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Effects of Mu- and Kappa-Opioid Receptor Agonists on Urinary Output in Mice

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RATHBUN, R. C., R. W. KATTAU AND J. D. LEANDER. *Effects of mu- and kappa-opioid receptor agonists on urinary output in mice.* PHARMACOL BIOCHEM BEHAV 19(5) 863–866, 1983.—The effects of ethylketazocine, morphine, bremazocine and naloxone were determined on urinary output and weight loss in Cox mice. Morphine and fentanyl were also studied in Harlan mice. Bremazocine, ethylketazocine and morphine markedly increased urinary output and weight loss within 5 hr after injection. Naloxone antagonized the diuretic actions of morphine (5 mg/kg) and bremazocine (0.06 mg/kg) over a similar dose range (0.3–10 mg/kg). By comparison with the other agonists, fentanyl had little effect on urinary output or weight loss. These results suggest that kappa agonist activity increases urinary output in mice just as reported for rats. The data also suggest that in mice morphine has some kappa agonist activity, whereas fentanyl does not.

Kappa-opioid agonists Bremazocine Ethylketazocine Urination Mice

MORPHINE-like compounds have usually been reported to produce a decrease in urinary output (an antidiuretic effect) [3]. However, in mice morphine and several cyclazocine-related benzomorphans increase the urinary output of mice [1]. Other benzomorphans, such as pentazocine, did not increase urination [1]. Later oxilorphan and butorphanol were shown to increase urination in the rat by decreasing vasopressin release [7], but the receptor at which this activity was produced was not identified.

Recently, it has been shown that increased urinary output in the rat is produced by kappa-opioid receptor agonist activity [5, 10, 14], whereas mu-opioid agonist activity does not increase urinary output [5,6]. The purpose of the present investigation was to compare the diuretic activities of prototype mu- and kappa-opioid agonists in the mouse. The compounds selected were morphine, a mu-opioid agonist, and the two kappa-opioid agonists, ethylketazocine and bremazocine [4,8]. When morphine was found to increase urinary output in the Cox mice initially studied, the effects of morphine and fentanyl were studied also in mice from Harlan. The objective of these comparisons was to evaluate the generality of the morphine effect across another strain of mice and to see if the increased urination could be produced by fentanyl, a more potent mu-opioid agonist.

METHOD

Male mice from two different suppliers [Lai:Cox (Standard) BR, hereafter called Cox, from Laboratory Supply Co., Inc., Indianapolis, IN, and Hsd:(CF1)BR, Harlan Sprague-Dawley, Inc., Indianapolis, IN] were used. Prior to testing, they had been housed for at least 3 days in group cages in a colony room which was illuminated between 7 a.m. and 7 p.m. each day, and had free access to food and water. Each mouse was only used once.

To measure urinary output, the mice, in groups of five, were marked for identification, weighed to the nearest 0.1 g, administered drug or saline and placed in metabolism cages. Three cages of five mice each were used for each treatment. Urine was collected in graduated centrifuge tubes, and the volumes were recorded at hourly intervals for five hours. At the end of the five hours, the individual animals were reweighed. Food and water were not available during the 5-hr period in the metabolism cage.

The drugs used and the forms in which the doses were calculated are morphine sulfate (Lilly), bremazocine hydrochloride (Sandoz Ltd., Basel, Switzerland), ethylketazocine methane sulfonate (Winthrop Research Institute, Rensselaer, NY), fentanyl citrate (McNeil Laboratories, Fort Washington, PA), and naloxone hydrochloride (Endo Laboratories, Inc., Garden City, NY). These drugs were dissolved in saline, and saline was used as a vehicle injection. The total volume of the SC injections was 0.01 cc/g of body weight.

RESULTS

Figure 1 shows the effects of morphine and ethylketazocine in Cox mice. At 1 hr and 2 hr after morphine injection there was an inverted U-shaped dose-effect curve

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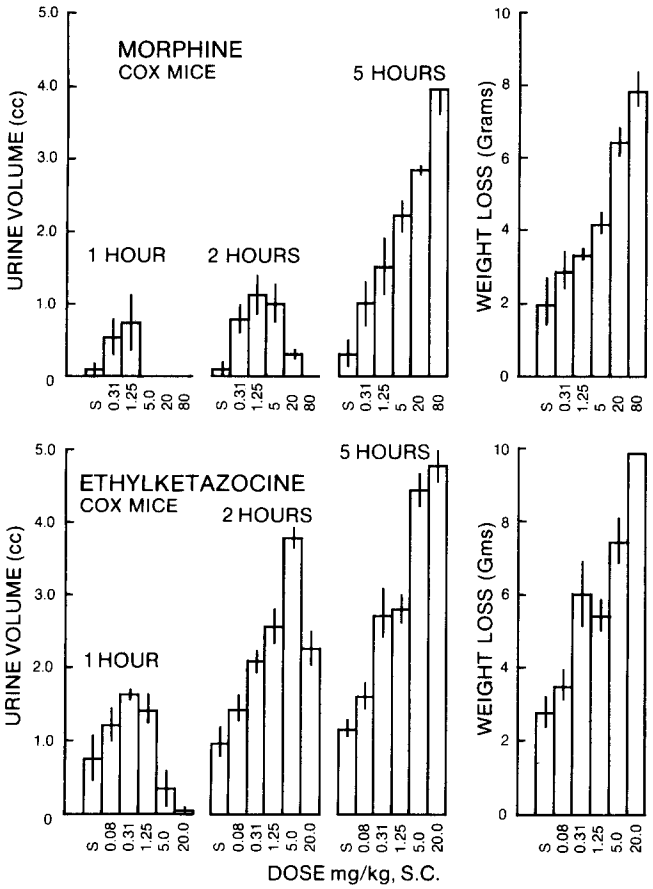

FIG. 1. The effects of morphine (top half of figure) and ethylketazocine (lower half) on cumulative urine output (at 1, 2 and 5 hr after injection) and on 5-hr weight loss as a function of dose in Cox mice. The points and brackets show the mean ± S.E. of 3 cages (each cage contained 5 mice). The results above S show the effects after saline injections.

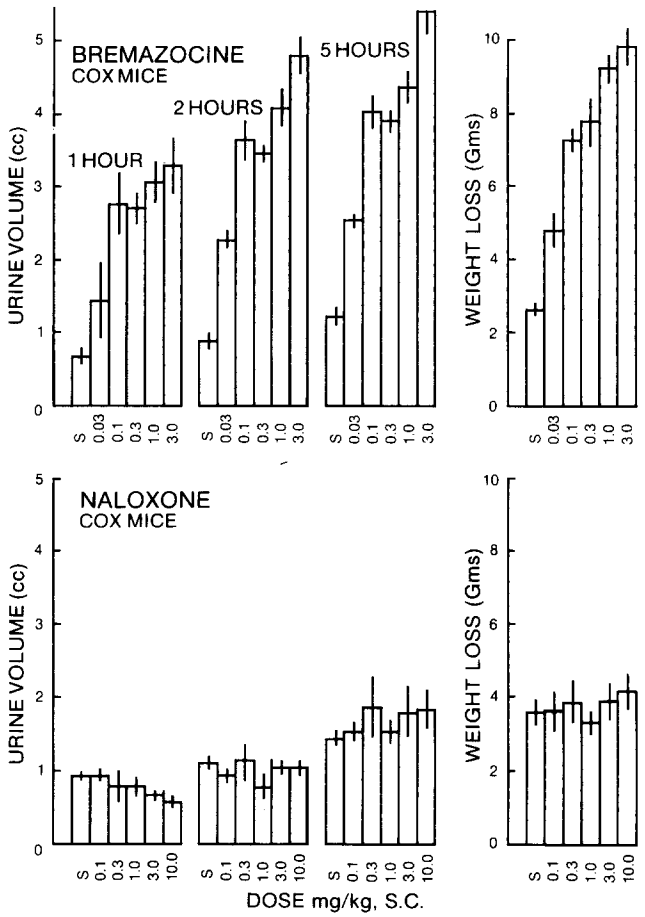

FIG. 2. The effects of bremazocine (top) and naloxone (bottom) on cumulative urine output and weight loss in Cox mice. Other details as in Fig. 1.

on urine output compared to the control value after saline administration. In contrast, at 5 hr after injection of morphine, there was a nice dose-related increase in cumulative urinary output, with 80 mg/kg of morphine producing an 8-fold increase in urine output compared to the controls. The frame to the right shows the weight loss that occurred per individual mouse as a function of morphine dose. There was a nice dose-related increase in weight loss, with the mice that received 80 mg/kg of morphine losing almost 4 times as much weight as the control mice.

Similar effects are shown in the lower part of Fig. 1 for ethylketazocine. The major difference from morphine was that ethylketazocine was at least 4-fold more potent than morphine. Ethylketazocine also clearly produced a larger peak increase in urine output at the 2-hour time than morphine had at that same time period.

The top of Fig. 2 shows the dose-effect data with bremazocine. In contrast to the effects seen with morphine and ethylketazocine, bremazocine produced clear dose-related increases at every time period. Likewise, there was also a dose-related increase in weight loss over the 5-hr period.

The lower part of Fig. 2 shows the effects of a range of doses of naloxone alone. It can be seen that naloxone alone had no effects on urinary output at any time period or on weight loss at 5 hr after injection. Although naloxone had no effects of its own on either urinary output or weight loss, it did antagonize the effects of morphine (5 mg/kg) and bremazocine (0.06 mg/kg) on both urine output and weight loss (Fig. 3). These antagonisms of the effects of morphine and bremazocine appeared to occur at relatively similar doses of naloxone. Note that in the first hour, low doses of naloxone (0.3 and 1.0 mg/kg) revealed a diuretic effect of 5 mg/kg of morphine, whereas this effect was antagonized by larger doses of naloxone.

Figure 4 shows the effects of morphine and fentanyl in mice from Harlan. Morphine decreased urinary output after higher doses at 1 and 2 hr after injection, and increased urinary output at the 5-hr time. This urinary increase at the 5-hr period was correlated with weight loss during the 5-hr time period. Fentanyl was relatively without effect except that the highest dose decreased urinary output at the 1- and 2-hr period, and increased urinary output at the 5-hr time. In contrast to the other drugs tested, fentanyl did not produce a dose-related increase in weight loss.

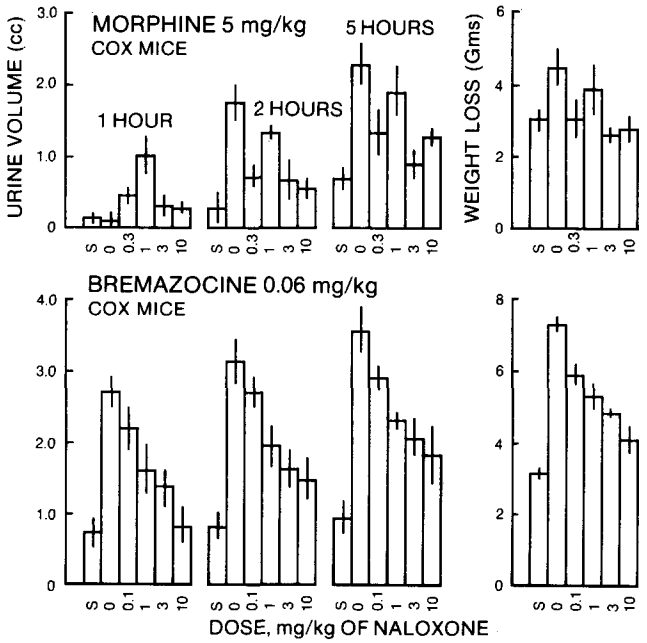

FIG. 3. The effects of various doses of naloxone on the increased urination effects of 5 mg/kg of morphine (top) and 0.06 mg/kg of bremazocine (bottom). The data above S show the effects of saline alone, whereas those above 0 show the effects of either morphine or bremazocine alone. The data above the naloxone doses show the effects of the dose of naloxone combined with morphine or bremazocine. Other details as in Fig. 1.

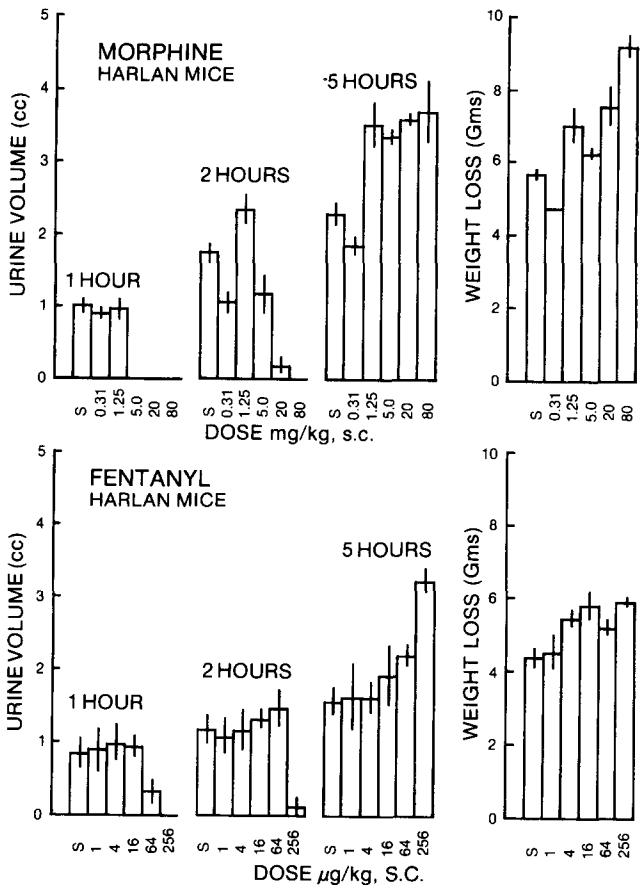

FIG. 4. The effects of morphine (top) and fentanyl (bottom of figure) on cumulative urine output and weight loss in Harlan mice. Note that fentanyl doses are in μg/kg, rather than mg/kg for all other doses previously. Other details as in Fig. 1.

DISCUSSION

The present results show that in mice the kappa agonists, ethylketazocine and bremazocine, and the mu-opioid agonist, morphine, increased urinary output within 5 hr after injection, and that this was correlated with a substantial weight loss during this period. Morphine also increased urine output and produced weight loss in the Harlan mice, whereas by comparison, fentanyl was relatively without effect. Only the highest dose of fentanyl affected urine output (decreases at 1 and 2 hr; increase at 5 hr), and no weight loss was produced by any dose of fentanyl. These data would suggest that increased urination in the mouse, as in the rat [5], is a result of agonist activity at kappa opioid receptors. This hypothesis is supported by the observation that the bremazocine- and morphine-induced increases in urinary outputs were decreased by similar doses of naloxone. Since naloxone has been shown to be at least 4–10 times more effective in antagonizing mu-opioid agonist actions than in antagonizing kappa-opioid agonist actions [5, 6, 9], the antagonism of both morphine- and bremazocine-induced urination over a similar naloxone dose range supports the view that these effects on urination were due to agonist actions at kappa-opioid receptors. The doses of naloxone which were effective in antagonizing the increased urinary output are much larger than the doses (0.02–0.32 mg/kg) which typically antagonize the analgesic effects of morphine [2, 11, 12, 13].

Two other observations support the hypothesis that increased urination was produced by kappa agonist action and not an action at a mu-opioid receptor. The first such observation is the fact that morphine and ethylketazocine produced an inverted U-shaped dose-effect curve in the early time periods after injection. This was previously reported for ethylketazocine and ketazocine in the rat [5], and was interpreted as being due to kappa agonist activity at intermediate doses, whereas the decrease after higher doses was due to mu-opioid actions relating to antidiuretic effects. The latter conclusion was due to the fact that low doses (0.01 and 0.1 mg/kg of naloxone antagonized the decrease (mu-opioid effect), whereas 10 mg/kg of naloxone was required to antagonize the diuretic effect (kappa-opioid effect) [5]. Bremazocine does not show this type of effect because, rather than having mu-agonist action as ethylketazocine and ketazocine have [5,14], bremazocine has mu-antagonist activity [5, 6, 14]. This interpretation is supported by the second observation which is that low doses of naloxone antagonized the decrease in urination produced by 5 mg/kg of morphine at the 1-hr time period and actually produced an increase in urination with the combination of 1 mg/kg of naloxone and 5 mg/kg of morphine compared to the saline-treated mice.

Thus the present results in mice show that kappa agonist activity increases urinary output and increases weight loss. These effects complement the previous results obtained in

rats [5]. The major difference between mice and rats is that in the mice, morphine appears to have kappa agonist activity, whereas in the rat, morphine only decreased urinary output.

The findings of the present study suggest that the kappa agonist activity of opioids can be identified in mice by measuring urinary output, weight loss, or both. Compounds which produced marked dose-related increases in urinary output or weight loss could be challenged with naloxone to determine if the effects were due to an action at kappa-opioid receptors. These studies can be conducted easily with a minimum of expense, and results are objectively quantifiable. Thus, the increased urination produced by kappa agonists can be used to study *in vivo* effects of manipulations at kappa receptors in mice.

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