Pharmacology Biochemistry & Behavior, Vol 38, pp 265–266 © Pergamon Press plc, 1991 Printed in the U S A 0091-3057/91 $3 00 + 00

Studies on the Involvement of Opioid Mechanism in the Locomotor Effects of Benzodiazepines in Rats

ELŻBIETA NOWAKOWSKA AND ALFONS CHODERA[1]

Department of Pharmacology, Medical Academy, Fredry 10 s., 61-701 Poznań, Poland

Received 26 February 1990

NOWAKOWSKA, E AND A CHODERA *Studies on the involvement of opioid mechanism in the locomotor effects of benzodiazepines in rats* PHARMACOL BIOCHEM BEHAV **38**(2) 265–266, 1991 —The influence of the opioid receptor antagonist naloxone upon the reduced locomotor activity after administration of nitrazepam (NTZ) and upon the increased locomotion after chronic nitrazepam administration was tested It was found that a single dose of naloxone counteracted both the reduced locomotion after acute administration of nitrazepam as well as the augmented locomotor activity after chronic application of nitrazepam It is assumed that opioid mechanisms are involved in the locomotor effects of benzodiazepines

Naloxone Nitrazepam Tolerance Locomotor activity

THE views on the mechanisms of tolerance formation to the sedative effect in rats (increased instead of reduced locomotion) after benzodiazepine treatment have not been definitely settled (6–8) Undoubtedly, functional changes of benzodiazepine receptors play some role (12), but other factors, e.g., changed GABA receptor function and involvement of the opioid receptor system (13), must be considered too

It is known that the opioid antagonist naloxone antagonizes a number of pharmacological effects induced by a single application of benzodiazepines, e.g., locomotor stimulation in mice (13). The aim of the investigation was to define the influence of a selective antagonist, naloxone, on the sedative effect of nitrazepam after a single application and on its activating effect after chronic administration.

METHOD

The experiments were carried out on white male Wistar rats weighing 180–200 g, with free access to standard granulated food for rodents (Bacutil) and tap water. The animals were housed in $37 \times 30 \times 15$ cm Plexiglas cages, 6–8 per cage, at the temperature of 18–20°C and humidity of 50–60%. The experimental groups contained 6–8 rats each. Nitrazepam (NTZ), Pharmaceutical factory "Polfa," Poznań, was dissolved in a 0.5% solution of methylcellulose (BDH) and injected intraperitoneally (IP). Naloxone hydrochloride (Endo Lab. Inc., New York) was dissolved in saline and injected subcutaneously (SC) in a volume of 1 ml/kg, 50 min before the test experiment in accordance with the procedure recommended by other authors (9). Locomotor activity was measured in treated and control groups using the Activity Meter type AM, 1 PAN Licence Cobrabid, Poland This apparatus enables automatic counts of single movements of the rat.

The animals were placed in the apparatus for a 5-min period twice. before and 25 min after the drug administration. The measurements were repeated after 1 week in animals treated with nitrazepam 10 mg/kg b wt. IP one dose daily. The significance of the differences between the groups in the locomotor activity test was assessed by the Student's *t*-test (16)

RESULTS

An introductory series of experiments carried out in the course of the sedative test in the control group of animals did not indicate any pharmacological activity of naloxone during a 60-min observation period The dose, the route, and the optimum time of naloxone injection were chosen according to Kuschinsky and Hornykiewicz (9) A single dose of NTZ 10 mg/kg IP, 25 min before the test administered to the control animals, caused a statistically significant decrease of locomotor activity of the rats Naloxone, 1 mg/kg SC, administered 50 min before the test and 25 min before NTZ application, evoked a slight inhibition of the NTZ sedative effect (Table 1).

In the group of animals with a 7-day treatment period, a 48-h interval was introduced after the last dose (wash-out period) and then a single dose of nitrazepam was administered 25 min before the locomotor activity test. In this case (group Ba, Table 1), a significant increase of locomotor activity as compared with the control group Aa (Table 1) was observed Naloxone administra-

[1]Requests for reprints should be addressed to Alfons Chodera

TABLE 1

THE INFLUENCE OF NALOXONE ON THE SEDATIVE EFFECT OF NITRAZEPAM AFTER A SINGLE DOSE AND ON THE STIMULATING EFFECT IN TOLERANT ANIMALS

Drug, Dose	Activity Counts ± SE: Before Drug Application	After Drug Application
A Single administration		
a) Control group (0 5% methylcellulose IP)	96 5 ± 14 0	49 0 ± 15 2
b) Naloxone 1 mg/kg SC	94 0 ± 14 5	40 0 ± 9 9
c) NTZ 10 mg/kg IP	85 7 ± 9 1	16 8* ± 5 2
d) Naloxone SC + NTZ IP	118 2 ± 7 5	45 5 ± 16 5
B After chronic (7 days) treatment‡		
a) NTZ 10 mg/kg IP	53 2 ± 14 6	121 4* ± 17 2
b) Naloxone 1 mg/kg SC + NTZ 10 mg/kg IP	40 8 ± 7 9	43 0† ± 14 9

*Statistically significant difference for $p<0$ 05 in comparison with control group Aa and the result before drug application

†Statistically significant difference for $p<0$ 05 in comparison with group Ba after drug application

‡After an interval of 48 h

tion in a dose 1 mg/kg SC 50 min before the test caused the reversal of the above-mentioned activating effect of NTZ (group Bb, Table 1).

DISCUSSION

In our recent papers we have shown that intraperitoneal administration of NTZ in dose of 10 mg/kg causes the development of tolerance to the sedative effect as early as after 7 days of treatment (10,11). Formerly, we also demonstrated that the increased locomotor activity in rats after chronic treatment with benzodiazepines is inhibited by the specific benzodiazepine antagonist RO 15-1788 (11). This fact corroborates a well-known hypothesis that the benzodiazepine receptor is involved in the activating effect appearing in rats during chronic treatment with benzodiazepines (3, 11, 12) In the activating effects of a single dose of benzodiazepines in mice the opioid receptors probably are of some importance (1). According to another report (13), the opioid antagonist naloxone prevents hyperactivity induced by a single dose of benzodiazepines. It is also a known fact that naloxone antagonizes some effects of benzodiazepines, e g , the anxiety-reducing effect indicating the possible involvement of opioid mechanisms (2, 4, 14, 15). It has been demonstrated recently that another opioid antagonist, naltrexone, prevents hyperactivity induced by the application of flunitrazepam (1).

It seems interesting that also the activating effect, which appears during chronic treatment with NTZ, is blocked by naloxone. Naloxone administered to rats in a dose of 1 mg/kg SC 50 min before the test did not change the locomotor activity of the animals, whereas the application 25 min before a single dose of NTZ caused an insignificant decrease of the sedative effect

In the group of animals treated with NTZ for 7 days an application of naloxone resulted in a statistically significant decrease of locomotor activity (suppression of the activating effect) as compared with the group treated with NTZ exclusively These results indicate that opioid mechanisms are involved in the activating effect, revealing itself in the course of tolerance formation to the sedative effect of NTZ. The mechanism of the antagonizing effect of naloxone in relation to single or chronic administration of benzodiazepines is unknown. However, an interaction of the opioid system with other receptor systems, e.g., GABA or benzodiazepine receptors, cannot be excluded.

REFERENCES

1 Castellano, C , Filibeck, V , Pavone, F Naltrexone-reversible effects of flunitrazepan on locomotor activity and passive avoidance behaviour in mice Eur J Pharmacol 104 111–116, 1984

2 Cooper, S J Benzodiazepine-opiate antagonist interactions in relation to anxiety and appetite Trends Pharmacol Sci 4 456–458, 1983

3 Crawley, J N , Marangos, P J , Paul, S M , Skolnick, P , Goodwin, F K Interaction between purine and benzodiazepine Inosine reverses diazepam-induced stimulation of mouse exploratory behavior Science 211 725–727, 1981

4 Duka, Th , Cumin, R , Haefely, W Naloxone blocks the effect of diazepam and meprobamate on conflict behaviour in rats Pharmacol. Biochem Behav 15 115–117, 1981

5 File, S , Pellow, S , Wilks, L The sedative effects of CL 218 872, like those of chlordiazepoxide, are reversed by benzodiazepine antagonist Psychopharmacology (Berlin) 85 295–300, 1985

6 File, S Behavioural pharmacology of benzodiazepines Prog Neuropsychopharmacol Biol Psychiatry 8 19–31, 1984

7 File, S , Pellow, S No cross-tolerance between the stimulatory and depressant actions of benzodiazepines in mice Behav Brain Res 17 1–7, 1985

8 Gardner, C R , Piper, D C Effects of agents which enhance GABA-mediated neurotransmission on licking conflict in rats and exploration in mice Eur J Pharmacol 83 25–33, 1982

9 Kuschinsky, K , Hornykiewicz, O Morphine catalepsy in the rat Relation to striatal dopamine metabolism Eur J Pharmacol 19 119–122, 1972

10 Nowakowska, E , Chodera, A , Cenajek, D , Szczawińska, K Differences in the development of tolerance to various benzodiazepines Pol J Pharmacol Pharm 39 27–30, 1987

11 Nowakowska, E , Chodera, A The influence of RO 15-1788 on the stimulating effect of some benzodiazepines in the state of tolerance Memoir Pharmaceut Red 1 1–5, 1989

12 Rosenberg, H C , Chiu, T H. Tolerance during chronic benzodiazepine treatment associated with decreased receptor binding Eur J Pharmacol 70 453–460, 1981

13 Sansone, M , Vetulani, J Effect of naloxone on the locomotor stimulatory action of chlordiazepoxide in mice Pharmacol Biochem Behav 31 371–373, 1988

14 Tripp, G , McNaughton, N Naloxone fails to block the effects of chlordiazepoxide on acquisition and performance of successive discrimination Psychopharmacology (Berlin) 91 119–121, 1987

15 Tripp, G , McNaughton, N , Oei, T P S Naloxone blocks the effects of chlordiazepoxide on acquisition but not performance of differential reinforcement of low rates of response (DRL) Psychopharmacology (Berlin) 91 112–118, 1987

16 Weber, E Grundrisse der Biologischen Statistic Jena, 1961