ANTAGONISTIC EFFECT OF BICUCULLINE AND THIOSEMICARBAZIDE ON THE TRANQUILIZING ACTION OF DIAZEPAM

R. U. Ostrovskaya and T. A. Voronina

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Bicuculline, a specific blocking agent of GABA-ergic receptors, in doses of 0.5 and 1 mg/kg (subcutaneously); and thiosemicarbazide, which inhibits GABA (γ-aminobutyric acid) synthesis in the brain, in doses of 5 and 8 mg/kg (subcutaneously); are antagonists of diazepam and weaken its tranquilizing action during conflict behavior in experimental rats. Bicuculline exhibits stronger antagonism toward diazepam than thiosemicarbazide. The results are evidence that GABA-ergic mechanisms may participate in the tranquilizing action of the benzodiazepines.

KEY WORDS: *diazepam; bicuculline; γ-aminobutyric acid; thiosemicarbazide.*

The ability of the benzodiazepines to increase the intensity of cortical inhibition is well known. Evidence has been obtained to show that this phenomenon is linked with potentiation of the inhibitory action of endogenous γ-aminobutyric acid (GABA) [11]. One possible cause of this effect has been shown to be an increase in the sensitivity of postsynaptic GABA-ergic receptors under the influence of diazepam [3], and, if large doses of diazepam are given, the slowing of its deactivation [4]. Diazepam is also known [8] to depress the activity of inhibitory GABA-ergic neurons in the cerebellum and to potentiate presynaptic inhibition in the spinal cord; the workers cited above suggest that this may lie at the basis of the mechanisms of the ataxic and muscle-relaxant action of diazepam.

TABLE 1. Effect of Bicuculline and Thiosemicarbazide on Tranquilizing Action of Diazepam under Conflict Conditions.

Drug and dose	Number of drinks of water	Number of trips to feeding bowl	Motor activity
Control (physiological saline)	1,8 (1,31—2,29)*	8,7 (6,01—11,39)	12,2 (9,02—15,38)
Diazepam (1 mg/kg)	9,3 (7,1 —11,5)	13,3 (9,63—16,97)	16,4 (10,52—22,28)
Bicuculline (1 mg/kg)	2,1 (1,61—2,59)	10,6 (6,19—15,01)	9,1 (5,92—12,28)
Thiosemicarbazide (5 mg/kg)	3,1 (2,12—4,08)	3,3 (1,83— 4,77)	18,5 (8,5 —28,5)
Diazepam (1 mg/kg) + Bicuculline (0.5 mg/kg)	5,5 (4,52—6,48)	10,6 (7,91—13,29)	12,1 (9,42—14,79)
Diazepam (1 mg/kg) + Bicuculline (1 mg/kg)	3,8 (2,82—4,78)	9,2 (7,48—10,91)	11 (7,08—14,92)
Diazepam (1 mg/kg) + Thiosemicarbazide (5 mg/kg)	6,1 (5,0 —7,2)	8,7 (7,8 — 9,6)	15,4 (10,86—19,94)
Diazepam (1 mg/kg) + Thiosemicarbazide (8 mg/kg	5,8 (3,88—7,72)	16,2 (12,08—20,32)	23 (14,21—31,79)

*Deviations from the mean for which P = 0.05 shown in parentheses.

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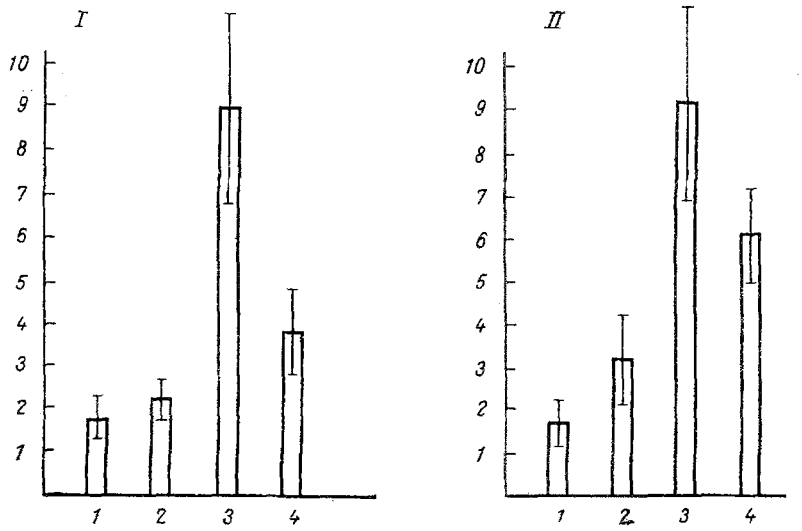

Fig. 1. Combined action of diazepam (1 mg/kg, intraperitoneally) with bicuculline (I) and thiosemicarbazide (II) on behavior of rats in conflict situation. Abscissa, I: 1) control (intact animals); 2) bicuculline (1 mg/kg, subcutaneously); 3) diazepam; 4) diazepam together with bicuculline; in II: 1) control; 2) thiosemicarbazide (5 μg/kg, subcutaneously); 3) diazepam; 4) diazepam together with thiosemicarbazide; ordinate, number of drinks of water despite negative stimulation.

Meanwhile, the question of the possible role of GABA-ergic mechanisms in the anxiety-relieving, tranquilizing action of diazepam remains unexplained. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

The tranquilizing action of the drugs was tested in experiments on male rats weighing 150-180 g by the conflict-situation method, one of the most selective models for the experimental assessment of tranquilizers [1, 5, 10]. The conflict situation was created by nociceptive stimulation of the animals (electric shock) in situations when a food reflex would normally be produced in the animals [2] (interaction between two opposite reflexes — food and defense). The action of the drugs under these circumstances was assessed from changes in the number of times the animals drank water (even though they received painful electric shocks at that moment), the number of approaches to the feeding bowl during which they did not take water, and a value reflecting total motor activity during recording for 20 min.

The substances chosen to interfere with GABA-ergic processes were bicuculline, a specific blocker of GABA-ergic receptors [7], and thiosemicarbazide, which lowers the GABA concentration in the brain [9]. These substances were injected subcutaneously in the subthreshold doses not causing convulsions in the animals. Considering the times taken for the action of these substances to reach its maximum, diazepam (1 mg/kg, intraperitoneally) was injected 3 min before, bicuculline (0.5 and 1 mg/kg) 5-8 min before, and thiosemicarbazide (5 and 8 mg/kg) 60 min before the experiments began. When a combination of the drugs was given, bicuculline was given 25 min after diazepam and thiosemicarbazide 30 min before diazepam. Altogether 8 series of experiments were carried out and 10 rats were used in each series.

EXPERIMENTAL RESULTS

Diazepam has a marked effect on the behavior of animals in a conflict situation and caused an increase in the number of times of taking water by 5.2 times compared with the control. Bicuculline did not significantly change the animals' behavior under conflict conditions, whereas under the influence of thiosemicarbazide there was a small increase, not statistically significant, in the number of times the animals took water and an increase, likewise not significant, in their motor activity (Table 1).

After a combination of bicuculline and diazepam, considerable weakening of the tranquilizing effect of the latter was observed (Fig. 1). There was, moreover, a definite relationship between the degree of this weakening and the dose of bicuculline given. For instance, in a dose of 0.5 mg/kg, bicuculline reduced the effect of diazepam by 41% with respect to the number of drinks of water, and in a dose of 1 mg/kg by 68%. The weakening of the action of diazepam by bicuculline was observed at all stages of development of the conflict situation and, in particular, during the first 10 min.

Significant weakening of the effect of diazepam on the behavior of the animals in a conflict situation also was observed when it was given together with thiosemicarbazide, although the antagonism of thiosemicarbazide to diazepam was less marked than that of bicuculline.

Tranquilizers in a conflict situation are known to have a specific, selective action which is manifested as normalization of the conflicting behavior and the relief of tension and anxiety, despite the action of negative stimuli [1, 10]. In the present experiments, a marked tranquilizing effect was shown by diazepam in the conflict situation. Neuroleptics, analgesics, and antidepressants are as a rule ineffective when this model is used [1, 2].

The fact that bicuculline and thiosemicarbazide can antagonize the effects of diazepam and reduce its tranquilizing action under experimental conflict conditions is evidence of a possible role of GABA-ergic mechanisms in the tranquilizing action of the benzodiazepines. The results do not support the hypothesis of Cook [6] who denies any role of GABA-ergic mechanisms in the tranquilizing action of the benzodiazepines on the basis of the absence of any such action on the part of aminohydroxyacetic acid (AHAA), an inhibitor of α-ketoglutarate-GABA transaminase.

Potentiation of the inhibitory action of GABA on cerebral cortical neurons, as shown by experiments to study the action of diazepam on the effects of this mediator when applied electrophoretically to a single neuron, is due to increased sensitivity of postsynaptic GABA-ergic receptors [3]. It is evident that an increase in the GABA level after administration of AHAA need not necessarily be accompanied by changes in behavior if the sensitivity of the postsynaptic receptors remains unchanged. This is confirmed by the results of the present investigation indicating stronger antagonism of bicuculline than of thiosemicarbazide toward diazepam.

LITERATURE CITED

1. Yu. I. Vikhlyaev and T. A. Klygul', Zh. Nevropat. Psikhiatr., No. 1, 123 (1966).
2. T. A. Klygul' and V. A. Krivopalov, Farmakol. Toksikol., No. 2, 241 (1966).
3. S. N. Kozhechkin and R. U. Ostrovskaya, Byull. Éksp. Biol. Med., No. 12, 1448 (1976).
4. R. U. Ostrovskaya, G. M. Molodavkin, R. P. Porfir'eva, et al., Byull. Éksp. Biol. Med., No. 3, 50 (1975).
5. L. Cook and A. Davidson, in: Benzodiazepines (ed. by S. Garattini et al.), Raven Press, New York (1973), p. 327.
6. L. Cook and J. Sepinwall, in: Sixth International Congress of Pharmacology. Abstracts, No. 963, Helsinki (1975).
7. D. R. Curtis and D. Felix, Brain Res., 34, 301 (1971).
8. W. Haefely, A. Kulcsar, H. Mohler, et al., in: Mechanism of Action of Benzodiazepines, edited by E. Costa and P. Greengard, New York (1975).
9. K. Kielam and J. Bain, J. Pharmacol. Exp. Ther., 119, 255 (1957).
10. L. Stein and B. Berger, Arzneimittelforsch., 21, 1073 (1971).
11. V. V. Zakusov et al., Arch. Int. Pharmacodyn., 214, 188 (1975).