EFFECT OF BENZODIAZEPINES IN 5'-NUCLEOTIDASE ACTIVITY OF RAT BRAIN

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Comprehensive clinical and experimental studies of the benzodiazepines suggest that a "GABA-benzodiazepine" receptor complex [2], including "GABA-modulin" [4, 7], participates in the mechanisms regulating GABA-ergic processes in the CNS. However, the molecular mechanisms of the receptor action of the benzodiazepines have not yet been finally elucidated, despite definite progress in this field [5, 12]. After the discovery and study of the mediator properties of ATP [1, 3] it became possible to study the role of purinergic mechanisms in the realization of the corresponding effects of benzodiazepines [6]. It has been found that adenosine A_2/R_a receptors, located on postsynaptic neuron membranes, are purine receptors which mediate inhibition of neurons [11].

To study the possible functional connection of these receptors with activity of the neuronal membrane marker enzyme [9, 10], in the investigation described below the effect of several benzodiazepines on 5'-nucleotidase activity was studied in the rat brain.

EXPERIMENTAL METHOD

Experiments were carried out on 330 noninbred male albino rats weighing on average 200 g. The animals were given one of the following tranquilizers of the benzodiazepine series intraperitoneally: phenazepam, diazepam, and Dormicum. Phenazepam was injected in doses of 2.5, 3.75, and 5 mg/200 g body weight. Crystalline phenazepam was suspended in physiological saline with the addition of the surfactant Span 80 (from Loba Chemie, Austria). The stable suspension contained 2.5 mg phenazepam in 1 ml. More concentrated (by 1.5 and 2 times) suspensions of phenazepam were prepared correspondingly. The experiments were carried out 1, 2, and 3 h after injection of the drug. Diazepam was injected in the form of its pharmacopoeal solution in ampuls containing 5 mg of the drug in 1 ml of solution (Seduxen, from Gedeon Richter, Hungary). Diazepam was injected in doses of 2, 3, and 4 mg/200 g body weight. Experiments were carried out 30 min and 1 and 2 h after injection of the drug. Dormicum (Hoffman–La Roche, Switzerland) was injected in the form of the pharmacopoeal solution in ampuls containing 5 mg of the active substance in 1 ml of solution. The dosage of the drug and times of its injection were the same as for diazepam. Animals receiving intraperitoneal injections of physiological saline in the corresponding volumes served as the control.

5'-Nucleotidase activity was determined by the method in [8] in a 10% rat brain homogenate in 0.001 M glycine buffer, pH 8.5. Activity of the enzyme was expressed in picomoles P_i/mg protein.

EXPERIMENTAL RESULTS

The 5'-nucleotidase activity of the control animals was 197.1 ± 3.61 pmole P_i/mg protein (Table 1). Injection of diazepam in a dose of 2 mg and phenazepam in a dose of 2.5 mg caused no significant changes in enzyme activity at any stage of the investigation. Dormicum in a dose of 2 mg depressed 5'-nucleotidase activity after 30 ming by 8.9% ($P < 0.05$) compared with the control. Changes in activity of the enzyme 1 and 2 h after injection of the initial dose of Dormicum were not significant.

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TABLE 1. Changes in 5'-Nucleotidase Activity (in pmoles P_i/sec/mg protein) in Rat Brain after Intraperitoneal Injection of Benzodiazepines (M ± m)

Benzodiazepine	Dose, mg/200 g body weight	Time after injection of drug			
		30 min	1 h	2 h	3 h
Diazepam	2	199,1±2,0 (6)	181,0±12,8 (18)	201,2±14,0 (6)	—
	3	199,8±13,2 (6)	156,1±11,2*(18)	170,0±10,0*(12)	—
	4	184,6±7,8 (6)	159,3±3,6* (12)	168,2±6,8 * (12)	—
Dormicum	2	179,6±6,3*(18)	214,6±7,6 (12)	190,8±6,8 (12)	—
	3	158,4±7,6*(12)	177,6±6,4* (12)	170,9±6,4* (12)	—
	4	179,4±4,0* (12)	164,4±10,8* (12)	161,6±9,2* (12)	—
Phenazepam	2,5	—	198,2±14,8 (12)	182,8±8,8 (12)	186,0±5,2 (12)
	3,75	—	182,1±6,4 (12)	165,8±2,0* (12)	207,6±4,8 (12)
	5	—	158,6±14,4* (12)	173,8±4,8* (12)	195,6±8,8 (12)

Legend. 5'-Nucleotidase activity in control 197.1 ± 3.6 pmoles P_i/sec/mg protein. Asterisk indicates statistically significant changes compared with control. Number of animals given in parentheses.

A 50% increase in the dose of diazepam reduced 5'-nucleotidase activity 1 h after injection of the drug by 20.8% ($P < 0.01$) and 2 h after injection by 13.8% ($P < 0.05$) compared with the control. In a dose of 3 mg, Dormicum reduced enzyme activity after 30 min by 19.6% ($P < 0.001$), and inhibition of enzyme activity after 1 and 2 h was somewhat weaker, by 10% ($P < 0.05$) and 13.4% ($P < 0.01$) respectively. An increase in the dose of phenazepam to 3.75 mg caused a decrease in enzyme activity by 15.8% 2 h after injection of the drug ($P < 0.001$).

Doubling the dose of all benzodiazepines used also was accompanied by inhibition of 5'-nucleotidase in rat brain homogenate. For instance, 1 h after injection of diazepam (4 mg) activity of the enzyme was reduced by 19.2% ($P < 0.001$), and after 2 h it was reduced by 14.7% ($P < 0.01$); when Dormicum was given in a dose of 4 mg the greatest decrease in enzyme activity was observed after 1 h (by 16.6%, $P < 0.02$) and after 2 h (by 18.8%, $P < 0.01$). If the dose of phenazepam was increaed to 5 mg, activity of the enzyme was reduced after 1 h by 19.5% ($P < 0.05$) and after 2 h by 11.8% ($P < 0.01$). Characteristically, the benzodiazepines studied had a consistently inhibitory action on 5'-nucleotidase when their dosage was increased by 50 and 100%. The most marked effect of inhibition of 5'-nucleotidase activity was observed 1 h after injection of 5 mg of phenazepam (by 19.5%, $P < 0.05$) and 3 mg of diazepam (by 20.8%, $P < 0.01$), and 30 min after injection of Dormicum in a dose of 3 mg (by 19.6%, $P < 0.001$). Consequently, with respect to strength of inhibition of the enzymic reaction of ATP hydrolysis in rat brain homogenate, all three benzodiazepines were about equal, but the manifestation of the effect and its duration differ somewhat. For example, in the case of Dormicum, its inhibitory action on the enzyme is already apparent after 30 min, whereas in the case of diazepam, the maximal effect of the drug is observed 1 h after injection into the animal. The duration of the action of phenazepam, depending on its dose, was 1-2 h, and 3 h after its injection, enzyme activity was restored virtually to the control level. This action of phenazepam can probably be explained by the effect not only of the drug itself, but also of its metabolites, which become inactive against 5'-nucleotidase after 3 h.

The decrease in activity of the enzyme catalyzing enzymic hydrolysis of AMP, discovered by these experiments, is evidence that benzodiazepines can interfere in adenosine metabolism.

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