# EFFECT OF BICUCULLINE, A BLOCKER OF GABA RECEPTORS, ON THE EFFECTS OF FENIBUT AND DIAZEPAM

L. Kh. Allikmets, L. K. Ryago, and A. M. Nurk

UDC 615.214.22.015.2:615.31:547.466.3].015.25

KEY WORDS: fenibut; diazepam; interaction with bicuculline.

Fenibut (β-phenyl-GABA), a compound similar in structure to the inhibitory mediator, GABA, is a tranquilizer whose clinical spectrum of action differs considerably from that of tranquilizers of the benzodiazepine series [2, 3]. Experiments with radioligand binding and electrophysiological investigations have now demonstrated the existence of a close link between GABA-ergic receptors and the action of benzodiazepine [7]. The role of GABA-ergic mechanisms in the action of fenibut remains unexplained.

It was accordingly decided to study the effect of bicuculline, which blocks GABA-receptors, on some behavioral and biochemical effects of fenibut and diazepam.

## EXPERIMENTAL METHOD

Male Wistar rats weighing 180-220 g were used. Fenibut (100 mg/kg, intraperitoneally) was injected 60 min, diazepam (2.5 mg/kg, intraperitoneally) 45 min, and bicuculline (1.25 mg/kg, subcutaneously) 10 min before the experiment began. Emotional behavior was assessed by a method of painful electrical stimulation [1]. The threshold of squeaking and the threshold of fighting were estimated in volts. Orienting motor activity was determined as the number of pulses (horizontal locomotion) in 2 min on an actometer. For the biochemical experiments the animals were decapitated and the brain quickly frozen in liquid nitrogen; the limbic structures (olfactory tubercle and adjacent nuclei) were separated and their content of GABA, homovanillic acid (HVA), and 3,4-dihydroxydiphenylacetic acid (DHPAA) determined spectrofluorometrically by the method in [5].

## EXPERIMENTAL RESULTS

Fenibut (100 mg/kg) inhibited orienting and motor activity and the emotional response (squeaking threshold) but did not affect aggressiveness (fighting threshold). Diazepam (2.5 mg/kg) increased locomotion and reduced aggressiveness in the experimental animals. Bicuculline (1.25 mg/kg) had no significant effect on the animals' behavior. However, its combined administration with fenibut did not abolish the behavioral effects of the latter but, on the contrary, potentiated them. Administration of bicuculline after diazepam prevented the appearance of the behavioral effects of the latter (Table 1). The biochemical tests showed that bicuculline did not abolish the increase in concentration of dopamine metabolites (HVA and DHPAA), but counteracted the increase in the GABA concentration after administration of fenibut. Diazepam, both alone and together with bicuculline, caused no significant change in the GABA, HBA, and DHPAA concentration in the limbic structures (Table 2). The results of this investigation show that the mechanism of action of fenibut is evidently through various neurochemical mechanisms. This conclusion is supported by the fact that bicuculline abolishes the effect of fenibut on the GABA concentration without any effect on dopamine metabolism. The action of fenibut on dopamine metabolism may perhaps be mediated through

Department of Pharmacology, Tartu University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 64-65, May, 1982. Original article submitted January 4, 1981.

0007-4888/82/9305-0621$07.50  

TABLE 1. Effect of Bicuculline on Behavioral Effects of Fenibut and Diazepam (10-12 animals in each group)

| Substance | Orienting and motor activity | Painful electrical stimulation, V | |
|---|---|---|---|
| | | squeaking threshold | fighting threshold |
| Control (physiological saline) | 71,6±6,2 | 20,8±1,1 | 25,8±1,4 |
| Fenibut (100 mg/kg) | 40,1±5,3* | 25,4±1,2* | 27,4±0,7 |
| Fenibut (100 mg/kg) + bicuculline (1.25 mg/kg) | 16,8±4,2† | 28,8±0,7† | 39,5±0,7† |
| Diazepam (2.5 mg/kg | 117,2±5,4* | 22,2±1,2 | 31,4±1,1† |
| Diazepam (2.5 mg/kg) + bicuculline (1.25 mg/kg) | 78,4±4,7 | 17,2±1,8 | 23,6±1,6 |
| Bicuculline (1.25 mg/kg) | 75,3±4,3 | 16,5±1,9 | 23,2±1,2 |

* $P<0,05$.
† $P<0,01$.

TABLE 2. Effect of Bicuculline on Biochemical Effects of Fenibut and Diazepam in Limbic Structures (10-12 animals in each group)

| Substance | GABA, μ moles/g | HVA, μg/g | DHPP, μg/g |
|---|---|---|---|
| Control (physiological saline) | 4,46±0,28 | 0,54±0,04 | 0,93±0,06 |
| Fenibut (100 mg/kg) | 6,34±0,37* | 0,68±0,05* | 1,18±0,06† |
| Fenibut (100 mg/kg) + bicuculline (1.25 mg/kg) | 3,98±0,47 | 0,69±0,05* | 1,22±0,08† |
| Diazepam (2.5 mg/kg) | 4,08±0,31 | 0,56±0,08 | 1,09±0,07 |
| Diazepam (1.5 mg/kg) + bicuculline (1.25 mg/kg) | 3,52±0,24 | 0,40±0,07 | 0,78±0,08 |
| Bicuculline (1.25 mg/kg) | 3,80±0,30 | 0,72±0,09 | 1,16±0,07* |

* $P<0,05$.
† $P<0,01$.

bicuculline-insensitive GABA receptors. This suggestion is confirmed by the results of an investigation [4] in which the effect of the chlorine analog of fenibut, baclofen, on dopaminergic processes is associated with bicuculline-insensitive GABA receptors. It is quite possible that this type of GABA receptor also is responsible for the behavioral effects of fenibut. Potentiation of the action of fenibut by bicuculline in the behavioral experiments is difficult to explain. We know that blockers of GABA receptors accelerate the release of GABA [6]. By analogy with baclofen which, according to data in the literature [8], accelerates GABA release, it can be postulated that fenibut acts in a similar way and that its action is potentiated by bicuculline. The increased liberation of GABA during combined administration of fenibut and bicuculline leads in turn to potentiation of all the behavioral responses mediated through bicuculline-insensitive GABA receptors studied. In the modern view, a complex of GABA receptors and benzodiazepine receptors participates in the mechanism of action of diazepam [7]. The effects of diazepam, as many workers have found [9], and as the results of the present investigations show, are linked with bicuculline-sensitive GABA receptors. This suggests the existence of different mechanisms of action of fenibut and diazepam on GABA-ergic processes and thus provides an explanation for the different clinical spectrum of action of these preparations.

## LITERATURE CITED


1. A. M. Zharkovskii, in: Proceedings of a Conference on Current Problems in Psychopharmacology [in Russian], Tartu (1976), pp. 28-30.
2. R. A. Khaunina, Novye Lekarstvennye Preparaty, No. 7, 2 (1978).
3. T. Ya. Khvilivitskii, in: Fenibut and Substituted Gamma-Aminobutyric Acid and Alpha-pyrrolidones [in Russian], Cherkassy (1981), p. 80.
4. N. G. Bowery, Nature, 283, 92 (1980).
5. C. J. Earley and B. E. Leonard, J. Pharmacol. Methods, 1, 67 (1978).
6. J. A. M. Heyden, K. van der Venema, and J.-J. Korf. J. Neurochem., 34, 1648 (1980).

7. L. L. Iversen, Nature, 285, 285 (1980).
8. P. J. Roberts, H. K. Gupta, and M. S. Shargill, Brain Res., 155, 209 (1978).
9. V. V. Zakusov, R. U. Ostrovskaya, S. N. Kozheshkin, et al., Arch. Int. Pharmacodyn., 229, 313 (1977).

# EFFECT OF ARECOLINE AND MUSCARINIC AND NICOTINIC CHOLINOLYTICS ON $^{22}$Na INCORPORATION INTO RAT BRAIN NEURONS

E. V. Semenov

UDC 612.822.014.462.1.014.46:[615.217.32+615.217.34

KEY WORDS: $^{22}$Na incorporation; muscarinic and nicotinic cholinolytics; cholinomimetics; rat brain.

Among the numerous effects of atropine-like substances in experimental animals, the appearance of slow-amplitude potentials similar to the waves found during natural sleep has been found on the EEG, and has been described as an EEG of "sleep" type [8, 9, 12]. However, the animals' behavior is characterized by wakefulness and increased motor activity. The reason for these effects may be that muscarinic cholinolytics disturb relations between excitation and inhibition among neurons, with the result that brain functions are disorganized, possibly because of a disturbance of ionic permeability of the neuron membranes.

The writer showed previously [4] that muscarinic cholinolytics and cholinomimetics modify the permeability of nerve cell membranes for monovalent cations.

In the present investigation the action of the cholinomimetic arecoline and of muscarinic and nicotinic cholinolytics on nerve cell membrane permeability was studied in different parts of the rat brain.

## EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-250 g, which received intraperitoneal injections of benactyzine (40 mg/kg), glypin (10 mg/kg), tropazine (40 mg/kg), adiphenine (40 mg/kg), and arecoline (2.5 mg/kg) made up in a volume of 0.1 ml solution/100 g body weight. Control animals received injections of water. An injection of $^{22}$Na (5 μCi) was given to the rats 30 min before sacrifice. The animals were decapitated after definite time intervals and the brain was removed and placed in a dish with ice. Separate parts of the brain were taken (hypothalamus, medulla and midbrain, basal ganglia, cortex) and hydrolyzed in 1N NaOH (0.7 ml) at 60°C for 30 min. The digest was neutralized with 1 ml of 0.67 N HCl. Incorporation of $^{22}$Na was determined by mixing 1 ml of the digest with 10 ml SM-7 scintillation solution in a liquid counter (from Packard, England) with a counting efficiency of 80%. The degree of incorporation of the isotope was estimated in cpm/mg protein. Protein was determined by the method of Lowry et al. [10].

## EXPERIMENTAL RESULTS

After injection of benactyzine incorporation of $^{22}$Na into hypothalamic neurons was increased (Table 1), but into neurons of the medulla—midbrain and cortex it was reduced. Incorporation of $^{22}$NA into nerve cells of the basal ganglia was increased a little after 1 h. Glypin increased $^{22}$Na incorporation into nerve cells of the basal ganglia but considerably reduced neuron membrane permeability in the cortex (by 39%) and in the medulla and midbrain (by 35% after 10 min).

Institute of Toxicology, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. N. Gilikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 66-68, May, 1982. Original article submitted March 30, 1981.

0007-4888/82/9305-0623$07.50  