EFFECT OF HORMONE LEVELS OF THE HYPOPHYSEO-ADRENAL SYSTEM ON METHIONINE-^{35}S INCORPORATION INTO BRAIN STRUCTURES

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Numerous investigations have shown that corticosteroids penetrate fairly rapidly into the brain where they accumulate selectively in the pituitary and in various other brain formations [11, 12, 16, 17]. Corticosterone accumulates most of all in the hypothalamus, hippocampus, septum, and amygdala, which suggests that adrenocortical hormones may act directly on these structures. Because of their effects on protein metabolism, some workers regard glucocorticoids as catabolic hormones [3, 19], on the basis of the increased protein breakdown, increased deamination of amino acids, and the appearance of a negative nitrogen balance after administration of large doses of ACTH or glucocorticoids. Other workers observed increased incorporation of methionine and glycine into liver proteins under the influence of cortisone and hydrocortisone [10, 13], indicating that glucocorticoids possess anabolic properties. Data on incorporation of various amino acids into individual brain formations also have been published [6, 9, 14, 15, 21, 22]. However, these data are very contradictory and difficult to analyze because the experiments were conducted on different species of animals, different amino acids were used, different brain formations were studied, at different times after injection of the labeled amino acids, and so on. Nevertheless systematic investigations of this sort are essential to explain the connection between nerve tissue protein metabolism and the functional state of the nervous system [1, 7, 18, 20].

This paper describes a comparative investigation of the degree of incorporation of a labeled amino acid into proteins of different brain formations closely connected with regulation of the hypophyseo-adrenocortical system, depending on the body levels of hormones of that system.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male rats weighing 200 g, divided into six groups: group 1 (control) – animals receiving 0.3-0.4 ml of physiological saline intraperitoneally daily for 1 week; group 2 – hypophysectomized animals; group 3 – adrenalectomized rats; group 4 – rats receiving ACTH-zinc-phosphate in a dose of 10 units/kg body weight intramuscularly daily; group 5 – animals receiving dexamethasone in a dose of 500 μg/kg intraperitoneally; group 6 – rats receiving ethimizole* (one of the most powerful stimulators of ACTH-glucocorticoid secretion) in a dose of 10 mg/kg subcutaneously. Hypophysectomy was performed on the rats by the transauricular route [8] and the adrenals were removed by the usual paraspinal method. The rats were used in the experiments 1 week after the operations.

The intensity of protein metabolism in the various brain formations was studied by histoautoradiography using methionine-^{35}S.† All rats of the control and experimental groups were given methione-^{35}S intraperitoneally in a dose of 0.5 mCi/g body weight (100 mCi per rat). The rats were decapitated 15-20 min after injection of the isotope. Histoautoradiographs were obtained by Zhinkin's method [2]. Regions of the brain including the hypothalamus (anterior or posterior), amygdala, and hippocampus, were fixed in Bouin's fluid and then de-

*1-ethylimidazole-4,5-dicarboxylic acid-bis-methylamide.

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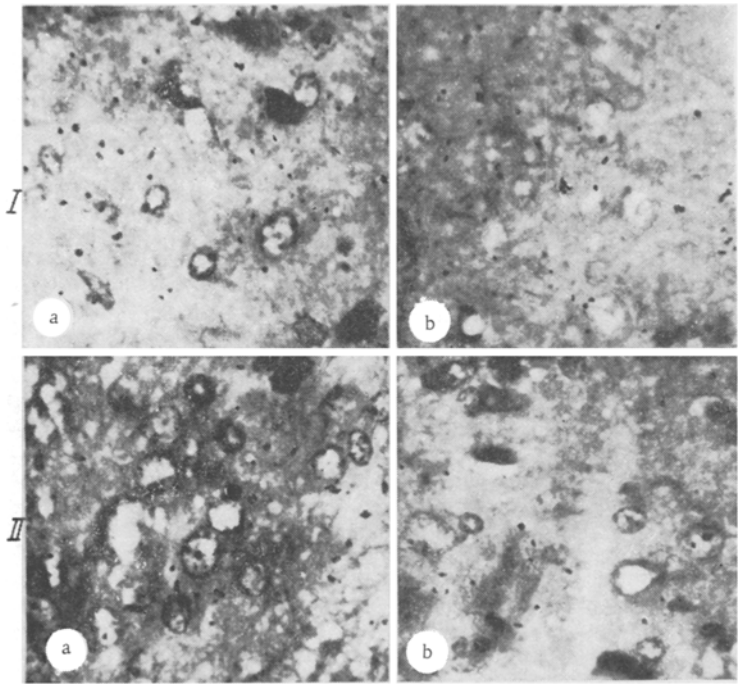

Fig. 1. Autoradiograph of rat hypothalamus 15 min after injection of methionine-^{35}S (812×). I) Anterior hypothalamus: a) intact rat, b) rat receiving dexamethasone (500 μg/kg in 1 week); II) posterior hypothalamus: a) intact rat, b) rat hypophysectomized 8 days before injection of label.

hydrated and embedded in paraffin wax in the usual way. Serial brain sections, 50 μ thick, cut on a microtome were fixed on slides coated with type R emulsion (from the Motion Picture Research Institute) and exposed in darkness for 10 days. After development and drying, the autoradiographs were stained with hematoxylin, counterstained with eosin, and mounted in Canada balsam. Quantitative analysis of incorporation of labeled methionine into cells of the anterior and posterior hypothalamic nuclei, into the dorsal hippocampus, and the mediobasal region of the amygdala was undertaken by counting tracks in the photographic emulsion with the aid of the grid of an ocular micrometer, followed by calculation of the mean value relative to an area of 100 μ^2. Sections from each part of the brain to be studied, 10-12 at a time, were distributed on five slides; tracks were counted in 750 squares of the ocular micrometer grid in three sample sections of each of five slides (i.e., in 15 sections from each part of the brain of the same animal). Data for each rat, averaged in this way, were subjected to statistical analysis. This value served as index of the intensity of incorporation of label by cells of the brain regions studied. Tracks were counted under the microscope with a magnification of 600 (objective 90, ocular 7).

EXPERIMENTAL RESULTS

Analysis of the autoradiographs obtained from the brains of the control rats showed that 15 min after injection of labeled methionine the isotope was incorporated with unequal intensity into neurons of the different brain formations (Figs. 1 and 2). Whereas the number of tracks (per 100 μ^2) in the anterior hypothalamic and mammillary nuclei of the hypothalamus, and also in the amygdala, varied from 1.4 to 2.25, in the dorsal hippocampus the intensity of incorporation of radiomethionine was over five tracks.

Counting the tracks (Fig. 3) showed that the intensity of incorporation of the label and, consequently, the rate of protein metabolism in nerve cells of the dorsal hippocampus, were considerably higher than in the other brain formations studied. The number of tracks above the nerve cells, it will be noted, was greater than the number above glial cells. The intensity of incorporation of radiomethionine into the supraoptic nuclei of the anterior hypothalamus was greater than the intensity of its incorporation into the parvocellular formations of the hypothalamus.

The way in which the degree of incorporation of methionine-^{35}S into the various brain formations changed as a result of extirpation of the endocrine glands and injection of the hormone is shown in Fig. 3. Clearly the

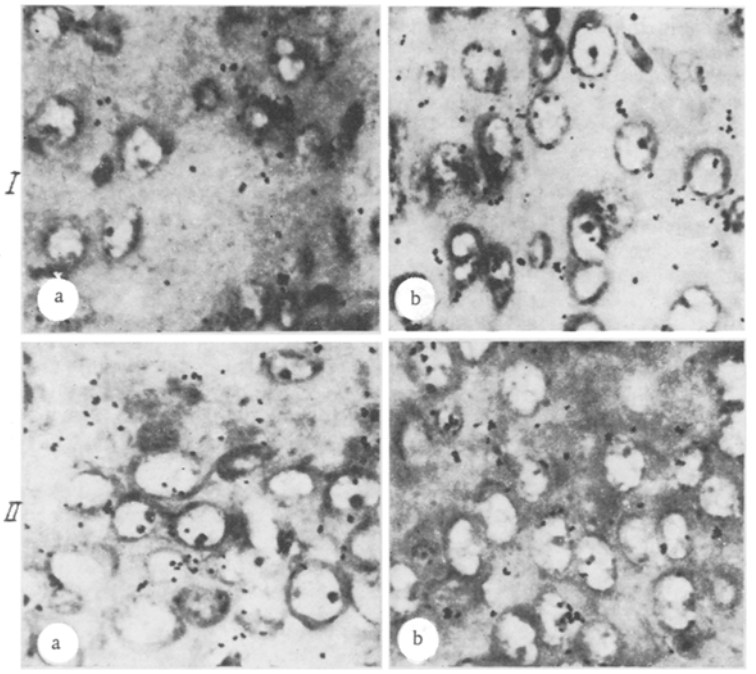

Fig. 2. Autoradiographs of limbic structures of rat brain 15 min after injection of methionine-^{35}S (812×). I) Mediobasal amygdala: a) intact rat; b) rat receiving ACTH (10 units/kg) for 8 days; II) dorsal hippocampus: a) intact rat, b) rat adrenalectomized 1 week before injection of label.

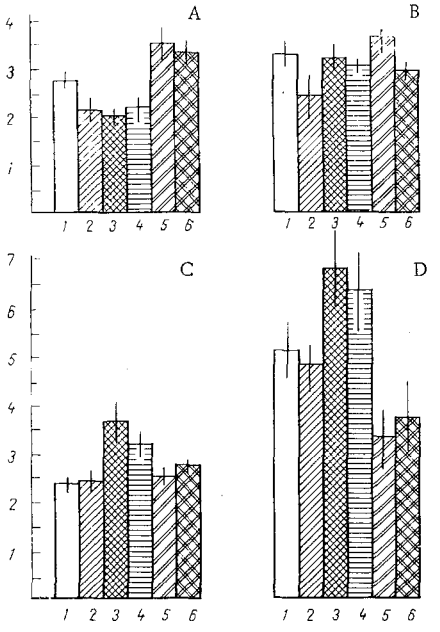

Fig. 3. Comparative intensity of methionine-^{35}S incorporation into brain structures (in tracks per 100 μ^2) 15 min after injection of label. A) Anterior hypothalamus; B) posterior hypothalamus; C) mediobasal part of amygdala; D) dorsal hippocampus. 1) Control; 2) hypophysectomy; 3) adrenalectomy; 4) injection of ACTH (10 units/kg daily for 8 days): 5) dexamethasone (500 μg/kg daily for 1 week); 6) ethimizole (10 mg/kg twice daily for 1 week).

intensity of incorporation of label into the anterior hypothalamus was significantly lower in the adrenalectomized or hypophysectomized animals and also after injection of ACTH; this process was definitely enhanced by chronic administration of dexamethasone or ethimizole. Changes in the same direction also were observed in the mammillary region of the posterior hypothalamus of the hypophysectomized animals and of rats receiving glucocorticoids. In the remaining groups of animals of this series the changes were not so clear. Incorporation of radiomethionine into nerve cells of the mediobasal amygdala was increased in the adrenalectomized rats and in animals receiving ACTH; this increase was less marked in rats receiving ethimizole. The intensity of incorporation of labeled methionine into the dorsal hippocampus rose considerably in the animals after adrenalectomy or administration of ACTH, and was reduced just as appreciably during chronic administration of dexamethasone or ethimizole.

Comparison of the intensity of methionine-^{35}S incorporation into the different brain formations shows that hypophysectomy led to a decrease in the number of tracks in the hypothalamic regions of the brain with no significant change of incorporation in the limbic structures. After adrenalectomy incorporation of the labeled amino acid into the anterior hypothalamus was reduced and the intensity of incorporation of the label into the amygdala and hippocampus was considerably increased. The same pattern was produced by injection of ACTH although, admittedly, the changes were less marked. Repeated injections of dexamethasone were accompanied by an increase in the number of tracks in the hypothalamic zones and a decrease in the intensity of incorporation of radioactive label into the dorsal hippocampus. After injections of ethimizole, incorporation of labeled methionine was increased in the anterior hypothalamic region and, to a lesser degree, in the amygdala, and was reduced in the dorsal hippocampus.

The changes in the intensity of incorporation of methionine-^{35}S observed as a result of the various treatments can hardly be attributed to changes in the permeability of the blood–brain barrier [5, 6], for Manina [4], in experiments with subcutaneous and intracerebral injection of radiomethionine into rats, showed that the intensity of incorporation of label into proteins was the same in the two groups of animals.

Changes in the intensity of incorporation of methionine-^{35}S in the present experiments, characterizing the degree of protein metabolism in nerve cells in different brain formations, evidently depends on the functional state and activity of neurons of these brain structures in response to changes in the level of hormones of the hypophyseo-adrenal system in the body as a result of extirpation of glands or administration of extrinsic hormones.

The results of the histoautoradiographic studies are evidence primarily of changes in the rate of protein synthesis in the different brain formations as a result of a change in the level of hormones of the hypophyseo-adrenal system in the animals. If adrenalectomy is regarded as a factor promoting accumulation of endogenous ACTH in the body, the following relationship can be sufficiently clearly discerned: Under the influence of a raised ACTH level the rate of protein metabolism is increased in the amygdala and hippocampus, whereas elevation of the corticosteroid level is accompanied by an increase in the number of tracks in the hypothalamic formations and by a decrease in their number in the hippocampus. Changes in protein synthesis in these structures, it can be tentatively suggested, are important in the mechanism of the change in excitability of brain structures under the influence of hormones of the hypophyseo-adrenal system. The results of the present investigation thus point to a certain selectivity of action of the hormones of this system on different parts of the brain and they are evidence of the different and unequal influence of ACTH and glucocorticoids on brain structures which control activity of the hypophyseo-adrenocortical system. It can be concluded from these results that all the brain formations studied participate in the mechanism of the negative feedback action of glucocorticoids, whereas mainly the amygdala and hippocampus are involved in the mechanism of the feedback action of ACTH.

According to some workers [16] corticosterone is concentrated mainly in the cell nuclei and to a lesser degree in the cytoplasm and is almost absent in the membranes. Hormones binding with the receptor macromolecule induced RNA and protein synthesis, thus leading to specific functional changes in all parts of the brain.

LITERATURE CITED

1. N. P. Elaev, Dokl. Akad. Nauk SSSR, 222, 1477 (1975).
2. L. N. Zhinkin (editor), Radioactive Indicators in Histology [in Russian], Leningrad (1959).
3. M. B. Lebedeva, Vopr. Med. Khim., No. 4, 278 (1956).
4. A. A. Manina, in: Radioactive Indicators in Histology [in Russian], Leningrad (1959), pp. 139-154.

5. I. V. Markova, "Relations between functions of the hypothalamic-hypophyseo-adrenal system, reactivity of the developing organism to pharmacological agents, and permeability of the blood–brain barrier," Author's Abstract of Doctoral Dissertation, Leningrad (1963).
6. E. F. Novikova, in: Abstracts of Scientific Proceedings of the 6th All-Union Conference on Neurochemistry [in Russian], Leningrad (1972), p. 107.
7. A. V. Palladin and Ya. V. Belik, in: Advances in Neurochemistry [in Russian], Leningrad (1974), pp. 19-28.
8. V. P. Fedotov and É. R. Bagramyan, Probl. Éndokrinol., No. 4, 114 (1968).
9. E. I. Chazov, S. P. Veselova, O. G. Krivosheev, et al., Probl. Éndokrinol., No. 1, 33 (1976).
10. N. A. Yudaev, M. B. Lebedeva, and N. P. Zav'yal'skaya, Probl. Éndokrinol., No. 6, 13 (1957).
11. F. Chytil and D. Toft, J. Neurochem., 19, 2877 (1972).
12. H. Knizley, Jr., J. Neurochem., 19, 2737 (1972).
13. K. Koike, T. Otaka, and S. Okui, J. Biochem. (Tokyo), 61, 679 (1967).
14. M. Litteria and M. W. Thorner, Brain Res., 90, 175 (1975).
15. J. M. Mattock and P. C. MacKinnon, Brain Res., 114, 166 (1976).
16. B. S. McEwen, C. Magnus, and G. Wallach, in: Steroid Hormones and Brain Function, Los Angeles (1971), pp. 247-258.
17. B. S. McEwen, G. Wallach, and C. Magnus, Brain Res., 70, 321 (1974).
18. B. W. O'Malley and R. E. Buller, in: The Year in Endocrinology, 1975-1976, New York (1976), pp. 277-315.
19. I. Miller, Proc. Natl. Acad. Sci. U.S.A., 57, 1959 (1967).
20. F. Schon and L. L. Iversen, Life Sci., 15, 157 (1974).
21. B. Semiginovsky, B. Jacoubek, M. Kraus, et al., Physiol. Bohemoslov., 23, 503 (1974).
22. R. B. Warner and A. R. Dick, J. Psychiatr. Res., 10, 263 (1974).