EMOTIONAL BEHAVIORAL DISORDERS IN RATS FOLLOWING INJECTION OF SUBCONVULSANT DOSES OF METRAZOL

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UDC 616.831.314

KEY WORDS: metrazol, subconvulsant doses, anxiety state, pathological system, pathological determinant, antagonistic regulation, locus coeruleus, striatum

Subconvulsant doses of the analeptic metrazol induce a state of anxiety and fear in man and behavioral changes in animals assessed as anxietylike in traditional tests for anxiety [10, 11, 14]. The writers showed previously that the pathogenesis of syndromes of emotional-behavioral disorders resembling depressive and psychotic states in rats is connected with the formation of a pathological system (PS) in the brain, with a pathological determinant (PD) in certain limbic structures [5, 6]. In the investigation described below an attempt was made to find corresponding behavioral changes and changes in electrical activity (EA) of various deep brain structures in rats following systemic administration of subconvulsant doses of metrazol.

EXPERIMENTAL METHOD

Experiments were carried out on 25 male Wistar rats weighing 250-350 g. Under hexobarbital anesthesia (150 mg/kg, intraperitoneally) nichrome electrodes (200 μ, insulated) were inserted stereotaxically [13] into the locus coeruleus (LC), the rostral part of the striatum (STR), the dorsal hippocampus, and the basomedial nucleus of the amygdala. Primary recording of EA of the deep brain structures, using a monopolar technique, was carried out 3-5 days after the operation, using an RM-86M polygraph (Nihon Kohden, Japan), for 3-4 h in the unrestrained animal. During the next 5-7 days, animals of the experimental group were given daily intraperitoneal injections of metrazol (10%, in ampules, USSR origin) in a dose of 10 mg/kg. Animals of the control group received injections of physiological saline. EA was recorded for 30-60 min before and 1-2 h after each injection of metrazol. The rats were kept in individual cages with free access to food and water and with natural alternation of daylight and darkness. At the end of the experiments, the location of the electrodes was verified histologically.

EXPERIMENTAL RESULTS

Injection of subconvulsant doses of metrazol into the rat caused characteristic behavioral changes which varied in severity. Starting with 5-20 min after each injection of the drug, periods of marked restlessness were observed for 2 h in the animals, in the form of increased alertness relative to the surrounding situation, and digging into the sawdust, accompanied by virtually continuous contractions of the muscles of mastication ("gnashing the teeth"). These periods of enhanced restlessness, which can be interpreted as periods of anxiety states, varied in duration from 1 to 45 min. They alternated with periods during which the locomotor activity of the animals ceased and the signs of restlessness mentioned above disappeared. These states of the animal could be defined as inactivation states. Sometimes the experimental animals gave inappropriate generalized responses to hitherto inert external stimuli relating to placement in the experimental chamber, fixation of the recording electrodes, the experimenter's voice, and so on, in the form of sudden sideways "leaps," sometimes accompanied by vocalization, or in the form

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0007-4888/91/0002-0135$12.50

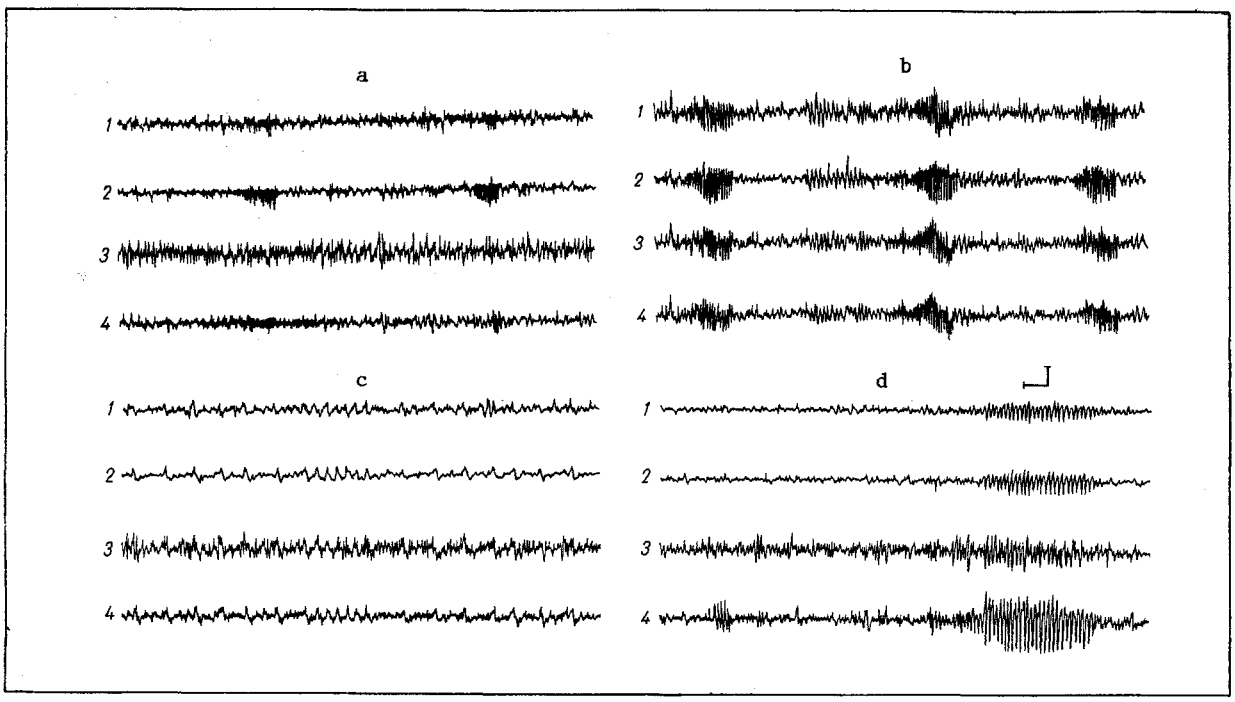

Fig. 1. Electrical activity in deep structures of the rat brain before and after systemic administration of metrazol (10 mg/kg). Before injection of metrazol: a) bursts of EA (10-11 Hz) accompanied by contractions of the muscles of mastication; after injection of metrazol: b) type I, bursts of EA (10-11 Hz) in the presence of signs of anxiety; c) type II, wavelike EA in the presence of signs of fear; d) type III, spindle-shaped EA (6 Hz), with disappearance of signs of anxiety and fear and absence of motor activity (inactivation state). 1) Amygdala, basomedial nucleus; 2) locus coeruleus; 3) dorsal part of hippocampus; 9) rostral part of striatum. Calibration: 200 μV, 1 sec.

of stepping backward, pressing the trunk against the floor, pressing back the ears (a passive defensive posture), followed by "freezing" in a tense posture, with increased muscle tone, exophthalmos, periodic vocalization, and sometimes these cases as a rule the rhythm of respiration was irregular, the frequency of the respiratory excursions was reduced to 40-60/min, mixed with brief (2-12 sec) or long (1-3 min, occasionally up to 45 min) periods of quickening of respiration to 90-150/min, with a corresponding increase or decrease in the amplitude of the respiratory excursions. These states could be assessed as states of pathologically enhanced fear, and they could last for a few minutes to 2 h (virtually throughout the period of observation after injection of metrazol).

The disturbances of the animals' behavior described above were observed as early as after the first injection of metrazol and they became more stable and more marked in character after subsequent injections of the drug. The intensity and duration of the anxiety states and of pathologically enhanced fear were greatest during 1-2 h after each successive injection of metrazol.

The periods of anxiety states were accompanied by bursts of EA, which were recorded in all the structures tested, with a frequency of 10-11 Hz, an amplitude of 200-500 μV, a duration of 1.5-4 sec, and a frequency of occurrence of 7-10 times per minute (Fig. 1b). The bursts were greater in amplitude and duration in LC and they occurred in it 0.5-1 sec earlier than in the other structures tested. We described this type of activity as type I EA.

Periods of pathologically enhanced fear were accompanied by a regular wavelike activity with a frequency of 0.7-1 Hz, alternating with complexes of wavelike EA with a frequency of 1.5-2.5 Hz and a duration of 2-12 sec (type II EA, Fig. 1c); periods (up to 45 min) of continuous wavelike EA with a frequency of 1.5-2 Hz also were observed. The frequency of occurrence of the waves corresponded to the animal's respiratory rhythm.

Inactivation states of the animals were accompanied by spindle-shaped EA with a frequency of 6 Hz, an amplitude of 600-800 μV, and a spindle duration of 3-6 sec (type III EA, Fig. 1d). This type of EA appeared in the rostral part of STR, and often was followed by generalization of this type of EA; the amplitude and duration of the spindles, however, remained maximal in STR.

Alternation of the anxiety state with outward tranquility was accompanied by a switch from type I to type II EA. The action of an external stimulus (if the experimenter's hands came close, a sudden sound, vocalization by another rat, and so on) led to restoration of the anxiety state in the rats with corresponding dominance of type I EA. The inactivation state in an animal, combined with an increased respiration rate, and characterized by the presence of EA of types II and III, was replaced under the influence of the external stimulus by pathologically enhanced fear, which corresponded on the EEG to the disappearance of type III EA and an increase in the amplitude of the type II EA.

Incidentally, in some animals, both of the experimental and of the control groups, the changes in behavior and EA described above could be observed in a mild form in the brain structures even before injection of the drug. Short periods of contractions of the muscles of mastication ("gnashing the teeth") and also transient episodes of "freezing" were usually induced by the action of an external stimulus. Periods of contraction of the muscles of .mastication corresponded to activity resembling type I EA, but the duration of the bursts in this case did not exceed 3 sec, and the frequency of their appearance was not more than 6/min. The bursts were most marked in LC (Fig. 1a). Transient episodes of "freezing" of the animals were accompanied by activity resembling type II EA, but the amplitude of this activity did not exceed 200 μV, and the periods of its occurrence did not exceed 1-2 min. These data indicate that the animals' emotional status differed initially.

Injection of physiological saline did not induce any marked changes in EA and behavior in animals of the control group.

Injection of subconvulsant doses of metrazol thus induced a complex series of changes in the rats' behavior, in which three main syndromes could be distinguished: anxiety states, pathologically enhanced fear, and an inactivation state. During the appearance of each of these syndromes, its characteristic pattern of brain EA was recorded.

The pathogenesis of anxiety and panic states in man, and also of increased alertness and disturbances of behavior of the anxiety and fear type in animals are connected with hyperactivity of the noradrenergic system of the brain, caused by enhanced neuronal activity of LC, the main source of noradrenergic innervation in the brain [9, 15]. Moreover, physiological tremor with a frequency of 8-12 Hz, arising in persons in a state of fear [12], also is connected with activation of the of the noradrenergic system. According to the results of the present investigation the amplitude and duration of generalized type I EA in animals with an anxiety state, accompanied by contractions of the muscles of mastication, were maximal in the region of LC. Additionally, the appearance of type I EA in LC was earlier than in other structures. It can be postulated that injection of subconvulsant doses of metrazol leads to hyperactivation of LC, which becomes the primary determinant, inducing the formation of a PS, manifested as anxiety. Meanwhile the possibility likewise cannot be ruled out that LC is part of the PS which is induced by another determinant, developing, for example, in one of the limbic structures of the brain.

In man, when intense fear gives way to torpidity, slow waves are observed on the EEG with a frequency of 2-3 Hz, and their appearance reflects the influence of the bulbar synchronizing system [8]. It has also been shown that the spread of ascending tonic influences of the respiratory center at the cortical and subcortical levels may be observed during changes in the functional state of the brain [2]. This effect probably takes place in animals at a time of pathological enhancement of fear. On the basis of the results described above it cannot be concluded which structure performs the role of determinant and forms a PS, manifested as the development of a state of pathological enhancement of fear in animals.

An inactivation state in animals associated with the appearance of characteristic EA in STR and its spread to other structures, with preservation of its maximal amplitude and duration in STR can be regarded as the result of hyperactivation of the limiting mechanisms of STR, expressed as the caudate response of movement delay and the appearance of accompanying spindle waves [7]. It has been shown that signs of marked akinesia are connected with the onset of hyperactivation of the caudate nuclei [4]. We also know that caudate stimulation can block phobic disorders in man for a long time and can induce inactivation states in animals [1, 7]. In our experiments the appearance of characteristic EA in STR was accompanied by quietening of the animal. It can be tentatively suggested that the hyperactive structures of STR become a determinant system, suppressing manifestations of alarm and phobic disorders in animals.

Thus injection of subconvulsant doses of metrazol into rats causes CNS changes realized as the appearance of various syndromes with accompanying PS [3] and involvement of GP, STR, the respiratory center and also, perhaps, other deep brain structures in the process. The antagonistic character of interaction of the systems of anxiety and inactivation states, determinants of which are evidently corresponding hyperactive structures of GP and STR, which we found is particularly interesting.

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EFFECT OF MICROINJECTION OF MORPHINE AND TRAMADOL INTO THE LOCUS COERULEUS ON NOCICEPTIVE RESPONSES OF SPINAL NEURONS AND ARTERIAL PRESSURE CHANGES

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UDC 612.884:612.831+612.143].014.46:[615.212.7:547.943].032.818.5

KEY WORDS: nociceptive stimulation; spinal neurons; locus coeruleus; arterial pressure; opiate analgesics.

It has been shown in recent years that analgesia arising after stimulation of the nuclei raphe, the periaqueductal gray matter, nucleus of the tractus solitarius, and certain other brain zones is accompanied by hypertension, tachycardia, and changes in the regional blood flow [6, 8, 10, 12]. It is considered that antinociceptive structures are involved in the regulation not only of pain, but also of the responses of the cardiovascular system, and that they may be the substrate for realization of both the pain-relieving and the hemodynamic action of narcotic analgesics. A special place in the integration of functional processes of different modalities during pain is played by the locus coeruleus in the medulla, which contains opiate peptides as well as noradrenalin, and which has multiple anatomical connections with antinociceptive and vasomotor structures of the brain, and also with the spinal cord [13]. However, changes in pain after injection of opiate analgesics into the locus coeruleus, their neurophysiological and neurochemical mechanisms, and their association with changes in nociceptive responses of the arterial blood pressure (BP) still remain completely unstudied.

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Department of Pharmacology, Academician I. P. Pavlov First Leningrad Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.*) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 2, pp. 126-128, February, 1991. Original article submitted June 22, 1990.

 0007-4888/91/0002-0138$12.50