Time Course of Hypothalamic CRF Activity after the Administration of Two Different Stresses

MUMEKI SAKAKURA1, YOSHIRO SAITO2, KAZUO TAKEBE1, ITARU YAMASHITA2, AND KANEI ISHI1

1Second Department of Medicine, Asahikawa Medical College, Asahikawa-shi 071-01 and 2Department of Psychiatry and Neurology, Hokkaido University School of Medicine, Sapporo-shi 060, Japan

Synopsis

The time course of hypothalamic corticotropin-releasing factor (CRF) activity after the administration of ether stress was different from that after immobilization stress. The maximal response of CRF activity was observed 2 min after ether stress, followed by a precipitous decrease 5 min after the stress. A gradual increase of CRF activity was subsequently observed for several minutes with fluctuated changes. Thus, response pattern was vibratory. But under immobilization stress, markedly fluctuant changes of CRF activity seen in the case of ether stress did not appear after the maximal response observed at 2 min, indicating that the response pattern was not vibratory. On the other hand, the concentration of plasma corticosterone increased significantly 5 min after the ether or immobilization stress with the peak value around 17 min.

Several investigations have indicated that the hypothalamic CRF activity is increased by various stimuli. Vernikos-Danellis (1965) demonstrated that CRF activity showed a marked and rapid increase after ether stress for 1 min. Furthermore, stress like ether-laparotomy or ether-laparotomy-intestinal traction provoked the rise of CRF activity in the hypothalamus within 2 min (Hiroshige et al., 1971; Takebe et al., 1971). Very few reports have been published about the time course of the hypothalamic CRF activity under stress (Hiroshige et al., 1971). However, few workers have performed the time course of the hypothalamic CRF activity after the administration of different stimuli in the same experimental schedule. Therefore, the response of hypothalamus to ether or immobilization stress was examined in order to make sure of the time course of the hypothalamic CRF activity and plasma corticosterone. A part of this work was preliminarily published elsewhere (Sakakura et al., 1976).

Materials and Methods

The experiments were performed with male rats of the Wistar strain, weighing between 180-220 g. They were housed at a constant ambient temperature of 22 ± 2°C and fed with rat biscuits, with water ad lib and acclimatized to a controlled lighting schedule (light on at 07:00, off at 19:00). Two rats were treated in one cage and were accustomed to manual handling by mock treatment at 16:00 at least for 7 days prior to sacrifice. As stimuli, ether anesthesia and immobilization were used in this experiment. Rats were exposed to ether vapour for 2 min and they were decapitated 1, 2, 3, 5, 7, 12, 17, 20 and 25 min after the start of the stress. Rats were also immobilized with a semi-cylindrical holder (15 x 7 cm, length x diameter) for 2 min and they were also sacrificed 2, 3, 5, 7, 12, 20 and 25 min after the start of the stress. Four rats were decapitated at 08:00 without any stress as the control group and other

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four rats were sacrificed 1 min after the onset of the stress for the decapitation-group at 1 min. All experiments were carried out from 08:00 to 09:00. At given times, the blood of rats was collected by decapitation and it was kept in a refrigerator till the determination of plasma corticosterone level. Plasma corticosterone was determined by the method of Zenker and Bernstein (1958). The median eminence of rats was quickly removed after the decapitation. Fine curved eye scissors were used to remove the median eminence (ME) in the shape of an oval piece of tissue of 2 to 3 mg in weight. Four pieces of the tissue obtained each time were homogenized and extracted with 40 µl of ice-cold 0.01 N acetic acid-saline solution for 1 hr. After centrifugation at 3000 rpm for 10 min, an aliquot of 0.4 µl of the supernatant was used for the determination of CRF activity in the crude extract of ME. In order to know the CRF activity in the hypothalamus, test materials in an amount of 0.4 µl were injected directly into the anterior pituitary exposed paraphryngeally, of dexamethasone-nembutal-chlorpromazine blocked female rats (Hiroshige et al., 1968). The microinjection for CRF assay was made regularly from 13:00 to 16:00 in the afternoon. An increase in the plasma corticosterone level (µg/100 ml) 20 min after the start of microinjection was considered as an index of endogenous ACTH activity and was designated as Δ20 in the original method (Hiroshige et al., 1968).

Results

In ether stress group, hypothalamic CRF activity showed a significant increase 1 min after the start of the stress and the peak (Δ20= 44.5±4.9 µg/100 ml) was observed at 2 min. CRF activity decreased precipitously to the control level 5 min after the onset of the stress. Thereafter, the hypothalamic CRF activity showed a gradual increase with two peaks. The peak value observed 12 min after stress was 19.0±1.4 µg/100 ml (Δ20) and another peak value at 20 min was 27.7±6.4 ml (Δ20) as shown in Fig. 1. The concentration of plasma corticosterone increased significantly 5 min after the start of the stress and continued to increase gradually for the next 15 min. The peak value (81.2±3.6 µg/100 ml) was observed 20 min after the stress.

Immobilization stress group in Fig. 2, the hypothalamic CRF activity increased significantly with a peak (Δ20=36.4±3.5 µg/100 ml) 2 min after the stress and then it decreased rapidly to the control level 7 min

Fig. 1. Time course of hypothalamic CRF activity (-----) and plasma corticosterone (-----) after the administration of ether stress. Cpd B is corticosterone. Vertical bars indicate the standard error.

For CRF assay: eight rats were used at 0, 2, 7, 12 and 25 min. Seven rats were used at 3, 5, and 20 min. Ten rats were used at 2 min.

For the determination of plasma corticosterone: four rats were used at 0, 1, 2, 3, 5, 12 and 17 min. Six rats were sacrificed at 7, 20 and 25 min.
The concentration of plasma corticosterone increased significantly 5 min after the onset of the stress and reached the peak (68.8 ± 2.1 µg/100 ml) 17 min after the start of the stress without vibratory changes.

Discussion

It is well known that the secretion of ACTH induced by the administration of stress is controlled by a specific neurohumoral substance of CRF. But very few studies have been performed about the time course of the hypothalamic CRF activity under stress. Redgate (1970) reported that the secretion of ACTH following electrical stimulation showed two different types: prompt and delayed. The prompt response or rapid rise of plasma ACTH level observed within a few minutes was elicited by stimulating the amygdaloid septal complex. Furthermore, Brodish and Long (1956) found that in the rat a severe stress caused a biphasic pattern of ACTH release. These data may also support our findings about the time course of the hypothalamic CRF activity after the administration of ether stress. Hiroshige et al., (1971) reported that the response pattern of hypothalamic CRF activity after the administration of ether-laparotomy stress was biphasic: rapid and slow phases. A rapid phase consisted of a prompt increase in CRF activity and a subsequent depletion lasting for 20 min after the onset of ether-laparotomy stress, while a slow phase appeared 40 min after the stress, showing a second peak value of CRF activity 80 min post stimulus. In our data, the peak of the hypothalamic CRF activity was observed 2 min after the onset of ether stress and the CRF activity showed a subsequent decrease to the control level at 5 min followed by a gradual increase after the decrease. There are some differences between our data and theirs. This dissociation might depend on the kind of stress. However, in either report, a biphasic change of CRF activity was observed after the administration of ether or ether laparotomy stress.

To the contrary, the peak appeared 2 min after the stress, but a biphasic change of CRF activity did not appear after the stress, followed by the nonsignificant rise of CRF activity in ME (Fig. 2).
decrease of initial increment. On the other hand, we could not demonstrate the different response patterns of the plasma corticosterone till 25 min after the administration of two different stimuli in spite of the different time course of CRF activity following these stimuli. The reason for this is not well known, but it may be possible that the response in plasma corticosterone concentration to a fluctuant change of CRF in the hypothalamus after ether might appear later than 25 min after ether stress.

This result suggests that the response to different stimuli may result in the different time courses in the hypothalamus-pituitary-adrenal axis, and the different responses of the hypothalamic CRF activity will be provoked by different stimuli under the control of a vibratory system (Szentagothai et al., 1968) in negative feedback mechanism. From our experiment, it may be imagined that the response pattern of CRF activity may depend on the kinds of stimuli. We must await further investigations to make clear the significance of the vibratory response in the hypothalamus-pituitary-adrenal axis.

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References