CHANGES IN GABA TRANSAMINASE ACTIVITY IN RAT BRAIN STRUCTURES INDUCED BY BENZODIAZEPINE TRANQUILIZERS

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Tranquilizers of the benzodiazepine series are agonists of γ-aminobutyric acid (GABA) [6]. The writers showed previously that diazepam and nitrazepam inhibit GABA transaminase (GABA-T) in the hippocampus and cerebellar cortex to different degrees [3]. However, no correlation was found between these differences and their pharmacological activity.

In the investigation described below the effect of benzodiazepines (BD) on GABA-T activity was studied in a number of brain structures and compared with their psychotropic activity.

EXPERIMENTAL METHOD

Experiments were carried out on 84 male albino rats weighing 150-200 g. Phenazepam (0.1-5 mg/kg), medazepam (0.5-5 mg/kg), diazepam (0.5-10 mg/kg), and nitrazepam (0.5-5 mg/kg) were injected intraperitoneally in a 20% solution of propylene glycol. Animals of the control group received an injection of the solvent. The rats were decapitated 30 min later. GABA-T was detected histochemically by the method in [12] modified by the present writers on cryostat sections (10 μ) of the hippocampus, frontal cortex, cerebellar cortex, and liver [4]. After incubation the sections were examined photometrically by the plug method at a wavelength of 546 nm, using an optical probe 1 μ in diameter. Activity of GABA-T was assessed from the increase in optical density compared with sections incubated in the presence of 1.1 mM aminohydroxyacetic acid, an inhibitor of GABA-T, and expressed in optical density units (o.d.u.). For each dose of the drug 300 measurements at least were made. The results were subjected to simultaneous automatic processing on the "Élektronika" TZ-16M computer [5].

The anxiolytic effect was evaluated by the method described previously [10] in the present writers' modification [1]. The sedative effect of the drugs was determined in an "open field" test [9].

EXPERIMENTAL RESULTS

The mean GABA-T activity in the control animals was 0.171 ± 0.008 o.d.u. in the frontal cortex and 0.362 ± 0.015 o.d.u. in the cerebellar cortex. Phenazepam led to a significant decrease in GABA-T activity in the neurons of the various structures and, in particular, of the hippocampus (Fig. 1). In the cerebral and cerebellar cortex phenazepam, irrespective of its dose, depressed enzyme activity by not more than 30-32%. Medazepam had a weak effect, on the whole not significant, on GABA-T in the hippocampus and frontal cortex, and only in doses of 2.5 and 5 mg/kg did it give rise to some degree (not more than 19.5%) of inhibition of its activity in the cerebellar cortex (Fig. 1).

Diazepam significantly inhibited GABA-T in neurons of the front cortex only in doses of 2.5 and 5 mg/kg (Fig. 1b). Tha maximal degree of lowering of enzyme activity was 49.5%. The effect of diazepam in a dose of 10 mg/kg in the cerebellar cortex was indistinguishable from its action in a dose of 5 mg/kg. Diazepam in this dose did not significantly change

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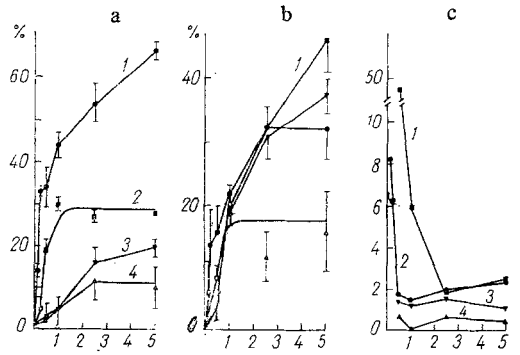

Fig. 1. Effect of BD on GABA-T activity. a and b: abscissa, dose of drugs (in mg/kg); ordinate, degree of inhibition of GABA-T activity (in %). In each group n = 4; filled symbols $P < 0.05$. a) Hippocampus and cerebellar cortex: 1 and 3; effects of phenazepam and medazepam respectively in hippocampus, 2 and 4) the same, in cerebellar cortex; b) frontal cortex: 1) diazepam, 2) nitrazepam, 3) phenazepam, 4) medazepam; c) selectivity of inhibition of GABA-T in hippocampus by BD: abscissa, dose of drugs (in mg/kg); ordinate, ratio of degrees of GABA-T inhibition in hippocampus and cerebellar cortex. 1) Diazepam; 2) phenazepam; 3) nitrazepam; 4) medazepam.

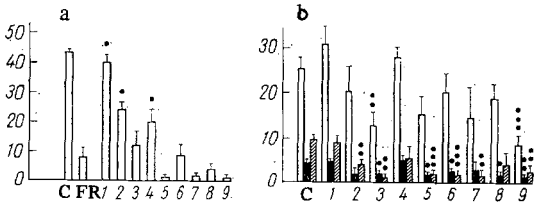

Fig. 2. Effect of BD on conditioned fear response (a) and behavior in open field test (b). Ordinate, number of motor acts. a) Number of squares crossed in 4 min (in 2 min in control). $P^* < 0.05$ compared with conditioned fear response. b: Unshaded columns — number of squares crossed; black columns — standing up on hind limbs, obliquely shaded columns — looking into holes in a period of 2 min. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; n = 5-6. C) Control; FR) fear response; 1, 2, 3) diazepam in doses of 2, 5, and 5 mg/kg respectively, 4 and 5) phenazepam 0.25 and 1 mg/kg; 6 and 7) nitrazepam 1 and 2.5 mg/kg; 8, 9) medazepam 1 and 2.5 mg/kg respectively.

GABA-T activity in the liver; in the control it was 0.175 ± 0.004 o.d.u., falling to 0.154 0.008 o.d.u. after administration of diazepam in a dose of 10 mg/kg (a decrease of 12%). The maximal degree of inhibition of GABA-T in the frontal cortex under the influence of nitrazepam was 37.3% (Fig. 1b).

Comparison of the effects of phenazepam and medazepam with those of diazepam and nitrazepam on GABA-T activity studied previously [3] showed that these drugs can be arranged in the following order of increasing strength of enzyme inhibition: phenazepam → diazepam → nitrazepam → medazepam. In addition, BD differed on GABA-T in the various brain structures tested. These differences were characteristic of phenazepam and diazepam in small doses and were exhibited particularly clearly when the degree of inhibition of GABA-T was compared in the hippocampus and cerebellar cortex (Fig. 1c). Clearly, by contrast with these drugs, selectivity of action on hippocampal GABA-T was not characteristic of nitrazepam and it was completely absent in the case of medazepam. Selectivity of inhibition of GABA-T correlated with their anxiolytic action. Diazepam, for instance, in a dose of 1 mg/kg and phenazepam in a dose of 0.25 mg/kg stimulated the motor activity of animals when depressed by fear (Fig. 2a). With an increase in the dose of diazepam to 2.5 and 5 mg/kg, and in the dose of phenazepam to 1 mg/kg the activating effect of the drugs was reduced. Investigations by the open field test also showed that diazepam in doses of 2.5 and 5 mg/kg and phenazepam in a dose of 1 mg/kg inhibited the animals' motor activity (Fig. 2b). Nitrazepam and medazepam, which did not cause selective inhibition of hippocampal GABA-T, did not prevent changes in behavior in the fear response (Fig. 2a). In a dose of 1 mg/kg they had little effect on the animals' motor activity, but in a dose of 2.5 mg/kg they depressed it (Fig. 2b). The weaker anxiolytic effect of these BD is confirmed by data in the literature [2].

The anxiolytic activity of the various BD studied in these experiments thus correlates with their ability to inhibit GABA-T, above all in the hippocampus. Differences in the effect of diazepam, nitrazepam, and medazepam on GABA-T also correspond to the degree of their affinity for benzodiazepine receptors [7]. The weak inhibition of enzyme activity in the liver tissue also indicates the importance of binding of BD with their own receptors in the realization of their inhibitory action on GABA-T. The maximal degree of inhibition of GABA-T in the hippocampus, frontal cortex, and cerebellar cortex corresponds to the distribution of one of the benzodiazepine receptor proteins (P_{55}) in these structures [11]. BD are probably weak inhibitors of GABA-T and the considerable inhibition of the enzyme in brain tissue is associated with selective accumulation of BD in the region of postsynaptic $GABA_2$-receptors, the regulatory units of which are benzodiazepine receptors [8]. $GABA_1$-receptors do not bind with the latter, and the GABA-T of $GABA_1$-receptor structures is inhibited only weakly by BD, and it is evidently this which explains the inability of BD, even in high doses, to cause complete inhibition of GABA-T.

The effect of tranquilizers of the BD series on GABA-T activity is thus an inseparable component of the neurochemical mechanism of realization of their anxiolytic effect.

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