receptors. The action of MA, a representative of this group belonging to the β-carboline family, on evoked activity of hippocampal neurons was opposite to the effects of BD which, with respect to this model, have a potentiating effect on depression of TS, and suppress seizure activity [7]. The results suggest that under the influence of MA and GA, GABA-ergic reciprocal inhibition in hippocampal slices is suppressed. With respect to their ability to inhibit specific binding of labeled flunitrazepam, the compounds tested can be arranged in the following order: MA > GA > LA [6]. It follows from the results described above that these compounds are arranged in the same order with respect to their ability to induce seizure discharges and to suppress inhibition. Correlation is thus observed between the affinity of β-carboline derivatives for BDR and their electrophysiological activity. This similarity of the electrophysiological effects of MA and GA permits the suggestion to be made that GA also possesses anxiogenic activity in vivo; LA evidently has no anxiogenic properties.

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EFFECT OF 1,4-BENZODIAZEPINE TRANQUILIZERS ON BRAIN XANTHINE OXIDASE ACTIVITY IN RATS

R. N. Korotkina, A. A. Papin,
T. A. Voronina, and A. A. Karelin

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Much interest has been aroused in recent years by the study of the molecular mechanisms of action of tranquilizers of the 1,4-benzodiazepine series, which have found extensive applications in clinical practice. An important role in the realization of the effect of benzodiazepines is played by purinergic mechanisms [5]. For instance, competitive relations have been found between benzodiazepines and adenosine for receptor binding sites in synaptosomes [4] and adenosine uptake is inhibited by benzodiazepines [5].

Skolnick and co-workers postulated that "endogenous benzodiazepines" of purine nature, especially products of adenosine-5'-monophosphate and adenosine metabolism, namely inosinic acid, inosine, and hypoxanthine, may be formed in nerve cells; these products may evidently function as endogenous ligands of benzodiazepine receptors [6].

The present writers [1, 2] have shown that administration of tranquilizers of the 1,4-benzodiazepine series inhibits activity of membrane-bound 5'-nucleotidase in brain tissue. It was also found that 1,4-benzodiazepines induce activity of cytosol enzymes — AMP deaminase and adenosine deaminase — to a considerable degree, so that the concentrations of inosinic acid and inosine in the brain cells increase after administration of tranquilizers.

Laboratory of Biochemistry and Department of Anesthesiology, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR. Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow.

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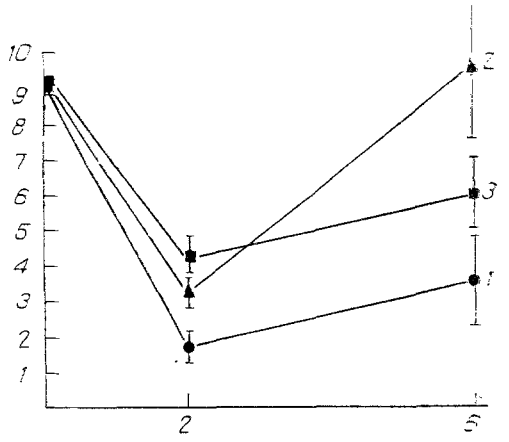

Fig. 1

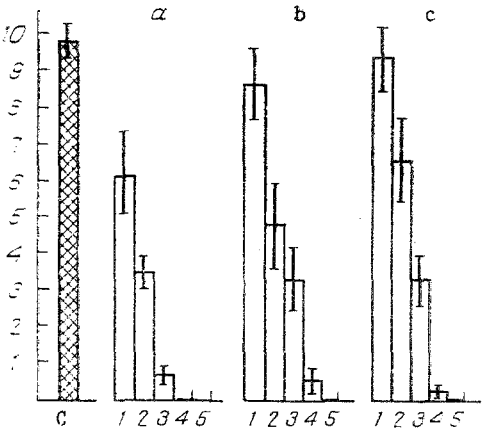

Fig. 2

Fig. 1. Xanthine oxidase activity in brain tissue of rats receiving benzodiazepines by intraperitoneal injection. Abscissa, time after injection, in h; ordinate (here and in Figs. 2 and 3), activity (in nmoles reduced cytochrome C/mg protein/min). Curves: 1) phenazepam, 2) nitrazepam, 3) diazepam.

Fig. 2. Effect of phenazepam (a), nitrazepam (b), and diazepam (c) in vitro on rat brain xanthine oxidase activity. C) Control, 1-5) 10^{-10}, 10^{-8}, 10^{-6}, 10^{-4}, and 10^{-3} M respectively.

In the investigation described below the effect of benzodiazepines were studied on activity of brain xanthine oxidase, an enzyme which oxidizes hypoxanthine with the formation of uric acid:

$$\text{hypoxanthine} + H_2O + 2O_2 \xrightarrow{\text{xanthine oxidase}} \text{uric acid} + H_2O.$$

EXPERIMENTAL METHOD

Experiments were carried out on 90 noninbred male albino rats weighing on average 200 g, into which one of the tranquilizers of the 1,4-benzodiazepine series, namely phenazepam, nitrazepam, or diazepam (USSR origin), was injected intraperitoneally in a dose of 5.0 mg/200 g body weight. For this purpose the above preparations in dry powder form were solubilized in 0.9% NaCl solution with the addition of the surfactant "Span-80" (Loba-Chemie, Vienna). The stable suspension contained 2.5 mg of the dry substance in 1 ml. Tests were carried out 2 and 6 h after injection of the tranquilizer. Animals receiving an injection of the same volume of 0.9% NaCl at the same times were used as the control.

Xanthine oxidase activity was determined in the supernatant of a 15% brain homogenate (centrifugation for 10 min in the cold at 3000g) by the method in [3].

Composition of the incubation samples: Na–phosphate buffer (pH 7.4) 0.1 M; cytochrome C 2.5×10^{-4} M, hypoxanthine 0.05 M, catalase 50 units/ml, bovine serum albumin 6.0 g/liter, $Na_2S_2O_4$ 1 mg, supernatant of 15% brain homogenate 0.04 ml, total volume of sample 1.55 ml.

In the experiments in vitro, brain supernatants were preincubated for 10 min at 37°C with shaking in a water bath in the presence of one of the tranquilizers (phenazepam, nitrazepam, or diazepam) in concentrations of 10^{-10}, 10^{-8}, 10^{-6}, 10^{-4}, or 10^{-3} M, after which enzyme activity in them was determined.

EXPERIMENTAL RESULTS

Data on the effect of benzodiazepines on brain xanthine oxidase activity under the experimental conditions in vivo are given in Fig. 1. It was found that 2 h after injection of phenazepam activity of the enzyme was reduced by 80.4% ($p < 0.01$), and 6 h after injection of the drug activity was still inhibited, although admittedly by a somewhat lesser degree (by 62.7%; $p < 0.02$).

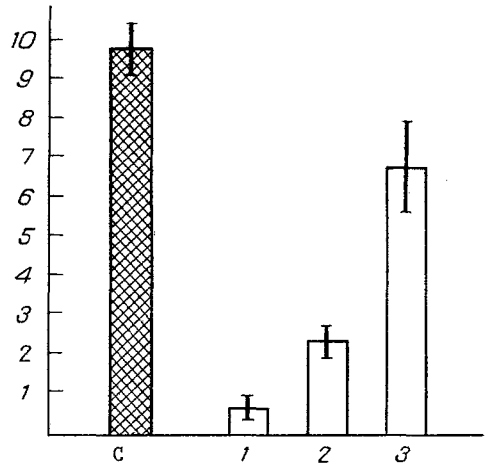

Fig. 3. Protective effect of hypoxanthine on inhibition of xanthine oxidase activity in the presence of phenazepam. C) Control, 1) phenazepam 10^{-6} M, 2) phenazepam 10^{-6} M + hypoxanthine 10^{-7} M, 3) phenazepam 10^{-6} M + hypoxanthine 10^{-6} M.

Injection of nitrazepam in vivo also inhibited xanthine oxidase activity: the reduction of activity after 2 h was by 64.3% ($p < 0.02$), but after 6 h, activity of the enzyme was back at the control level. A similar picture was observed with diazepam: after 2 h activity was inhibited by 55.8% ($p < 0.05$), but after 6 h xanthine oxidase activity was increased.

To determine whether the inhibition of xanthine oxidase activity observed was due to the direct action of these tranquilizers, or to a chain of neurochemical reactions induced by the injected tranquilizers, a series of experiments was carried out in vitro and their results are given in Fig. 2.

In a concentration as low as 10^{-10} M phenazepam was found to inhibit xanthine oxidase activity by 36.6% ($p < 0.05$), and with an increase in its concentration to 10^{-8} M activity of the enzyme was inhibited by 64.7% ($p < 0.001$); in a concentration of 10^{-6} M phenazepam inhibited activity of the enzyme almost completely (by 93.7%, $p < 0.001$), and with a further increase in concentration, enzyme activity fell to 0.

Nitrazepam in a concentration of 10^{-10} M had no effect on xanthine oxidase activity; an increase in its concentration to 10^{-8} M led to inhibition of enzyme activity by 51.5% ($p < 0.01$) and with a concentration of 10^{-6} and 10^{-4} M inhibition of activity reached 65.6% ($p < 0.05$) and 95.1% ($p < 0.02$) respectively. Complete inhibition of activity was observed by nitrazepam in a concentration of 10^{-3} M.

The effect of diazepam on xanthine oxidase activity was identical in character: with a concentration of 10^{-8} M enzyme activity was reduced by 33.2% ($p < 0.05$). In concentrations of 10^{-6} and 10^{-4} M diazepam inhibited activity by 67.6% ($p < 0.01$) and 99.0% ($p < 0.001$) respectively. As in the case of nitrazepam, complete inhibition of enzyme activity was observed with a concentration of 10^{-3} M.

The experiments thus showed that the action of the benzodiazepines tested on xanthine oxidase was uniformly inhibitory in character. The results of the experiments in vitro indicate a direct inhibitory effect of the tranquilizers on xanthine oxidase activity with the result that the hypoxanthine concentration in the brain cells rises.

It will be noted, however, that the degree of inhibition of xanthine oxidase activity by benzodiazepines differed in the case of different compounds. For instance, of all the drugs tested, phenazepam gave the strongest inhibitory effect: a decrease in enzyme activity was observed with a concentration as low as 10^{-10} M, at which neither nitrazepam nor diazepam had any effect on activity of the enzyme. If it is recalled that the tranquilizing and sedative activity of phenazepam is ten times stronger than that of the diazepam, the degree of inhibition of xanthine oxidase by benzodiazepines can be regarded as a criterion of the tranquilizing and sedative effect of these drugs.

To study the mechanism of the inhibitory effect of benzodiazepines on xanthine oxidase activity, a special series of experiments was carried out in vitro in which supernatants of brain homogenates were preincubated for 5 min in the presence of hypoxanthine in final concentrations of 10^{-7} and 10^{-6} M, after which phenazepam was added to all samples in a final concentration of 10^{-6} M and the samples were reincubated for 10 min at 37°C with shaking.

It will be clear from Fig. 3 that in the presence of hypoxanthine in a concentration of 10^{-7} M xanthine oxidase activity was appreciably inhibited by phenazepam (by 75.9%, $p < 0.001$) although, admittedly, by a rather lesser degree than when the samples were incubated in the presence of phenazepam alone in the same concentration (93.7%, $p < 0.001$). Increasing the hypoxanthine concentration in the sample to 10^{-6} M led to inhibition of enzyme activity in the presence of phenazepam by only 30.4% ($p < 0.05$).

Incubation of the supernatant of the brain homogenate (the source of xanthine oxidase) with the substrate of this enzyme, namely hypoxanthine, in a concentration of 10^{-6} M in fact restored the initial level of xanthine oxidase activity corresponding to the control.

This effective screening (protection) of the active sites of the xanthine oxidase molecule by the enzyme substrate (hypoxanthine) is evidence more in favor of direct competitive displacement of the inhibitor from its binding with the active site of the xanthine oxidase molecule or that a conformational change in the enzyme molecule itself is essential for the catalytic act.

The fact that xanthine oxidase is protected by its substrate (hypoxanthine) against the inhibitory effect of phenazepam indicates that phenazepam corresponds most closely in its structure (compared with nitrazepam and diazepam) to the active site of the xanthine oxidase molecule.

On this basis phenazepam can be regarded as a more or less selective inhibitor of rat brain xanthine oxidase.

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