# ANTAGONISM OF RO 15-1788 WITH BENZODIAZEPINES WITH RESPECT TO EFFECT ON MOTIVATED AGGRESSION AND ANALGESIC ACTION

R. U. Ostrovskaya and G. M. Molodavkin

UDC 615.214:547.891.2].015.23:615.31:547.466.3].015.4:612.821.3

KEY WORDS: GABA interaction; benzodiazepines; opiates; RO 15-1788, an antagonist of benzodiazepine receptors; bicuculline.

In the modern view benzodiazepines (BD) simulate effects of the hypothetical endogenous ligand that is complementary to their binding sites, the so-called BD receptors [6]. BD inhibit activity of a modulator peptide (GABA-modulin), which reduces the affinity of the GABA-ergic receptor for its ligand [8]. Meanwhile evidence has been obtained of the existence of a group of BD-receptors that are not modulated by GABA [12]. Although the importance of this heterogeneity for the ensuring of a wide spectrum of pharmacological activity of the BD has not yet been finally settled, some data on this problem already exists. Opportunities in this direction are provided by the use of RO 15-1788 [ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-(1, 5a)(1, 4)-benzodiazepine-3-carboxylate] [9], an antagonist of central BD-receptors. It has been shown [2] that the antihypoxic effect of the BD, which can be reproduced by the hypothetical ligands of central, but not peripheral, BD receptors, is abolished by compound RO 15-1788, but not by bicuculline. It is evidently connected with a variety of central BD-systems that is not modulated by $GABA_A$-receptors. There is evidence that RO 15-1788 can abolish the muscle-relaxant, sedative, and anticonvulsant activity of the BD, without any influence on the analogous effects of GABA-ergic drugs [5, 9]. Data on selectivity of antagonism of RO 15-1788 as regards the tranquilizing properties of BD and of GABA-ergic drugs is contradictory [4, 9, 10, 13], and this may be due to the use of different tests.

Accordingly, in the investigation described below interaction of BD and GABA-ergic substances with blockers of the corresponding receptors (RO 15-1788 and bicuculline) on one model of intraspecific aggression was compared. On the basis of data on interaction of the BD-GABA system with opiates [3, 7, 11, 14], and in view of the absence of information on the effect of RO 15-1788 on it, this problem also was studied.

## EXPERIMENTAL METHOD

A method of motivated intraspecific aggression [1] was used. Experiments were carried out on male albino rats weighing 200-250 g in a chamber (60 × 50 × 55 cm) with a small bench in the center (8 × 8 × 6 cm — the minimal area accommodating two rats). Shocks of alternating current 1 sec in duration, with a frequency of one shock every 3 sec, and with an amplitude of 60-70 V, were applied to the electrode floor. A reflex of avoidance of electronociceptive stimulation of these limbs by jumping up on the bench was formed in each rat separately. Integrity of the avoidance reflex was verified the next day, after which the effect of one of the test drugs was studied on these rats, taken in pairs. The presence or absence of combined avoidance by two rats jumping on the bench for 1 min, its duration, the number of fighting cycles, and their total duration were recorded. The criterion of avoidance was the presence of both rats on the bench for at least 10 sec. To prevent the rats from learning combined avoidance on the bench each rat was used only once in the experiment. In experiments with unavoidable stimulation (without the bench) the number of fighting cycles in 1 min and their total duration were recorded. All substances were injected intraperitoneally: the cetyl ester of GABA (CEGABA, 10 mg/kg) and muscimol (1 mg/kg) 60 min, Depakine (sodium valproate) (300 mg/kg), and pyracetam (GABA analog) (500 mg/kg) 30 min before testing. Diazepam (1 mg/kg daily) and phenazepam (0.3 mg/kg daily) were injected for 3 weeks, the last dose 30

Laboratory of Pharmacology of the Nervous System, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 4, pp. 448-450, April, 1985. Original article submitted June 15, 1984.

0007-4888/85/9904-0471$09.50  

TABLE 1. Effect of GABA-Ergic Substances and of Antagonists of GABA and Benzodiazepine Receptors on Motivated Agression (M ± m)

| Substance or combination of substances | Motivated aggression | | | Unmotivated aggression | |
|---|---|---|---|---|---|
| | duration of joint avoidance, sec | number of fighting cycles | total duration of fighting, sec | number of fighting cycles | total duration of fighting, sec |
| Control | 10,9±7,5 | 11,4±3,5 | 49,7±8,4 | 10,9±4,1 | 45,4±9,2 |
| CEGABA | 52,2±6,4* | 1,6±1,1* | 5,5±2,8* | 11,4±3,2 | 50,8±6,1 |
| Depakine | 48,6±8,1* | 1,2±0,8* | 5,2±3,4* | 10,5±4,2 | 42,9±11,2 |
| Pyracetam | 53,6±5,4* | 0,9±0,4* | 4,1±3,2* | 9,4±3,1 | 43,5±9,4 |
| Muscimol | 51,4±7,5* | 1,7±0,8* | 3,9±2,6* | 8,6±4,7 | 46,7±8,5 |
| Phenazepam | 45,4±12,6* | 1,9±0,7* | 4,6±2,8* | 12,1±2,5 | 52,9±3,3 |
| Diazepam | 48,6±14,5* | 1,7±0,9* | 3,6±1,9* | 10,1±3,5 | 42,7±10,4 |
| CEGABA + bicuculline | 32,4±6,2* | 4,1±1,2* | 12,9±3,2* | 10,4±2,9 | 51,2±6,6 |
| Pyracetam + bicuculline | 11,2±6,4 | 9,4±3,7 | 45,4±9,2 | — | — |
| Phenazepam + bicuculline | 15,8±9,1 | 12,6±5,8 | 45,6±8,1 | — | — |
| Diazepam + bicuculline | 19,8±7,2 | 10,4±3,1 | 48,5±7,8 | — | — |
| CEGABA + RO 15-1788 | 18,4±10,5 | 11,5±4,2 | 50,7±6,5 | — | — |
| Phenazepam + RO 15-1788 | 53,1±4,9* | 1,2±0,5* | 4,8±3,1* | — | — |
| Diazepam + RO 15-1788 | 16,8±8,2 | 12,4±3,9 | 51,6±5,9 | — | — |
| | 11,4±6,5 | 10,7±4,1 | 46,9±8,1 | — | — |

Legend. *P < 0.05 compared with control.

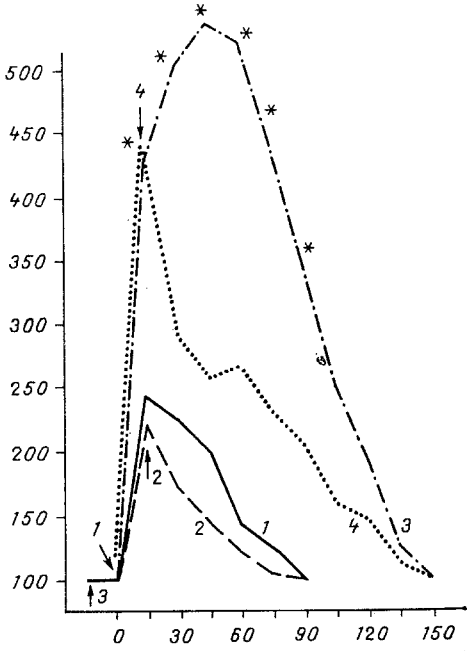


Fig. 1. Effect of RO 15-1788, an antagonist of benzodiazepine receptors, on the potentiating action of diazepam against the analgesic effect of morphine. Abscissa, time (in min); ordinate, elevation of threshold of nociceptive response (in % relative to initial level, taken as 100). 1) Morphine (2.5 mg/kg), 2) morphine (2.5 mg/kg) and RO 15-1788 (6 mg/kg), 3) diazepam (10 mg/kg) and morphine (2.5 mg/kg), 4) diazepam (10 mg/kg), morphine (2.5 mg/kg), and RO 15-1788 (6 mg/kg). Arrows indicate order of administration of drugs. *P < 0.05 compared with effect of morphine.

min before testing. Bicuculline (1 mg/kg) and RO 15-1788 (5 mg/kg) were injected against the background of the agonists. For each substance or their combinations two groups each of seven pairs of animals were used: Rats of one group were kept in pairs in the chamber with a bench, rats of the other group were kept in the chamber without a bench.

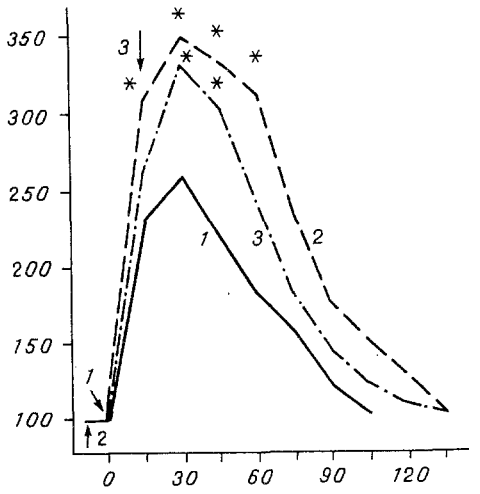


Fig. 2. Effect of compound RO 15-1788 on potentiating action of muscimol against analgesic effect of morphine. 1) Morphine (2.5 mg/kg), 2) muscimol (1 mg/kg) and morphine (2.5 mg/kg), 3) muscimol (1 mg/kg), morphine (2.5 mg/kg), and RO 15-1788 (6 mg/kg). Remainder of legend as to Fig. 1.

In experiments to study the effect of RO 15-1788 bicuculline on the action of opiates and their relationship with BD, the analgesic effect was estimated on rats by the vocalization test during electronociceptive stimulation of the tail. The threshold of the response was determined before administration of morphine and against the background of morphine, injected 15 min after isotonic NaCl solution (control group), of diazepam (10 mg/kg), or of muscimol (1 mg/kg). In experiments with antagonists of the corresponding receptors, they were injected 15 min after morphine: RO 15-1788 (6 mg/kg) intravenously, bicuculline (1 mg/kg) subcutaneously. The numerical data, including those on the change of threshold of the nociceptive response (relative to its initial value, taken as 100%), were analyzed by Student's method.

## EXPERIMENTAL RESULTS

Control animals kept in the chamber after application of electric shocks to the electrode floor fought for possession of the bench. CEGABA, muscimol, Depakine, and pyracetam weakened the animals' motivated agressiveness, and as a result the rats occupied the bench jointly. Diazepam and phenazepam had a similar action. The effects of the substances described were specific against motivated aggression, for in a chamber without bench (unmotivated aggression) the rats behaved like the control animals. Bicuculline, which blocks GABA-ergic receptors, although not affecting manifestation of motivated aggression, abolished the effects of CEGABA and pyracetam, and also those of diazepam and phenazepam. Unlike bicuculline the benzodiazepine antagonists RO 15-1788 abolished the antiaggressive action of the BD-tranquilizers but did not affect the effects of the GABA-mimetic CEGABA (Table 1). Compound RO 15-1788 had a weak antiagressive action, in agreement with data obtained by other methods [4].

In the model of motivated aggressiveness which was used, a hitherto unknown effect of GABA-ergic compounds such as CEGABA, muscimol, Depkine, and pyracetam, was demonstrated. It was also shown that RO 15-1788 is an antagonist against effects only of BD, but not of GABA-positive substances, whereas bicuculline abolishes the antiaggressive action of both groups of compounds.

In agreement with data in the literature [11, 14], diazepam in the present experiments increased the strength and duration of the analgesic action of morphine. This effect of diazepam was abolished by compound RO 15-1788, but under these circumstances activity of the antagonist against a combination of diazepam with morphine was much stronger than against morphine alone (Fig. 1).

Muscimol, as was described previously [14], also potentiates the analgesic action of morphine; compound RO 15-1788, however, does not cause statistically significant weakening of the effect of muscimol (Fig. 2). It was shown previously that bicuculline weakens the effect of diazepam and muscimol [14], and it also abolishes the analgesic action of inhibitors of α-ketoglutarate-GABA transaminase [7]. The same rule was thus observed on the model of potentiation of action of the analgesics as in the study of motivated agression: RO 15-1788 abolishes the effect of BD, but not of GABA-positive substances, whereas bicuculline weakens the action of both types of compounds. These facts confirm data on the selectivity of antagonism of RO 15-1788 against BD. At the same time, they are evidence that although the antiagressive effect of BD is realized through their direct interaction with specific receptors, the latter are under the control of bicuculline-sensitive ($GABA_A$) receptors. This system evidently plays a modulating role in the actions of BD on the analgesic effect of opiates.

LITERATURE CITED


1. Yu. V. Burov and R. M. Salimov, Byull. Éksp. Biol. Med., No. 5, 64 (1975).
2. R. U. Ostrovskaya, in: Abstracts of Proceedings of a Soviet–Finnish Symposium on Neuropsychopharmacology and Biological Aspects of Alcoholism [in Russian], Moscow (1983), p. 45.
3. R. U. Ostrovskaya and G. M. Molodavkin, Byull. Éksp. Biol. Med., No. 3, 313 (1980).
4. L. H. Allikmets and L. K. Rago, Naunyn-Schmiederberg's Arch. Pharmakol., 324, 235 (1983).
5. E. P. Bonetti, R. Schaffner, R. Cumin, et al., Naunyn-Schmiederberg's Arch. Pharmakol., 316, Suppl. 69 (1981).
6. C. Braestrup and R. F. Squires, Proc. Natl. Acad. Sci. USA, 74, 3805 (1977).
7. F. V. De Feudis, Trends Pharmacol. Sci., 3, 444 (1982).
8. A. Guidotti, G. Toffano, and E. Costa, Nature, 275, 553 (1978).
9. W. Hunkeler, H. Mohler, L. Pieri, et al., Nature, 290, 514 (1981).
10. S. Liljequist and J. Engel, Acta Pharmacol. Toxicol., 51, Suppl. 1, Abstr. 5 (1982).
11. R. Morichi and G. Pepeu, Pain, 7, 173 (1979).
12. J. Patel, P. J. Marangos, and F. Goodwin, Eur. J. Pharmacol., 72, 419 (1981).
13. M. Schwartz, L. Turski, and K. H. Sontag, Eur. J. Pharmacol., 90, 139 (1983).
14. V. V. Zakusov, R. U. Ostrovskaya, and V. M. Bulyaev, Arch. Inst. Pharmacodyn., 265, 61 (1983).