents and, in exerting their non-enzymatic fibrinolytic influence, are of adaptive importance. They inhibit hypercoagulation, lyse unstabilized fibrin clots, and thus, by maintaining the fluidity of the circulating blood, ensure normal functioning of the body in stressful situations.

The question naturally arises as to whether or not the process of complexing in adrenalectomized animals during stress is similar to that which occurs with other parameters characterizing the condition of the anticoagulation system. This work was devoted to clarification of this question.

Methodology /1109

The work was carried out using non-thoroughbred female rats weighing 170-200 grams. The lytic activity of complex heparin compounds was determined 8-12 days after adrenalectomy, when, judging from a sharp decrease in blood corticosteroid content and increase in ACTH secretion, the results of adrenalectomy were sufficiently pronounced [9]. Intact rats were used as controls. Stress was induced through 30-minute immobilization (tying to a peg). Blood was taken from the jugular vein and mixed 9:1 with sodium citrate. Total blood fibrinolytic activity was determined using the method of Astrup and Müllertz [10], and non-enzymatic fibrinolysis caused by the complex heparin compounds was determined using the method of Kudryashov and Lyapina with ε-aminocaproic acid (EACA), which suppresses enzymatic fibrinolysis [6]. Fractional separation of the following complex heparin compounds was carried out: with fibrinogen (FG) -- using the method of Kudryashov, Kalishevsky, and Lyapina [2], epinephrine (ENP) -- using the method of Kudryashov and Lyapina [5], plasminogen (PMG), and plasmin (PM).

Complexes of PMG and PM were separated out after obtaining FG and ENP. To accomplish this, 53.3% ethyl alcohol was added to the supernatant liquid after precipitation of the ENP complex until it constituted 20% in the system and pH=6.5. The precipitate of the 2nd

and 3rd fractions was separated out by Cohn's method [11] using a centrifuge at 0° and dissolved into a physiologic salt solution. Alcohol content in the solution of the 2nd and 3rd fractions was brought to 25% and pH=7.2. The 3rd fraction was then separated from the precipitate of the 2nd and 3rd fractions by centrifugation at 0°, dissolved into a physiologic salt solution, and the alcohol content of the system was brought to 17% and pH=5.2. The precipitate of the 3rd fraction (PMG, PM + plasminogen) was collected by centrifugation and dissolved in a volume of borate solution equal to the initial plasma volume.

Determination of FG, ENP, and PMG complex activity was carried out on plates of fibrin not stabilized by factor XIII. Activity of the PM complex, in distinction to that of the other complexes, was determined on plates of stabilized fibrin. The activity of PMG and PM complexes was determined on fibrin plates heated for 30 minutes at 85° [12].

To determine activity on the plates, 0.05 ml plasma or complex was taken and incubated for 24 hours at 37°. The amount of fibrinolytic activity, non-enzymatic fibrinolysis, and FG, ENP, PMG, and PM activity was judged according to the size of the zones of lysis on the plates of fibrin in mm^2.

Results of the Study and Discussion

Before studying the lytic activity of complex heparin compounds in adrenalectomized animals during stress caused by 30-minute immobilization, it was necessary to establish how this stress affects complexing in intact animals. To do this, comparison was made of total blood non-enzymatic fibrinolytic activity conditioned by complex heparin compounds and the activity of certain complexes (FG, ENP, PMG, and PM) in intact animals subjected to and not subjected to stress effects. Results of this comparison are shown in tables 1 and 2.

As can be seen from the data presented in table 1, the stress we applied caused a considerable increase in total non-enzymatic fibrinolytic activity: its absolute value increased more than three times (32.9 ± 4.0

instead of 9.5 ± 2.3 mm^2) and its share in overall blood fibrinolytic activity doubled (40.8 ± 4.0 instead of 20.7 ± 5.0%). ENP complex activity grew most sharply here -- more than 9 times, while the activity of FG, PMG, and PM increased more than 2-5 times (table 2). /1110

TABLE 1. TOTAL NON-ENZYMATIC FIBRINOLYTIC ACTIVITY ZONE OF LYSIS SIZE (IN MM^2)

Animal Group	Number of animals	Zone of lysis (A)	t	Zone of lysis with EACA (B)	t	B X 100 / A
Intact, no immobilization	21	40.0±5.4	4.4	9.5±2.3	5.1	20.7±5.0
Intact, with immobilization	29	76.0±6.2	1.8	32.9±4.0	1.36	40.8±4.0
Adrenalectomized, with immobilization	21	54.0±5.5		15.0±3.4		25.8±6.0

TABLE 2. LYTIC ACTIVITY OF COMPLEX HEPARIN COMPOUNDS ZONE OF LYSIS SIZE (IN MM^2)

Animal Group	Number of animals	FG	ENP	PMG	PM
Intact, no immobilization	23	44.6±10.9	6.7±2.6	17.1±7.0	5.6±1.2
Intact, with immobilization	22	98.0±17.9	56.7±10.9	55.6±12.8	26.2±6.1
Adrenalectomized, with immobilization	23	27.4±6.9	19.5±5.5	17.0±1.6	4.8±1.0

It is thus quite clear that a stressful state caused by 30-minute immobilization was accompanied in intact rats by a sharp increase in blood non-enzymatic fibrinolytic activity conditioned by the lytic properties of complex heparin compounds.

When adrenalectomized animals were subjected to such stress, complexing actually occurred no more intensely in them than in intact animals not subjected to stress. Despite some increase in total

non-enzymatic fibrinolytic activity noted in adrenalectomized animals during stress (15.0 ± 3.4 instead of 9.5 ± 2.3 mm^2), there was no statistically significant difference between them and intact animals not subjected to stress (t = 1.36). At the same time, non-enzymatic fibrinolytic activity in adrenalectomized animals during stress differed significantly from fibrinolytic activity in intact animals which had been subjected to this stress (15.0 ± 3.4 versus 32.9 ± 4.0 mm^2; t = 3.4) (table 1).

The same picture may be seen in reference to the activity of certain other complexes (table 2). If, as has been indicated above, the activity of all complexes sharply increases in intact animals during stress, the activity of PMG and PM complexes in adrenalectomized rats in the same situation remains the same as in intact ones free of stress, ENPN complex activity is three times higher (in intact animals more than 9 times higher), and the activity of the FG complex even decreases 1.5 times.

These data are evidence of the fact that disturbance of the body's adaptive capabilities after adrenalectomy also includes disturbance of the process of heparin complex formation and, consequently, the defense reaction of the anticoagulation system in response to stress cannot (in this case) be fully implemented.

Such a reduction in adaptive resources after adrenalectomy, as we know, is conditioned by inadequate body corticosteroids, primarily glucocorticoids. Hence, there is every reason to believe that injection of the corresponding hormone preparations can normalize the process of complexing in response to stress.

To test this, hydrocortisone (1 mg/100g of weight: 0.5 ml volume) was injected intraperitoneally into adrenalectomized rats 30 minutes before tethering, thereby compensating for their lack of natural corticosteroid output in response to the stimulation of stress [8].

Adrenalectomized and intact animals which had received intraperitoneally/llll equal volumes of physiologic salt solution served as controls. The data obtained are shown in tables 3 and 4.

TABLE 3. TOTAL NON-ENZYMATIC FIBRINOLYTIC ACTIVITY
ZONE OF LYSIS SIZE (IN MM^2)

Animal Group	Number of animals	Lysis zone (A)	Lysis zone w/ EACA(B)	B X 100 / A
Intact, intraperitoneal salt solution, with immobilization.....	38	126.8±4.0	82.6±2.6	65.3±2.1
Adrenalectomized, intraperitoneal salt soln, with immobilization.....	17	50.5±5.9	23.6±3.5	44.6±3.5
Adrenalectomized, intraperitoneal hydrocortsn with immobilization.....	24	109.5±6.2	73.0±0.1	60.8±1.7

TABLE 4. LYTIC ACTIVITY OF COMPLEX HEPARIN COMPOUNDS
ZONE OF LYSIS SIZE (IN MM^2)

Animal Group	Number of animals	FG	ENP	PMG	PM
Intact, intraperitoneal salt solution, with immobilization.....	25	100.5±8.6	68.0±9.0	81.5±5.6	19.0±8.9
Adrenalectomized, intraperitoneal salt sol'n with immobilization.....	17	27.3±5.8	18.7±2.7	18.3±4.1	3.9±1.7
Adrenalectomized, intraperitoneal hydrocortsn. with immobilization.....	24	142.5±8.9	64.5±7.3	83.2±5.9	18.7±5.2

While reviewing the data cited in these tables, it is most important to bear in mind the following fact. Intact and adrenalectomized animals reacted to additional stress, such as intraperitoneal injection of the physiologic salt solution, with a more significant increase in non-enzymatic fibrinolytic activity and, consequently, a more intensive process of complexing, than they did to stress caused only by immobilization. In the intact animals, the absolute value of non-enzymatic fibrinolytic activity and its share in overall

blood fibrinolytic activity rose significantly ($t = 10.30$ and $t = 5.30$) in comparison to the corresponding indices in intact animals which were subjected to immobilization stress alone. ENP and PMG complex activity was especially elevated here. The proportion of non-enzymatic fibrinolytic activity in overall fibrinolytic activity for adrenalectomized animals also increased with some degree of significance in comparison to that which occurred in them after immobilization alone. This increase of complexing after additional stress, however, proceeded at /1112 a markedly lower level in them than in the intact animals. In fact, only in the presence of additional stress did this level approach that which occurred in intact animals which had been subjected only to immobilization (cf. tables 1 and 3).

It is interesting to note that the activity of FG, ENP, PMG, and PG complexes did not change with additional stress (table 4).

The picture changes completely when adrenalectomized animals were injected intraperitoneally with hydrocortisone rather than a physiologic salt solution. After this all indices were comparable with those from intact animals which had been subjected to similar combined stresses. Both the absolute value of total non-enzymatic fibrinolytic activity and its share in overall blood fibrinolytic activity increase to levels in intact animals, and the activity of FG, ENP, PMG, and PM complexes reach control values. In other words, when blood corticosteroid levels are raised, the adrenalectomized animals begin to react to stress with as intensive a process of heparin complex formation as intact animals do. This is particularly graphically illustrated by figures 1 and 2, in which changes in non-enzymatic fibrinolytic activity

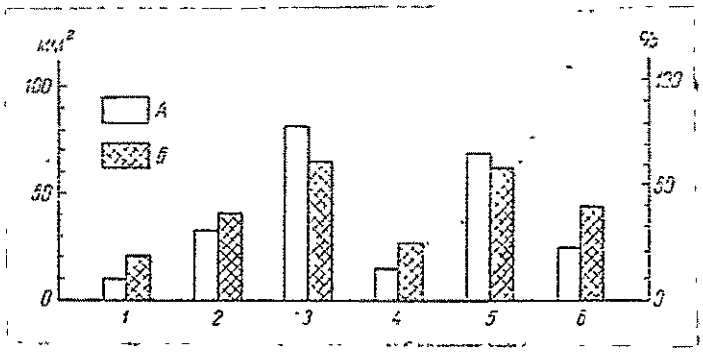

Fig. 1. Total non-enzymatic fibrinolytic activity

1 -- intact, no immobilization; 2 -- intact, immobilization; 3 -- intact, intraperitoneal physiologic salt solution; with immobilization; 4 -- adrenalectomized, with immobilization; 5 -- adrenalectomized, intraperitoneal hydrocortisone, immobilized; 6 -- adrenalectomized, intraperitoneal salt solution, immobilized. A -- absolute value; B -- % of overall activity.

and the activity of certain heparin complexes in all groups of animals are presented in comparison with corresponding indices in intact animals which had not been subjected to stress (in fig. 2 these indices are taken as the initial level).

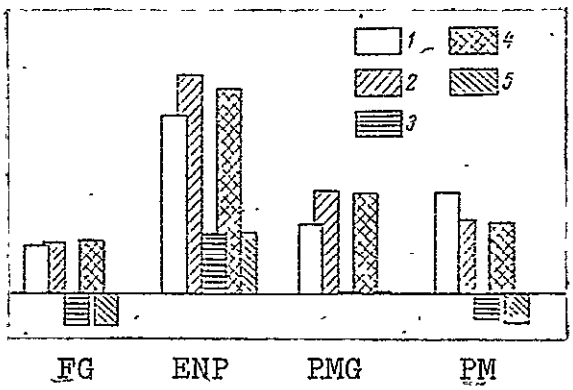

Fig. 2. Activity of complex heparin compounds.

1 -- intact, with immobilization; 2 -- intact, intraperitoneal physiologic salt solution, with immobilization; 3 -- adrenalectomized, with immobilization; 4 -- adrenalectomized, intraperitoneal hydrocortisone, with immobilization; 5 -- adrenalectomized, intraperitoneal physiologic salt solution, with immobilization.
Horizontal line -- activity level for complexes in intact rats without stress.

Normalization of the complexing process during stress in adrenalectomized animals with substitution therapy using hydrocortisone is evidence of the re-establishment of the functioning of the anticoagulation system. This coincides with data from works in which it was shown that disturbance of the coagulation and anticoagulation potentials of the blood, acutely manifested during stress in adrenalectomized animals and animals with impeded hypophysial ACT function, returns to normal under the influence of the corresponding exogenous corticosteroid and ACTH [7, 8].

In conclusion, we should also note that the formation of heparin complexes, which are active agents in the anticoagulation system, is a process which is sensitive to stress; intensification of stress immediately entails intensification of complexing. In a body which has been deprived of the capability of mobilizing proper defensive mechanisms in response to stress because of adrenalectomy, the process of heparin complex formation is not only significantly weakened, but quite disrupted. Increasing the level of corticosteroids through exogenous hormone introduction into adrenalectomized animals normalizes the process of heparin complex formation.

REFERENCES

[1] Kudryashov, B. A., T. M. Kalishevskaya, L. A. Polyakova, Vopr. meditsinsk. khimii, 12, 114, 1966.

[2] Kudryashov, B. A., T. M. Kalishevskaya, L. A. Lyapina, Vopr. meditsinsk. khimii, 14, 217, 1968.

[3] Kudryashov, B. A., Usp. fiziolog. nauk, 1, 17, 1970.

[4] Kudryashov, B. A., Arkhiv patologii, 33, 3, 1971.

[5] Kudryashov, B. A., L. A. Lyapina, Vopr. meditsinsk. khimii, 17, 46, 1971.

[6] Kudryashov, B. A., L. A. Lyapina, Labor. delo, 6, 326, 1971.

[7] Lomovskaya, E. G., F. B. Shapiro, Biolog. nauki, 6, 38, 1969.

[8] Shapiro, F. B., E. G. Lomovskaya, Biolog. nauki, 5, 42, 1972.

[9] Shchedrina, R. N., Problemy endokrinolog., 17, 58, 1971.

[10] Astrup, T., S. Müllertz, Archiv. Bioch. a. Biophys., 40, 346, 1952.

[11] Cohn, W., L. Strong, N. Hughes, J. Amer. Chem. Soc., 68, 459, 1946.

[12] Lassen, M., Acta Physiol. Scand., 27, 371, 1952.