

## Morphine Tolerance Decreases the Analgesic Effects of Ketamine in Mice

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Previous studies have shown that ketamine interacts with opiate receptors, and it has been suggested that ketamine-induced analgesia is mediated through opiate receptors. If so, ketamine should produce less analgesia in morphine tolerant animals, just as morphine does. To test this hypothesis, the analgesic effects of ketamine were tested in mice implanted with placebo pellets and in mice made tolerant to morphine through implantation of morphine pellets, using the abdominal constriction test. The test consisted of ip injection of 1% acetic acid, which caused stretching of hind limbs and constriction of abdominal muscles, also called writhing. The number of writhes was counted for each mouse 10–15 min following acetic acid injection. Morphine pellet implanted mice treated with saline writhed  $12.2 \pm 0.8$  times (mean  $\pm$  SEM), not significantly different from  $9.8 \pm 0.9$  times seen in placebo pellet implanted mice. Treatment of the animals with ketamine at three doses of 20, 25, and 30 mg/kg, subcutaneously (sc), reduced the number of writhes in the placebo pellet implanted group to  $5.8 \pm 0.8$ ,  $4.2 \pm 0.7$ , and  $1.3 \pm 0.3$ , respectively. In the morphine pellet-implanted group, with the same doses of ketamine, the numbers of writhes were  $10 \pm 0.9$ ,  $9.3 \pm 1.1$ , and  $5.2 \pm 0.9$ , respectively. Morphine-tolerant animals writhed significantly more at each dose of ketamine, indicating that they were cross tolerant to the analgesic effects of ketamine. (Key words: Analgesia, mechanisms. Analgesics, morphine: tolerance. Anesthetics, intravenous: ketamine; morphine. Receptors, opiate: delta; mu. Receptors, PCP/sigma. Tolerance: morphine.)

ANALGESIA PRODUCED BY ketamine has been ascribed in part to its interaction with opiate receptors. Initially, it was observed that ketamine analgesia was partially antagonized by the narcotic antagonist, naloxone.<sup>1,2</sup> Subsequently, ketamine was found to bind to opiate receptors *in vitro*<sup>3-5</sup> and to displace radioactively labeled etorphine, a potent narcotic, from brain opiate receptors *in vivo*.<sup>5</sup>

To elucidate further the mechanism of analgesic action of ketamine, we reasoned that, if ketamine produces analgesia through binding to opiate receptors, it should be, as morphine is, less effective as an analgesic in morphine-tolerant animals. Accordingly, we examined the analgesic effects of ketamine in mice made tolerant to morphine.

### Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee, Health Sciences, Columbia University.

### ANALGESIA TESTING

An acetic acid-induced abdominal constriction test in the mouse was used to assess analgesia as previously described.<sup>6</sup> Briefly, male Swiss-Webster mice (CF1 strain) weighing 22–26 grams, in groups of five, were injected, ip, with a 1.0% acetic acid in 0.9% NaCl solution at a dose of 0.01 ml/gram body weight. After injection, animals were placed in individual wire cages for ease of identification. In response to the ip injection of acetic acid, the mouse stretched its hind limbs with constriction of abdominal muscles at intervals; the response is also known as writhing. Each animal was used once only. All results are based on the number of writhes per mouse during the 5-min interval from 10–15 min after acetic acid injection. The observer was uninformed as to drug treatment and pretreatment of the animals.

### INDUCTION OF MORPHINE TOLERANCE AND ANALGESIC EFFECTS OF KETAMINE AND MORPHINE

Seventy-four hours prior to analgesia testing, mice were briefly (2–3 min) anesthetized with isoflurane (2.0–2.5 vol%) in oxygen. Experimental animals received pellets containing 75 mg of morphine base, implanted subcutaneously in a flank. Control animals received placebo pellets. (Both sets of pellets were a gift from the Research Technology Branch, NIDA, Rockville, MD.) Two hours prior to analgesia testing, all pellets were removed, again under brief isoflurane anesthesia. Five minutes before the ip injection of acetic acid, each animal received an injection, subcutaneously (sc), containing either normal saline, ketamine HCl at one dose of 20, 25, or 30 mg/kg, or morphine HCl, 1 mg/kg. After analgesia had been assessed, those animals which had been implanted with a morphine pellet were given a further injection, sc, of naloxone, 1 mg/kg, in 0.25 ml normal saline, and observed for an additional 10 min for signs of narcotic withdrawal. (Naloxone was a gift from DuPont Pharmaceuticals, Garden City, NY.)

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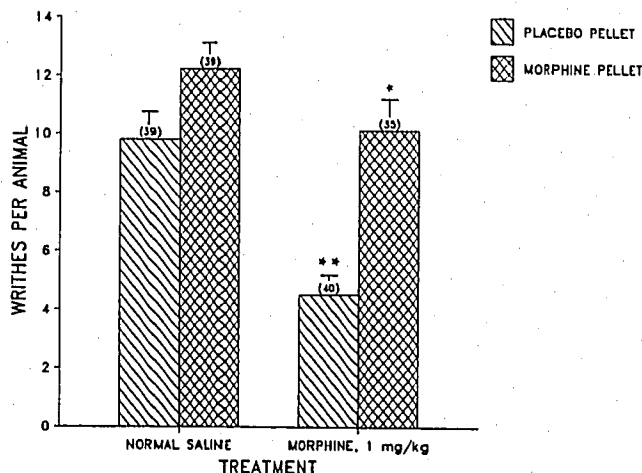


FIG. 1. Effect of morphine on abdominal constriction (writhing) in placebo pellet-implanted *versus* morphine pellet-implanted mice. Number of animals in each group is shown in parentheses at top of each bar, along with SEM. \*Significantly greater compared with placebo pellet-implanted animals treated with morphine ( $P < 0.001$ ), and not different from morphine pellet-implanted animals treated with saline ( $P > 0.05$ ). \*\*Significantly less compared with placebo pellet-implanted animals treated with saline ( $P < 0.0001$ ).

#### STATISTICS

Student's  $t$  test for unpaired data was used to compare the number of writhes per mouse in the morphine pellet-implanted *versus* the placebo pellet-implanted groups when these mice were treated either with normal saline (control number of writhes) or with morphine HCl, 1 mg/kg, prior to analgesia testing.

For the experiments in which the animals were given

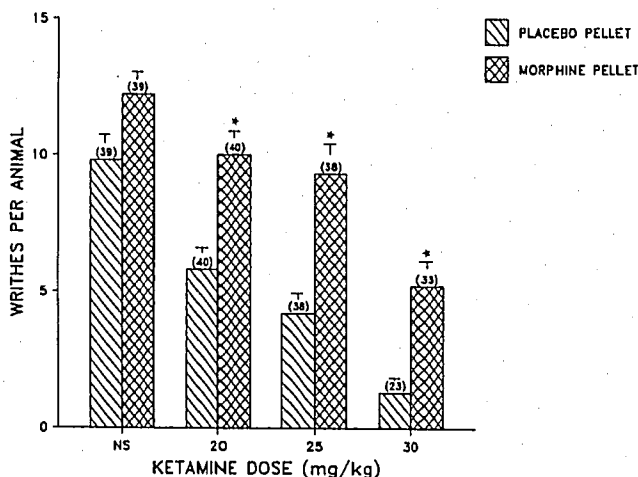


FIG. 2. Effect of three doses of ketamine on abdominal constriction (writhing) in placebo pellet-implanted *versus* morphine pellet-implanted mice. Number of animals in each group is shown in parentheses at top of each bar, along with SEM. \*Significantly greater compared with the corresponding placebo pellet-implanted group ( $P < 0.05$ ).

a dose of ketamine prior to analgesia testing, the number of writhes per mouse in the morphine pellet-implanted group were compared with those in the placebo pellet-implanted group at each of the three doses of ketamine using a two-way factorial analysis of variance (ANOVA) with unequal, but proportional, replication.<sup>7</sup>

Significant differences were compared using the Newman-Keuls test.

#### Results

Placebo pellet-implanted animals injected with normal saline prior to analgesia testing writhed  $9.8 \pm 0.9$  times (mean  $\pm$  SEM,  $n = 39$ ). Morphine pellet-implanted animals injected with normal saline writhed  $12.2 \pm 0.8$  times ( $n = 39$ ). These two values were not significantly different ( $P > 0.05$ ).

When animals from the placebo pellet-implanted group were treated with morphine, 1 mg/kg, prior to analgesia testing, the number of writhes was reduced to  $4.5 \pm 0.6$  ( $n = 40$ ). However, when animals from the morphine pellet-implanted group were treated with the same dose of morphine, they writhed  $10.1 \pm 1.0$  times ( $n = 35$ ), a significantly greater number than in the placebo pellet-implanted animals ( $P < 0.001$ ), and not significantly different from morphine pellet-implanted animals treated with saline ( $P > 0.05$ ). These results are shown in figure 1.

At the dose of 20 mg/kg of ketamine, placebo pellet-implanted animals writhed  $5.8 \pm 0.8$  times ( $n = 40$ ), whereas the morphine pellet-implanted animals writhed  $10.0 \pm 0.9$  times ( $n = 40$ ). At the dose of 25 mg/kg of ketamine, the respective values were  $4.2 \pm 0.7$  times ( $n = 38$ ), and  $9.3 \pm 1.1$  writhes ( $n = 38$ ). In morphine pellet-implanted animals, these two doses of ketamine did not significantly decrease the numbers of writhes, compared with controls. At the dose of 30 mg/kg of ketamine, the placebo pellet-implanted group writhed  $1.3 \pm 0.3$  times ( $n = 23$ ), whereas the morphine pellet-implanted animals writhed  $5.2 \pm 0.9$  times ( $n = 33$ ). Results are shown in figure 2.

ANOVA testing of the data showed that ketamine had a significant effect on the number of writhes in both groups of animals ( $F_{(1),2,132} = 12.73$ ,  $P < 0.0005$ ), and that morphine tolerance also had a significant effect on the number of writhes as affected by ketamine, ( $F_{(1),1,132} = 16.50$ ,  $P < 0.0005$ ). However, there was no interaction between the effect of morphine tolerance and the effect of ketamine ( $F_{(1),2,132} = 0.02$ ,  $P > 0.25$ ). Multiple comparisons of the mean number of writhes indicated that, at each of the three doses of ketamine used, the number of writhes in the morphine pellet-implanted group was significantly greater than in the placebo pellet-implanted group ( $P < 0.05$ ).

All of the morphine pellet-implanted animals demonstrated withdrawal jumping, increased defecation and micturition, with an average weight loss of 2 grams, and increased locomotor activity within a few minutes after the injection of naloxone. Several of the placebo pellet-implanted animals were also injected with naloxone after analgesia testing, and demonstrated none of these behavioral effects.

### Discussion

The results demonstrate that the morphine pellet-implanted animals were tolerant to morphine at the time of analgesia testing. In addition, the development of withdrawal symptoms following a challenge dose of naloxone indicates that the animals were morphine dependent as well. Ketamine was found to be much less effective as an analgesic agent in the morphine tolerant mice, indicating a state of cross-tolerance between morphine and ketamine.

Cross tolerance between ketamine and morphine has also been examined in rats by De Simoni *et al.*<sup>8</sup> In their study, 100 mg/kg of ketamine was injected, ip, and analgesia was assessed by tail compression and observing one of three responses (either vocalization or biting the tail, or, for animals which had no righting reflex, slight abdominal contraction). They found no evidence of cross tolerance to the analgesic effects of the two drugs, although cross tolerance to the stimulatory effect of the two drugs on dopamine metabolism was demonstrated.

Our results would seem to conflict with those of De Simoni *et al.* However, our results are based on the use of subanesthetic analgesic doses of ketamine (none of the animals lost their righting reflex). Also, our results showed the analgesic action of ketamine is dose dependent in placebo pellet-implanted animals. In morphine-tolerant animals, ketamine, in doses of 20 and 25 mg/kg, did not produce analgesia (fig. 2). De Simoni *et al.* used an anesthetic dose of ketamine, as all of the animals lost their righting reflex for an average of 36–61 min. As only this one dose of ketamine was used, one cannot determine on a dose-response curve for analgesia where the response was occurring; *i.e.*, the dose of ketamine used may have been high enough to be supramaximal, which would tend to obscure any decreased analgesic effects of ketamine in morphine-tolerant animals. In addition, using other paradigms to quantitate analgesia in the rat, *e.g.*, the tail-flick test, graded analgesic responses have been found at much lower doses of ketamine (30–45 mg/kg, ip).<sup>2</sup>

Events occurring at opiate receptors during the development of tolerance and maintenance of the tolerant state are not well understood. Early studies of this sub-

ject, which did not differentiate among opiate receptor subtypes, led to conflicting results.<sup>9–13</sup>

More recent studies of the effects of morphine tolerance on opiate receptor subtypes using subtype selective ligands seem to show an upregulation of mu and/or delta receptors, manifested as an increase in apparent receptor density ( $B_{max}$ ), without any change in receptor affinity for ligands ( $K_d$ ).<sup>14,15</sup> We did not study the opiate receptor characteristics in animals implanted either with morphine or placebo pellets, but results presented here clearly demonstrate cross tolerance between morphine and ketamine, whatever the state of opiate receptors.

Sircar *et al.*<sup>16</sup> reported that ketamine displaces <sup>3</sup>H-SKF 10,047, a PCP/sigma receptor selective ligand, from rat brain with an  $IC_{50}$  (concentration which inhibits binding of radioligand by 50%) value in the micromolar range, similar to the concentrations at which ketamine displaces <sup>3</sup>H-dihydromorphine (a preferential mu-receptor ligand) from binding sites in rat brain homogenates.<sup>5</sup> However, the analgesic effects of SKF 10,047 and PCP are not antagonized by the opiate antagonist, naltrexone,<sup>17</sup> whereas the analgesic effects of ketamine are partially antagonized by naloxone.<sup>1,2</sup> This suggests that the analgesic effects of ketamine are not due to an interaction of the drug with the PCP/sigma receptor, even though ketamine binds to this site, which might account for some of the psychotomimetic effects of this drug.

In summary, the present study demonstrates that morphine tolerant animals are cross tolerant to the analgesic effects of ketamine. These results suggest that the analgesic effects of ketamine are mediated by mu and/or delta receptors.

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