Pharmacology Biochemistry & Behavior, Vol. 35, pp. 85–88. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Anxiolytic and Sedative Properties of BW A78U, a Novel Anticonvulsant Adenine Derivative

MARC WILLARD, RENE MISSLIN, ELISE VOGEL, LAURENT DESAUBRY,* CAMILLE GEORGES WERMUTH* AND JEAN-JACQUES BOURGUIGNON*

Laboratoire de Psychophysiologie, 7 rue de l'Université, 67000 Strasbourg, France
**Département de Pharmacochimie Moléculaire, Centre de Neurochimie*
5 rue Blaise Pascal, 67084 Strasbourg Cédex, France

Received 19 May 1989

WILLARD, M., R. MISSLIN, E. VOGEL, L. DESAUBRY, C. G. WERMUTH AND J.-J. BOURGUIGNON. *Anxiolytic and sedative properties of BW A78U, a novel anticonvulsant adenine derivative.* PHARMACOL BIOCHEM BEHAV **35**(1) 85–88, 1990.—The anticonvulsant BW A78U, tested in a free mouse exploratory situation, reduced in a dose-dependent fashion the locomotion and the number of rearings, this sedative effect being significant up to a dose of 15 mg/kg (IP, 20 min before testing). In an unconditioned conflict test, the light/dark box choice situation, specific for anxiolytics, low doses of BW A78U increased the time spent by mice in the lit box as well as the number of transitions between the two boxes. Finally, we demonstrated that this drug was able to protect mice against pentylenetetrazole-induced convulsions. Our data show that BW A78U possesses some of the characteristic properties of the minor tranquilizers. However, since this compound binds to the benzodiazepine receptor with a very low affinity ($IC_{50} = 13.6$ μM), it can be assumed that this drug does not exert its behavioral effects through these receptors. It may interfere with other targets involving adenosine, another potent physiological regulator of neuronal excitability.

BW A78U Light/dark choice procedure Staircase test Locomotion Mice Convulsions Sedation Anxiolysis Adenosine

THE need of new antiepileptic agents led to the discovery of the compound BW A78U [9-(2-fluorobenzyl)-6-(methylamino)-9H-purine], a potent, orally active anticonvulsant against electroshock-induced seizures in rats and mice as well as audiogenic convulsions in mice (5–8). It has been described to bind to the benzodiazepine receptor (9). Thus, it seemed to be interesting to examine the possible sedative and anticonflict effects of BW A78U. A series of investigations were undertaken to further evaluate the neuropharmacological properties of BW A78U. Experiment 1 was designed to investigate the effects of several doses of BW A78U on locomotion and rearing behavior in mice confronted to a free exploration test especially able to reveal potential sedative properties. Experiment 2 was aimed at examining possible anxiolytic effects of this compound using the staircase test described by Thiébot *et al.* (15) and modified for use in mice by Misslin *et al.* (11): it was shown that anxiolytic drugs tend to increase the number of steps climbed and the number of rears. In order to test the possible anticonflict properties of BW A78U, we used an unconditioned conflict situation, the light/dark choice procedure, described by Crawley and Goodwin (3) and modified by Belzung *et al.* (1) (Experiment 3). Experiment 4 was aimed at confirming the anticonvulsant properties of BW A78U.

GENERAL METHOD

Animals

Male Swiss albino mice from Centre d'Elevage R. Janvier, 12 weeks of age at time of testing, were used. Prior to experimental testing, they were housed five to a standard cage containing a constant supply of food pellets and water, and kept on a 12/12-hr light-dark cycle with light on at 1 a.m. in order to observe animals in their high activity period, that is, when light is off.

Drug

The compound BW A78U was synthesized following a modified literature procedure (12). The methanesulfonate salt was prepared by adding one equivalent of methanesulfonic acid to a solution of the free base in isopropanol. The crude salt was recrystallized from isopropanol (m.p. 198°C).

[1]Requests for reprints should be addressed to Rene Misslin.

EXPERIMENT 1

Apparatus

The apparatus consisted of a polyvinyl chloride box (30 × 20 × 20 cm) subdivided into six equal, square exploratory units and covered with Plexiglas. It could be divided in half by means of three temporary partitions. The apparatus was kept on a stand in the room which housed the mice. During observation, the experimenters stood next to the boxes always at the same place.

Procedure

Each subject was placed in one-half of the apparatus with the temporary partitions in place, in order for familiarization to occur. Approximately 24 hr after being placed in the apparatus, the subject was exposed to the familiar and novel environment by the removal of the temporary partitions. The subject was then observed, in red light, for ten minutes. The number of units entered by the subject as well as its number of rearings were respectively recorded as locomotion and rearing behavior.

Drug was administered intraperitoneally, 20 minutes before testing, in concentrations giving an injection volume of 10 ml/kg of mouse. Mice were randomly allocated to the following groups: a) vehicle control (saline: n = 15) and BW A78U (5, 15, 25 and 35 mg/kg in saline; n = 15); b) vehicle control (saline; n = 15) and BW A78U (10, 20, 30 and 40 mg/kg in saline; n = 15).

EXPERIMENT 2

Apparatus

The apparatus was a staircase that consisted of a white PVC enclosure with five steps (3.0 cm high, 10.0 cm wide, 7.5 cm deep) surrounded by a wall 23 cm high. A light from a 100-watt desk lamp above the staircase provided the only room illumination.

Procedure

Mice were placed singly on the floor of the box, facing the steps. Two behavioral parameters were measured: the number of rears and the number of steps climbed; a step was considered as climbed only if the animal placed its four paws on it. The duration of the test was 5 min.

Drug was administered as in Experiment 1. Mice were randomly allocated to the following groups: vehicle control (saline; n = 15) and BW A78U (7.5, 10 and 12.5 mg/kg in saline; n = 15).

EXPERIMENT 3

Apparatus

The apparatus consisted of two polyvinylchloride boxes (20 × 20 × 14 cm) covered with Plexiglas. One of these boxes was darkened. A light from a 100-watt desk lamp above the other provided the only room illumination. An opaque plastic tunnel (5 × 7 × 10 cm) separated the dark box from the lit one. During observation the experimenter always sat at the same place, next to the apparatus.

Procedure

The subjects were individually tested in five-minute sessions in the apparatus described above. All mice were placed in the lit box to initiate the test session. The amount of time spent by mice in the lit box and the number of transitions across the tunnel were recorded, minute by minute, during 5 minutes after the first entry in the dark box. A mouse whose four paws were in the new box was considered as having changed boxes.

Drug was administered as in Experiment 1. Mice were randomly allocated to the following groups: vehicle control (saline; n = 36) and BW A78U (5, 7.5, 10 and 12.5 mg/kg in saline; respectively n = 21, 36, 36 and 21).

EXPERIMENT 4

Procedure

BW A78U (0, 5, 10, 15 and 20 mg/kg) was administered intraperitoneally to mice (n = 10) 20 min before intraperitoneal injection of pentylenetetrazole (60 mg/kg) in concentrations giving an injection volume of 10 ml/kg of mouse. The number of mice showing full tonico-clonic seizures in a 5-min period after injection of pentylenetetrazole was recorded.

Statistical Analysis

Statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance followed by the Dunnett test in Experiment 1, 2 and 3. The χ^2 test was used in Experiment 4.

RESULTS

Experiment 1

Analysis of variance revealed significant differences among the groups with respect to locomotion [a: $F(4,70) = 32.48$, $p<0.001$; b: $F(4,70) = 23.85$, $p<0.001$] and to the number of rearings [a: $F(4,70) = 32.14$, $p<0.001$; b: $F(4,70) = 58.10$, $p<0.001$]. Figure 1a and b show that BW A78U produced a dose-dependent decrease in the two behavioral parameters; this effect reached significance from the dose of 15 mg/kg.

Experiment 2

There were no significant differences between control mice and BW A78U-treated mice with respect to the number of steps climbed, $F(3,56) = 0.054$, nor to the number of rears, $F(3,56) = 0.88$ (Fig. 2).

Experiment 3

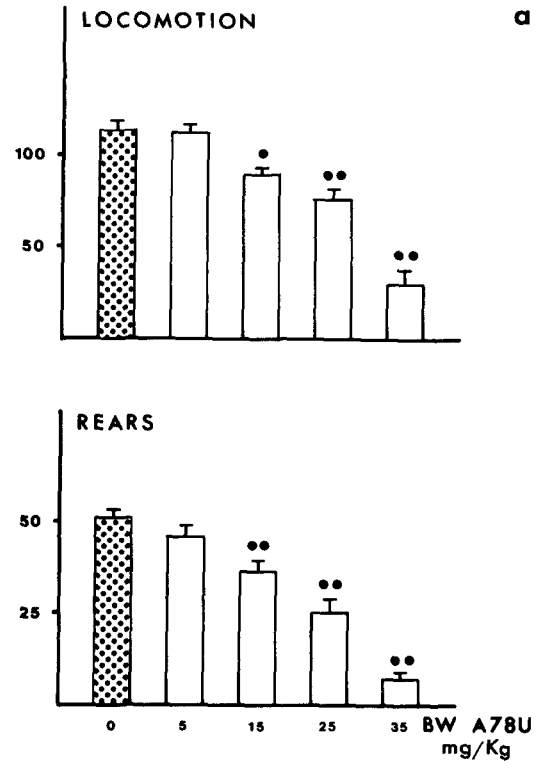
Analysis of variance revealed significant differences among groups with respect to the time spent by mice in the lit box, $F(4,145) = 3.87$, $p<0.005$, and to the number of transitions, $F(4,145) = 4.26$, $p<0.003$. Figure 3a shows that BW A78U significantly increased the time spent in the lit box at doses of 7.5 and 10 mg/kg, while it increased the number of transitions at a dose of 10 mg/kg (Fig. 3b).

Experiment 4

A dose of 60 mg/kg of pentylenetetrazole induced full tonico-clonic convulsions in 10 out of 10 animals 5 minutes after injection. In contrast, this drug injected 20 minutes after BW A78U dosed at 5, 10, 15 and 20 mg/kg induced respectively in 7, 6, 4 and 2 mice out of 10 full tonico-clonic convulsions. There were significant differences between the mice treated with pentylenetetrazole alone and those pretreated with BW A78U at 10, 15 and 20 mg/kg ($\chi^2 = 5$, $p<0.05$; $\chi^2 = 10.76$, $p<0.01$; $\chi^2 = 13.33$, $p<0.001$, respectively).

GENERAL DISCUSSION

The BW A78U has been described as a potent orally active



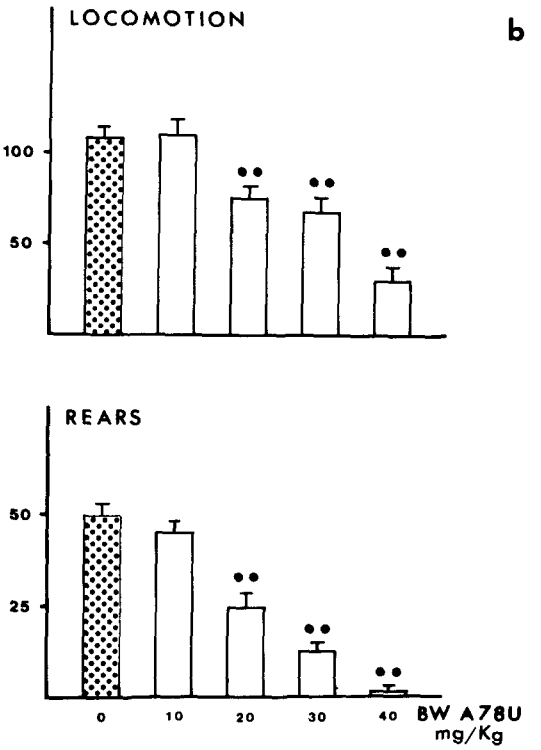


FIG. 1. (a) Effects of several doses of BW A78U on locomotion and rears of mice confronted with a free exploratory test. $p<0.02$; $p<0.005$. (b) Effects of several doses of BW A78U on locomotion and rears of mice confronted with a free exploratory test. $p<0.005$.

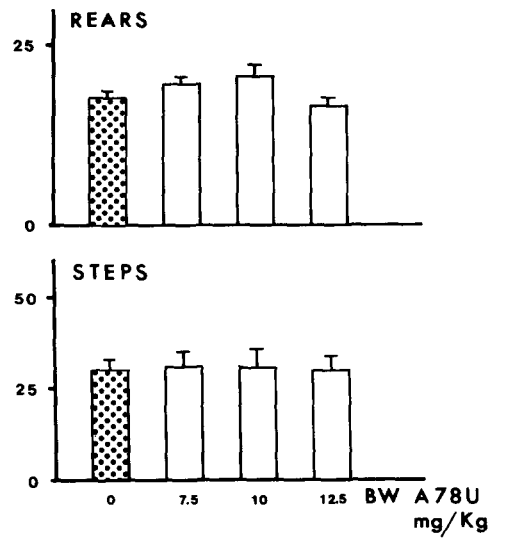


FIG. 2. Effects of several doses of BW A78U on rears and steps climbed by mice confronted with a staircase test.

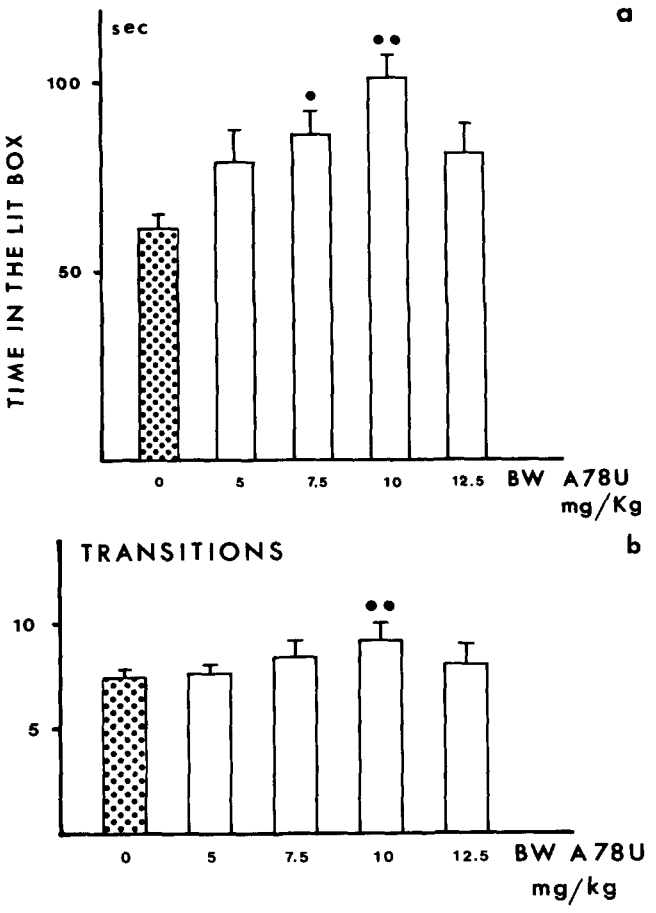


FIG. 3. (a) Effects of several doses of BW A78U on the time spent by mice in the light/dark choice procedure. $p<0.05$; $p<0.005$. (b) Effects of several doses of BW A78U on the number of transitions in the light/dark choice procedure. $p<0.005$.

anticonvulsant drug against maximal electroshock-induced seizures in rats ($ED_{50} = 1.7$ mg/kg, IP) and in mice ($ED_{50} = 5$ mg/kg, IP) (5–8). In the present study, we found that this drug is also efficient against pentylenetetrazole-induced convulsions in mice. Thus, BW A78U appears to exhibit the same protective activity as valproic acid and benzodiazepine agonists which are widely used as antiepileptics. Furthermore, the present results show that BW A78U was able to depress, in a dose-dependent manner, both locomotion and rearing behavior in mice confronted with a free exploratory situation, these effects being significant up at a dose of 15 mg/kg IP. When given at doses lacking in sedative effects, BW A78U induced in mice confronted with a light/dark choice procedure an increase in the time spent by the animals in the lit box as well as in the number of transitions between the two boxes. These latter data closely resemble those obtained by some of us in the same behavioral test with benzodiazepine receptor agonist clorazepate (1). The results we obtained using the staircase test in mice, another procedure for the screening of anxiolytic agents, show that BW A78U did not significantly modify the behavior of animals. However, it has been reported that in mice, the increased climbing produced by some anxiolytics is often less pronounced than in rats, the mouse having an average control baseline for this variable much higher than the rat (14).

Taken together,the present findings suggest that the anticonvulsant BW A78U appears to possess, like several other anticonvulsant drugs, sedative and anxiolytic effects. Such pharmacological properties are characteristic of the "typical" anxiolytics such as the classical benzodiazepines and nonbenzodiazepine agents displacing (^{3}H)benzodiazepines from their binding sites, but also of "atypical" agents such as meprobamate, phenobarbital, tracazolate or fenobam which do not displace benzodiazepines and for which the biological target remains unknown (13). However, we found that BW A78U binds to the benzodiazepine receptor with a very low affinity [(^{3}H)flunitrazepam binding, $IC_{50} = 13.6$ μM] (unpublished data). More recently, the parent 9-benzylpurine has been shown to present about the same affinity (9). It is unlikely that the behavioral effects of BW A78U can be related to its affinity with the GABA receptor-benzodiazepine-Cl channel complex.

Attention has been recently focused on the evidence supporting a neuromodulatory role for adenosine in the CNS (16). Adenosine presents hypnotic, anticonvulsant, muscle relaxant and putative anxiolytic activities (4,10). On the other hand, adenosine receptor antagonists, such as caffeine and theophylline, cause anxiety and even panic attacks (2). As the compound BW A78U structurally resembles adenosine, it can be suggested that the behavioral effects of BW A78U may be related to its putative action on the adenosinergic neuromodulatory system.

Further investigations are in progress for a better characterization of the anxiolytic effects of BW A78U and for the identification of the neurotransmission system involved in the CNS-properties of this compound and its analogues.

ACKNOWLEDGEMENTS

We thank Dr. P. Keane from the Sanofi Company, Toulouse, France, for the determination of the IC_{50} value of BW A78U for benzodiazepine receptors.

REFERENCES

1. Belzung, C.; Misslin, R.; Vogel, E.; Dodd, R. H.; Chapouthier, G. Anxiogenic effects of methyl-β-carboline-3-carboxylate in a light/dark choice situation. Pharmacol. Biochem. Behav. 28:29–33; 1987.
2. Charney, D. S.; Galloway, M. P.; Heninger, M. P. The effects of caffeine on plasma MHPG, subjective anxiety, autonomic symptoms and blood pressure in healthy humans. Life Sci. 35:135–144; 1984.
3. Crawley, J. N.; Goodwin, F. K. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol. Biochem. Behav. 13:167–170; 1980.
4. Dragunow, M. Purinergic mechanisms in epilepsy. Prog. Neurobiol. 31:85–108; 1988.
5. Kelley, J. L. Heterocyclic compounds useful in the treatment of CNS disorders, their pharmaceutical compositions, and their use. Eur. Pat. Appl. EP 157,637 (Wellcome Foundation Limited, 9 October 1985).
6. Kelley, J. L.; Soroko, F. E. 9-(2-Fluorobenzyl)-6-(methylamine)-9H-purine hydrochloride. Synthesis and anticonvulsant activity. J. Med. Chem. 29:1133–1134; 1986.
7. Kelley, J. L.; Krochmal, M. P.; Linn, J. A.; McLean, E. W.; Soroko, F. E. 6-(Alkylamino)-9-benzyl-9H-purines. A new class of anticonvulsant agents. J. Med. Chem. 31:606–612; 1988.
8. Kelley, J. L.; Krochmal, M. P.; Linn, J. A.; McLean, E. W.; Soroko, F. E. 9-(2-Fluorobenzyl)-6-(alkylamino)-9H-purines. A new class of anticonvulsant agents. J. Med. Chem. 31:1005–1009; 1988.
9. Kelley, J. L.; McLean, E. W.; Ferris, R. M.; Howard, J. L. Benzodiazepine receptor binding activity of 6,9-disubstituted purines. J. Med. Chem. 32:1020–1024; 1989.
10. Marangos, P. J.; Boulenger, J. P. Basic and clinical aspects of adenosinergic neuromodulation. Neurosci. Biobehav. Rev. 9:421–430; 1985.
11. Misslin, R.; Ropartz, P.; Mandel, P. Etude comparée du di-n-propylacétate et de l'oxazépam sur l'activité spontanée de la souris. CR Acad. Sci. (Paris) 281:175–1178; 1975.
12. Montgomery, J. A.; Temple, C., Jr. Synthesis of potential anticancer agents. XXVI. The alkylation of 6-chloropurine. J. Am. Chem. Soc. 83:630–635; 1961.
13. Patel, J. B.; Martin, C.; Malik, J. B. Differential antagonism of the anticonflict effects of typical and atypical anxiolytics. Eur. J. Pharmacol. 86:295–298; 1983.
14. Simiand, J.; Keane, P. E.; Morre, M. The staircase test in mice: a simple and efficient procedure for primary screening of anxiolytic agents. Psychopharmacology (Berlin) 84:48–53; 1984.
15. Thiébot, M. H.; Soubrié, P.; Simon, P.; Boissier, J. R. Spécificité d'action des tranquillisants mineurs dans le test de l'escalier. Relation entre ces effets et leurs propriétés anxiolytiques. J. Pharmacol. (Paris) 7:87–102; 1972.
16. Williams, M. Adenosine—a selective neuromodulator in the mammalian CNS? Trends Neurosci. May:164–168; 1984.