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EFFECT OF PHENAZEPAM ON ETHANOL INTAKE IN RATS

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The new Soviet tranquilizer phenazepam, if given by daily intraperitoneal injection to rats in a dose of 1 mg/kg for 3 weeks, can depress the craving for ethanol developed beforehand by administration of a 5% solution of alcohol for 2 months as the only source of fluid. The mechanism of this effect is probably connected with changes in the activity of the hypothalamic neurosecretory centers observed under these conditions. The property thus revealed evidently also explains the efficacy of phenazepam in the treatment of patients with chronic alcoholism.

KEY WORDS: tranquilizers; treatment of alcoholism; hypothalamic neurosecretion.

Phenazepam (7-bromo-5-o-chlorophenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one) is the first Soviet tranquilizer of the benzodiazepine series, synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR, and the Physicochemical Institute, Academy of Sciences of the Ukrainian SSR. Clinical trials have demonstrated its great efficacy in the treatment of patients with chronic alcoholism [2]. However, the mechanism of its antialcoholic action has not yet been established, and the investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male albino rats weighing initially 140-160 g, in which alcohol dependence was formed by the method described previously [1]. For 3 weeks one group of these animals received phenazepam in a near-therapeutic dose (1 mg/kg). The drug was given by intraperitoneal injection, as a suspension with Tween-80, 30 min before the animal was placed in an individual cage. The rats of the other group served as the control and received injections of water (with Tween-80) under similar experimental conditions. Observations on these animals continued for 3 weeks, during which they were allowed free choice of fluid for drinking. The effects of phenazepam on the water intake of previously intact rats was studied at the same time. The experimental conditions, the time of the last observations, and the control corresponded to those for the animals receiving ethanol. To study the mechanism of action of phenazepam under conditions of alcohol dependence, parallel with observations on the ethanol and water intake of the animals of the above groups, the state of the hypothalamic-hypophyseal neurosecretory system (HHNS), which plays the leading role in the formation of adaptive reactions [4], was studied. For this purpose, the experimental, control, and intact animals were decapitated at times of the experiments characterized by the greatest changes in the intake of the various fluids, i.e., 24 h before beginning or 1 and 21 days after the end of administration of the drug or water. The brain and pituitary were fixed in 96% ethanol or Bouin's fluid and embedded in paraffin wax. Sections of the hypothalamus and pituitary were stained with toluidine blude by Nissl's method and with paraldehyde-fuchsin by the Gomori—Gabe method, with counterstaining with Halmi's mixture. The morphological and

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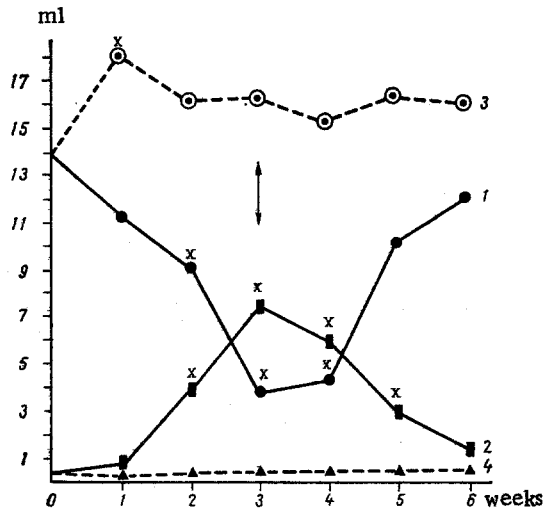

Fig. 1. Effect of phenazepam (1 mg/kg daily for 3 weeks) on ethanol and water intake by rats with preformed preference for ethanol. Abscissa, time of observation (in weeks); ordinate, volume of fluid per animal (in ml). Experiment: 1) ethanol consumption; 2) water consumption. Control: 3) ethanol consumption, 4) water consumption. x) Value differs significantly from initial ($P < 0.05$). Arrow marks time of discontinuing phenazepam.

functional state of the supraoptic (SON) and paraventricular (PVN) hypothalamic nuclei was assessed by counting the relative numbers of different types of neurosecretory neurons [3]. The content of neurohormones in the median eminence (ME) and the principal posterior lobe (PPL) of the neurohypophysis was assessed visually by a 5-point system [3].

EXPERIMENTAL RESULTS

Prolonged forced consumption of ethanol by the rats was found to lead to the development of a marked and lasting preference for the narcotic in some of them. A course of phenazepam in a daily dose of 1 mg/kg significantly altered the character of the motivation formed under these conditions. As Fig. 1 shows, starting from the second week of administration of diazepam the ethanol consumption fell progressively and the water consumption rose correspondingly. In the third week of the experiment the previous motivation was reversed and water was selectively preferred. This new motivation likewise was stable, for it continued for a week after discontinuation of the diazepam. Preference for ethanol then gradually returned, indicating that the observed effect depended on the action of phenazepam. This was also confirmed by the results of the control experiments which showed that injections of water to animals preferring ethanol increased the consumption of the narcotic, although this continued only (statistically significant changes) for 1 week of observation.

When the effect of phenazepam on the water intake of rats not receiving ethanol was studied, a moderate and reversible increase in its consumption was recorded only in the third week of administration of phenazepam. The water intake of the animals receiving injections of water under identical conditions was unchanged throughout the period of observation.

Significant changes also occurred under these experimental conditions in the morphological and functional state of the HHNS of the experimental animals. Compared with intact rats, the number of actively functioning type Ia neurons was sharply reduced in SON and PVN ($P \leq 0.001$), so that the predominant cells in their morphological picture became inactive neurons of types Ic and II, some of which, as a result of rapid degenerative changes, were converted into pycnomorphic type III cells. Inhibition of synthesis of neurohormones was combined with delay in their transport along the hypothalamo-hypophyseal tract (HHT), as shown by the appearance of numerous axons of neurosecretory cells forming large and giant expansions, tightly

packed with homogeneous masses of neurosecretion, in the morphological picture of the hypothalamus of these animals. Meanwhile evidence of blockade of liberation of neurohormones from ME and PPL of the neurohypophysis were observed, namely accumulation of large quantities of neurosecretion in the HHT endings located here, in the form of large Herring's bodies, and also reduction of the capillary network in these parts of the brain.

The study of the brain of rats receiving phenazepam for 3 weeks after ethanol for 2 months showed that the state of the HHNS of these animals was practically indistinguishable from that of the rats studied before taking phenazepam. A 3-week course of injections of water likewise had no significant effect on most of the indices of the HHNS tested in rats preferring ethanol. The characteristics of HHNS remained the same also in the animals of this series of experiments which were tested 3 weeks after discontinuation of phenazepam and water.

However, the study of the effect of the above factors on the HHNS of previously intact animals showed a number of fundamental differences. Compared with the latter, a marked decrease in neurohormone synthesis was observed in SON and PVN of the rats tested after a 3-week course of phenazepam, as shown by the predominance of inactive neurons of types Ib, Ic, and II in their morphological picture. Meanwhile, delay in transport and a decrease in liberation of neurohormones from ME and PPL of the neurohypophysis were observed. Changes in HHNS found in rats tested after the end of a 3-week course of water injections were directly opposite in character. Highly active neurons of type Ia, the psychological characteristics of which (vacuolation of the cytoplasm, absence of visible granules of neurosecretion in the cytoplasm, a large nucleus, often with a double nucleolus) reflects marked activation of neurohormone synthesis, predominated in SON and PVN of these animals. The virtual absence of axons of cells containing visible granules of neurosecretion on the territory of the hypothalamus and the sharp decrease in reserves of neurohormones deposited in ME and PPL of the neurohypophysis, combined with hyperemia of the vascular network of this region of the brain, indicate that besides an increase in the production of neurohormones, their transport along HHT was accelerated and they were liberated in large amounts into the blood stream.

Comparison of these results showing the effect of the experimental factors used on the attitude of the animals to ethanol with the characteristics of their HHNS reveals certain general rules. For instance, in the experiments in which phenazepam and water were given to animals with a preformed dependence on alcohol, despite marked and opposite changes in the quantity of ethanol consumed, the indices of the state of the HHNS were virtually indistinguishable from each other and, moreover, they agreed with those obtained in rats before the administration of diazepam water began. Completion of the opposite process, in the form of restoration of preference for ethanol after discontinuation of phenazepam, likewise was not reflected in the preformed characteristics of the HHNS. Meanwhile, in previously intact animals, obvious dissociation was observed between the decrease in activity of HHNS under the influence of phenazepam and its sharp increase after a course of water injections given under similar conditions.

The explanation of these outwardly contradictory facts must begin with a consideration of the role of HHNS and the central effector component triggering the neurohormonal mechanisms of formation of the integral response of the animal to stress [4]. According to these views, the marked activation of HHNS observed in hitherto intact rats under the influence of water injections must be regarded as a manifestation of emotional stress, developing during prolonged and regularly repeated nociceptive stimulation. From the same standpoint, the decrease in activity of HHNS observed under similar experimental conditions under the influence of phenazepam can be regarded as a manifestation of the antistressor effect of the drug. Since differences in the state of HHNS arising during separate administration of phenazepam and ethanol are only quantitative in character, it can tentatively be suggested that as regards the direction of their action of the trigger neurohormonal stage of the general mechanism of realization of the stress response these substances are agonists. Accordingly, the increased ethanol consumption of the animals with preformed dependence on alcohol, observed during the first week of water administration, can be regarded as a factor whose action prevents the development of a negative emotional-stress response during exposure to repeated adversive influences, as shown by maintenance of the indices of the state of HHNS recorded before the beginning and after the end of this exposure at a stable level. However, in its antistressor action, phenazepam evidently is much superior to ethanol, as shown by maintenance of the stable state of the HHNS of the rats receiving phenazepam despite the sharp reduction in the volume of ethanol consumed by them. This property of phenazepam, combined with its

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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