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The Effect of Cumulative Dosing on the Analgesic Potency of Morphine in Mice

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DUTTAROY, A., R. KIRTMAN, F. FARRELL, M. PHILLIPS, J. PHILIPPE, T. MONDERSON AND B. C. YOBURN. *The effect of cumulative dosing on the analgesic potency of morphine in mice.* PHARMACOL BIOCHEM BEHAV **58**(1) 67–71, 1997.—Opioid analgesic potency can be evaluated using cumulative dosing, in which subjects are repeatedly administered a drug and tested after each dose until a criterion effect is reached. Although many laboratories use cumulative dosing, the effects of varying the starting dose and the magnitude of the increment dose on morphine analgesia (tail flick) in mice have not been evaluated. In experiment 1, mice were injected with the same starting dose [0.5 mg/kg subcutaneously (SC)] and 30 min later were tested for analgesia. Mice that were not analgesic were administered an increment dose (0.5, 1.0, 2.0, 2.5, or 3.0 mg/kg) and retested. The process was continued until all mice were analgesic. There was a significant effect of increment dose on morphine potency, with the relative potency increasing as the increment dose was increased. In experiment 2, different starting doses (0.5, 1.0, 2.0, or 3.0 mg/kg) were used with a constant increment dose of 1.0 mg/kg. There was a significant effect of starting dose on the potency of morphine, with the relative potency increasing as the starting dose increased. To determine if increment and starting dose affect tolerance estimates, mice were implanted SC with a 25- or 75-mg morphine or placebo pellet for 7 days and then tested using cumulative dose–response. Changes in the increment dose significantly affected the degree of tolerance for mice implanted with a 25-mg morphine pellet but not for mice implanted with a 75-mg morphine pellet. Changes in the starting dose did not significantly alter estimates of tolerance. Overall, these data indicate that the starting dose and increment dose can impact on morphine's potency determined by cumulative dosing protocols. Furthermore, estimates of tolerance can be affected by dosing parameters in the cumulative dosing protocol. These results suggest that cumulative dosing procedures should be standardized across experiments. © 1997 Elsevier Science Inc.

Opioid Cumulative dose–response Analgesia ED_{50} Tolerance Relative potency Shift in ED_{50} Morphine

THE POTENCY of opioid analgesics is evaluated in dose–response studies (e.g., 1,3,11) that can be performed using either a standard dose–response approach or a cumulative dose–response protocol (1,3,9). In a standard dose–response study, mice are divided into several groups and each group is injected with a different dose of drug so that each group gets only a single dose. If many doses are evaluated, this method can require a large number of animals and a substantial supply of the test drug. Conversely, by employing a cumulative dose–response protocol, the number of animals and drug used can be dramatically reduced (3,10,12). Typically, in a cumulative dose–response protocol, animals are injected with an initial dose (i.e., starting dose) of a drug and tested for some criterion effect. Animals that do not reach the criterion effect are given a second dose (i.e., increment dose) of the same drug and then retested. This dosing procedure is continued until a predetermined percentage of animals respond. As with the standard dose–response, an ED_{50} and relative potency estimates can be calculated.

Although cumulative dose–response protocols have been used to evaluate opioid analgesia (1,3,4,7,10), there is no customary protocol, which has made it difficult to compare data. The two basic parameters of the cumulative dose response protocol are the starting dose and the increment dose. However, it is not clear what effect changes in either of these variables would have on opioid potency. In the present experi-

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ments, we determined the effect of the magnitude of the starting dose and of the increment dose on morphine's analgesic potency in mice. In addition, the effect of the starting dose and increment dose on estimates of tolerance to morphine were ascertained.

METHODS

Subjects

Male Swiss–Webster mice (22–44 g) (Taconic Farms, Germantown, NY, USA) were housed 10 per cage with free access to food and water. Mice were used only once.

Antinociception

Antinociception (i.e., analgesia) was determined with the tail flick assay (2). A beam of light was focused on the dorsal surface of the tail of the mouse and the apparatus adjusted so that baseline tail flicks occurred within 2–4 s. In dose–response studies, a cutoff tail flick latency (10 s) was used to avoid tissue damage. Mice that did not flick their tails within 10 s were considered analgesic.

Cumulative Dose–Response Protocol

General procedure. Mice (n = 6–11/group) were injected with a starting dose of SC morphine and tested for antinociception 30 min after administration of the drug. Mice that were not analgesic were given a second dose (i.e., increment dose) of morphine within 5 min of testing and then were retested for antinociception. This cumulative dose–response protocol was continued until all mice were analgesic. Each experiment was repeated three to seven times by a tester who was blind to treatment group.

Effect of increment and starting dose. Five different increment doses were examined. Five groups of mice were injected with a starting dose of 0.5 mg/kg morphine and 30 min later were tested for antinociception. Mice in each group that were not analgesic were given a different increment dose (0.5, 1.0, 2.0, 2.5, or 3.0 mg/kg) and retested 30 min later. The increment doses were doubled for the 0.5- and 1.0-mg/kg groups after a cumulative dose of 8.5 mg/kg. For the other groups (2.0, 2.5, and 3.0 mg/kg), the increment was held constant throughout.

Four different starting doses were examined. Four groups of mice were injected with various starting doses (0.5, 1.0, 2.0, or 3.0 mg/kg) of morphine and 30 min later were tested for antinociception. Mice that were not analgesic were given an increment dose of 1.0 mg/kg and retested 30 min later. The increment dose was held constant throughout.

For these dose–response studies, the group of mice receiving a starting dose of 0.5 mg/kg followed by an increment dose of 1.0 mg/kg was taken as the reference dosing protocol. Data are presented as the mean (±SEM) relative potency (ED_{50} for reference group/ED_{50} for experimental group) such that an increase in relative potency indicates a decrease in the ED_{50}.

Effect of increment dose and starting dose on morphine tolerance. Tolerance was induced by SC implantation of a 25- or 75-mg morphine pellet for 7 days. Control mice were implanted with placebo pellets. On day 7 following implantation, mice were tested for antinociception. Mice with implanted morphine pellets (25 or 75 mg) were divided into three groups and injected with a constant starting dose of 0.5 mg/kg morphine and different increment doses (1.0, 2.0, or 3.0 mg/kg). The increment doses were doubled after a cumulative dose of 8.5 or 9.5 mg/kg. In another study, three groups of mice implanted with morphine pellets (25 mg) were injected with different starting doses of morphine (0.5, 2.0, or 4.0 mg/kg) and a constant increment dose of 1.0 mg/kg. Placebo mice for both experiments were injected with a starting dose of 0.5 mg/kg and a constant increment dose of 1.0 mg/kg. The shift in the ED_{50} was calculated with reference to the placebo group. For tolerance studies, data are presented as the mean (±SEM) shift in the ED_{50} (ED_{50} for morphine group/ED_{50} for placebo group) such that increases indicate more tolerance.

Drugs

Morphine sulfate was supplied by Penick Corporation (Newark, NJ, USA). The drug was dissolved in 0.9% NaCl, and doses are expressed as the base. Morphine and corresponding placebo pellets were obtained from Research Triangle Institute (Research Triangle Park, NC, USA) through the Research Technology Branch of the National Institute on Drug Abuse. Pellets were wrapped in nylon mesh before SC

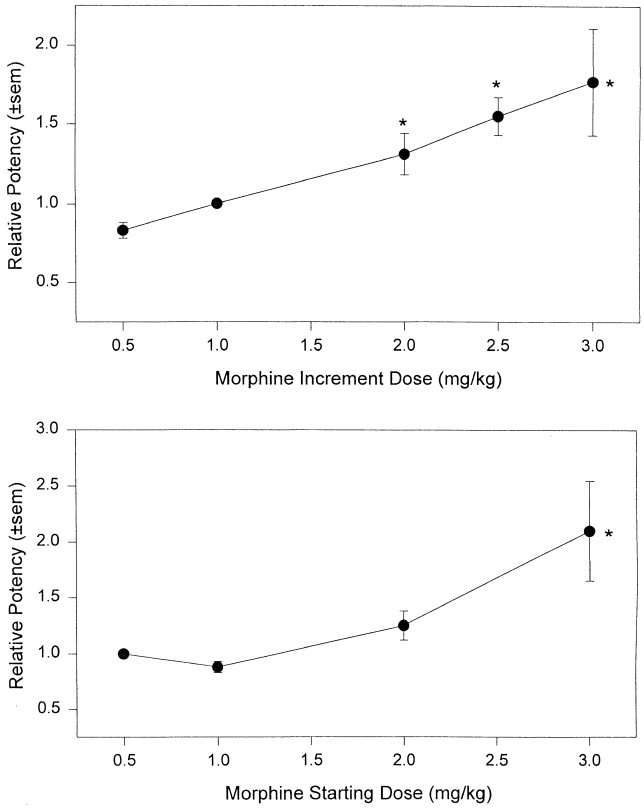

FIG. 1. Effect of increment dose and starting dose on morphine potency. (Top panel) Increment dose. Mice were injected with the same starting dose (0.5 mg/kg) and different increment doses (0.5, 1.0, 2.0, 2.5, or 3.0 mg/kg) of morphine and tested for analgesia. (Bottom panel) Starting dose. Mice were injected with different starting doses (0.5, 1.0, 2.0, or 3.0 mg/kg) and the same increment dose (1.0 mg/kg) of morphine and tested for analgesia. Data are plotted as mean relative potencies (±SEM) vs. dose for three to seven separate experiments. The group of mice receiving a starting dose of 0.5 mg/kg followed by an increment dose of 1.0 mg/kg was taken as the reference (relative potency = 1) for both studies, and relative potencies were calculated relative to this group. The mean (±SEM) ED_{50}s for the reference groups were 3.4 (±0.3) mg/kg and 2.7 (±0.3) mg/kg for the top and bottom panels, respectively. *$p < 0.01$, significantly different from the reference group.

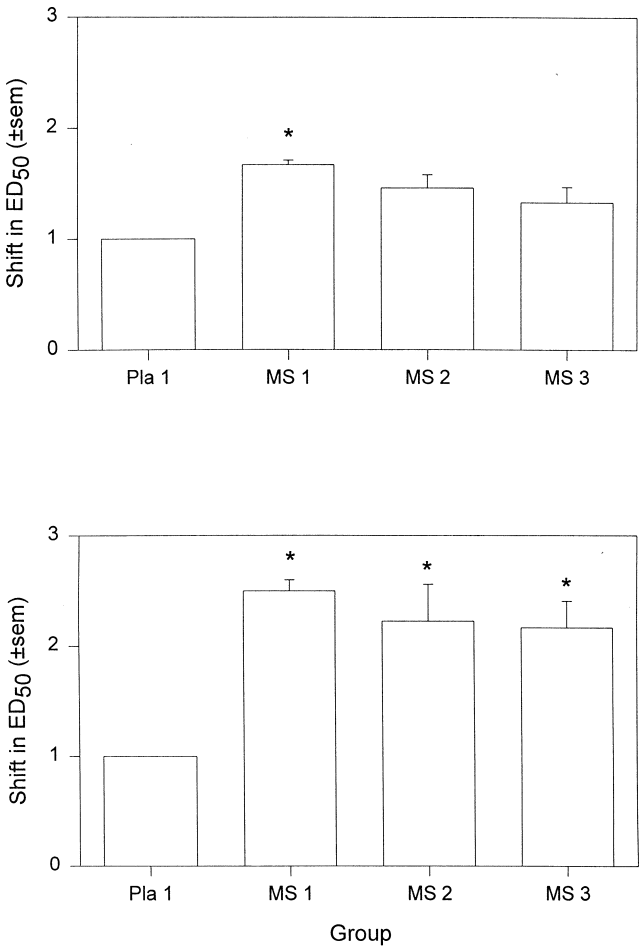

FIG. 2. Effect of the magnitude of the increment dose on morphine tolerance (shift in ED_{50}). Mice were implanted with a placebo or a 25-mg (top panel) or 75-mg (bottom panel) morphine pellet for 7 days. On day 7, mice were injected with the same starting dose (0.5 mg/kg) and different increment doses (1.0, 2.0, or 3.0 mg/kg) of morphine and tested for analgesia. A starting dose of 0.5 mg/kg and an increment dose of 1.0 mg/kg was employed for the placebo control group (Pla 1), which served as the reference (potency = 1) for all treatments. Data are plotted as mean (±SEM) shift in ED_{50} vs. increment dose for three to five separate experiments. The mean (±SEM) ED_{50}s for the placebo control groups were 2.8 (±0.4) mg/kg and 3.3 (±0.2) for the top and bottom panels, respectively. The shift in ED_{50} = (ED_{50} for the morphine group/ED_{50} for placebo). *$p < 0.05$, significantly different from corresponding placebo control group. Pla, placebo; MS, morphine; 1, 2, 3 indicate magnitude of increment dose (mg/kg).

implantation in the nape of the neck of the mouse. Pellets were implanted while mice were lightly anesthetized with halothane/oxygen (4:96).

Data Analysis

Quantal dose–response data were analyzed by probit analysis (5) using software (BLISS 21, Department of Statistics, University of Edinburgh, Edinburgh, Scotland) that estimates ED_{50}s, 95% confidence limits, and relative potencies. Significant differences between potency estimates and ED_{50}s were determined by ANOVA. Significant differences between groups were determined by post hoc tests (Bonferroni's method).

RESULTS

Increasing the increment dose significantly increased the relative analgesic potency of morphine [$F(4, 26) = 5.9$, $p < 0.01$] (Fig. 1, top panel). Relative potencies dose-dependently increased from 0.8 to 1.8 as the increment dose was increased from 0.5 to 3.0 mg/kg. Post hoc tests indicated that only the 2.0-, 2.5-, and 3.0-mg/kg increment doses significantly increased morphine potency compared with the reference group (1.0 mg/kg increment). An increase in the starting dose also significantly increased the relative potency of morphine [$F(3, 21) = 5.9$, $p < 0.01$] (Fig. 1, bottom panel). Post hoc tests indicated that the approximately twofold relative potency increase for the 3.0-mg/kg starting dose was significantly different from the reference group. Therefore, increases in increment dose as well as starting dose significantly increased morphine's relative potency.

A significant effect of treatment [$F(3, 8) = 6.0$, $p < 0.05$] was found in the 25-mg morphine pellet and increment dosing study (Fig. 2, top panel). Post hoc tests indicated significant tolerance in mice in the 1.0-mg/kg increment dose group implanted with a 25-mg morphine pellet (MS 1) compared with placebo-treated mice in the 1.0-mg/kg increment dose group (Pla 1). However, when the increment dose was increased to 2.0 and 3.0 mg/kg (MS 2, MS 3), there was no significant effect

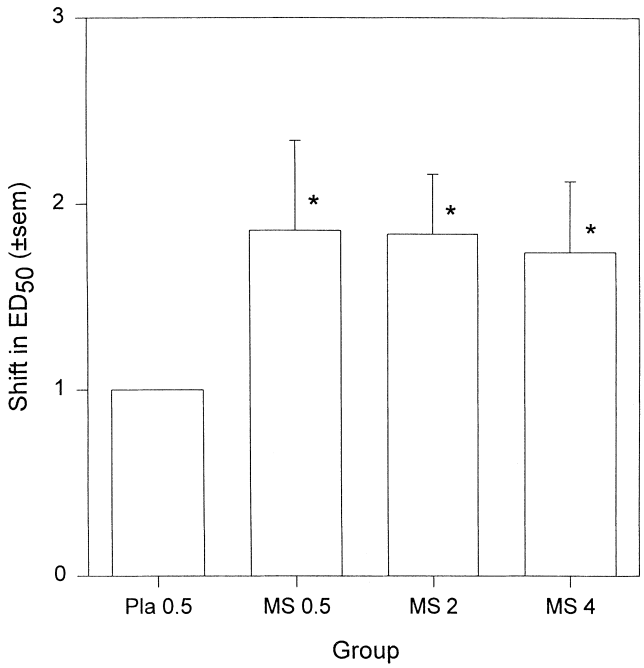

FIG. 3. Effect of the magnitude of the starting dose on morphine tolerance. Mice were implanted with placebo or a 25-mg morphine pellet for 7 days. On day 7, mice were injected with different starting doses (0.5, 2.0, or 4.0 mg/kg) and the same increment dose (1.0 mg/kg) of morphine and tested for analgesia. A starting dose of 0.5 mg/kg and an increment dose of 1.0 mg/kg was employed for the placebo control group (Pla 0.5), which served as the reference (potency = 1) for all treatments. Data are plotted as mean (±SEM) shift in ED_{50} vs. starting dose for six separate experiments. The shift in ED_{50} = (ED_{50} for the morphine group/ED_{50} for placebo). The mean (±SEM) ED_{50} for the placebo control group was 2.6(±0.5)mg/kg. *$p < 0.05$ significantly different from placebo control group. Pla, placebo; MS, morphine; 0.5, 2, 4 indicate magnitude of starting dose (mg/kg).

of morphine pellet implantation relative to the placebo reference group (Pla 1).

Mice implanted with a 75-mg morphine pellet showed greater tolerance [$F(1, 24) = 14.41$, $p < 0.001$] than the 25-mg morphine pellet groups, as shown by the greater shift in ED_{50}s (Fig. 2). A significant effect of treatment with a 75-mg morphine pellet [$F(3, 16) = 7.0$, $p < 0.05$] was found in the increment dosing study (Fig. 2, bottom panel). Post hoc tests indicated that all morphine groups (MS 1, MS 2, MS 3) differed from the placebo reference group (Pla 1).

Finally, the effect of an increase in the starting dose was assessed in mice implanted with 25-mg morphine pellets (Fig. 3). There was a significant group effect [$F(3, 15) = 4.4$, $p < 0.05$], and post hoc tests indicated a significant shift in the ED_{50} for all morphine groups (MS 0.5, MS 2, MS 4) compared with the placebo reference group (Pla 0.5).

DISCUSSION

In the present experiments, we examined two important parameters in the cumulative dose–response protocol using morphine analgesia in the mouse as an outcome variable. Our results show that a change in the increment dose as well as changes in the starting dose altered the estimated analgesic potency of morphine in untreated mice. Therefore, it is important to perform cumulative dose–response studies using a protocol with defined increment and starting doses to obtain consistent ED_{50}s across experiments. It should be noted that in these studies, the time between injections for cumulative dosing was held constant at 5 min. It would be expected that variations in this interval would also impact on the estimated ED_{50}.

In all studies, a reference group was employed to estimate the effects of starting dose and increment dose. We employed this group's starting dose (0.5 mg/kg) and increment dose (1.0 mg/kg) as a control group in studies when using cumulative dose–response. The mean ED_{50} for all reference groups across all studies in the present paper was 3.1 (±0.2 SEM) mg/kg. The ED_{50} for morphine analgesia in mice determined in cumulative dosing studies tends to be somewhat greater than that determined with standard dosing. For example, in recent studies from our laboratory, the range of morphine ED_{50}s has been 1.3–2.7 mg/kg (3,6,8,10) using standard dosing, whereas the range in this and a previous study (3) using the cumulative protocol was 2.4–3.9 mg/kg. Similarly, ED_{50}s calculated for other opioid agonists (e.g., etorphine, fentanyl, meperidine, oxycodone) using cumulative dosing (3,10,12) tend to be greater compared with the standard dosing protocols, although they are in the same general range. Taken together, these data suggest that the cumulative dosing protocol results in ED_{50} estimates that are roughly comparable to those determined in standard dose–response protocols. Most importantly, however, the relative relationships between treated and control groups using cumulative dosing (in which increment and starting dose are held constant) are quite similar to those under standard dosing, with effects such as tolerance and functional supersensitivity being readily noted (3,6,10,11,12).

In the tolerance study, we examined the effect of both the starting dose and the increment dose on the degree of tolerance following implantation of morphine pellets. The reference group for the magnitude of tolerance in each of these experiments was a placebo-implanted group that received a starting dose of 0.5 mg/kg and an increment dose of 1.0 mg/kg. It might be assumed a priori that tolerance would increase the ED_{50}, and consequently investigators might attempt to increase efficiency (i.e., reduce the time required for the whole experiment) by using a higher starting dose or larger increment dose in the tolerant group. Therefore, we compared the effect of increasing the starting dose and increment dose on the calculated shift in the ED_{50} for morphine. Interestingly, the effects of starting dose and increment dose on estimates of tolerance were rather small. For the 25-mg morphine pellet treatment, the size of the increment dose produced a small, yet significant, decrease in the estimated degree of tolerance (Fig. 2, top panel). When a 75-mg morphine pellet was used, more tolerance was observed than with the 25-mg morphine pellet, but the increment dose did not significantly affect the degree of tolerance (Fig. 2, bottom panel). The magnitude of the starting dose did not affect tolerance estimates for the 25-mg morphine pellet treatment (Fig. 3), and consequently we did not perform any experiments on starting dose employing 75-mg pellets. Thus, we found that the increment dose, but not the starting dose, produced an effect on the estimate of tolerance, although the magnitude of the effect was small. However, given the possibility that in some experiments both parameters may be important and may affect potency estimates more dramatically, we recommend that both the starting dose and increment dose be held constant for both control and treated groups.

Overall, these results argue that the protocols for cumulative dose–response studies should be standardized. Specifically, the same starting dose, set of increment doses, and interdose interval should be used for all groups in any experiment. We would also recommend that a common protocol be used across different studies to facilitate comparisons. Although the effects of changing cumulative dose–response parameters were not dramatic in the present study, it is possible that in some cases that they may make a critical difference. Consequently, it is prudent to standardize cumulative dosing.

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