THE CORTICOSTERONE LEVEL AND BINDING POWER OF THE PLASMA PROTEINS DURING EXPOSURE TO HEAT

O. V. Molotkov and N. B. Kozlov

UDC 612.12.018:612.453 + 612.124]-06:612.591

Exposure of rats to a high external temperature (45°C) for 20-60 min led to an increase in the level of total and protein-bound corticosterone in the peripheral blood plasma. The concentration of free corticosterone was increased 20 min after the beginning of exposure to heat and fell to its initial level during the subsequent times of observation. The development of heat shock was accompanied by an increase in the ability of the blood plasma to bind glycocorticoids.

The endocrine component (especially the state of the pituitary–adrenocortical system) plays an important role in the response of the organism to heat. However, the data in the literature on this question are contradictory and in some cases have been obtained by indirect methods of determination of the state of adrenal function [1, 4, 6, 8, 12, 16, 20]. Meanwhile, having regard to the importance of these glands in the maintenance of homeostasis, it is essential to study the dynamic of their function during exposure to heat as precisely as possible.

In the investigation described below the concentration of adrenocortical hormones (corticosterone) in the peripheral blood of rats was studied at various times after exposure to a high external temperature (45°C).

EXPERIMENTAL METHOD

Altogether 85 noninbred albino rats of both sexes weighing 180-220 g were used. The animals were heated in a special incubator at 45°C and at a relative humidity of 30%. Under these conditions, after 40-70 min the rats developed the typical picture of heat shock: a passive, flaccid position of the body with no response to a change in posture or to nociceptive stimulation; the corneal reflex was clearly marked. The rectal temperature rose on the average from the normal level of 36.7°C to 43.1°C.

Blood for investigation was taken from the experimental animals 20 min after the beginning of exposure to heat (the stage of restless movements), during heat shock, and 20 min thereafter. It should be emphasized that after the onset of heat shock the animals were quickly removed from the incubator. As a rule the rats developing heat shock died during the next 30-60 min.

The concentrations of protein-bound, free, and total corticosterone were determined in the peripheral blood plasma by a fluorometric method (the method of de Moor [19] in the modification of Pavlikhina et al. [5]. The ability of the plasma to bind corticosteroids also was determined by the addition of a known quantity of corticosterone in vitro. The total plasma protein concentration was studied by Lowry's method [17].

EXPERIMENTAL RESULTS AND DISCUSSION

The total corticosterone concentration in the blood plasma was increased by about 37% 20 min after the beginning of hyperthermia, and at the moment of development of heat shock it was increased by 65% (Table 1). A high level of corticosterone remained in the blood 20 min after heat shock.

Department of Biochemistry, Smolensk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 9, pp. 44-47, September, 1972. Original article submitted November 18, 1971.

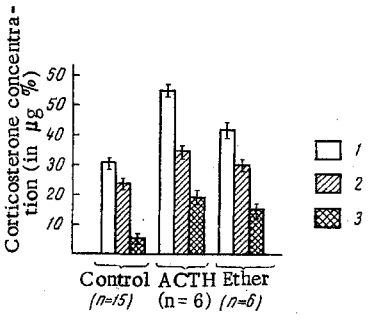

Fig. 1

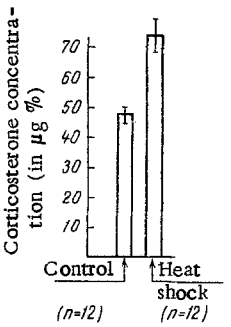

Fig. 2

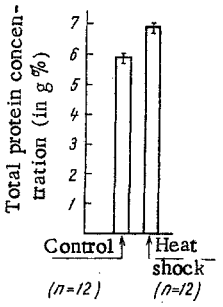

Fig. 3

Fig. 1. Effect of ACTH and ether anesthesia on the corticosterone level in the blood plasma: 1) total corticosterone, 2) protein-bound, 3) free corticosterone.

Fig. 2. Changes in ability of blood plasma proteins to bind corticosterone during exposure to heat.

Fig. 3. Effect of a high temperature on total protein concentration in blood plasma.

TABLE 1. Corticosterone Concentration in Peripheral Blood Plasma of Rats during Exposure to Heat (in $\mu g\%$)

Type of corticosterone	Control rats	20 min after beginning of exposure to heat	Moment of heat shock	20 min after heat shock
Total	30,0±1,2 n=15	41,0±2,5 n=7 P<0,001	49,1±2,7 n=15 P<0,001	43,3±3,6 n=5 P<0,001
Protein-bound	24,0±2,2 n=15	34,5±3,1 n=12 P<0,001	44,1±2,6 n=15 P<0,001	39,0±4,0 n=5 P<0,02
Free	5,1±0,5 n=15	6,8±0,41 n=12 P<0,05	5,5±0,44 n=15 P>0,5	4,9±0,53 n=5 P>0,5

The concentration of protein-bound corticosterone also rose during exposure to heat, and the higher the degree of hyperthermia the higher the level of protein-bound corticosterone in the blood plasma. Meanwhile the concentration of free corticosterone in the peripheral blood plasma was significantly increased only 20 min after the beginning of exposure to heat and it was practically indistinguishable from the control levels at subsequent periods of the investigation.

It can be concluded from the results of determination of the total corticosterone concentration in the plasma that during exposure to a high external temperature the activity of the adrenal cortex is stimulated. However, it is very interesting to note that after its increase 20 min after the beginning of exposure to heat, the concentration of free corticosterone thereafter fell to its initial level. This fact must be particularly emphasized since it is the free form of corticosterone, as it is now generally recognized, which plays the decisive role in the mechanism of action of glucocorticoid hormones [3, 9, 11, 21].

Stimulation of adrenocortical function is usually accompanied by an increase in the blood concentration of the free form of glucocorticoids. Special control experiments showed that procedures such as ether anesthesia or intraperitoneal injection of 5 units ACTH in fact give rise to a marked increase in the blood concentrations of both protein-bound and free corticosterone (Fig. 1).

Corticosterone, like hydrocortisone, is bound in the plasma mainly with transcortin, a protein belonging to the α-globulin fraction [2, 9, 13, 14]. It has also been shown that the binding power of this protein may vary in different states of the organism [7, 15, 18].

Since, as was shown above, heat shock was accompanied by a marked increase in the total corticosterone concentration in the plasma without any increase in the level of the protein-bound form of the hormone at that time, it was postulated that at this period the binding power of transcortin is sharply increased.

To test this hypothesis, the ability of the plasma of control animals and of animals heated to a stage of heat shock to bind adrenocortical hormone was determined in special experiments. For this purpose, before fractionation of the corticosterone by means of Sephadex G-50 into protein-bound and free forms, 100 μg corticosterone was added to the blood plasma. The results given in Fig. 2 show that at the time of onset of heat shock the binding power of the blood plasma proteins was considerably increased. This increase evidently explains the absence of any marked increase in the free form of corticosterone at these periods of investigation.

In view of reports in the literature that adrenocortical hormones can be bound (although less stably) not only by the specific protein transcortin, but also by other proteins and, in particular, by albumins [2, 11, 13, 22], the concentration of the total blood plasma proteins was determined in the control and heated rats (Fig. 3). The results showed that there was a marked increase in the total plasma protein concentration in the experimental animals at the moment of onset of heat shock from the normal level of 5.9 up to 6.8 g% ($P < 0.01$).

However, comparison of the figures reflecting the increase in the binding power of the plasma and the total concentration of plasma proteins shows that simple hyperproteinemia cannot explain the observed increase in the binding power of the plasma. The possibility cannot be ruled out that in this case, superposed on the increased total protein concentration in the blood, the synthesis of transcortin was increased or a change occurred in its conformation, giving the protein greater affinity for corticosterone.

LITERATURE CITED

1. F. T. Agarkov, V. A. Maksimovich, and A. N. Namyatnyi, Probl. Éndokrinol., No. 5, 15 (1962).
2. S. G. Genes, Pat. Fiziol., No. 6, 7 (1966).
3. P. D. Gorizontov and T. N. Protasova, The Role of ACTH and of Corticosteroids in Pathology [in Russian], Moscow (1968), p. 36.
4. A. N. Namyatnyi, F. T. Agarkov, and V. A. Maksimovich, in: Proceedings of a Conference on Adaptation, Training, and Other Methods of Increasing the Resistance of the Organism [in Russian], Donetsk (1960), p. 104.
5. L. V. Pavlikhina, I. Ya. Usvatova, and V. F. Bunatyan, in: Methods of Investigation of Some Systems of Humoral Regulation [in Russian], No. 5, Moscow (1967), p. 50.
6. É. L. Perel'man and F. F. Sultanov, Izvest. Akad. Nauk Turkmensk. SSR, Seriya Biol. Nauk, No. 1, 48 (1962).
7. V. B. Rozen and G. Ya. Riekstinya, Probl. Éndokrinol., No. 5, 75 (1970).
8. F. M. Shleifman, Gig. i San., No. 5, 14 (1961).
9. N. A. Yudaev, Vestn. Akad. Med. Nauk SSSR, 7, 12 (1969).
10. C. J. Beling, Acta Endocrinol. (Copenhagen), 79, 23 (1963).
11. M. Booth, P. F. Dixon, C. H. Gray, et al., J. Endocrinol., 23, 25 (1961).
12. K. J. Collins, J. D. Few, T. G. Fornard, et al., J. Physiol. (London), 194, 33 (1968).
13. W. H. Daughaday, J. Lab. Clin. Med., 48, 799 (1956).
14. W. H. Daughaday, Arch. Intern. Med., 101, 286 (1958).
15. R. R. Gala and U. Westphal, Endocrinology, 75, 1079 (1965).
16. H. B. Hole, G. Sayers, K. L. Sydnor, et al., J. Clin. Invest., 36, 1642 (1957).
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
18. I. H. Mills, H. P. Schadl, P. S. Chen, et al., J. Clin. Endocrinol., 20, 515 (1960).
19. P. de Moor, K. Heirweg, J. F. Heremans, et al., J. Clin. Invest., 41, 816 (1962).
20. O. O. Pylkkö and N. E. Törnblom, Acta Endocrinol. (Copenhagen), 28, 251 (1958).
21. A. A. Sandberg and W. R. Slaunwhite, J. Clin. Invest., 42, 51 (1963).
22. U. Westphal, Arch. Biochem., 66, 71 (1957).