THE FUNCTIONAL RELATIONSHIP BETWEEN THE HYPOTHALAMUS AND THE ANTERIOR PITUITARY-ADRENAL SYSTEM

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EIGHT FIGURES

It is a well known fact that the hypothalamus plays an important role in the regulation of the endocrine function through the anterior pituitary gland. This is widely acknowledged under the concept of "hypothalamo-pituitary system". The connecting pathway, however, between the hypothalamus and the anterior pituitary gland still remains unknown. Recently, as a result of extensive research on this problem, the theory of "neural mechanism" has gradually come to be disapproved, and the theory of so-called "neuro-humoral regulation system", which advocates that the two are connected humorally through the hypophyseal portal vessels, has come to be dominant.

The region of the hypothalamus relating with the anterior pituitary gland in regard to the adrenocortical regulation has been described respectively to be the anterior part by Hume and Wittenstein (1951) or to be the posterior part by de Groot and Harris (1950, 1952) and Porter (1953) from their experimental findings. And as to the so-called neuro-chemical transmitter which reaches the anterior pituitary gland from the hypothalamus via the portal vessels, it has been variously presumed in spite of the studies by Sawyer (1952) and Harris, Jacobsohn and Kahlson (1952) etc.

Catechol and chemocorticoid (Ch. C.) contained in the adrenal vein blood, sudanophile substance in the adrenal cortex and potassium contained in the blood serum are known to be closely related with the function of the hypothalamo-pituitary system.

The purpose of this report is, by observing the changes of these substances, to investigate the location of the hypothalamus which control the anterior pituitary-adrenal system and to study the mechanism of the so-called neuro-chemical transmission.

METHODS

In cats, anesthetized with ouropan-natrium, the adrenal vein blood was collected by means of the vena cava pocket method. Ch. C. content was estimated by means of Nakao-Aizawa's

method and catechol content by the rabbits intestinal segment method, and for separating estimation of adrenaline and noradrenaline, rabbits intestine and fowls rectal coecum were used. Potassium content in the blood serum was estimated by King's modified method in nonanesthetized rabbits. Hypothalamus was stimulated with square wave (Frequency: 60 cycles per second, voltage: 4-6 volts, pulse duration: 8.3 msec.) for ten minutes, and the stimulated points were ascertained by serial sections of the brain. Adrenal cortex was studied histologically by sudan III staining.

EXPERIMENTAL RESULTS

1) *Chemocorticoid content*

Stimulation of the hypothalamus caused the increase of Ch. C. content in the adrenal vein blood in 18 cases out of 28, decrease in 4 cases, but in the other 6 cases no change was observed. Stimulated points which showed increase of Ch.C. were widely extended from the level of the optic chiasma to the level of the mammillary body, and the remarkable increase (over $+90\gamma/10\text{cc.}$) was obtained in 5 cases from the posterior part of the hypothalamus, that is, the posterior hypothalamic nucleus, mammillary body and their neighbouring regions. From middle and the anterior part of the hypothalamus increase over $+50\gamma/10\text{cc.}$ was obtained in 2 cases but the other cases did not show any marked increase. The relation between the stimulated points and the change of Ch.C. content is shown in Figure 1. The histological findings of adrenal cortex supported such changes of Ch.C., that is in cases which showed the marked increase of Ch.C. content by stimulation of the posterior part of the hypothalamus marked decrease of sudanophile substance was observed (Fig. 2).

Fig. 1. Influence of hypothalamic stimulation upon the chemocorticoid content in the adrenal vein blood.

Abbreviations in Figures, 1, 3 and 6 are as follows:

III.: Ventriculus tertius, M.i.: Massa intermedia, C.f.d.: Columna fornicis descendens, C.a.: Commissura anterior, C.m.: Corpus mamillare, Ch.: Chiasma opticum, H.a.: Hypophysis anterior, H.p.: Hypophysis posterior, P.: Pons

Fig. 2. The change of sudanophile substance in the adrenal cortex, following hypothalamic stimulation.

A: Control (normal accumulation of sudanophile substance).

B: Stimulation of the supramammillary nucleus (marked decrease of sudanophile substance).

C: In hypophysectomized cat, stimulation of the supramammillary nucleus (almost no change of sudanophile substance).

In 3 of 4 hypophysectomized cats, stimulation of the posterior part of the hypothalamus did not cause increase of Ch.C. and the changes of the sudanophile substance. While in the other one case slight increase of Ch.C. and slight decrease of sudanophile substance were observed.

2) Catechol content

Catechol content was increased after stimulation of the hypothalamus in 8 cases out of 17, especially the remarkable one was frequently obtained by stimulation of the ventromedial and dorsomedial hypothalamic nuclei and their neighbourhood (Fig. 3). In 3 out of 4 hypophysectomized cats stimulation of the hypo-

Fig. 3. Influence of hypothalamic stimulation upon the catechol content in the adrenal vein blood.

thalamus caused the increase of catechol, both adrenaline and noradrenaline, as in normal animals.

3) *Relation between Ch.C. and Catechol*

In the above mentioned experiments, Ch.C. and catechol contents were estimated simultaneously in the same animal, and it was found that in some cases the increase of the two substances paralleled each other but in the other cases they did not at all. As shown in Figure 4, a remarkable increase of Ch.C. content

Fig. 4. The changes of adrenaline and chemocorticoid content in the adrenal vein blood, following hypothalamic stimulation.

A: Stimulation of the dorsal part of the dorsomedial hypothalamic nucleus.

B: Stimulation of the dorsal part of the supramammillary nucleus.

was mainly obtained from the posterior part of the hypothalamus but that of catechol from the middle part.

4) Potassium content

After administration 5 mg. of ACTH or 1 mg. of DOCA in nonanesthetized rabbits, we also confirmed as did the previous investigators the decrease of serum potassium content (Fig. 5). Then, the influence of the hypothalamic stimulation upon the potassium content was studied with 20 rabbits. From the anterior and middle parts of the hypothalamus, especially the ventromedial hypothalamic nucleus remarkable increase of potassium content was most frequently obtained, and from the posterior part, i. e. mammillary body, indefinite response, but somewhat decreasing tendency was observed. In other words, the response from the posterior part of the hypothalamus was not the same as that from the anterior

Fig. 5. The change of potassium content in the blood serum, following administration of 5 mg. of ACTH and 1 mg. of DOCA.

Fig. 6. Influence of hypothalamic stimulation upon the potassium content in the blood serum.

part (Fig. 6). Moreover, in hypophysectomized or adrenalectomized rabbits, the response caused from the posterior part of the hypothalamus reversed, that is, decrease occurred no more but marked increase was obtained. The latter fact may be assumed as the result of absence of the potassium decreasing effect of ACTH or DOCA from the anterior pituitary or adrenal gland.

5) *Influence of acetylcholine on the ACTH secretory function of the anterior pituitary gland*

Direct electrical stimulation of the anterior pituitary gland did not cause the increase of Ch. C. content in the adrenal vein blood. Injection of minimal doses

Fig. 7. The change of chemocorticoid content in the adrenal vein blood, following administration of acetylcholine and adrenaline into the anterior pituitary gland directly (thick line) and into the vertebral artery (fine line).

Fig. 8. The change of potassium content in the blood serum, following local application of acetylcholine and adrenaline into the anterior pituitary gland.

of acetylcholine (0.15 mg.) into the anterior pituitary gland caused the increase of Ch. C. content and decrease of potassium content. Injection of adrenaline (10 γ) into the anterior pituitary gland caused no change of Ch. C. or potassium (Figs. 7 and 8). The same amount of acetylcholine or adrenaline doses administered into the vertebral artery but did not cause increase of Ch. C. content.

COMMENT

The close connection between the hypothalamus and ACTH secretion is becoming evident recently, but the detailed mechanism has not yet been clarified. de Groot and Harris (1950, 1952) have found that stimulation of the posterior part of the tuber cinerium or mammillary region resulted in a lymphopenia similar to that of following the intravenous injection of an appropriate dose of ACTH and have concluded that the secretion of ACTH by the pituitary gland seems to be under neural control via posterior hypothalamus. Porter (1953) has supported Harris' opinion by his experiments in which stimulation of the posterior hypothalamus caused the eosinopenia and posterior hypothalamic lesions prevented the normal eosinopenic response to epinephrine, histamine and formalin.

On the contrary, Hume and Wittenstein (1951) described that in dogs, the lesion which prevent the eosinopenic response to adrenaline was in the supraoptic or paraventricular nuclei.

McCann (1953) found that the lesions of the median eminence in the rat completely blocked the adrenal ascorbic acid depletion caused by administration of adrenaline and thought that this result was due to the lack of ACTH. The fact that an increase in adrenal size and histological evidence of adrenocortical hypertrophy caused by repeated trauma are prevented by destruction of median eminence has been reported by Hume and Ganong (1954).

Thus the hypothalamus has been attached importance in secretion of ACTH, and we also reached the same conclusion from our own experiments in which the stimulation of the hypothalamus especially of the posterior part caused increase of Ch. C. content in the adrenal vein blood and decrease of sudanophile substance in the adrenal cortex, and no such changes in the hypophysectomized animals. As to the secretory mechanism of ACTH from the pituitary gland by the hypothalamic stimulation, it may be thought that ACTH produced under neural control from the posterior part of the hypothalamus activated the adrenal cortex and the latter secondarily caused the increase of Ch. C. in the adrenal vein blood.

There is another possibility of secretory mechanism of ACTH, namely that the increase of Ch. C. content caused from the stimulation of the medial part of the hypothalamus, especially from the ventromedial hypothalamic nucleus and its neighbourhood, might be due to the secondary effect of the increased secretory function of the adrenal medulla. The stimulation of this region caused the marked increase of adrenaline content in the adrenal vein blood, and the mode of increase of Ch. C. was similar to that caused by stimulation of the splanchnic nerve or administration of adrenaline, so the participation of adrenaline must be considered as a causative factor of the adrenal-cortical activation. In connection

with this problem, McDermott, *et al.* (1950) and others described that, in hypophysectomized rats, administration of minimal dose of adrenaline in the anterior chamber of the eye where the pituitary tissue had been grafted, caused the decrease of ascorbic acid in the adrenal gland and circulating eosinophiles, but these responses were not obtained by the administration in the other normal eye or after the eyes containing the grafts were enucleated, and that ACTH which secreted from the anterior pituitary tissue activated by adrenaline caused the hypersecretion of the adrenalcortical hormone. Kawahara, *et al.* (1954) who estimated ascorbic acid, cholesterol in the adrenal gland and observed the respiration of the adrenal tissue stated that adrenaline acted on the adrenal cortex via an anterior pituitary gland. On the contrary, Hugerford (1949) obtained a decrease in the number of circulating mononuclear leucocytes by administration of epinephrine in hypophysectomized rats but not in adrenalectomized rats, and concluded that at least epinephrine acts directly on the adrenal cortex. Ishibashi (1955) also proved the increase of Ch. C. content by administration of adrenaline in hypophysectomized or splanchnectomized animals and concluded that adrenaline influenced directly on the adrenal cortex. Speirs and other (1949, 1954) reported that in hypophysectomized mice, an eosinopenic response occurred following an epinephrine injection and that the mode of eosinopenic response to ACTH differed from that of adrenaline and assumed the direct action of the epinephrine on the adrenal cortex.

Thus, as to the mechanism of adrenaline action on the hypothalamo-pituitary-adrenal system, there are two theories, namely; direct action on the adrenal cortex, or indirect action via the anterior pituitary gland. The present experiment may elucidate this problem, that is, as described previously, the mode of increase of Ch. C. content caused from the middle part of the hypothalamus differed from that caused from the posterior part but was similar to that caused by stimulation of the splanchnic nerve or adrenaline injection. From these results it may be considered that the impulse from the sympathetic center in the middle part of the hypothalamus reaches the adrenal medulla via the splanchnic nerve and adrenaline secreted from the latter acts on the adrenal cortex and consequently causes the hypersecretion of Ch. C..

It has been described that the hypothalamus, especially the posterior part is playing an important role in the secretory mechanism of ACTH from the anterior pituitary gland, but its pathway has not been clarified yet. Since the discovery of a circulatory system called "hypophyseal portal vessels" by Lieutaud, the theory that the impulse from the hypothalamus reaches the anterior pituitary gland through this system and consequently ACTH is secreted has been advanced. Many investigations have been tried to prove the functional connection between the hypothalamus and the anterior pituitary gland, assuming that there is a neuro-chemical transmitter. de Groot and Harris (1952) described that an unknown neuro-chemical substance liberated from the nerve ending of posterior tuberal region to the neighbourhood of the portal vessels stimulated the anterior pituitary cell, and consequently the secretion of ACTH occurred. McCann and others (1953) described that the lesions of the median eminence in rats completely blocked the adrenal ascorbic acid depletion which occurs in response to epinephrine and

that the neuro-chemical transmitter produced in the hypothalamus called forth the ACTH secretion through the portal vessels. As to the neuro-chemical transmitter, it has been presumed variously, that is, as histamine-like substance (Harris, Jacobsohn and Kahlson, 1952) antihistamine (Grillemin and Fortier, 1955) or noradrenaline (Vogt). Recently Shibusawa (1955) observed the effects of vasopressin or ADH in releasing ACTH.

This investigation has been attempted to clarify this neuro-chemical transmitter, and the following results were obtained. Direct application of 10 γ of adrenaline in the anterior pituitary gland and the administration in the vertebral artery which was considered to distribute the hypothalamus and pituitary gland, did not cause any evident change of Ch. C. content in the adrenal vein blood and potassium content in the blood serum. These results did not agree with that of Sawyer (1952) and others who stated that adrenaline-like substance acted on anterior pituitary gland via the portal vessels, and with that of McDermatt, *et al.* (1950), Selye and Fortier (1949) who described that adrenaline directly acted on anterior pituitary gland. But direct administration of acetylcholine (0.15 mg.) in the anterior pituitary gland caused increase of Ch. C. content and decrease of potassium content. This dose of acetylcholine administered into the vertebral artery did not cause increase of Ch. C. content. It may be considered, from these results, that acetylcholine-like substance which is cholinergic agent produced at hypothalamic nerve ending reached the anterior pituitary cells as a neuro-chemical transmitter via the portal vessels and caused increase of secretion of ACTH, and consequently increase of Ch. C. and decrease of potassium content occurred.

Recently acetylcholine is attached importance as mediator at the synapse, so the authors' results support the opinion that acetylcholine-like substance is playing an important role in connecting the posterior part of the hypothalamus with anterior pituitary gland as a neuro-chemical transmitter.

SUMMARY

- 1) Stimulation of the posterior part of the hypothalamus caused increase of Ch. C. content in the adrenal vein blood and decrease of sudanophile substance in the adrenal cortex. In hypophysectomized cat, these changes were not obtained.
- 2) Catechol content in the adrenal vein blood was remarkably increased by stimulation of the middle part of the hypothalamus in most cases.
- 3) On the influence of the hypothalamic stimulation upon the potassium content in the blood serum, from the anterior and middle parts of the hypothalamus, remarkable increase was obtained, and from the posterior part the tendency to decrease was generally observed. In hypophysectomized or adrenalectomized rabbits, the response from the posterior part reversed, namely potassium content markedly increased.
- 4) Direct electrical stimulation of the anterior pituitary gland did not cause increase of Ch. C. content in the adrenal vein blood. Local application of minimal doses of acetylcholine (0.15 mg.) into the anterior pituitary gland caused the increase of Ch. C. content and decrease of potassium content, but

the application of adrenaline (10 γ) did not cause the change.

From the above results it may be considered that acetylcholine-like substance liberated at nerve ending by excitation of nerve cells in the posterior part of the hypothalamus reaches the anterior pituitary cells as a neuro-chemical transmitter via the portal vessels and causes increase of secretion of ACTH, and consequently increase of Ch. C. content in the adrenal vein blood and decrease of serum potassium content occurs.

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