Pharmacology Biochemistry & Behavior, Vol. 26, pp. 705–707. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Delineation of Mu-Antagonist, Partial Kappa Agonist and Non-Opioid Agonist Activity of Cyclazocine Using Urinary Output of Rats

J. DAVID LEANDER

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285

Received 5 December 1986

LEANDER, J. D. *Delineation of mu-antagonist, partial kappa agonist and non-opioid agonist activity of cyclazocine using urinary output of rats.* PHARMACOL BIOCHEM BEHAV **26**(4) 705–707, 1987.—The effects of cyclazocine (SC) were studied under a variety of test conditions to determine its various activities on urinary output. Cyclazocine antagonized the morphine-induced (20 mg/kg) antidiuretic effect in water-loaded (3 ml/100 g b.wt.) rats at low doses (0.08, 0.16 and 0.32 mg/kg). At higher doses (1.25, 5 and 20 mg/kg), cyclazocine caused diuresis in normally hydrated rats. The diuresis produced by 1.25 and 5, but not 20 mg/kg, was antagonized by naloxone (10 mg/kg). In water-deprived rats and in normally hydrated rats, cyclazocine, in a dose-related fashion, antagonized the diuretic effects of 0.08 mg/kg of bremazocine. These results are compatible with the interpretation that cyclazocine has mu-antagonist activity at low doses, then partial kappa-agonist activity at intermediate doses, followed by non-opioid agonist activities at the higher doses.

Mu Kappa Non-opioid Cyclazocine Diuresis

OVER the years, opioids have generally been known to have antidiuretic effects; that is, opioid administration has usually led to a decrease in urinary output (e.g., [1]). Recently, full agonists or partial agonists at kappa-opioid receptors have been shown to cause increased urinary output, i.e., a diuretic effect [3–5, 13]. This increased urinary output effect appears to be due to suppression of plasma vasopressin levels [8,13].

Cyclazocine, a mixed agonist/antagonist, has been described as having morphine-antagonist actions [11], but also has been described as sharing common drug discriminative properties with ketazocine, oxilorphan, ethylketazocine and butorphanol [12,14]. Of the above compounds, ketazocine and ethylketazocine are full kappa agonists on the diuretic test, whereas oxilorphan and butorphanol are partial kappa agonists [3,4]. However, when cyclazocine was previously studied as to its diuretic effects, it was not characterized as to whether its effect was similar to a full or partial kappa agonist [3]. The purpose of the present experiments was to characterize more fully the effects of cyclazocine on urinary output.

METHOD

The subjects were a pool of 48 Long-Evans hooded male rats (Charles-River Breeding Laboratories, Inc., Portage, MI) weighing 400 to 500 g. They were used repeatedly in experiments on urinary output, but no more frequently than twice a week. The rats usually had free access to lab chow, and to tap water at all times except during the times when urinary output was being measured. The only exception to the above statement was when the effects of drugs were studied in rats that had been deprived of water for 24 hr before drug administration. The interaction of cyclazocine with morphine was studied in rats that were administered a water-load of 3 ml/100 g of body weight simultaneously with injection of drugs or vehicle. This volume of water-load is within the range others have used to measure antidiuretic effects of mu agonists such as morphine [1]. These procedures are similar to those previously used to study kappa agonists, partial agonists and antagonists, as well as mu agonists and antagonists [3–5, 9, 10].

All injections were administered SC in a volume of 1 ml/kg. Cyclazocine (Sterling-Winthrop Research Institute, Rensselaer, NY) was administered as the free base by dissolving in a minimally dilute lactic acid solution. Bremazocine HCl (Sandoz, Ltd., Basel, Switzerland) and naloxone HCl (Endo Laboratories, Garden City, NY) were dissolved in distilled water. When two injections were scheduled, they were administered into opposite sides of the lower abdominal area almost simultaneously.

RESULTS AND DISCUSSION

Figure 1 shows the effects of various doses of cyclazocine alone and in combination with a diuretic dose (0.08 mg/kg) of bremazocine in animals that were 24-hr water-deprived

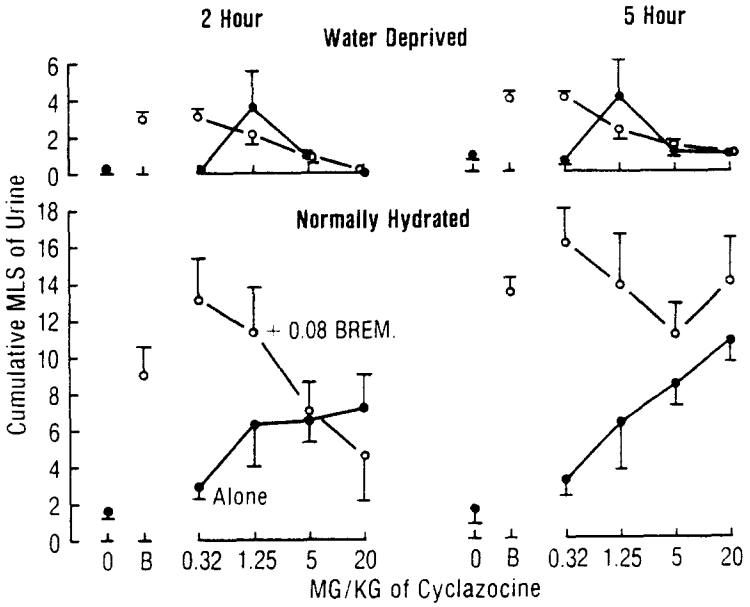

FIG. 1. Effects of various doses of cyclazocine alone (●) and in combination with 0.08 mg/kg of bremazocine (○) in animals that were 24-hr water-deprived (top) or normally hydrated (bottom) on cumulative urinary output at 2 (left) and 5 (right) hours. Each point is the mean (±S.E.M.) of 4–5 rats.

(upper) or normally hydrated (lower). Cyclazocine alone produced a dose-related increase in urinary output in normally hydrated rats. At 2 hr, the effect appeared to plateau over the doses of 1.25 to 20 mg/kg; whereas, at 5 hr it was more clearly dose-related over that dose range. In water-deprived rats, cyclazocine did not produce dose-related increases in urinary output. When cyclazocine was combined with bremazocine in normally hydrated rats, there was some antagonism of bremazocine at 2 hr but not at 5 hr after administration (Fig. 1). However, in the water-deprived rats, cyclazocine produced clearly dose-related antagonism of bremazocine's diuretic effect at both 2 and 5 hr.

Previous work has shown that the partial kappa agonist butorphanol does not produce diuretic effects in water-deprived animals, whereas the full kappa agonist bremazocine does [6]. This difference between partial and full kappa agonists has been replicated in unpublished experiments with the partial agonist nalorphine and the full agonists U-50,488H and ethylketazocine. Thus, the present data can be interpreted to mean that cyclazocine functions as a partial kappa agonist over the dose range of 1.25 mg/kg and above. The antagonist actions of cyclazocine on bremazocine's diuretic effect, specially in the water-deprived condition, support that interpretation. Also, the tendency for the magnitude of the diuretic effect to plateau, as seen at the 2-hr time period in normally hydrated rats, has been characteristic for other partial kappa agonists, such as nalorphine, butorphanol and oxilorphan [4].

Figure 2 shows the time course of the diuretic effects of three doses of cyclazocine with and without simultaneous administration of 10 mg/kg naloxone. The 10 mg/kg dose of naloxone has previously been shown to markedly antagonize the diuretic effects of kappa agonists [3,4]. In this experiment, naloxone markedly antagonized the diuretic effect of the 1.25 mg/kg dose of cyclazocine, partially antagonized the diuretic effect of 5 mg/kg after the first hour, and did not antagonize the effect of 20 mg/kg of cyclazocine at all. These results mean that the diuretic effect of 1.25 mg/kg of cyclazocine is completely due to an agonist action at some opioid receptor, whereas the diuretic effect of 20 mg/kg of cyclazocine is not the result of an agonist action at an opioid receptor, but rather due to some other pharmacological activity unrelated to opioid receptors.

Figure 3 shows the mu antagonist action of cyclazocine. These data were obtained in rats that were normally hydrated before drug administration and then administered a water-load of 3 ml/100 g simultaneously with drug injections. The points on the far left show the effects of 20 mg/kg of morphine plus cyclazocine vehicle. Morphine alone produced a marked antidiuretic effect evident at 2 hr which was gone at 5 hr. That antidiuretic effect of morphine was antagonized by lower doses of cyclazocine (0.16 and 0.32 mg/kg) than those which produce diuretic effects (Fig. 1). Note that the diuretic effects produced by the larger doses of cyclazocine, coupled with the mu antagonism, result in urinary outputs above the parallel lines (1.25 and 5 mg/kg).

These data show that cyclazocine produces mu-receptor antagonist activity beginning at low doses (0.16 and 0.32 mg/kg). At slightly higher doses (>0.32 mg/kg), cyclazocine produces partial kappa agonist activity. At the 20 mg/kg dose cyclazocine alone, there is a diuretic effect produced which is not related to an agonist action at opioid receptors, since it was not blocked by a large (10 mg/kg) dose of naloxone. This non-opioid agonist action could be the phencyclidine-like action of cyclazocine observed in other experimental paradigms [2, 7, 14]. Under similar test conditions, phencyclidine produced a marked diuretic response which was unaffected by naloxone (10 mg/kg) administration [3]. The diuretic effect of phencyclidine is eliminated by water deprivation (unpublished study), just as was the diuretic effect of the non-opioid agonist action of cyclazocine in the present study.

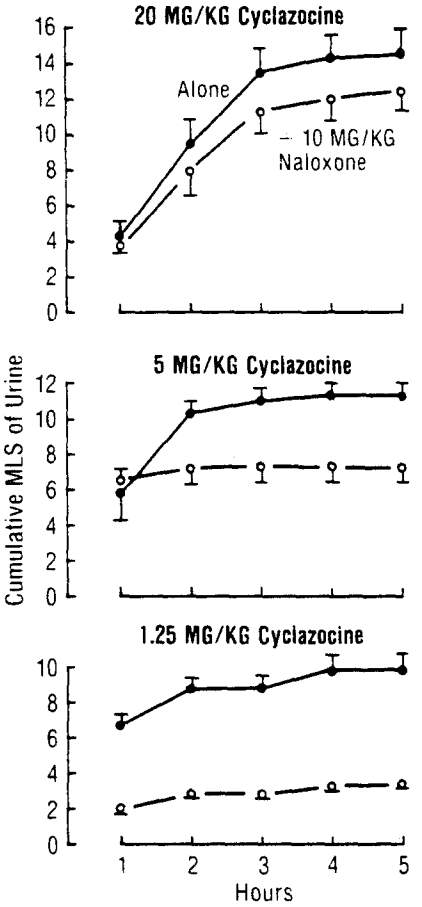

FIG. 2. Time course of the diuretic effect of 3 doses of cyclazocine with (○) and without (●) simultaneous administration of 10 mg/kg of naloxone. Each point is the mean (±S.E.M.) of 4–5 rats.

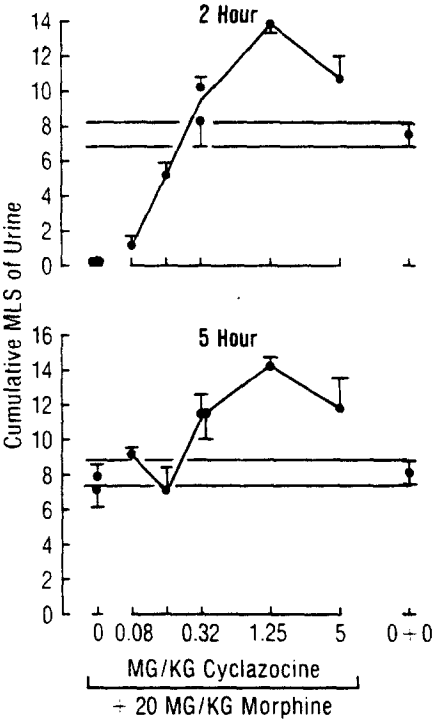

FIG. 3. Effects of various doses of cyclazocine on the antidiuretic effect of 20 mg/kg of morphine in water-loaded rats at 2 (top) and 5 (bottom) hours after injection. The parallel lines in each frame shown ±1 S.E.M. around the mean (shown on far right) urine output of rats water-loaded (3 ml/100 g) and administered both the cyclazocine vehicle and the morphine vehicle. Each point is the mean±S.E.M. of 4–5 rats. When a point was multiply determined, both values are shown.

ACKNOWLEDGEMENTS

I thank the pharmaceutical companies that donated drugs used in this study, Mr. John Hart for technical assistance, Mr. Jack Clary for artwork, and Mrs. Joan Hager for manuscript preparation.

REFERENCES

1. Huidobro, F. Antidiuretic effect of morphine in rats: Tolerance and physical dependence. *Br J Pharmacol* **64** 167–171, 1978.
2. Leander, J. D. Comparison of phencyclidine, etoxadrol, and dexoxadrol in the pigeon. *Subst Alcohol Actions Misuse* **2**: 197–203, 1982.
3. Leander, J. D. A kappa opioid effect: Increased urination in the rat. *J Pharmacol Exp Ther* **224**: 89–94, 1983.
4. Leander, J. D. Evidence that nalorphine, butorphanol and oxilorphan are partial agonists at a κ-opioid receptor. *Eur J Pharmacol* **86**: 467–470, 1983.
5. Leander, J. D. Further study of kappa opioids on increased urination. *J Pharmacol Exp Ther* **227**: 35–41, 1983.
6. Leander, J. D. Kappa opioid agonists and antagonists: Effects of drinking and urinary output. *Appetite* **5**: 7–14, 1984.
7. Leander, J. D., C. R. Wood, D. M. Zimmerman and L. A. Dykstra. Phencyclidine-type catalepsy in the pigeon: An update on Chen's work. *Drug Dev Res* **7**: 75–85, 1986.
8. Leander, J. D., R. L. Zerbe and J. C. Hart. Diuresis and suppression of vasopressin by kappa opioids: Comparison with mu and delta opioids and clonidine. *J Pharmacol Exp Ther* **234**: 463–469, 1985.
9. Leander, J. D. and D. M. Zimmerman. Picenadol, a mixed opioid agonist/antagonist without kappa opioid agonist activity in the rat urination test. *Drug Dev Res* **3**: 483–488, 1983.
10. Leander, J. D. and D. M. Zimmerman. Antagonism of bremazocine-induced urination as a test for kappa-opioid receptor antagonists within the phenylpiperidine series. *Drug Dev Res* **4**: 421–427, 1984.
11. McMillan, D. E. and L. W. Harris. Behavioral and morphine-antagonist effects of the optical isomers of pentazocine and cyclazocine. *J Pharmacol Exp Ther* **180**: 569–572, 1972.
12. Schaefer, G. J. and S. G. Holtzman. Disriminative effects of cyclazocine in the squirrel monkey. *J Pharmacol Exp Ther* **205**: 291–301, 1978.
13. Slizgi, G. R. and J. H. Ludens. Studies on the nature and mechanism of the diuretic activity of the opioid analgesic ethylketocyclazocine. *J Pharmacol Exp Ther* **220**: 585–591, 1982.
14. Teal, J. J. and S. G. Holtzman. Discriminative stimulus effects of cyclazocine in the rat. *J Pharmacol Exp Ther* **212**: 368–376, 1980.