Changes in the Activity of the Limbic-Hypothalamic Neural Pathways Under the Repeated Immobilization Stress

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Synopsis

Changes in the electrical activity of the limbic-hypothalamic neural pathways during the process of adaptation to the immobilization stress were studied in the rabbit with chronically implanted electrodes. Both the hippocampally and the amygdaloidly evoked potentials in the medial basal tuberal region revealed decreases in their amplitudes during exposure to the 1st immobilization stress. Following the repeated exposure to the stress, the resting levels of their amplitudes decreased, and thus the decrease of amplitude in response to immobilization stress was reduced. To administered exogenous ACTH or cortisol acetate, those evoked potentials were significantly sensitive, and in the rabbit having been exposed to the repeated immobilization stress, the response to exogenous ACTH was observed to be altered.

It was considered that the limbic-hypothalamic neural pathways played a role in the establishment of adaptation mechanism by changing their responsiveness to the stressful stimuli and to ACTH and/or adrenal corticoid during the repeated exposure to stress.

It is now generally recognized that the central nervous system plays an essential role in the regulation of pituitary-adrenal axis, both in basal conditions and during the response to stress. However, in spite of the accumulated data, little is known about the buffer action of the brain mechanisms to stress stimuli by which the animal can adapt itself to external and internal stimuli.

Recently, Kawakami *et al.* (1971) have suggested that the central nervous system adapts to the repeated stress by establishing an inhibitory mechanism of ACTH through the hippocampus and the amygdala. Thus it was supposed that the limbic structures function as an important modulator of hypothalamo-hypophyseal activity in the adaptive process.

The present experiment was designed in order to elucidate the role of the limbic-

hypothalamic neural pathways during the process of adaptation by observing changes of the evoked potentials (EVPs) in the medial basal tuberal region including the median eminence elicited by the hippocampal or the amygdaloid stimulation.

Materials and Methods

Experiments were performed on 30 rabbits (female adult New Zealand white rabbits) weighing 2.5–3.5 kg. Coaxial bipolar electrodes were stereotactically implanted into the medial basal tuberal region (including the arcuate nucleus, ARC, median eminence region, ME) of the hypothalamus, the dorsal hippocampus (HPC, cornu ammonis) and the medial part of the amygdala (AMYG) by the atlas of rabbit brain of Sawyer *et al.* (1954). The distance between the bared portion of tip and the end of barrel was 0.5–0.8 mm. These experimental rabbits were used at least three weeks after recovery from the operative procedure. The recording experiment was performed

Fig. 1. Representative example of changes in the evoked potentials during the repeated immobilization stress. The amygdaloidly evoked potential in the arcuate nucleus (AMYG-ARC) decreased remarkably in amplitude and in latency following application of the 1st immobilization stress. Under the 7th immobilization, these changes decreased. The hippocampally evoked potential (HPC-ARC) decreased in amplitude by the 1st immobilization stress, while almost no change was observed under the 7th immobilization.

while the rabbit was at rest either on its behavior or on its cortical EEG. Single shock stimuli (8–18 V; 0.01–0.1 msec duration) were delivered to the HPC or the AMYG during EEG synchronized phase but just after the appearance of a spindle burst. The minimum interval between stimuli was 60 sec. Hypothalamic potentials were recorded monopolarly with standard oscilloscope techniques. Each study involved 10 potentials recorded to calculate the mean amplitude and latency. Immobilization stress was given for 6 hours a day, from 12:00 to 18:00. During the exposure to stress, food and water were restricted. Rabbits were exposed to the immobilization repeatedly for successive 7 days. The dosages of hormones were: ACTH—1 mg/kg (Acthar, Armour) and cortisol acetate—2

mg/kg (Nihon Merk Banyu). At the end of the experiment, the electrode placements were verified histologically.

Results

Effects of the 1st immobilization stress

The EVPs in the medial basal tuberal region (including the ARC-ME) induced by the stimulation of the HPC (hippocampally EVP) or the AMYG (amygdaloidly EVP) had a consistent wave form, latency and

Fig. 2. Changes of the evoked potentials in the arcuate nucleus induced by the stimulation of the hippocampus (HPC-ARC) and the amygdala (AMYG-ARC), at 45 or 30 min after the intravenous injection of ACTH (1 mg/kg), and its graphical manifestation in percentage in relation to the amplitude of the potential before injection. Open circle with broken lines; the 1st negative response. Filled circle with solid lines; the 2nd positive response. Further explanation in the text.

amplitude in normal intact rabbits: the hippocampally EVP consisted of the 1st negative (latency: 4–6 msec) and the 2nd positive (8–14 msec) responses, and the amygdaloidly one consisted of the 1st negative (8–14 msec) and the 2nd positive (18–22 msec) responses. The short-latency positive response (4.5–5 msec) was recorded inconsistently in the amygdaloidly EVP.

Typical responses of the EVPs recorded in the ARC to the immobilization stress are illustrated in Figure 1. In the hippocampally EVP, both the 1st and the 2nd responses decreased in amplitudes immediately after the onset of immobilization on the 1st day.

The depression was more remarkable in the 1st response than in the 2nd one. The decreased levels recovered within 2 hr after the release from immobilization. Both responses (8–10, 18–22 msec) of the amygdaloidly EVP decreased markedly in amplitudes immediately after starting the immobilization, and the latency of the 2nd positive response (18–22 msec) was lengthened by 2 msec.

Effects of ACTH in intact rabbits

Recording experiments were conducted on 5 rabbits with intact adrenal glands. Figure 2 illustrates typical responses of the ARC EVP to ACTH injection (shown left): the 1st

Fig. 3. Responses of the evoked potentials to cortisol acetate. The amygdaloidly evoked potential in the arcuate nucleus (AMYG-ARC) decreased in amplitude of the 2nd component (18–22 msec) after the injection of cortisol acetate (2 mg/kg). The hippocampally one (HPC-ARC) increased in amplitude of the 1st component (4–6 msec).

negative response of the hippocampally EVP started decreasing at 5 min and reached its peak at 45 min after ACTH injection. The depression recovered the control level at 90 min, though followed by a gradual decrease. The 2nd positive response began to decrease in amplitude immediately after ACTH injection and reached a minimum level at 30 min after treatment with ACTH. The depression lasted for about 4 hr. In the amygdaloidly EVP, the amplitudes of the 1st and 2nd responses decreased in a very short latency and reached the bottom at 60 min after the injection. These changes recovered the control levels at 3 to 4 hr. The latency was not influenced either in the hippocampally or in the amygdaloidly EVP.

Effects of cortisol acetate in intact rabbits

Figure 3 is typical of the responses of the EVPs in the ARC in the rabbits with intact adrenal glands to cortisol acetate: in the hip-

pocampally EVP, the 1st negative response increased markedly in amplitude within 1 hr and its level continued for about 5 hr. The 2nd positive response showed no remarkable changes in both the amplitudes and latency following treatment with cortisol acetate. The marked depression of the amplitude in the amygdaloidly 2nd positive response was observed within 3 hr after cortisol treatment. The 1st negative response showed no remarkable change.

Effects of the 7th immobilization stress

The initial levels of amplitudes of both the hippocampally and the amygdaloidly EVP, which were recorded before daily exposure to the immobilization (daily control level) showed a gradual decrease, while almost no change was shown in the latency. That is, the daily control level of the hippocampally EVP decreased gradually throughout 7 days. That of the amygdaloidly one showed also a

Fig. 4. The initial levels of amplitudes of the evoked potentials before daily exposure to the immobilization stress (daily control level). The amygdaloidly evoked potential in the arcuate nucleus (AMYG-ARC) showed a decrease on the 3rd day, and on the 7th day it returned to the level of the 1st day. The hippocampally one (HPC-ARC) decreased gradually throughout 7 days.

gradual decrease until the 3rd day of the repeated immobilization stress, while showing a tendency on the 7th day to recover the initial observed on the 1st day (Fig. 4).

Typical responses to the ARC EVP to the 7th immobilization are illustrated in Figure 1. The decrease of amplitude in the hippocampally EVP during response to stress lessened gradually throughout 7 days and the decreased level returned to the control level before the release from the immobilization. In the amygdaloidly EVP, the decrease of amplitude in response to the 7th immobilization was almost similar to that on the 1st day, while until the 3rd day it showed a lessened one. However, the prolongation of the latency noticed in the 2nd positive response on the 1st day was no longer observed on the 7th day.

Effects of ACTH after 7 days of repeated immobilization stress

The experiment was performed on the day

following the last day of repeated immobilization stress. An example of responses of EVPs in the ARC, which will be illustrated in Figure 2 (shown right), was recorded from the identical rabbit in which was examined the response to ACTH before the application of the repeated stress (shown right).

The 1st response in the hippocampally EVP increased in its amplitude immediately after ACTH injection and reached its peak at 30 min, with recovery in less than 2 hr. It increased again and lasted for 4 to 6 hr. The 2nd positive response showed no remarkable rapid changes, but was followed by a slight increase in amplitude continuing for about 5 to 6 hr.

Decrease of amplitude in response to ACTH was also noted in the amygdaloidly EVP in the ARC. The 1st negative response recovered the control level at 1 to 2 hr after injection, and then its amplitude increased slightly, maintaining the level for 3 to 4 hr. The 2nd positive response recovered the control level

at 3 to 4 hr after ACTH treatment with 2 hr delay in comparison with the recovery time of the 1st negative response.

Discussion

The short-latency negative response (4–6 msec) of the hippocampally EVP which was consistently recorded and the short-latency positive response (4.5–5 msec) of the amygdaloidly EVP which was not always consistently recorded, are considered to be involved in a direct or a very pauci-synaptic pathway (Kawakami *et al.*, 1970). However, only the changes of the evoked responses which were consistently recorded will be discussed.

Following the exposure to immobilization stress, the hippocampally and amygdaloidly EVPs showed decrease in their amplitudes. It is acceptable that the immobilization stress, as an emotional stress, is able to affect such limbic structures with a neurogenic component. The changes of EVPs during the exposure to immobilization stress are considered partially due to neurogenic effect of the stress.

Furthermore, it was found that the electrical stimulation of the hippocampus or the amygdala induced an increase of biosynthesis of adrenal steroid hormones and blood corticosterone concentration in the non-stressed rabbit (Kawakami *et al.*, 1968a). This result may suggest that the hippocampus or the amygdala is closely related with the hypophyseal activation to release ACTH. It was observed, also, that the hippocampal stimulation inhibited ACTH release following exposure to stressful stimuli such as the immobilization (Kawakami *et al.*, 1968a) or the painful electrical stimulation (Endröczy *et al.*, 1959) and the amygdaloid stimulation exerted a slight additional release of ACTH. On the other hand, the electrical stimulation of the medial basal hypothalamus induced a facilitation of ACTH release under stressed condi-

tion as well as under non-stressed one (Kawakami *et al.*, 1971). Thus the decreased activity of the hippocampally and amygdaloidly EVP in the medial basal tuberal region during exposure to immobilization stress might be interpreted as indicating that, under the acute stress, the medial basal hypothalamus worked dominantly to release ACTH in the so-called deafferented condition.

Moreover, the result of experiments on ACTH or corticoid treatment indicates a possibility that the changes of EVPs during response to a single exposure to immobilization stress may depend on the feedback effect of the high blood levels of ACTH and/or adrenal corticoids. That is, the patterns of changes in the hippocampally or the amygdaloidly EVP after ACTH administration were very similar to those during the immobilization. After the administration of cortisol acetate, the hippocampally EVP showed an almost opposite change to that during the immobilization, while the amygdaloidly EVP showed a similar change as seen during immobilization. These results suggest that those neural pathways have a significant sensitivity to exogenous ACTH and corticoid. Feldman *et al.* (1961) and Endröczy (1969) also reported that adrenocortical hormones caused a change in the evoked potentials shortly after treatment with them. Furthermore, Kawakami *et al.* (1968b) examined the effects of a minute crystalline corticosterone implanted into the hippocampus or the amygdala upon the formation of adrenocorticoids. Formation of adrenal steroid hormones increased, when they were implanted in the hippocampus and decreased in the amygdala. From these results and the previous findings (Kawakami *et al.*, 1968a), Kawakami *et al.* (1968b) proposed that a part of the hippocampus might be related with the positive feedback mechanism of adrenal corticoid and the amygdala might be related with the negative feedback mechanism of it.

It is not clear, however, whether the same nervous pathways that control ACTH secre-

tion through the steroid feedback mechanism are also involved in the activation of the pituitary during stress. Although ample evidences have proved that different mechanisms are involved in the control of the pituitary-adrenal axis, the participation of those neural pathways in both mechanisms is clear. It seems likely that the changes of hippocampally and the amygdaloidly EVP during the stress may represent the summation of both ACTH regulating activities. Those neural pathways may modulate the ACTH secretion of the pituitary by sending facilitatory or inhibitory impulses impinging on the basal hypothalamus through mechanism which is involved in the stress-induced release of ACTH and through the steroid feedback mechanism.

In the rabbit having been exposed to the repeated immobilization stress, the hippocampally EVP in the medial basal tuberal region revealed a decreased initial level of amplitude on the 7th day, and thus resulted in a lessened decrease in its amplitude during response to the 7th stress. The amygdaloidly EVP showed a decreased initial level, and then resulted in a lessened decrease in response to stress on the 3rd day. On the 7th day, it represented a slight recovery in its responsiveness to the stress, showing a recovered initial level. These findings may indicate that the neural pathway from the hippocampus or the amygdala to the medial basal hypothalamus resulted in the so-called "habituation" to the stress stimuli, and further its resting activity was decreased following the repeated exposure to stress. It is not clear, however, why the initial level of amplitude resulted in the decrease. A possible explanation proposed is that it resulted at least partially from the feedback effect of the high blood levels of ACTH and/or adrenal steroids brought about by the repeated stress.

It has been observed that after the repeated application of the same stress the stress no longer caused the release of ACTH (Kawakami *et al.*, 1971). Namely, there was a con-

siderable increase in the biosynthesis of adrenocorticoids and plasma corticosterone noticed during the first immobilization while the 7th immobilization induced hardly any change or a slight decrease in the biosynthesis of adrenocorticoids. The electrical stimulation of the hippocampus or the amygdala during the 7th immobilization resulted in a further slight decrease of the biosynthesis of adrenocorticoids, although stimulation of the arcuate nucleus-median eminence region still exerted a facilitatory effect upon it. These findings suggested to us that the inhibiting impulses from the hippocampus and the amygdala might play an important role to prevent ACTH secretion in the condition of repeated exposures to the same stress, and further that the brain mechanism adapted itself to the stress not by diminishing the response to stress but by changing it. Thus the decreased change of the EVP in response to stress may not merely mean the habituation but it may mean actually an altered response to stress. Altered responsiveness to exogenous ACTH was also observed in the hippocampally EVP, that is, changes of the hippocampally EVP to administered ACTH after the repeated application of stress were almost opposite to those before the application of stress.

It is considered that the central nervous system makes adaptation mechanism by changing its responsiveness to the stressful stimuli and to ACTH or corticoid during the repeated exposures to stress.

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