7. V. I. Metelitsa, The Cardiologist's Guide to Clinical Pharmacology [in Russian], Moscow (1980).
8. V. K. Piotrovskii, E. B. Smirnova, and O. S. Ryabokon', in: Abstracts of Proceedings of an All-Union Symposium on the Oriented Search for New Physiologically Active Substances [in Russian], Riga (1979), p. 33.
9. N. T. Pryanishnikova and N. A. Sharov, Trimecaine. Pharmacology and Clinical Use [in Russian], Leningrad (1967).
10. E. I. Chazov and O. M. Eliseev, Ter. Arkh., No. 1, 3 (1981).
11. B. Calesnick, M. Smith, and R. Beutner, J. Pharmacol. Exp. Ther., 102, 138 (1951).
12. H. R. Kaplan, J. N. Barker, D. Dagau, et al., Arch. Int. Pharmacodyn., 207, 28 (1974).
13. F. J. Kniffen, T. E. Zomas, N. Z. Nohel-Allen, et al., Circulation, 49, 264 (1974).
14. E. R. Smith, B. R. Duce, and R. N. Boger, Am. Heart J., 83, 365 (1972).
15. E. Wick and J. Dörner, Z. Kreislaufforsch., 61, 1 (1962).
16. M. M. Winbury and M. Z. Hemmer, J. Pharmacol. Exp. Ther., 113, 403 (1955).

FENIBUT BINDING WITH BICUCULLINE-INSENSITIVE GABA RECEPTORS IN THE RAT BRAIN

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Fenibut (β-phenyl GABA) has a sedative and tranquilizing action [5]. Many of the effects of fenibut have been shown to resemble those of GABA-mimetic substances [4]. Because of the structural similarity of GABA and fenibut it can be postulated that fenibut affects GABA-ergic processes in the CNS. Fenibut has not yet been found to affect the activity of enzymes participating in GABA metabolism [2] and, in addition, fenibut has no action on reassimilation of GABA [3].

Since the effect of fenibut on GABA receptors has received little study, it was decided to investigate the action of fenibut on GABA binding with GABA receptors in the rat brain.

EXPERIMENTAL METHOD

Male Wistar rats weighing 250-300 g were used. Binding of [^{3}H]-GABA was determined in the corpus striatum. The membrane fraction was obtained as follows: the rat was decapitated and the corpus striatum quickly removed in the cold and homogenized in 60 volumes of cold Tris-HCl buffer, pH 7.4, with a knife homogenizer (8000 rpm, 60 sec). The resulting suspension was centrifuged at 30,000g for 30 min at 4°C. The residue was left to stand overnight at −20°C. Next day the residue was kept for 15 min at room temperature, then suspended in the initial volume of the same buffer, and again homogenized and centrifuged at 30,000g. The residue was again allowed to stand overnight at −20°C. The residue was then rehomogenized and centrifuged another four times at 30,000g. After the last centrifugation the residue was rehomogenized in the same volume of buffer. The reaction of binding of [^{3}H]-GABA with receptors was carried out in the absence of NaCl at 0°C for 10 min and in the presence of 2.5 μM $CaCl_2$ and 50 μM (+)-bicuculline at 20°C (10 min). The reaction mixture contained: 0.9 ml of membrane protein suspension, 6 nM labeled GABA (50 Ci/mmole, from Amersham Corporation, England). Specific binding of the label with GABA receptors was determined from the difference between binding of the label in the presence and absence of 100 μM unlabeled GABA in the reaction mixture. The reaction was stopped by addition of 4 ml of cold Tris-HCl buffer, pH 7.4 (2°C), and rapid filtration through glass filters of the GFB type (from Whatman, England). The filters were washed three times with 4 ml of the same buffer and transferred

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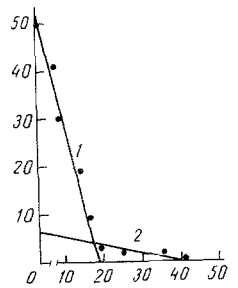

Fig. 1

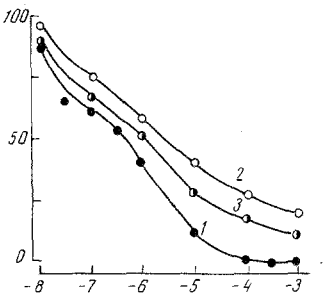

Fig. 2

Fig. 1. Analysis of GABA binding with rat brain membranes. To 0.9 ml of membrane suspension (0.5 mg protein) containing 2.5 mM $CaCl_2$ and 50 μM (+)-bicuculline different concentrations of GABA in 50 μl of Tris-HCl buffer, pH 7.4, and 6 nM [^{3}H]-GABA in 50 μl of Tris-HCl buffer were added. The "free" concentration was compared with the total concentration of added GABA. Abscissa, binding (in pmoles/g protein); ordinate, ratio of bound GABA (in pmoles/g protein) to free GABA (in nmoles), multiplied by 100. 1) K_{diss} = 37 nM, maximal binding 19 pmoles/g protein; 2) K_{diss} = 667 nM, maximal binding 40 pmoles/g protein.

Fig. 2. Bicuculline-independent binding of [^{3}H]-GABA in the presence of different concentrations of GABA (1), (±)-fenibut (2), and (±)-baclofen (3). Binding carried out in the presence of 2.5 mM $CaCl_2$ and 50 μM (+)-bicuculline to inhibit Ca-independent, bicuculline-sensitive binding of [^{3}H]-GABA. Fraction (48 ± 3%) which displaced the label with unlabeled GABA (100 μM) had the property of specific binding of [^{3}H]-GABA. Concentration of ligand ([^{3}H]-GABA) 6 nM. Displacement of (+)-fenibut and (±)-baclofen shown as percentage of displacement observed when 100 μM GABA was used. Abscissa, log of molar concentration of displacer; ordinate, specific binding of [^{3}H]-GABA (in percent).

to flasks containing scintillator. A dioxane scintillator was used: 60 g naphthalene, 4 g 2,5-diphenyloxazole (PPO), 0.2 g n-bis-2,5-phenyloxazolylbenzene (POPOP), 120 ml methanol, and dioxane up to 1 liter (mean efficiency 32%). Radioactivity was measured on an "Ultrabeta 1210" counter (LKB, Sweden). Protein in the samples was determined by the method of Lowry et al. [8].

EXPERIMENTAL RESULTS

The results showed that fenibut does not bind with Na-independent GABA receptors (IC_{50} > 250 μM). In view of data in the literature showing that some effects of baclofen (a chlorine analog of fenibut) are bicuculline-insensitive [6, 9] and are connected with Ca-dependent GABA receptors [7], a series of experiments was carried out to study binding of [^{3}H]-GABA in the presence of 2.5 mM $CaCl_2$ and 50 μM (+)-bicuculline. Analysis of [^{3}H]-GABA binding under these conditions showed the presence of low-affinity (dissociation constant K_{diss} = 667 nM) and high-affinity (K_{diss} = 37 nM) GABA binding sites (Fig. 1). The presence of two binding sites is difficult to explain under these conditions and elucidation of this problem requires further research. The study of (±)-fenibut and (±)-baclofen showed that these stereoisomers bind, depending on molar concentration, with Ca-dependent GABA receptors in spite of the presence of 50 μM (+)-bicuculline, which blocks GABA receptors (Fig. 2). (±)-Fenibut displaces [^{3}H]-GABA from receptor sites less strongly than (±)-baclofen, in agreement with their biological activity *in vivo*. We know [6, 7] that Ca-dependent bicuculline-insensitive GABA receptors are located on neuron terminals and that they take part in the regulation of mediator secretion. The dopaminergic effects of fenibut, which have been described [1], may perhaps be connected with the action of this drug on Ca-dependent GABA receptors.

It can be tentatively suggested that (–)-fenibut is bound more firmly by Ca-dependent GABA receptors than the (+)-isomer, since (–)-baclofen has been shown to be more active toward the Ca-dependent GABA receptor [7].

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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