ability to increase the water consumption of hitherto intact rats, probably lies at the basis of the reversal of the alcohol motivation observed in the third week of its administration.

During repeated exposure to adversive influences, phenazepam can thus depress the craving for ethanol formed by prolonged alcohols consumption. The mechanism of this effect is connected with changes in the activity of motivation-adaptation centers of the hypothalamus. This property evidently also explains the great efficacy of phenazepam when used clinically for maintenance therapy under conditions creating the threat of recurrence of alcoholism.

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EFFECT OF GABA-ERGIC AGENTS ON THE ANALGESIC EFFECT OF MORPHINE IN RATS

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In order to detect possible interaction between GABA and opiates, the effects of GABA-ergic drugs on analgesia induced by morphine were studied. The vocalization response to electrical stimulation of the tail in rats was used as an index of the action of morphine. Thiosemicarbazide, an inhibitor of glutamate decarboxylase, and bicuculline, which blocks GABA-ergic receptors, drugs which, it is suggested, can be considered as a group of GABA-negative compounds, weaken and shorten the effect of morphine. Depakine, an inhibitor of α-ketoglutarate-GABA-transaminase, like GABA itself, given in large doses (GABA-positive effects) strengthens morphine analgesia and prolongs its effect. The possible causes of these relations between GABA and opiates are discussed.

KEY WORDS: GABA; morphine; cyclic nucleotides; opiate receptors; bicuculline; thiosemicarbazide; analgesia.

Research workers studying the mechanism of action of analgesics have recently turned their attention to the possible participation of GABA-ergic mechanisms in the realization of their effect. Information on this problem in the literature is contradictory. Besides reports that the GABA-mimetic muscimol can potentiate the analgesic effect of morphine [3], there is also evidence that muscimol has no such effect [7]. According to some observations, a substance causing accumulation of GABA in brain tissue, namely aminohydroxyacetic acid (AHAA), weakens the analgesic effect of morphine [10] and the stimulation of motor activity induced by it [6]; meanwhile, according to another report, AHAA can potentiate morphine analgesia [11]. Experiments to determine the GABA concentration in brain tissue have shed no light on this problem. According to some workers [14], morphine has no effect on this index; others [12], however, found that morphine causes GABA to accumulate in structures specifically connected with the conduction of nociceptive impulses at the level of the spinal cord and thalamus.

In the light of these contradictions there is an obvious need to use methods of pharmacological analysis in order to resolve this problem of the possible role of GABA in the mechanism of action of analgesics. The object of the present investigation was to study the in-

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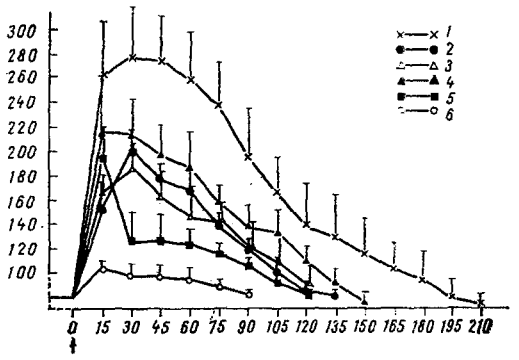

Fig. 1. Changes in threshold of nociceptive response in rats under the influence of morphine (2.5 mg/kg) in control (2) and in conjunction with GABA-ergic agents (1, 3-6). Abscissa, time (in min); ordinate, threshold of nociceptive response (in %; threshold of control response taken as 100%). 1) Depakine (300 mg/kg) + morphine; 2) morphine alone; 3) GABA (300 mg/kg) + morphine; 4) GABA (1000 mg/kg) + morphine; 5) bicuculline (2 mg/kg) + morphine; 6) thiosemicarbazide (5 mg/kg) + morphine. Arrow indicates time of injection of morphine.

fluence of substances acting on different stages of GABA-ergic transmission on the analgesic effect of morphine.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 150-180 g; the test for assessing the analgesic effect of morphine (2.5 mg/kg intravenously) was the vocalization response to electrical stimulation of the skin of the tail. The threshold of this response was determined in control experiments, and again every 15 min until the initial response was restored. Drugs with a specific effect on GABA-ergic processes were conventionally divided into two groups: GABA-negative and GABA-positive. The first group included substances delaying GABA formation from glutamate by inhibiting glutamate decarboxylase activity (in this investigation thiosemicarbazide was used), and also the sustance bicuculline, a specific blocker of GABA-ergic receptors. The GABA-positive substances included compounds with the effect of GABA-mimetics (muscimol, lipophilic derivatives of GABA) or causing accumulation of GABA in brain tissue by reducing the activity of α-ketoglutarate-GABA-transferase (GABA-T). One of the most active GABA-T inhibitors, ABAA, which was used in a preliminary series of experiments, has not since been studied: administration of morphine after AHAA led to death of the majority of the animals, possibly because of potentiation of the depriming effect of morphine on respiration. Instead, n-dipropyl acetate (depakine), a fairly active GABA-T inhibitor without some of the defects of AHAA, was therefore used. Depakine was injected intraperitoneally in a dose of 300 mg/kg; since maximal accumulation of GABA occurs 20 min after administration of depakine, this substance was injected simultaneously with morphine.

A separate series of experiments was carried out with GABA itself, injected intraperitoneally in doses of 300 and 1000 mg/kg. Despite the low permeability of the blood–brain barrier for GABA, in high doses it nevertheless exhibits neurotropic activity [2]. The impermeability of the blood–brain barrier for GABA is evidently only relative. Administration of GABA in large doses can itself be regarded as a GABA-positive procedure. Thiosemicarbazide and bicuculline were injected subcutaneously in doses of 5 and 2 mg/kg respectively, which have been shown to have a clear GABA-negative effect [1]. Since the maximal effect of morphine occurs 20-30 min after intravenous injection, and the maximal effects of thiosemicarbazide and bicuculline occur about 70 and 15-20 min respectively after administration, the thiosemicarbazide was given 40 min before morphine and bicuculline at the same time as morphine.

EXPERIMENTAL RESULTS AND DISCUSSION

In a dose of 2.5 mg/kg morphine raises the threshold of pain sensation to reach a maximum (on average 220% compared of the initial level, taken as 100%) between 15 and 30 min after injection, with a return to the original threshold after 120-135 min. The GABA-ergic substances studied had a definite influence on the effect of morphine; the GABA-negative and GABA-positive substances changing it in opposite directions. On the one hand bicuculline and, still more, thiosemicarbazide weakened the effect of morphine, reducing the peak of its analgesic action and shortening its duration (Fig. 1: 5, 6). On the other hand, depakine sharply increased the intensity of the maximal effect of morphine and prolonged it (Fig. 1: 1). GABA, injected in a relatively small dose (300 mg/kg) caused no significant change in the effect of morphine, whereas in a dose of 1000 mg/kg it caused the effect of morphine to develop earlier and to last longer (Fig. 1: 3, 4).

The results thus indicate that all GABA-negative agents tested weakened the analgesic effect of morphine, whereas GABA-positive agents strengthened it. The character of these experiments does not allow any conclusion to be drawn regarding the mechanism of these relationships, but analysis of the data, combined with facts described in the literature, do permit certain suggestions to be put forward. In experiments with iontophoretic application of naloxone to neurons, this drug was found to antagonize the inhibitory effect of GABA selectively, i.e., it had a bicuculline-like action. This suggested that naloxone can block GABA-ergic receptors [8]. If it is recalled that naloxone is an antagonist of morphine and endogenous opiates, the possibility cannot be ruled out that there is some similarity between the hypothetical GABA-ergic receptors and opiate receptors, and consequently, that GABA-ergic agents are mutually complementary to opiate receptors. The ability of GABA-positive compounds to potentiate, and of GABA-negative to weaken the effect of morphine, described above, may be explained not only by the similarity of the receptor mechanisms, but also by the uniformity of the secondary changes in the biochemical properties of the neurons produced by GABA and morphine and, in particular, changes in the system of cyclic guanosine-3,5-monophosphate (GMP), a "second transmitter." There is evidence [13] of negative feedback between GABA and cyclic GMP. It has been shown that substances causing accumulation of GABA in brain tissue reduced the cyclic GMP concentration in it. Benzodiazepines [5], whose GABA-positive action can now be regarded as sufficiently firmly established [5, 9, 16], have the same property. On the other hand isoniazid, which lowers the GABA concentration, and also picrotoxin, which blocks GABA-receptors, increase the cyclic GMP concentration [5]. It has also been shown [4] that morphine reduces the cyclic GMP concentration in brain tissue.

A shift in the ratio between cyclic AMP and cyclic GMP, causing changes in the activity of the corresponding protein kinases and, consequently, in the rate of phosphorylation or dephosphorylation of the corresponding proteins, can change membrane resistance. The fact that activation of GABA-receptors, as well as of opiate receptors, is accompanied by membrane hyperpolarization may be based on the changes in the cyclic nucleotide system described above, and may be the cause of the potentiating effect of GABA on morphine. Although the functional role of these changes in the realization of the effects of analgesics has not yet been explained, the decrease in the cyclic GMP concentration may perhaps reflect processes on which morphine acts in the same way as GABA-positive and in the opposite way to GABA-negative substances.

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EFFECT OF PSYCHOTROPIC DRUGS ON BEHAVIOR OF INBRED MICE UNDER EMOTIONAL STRESS

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The behavior of C57BL/6, CBA, and BALB/c mice in an "open field" test was studied after administration of phenazepam in doses of 0.05, 0.075, and 0.1 mg/kg and of sydnocarb in doses of 6, 12, and 24 mg/kg. The initial response to emotional stress was characterized by greatest motor activity (MA) in C57BL/6 mice and minimal in BALB/c mice. Phenazepam lowered MA in C57BL/6 mice proportionally to the dose. A biphasic effect of the tranquilizer was found in BALB/c mice. Depending on the dose, sydnocarb stimulated MA of C57BL/6 mice, did not effect the behavior of CBA mice, and in a dose of 24 mg/kg, it increased MA of BALB/c mice.

KEY WORDS: emotional stress; psychotropic agents.

Clinical and experimental investigations have shown that responses to psychotropic drugs reflect individual differences [1, 6].

The object of this investigation was to study genetically determined differences in the action of new Soviet preparations phenazepam and sydnocarb on the behavior of mice under emotional stress.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/6 (B6), CBA, and BALB/c (C) mice weighing 18-20 g (from the Stolbovaya nursery, Academy of Medical Sciences of the USSR), which were kept on a standard diet and with 12-hour periods of daylight for 21 days before the experiments, in cages accommodating three mice.

A stressor situation was simulated in an "open field" test by switching on four 60-W lamps creating an intensity of illumination of 1500 lx on the cage floor.

The preparations were injected intraperitonally in a suspension of Tween-80 with water 30 min before the beginning of the experiments: phenazepam was given in doses of 0.05, 0.075, and 0.1 mg/kg and sydnocarb in doses of 6, 12, and 24 mg/kg. The corresponding volume of a suspension of Tween-80 in water were injected into the control animals.

The behavior of the mice in an open field was observed for 3 min. Total motor activity (MA) was recorded (as the number of crossings of sectors over the whole area, the number of times the animals stood on their hind limbs), the horizontal MA (the total number of sector crossings), the peripheral MA (the number of peripheral sectors crossed), and the central MA (the number of sectors crossed in the center) were recorded. Each animal was used once only in the experiments. Student's method for dependent samples was used for the statistical analysis.

EXPERIMENTAL RESULTS AND DISCUSSION

The study of the open field behavior of inbred mice showed that their response to emotional stress is genetically dependent. B6 mice showed the highest activity for all indices

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