CHANGES IN THE EEC OF THE HYPOTHALAMUS
AND LIMBIC SYSTEM AFTER ADMINISTRATION
OF ACTH, SU-4885 AND ACH IN RABBITS
WITH SPECIAL REFERENCE TO NEURO-
HUMORAL FEEDBACK REGULATION
OF PITUITARY-ADRENAL SYSTEM

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Interactions between the endocrine and the central nervous systems have a great physiological significance in the maintenance of homeostasis within an organism, and a considerable number of studies have contributed to our understanding of the functions of the central nervous system in regulating hormonal activity. However, most of these works have dealt only with the direct hormonal changes caused by alterations in the state in the brain, and the problem of influences by the sequential alterations in the hormonal balance accompanying the initial changes has been left almost untouched. In general only a few works have been done on the possible role played by such humoral factors in controlling the central nervous system^{1,2,3,4}).

The present investigation attempted to elucidate the dynamic features of the interactions among the central nervous system, the adrenocorticotrophic hormone and the adrenocortical hormone. The pituitary gland had previously been regarded as an exclusive focal point in the feedback control of these hormones, and the involvement of the central nervous system had been neglected. But recently some evidence has been obtained showing that the basal hypothalamus also participates in the feedback control of the adrenocorticotrophic and adrenocortical hormones as well as of all other trophic hormones in a critical manner. The present work tries to make clear the role of the basal hypothalamus and those areas closely connected to it in the feedback control of the secretion of these hormones.

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Many previous investigations have made an approach to the problem of the hypothalamic control of the secretion of various trophic hormones including corticotrophin by making localized destructions or applying electrical stimulation in the basal hypothalamus and observing the resulting alterations in the size of the target organs or in the biochemical constitution of the blood or the tissues^{5, 6, 7, 8, 9, 10, 11}. The present experiment, on the other hand, was based mainly upon the EEG recording, which enabled observations of the brain activity changing every moment along with the alterations in the hormonal condition. Hence a clarification of sequential neurohumoral interactions in the feedback control of the homeostasis of the adrenocorticotrophic and adreno-cortical hormones was made possible.

MATERIALS AND METHODS

Fifty-three mature New Zealand White rabbits (3-4 kg) were used in this study. All experiments were performed on chronic preparations.

The EEG from different regions of the brain was recorded through permanent bipolar concentric electrodes made of 26 gauge stainless steel tubes and stainless steel cores, insulated with Formvar except at their tips, which were embedded in the brain. These electrodes were fixed in position by lucite dental cement attached to the calvarium and to screws embedded in the skull. Silver-ball electrodes resting on the dura were used for cortical records. The leads were soldered to a 9-place Winchester receptacle which was fixed into the surface of the skull. On recording, a 9-prong Winchester element with long wire leads was plugged into the receptacle. During the EEG recording the rabbit was free to move around on a table in the shielded, sound proof recording chamber, to eat, drink, and sleep, thus excluding the acute stress factors which usually disturb the EEG pattern. Continuous recording was made for periods of 2 hours to 6 hours after the administration of ACTH, SU-4885 and hydrocortisone acetate. Frequency analysis was made using a band-pass type analyzer (7 or 5 bands). The EEG patterns and the average integrated band values over 10 epochs (each epoch being 10 seconds) were compared between before and after injection. The comparison was made only with respect to the EEG state when the animal was sitting still showing a sporadic spindle burst in the neocortical EEG. In this experiment, the administration of ACTH, SU-4885 and hydrocortisone acetate was performed on all cases as follows. Adrenocorticotrophin (Cortrophine; N. V. Organon-Holland) at a dose level of 0.3 I.U./kg and SU-4885 (Metopirone; Ciba-Switzerland) at 75 mg/kg body weight were injected into the ear vein in saline solution. Hydrocortisone acetate (Merck & Co., Inc.-U.S.A.) was used at a dose level of 1 mg/kg body weight in the same way.

Hydrocortisone implantation was performed bilaterally by the method of SMELIK and SAWYER¹¹. Localized lesions were made bilaterally in certain regions of the brain by a high frequency electrolytic lesion-maker using the coordinates from the stereotaxic atlas prepared by SAWYER, EVERETT and GREEN¹². The lesioning electrode was made of stainless steel tube (26 gauge) for the hypothalamus and of 23 gauge for the septum, central grey and the midbrain reticular formation, insulated to within 1.0 mm of its tip. Six hours' immobilization was employed as a stressor from 8:00 a.m. to 2:00 p.m. The stress response was measured as indicated by the decrease of blood lymphocytes and by the increase of urinary Porter-Silber chromogens. The initial blood sample was

taken just before the onset of the immobilization and the final sample just after the release from stress. Serial 24 hour urine collections were started at the onset of the immobilization and continued for 3 days. The resting level of the steroid excretion was determined in two 24 hour urine collections before the start of stress. Blood lymphocytes (N/mm^3) were counted by the method of COLFER, DE GROOT and HARRIS¹³⁾ and the differential count was stained with May-Giemsa's method. Urinary Porter-Silber chromogens ($\mu g/24$ hours) were determined by a modification of the method of GLENN and NELSON with the purification procedure by Florosil column chromatography¹⁴⁾. The immobilization was performed before and 14-16 days after hydrocortisone implantation or electrolytic lesioning.

In order to determine the locations of implanted hydrocortisone or electrolytic lesion, the rabbits were later killed with air-embolism under pentobarbital anesthesia, and 10% formalin injected into the carotid arteries for intracranial circulation. Then the heads were removed, trimmed and placed in 10% formalin. After complete fixation, the brains were embedded in paraffine and serial sections (30μ .) were cut and stained with the thionin staining.

RESULTS

A. *Effects of adrenocorticotrophic hormone, SU-4885 and hydrocortison acetate on the electrical activity of the basal hypothalamus and the limbic system.*

1. *Effects of adrenocorticotrophic hormone (ACTH).*

a) *Hippocampus (HPC)*: In the control state prior to injection when the animal was sitting still showing sporadic spindle bursts in the neocortical EEG, the EEG activity of HPC (alveus) was characterized by the 4-8 cps waves riding on rather irregular fast waves. Starting at about 30 minutes after injection, the 4-8 cps component gradually decreased and did not return to the control level for 2 hours. The fast component of 20-60 cps showed a similar change (FIG. 1-A).

b) *Amygdala (AMYG)*: The amygdalar (lateral basal portion) EEG before injection also showed the 4-8 cps waves intermixed with both higher and lower components. The 4-8 and 8-13 cps components gradually increased after injection and also did not come down to the control level for 2 hours. Moreover in the subjects which always showed relevant spindle-like bursts of 40 cps sinusoidal wave when aroused throughout the experiment, the 20-60 cps component greatly rose starting 15 minutes after injection up to 150 per cent of the control value, although the animal was sitting still showing the light-sleep spindle in the neocortical EEG. Thereafter it slightly declined, though still keeping a value higher than the control level by no less than 20 per cent (FIG. 1-B, FIG. 2).

c) *Hypothalamic nuclei*: i) Anterior hypothalamic area (AHA). The EEG of the AHA in the control state was dominated by slower components in the range of 2-13 cps. The amplitude of the signals was quite high, around 300 μV . The 8-13 cps component increased considerably 1 hour after injection, but decreased from 1.5 to 2 hours after injection. The 4-8 cps component also

FIG. 1. The changes in EEG activity of the dorsal hippocampus (DHPC)-(A) and the amygdala (AMYG)-(B), after intravenous injection of adrenocorticotrophic hormone (ACTH, 0.3 I.U./kg), as shown by sample sections of the original EEG traces (left) and by integrated frequency-band values averaged for 10 epochs, each epoch being 10 seconds (right).

FIG. 2. The EEG recording of the frontal cortex (FC), DHPC and AMYG taken 15 minutes after intravenous injection of ACTH (0.3 I.U./kg).

FIG. 3. The changes in EEG activity of the anterior hypothalamic area (AHA)-(A) and the medial preoptic area (MPO)-(B), after intravenous injection of ACTH (0.3 I.U./kg).

increased slightly 1 hour after injection (FIG. 3-A). ii) Medial preoptic area (MPO). The control EEG pattern of the MPO was dominated by 2-8 cps components. These slower components were considerably influenced by ACTH injection varying biphasically during the time course of the experiment. They were greatly enhanced directly after injection for 20 minutes, but, then they were remarkably depressed for the next 1 to 1.5 hour. Thereafter, they gradually recovered and finally returned to the control level about 2 hours after injection (FIG. 3-B). iii) Median eminence and periventricular arcuate nucleus (ME-ARC). The EEG activity of the ME-ARC in the control state showed waves of 4-13 cps with some clear irregular slow components of 2-4 cps and a faster component of 13-20 cps. The amplitude of the EEG was quite high (about 300μ V). Directly after injection, almost all the components observed in the control state were depressed and did not recover until 3 hours had passed (FIG. 4-A). iv) Posterior hypothalamic area (PHY). The EEG of the PHY before injection consisted mainly of 4-8 cps waves, which showed no remarkable change after injection (FIG. 4-B). v) Mammillary body (MM). In the MM, in the control state slower components of 2-4 and 4-8 cps were more marked than the other components. The change produced by the injection was not remarkable, but a slight tendency to increase was observed from 15

FIG. 4. The changes in integrated frequency-band values of analyzed EEG in the periventricular arcuate nucleus (ARC)-(A), the posterior hypothalamus (PHY)-(B), the mammillary body (MM)-(C) and the midbrain reticular formation (RF)-(D), after intravenous injection of ACTH (0.3 I.U./kg).

minutes to 1 hour after injection (FIG. 4-C).

d) Midbrain reticular formation (RF): In the RF, 4-8 cps waves prevailed in the control state. These waves increased slightly from 15 minutes to 1 hour after injection (FIG. 4-D).

The above observations may be interpreted as showing: i) depressed EEG activity of the HPC and the ME-ARC, ii) enhanced EEG activity of the AMYG, AHA, MM and the RF, and finally, iii) the initially enhanced and then depressed activity of the MPO, by ACTH administration.

These facts agree with the observations made by KAWAKAMI⁸⁸⁾ that in the adrenalectomized animals the rate of the unit discharge increased in the ARC,

VMH and the dorsal nucleus of the hypothalamus following the rise of ACTH in the blood, while it decreased in the posterior nuclei of the hypothalamus and in the RF.

2. Effects of SU-4885.

SU-4885 has been known to act directly on the adrenal cortex to inhibit corticoid formation^{15,16}. Therefore the changes following SU-4885 administration as described below will be interpreted as being due to the decrease of corticoid level in the circulating blood and the resulting secondary increase of ACTH.

a) *HPC* (alveus): The dominant component of 4-8 cps gradually increased. The increasing tendency could be still observed even 5 hours after injection.

b) *AMYG* (lateral basal portion): The dominant component of 4-8 cps was considerably decreased 15 minutes after injection and remained at lowered values till 1.5 hour. Then it gradually recovered and took values higher than the control level from 2 to 4 hours after injection. Thereafter it began to decline and returned to the control level. In the subjects in which 40 cps spindle occurred in the AMYG in arousal, 15 minutes after injection, the following EEG pattern were observed simultaneously. In the AMYG, the 30-

FIG. 5. The changes in EEG activity of AHA-(A), ARC-(B) and RF-(C), after intravenous injection of SU-4885 (75 mg/kg).

60 cps component showed an abrupt rise, while in the neocortex the spindle bursts appeared along with the decrement in the 4-8 cps component. Thereafter the former declined a little but still kept higher values than the control by 15 per cent till 6 hours later.

c) *Hypothalamic nuclei*: i) AHA. All frequency components decreased 5 minutes after injection, but quickly recovered and took higher values than in the control state from 1 hour to 5 hours after injection (FIG. 5-A). ii) ME-ARC. All components below 20 cps decreased 10 minutes after injection, but soon recovered. The lower waves, especially, in the range of 2-8 cps began to increase gradually. They kept increasing till 6 hours after injection (FIG. 5-B). iii) PHY. The 4-8 cps waves together with the faster components showed a slight increase.

d) RF: Almost all components were equally reduced from 5 minutes after injection to 1.5 hour, but they were enhanced thereafter till 4 hours after injection. They returned to the control level at about 5 hours after injection (FIG. 5-C). In all, SU-4885 can therefore be regarded to have a facilitatory

FIG. 6. The changes in EEG activity of DHPC-(A) and AMYG-(B), after intravenous injection of hydrocortisone acetate (ACH, 1 mg/kg).

action on the HPC, AMYG and the PHY, and also on the ME-ARC and the AHA except for the transient inhibitory effect. The reticular formation revealed a biphasic influence, first inhibitory then facilitatory. The changes are all attributable to the decrease in adrenocortical hormone in the blood following the suppression of its production in the adrenal cortex by SU-4885.

3. Effects of hydrocortisone acetate.

a) *HPC* (alveus): The 4-8 cps components gradually increased after injection. This upward tendency could be still observed even 6 hours after injection (FIG. 6-A).

b) *AMYG* (lateral basal portion): The 2-8 cps components showed lower values from 1 to 2 (or 3) hours after injection, but then they gradually increased up to a value higher than the control level (FIG. 6-B).

c) *Hypothalamic nuclei*: i) *AHA*. All frequency components decreased directly after injection and remained lower than the control level over 6 hours (FIG. 7-A). ii) *ME-ARC*. All frequency components gradually increased after injection. The increment was seen even 6 hours after injection (FIG. 7-B).

d) *RF*: All components decreased soon after injection and kept low

FIG. 7. The changes of EEG activity of AHA-(A), ARC-(B) and RF-(C), after intravenous injection of ACH (1 mg/kg).

values thereafter at least until 3 hours later, but then gradually returned to the control level (FIG. 7-C).

The administration of hydrocortisone acetate can be concluded to have a facilitatory effect on the HPC and the ME-ARC, while it seemed to exert an inhibitory action on the AMYG, AHA and the RF.

B. Effects of hydrocortisone implantation or electrolytic lesions in various regions of the brain on the stress response.

In this procedure the ACH-sensitive areas in the brain were explored by implanting hydrocortisone in some brain structures and observing the resulting changes in the responses to immobilization stress as described below. However, one may suppose that a stress situation will elicit very much complicated patterns of responses in the nervous and the humoral systems and the results will be hardly comparable with those obtained in the first part of this investigation by mere injections of hormones into the blood. However, according to Kawakami's previous report³⁸⁾ it seems safe to assume that our two results are well comparable. It was reported, that after forcing immobilization stress to rabbit, the hippocampal EEG showed an increase in the 4-8 cps component, while almost all frequency components were depressed in the amygdala EEG and the reticular EEG revealed an increase in the 2-4 cps component but a decrease in all other components. These changes were the opposite to those observed after ACTH injection and analogous to those after ACH injection in first part of our present experiment. Therefore in the immobilization stress, the other factors, such as emotional, other than the changes in the ACTH or ACH concentration in blood can be considered to have little, if any, effect on the brain activity.

The rabbits were forced to sustain 6 hours' immobilization from 8:00 a. m. to 2:00 p. m. on the day of experiment, and the stress responses as indicated by the decrease of blood lymphocytes and the increase of urinary Porter-Silber chromogens were measured.

The level of blood lymphocytes in 5 control rabbits led to marked lymphopenia with the maximum drop at about 4 to 6 hours after the onset of the immobilization (FIG. 8, left). The average decrease of blood lymphocytes among 22 control rabbits was 34.3 ± 9.0 per cent of the initial count at the end of the immobilization. The urinary Porter-Silber chromogens ($\mu\text{g}/24$ hours) of 14 control cases showed marked increases in the urine on the day of the immobilization (FIG. 8, right). The increase of urinary Porter-Silber chromogens on the day of the immobilization was shown with the percentage of the resting level described in MATERIALS AND METHODS of this report.

In the experimental animals with hydrocortisone implantation or lesions in the brain, 5 rabbits with hydrocortisone in the ME-ARC showed inhibition of the stress response to 6 hours' immobilization. The level of blood lympho-

FIG. 8. The changes of blood lymphocytes (N/mm³) in control rabbits (left) and of urinary Porter-Silber chromogen (ug/24 hours) in control rabbits (right) by 6 hours' immobilization.

cytes at the end of the immobilization was 69.1 to 100 per cent of the initial count after implantation as compared with 30.6 to 45.2 per cent before implantation. At the same time, the level of urinary Porter-Silber chromogens on the day of the immobilization was 47.6 to 116 per cent of the resting level after implantation and 153 to 239 per cent before implantation.

Also, in 5 rabbits with implanted hydrocortisone at the nucleus ventromedialis hypothalami, the level of blood lymphocytes at the end of the immobilization was 63.4 to 93.4 per cent of the initial count after implantation with 32.4 to 53.0 per cent before implantation; and the amount of urinary Porter-Silber chromogens on the day of the immobilization was 64.2 to 114 per cent of the resting level after implantation while 150 to 200 per cent before implantation. In 6 rabbits with electrolytic lesions in the same area, marked inhibition of the stress response was seen after lesioning. The level of blood lymphocytes at the end of the immobilization after lesioning was 63.8 to 90.6 per cent of the initial count as compared with 27.3 to 48.2 per cent before making electrolytic lesions. At the same time the urinary Porter-Silber chromogens also revealed 27.0 to 111 per cent after and 147 to 209 per cent before.

However, in the other regions, such as the septum pellucidum, PHY, central

FIG. 9. Location of hydrocortisone implanted in the brain. Solid circles represent the cases with inhibitory effect on the stress response. A, B, C, D: frontal sections at A1, AP0, P1, P6 and P9 according to the atlas of SAWYER et al. E: sagittal section through the brain stem. Abbreviation: Cort, cerebral cortex; VEN, ventricle; SP, septum pellucidum; OT, optic tract; DMH, nucleus dorsomedialis hypothalami; VMH, nucleus ventromedialis hypothalami; ARC, nucleus periventricularis arcuatus; HPC, hippocampus; PHY, posterior hypothalamus; RF, midbrain reticular formation; M, massa intermedia; OCH, optic chiasma; MM, nucleus mammillaris medialis; IPN, nucleus interpeduncularis.

FIG. 10. Localized lesions in the brain. A: transverse and sagittal sections through the septum pellucidum showing lesioned areas in stipple. B, C, D: transverse and sagittal sections through the hypothalamus showing lesioned areas in stipple. E, F: transverse and sagittal sections through the midbrain showing lesioned areas in stipple. Abbreviations: FF, fimbria of fornix; SC, superior colliculus.

grey and the RF, no remarkable changes of stress responses were seen, either by implanting hydrocortisone or by making lesions.

From the facts described above, the ME-ARC and the nucleus ventromedialis hypothalami might be regarded as the corticoid sensitive areas in the brain, which might produce the factor effecting ACTH discharge from the pituitary.

DISCUSSION

The results described above have shown that the activity of the mesencephalic reticular formation is slightly enhanced phasically by the rise of the ACTH concentration in blood or by exposing the animal to a stressor, but is strongly depressed by the increase of the ACH occurring secondarily to the rise of the ACTH. The fact that the activity of the midbrain reticular formation declined in accordance with the increase of ACH suggests that, when the animal is confronted with a stress, this structure functions to suppress the inflow of external stimuli, thus playing the important role of preventing

hyper-excitation of higher brain centers. It should be remembered that this structure was conceptualized by MAGOUN^{17,18)} as a high gain amplifier for the higher brain centers, especially for the hypothalamus and the pituitary body. It is a well established fact that adrenaline release occurs directly after a stress, and also that adrenaline activates the midbrain reticular formation^{19, 20, 21)}. The apparent contradiction between this fact and the present result might be understood by hypothesizing that initially the midbrain reticular formation is activated by adrenaline and ACTH but then it reacts so as to restore the hyper-activated state of the brain to a more normal level. Taking into consideration the report by SLUSHER and HYDE²²⁾ that an inhibitory area of adrenal corticosteroid release exists in the posterior ventral midbrain tegmentum, the role of the reticular formation in controlling the ACTH secretion is expected to be of a much more complicated nature than described here.

ANDERSON et al.²³⁾, and ROYCE and SAYERS²⁴⁾ have implicated a mesencephalic-hypothalamic pituitary activating system in the increase in urinary corticoids following stress. On the basis of neurological investigation on rat by NAUTA²⁵⁾ it appears that ascending mesencephalic pathways send collaterals into the hypothalamus. These physiological and anatomical finding, together with the conceptualization by MAGOUN^{17,18)} of the reticular functions as a high gain amplifier for the hypothalamus and the pituitary body as well as for other higher brain structures, stress the significance of the connection between the midbrain reticular formation and the hypothalamus in the feedback control of ACTH and ACH as assumed above.

With respect to the physiological role played by the limbic system which has been considered to be among other functions, a higher center regulating the hypothalamic activity and to have some significance in emotional stress, MASON^{26, 27)} has already reported that an increase occurred in the 17-OHCS level in the blood after amygdalar stimulation, but it decreased after hippocampal stimulation, which suggests that the enhanced activity of the amygdala facilitates the release of ACTH, while that of the hippocampus inhibits the release of ACTH.

The present experiment have revealed that, 1) the increase of ACTH enhanced the amygdalar (lateral basal portion) activity and depressed the hippocampal (alveus) activity, 2) the increase of corticoid depressed the amygdalar activity and enhanced the hippocampal activity. From these facts an existence of an antagonistic relationship, which work in an opposite fashion in regulating the secretion of ACTH and of ACH, is suggested between the hippocampus and amygdala.

In the basal hypothalamus, which has been conceptualized in the recent works as a focus in the negative feedback regulation of the interaction between ACTH and ACH, the median eminence known as the final common pathway for the ACTH release has been found to respond in a peculiar

manner to the changes in ACTH and ACH in the blood. Namely, the activity of the median eminence including the periventricular arcuate nucleus was depressed by the increase of ACTH and enhanced by the decrease of ACH. This structure has proved to be the most sensitive area for the inhibitory action of hydrocortisone upon ACTH secretion as shown by the latter part of this experiment. These results agree well with the observation by SMELIK and SAWYER^{11,28)} who had found similar inhibitory influences of hydrocortisone implantation in these areas taking the increasing rate of plasma corticosterone level in a stress as an index. This structure is generally accepted to produce a neurosecretory material somehow associated with the specific ACTH-releasing factor^{29,30,31)}. It would then be safe to assume that the elaboration or release of such a factor is suppressed by corticoids by inhibiting the activity of this structure. Putting together this assumption and the results obtained by EEG observation as above, a more complete figure can be developed of neurohumoral interaction in the negative feedback control of ACTH and ACH secretion, in which the median eminence works as the focal structure. That is, this structure may, perhaps, play the role of maintaining the homeostasis of the ACTH and ACH. In other words, it works against the raised ACTH release from the anterior pituitary gland when ACTH is increased in the blood, or elevates the ACH secretion, when ACH in the blood is decreased, by facilitating the ACTH release.

On the contrary, the posterior portion of the hypothalamus, which has been reported to be strongly activated by adrenaline^{32,33,34,35,36,37)}, a substance released by stress, was little, effected by the increase of ACTH and ACH in the blood. Therefore it is assumed that although this portion of the hypothalamus is supposed to have a facilitatory effect through the pituitary-adrenal system on the ACTH and ACH when the adrenaline increase occurs in the blood as in a stress, there is little feedback reaction to this structure from the increase or decrease in these hormones.

The anterior hypothalamic area took the same attitude as the reticular formation, showing an enhanced activity by the increase of ACTH and a depressed activity by the increase of ACH.

From these considerations it may be concluded that the basal hypothalamus works as a focal point in the negative feedback control of the maintenance of the dynamic equilibrium of ACTH and ACH in an organism. It may also be assumed that there are several structures in the brain surrounding the basal hypothalamus which control ACTH secretion, and exerting facilitatory or inhibitory actions on the control function of the basal hypothalamus. Above all the hippocampus (alveus) and the lateral basal amygdala are found to react to the rise of ACTH in the blood with a depression and an enhancement of electrical activity, which in turn facilitate the ACTH release by activating the basal hypothalamus, thus working in a positive feedback control of ACTH

secretion. On the other hand, the activation in the posterior hypothalamus and the reticular formation in response to the increase or decrease in ACTH and ACH should be a secondary change to the increase of adrenaline or antidiuretic hormone caused by a stress, rather than a direct effect of ACTH and ACH.

SUMMARY

The neurohumoral interaction in the feedback control of ACTH secretion was studied by means of EEG observation before and after intravenous administration of adrenocorticotrophic hormone, SU-4885 which is an inhibitory substance for the adrenocortical hormone production by the adrenal cortex, and hydrocortisone acetate in rabbit. For further clarification of this problem, crystalline hydrocortisone acetate was implanted or a localized lesion was made in the brain and alterations in the stress response were observed.

The results were as follows:

A. The EEG activity showed the following alterations after injection of adrenocorticotrophic hormone.

1. Injection of adrenocorticotrophic hormones brought about a decrease in slower components of 2-13 cps in the dorsal hippocampus (alveus) and the median eminence-periventricular arcuate nucleus, and an increase in the amygdala (lateral basal portion), anterior hypothalamic area, mammillary body and the midbrain reticular formation. In some cases 40 cps spindle-like burst increased after injection. The medial preoptic area revealed a diphasic alteration by injection, first an increase and then a decrease in the slower components. Little change occurred in the activity of the posterior hypothalamic area.

2. With administration of SU-4885, there was an increase in the slower frequency components in the dorsal hippocampus, anterior hypothalamic area, median eminence-periventricular arcuate nucleus and the posterior hypothalamic area. In the amygdala these components first decreased but from 2-4 hours after injection then increased. In some cases the 40 cps waves showed a remarkable increase. In the midbrain reticular formation the slower components showed a diphasic change, first a decrease and then an increase.

3. With hydrocortisone acetate injection, there was an increase in the slower components of the dorsal hippocampus and the median eminence-periventricular arcuate nucleus, but these components markedly decreased in the amygdala, anterior hypothalamic area and the midbrain reticular formation.

B. After implantation of hydrocortisone acetate or localized electrolytic destruction in the median eminence-periventricular arcuate nucleus and the nucleus ventromedialis hypothalami, the stress response of the rabbit, as

indicated by the decrease of blood lymphocytes or the increase of urinary Porter-Silber chromogen excretion, showed a depression. In cases of the septum pellucidum, posterior hypothalamic area, central grey and the mid-brain reticular formation, no remarkable change occurred following these treatments in the stress response.

On a basis of these results it was suggested that the basal hypothalamus might be a focus for the negative feedback control for the maintenance of homeostasis of adrenocorticotrophic hormone and adrenocortical hormone, and that the hippocampus and the amygdala might participate in this mechanism by acting on the basal hypothalamus when their activities were influenced by the changes in adrenocorticotrophic or adrenocortical hormone level.

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