MECHANISM OF THE ACTION OF DIAZEPAM ON THE BRAIN ACETYLCHOLINE LEVEL IN MICE

V. D. Tonkopii, G. A. Sofronov, I. E. Aleksandriiskaya, and L. M. Brestkina

UDC 615.214.22:547.891.2].015.4:612.822.1:577.175.822

Administration of diazepam (20 mg/kg) leads to an increase in the free and bound acetylcholine concentrations in the mouse brain. Preliminary administration of diazepam (20 mg/kg) potentiates the anticholinesterase action and toxicity of galanthamine. The toxicity of eserine, armin* and phosphacol† is unchanged under these conditions. It is suggested that diazepam blocks the liberation of acetylcholine from cholinergic nerve endings, which must lead to a decrease in the concentration of functional acetylcholine in the synaptic space.

KEY WORDS: diazepam; brain; acetylcholine; cholinesterase inhibitors.

The benzodiazepines, which are widely used in clinical practice as tranquilizers and anticonvulsants, are known to reduce the rate of acetylcholine turnover and to increase its concentration in the brain of animals [6, 8, 13].

This effect is linked with the blocking of acetylcholine liberation from presynaptic nerve endings by the diazepines, although this has not been demonstrated experimentally [8].

The object of the present investigation was the pharmacological analysis of the mechanism of the effect of diazepam on the acetylcholine concentration in the mouse brain.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 19-23 g. The concentrations of free and bound acetylcholine were determined by the method of Crossland and Slater [9] in the whole brain (without the cerebellum) of the animals 30 min after administration of diazepam in doses of 2.5 and 20 mg/kg. The toxicity of the cholinesterase inhibitors eserine, armin, and phosphacol was determined after preliminary administration of diazepam in a dose of 20 mg/kg 30 min before the inhibitors, and the toxicity of galanthamine was determined under the same experimental conditions after administration of diazepam in doses of 2.5, 5, 10, and 20 mg/kg. The values of LD_{50} were calculated by probit analysis of the mortality curves, using the method of least squares [3].

The effect of galanthamine (1 and 4 mg/kg), eserine (0.4 mg/kg), and armin (0.35 mg/kg) on the inhibition of acetylcholinesterase activity was investigated in the brain of intact mice and of mice receiving diazepam in doses of 2.5 and 20 mg/kg 30 min before administration of the inhibitors. The acetylcholinesterase activity was determined by Hestrin's method [11] 30 min after injection of cholinesterase inhibitors. Preliminary experiments showed that diazepam itself, in the doses used, did not affect acetylcholinesterase activity. The degree of inhibition of enzyme activity after administration of the reversible inhibitors – galanthamine and eserine – was determined by the method suggested by Tonkopii et al. [5]. All drugs were dissolved in water and injected intraperitoneally in a dose of 0.1 ml/10 g body weight. Tween-80 was added to the weighed samples of diazepam, and aqueous suspensions of the drug were then prepared.

EXPERIMENTAL RESULTS AND DISCUSSION

Administration of diazepam in a dose of 2.5 mg/kg did not change the acetylcholine concentration in the mouse brain. Increasing the dose of the drug to 20 mg/kg led to a significant ($P < 0.01$) increase in the levels

*The ethyl-p-nitrophenyl ester of ethylphosphinic acid.
†Synonymous with paraoxan.

S. M. Kirov Military Medical Academy, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. N. Golikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 7, pp. 38-40, July, 1978. Original article submitted December 21, 1977.

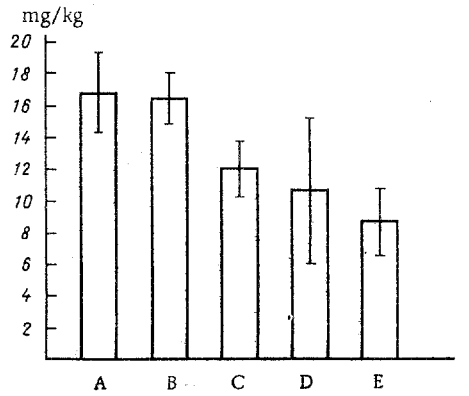

Fig. 1. Changes in LD_{50} of galanthamine after preliminary (30 min beforehand) administration of diazepam to mice. A) Control (without diazepam), B, C, D, E) after preliminary administration of diazepam in doses of 2.5, 5, 10, and 20 mg/kg respectively.

TABLE 1. Acetylcholine Concentration (in μg/g wet weight of tissue) in Mouse Brain after Administration of Diazepam (M ± m)

Dose of diazepam, mg/kg	Free acetylcholine	P	Bound acetylcholine	n	P
Control	2,10±0,02	—	2,41±0,04	15	—
2,5	2,07±0,02	>0,5	2,39±0,04	7	>0,5
20,0	2,77±0,04	<0,01	2,91±0,06	8	<0,01

TABLE 2. LD_{50} (in mg/kg) of Cholinesterase Inhibitors in Mice after Preliminary (30 min beforehand) Injection of Diazepam (20 mg/kg) (M ± m)

Cholinesterase inhibitor	Control	n	After preliminary diazepam	n	P
Galanthamine	16,66±1,22	42	7,89±0,65	36	<0 01
Eserine	1,03±0,05	30	0,905±0,013	42	>0,5
Phosphacol	2,05±0,18	36	2,04±0,08	36	>0,5
Armin	1,55±0,008	36	1,65±0,036	36	>0,5

of both free and bound acetylcholine (Table 1). The toxicity of eserine, phosphacol, and armin was unchanged in the animals receiving a preliminary injection of diazepam in a dose of 20 mg/kg, whereas the toxicity of galanthamine under these conditions was more than doubled (Table 2). The values of LD_{50} for galanthamine fell with an increase in the dose of diazepam (Fig. 1). In a dose of 2.5 mg/kg diazepam caused no changes in the acetylcholine concentration and did not affect the toxicity of galanthamine.

The degree of inhibition of acetylcholinesterase activity in the mouse brain after administration of armin and eserine was unchanged by preliminary administration of diazepam in doses of 2.5 and 20 mg/kg. Potentiation of the anticholinesterase action of galanthamine ($P < 0.01$) was found after preliminary administration of diazepam in a dose of 20 mg/kg (Table 3). Injection of diazepam into mice in doses causing changes in the brain acetylcholine level thus was shown to potentiate the acetylcholinesterase action and toxicity of galanthamine.

The increase in the acetylcholine concentration in the mouse brain after injection of diazepam may have been the result of a decrease in the intensity of liberation of the mediator from cholinergic nerve endings. In that case the concentration of functional acetylcholine in the synaptic space would have fallen significantly. The validity of this hypothesis is confirmed by the increase in anticholinesterase activity and toxicity of galanthamine under the influence of diazepam but the absence of this effect when the other cholinesterase inhibitors

TABLE 3. Inhibition of Acetylcholinesterase Activity (in %) in Mouse Brain 30 Min after Injection of Cholinesterase Inhibitors, Preceded by Administration of Diazepam (M ± m)

Cholinesterase inhibitor	Dose, mg/kg	Control	n	2.5 mg/kg diazepam	n	P	20 mg/kg diazepam	n	P
Galanthamine	4	70,6±1,27	10	71,5±1,41	6	>0,5	86,2±0,60	10	<0,01
Phosphacol	1	23,5±0,92	8	25,3±1,21	6	>0,5	42,3±0,41	8	<0,01
Armin	0,3	83,6±1,82	12	84,6±3,21	10	>0,5	80,0±1,85	10	>0,5
Eserine	0,4	79,9±3,43	6	76,1±2,71	8	>0,5	81,7±1,71	10	>0,5

were used. The anticholinesterase action of galanthamine, which is a competitive reversible inhibitor, is known to be extremely sensitive to changes in the acetylcholine concentration in the medium [4]. For instance, even a small decrease in the acetylcholine concentration leads to an increase in the degree of inhibition of acetylcholinesterase activity by the reversible inhibitor [1]. The anticholinesterase activity of the other cholinesterase inhibitors used, however, can be significantly cahnged only by high concentrations of acetylcholine [2], many times higher than the concentration of functional mediator in the synaptic space [10].

In connection with the use of reversible cholinesterase inhibitors and, in particular, of eserine as antidotes in the treatment of benzodiazepine poisoning [7, 12], the results of the present experiments are of practical importance. Great care must evidently be taken when galanthamine is used in combination with large doses of benzodiazepines.

LITERATURE CITED

1. E. T. Vasilenko and V. D. Tonkopii, Biokhimiya, No. 4, 701 (1974).
2. R. I. Volkova, Biokhimiya, No. 2, 282 (1965).
3. V. B. Prozorovskii, Farmakol. Toksikol., No. 1, 15 (1962).
4. V. D. Tonkopii and V. B. Prozorovskii, Byull. Éksp. Biol. Med., No. 7, 823 (1976).
5. V. D. Tonkopii, N. V. Savateev, A. P. Brestkin, et al., Dokl. Akad. Nauk SSSR, 273, 576 (1972).
6. C. Bianchi, L. Beani, and A. Bertellini, Neuropharmacology, 14, 327 (1975).
7. C. D. Blitt and W. C. Petty, Anesth. Analg. Curr. Res., 54, 607 (1975).
8. S. Consolo, H. Ladinsky, G. Peri, et al., Europ. J. Pharmacol., 27, 266 (1974).
9. J. Crossland and P. Slater, Brit. J. Pharmacol., 33, 42 (1968).
10. S. Ehrenpreis, Drugs Affecting the Peripheral Nervous System, New York (1967).
11. S. Hestrin, J. Biol. Chem., 181, 180 (1948).
12. B. D. Snyder et al., J. A. M. A., 230, 1433 (1974).
13. G. Zsilla, D. L. Chenney, and E. Costa, Arch. Pharmak. Exp. Path., 294, 251 (1976).