LITERATURE CITED

1. A. M. Zharkovskii, L. K. Ryago, and L. Kh. Allikmets, in: Development of Psychiatry and Psychopharmacology in Tartu University [in Russian], Tartu (1981), pp. 111-119.
2. G. I. Kovalev, "Neurochemical study of structural analogs and derivatives of gamma-aminobutyric acid," Candidate's Dissertation, Moscow (1980).
3. N. I. Maisov, M. D. Chipalivili, N. G. Aleksidze, et al., Byull. Éksp. Biol. Med., No. 5, 561 (1977).
4. L. K. Ryago, L. Kh. Allikmets, and A. M. Zharkovskii, in: Abstracts of Proceedings of the 4th All-Union Symposium on the Oriented Search for Physiologically Active Substances [in Russian], Riga (1981), p. 47.
5. R. A. Khaunina, Nov. Lekarst. Prep., No. 7, 2 (1978).
6. N. G. Bowery, A. Doble, D. R. Hill, et al., Eur. J. Pharmacol., 71, 53 (1981).
7. D. R. Hill and N. G. Bowery, Nature, 209, 149 (1981).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol., 193, 265 (1951).
9. J. Sawynok and F. S. Labella, Eur. J. Pharmacol., 70, 103 (1981).

PRESYNAPTIC COMPONENT IN THE MECHANISM OF ACTION OF FENIBUT

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Fenibut (β-phenyl GABA) has found clinical application as a tranquilizer and sedative [5]. Despite its structural similarity with GABA, fenibut evidently differs significantly from it in the mechanism of its action. Neurochemical studies of the action of fenibut on GABA metabolism in brain tissue have not revealed sufficiently clear changes. Fenibut is known to cause a moderate increase in the GABA content in the brain, to modify the activity of enzymes involved in GABA metabolism, namely glutamate decarboxylase and GABA transaminase [1, 2], but not to possess affinity for systems of reassimilation [3] and receptor binding of [^{3}H]-GABA [7]. Meanwhile evidence has been obtained that fenibut and baclofen (the chlorophenyl analog to fenibut) possess GABA-like activity [8, 12]. Considering the important role of mediator release as one of the neurochemical stages of synaptic transmission, it was decided to study the possible action of fenibut on this presynaptic mechanism of function of the GABA system.

EXPERIMENTAL METHOD

The method of superfusion [9] of the fraction of "coarse" synaptosomes isolated from rat brain was used to study the release of labeled [^{3}H]-GABA. Wistar rats weighing 180-200 g were decapitated and the cerebral cortex was quickly removed in the cold and homogenized in 0.32 M sucrose (in the ratio 1:10); the homogenate was certrifuged at 1000g for 10 min. The supernatant was again centrifuged at 20,000g for 25 min. The resulting residue of "coarse" synaptosomes (the P2 fraction) was suspended in 0.32 M sucrose (the protein concentration in the resulting suspension of synaptosomes was 7-10 mg/ml). After 30 min 50 µl of suspension was added to 2 ml of incubation medium containing 124 mM NaCl, 5 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM glucose, 20 mM NaH_2PO_4, 1.2 mM KH_2PO_4, and 0.2 mM aminohydroxyacetic acid, pH 7.35. After 2 min [^{3}H]-GABA was added up to a final concentration of 10^{-4} mM. The samples were incubated for 5 min at 37°C, after which protein was precipitated on a Whatman GF/C glass-fiber filter, fixed in a thermostatically controlled chamber. The synaptosomes were washed with 15 ml of incubation medium. The rate of superfusion during the experiments was 0.6 ml/min. Every minute samples of superfusion fluid were collected in flasks containing 10 ml of Bray's scintillation fluid. Radioactivity of the samples was measured with an Inter-

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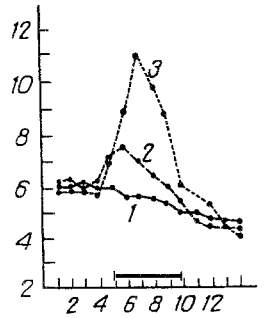

Fig. 1

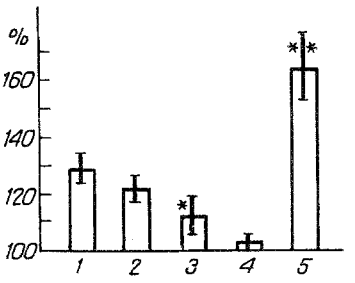

Fig. 2

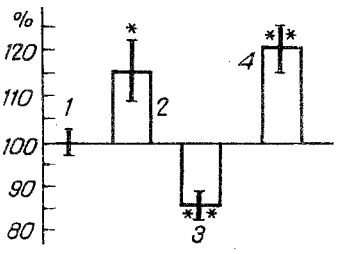

Fig. 3

Fig. 1. Effect of GABA and fenibut on spontaneous release of [^{3}H]-GABA from rat cerebral cortical synaptosomes: 1) spontaneous release; 2) release under the influence of 100 μM fenibut; 3) release under the influence of 100 μM GABA. Abscissa, fraction Nos.; ordinate, radioactivity in each fraction in % of total radioactivity in all fractions and residue on filter.

Fig. 2. Effect of bicuculline and picrotoxin on [^{3}H]-GABA release induced by fenibut: 1) fenibut (100 μM), 2) bicuculline (100 μM), 3) fenibut (100 μM) + bicuculline (100 μM), 4) picrotoxin (100 μM), 5) fenibut (100 μM) + picrotoxin (100 μM). Basal release of [^{3}H]-GABA from 5th through 10th minute taken as 100%; *$P < 0.01$; **$P < 0.05$ compared with 1.

Fig. 3. Effect of fenibut and GABA antagonists on K^+- stimulated [^{3}H]-GABA release. 1) K^+ (28 mM), 2) K^+ + fenibut (100 μM), 3) K^+ + fenibut (100 μM) + bicuculline (100 μM), 4) K^+ + fenibut (100 μM) + picrotoxin (100 μM). Quantity of [^{3}H]-GABA released through the action of 28 mM KCl taken as 100%. *$P < 0.1$, **$P < 0.05$ compared with 1.

technique (France) liquid scintillation counter. The amount of radioactivity released during superfusion with buffer solution not containing any of the test substances, during an interval of 5-10 min, was taken as 100%. The results of 4-6 experiments were subjected to statistical analysis with calculation of the means and their confidence intervals by Student's method.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of substances added to the superfusion medium on spontaneous release of labeled [^{3}H]-GABA was studied. Under these conditions fenibut caused a significant increase, depending on concentration, in the yield of radioactive label: up to 130.7 ± 5.9% in a concentration of 100 μM (Fig. 1) and 115.9 ± 4.3% in a concentration of 50 μM. The effect of addition of 100 μM GABA was manifested as a marked increase in the quantity of tritium label released from the synaptosomes (268.2 ± 20.0%), approximately five times greater than the effect of fenibut in an equimolar concentration. The effect observed can be attributed, at least in part, to the ability of GABA to stimulate the release of accumulated [^{3}H]-GABA through a homologous exchange mechanism [11]. To analyze the possible mechanism of this interaction the effect of bicuculline (BCC) and picrotoxin (PT), antagonists of GABA receptors, on the liberation process was studied. In a concentration 100 μM BCC showed a moderate stimulating action (123.6 ± 4.9%) on spontaneous release, comparable with the effect of fenibut (Fig. 2). PT did not change the basal level of GABA release (102.3 ± 2.3%).

Affinity for the binding site of the ligand, which is well marked in the case of BCC, but not affinity for the chlorine ionophore, with which PT interacts, evidently plays an important role in the mechanism of action of GABA-ergic drugs on spontaneous release of [^{3}H]-GABA. Simultaneous addition of fenibut and BCC to the superfusion medium led to some weakening of the effect of fenibut (to 113.0 ± 6.3%), whereas when fenibut was given together with PT, the effect was appreciably potentiated (to 1.65.2 ± 12.4%).

In a concentration of 50 μM fenibut did not change release of the label due to potassium (28 mM) depolarization, but in a concentration of 100 μM this effect was significantly potentiated (Fig. 3). It is interesting to note that antagonists of GABA receptors were similar

in their action on the effect of fenibut in relation to K^+-depolarization. PT did not alter the level of depolarization, whereas BCC reversed it.

The experiments thus showed that fenibut, a β-phenyl derivative of GABA, has a presynaptic GABA-ergic component in the spectrum of its action. Under these experimental conditions fenibut can increase both spontaneous and K^+-stimulated release of [^{3}H]-GABA from rat cerebral cortical synaptosomes. BCC and PT, antagonists of GABA receptors, have opposite actions on the effect of fenibut: the first reverses the action of the drug on spontaneous and K^+-stimulated release, whereas PT potentiates the effect of fenibut on spontaneous mediator release but does not change the effect of combined administration of fenibut and K^+.

It is interesting to compare the BCC-dependent effect of fenibut on spontaneous and K^+-stimulated release of [^{3}H]-GABA, revealed by these experiments, with the antagonistic action of BCC on the fenibut-induced increase in the GABA concentration in the rat corpus striatum [4]. In a study with (−)-baclofen, structurally similar to fenibut, the effect observed was a decrease in synaptosomal release of [^{3}H]-GABA, opposite to that observed in the present experiments and not abolished by BCC [6]. The existence of BCC-insensitive receptors for (−)-baclofen, postulated in [6], may be extended by the suggestion of a possible role of BCC-sensitive receptors, whose activation by fenibut potentiates the release of GABA, in the regulation of mediator release.

LITERATURE CITED

1. N. P. Bobrova, "Gamma-aminobutyric acid metabolism in the brain under the influence of some neurotropic drugs," Candidate's Dissertation, Minsk (1975).
2. G. I. Kovalev, "Neurosurgical study of structural analogs and derivatives of gamma-aminobutyric acid," Candidate's Dissertation, Moscow (1980).
3. I. S. Morozov, G. I. Kovalev, N. I. Maisov, et al., Khim.-farm. Zh., No. 1, 13 (1977).
4. L. K. Ryago and A. M. Zharkovskii, in: Mechanism of Action of Psychotropic Drugs [in Russian], Tartu (1982), p. 50.
5. R. A. Khaunina and I. P. Lapin, Khim.-farm. Zh., No. 12, 125 (1976).
6. N. G. Bowery, et al., Nature, 283, 92 (1980).
7. A. Galli et al., J. Neurochem., 32, 1123 (1979).
8. R. Kerwin and C. Pycock, J. Pharm. Pharmacol., 30, 662 (1978).
9. J. Levi and M. Raiteri, Eur. J. Pharmacol., 25, 411 (1974).
10. R. W. Olsen et al., in: Amino Acids as Chemical Transmitters, F. Fonnum, ed., New York (1978), p. 467.
11. M. Raiteri et al., J. Neurochem., 24, 1243 (1975).
12. P. Roberts et al., Brain Res., 155 209 (1978).