ACETYLCHOLINESTERASE ACTIVITY IN THE SUPRAOPTIC AND PARAVENTRICULAR HYPOTHALAMIC NUCLEI DURING SUPPRESSION OF PITUITARY ADRENOCORTICOTROPIC FUNCTION

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If the synthesis of endogenous ACTH in rats is suppressed by repeated injections of hydrocortisone solution acetylcholinesterase activity in the neurosecretory neurons of the supraoptic and paraventricular nuclei is not significantly changed. The absence of regular changes in acetylcholinesterase activity in the magnocellular neurosecretory nuclei confirms the hypothesis that they are not the structures responsible for synthesis of hypophyseotropic corticotropin releasing factor.

Acetylcholinesterase (ACE) plays an important role not only in the transmission of the nervous impulse, but also in neurosecretion [5]. This enzyme has been discovered in neurons of the supraoptic and paraventricular nuclei of the hypothalamus under normal conditions in various experimental animals [6, 9, 10, 13, 14]. An increase in the production of neurosecretion evoked in animals by salt loading led to increased ACE activity in the neurosecretory neurons of these nuclei [8, 14].

The author has shown previously that blocking the adrenocroticotropic function of the pituitary by injection of hydrocortisone induces a marked decrease in ACE activity in the system of parvocellular hypothalamic nuclei (ventromedial and arcuate nuclei), and this possibly reflects changes in their level of synthesis of hypophyseotropic corticotropin releasing factor [2].

Since no general agreement has been reached on the role of the magnocellular neurosecretory system in the synthesis of corticotropin releasing factor, it was important to study whether changes in ACE activity take place simultaneously in the magnocellular neurosecretory nuclei of the hypothalamus under the same conditions of suppression of ACTH production.

It was accordingly decided to investigate the character of distribution and the level of ACE in the neurosecretory neurons of the supraoptic and paraventricular nuclei during blocking of pituitary adrenocorticotropic function.

EXPERIMENTAL METHOD

Sixteen male albino rats weighing 120-140 g were used. The animals were divided into two groups, experimental and control, with eight rats in each group. Each day the rats of the experimental group received a subcutaneous injection of hydrocortisone solution in a dose of 10 mg/100 g body weight. The control rats received physiological saline. Ten days after the beginning of the experiment the animals were decapitated. The criterion of blocking of ACTH synthesis in the pituitary was a decrease in the absolute and relative weight of the adrenals of the experimental rats. ACE activity in the hypothalamus was tested histochemically by the method described previously [1, 2]. The hypothalamus was fixed in 12% formol-calcium mixture for 6 h at 4°C. Serial freely-floating sections, 20μ in thickness, were cut simultaneously

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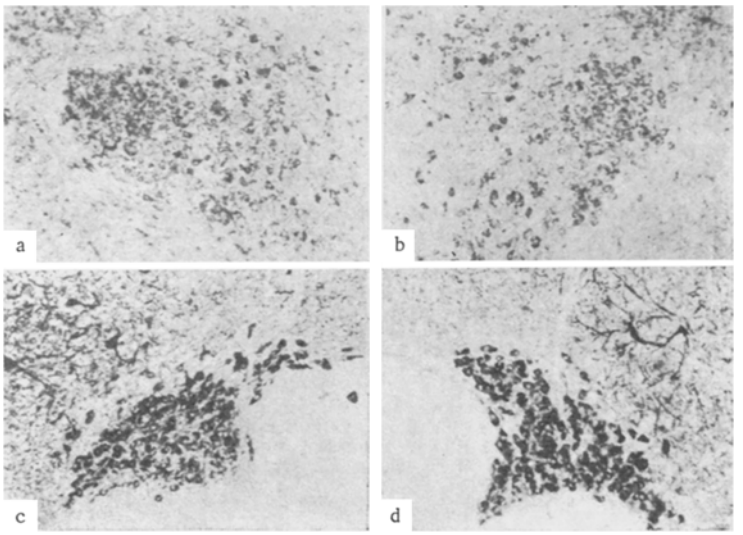

Fig. 1. ACE activity in neurosecretory neurons on paraventricular (a, b) and supraoptic (c, d) nuclei of rats under normal conditions (a, c) and after blocking of ACTH synthesis by hydrocortisone (b.d), 120 ×.

from 2 pieces of brain (taken from experimental and control animals), each pair of the series was incubated by Karnovsky's method [11], postfixed in 3% formalin, straightened out on slides, and mounted in glycerol–gelatin.

Differentiation between specific and nonspecific cholinesterase was carried out by the method of comparing two neighboring sections after incubation of one of them in medium with butyrylthiocholine and the other in medium with acetylthiocholine [4]. A 0.1% solution of neostigmine was used as the inhibitor of total cholinesterase activity. The levels of ACE activity in the nervous structures were judged from the intensity of specific precipitation at the sites of enzymic activity.

EXPERIMENTAL RESULTS

The results of these experiments showed that under normal conditions the neurosecretory neurons of the rat supraoptic nuclei have a high ACE content; the reaction for ACE is less strong in neurosecretory neurons of the paraventricular nuclei. The enzyme was clearly detected in the cytoplasm of the neurosecretory neurons, but the cell nuclei did not contain the enzyme. In the overwhelming majority of neurosecretory cells in the paraventricular nucleus the reaction for ACE was ill-defined: the intensity of staining of the cells was average, although individual neurosecretory neurons were intensively stained (Fig. 1a). While many neurosecretory cells of the supraoptic nucleus were intensively stained, some were only moderately stained and others were pale (Fig. 1c). Differences between the intensity of the reaction for ACE in cells of the same nucleus possibly reflect different states of function of the neurosecretory cells depending on the stage of production, accumulation, and liberation of the neurosecretory material.

After synthesis of ACTH in the pituitary had been blocked by hydrocortisone injection the neurosecretory neurons of the paraventricular nucleus in all the experimental animals were indistinguishable from neurons of the corresponding nucleus of the control animals as regards the intensity of the reaction for ACE (Fig. 1a, b).

No changes in ACE activity in the neurosecretory cells of the paraventricular nucleus were therefore found under these experimental conditions after hydrocortisone administration.

By contrast with the paraventricular nucleus, in the supraoptic nucleus of two of the eight experimental animals there was some increase in the number of cells with intensively stained cytoplasm. In the remaining six experimental animals the intensity of staining of the cytoplasm of the neurosecretory neurons of the supraoptic nucleus was indistinguishable from that in the control (Fig. 1c, d). In some experimental animals, a tendency toward an increase in size of the neurosecretory cells of the supraoptic nucleus was

observed after injection of hydrocortisone. A slight increase in size and in the density of distribution of the cells or an increase in the intensity of staining of the cytoplasm of the neurosecretory neurons in the supraoptic nucleus was observed only in single experimental animals, and it evidently reflects a nonspecific reaction of the supraoptic nucleus to injection of hydrocortisone.

It was accordingly concluded from the results of these experiments that under the conditions used there is no significant change in ACE activity in the neurosecretory neurons of the paraventricular and supraoptic nuclei of rats if the synthesis of endogenous ACTH is blocked.

Insufficient information on the specific increase in functional activity of cells of the supraoptic and paraventricular nuclei in response to injection of hydrocortisone into animals was found in the literature. Some workers, for instance, observed an increase in the amount of Gomori-positive material in the supraoptic and paraventricular nuclei of rats receiving injections of cortisone [3, 7, 12]. However, as De Groot [7] points out, under these conditions the content of neurosecretory material in the posterior lobe of the pituitary was unchanged. According to Szentagothai et al. [15], the dimensions of the nuclei of the neurosecretory neurons (used as a criterion of their functional activity) were unchanged after administration of cortisone, while the dimensions of the nuclei of the neurosecretory cells of the paraventricular nucleus increased during all types of endocrine disturbances, including after administration of cortisone. These workers consider that this reaction of the paraventricular nucleus to injection of cortisone is not specific. The absence of regular changes in ACE activity in the neurosecretory neurons of the supraoptic and paraventricular nuclei in the present experiments suggests that during blocking of the synthesis of endogenous ACTH the cholinergic mechanism in these cells remains intact.

The absence of data in the literature on a specific increase in functional activity of the neurosecretory cells in response to injection of hydrocortisone, on the one hand, and the absence of regular changes in the acetylcholine–acetylcholine esterase system in these cells in response to administration of hydrocortisone to the animals, on the other hand, suggest that the supraoptic and paraventricular nuclei are not among those hypothalamic structures which are responsible for the production of hypophyseotropic corticotropin releasing factor. In all probability the structures responsible for this function are the ventrome medial and arcuate nuclei, components of the hypophyseotropic region in which, as the writer has shown previously [12], ACE activity is sharply reduced under these same conditions.

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