EFFECT OF GABA-NEGATIVE DRUGS ON ANXIOLYTIC AND SEDATIVE EFFECTS OF DIAZEPAM

B. V. Andreev and Yu. D. Ignatov

UDC 615.214.22.015.2:615.31:547.466.3

KEY WORDS: diazepam; bicuculline; picrotoxin; GABA-ergic mechanisms.

There is still no general agreement on the role of GABA-ergic mechanisms in the psychotropic effect of the benzodiazepines (BDZ). According to some investigations, these processes are very important in the mechanisms of both the anxiolytic [3, 4, 12] and the sedative action of BDZ [4], as is confirmed, in particular, by the weakening of these effects of BDZ by GABA-negative drugs. However, no such action of blockers of GABA-receptors could be found in some investigations [8, 11]. Moreover, more recent studies have led to the formulation of a well-argued hypothesis, which explains the origin of the anxiolytic and sedative effects of BDZ from the standpoint of their action on different types of BDZ receptors, and they postulate that GABA-ergic mechanisms participate in the mechanism of the sedative effect only, and not of the anxiolytic action of BDZ [7, 9].

Accordingly the aim of the investigation described below was to study the effect of bicuculline and picrotoxin, which block GABA-receptors, on exhibition of the anxiolytic and sedative effects of diazepam.

EXPERIMENTAL METHOD

Experiments were carried out on 82 male albino rats weighing 200-250 g. The anxiolytic effect of diazepam was measured by a modified technique of Jacobs and Cohen [5], based on inhibition of the natural investigative behavior of animals by anxiety before painful electrical stimulation received by them in that situation. The rats were kept in a chamber measuring $40 \times 50 \times 55$ cm and their investigative activity was studied for 2 min; painful electric shocks were then applied to them through the electrode floor (60 Hz, 0.5 msec, 60 V, 3 sec), after which the animals remained in the chamber for a further 15 sec. Seven days later, after preliminary ranking of the rats in groups depending on their original investigative activity, the animals were replaced in the same experimental situation and the number of times they crossed the squares, the number of defecations, and the emotional reactivity (ER) of the animals on being picked up by the hand, were estimated. ER was determined in points according to the scale suggested by Allikmets and Zharkovskii [1]. The sedative effect was assessed by the "open field" test (an area $100 \times 100 \times 45$ cm) on the basis of inhibition of spontaneous locomotor activity in the course of 2 min. Diazepam, the GABA-receptor blockers picrotoxin and bicuculline, and the GABA-positive drug γ-acetylene-GABA (GA-GABA) (from Centre de Recherche Merrell International, France) were injected intraperitoneally 30, 20, and 5 min and 4 h respectively before the experiment. When the drugs were used in combination, bicuculline was injected 25 min after diazepan and picrotoxin 10 min after diazepam and 3 h 40 min after GA-GABA.

EXPERIMENTAL RESULTS

Replacing the animals in a situation in which they had previously been subjected to aversive action caused inhibition of their investigative behavior and an increase in ER and the number of defecations compared with intact rats of the control group (Fig. 1). Diazepam, in a dose of 2.5 mg/kg body weight, had a significant disinhibiting action under these conditions and also lowered ER and the number of defecations, evidence of an anxiolytic effect of the drug. Spontaneous motor activity was unchanged under these circumstances (Fig. 2). In a dose of 5 mg/kg diazepam had no disinhibiting effect, but completely suppressed ER and defecation. In this dose diazepam also inhibited spontaneous locomotor activity, an effect which can be regarded as due to its sedative action [2].

Department of Pharmacology, Academician I. P. Pavlov First Medical Institute, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 6, pp. 685-687, June, 1981. Original article submitted December 12, 1980.

0007-4888/81/9106-0756$07.50

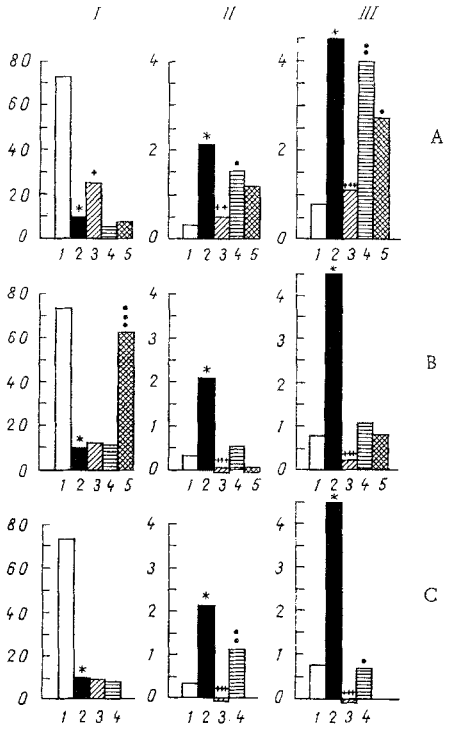

Fig. 1

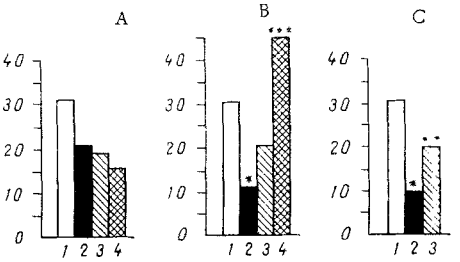

Fig. 2

Fig. 1. Effect of diazepam (A, B), GA-GABA (C) alone and in combination with GABA-negative drugs on manifestation of emotional response of anxiety. 1) Control without aversive stimulation, 2) control with aversive stimulation, 3) diazepam, 4) diazepam + bicuculline (2 mg/kg), 5) diazepam + picrotoxin (2 mg/kg); A) diazepam 2.5 mg/kg, B) 5 mg/kg. C: 3) GA-GABA (100 mg/kg), 4) GA-GABA + picrotoxin (2 mg/kg). *P < 0.001 compared with control without aversive stimulation. +) P < 0.05, ++) P < 0.01; +++) P < 0.001 compared with control with aversive stimulation. .) P < 0.05; ..) P < 0.01; ...) P < 0.001 compared with effect of diazepam in doses of 2.5 and 5 mg/kg and GA-GABA in a dose of 100 mg/kg. Ordinate: I) mean number of squares crossed, II) mean level of ER (in points), III) mean number of defecations.

Fig. 2. Effect of diazepam (A, B) and GA-GABA (C), alone and in combination with GABA-negative drugs on spontaneous motor activity. 1) Control with aversive stimulation, 2) diazepam, 3) diazepam + bicuculline (2 mg/kg), 4) diazepam + picrotoxin (2 mg/kg); A) diazepam 2.5 mg/kg, B) 5 mg/kg. C: 2) GA-GABA (100 mg/kg), 3) GA-GABA (100 mg/kg) + picrotoxin (2 mg/kg). *P < 0.05 compared with control; **P < 0.05, ***P < 0.01 compared with effect of diazepam in a dose of 5 mg/kg and GA-GABA in a dose of 100 mg/kg.

Picrotoxin and bicuculline in a dose of 2 mg/kg did not change the emotional tension but reduced the disinhibiting effect of diazepam in a dose of 2.5 mg/kg and its inhibitory effect on ER and defecation (Fig. 1). Meanwhile injection of picrotoxin against the background of diazepam in a dose of 5 mg/kg not only abolished its inhibitory action on investigative behavior, but also led to the appearance of a marked disinhibiting effect, greater than the corresponding action of diazepam in a dose of 2.5 mg/kg by 2.6 times (P < 0.001). Under these circumstances picrotoxin caused no significant change in the inhibitory action of diazepam (5 mg/kg) on ER and the number of defecations (Fig. 1). Picrotoxin reversed the effect of diazepam (5 mg/kg) in the open field test also, as was shown by an increase in locomotion on average by 40% compared with the control (Fig. 2). Meanwhile the number of holes investigated also was increased (by 77%), a characteristic feature of the activating effect of BDZ [13]. Unlike picrotoxin, bicuculline (2 and 4 mg/kg) caused no significant change in the inhibitory effect of diazepam in a dose of 5 mg/kg on any of the test indices (Figs 1 and 2). Only a tendency was observed for the effect of diazepam to decrease, as reflected in the ER, number of defecations and spontaneous motor activity.

GA-GABA in a dose of 100 mg/kg had a distinct sedative action, similar to the effect of diazepam in a dose of 5 mg/kg (Fig. 2). However, the effect of picrotoxin on the sedative action of GA-GABA and diazepam in these doses differed significantly. First, picrotoxin abolished the depriming action of GA-GABA on ER and

defecation but did not cause the appearance of a disinhibiting effect, as in the experiments with diazepam. Second, picrotoxin did not reverse the action of GA-GABA on spontaneous motor activity but merely reduced it significantly (Figs. 1 and 2).

Weakening of the general depriming action of diazepam by picrotoxin and, to a certain extent, by bicuculline confirms the important role of GABA-ergic mechanisms in the realization of the sedative effect of BDZ. Meanwhile reversal of the sedative effect of diazepam and the appearance of a marked anxiolytic effect when it was given together with picrotoxin may be evidence that the tranquilizing action of BDZ is not necessarily effected through GABA-ergic mechanisms. That this hypothesis is correct is confirmed by the results of experiments with GA-GABA, causing activation of GABA receptors only [6]. Under these conditions picrotoxin merely weakened the sedative effect of GA-GABA. A sufficiently convincing explanation of these facts is provided by the hypothesis that there are two types of benzodiazepine receptors [7, 9], one of which is unconnected with GABA receptors and is responsible for the anxiolytic action of BDZ. The other type is interlinked with GABA-ergic receptors and the chloride ionophore and mediates the sedative effect of BDZ. Picrotoxin, which blocks conduction in chloride channels, disturbs the functioning of benzodiazepine receptors of the second type [9]. In that way the sedative effect masking the anxiolytic action of diazepam is abolished.

LITERATURE CITED

1. L. Kh. Allikmets and A. M. Zharkovskii, Zh. Vyssh. Nerv. Deyat., No. 6, 1284 (1974).
2. Yu. I. Vikhlyaev and T. A. Voronina, in: Minor Tranquilizers in the Treatment and Rehabilitation of Patients with Psychoneurological and Psychosomatic Diseases [in Russian], Leningrad (1979), pp. 5-14.
3. R. U. Ostrovskaya and T. A. Voronina, Byull. Éksp. Biol. Med., No. 3, 292 (1977).
4. M. L. Billingsley and R. K. Kubena, Life Sci., 22, 897 (1978).
5. B. L. Jacobs and A. Cohen, J. Comp. Physiol. Psychol., 90, 102 (1976).
6. J. Jung and B. Lippert, J. Neurochem., 28, 717 (1977).
7. C. A. Klepner, A. S. Lippa, D. I. Benson, et al., Pharmacol. Biochem., 11, 457 (1979).
8. A. S. Lippa, E. N. Greenblatte, and R. W. Pelham, in: Animal Models in Psychiatry and Neurology, Oxford (1977), pp. 279-292.
9. A. S. Lippa, D. Critchett, M. C. Sano, et al., Pharmacol. Biochem., 10, 831 (1979).
10. N. A. Norma and M. W. Parkes, Psychopharmacologia, 29, 277 (1973).
11. P. Soubrie, P. Simon, and J. R. Boissier, Neuropharmacology, 15, 773 (1976).
12. L. Stein, C. D. Wise, and J. D. Belluzi, in: Mechanism of Action of Benzodiazepines, New York (1975), pp. 29-44.