MET-ENKEPHALIN RESTORES SELF-STIMULATION BEHAVIOR IN RABBITS AFTER DESTRUCTION OF HYPOTHALAMIC NUCLEI

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The method of functional blocking of brain structures whose neurons are responsible for reception, afferent and efferent functions, or synthesis of endogenous biologically active substances, and in particular, of oligopeptides, is widely used in order to study brain functions.

Meanwhile it has been shown that oligopeptides can repair disturbances of motivational-emotional reactions and memory caused by trauma, or by therapeutic and experimental intracranial intervention [1-5, 7].

Experiments with bilateral coagulation of hypothalamic motivational food centers and also changes in dopaminergic mechanisms in animals in the recovery period, with a lateral hypothalamus syndrome [8, 13] led to enunciation of the hypothesis that mechanisms of chemical compensation of functions exist in the brain [6]. It is important to emphasize that even in the absence of hypothalamic motivational pacemakers, behavior aimed at satisfaction of vitally important needs can be restored through the reorganization of cortico–subcortical integration and of the chemical mechanisms of the brain in animals [5, 9, 10, 12].

Compensatory effects after destruction of the various motivation-inducing zones of the hypothalamus have been found by the use of several oligopeptides, such as: substance P, ACTH, and β-lipotropin [1, 3-5].

Oligopeptides have been shown to restore physiological reactions induced by stimulation of motivation-inducing centers of the hypothalamus, during exposure to blockers of protein synthesis [9, 10].

The aim of this investigation was to study the role of Met-enkephalin (ME) in the mechanisms of compensation of self-stimulation (SS) behavior in rabbits after destruction of individual hypothalamic nuclei.

EXPERIMENTAL METHOD

Experiments were carried out on 51 male chinchilla rabbits weighing 3-3.5 kg. A few days before the experiments began, each animal, after preliminary scalping under local anesthesia with 2% procaine solution, had bipolar needle electrodes with a tip 0.12 mm in diameter implanted at different points of the hypothalamus. The electrodes were fixed at points of the brain, electrical stimulation of which led to the appearance of SS in the rabbits.

In the course of the experiments the animals were given free access to water and food. The unrestrained animals were quickly taught to close an electric circuit in order to obtain stimulation of the lateral hypothalamus by touching a metal lever with their nose or lips. The parameters of stimulation were: square pulses, frequency 100 Hz, duration of stimulation 0.3 sec, strength from 40 to 120 μA, pulse duration 1.4 msec. The strength of the current in each individual case corresponded to the threshold of appearance of a behavioral SS reaction. Experiments were carried out daily for 7-10 days. The frequency of SS was tested every 10 min, with a 5-min interval, during a 2-hourly experiment. Absolute background values of the frequency of SS were taken to be 100%. Changes in the frequency of SS were examined relative to the background. A cannula for injection of ME was implanted into the lateral cerebral ventricles.

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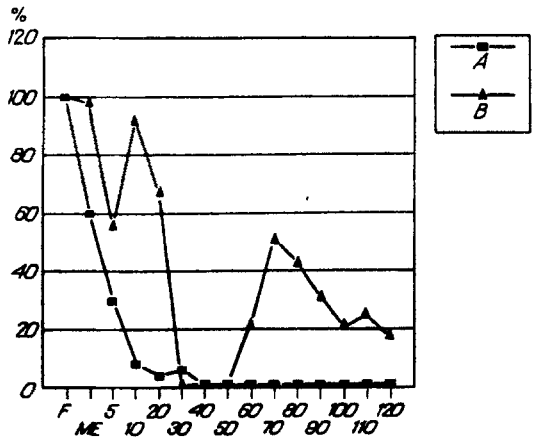

Fig. 1

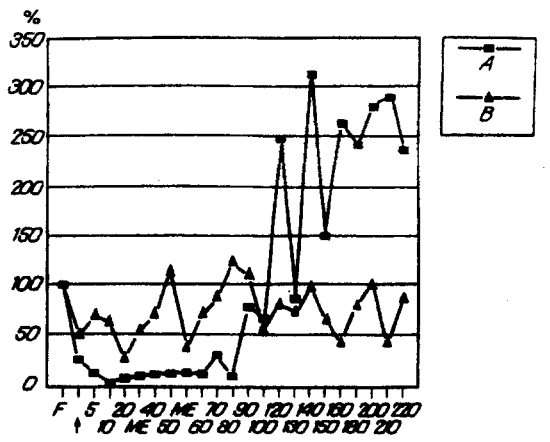

Fig. 2

Fig. 1. Injection of Met-enkephalin into rabbits with initially low level (A) and with initially high level of self-stimulation (B). F) Background level of SS, taken as 100%. ME) Intraventricular injection of Met-enkephalin in a dose of 0.1 μg/kg. Abscissa, time (min); ordinate, frequency of SS, %.

Fig. 2. Intraventricular injection of Met-enkephalin against the background of destruction of ventromedial hypothalamic nucleus in rabbits with initially low frequency (A) and with initially high frequency of self-stimulation (B). F) Background level of SS taken as 100%. I) Destruction of ventromedial hypothalamic nucleus, ME) intraventricular injection of Met-enkephalin in a dose of 0.1 μg/mg. Abscissa, time (min); ordinate, frequency of SS, %.

Histograms of the distribution of intertrial intervals were analyzed. The mean duration (T_x) and the duration of the leading, preferred intertrial interval (T_m), in msec, were examined.

In the 1st series of experiments the dynamics of SS was studied in response to injection of ME into the lateral cerebral ventricles in doses of 0.1, 1, and 10 μg in 10 μl of sterile physiological saline.

In the 2nd series of experiments SS was analyzed after electrolytic destruction and blocking of the hypothalamic structures by 2% procaine solution followed by injection of ME in the doses stipulated above.

Each experimental group was divided into two subgroups: animals with a high frequency of SS – up to 300 SS during a 5-min interval, and animals with a low frequency of SS – up to 100 SS during a 5-min interval.

Hypothalamic structures were coagulated unilaterally, contralaterally relative to the stimulating electrode, for 60 sec, by a direct current 2 mA in strength. After the end of the experiments the animals' brain was fixed in 10% formalin solution, frozen, and the localization of the lesions determined by reconstruction of frontal sections, using maps in stereotaxic atlases. The experimental results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

After injection of ME into the lateral cerebral ventricles in doses of 0.1, 1, and 10 μg/kg, ME was shown to reduce the frequency of SS.

The greatest inhibitory effect on the frequency of SS following injection of ME was observed in a dose of 0.1 μg/kg in seven rabbits with a low initial level of SS, whereas ME in a dose of 0.1 μg/kg inhibited the frequency of SS highly significantly ($p < 0.001$), which was 6.9 ± 4.2% of the background level, taken as 100% (Fig. 1A).

In six rabbits with an initially high level of frequency of SS, ME in the same dose of 0.1 μg/kg caused less marked inhibition of the frequency of SS, namely 55.66 ± 12.7% of the background level, taken as 100% (Fig. 1B).

The inhibitory effect of ME was exhibited 4-6 min after its injection into the lateral cerebral ventricles, to reach a maximum 20-45 min after injection. Meanwhile the duration of the intertrial intervals was increased. In the background, the leading – predominant (T_m = modal) intertrial intervals were 0.2 sec in duration (34%), whereas after injection of ME in a dose of 0.1 μg/mg T_m increased (0.6 sec) to 47%. From 45 to 90 min after injection of ME, T_m fell to its initial level. After 15-45 min the long intertrial intervals increased in duration to 0.6 sec. Compared with the background, their number increased by 19%. After 90 min the frequency of SS increased to reach 77% of the background frequency, taken as 100%, SS became more regular. The number of long intertrial intervals fell to the background value. Under these circumstances the duration of the modal intervals equaled the mean values of the intertrial intervals, namely 0.2 sec.

Injection of ME in a dose of 1 μg/kg caused inhibition of the frequency of SS in five rabbits with a low initial level of SS, which amounted to 58 ± 11.4%. In four rabbits with an initially high frequency of SS, namely 68.3 ± 5.9% of the background level, taken as 100%, after 15-30 min the frequency of T_m was increased by 25%, and the number of long intertrial intervals also increased. By 60-90 min the frequency of SS rose by 15-20%.

An inhibitory effect also was observed after injection of ME in a dose of 10 μg/kg. In six rabbits with a low initial level of SS its frequency was 29 ± 3.5%. In five rabbits with an initially high level of SS it was 48.58 ± 9.8% of the background level of SS, taken as 100%. Changes in patterns of SS in response to injection of 1 and 10 μg/kg were similar to changes in the patterns of SS following injection of ME in a dose of 0.1 μg/kg. In all the animals the frequency of T_m, which increased after injection of ME, fell after 60-90 min to the background level. Under these circumstances, one leading intertrial interval (0.2 sec) predominated, and SS became regular and amounted to 60-80% of the background value, taken as 100%.

A study of the dynamics of SS after injection of ME and after destruction of the ventromedial hypothalamic nucleus (VMN), taking account of the original frequency of SS, gave the following results.

In five rabbits with a low initial frequency of SS, after destruction of VMN the frequency of SS fell sharply to 12.27 ± 5.85% of the background value. Injection of ME in a dose of 0.1 μg/kg led to restoration of the initial frequency of SS, which actually exceeded by 8.9% the background level taken as 100% (Fig. 2B).

In six rabbits with a high background frequency of SS, and in which destruction of VMN inhibited SS to 36.42 ± 13.4%, injection of ME in a dose of 0.1 μg/kg caused partial recovery of SS, to 64.75 ± 15.5% of the initial background value, taken as 100%. On the 2nd day, SS was restored to its initial level (Fig. 2A).

Our experiments thus showed that after destruction of VMN, a more marked effect was obtained when ME was injected into animals with an initially low frequency of SS.

Injection of ME in a dose of 0.1 μg/kg in four rabbits with an initially low frequency of SS and with destruction of the lateral hypothalamic nucleus (the frequency of SS was depressed under these circumstances by 25% compared with the background value, taken as 100%) caused complete restoration of the frequency of SS. On the 2nd day the frequency of SS in these animals was 5.7% higher than the background value, which was recorded in these rabbits before destruction of the lateral hypothalamic nucleus. After injection of ME in a dose of 0.1 μg/kg, the frequency of SS rose from 75% after destruction to 82.4% after injection of ME in three rabbits with an initially high frequency of SS and with a destroyed lateral hypothalamic nucleus. Not until the 2nd day did SS return to its initial level.

Evaluation of the results shows that ME alone, after additional injection into the brain, inhibits SS, meanwhile ME restores the behavior of SS when suppressed after destruction of various hypothalamic structures. The compensatory effect of ME was manifested most strongly in animals with a low initial frequency of SS. After destruction of hypothalamic motivation-related structures, responsible for the formation of SS behavior, a deficit of opioid peptides and, in particular, of ME, is evidently created, for it is in these structures that ME is produced and stored [6, 8, 13]. It can accordingly be suggested that following local destruction of hypothalamic structures, receptors for ME are preserved in other parts of the brain, and for that reason following injection of ME into the lateral ventricles of the brain, SS is restored.

Consequently, the absence of SS following destruction of hypothalamic structures is connected, not with defects of those structures, but with a disturbance of their neurosecretory function.

The results described above are further evidence of the possibility of chemical compensation of lost brain functions. The role of oligopeptides in compensation of disturbed brain functions also was demonstrated in research by F. I. Dzhafarov (1988), R. A. Burchuladze (1990), Sandyh Reuven (1988), D. De Wied (1988), and G. Wolterink (1988).

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