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Physical Dependence Induced by the Voluntary Consumption of Morphine in Inbred Mice

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BELKNAP, J. K. *Physical dependence induced by the voluntary consumption of morphine in inbred mice.* PHARMACOL BIOCHEM BEHAV **35**(2) 311–315, 1990.—When given a two-bottle choice between gradually increasing morphine concentrations (in 0.2% saccharin) and plain tap water, C57BL/6J mice consumed almost 90% of their daily fluid intake from the morphine-saccharin bottle, while the DBA/2J strain, in contrast, consumed 13% or less from the morphine-saccharin solution. The C57BL/6J strain consistently consumed mean daily doses of morphine sulfate in excess of 200 mg/kg, which was sufficient to induce an easily discernable withdrawal syndrome upon removal of the morphine solution, either with or without naloxone challenge. Hypothermia, tremor, wet dog shakes, jumping, and diarrhea were prominent withdrawal signs. In separate experiments, the saccharin was removed from the morphine-containing bottle, yet the C57BL/6J mice continued to prefer the morphine solution over tap water. In complete contrast to the above, mice of the DBA/2J strain rejected the morphine-saccharin solution at the lowest concentration employed, and at no time did their mean daily morphine dose exceed 20 mg/kg. Thus, morphine-saccharin preference is strongly genetically determined, and a high degree of physical dependence can result in the morphine-preferring strain. Palatability differences appear not to be the predominant explanation for these differences in morphine-saccharin consumption.

C57BL/6J DBA/2J Morphine Drug self-administration Opioid physical dependence

FOLLOWING up on the earlier work of Khavari and Risner (8) in rats, Horowitz *et al.* (6) and Horowitz (7) demonstrated that a normally avoided morphine solution (0.375 mg/ml) could be made highly preferred vs. tap water in some inbred strains of mice by the addition of saccharin. In a series of 9 inbred mouse strains given a two-bottle ad lib free choice situation (tap water vs. morphine solution), all but the C57BL/6JA (Alberta) strain avoided the morphine solution in the absence of saccharin, including the C57BL/6J and DBA/2J strains. With the addition of 0.06% saccharin, however, which presumably masked the bitter taste of morphine, a dramatic increase in morphine consumption was seen in some strains, but no change in others. A very wide range of preference ratios resulted among the 9 inbred strains, from a low of 4% (DBA/2J) to a high of 98% (C57BL/6J) for the morphine-saccharin solution vs. tap water. In the C57BL/6J strain, daily consumption of morphine reached 140 mg/kg, while the DBA/2J strain consumed less than 10 mg/kg per day under the same conditions (7). These workers also showed that the removal of the saccharin from the C57BL/6J mice after 10 days of free choice morphine-saccharin led to only a moderate reduction in morphine consumption in the C57BL/6J strain. The morphine sulfate solution alone (0.375 mg/ml) continued to be preferred over tap water (75% preference ratio), suggesting that the morphone itself can be reinforcing to mice of this strain if their consumption of morphine had been earlier enhanced by saccharin (6). The saccharin alone was reported to be highly preferred over tap water to a similar extent in both strains. These two strains have also been shown to differ in the voluntary consumption of etonitazene, although the strain differences were smaller than with morphine (4). Our objectives in the present studies were to modify this two-bottle choice procedure to produce even greater morphine intakes by means of gradually ascending morphine concentrations in the drinking fluid in C57BL/6J and DBA/2J mice, and to systematically assess the degree of physical dependence produced by this regimen.

METHOD

Male mice from the C57BL/6J and DBA/2J inbred strains were purchased from the Jackson Laboratories, Bar Harbor, ME, and

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used in our experiments at 2.5–5 months of age. The animals were housed singly throughout each experiment, and fluids were presented in the home cage using inverted 25 ml graduated cylinders. Daily fluid consumption was routinely monitored at 1–2 hours after light onset each day, and fresh fluids provided on alternate days, except Experiment 5, 4–5 days. In the two-bottle choice studies, the positions of the two bottles were reversed at these times to eliminate position preference effects. All morphine concentrations and doses are expressed in terms of the sulfate salt. Ambient temperatures were maintained at 22–24°C.

In Experiment 1, 6 mice of each inbred strain were presented with a two-bottle choice: one bottle always contained plain tap water and the other bottle contained 0.3 mg/ml of morphine sulfate on Days 0–4, 0.5 mg/ml on Days 5–10 and 0.75 mg/ml on Days 11–16 in 0.2% sodium saccharin. This concentration of saccharin was chosen because it is maximally preferred in both strains based on our pilot work, and the data of Fuller (5). An additional 6 mice per strain received the same morphine vs. tap water regimen, but without saccharin. At the end of Day 16, the saccharin was omitted from the morphine solution for the next 5 days (Days 17–21).

In Experiment 2, we assessed morphine intake with gradually increasing morphine concentrations in the drinking fluid as in Experiment 1, compared to the use of a fixed concentration, as in the studies of Horowitz and co-workers (6,7). One group of 8 C57BL/6J mice received the same gradually increasing morphine schedule as in Experiment 1, except that a 14-day morphine-saccharin exposure period was used instead of 16 days. A second group of 8 C57BL/6J mice received a fixed 0.75 mg/ml morphine concentration in saccharin for the same period. The mice receiving the ascending schedule were challenged with naloxone (5.0 mg/kg, IP) at the end of Day 14, and the withdrawal syndrome monitored at 10-min intervals up to 40 min postinjection. At 10-min intervals, each mouse was placed on a small 10×10 cm elevated platform for one min, and the presence or absence of tremor, wet dog shakes, diarrhea (soft stool), jumping and lacrimation noted.

In Experiment 3, we used a single-bottle (no choice) paradigm where only morphine-saccharin was available as the sole fluid source in 8 mice of each strain. An additional 6 mice of each strain received only tap water in the single bottle (controls). Gradually increasing morphine concentrations in 0.2% saccharin was used for 14 days as in Experiment 1, while only tap water was used in the control group.

In Experiment 4, 6 C57BL/6J mice were exposed to a two-bottle choice paradigm using the same ascending morphine concentrations as in the above experiments, except that these mice had radio telemetry implants (Mini-mitter models X and XM), as previously described (2). An additional 6 implanted mice of this strain were exposed only to the tap water vehicle (controls). At the end of the 14-day morphine-saccharin treatment, the morphine was withdrawn, and the ensuing withdrawal syndrome monitored for the next 36 hours without the use of an antagonist.

In Experiment 5, we assessed strain differences in saccharin preference alone, and also the ability of saccharin to mask a bitter alkaloid taste. Quinine sulfate (QS) was used because this alkaloid has a similar bitter taste compared to morphine, but lacks opioid effects (4). Groups of 7–8 mice of each strain were given a two-bottle choice between tap water in one bottle, and 0.2% saccharin in the other bottle. The saccharin-containing bottle also contained the following according to the following schedule: Days 1–7, 0.2% saccharin alone; Days 9–14, 0.2 mg/ml QS; Days 15–18, 0.3 mg/ml QS; Days 19–23, 0.4 mg/ml QS. The two bottles were alternated in position at each concentration change. Only data from the last two days of each 4–7 day block was used for data analysis, when choice behavior was most stabilized. The quinine concentration (mg/ml) in 0.2% saccharin necessary to reduce consumption below that of the tap water alternative (preference ratio below 50%) was determined for each mouse, and used as an index of palatability of bitter alkaloid-saccharin mixtures. Mice continuing to prefer QS-saccharin at the highest QS concentration employed (0.4 mg/ml) were arbitrarily given a value of 0.5 mg/ml (3 mice of each strain).

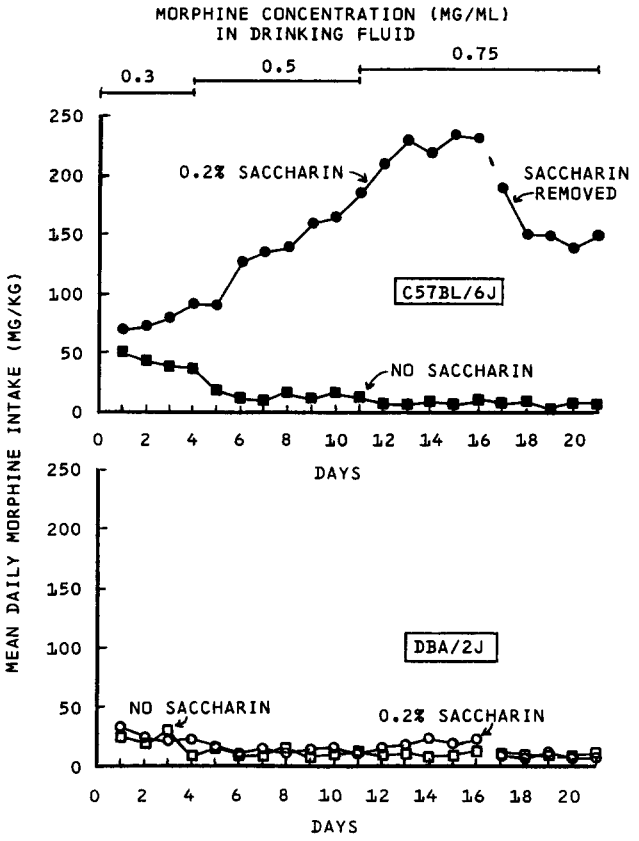

FIG. 1. Mean daily morphine intake (mg/kg) in C57BL/6J and DBA/2J male mice exposed to a two-bottle choice situation for either morphine mixed with 0.2% saccharin (morphine-saccharin) or morphine alone (no saccharin) in one bottle vs. plain tap water in the second bottle. For the first 16 days, the animals were exposed to gradually increasing morphine sulfate concentrations in 0.2% saccharin as shown at the top of the figure. On Days 17–22, the saccharin was removed from the morphine solutions in both strains. Each point represents the mean of 6 mice. For the morphine-saccharin groups, the C57BL/6J mice consumed significantly more morphine than the DBA/2J mice for each day of the experiment ($p<0.01$, two-tailed t-test).

RESULTS

The results for Experiment 1 in terms of daily morphine intake (mg/kg/mouse) for the 6 C57BL/6J and 6 DBA/2J male mice are shown in Fig. 1. Mean daily morphine intake reached a value of 216 mg/kg/day/mouse in the C57BL/6J strain on Days 11–16. When the saccharin was omitted on Days 17–21, morphine consumption declined, but still remained high at 153 mg/kg/day. In contrast, mice of both strains exposed to the same regimen of morphine without saccharin consistently avoided the morphine solution. Morphine consumption without saccharin in the C57BL/

6J strain did not exceed 20 mg/kg/day in the latter half of this experiment, a value less than ⅛ that seen in the morphine-saccharin group (Fig. 1). In contrast, the morphine-saccharin group showed morphine vs. tap water preference ratios of 88% or greater on all days when saccharin was added (Days 0–16), and a decline to 70% was seen when the saccharin was omitted on Days 17–21. Straub tail, an index of morphine intoxication, was seen in the majority of the C57BL/6J mice throughout the experiment. The DBA/2J mice, in contrast, consistently and uniformly avoided the morphine-saccharin solution during all phases of this experiment, never exceeding a mean morphine intake of 20 mg/kg/day. Morphine preference ratios for this strain were highest during Days 0–4 (13% preference ratio), and declined to 5% or less for the remainder of the experiment. The presence of saccharin in the morphine solution caused only a small increase in morphine consumption (12%) compared to the morphine alone. No Straub tails were seen in mice of this strain.

In Experiment 2, the gradually ascending morphine concentrations in the drinking fluid resulted in a 41% higher morphine intake in the final stages of this experiment (Days 11–14) when both groups were at the 0.75 mg/ml concentration (269 ± 46 vs. 191 ± 33 mg/kg/day, $p<0.05$), indicating that gradually increasing morphine concentrations are more effective in inducing high levels of morphine intake in C57BL/6J mice than a single fixed concentration at the highest concentration employed. These results are shown in Fig. 2. Those mice given the ascending series were then challenged with naloxone (5.0 mg/kg) to assess physical dependence development. The incidence of precipitated withdrawal signs is shown in Fig. 3.

In Experiment 3 (single bottle, no choice), mean daily fluid consumption of morphine-saccharin for the C57BL/6J strain in the latter stages of the experiment (Days 11–14) was 274 mg/ml, which is slightly higher than that seen in two-bottle choice conditions, indicating that the choice of tap water does not greatly alter morphine-saccharin consumption compared to single-bottle (morphine-saccharin only) conditions. The mean daily drug consumption for the C57BL/6J mice on Days 11–14 was typical of the four experiments we have performed using the single-bottle (no choice) paradigm (unpublished). In this experiment, fluid consumption from the morphine-saccharin bottle approximately doubled what was seen in the control group given tap water alone

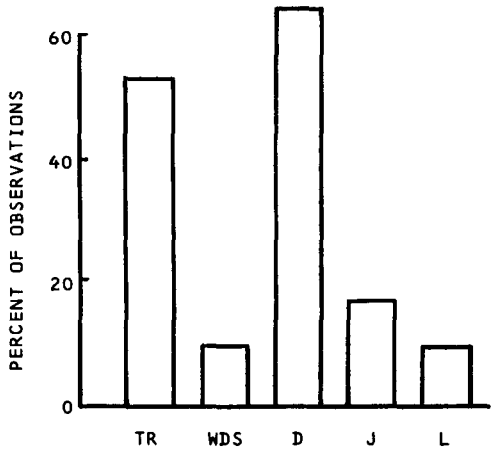

FIG. 3. Incidence of withdrawal signs in 8 C57BL/6J mice exposed to an ascending series of morphine concentrations in the drinking fluid (Experiment 2), and challenged with naloxone (5.0 mg/kg, IP) at the end of Day 14. Each animal was observed for the presence or absence of tremor (TR), wet dog shakes (WDS), diarrhea (D), jumping (J) or lacrimation (L) for a one-min period at ten-minute intervals following the naloxone injection, for a total of 4 observations per mouse or 32 observations overall. Withdrawal sign incidence is expressed as a percent of total observations in which a given withdrawal sign occurred.

(11.2 ± 2.4 vs. 5.4 ± 1.2 ml per day, means ± SD), indicating that these animals were clearly consuming more fluid than required to meet the needs for thirst. This is presumably a reflection of the positively reinforcing effects of morphine-saccharin consumption in C57BL/6J mice. In contrast, all of the 8 DBA/2J mice tested under the same conditions rejected comparable morphine-saccharin solutions as the sole fluid source to the point where the experiment had to be terminated for these mice due to inadequate fluid intake (2.4 ± 1.1 ml/day vs. control values of 5.1 ± 1.6 ml/day) during Days 5–10, when the morphine concentration was 0.5 mg/ml. This level of fluid intake was judged to be inadequate to maintain the health of the animals, as reflected in an average 13% body weight loss in these mice compared to a 2% loss in the

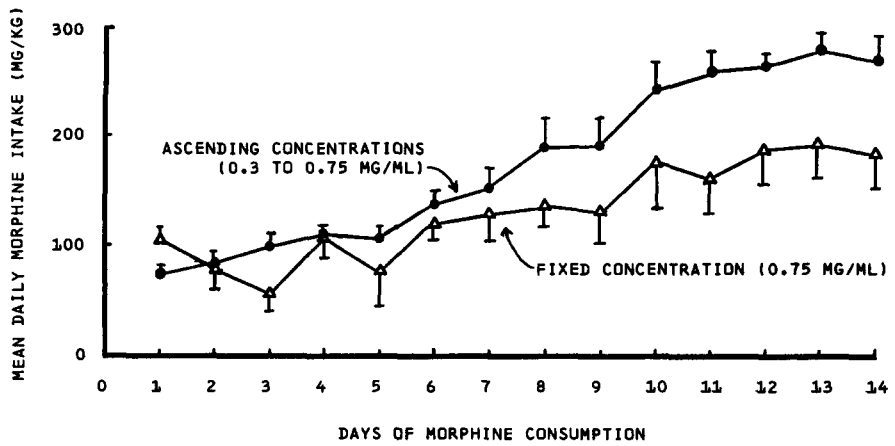

FIG. 2. Mean daily morphine intake (mg/kg) in two groups of C57BL/6J mice, one receiving an ascending series of morphine concentrations as in Experiment 1, and the other receiving a fixed concentration of 0.75 mg/ml morphine sulfate in 0.2% saccharin for the same 14-day period. N = 8 per point (means ± SE). The ascending series group consumed significantly more morphine than did the fixed concentration group ($p<0.01$, two-tailed t-test).

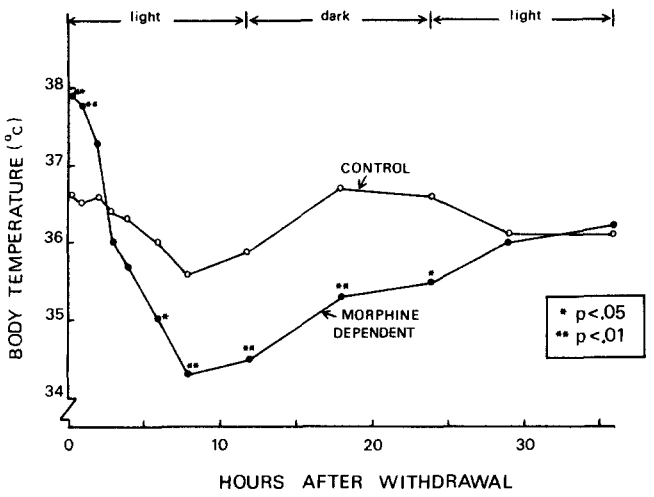

FIG. 4. Time course of core body temperatures in C57BL/6J mice exposed to an ascending series of morphine concentrations in the drinking fluid or tap water alone (controls) in Experiment 4. Body temperatures were monitored by radio telemetry, which allowed determinations to be made without handling or otherwise disturbing the animals. Each point represents 6 mice. The phase of the light cycle is also shown, since control (tap water only) body temperatures were seen to vary almost 1.0°C during a normal diurnal cycle. Withdrawal was effected by removing the morphine from the drinking fluid at the end of Day 14 of morphine exposure as described in Experiment 1. Peak hypothermia (−1.4°C vs. controls, $p<0.01$, ANOVA) was seen at 8 to 18 hours after withdrawal. No antagonists were used.

C57BL/6J mice. Despite apparent thirst and dehydration, mice of the DBA/2J strain showed a pronounced rejection of the sweetened morphine solution. These results underscore the profound differences that exist between these two strains in their acceptance of morphine solutions.

In Experiment 4, we sought to assess the withdrawal syndrome without the use of an antagonist in C57BL/6J mice. Following removal of the morphine-saccharin fluid, hypothermia developed in the morphine-exposed group compared to the control (no morphine) group, reaching a maximum of −1.4°C at 8 to 18 hours postwithdrawal relative to controls, as shown in Fig. 4. Diarrhea and tremor were observed in all but one of the eight morphine-saccharin-treated C57BL/6J mice. No incidence of jumping or wet dog shakes was seen.

In Experiment 5, C57BL/6J mice consumed almost twice as much 0.2% saccharin than did DBA/2J mice (9.1 ± 1.2 vs. 4.7 ± 1.7 ml) during the last two days of saccharin alone presentation under two-bottle choice conditions, indicating that there are sizeable palatability differences for 0.2% saccharin between these two strains. This has also been noted by Fuller (5) and Forgie *et al.* (4). The saccharin preference ratios (vs. tap water) were also greater in C57BL/6J mice (93 vs. 77%), although the latter comparison just failed significance ($p<0.07$, two-tailed t-test). In terms of explaining the marked differences between these two strains in morphine-saccharin consumption (Experiment 1 and 2), the palatability of bitter alkaloid-saccharin mixtures is of greater relevance than that of saccharin alone. This was assessed in terms of the quinine sulfate (QS) concentration needed to reduce quinine-saccharin consumption to less than the tap water alternative. This value was very similar in the two strains (C57BL/6J, 0.33 ± 0.13 mg QS/ml; DBA/2J, 0.31 ± 0.15 mg QS/ml), indicating that there were no palatability differences between the strains for bitter alkaloid-saccharin mixtures. Quinine consumption in 0.2% saccharin, expressed in mg/kg/day, was not significantly different between the strains at any concentration of QS employed, which stands in marked contrast to morphine consumption under similar conditions (Experiment 1 and 2). These QS consumption values (mean ± SE) for the DBA/2J mice were 31 ± 8, 50 ± 11 and 49 ± 14 for the 0.2, 0.3 and 0.4 mg/ml QS concentrations, respectively; for the C57BL/6J mice the corresponding values were 35 ± 5, 41 ± 5 and 46 ± 6 mg/kg/day of QS consumption.

DISCUSSION

The addition of saccharin is a remarkably effective means of inducing the voluntary consumption of large doses of morphine sufficient to produce an identifiable withdrawal syndrome in C57BL/6J mice, either with or without the use of an antagonist. The use of an ascending schedule of morphine concentrations in the drinking fluid, rather than a fixed concentration, further enhanced morphine intake. In mice of this strain, it matters little in terms of morphine intake whether a second bottle containing tap water is also freely available, since their consumption of tap water was quite small, representing less than 13% of their total fluid intake. During withdrawal, we were able to elicit all the classical withdrawal signs typically seen in laboratory rodents, including hypothermia, tremor, wet dog shakes, diarrhea (soft stool), jumping and lacrimation. Further, these physically dependent C57BL/6J mice will prefer to drink morphine alone (no saccharin) over tap water once the animals had been previously induced to consume large doses of morphine via the use of 0.2% saccharin (Experiment 1). Without the use of saccharin, mice of this strain avoided morphine from the outset, presumably because of its bitter quinine-like taste. In complete contrast to C57BL/6J mice, DBA/2J mice consistently rejected morphine-saccharin solutions offered under the same conditions, and when forced to consume this fluid, they restricted their intake so drastically that serious body weight loss and dehydration resulted. The dramatic differences between these two strains indicates a strong degree of genetic determination of morphine consumption and acceptance under our test conditions. It is of interest that mice of the C57BL/6J strain also show a preference for 6 to 10% ethanol concentrations in the drinking fluid vs. tap water, while DBA/2J mice strongly avoid such ethanol solutions in two-bottle choice situations (1,3).

We believe that the major weakness of two-bottle choice paradigms, namely the low dosages that usually result, has been overcome by the addition of the highly preferred masking taste of saccharin, and an ascending schedule of morphine concentrations. This is effective, however, only for the strain that shows a high acceptance of morphine (C57BL/6J) and not for the strains that do not (e.g., DBA/2J). One possible explanation for this may be strain differences in palatability for saccharin, where a 2-fold difference was found in the consumption of 0.2% saccharin consumption when tap water was also freely available (Experiment 5). However, the strain differences in voluntary consumption when morphine was added to the saccharin were over 10-fold (Experiment 1 and 2), indicating that the morphine itself is a very important factor determining strain differences in morphine-saccharin drinking. Of probably greater relevance than saccharin palatability is the palatability of bitter alkaloid-saccharin mixtures in the two strains, as when morphine and saccharin are administered together (Experiments 1 and 2). When quinine was used as the bitter tasting agent, because of its similar taste to morphine, no strain differences were noted in quinine consumption in 0.2% saccharin, or in the concentration of quinine necessary to cause the animals to prefer the tap water alternative. Similar findings in these two strains have been reported by Forgie *et al.* (4) for

quinine, with or without saccharin. These findings stand in marked contrast to the very large strain differences when morphine was used, and presumably reflect the great importance of opioid effects subsequent to morphine drinking, e.g., its reinforcing properties.

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