HYPERACTIVITY OF HYPOTHALAMIC NEUROSECRETION AND COINCIDENTAL OCCURRENCE OF THYROID ENLARGEMENT FOLLOWING ADMINISTRATION OF METHYLTHIOURACIL

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At the present time it is strongly postulated, depending on various experimental data of stalk section or of hypothalamic lesion, that some neural mechanism in the hypothalamus is largely responsible for maintaining and regulating the secretion of so-called trophic hormone by the adenohypophysis (Cahne and Cahne, 1938; Westman and Jacobsohn, 1938; Uotila, 1939; Heinbecker, 1944, 1949; Brolin, 1945, 1947; Greer, 1951, 1952), and that hypothalamic nerve fibers liberate some chemical transmitters into the hypophysial portal vessel which are carried to the anterior pituitary to exert a specific influence over the activity of the gland (Harris, 1937; Harris and Jacobsohn, 1952; Benoit and Assenmacher, 1953).

In connexion with these facts it should be mentioned that stalk section induces a change in the cytology of the basophile cells, and prolonged administration of posterior pituitary hormone results in an increase of well granulated hypophysial basophile cells which are responsible for the production of thyrotrophic hormone (Heinbecker, 1949).

Since it seemed, from the above mentioned points, very likely that thyroid activity is under at least indirect control of neurosecretory activity of hypothalamic nuclei, several workers have studied the effect of thiouracil on hypothalamic neurosecretion and thyroid gland (Fontaine and Gabe, 1956; Shibusawa *et al.*, 1956), but the results were inconsistent and further investigation is required for the substantiation of this view.

This paper is a part of a series of experiments designed to clarify the problems related to the mechanism regulating the pituitary-thyroid axis.

MATERIALS AND METHODS

Forty four adult albino rats weighing 95-150 g were divided into four groups according to duration of treatment: (1) 11 animals that received daily injection of 20 mg of methylthiouracil (M.T.U.) for two consecutive days with 3 animals representing controls that were injected with physiological saline; (2) 7 animals injected with M.T.U. for 6 days with 3 controls; (3) 8 animals injected with M.T.U. for 13 days with 3 controls; (4) 9 experimentals with 3 controls treated for 24 days.

All the animals were killed by decapitation at the end of 24 hours following the final injection, and the brains were fixed with Helly's fluid, cut in $6\ \mu$ thickness in sagittal planes after the routine paraffin technique. On the other hand, measurements were made on the weight of thyroid and the body weight, and the degree of thyroid enlargement was expressed in terms of thyroid weight (mg)/body weight (g).

OBSERVATIONS

I. Secretory activity of hypothalamic nuclei in the course of administration of M.T.U.

In contrast to the normal appearance of the cells in supraoptic nucleus, remarkable occurrence of the so-called red pyknosis of the nucleus was observed in the majority of the nuclei in question in animals injected with M.T.U. for 2 days (Fig. 1 A, Fig. 2). However such was not the case with paraventricular nucleus, only a few of the cells showing signs of red pyknosis (Fig. 1B). Some fractions of the cells of supraoptic nucleus showed significant acidophily on the whole neuron somata, which obscured details of production of chrome haematoxylin (C.H.) positive secretory material. In other cells showing apparent sign of red pyknosis but not significant acidophily over the whole cytoplasm, it was observed that production of C.H. positive material was not affected in remarkable measure as compared with that in control animals. Likewise there was no marked difference in cytological as well as histological pictures with respect to the axons originating from the supraoptic nucleus, and in the overall pictures of neurohypophysis as compared with those in the control animals (Fig. 1C).

In animals that received M.T.U. for 6 successive days the pyknotic change of nucleus together with the acidophily of the cytoplasm became less marked, and production of C.H. positive material was observed relatively increased in the supraoptic nucleus as compared with that in the control animals. It was of interest to observe that no marked change took place in the neurons of paraventricular nucleus. In the area of neurohypophysis including the median eminence and the posterior lobe of the pituitary, considerable increase of the presence of C.H. positive material and the so-called Herring bodies were noticeable.

In animals treated with M.T.U. for 13 days, it was of interest to observe that a number of cells with red pyknosis went into the normal range (Fig. 2), whereas signs of hyperactivity of the neurosecretory phenomenon in supraoptic nucleus were much more evident, which showed a number of axons charged with significant amount of C.H. positive material (Fig. 1D), though occurrence of the material within the neuron somata varied from animal to animal. On the other hand, it was observed that a kind of C.H. positive material was elaborated through the agency of nuclear activity instead of cytoplasm in the cells with poor Nissl substance and basophilic substance, which were said to be responsible for the formation of neurosecretory material, in supraoptic nucleus (Fig. 3), and that, as will be seen below, the animals with such a peculiar picture of nuclear secretion had a very slight rate of thyroid enlargement. In line with such a significant occurrence of C.H. positive material along the axons of supraoptic nucleus, the neurohypophysis became much more abundant (Fig. 1F). Here it is to be mentioned that, in contrast to such hyperactivity of the supraoptic neurons, cytological

Fig. 1. A, D and G show the cells and axons in supraoptic nucleus; B, E and H show the cells and axons in paraventricular nucleus; C, F and I show the neurohypophysis following M.T.U. administration for 2 days, 13 days, and 24 days respectively. A show marked red pyknosis. D shows the picture of augmented secretory activity and of full filled axons with C. H. positive material. G shows the returning to normal. E shows slightly increased secretory activity, and H shows returning to normal secretory activity in paraventricular nucleus. F shows significant increase of C.H. positive material and Herring bodies in neurohypophysis. I shows returning to normal state.

pictures of the paraventricular neurons showed relatively less increased secretory activity (Fig. 1E).

In animals injected with M.T.U. for 24 days, apparent tendency of recovery of the secretory activity to the normal level was observed to be approximating

the normal state too (Fig. 1 G,H). In parallel with such a decrease of secretory activity of the two hypothalamic nuclei, the amount of C. H. positive materials in the neurohypophysis became more or less decreased, approximating nearly that in the control animals (Fig. 1 I).

Fig. 2. Usual occurrence of red pyknosis less than 20 per cent in normal animals, marked increase in response to administration of M.T.U. in supraoptic nucleus.

II. Development of goiter in the course of administration of M.T.U.

In the course of administration of M.T.U. as shown by Fig. 4, the thyroid became gradually enlarged in response to administration of M.T.U. At the end of 2 days the growth curve did not show any remarkable difference from the normal level, but it attained a significantly higher level at the end of 6 days. At the end of 13 days the growth curve attained the maximum, then gradually declined in the course of following periods of long-term administration. In the present long-term observation it appeared much significant that, in spite of the common concept of goiter development, M.T.U. called forth suppression after the initial acceleration of the thyroid development.

Fig. 3. Various pictures of nuclear secretion of the cells with poor Nissl substance and basophilic substance in supraoptic nucleus. Arrow shows the C.H. positive material. F and G show the plasma nucleolus surrounded by C.H. positive material. H shows normal secretory cell with rich Nissl substance.

Fig. 4. Growth curve of goiter showing possible presence of acute phase of goiter development in response to administration of M.T.U.

COMMENTS AND CONSIDERATIONS

In the recent progress of neuroendocrinology it has increasingly been substantiated that the pituitary-thyroid axis is under the control of hypothalamic activity. Lesions applied at various level of the hypothalamus or section of the pituitary stalk have been found to cause more or less marked inhibition of release of thyrotrophic hormone from the adenohypophysis (Westman and Jacobsohn, 1938; Uotila, 1939; Heinbecker, 1949; Greer, 1951, 1952; Bogdanove and Halmi, 1953).

However, there is less evidence pointing to the conception on the basis of studies of hypothalamic neurosecretion in comparison with thyrotrophic secretion. The notable classic of Herring (1908) has shown that total thyroidectomy is followed by increase of the "colloids" in neurohypophysis on one side and by remarkable apparition of granulus in the cells of anterior hypothalamus on the other side. Very recently Shiozaki (1956) made a similar line of study, showing that the accumulation of C.H. positive materials in neurohypophysis resulted following temporary hyperactivity of the paraventricular neuron as the consequence of total thyroidectomy. He showed also that the condition of hyperactivity of neurons in question was closely related to the occurrence of the red pyknotic

change of the nucleus and the remarkable acidophily of the cytoplasm in the neuron somata.

As regards the functional co-ordination between the hypothalamic neurosecretion and adenohypophysis, Benoit and Assenmacher (1953) have rendered evidence proving migration of neurosecretory principle via the pituitary portal vessel to the adenohypophysis to exert a regulatory influence over the pituitary activity of gonadotrophic production therein.

Martini (1955), Fontaine and Gabe (1956) and Shibusawa *et al.* (1956) have shown marked effect of thiouracil on the hypothalamic histological picture on one side and on the pituitary-thyroid axis on the other side, but the results are concerned with cytological changes in the hypothalamo-hypophyseal system with contradictory results in several respects.

In the present study it was learned that, in line with the observation of Shiozaki (1956), hyperactivity of hypothalamic neurosecretion was preceded by a significant occurrence of nuclear red pyknosis and acidophily of the neuron somata in response to administration of M.T.U., though the drug predominantly affected the supraoptic neurons in contrast to the effect of total thyroidectomy which influenced the paraventricular neurons. According to Hagen (1955) the so-called red pyknosis of the nucleus in the supraoptic as well as paraventricular neurons was taken as a characteristic sign of pathologic or degenerative process, but, on the basis of lines of evidence showing that a face of significant hyperactivity follows such a peculiar phenomenon, I am of opinion that it would represent an

Fig. 5. Interrelation among goiter development, red pyknosis and neurosecretory activity of supraoptic nucleus

unusual change of the nuclear component under a certain acute demand of hyperproduction of secretory material in a given neuron soma.

As a matter of fact the red pyknosis is observable even in normal animals (Ortmann, 1951; Mosinger, 1954; Shiozaki, 1956; Imai, 1956) though the rate of occurrence is, according to the present observation, always less than 20 per cent among the cells of supraoptic nucleus in the rat (Fig. 2). Yamada *et al.* (1956) have shown that the phenomenon is observed in early post natal development of the dog.

Presently it was observed that the cells of supraoptic nucleus exhibited remarkable pyknotic change of nucleus in the course of treatment with M.T.U., the rate of occurrence rising as higher as 60 per cent shortly after administration, while the cells regained a normal cytological picture in less than 10 days. Such observations seem to substantiate the concept that the remarkable acidophily of the nucleus as a whole as well as of cytoplasm would represent physiological change of the neuron somata, not any pathologic or degenerative features of the neurons under consideration.

It is generally accepted that the development of goiter in administration of M.T.U. follows the course of linear increase, attaining as large as ten fold in weight in order to grow up for satisfactory compensation. As mentioned above, the present results have shown that the goiter development comes to decline in the course of long-term treatment with the drug, after attaining the maximum

Fig. 6. Fate of neurosecretory activity and of contents of basophilic and Nissl substance in the face of acute demand for neurosecretory substance in the cells in supraoptic nucleus. A: normal state, B: hypersecretory activity of cells, C: shows nuclear secretion in the cell with poor basophilic substance in 13 days D: the cell in 24 days. NN, N, BA, NS and AO show nucleolus, nucleus, basophilic substance, neurosecretory substance and axon respectively.

towards 13 days. This implies that there would be phases, viz., an acute phase and a chronic phase of the goiter development, in the course of administration of M.T.U. on rats (Fig. 5).

In comparison with the progress of so-called acute phase of goiter development, the changes in hypothalamic neurosecretion appear much significant when brought into comparison with the growth curve of the thyroid, the sequence of hyperactivity of supraoptic nucleus provides a marked parallelism (Fig. 5). In animals showing only slight degree of goiter development a characteristic sign of nuclear secretion was sometimes observed (Fig. 3). The nature and the functional significance of such a phenomenon is yet unknown, but, on account of the facts that a similar phenomenon takes place in the cells of supraoptic as well as paraventricular nuclei in the course of early post natal development of the dog (Yamada *et al.*, 1956), it might be presumed that the secretory neurons, after being exhausted of materials of the synthesis of neurosecretory substances in the pericarion, would be forced to perform such nuclear secretion in the face of an acute demand for secretory activity (Fig. 6).

On the whole, it appears likely that the development of the goiter, at least in the acute phase of the development, under the influence of M.T.U. would depend on temporary hyperactivity of the hypothalamic nuclei, especially of supraoptic nucleus.

SUMMARY

Changes in hypothalamic neurosecretion were observed and brought into comparison with the development of goiter in response to the administration of methylthiouracil.

Development of goiter followed two phases, viz., acute initial phase and later chronic phase. In the acute phase of goiter development, thyroid does not grow lineally, but shows a temporary growth followed by gradual decline. Such a condition was learned to develop in parallel with a temporary increase in the secretory activity in hypothalamic nuclei, especially in the supraoptic nucleus.

Hyperactivity of supraoptic neurons was observed to be preceded by a remarkable occurrence of red pyknosis of the nucleus and significant acidophilia of the perikaryon, the phenomenon being considered to represent certain physiological processes called forth in the face of acute demand of neurosecretory activity. In some of the cells, in which cytoplasmic basophilia are lacking as the consequence of hyperactivity, a characteristic activity of nuclear secretion was observed.

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