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Effects of Kappa-Opioid Receptor Agonists and Morphine on Food Intake and Urinary Output in Food-Deprived and Nondeprived Rats

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RAMARAO, P. AND H. N. BHARGAVA. *Effects of kappa-opioid receptor agonists and morphine on food intake and urinary output in food-deprived and nondeprived rats.* PHARMACOL BIOCHEM BEHAV 33(2) 375–380, 1989.—The effects of kappa-opioid receptor agonists, bremazocine, U-50, 488H and tifluadom and of a mu-opioid receptor agonist, morphine, on food intake and urinary output in food-deprived and nondeprived Sprague-Dawley rats was determined. In food-deprived animals, intraperitoneal administration of bremazocine at 0.1 mg/kg increased food intake but at 1.0 and 10.0 mg/kg doses decreased it. Tifluadom (0.1–10.0 mg/kg) had no effect on food intake. U-50,488H at 1.0 mg/kg increased food intake, whereas 10.0 mg/kg dose decreased the food consumption. In nondeprived rats, the kappa-opioid receptor agonists failed to produce any effect on food consumption. In food-deprived rats, all the three kappa-opioid receptor agonists increased the urinary output at the highest dose (10 mg/kg). In nondeprived rats similar effects as in food-deprived rats were observed except bremazocine increased urinary output at all the doses used. These results with kappa-opioid agonists may be related to either the existence of more than one population of kappa-opioid receptors or their differential actions at the opioid receptor types.

Kappa opioid receptor agonists Bremazocine Tifluadom U-50,488H Food intake Urinary output Food deprivation Sprague-Dawley rats

THE concept of multiplicity of opioid receptors is well recognized (35). Although five major opioid receptors namely μ, δ, κ, σ, ϵ have been postulated, studies have been directed to understand the role of μ, δ and κ- receptors in various physiological and pathophysiological states. Opioid receptors have been implicated in a variety of functions including ingestive behavior. Thus drugs acting at μ-, δ- and κ- receptors appear to increase feeding behavior in satiated animals (16). However, in some cases opposite effects of μ- and κ-opioid drugs have been reported. For example, μ-agonists decrease while κ-opioid drugs have been reported. For example, μ-agonists decrease while κ-agonists increase the urinary output. Several factors such as food deprivation, water deprivation, stress and circadian rythms, which influence the endogenous opioid system, can modify the pharmacological effects of exogenously administered opiates.

The effect of morphine on food intake has been controversial since factors like food deprivation or nondeprivation, doses used and the duration for which measurements made, have produced differential results (16). In general, morphine reduces food intake in food-deprived rats and increases it in food-nondeprived rats (29). Higher doses, which produce general depressant effects reduce food intake, while lower doses of morphine increase food intake (10,15).

Similarly, evidence has also been accumulated which suggests a role of the κ-opioid receptor system in the maintenance of feeding behavior. A variety of κ-agonists, such as tifluadom, butorphanol, bremazocine and U-50,488, have all been demonstrated to increase feeding (1, 2, 14, 17, 19–21, 28). Although dynorphin (1–13), an endogenous ligand for the κ-opioid receptor has been suggested to be the major mediators of the opioid feeding drive, other peptides, such as β-endorphin (13) and D-Ala^2-D-Leu^5-enkephalin (31) have also been shown to stimulate feeding after central administration.

Mu-agonists like morphine cause antidiuresis (6), whereas κ-agonists produce diuresis (11,12). The antidiuretic effect of μ-agonists has been suggested to be due to the release of antidiuretic hormone (ADH) (34). Kappa-opioid receptor agonists produce diuresis in normally-hydrated rats (30) as a result of inhibition in the release of ADH from the neurohypophysis (11,12).

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The effect of food deprivation on the levels of endogenous opioids and their receptors has also been studied. The levels of immunoreactive (ir)-dynorphin were shown to be altered under conditions which alter the feeding drive (18,22). Food deprivation increases ir-β-endorphin in plasma (3) and decreases it in the brain (4). Other studies have shown the brain β-endorphin levels are increased with food deprivation (9,22). Finally, food deprivation has been shown to increase the binding of ^{3}H-dynorphin (1–8) within the central nervous system (32).

The actions of morphine, a μ-agonist, on food and water intake are affected by food deprivation (29). However, little is known about the effect of food deprivation on κ-opioid receptor agonist-induced ingestion of food and output of urine.

Studies form this laboratory indicate that kappa opioid receptor agonists, U-50,488H, bremazocine and tifluadom produce differential effects on morphine-induced analgesia in morphine-naive and morphine-tolerant rats (7,24). Further, Cooper *et al.* (2) reported that the effects of kappa-opioid receptor agonists in feeding were not uniform. In view of these results, the present studies were undertaken to determine the effects of three kappa-opioid agonists bremazocine (25), U-50,488H (33) and tifluadom (26,27) and a μ-opioid agonist morphine, on food intake in food-deprived and food-nondeprived rats. Since κ-agonist also produce diuresis, the effects of kappa agonists on urinary output in normally hydrated rats which were either deprived of food or were given food ad lib have also been determined.

METHOD

Animals

Male Sprague-Dawley rats (200–250 g) obtained from the King Animal Co., Oregon, WI were housed three to a cage, in a room with controlled temperature (23 ± 1°C), humidity (50 ± 10%) and artificial light (0600–1800 hr). The animals had continuous access to food (Purina rat chow) and water. Experiments were carried out only after the animals had been acclimated to the above environment for at least four days.

Drugs

Morphine sulfate was obtained form Mallinckrodt Chemical Co., St. Louis, MO. Tifluadom [1-methyl-2(3-thienyl carbonyl) amino methyl-5-(2-fluorophenyl) H-2,3-dihydro-1,4-benzodiazepine] and bremazocine hydrochloride were obtained as gifts from Dr. D. Romer of Sandoz Pharmaceuticals, Basel, Switzerland and Dr. H. Zeugner of Kali-Chemie Pharma Ltd., Hanover, FRG. U-50,488H [trans-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide methane sulfonate) was gift from Dr. Philip von Voigtlander of Upjohn Company, Kalamazoo, MI. The drugs were dissolved in distilled deionized pyrogen free water and injected intraperitoneally in a volume such that each rat received 1 ml/kg of the drug solution. The doses represent the salt form of the drugs.

Experimental Procedures

Measurement of food intake. Before the actual experiments, all rats were placed individually in metabolism cages every day for six hours for at least three days to habituate them. On the day of the experiment, rats were housed individually in clear plastic rat metabolism cages (Nalgene, Italy). Preweighed amount of powdered food (Purina rat chow) was provided in the food hopper. The animals readily consumed the powdered food by licking. Water was withheld during the experimental period. The amount of food consumed was determined by weighing the residual powder to the nearest 0.1 g at two hourly intervals. Food that was lost by spillage was collected and appropriate corrections were made. Determinations were made of the amounts of food ingested every two hours in six-hour blocks in the light period (1000–1600 hr) following intraperitoneal injections of the kappa-opioid agonists or the saline. To avoid possible effects of diurnal rythms, drugs were administered at 1000 hr. each time the experiment was performed. The amount of food consumed in 2, 4 and 6 hr was expressed as g/kg of body weight. Six rats were used for each treatment group.

The experiments were conducted with rats which were either deprived of food or had free access to food. In food-nondeprived protocol, the animals had continuous access to the food and water before the experiment. In food-deprived protocol, the food was withheld for 24 hr before the experiment, however, water was available continuously.

Measurement of urinary output. The volume of urine voided was collected in graduated cylinders attached to the metabolism cages and measured. The rats were injected with either the vehicle or an appropriate dose of the drug. The urinary output for a 4-hour period was recorded and expressed as mean volume (ml) ± S.E.M. Six rats were used for each treatment group.

Statistics

Mean group food intake was calculated and statistical evaluation between the groups was made using analysis of variance followed by Scheffe's S-test. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Food Consumption in Food-Deprived and Nondeprived Rats

The food intake in the food-deprived and food-nondeprived animals which were injected with the vehicle significantly differed at all the intervals studied ($p<0.05$). In food-nondeprived animals the food consumption was significantly ($p<0.005$) lower than that of food-deprived animals.

Effect of Opioid Receptor Agonists on Food Intake in Food-Deprived Rats

Morphine (5 mg/kg, IP) significantly ($p<0.01$) increased food consumption at all intervals of the study in food deprived animals (Fig. 1). As the time progressed after the administration of drug the level of significance decreased. At 10 mg/kg dose, morphine did not affect the food intake. At the highest dose studied (20 mg/kg) morphine tended to decrease the food consumption though the effect was not statistically significant.

Two hours after the administration, bremazocine produced differential effects on food consumption which depended upon the dose. At the lowest dose (0.1 mg/kg, IP), bremazocine significantly increased food intake, whereas 1.0 and 10.0 mg/kg doses significantly attenuated the food intake. The food intake caused by 0.1 mg/kg of bremazocine was significantly greater than the control at all time intervals of the study. The inhibitory effect of bremazocine (10 mg/kg) was significant up to 4 hours.

U-50,488H (1 mg/kg) significantly enhanced the food ingestion at 2 and 4 hr after its administration, however, at 6 hr the effect was not significant. At 10 mg/kg dose, U-50,488H decreased the food consumption. At the lowest dose (0.1 mg/kg) it did not produce any effect. This indicates that the dose-response curve for U-50,488H on food intake is U-shaped.

Tifluadom, at all the doses studied did not produce any significant effect on food consumption.

Effect of Opioid Agonists on Food Intake in Food-Nondeprived Animals

The effect of morphine and the kappa-opioid agonists on the

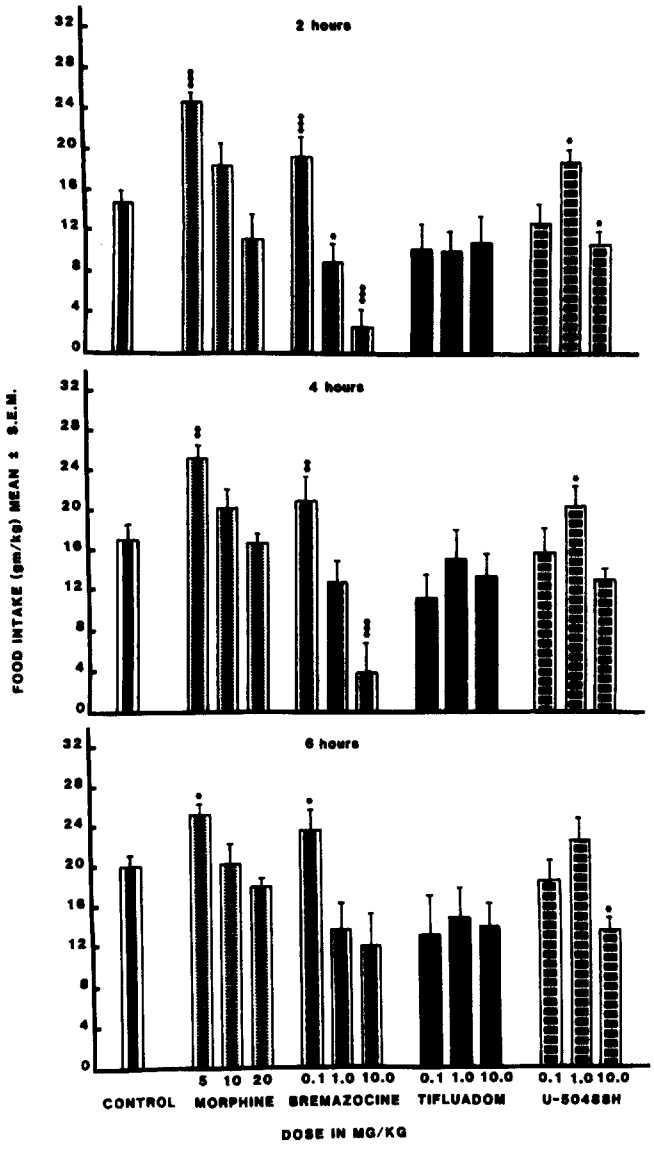

FIG. 1. Effect of morphine and kappa-opioid agonists on 2-, 4- and 6-hour food intake in 24-hr food-deprived rats. The vertical bars represent the S.E.M. * denotes $p<0.05$; ** denotes $p<0.01$; *** denotes $p<0.001$.

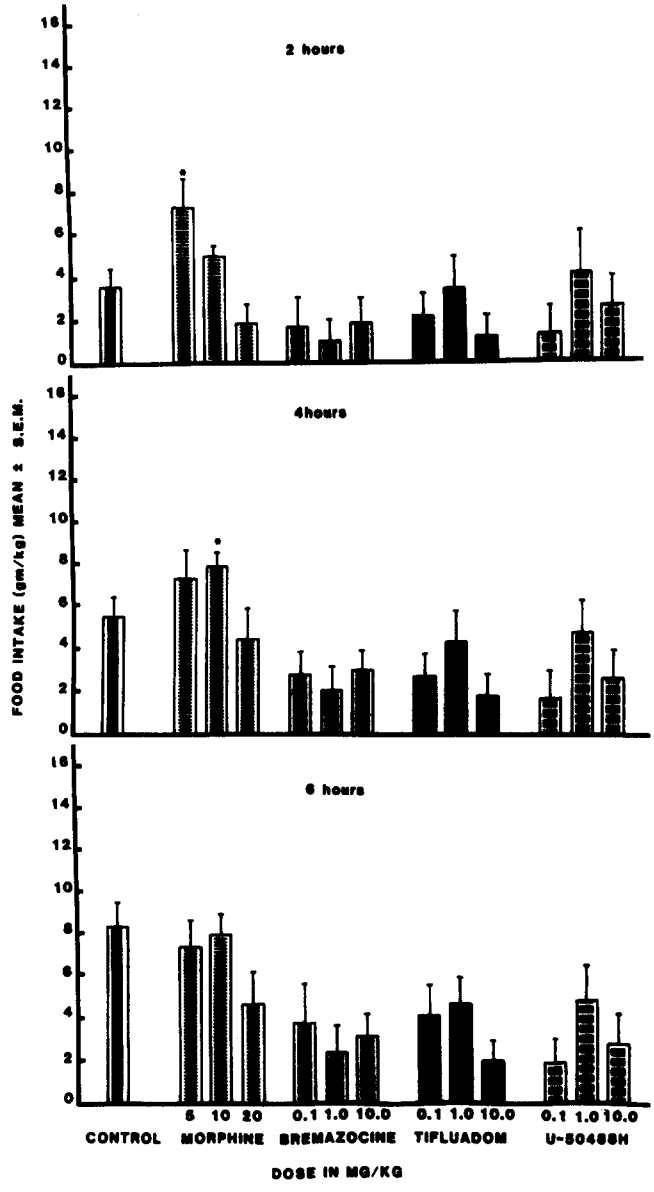

FIG. 2. Effect of morphine and kappa-opioid agonists on the 2-, 4- and 6-hour food intake in food-nondeprived rats. The vertical lines denote the S.E.M. * denotes $p<0.05$.

cumulative food intake at 2, 4 and 6 hr in nondeprived animals is shown in Fig. 2.

Morphine at low doses (5 mg/kg, IP) significantly increased the food consumption at 2 hr. The 10 mg/kg dose enhanced the food intake at 4 hours after its administration. However, at the highest dose, morphine decreased the food consumption though it was not significant. None of the kappa-agonists increased the food intake when administered intraperitoneally in nondeprived animals.

Effect of Opioid Agonists on Urinary Output in Food-Deprived and Nondeprived Animals

The effect of morphine and kappa agonists on cumulative urinary output 4 hr after their administration in food-deprived and food-nondeprived animals is shown in Fig. 3 and Fig. 4, respectively.

The volume of urine voided in control animals during 4-hour period was 2.0 ± 0.17 ml. Morphine at all doses failed to produce any effect on urinary output in food-deprived and food-nondeprived animals. Bremazocine, tifluadom and U-50,488H at the highest dose significantly increased the urinary output in food-deprived animals. Bremazocine, at all doses, significantly increased urinary output in food-nondeprived animals. In general, the urinary output was more ($p<0.01$) in food-nondeprived animals than food-deprived animals.

DISCUSSION

The present studies clearly indicate that morphine at low doses (5 mg/kg) increases food consumption in food-deprived and nondeprived animals, but at high dose (20 mg/kg) tended to

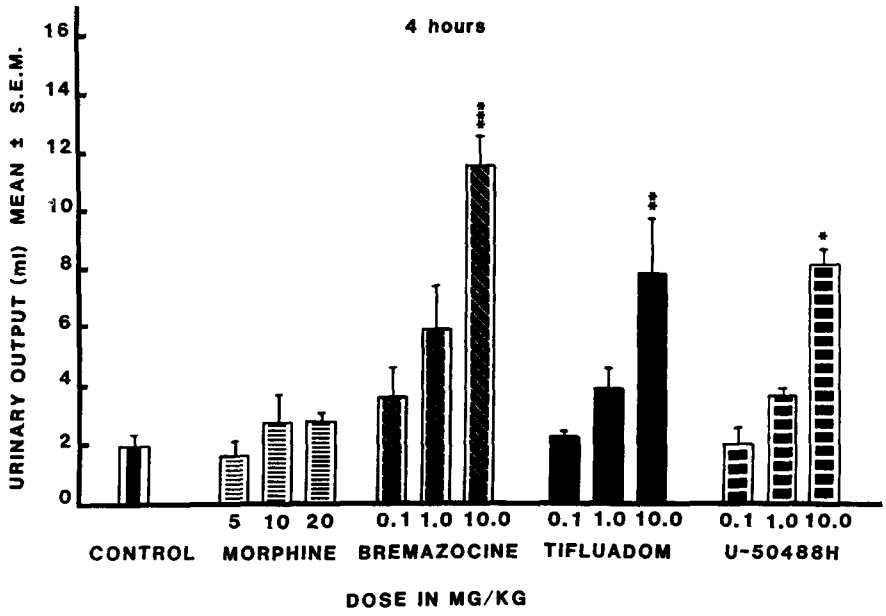

FIG. 3. Effect of morphine and kappa-opioid agonists on the 4-hour urinary output in 24-hour food-deprived rats. The vertical lines represent the S.E.M. * denotes $p<0.05$; ** denotes $p<0.01$; *** denotes $p<0.001$.

decrease the food consumption. The results obtained in food-deprived rats are in agreement with those of Sanger and McCarthy (29). The decrease in the food consumption observed at high doses of morphine may be due to the masking of feeding effects by the other opioid behavioral effects such as sedation.

Administration of kappa-agonists also modified food intake behavior differentially in food-deprived and nondeprived rats. In rats which were nondeprived none of the kappa-opioid receptor agonists injected intraperitoneally produced any effect on food intake at any of the doses used. Kappa agonists in the same dose range, however, when injected subcutaneously, enhanced the food intake (16). The results suggest that the route of administration can influence the outcome of such experiments. Differential effects of food intake have been observed with tifluadom when administered

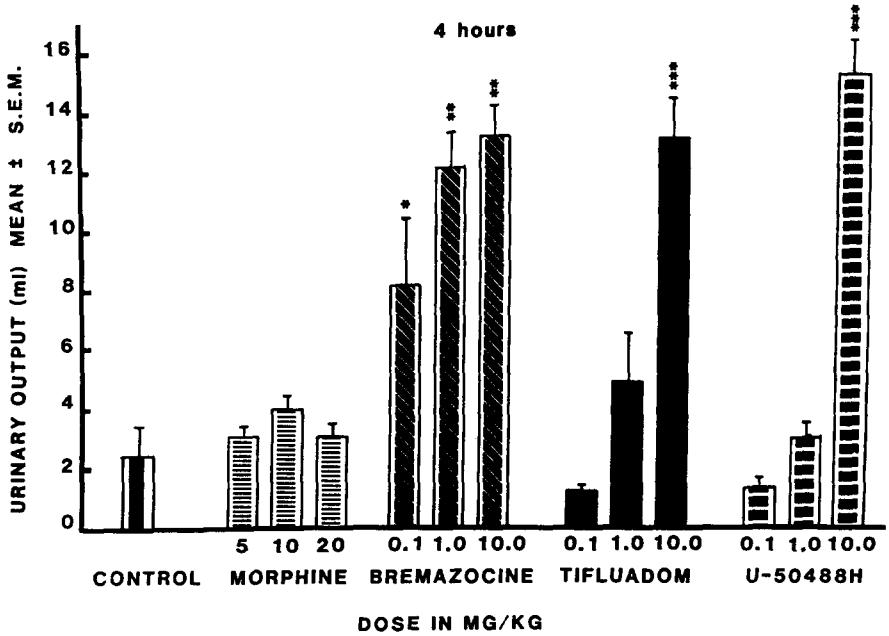

FIG. 4. Effect of morphine and kappa-opioid agonists on the 4-hour urinary output in food-nondeprived rats. The vertical lines represent the S.E.M. * denotes $p<0.05$; ** denotes $p<0.01$; *** denotes $p<0.001$.

subcutaneously and intraperitoneally. In food-nondeprived rats, subcutaneous injections of tifluadom increased food intake, whereas intraperitoneal injections decreased food intake (2). Other reasons for such differences may include the use of powdered food in the present study. Most other investigators have used food pellets. Another reason may be the time of day when the studies were conducted. In the present investigation, all experiments were done at the same time of the day to avoid any effect of diurnal rhythms. In food-deprived rats, kappa-opioid receptor agonists produced differential effects. Bremazocine, at low doses, increased food intake but at higher doses inhibited it. Tifluadom had no effect at any of the doses used. U-50,488H at the lowest doses (0.1 mg/kg) used had no effect, 1.0 mg/kg dose increased it and high dose (10 mg/kg) inhibited it. The effects of kappa-opioid drugs on food intake in food-deprived animals has not been determined before.

Although the effects of morphine and other kappa opioid agonists on urinary output in normally-hydrated animals has been determined (11,12), studies have not been done to examine the effect of these drugs in normally-hydrated rats which were either deprived or nondeprived of food. Morphine did not affect the urinary output in either the food-deprived or nondeprived rats. All the kappa-opioid agonists produced a dose-dependent effect on urinary output in both food-deprived and nondeprived rats. Tifluadom, bremazocine and U-50,488H only at the highest dose (10 mg/kg) increased the urinary output in food-deprived and nondeprived rats. The lower dose (1.0 mg/kg) of tifluadom and U-50,488H did not significantly increase the urinary output. On the other hand, bremazocine increased urinary output in nondeprived rats at all doses used. In general, the increase in urinary output by kappa agonists was greater in nondeprived rats.

Several things are evident from the present studies. The effects of kappa opioid receptor agonists on food intake in food-deprived and nondeprived rats are complex and depend on the state of the animal and the dose used. It has been shown that the hyperphagic response to many opioid drugs appear to have a very narrow range since at lower doses the effects are not evident and at higher doses, perhaps due to the sedative action of the drug the food intake is inhibited. This then would lead to an inverted U-shaped dose-response curve, an effect observed in the present studies and also with another kappa drug PD 117302 (5). It is interesting to note that PD 117302, a kappa-opioid agonist, decreased the food intake without affecting the water intake (5). Thus, the effect on the food intake were dependent on the sedative properties of the drugs. The kappa-agonists produced a dose-dependent increase in the urinary output in both food-deprived and nondeprived rats.

The differences in the actions of various opioid agonists on food intake can be accounted for by their selectivity at the opioid receptor subtypes. Studies by Wood (36) and by James and Golstein (8) indicate that U-50,488H is the most selective ligand for the kappa opioid receptor, although it does have action at the μ- and δ-opioid receptors. Tifluadom, on the other hand, was equally potent at the μ- and κ-opioid receptor and less active at the δ-site. Bremazocine is not selective at all.

In summary, kappa-opioid agonists produce complex actions on food intake which are dictated upon by the fed state of the animal, dose and selectivity of the agent used and the sedative effect of the drug. However, the effect on urinary output produced by kappa-opioid agonists are independent of the fed state in animals which are normally hydrated. It appears that the receptor systems controlling the ingestive behavior and diuresis may be different.

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