Physical dependence and tolerance development to sustained low concentrations of morphine in mice

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The subcutaneous implantation of morphine pellets is the most widely used method for the rapid induction of physical dependence and tolerance to morphine in mice (Maggiolo & Huidobro 1961; Way et al 1969). The pellets used have mostly contained 75–100 mg morphine alkaloid/pellet. We have used naloxone-induced jumping and changes in hot-plate latency to examine the development of physical dependence and tolerance in mice to relatively low concentrations of morphine from a newly developed sustained-release preparation (Howes & Schwope 1976; Kosersky & Ryan 1978).

Male, albino Charles River mice (Charles River laboratories, Wilmington, Mass., U.S.A.), 20–25 g, were used, had implanted subcutaneously in the dorsal scapular region a small rod (25 mg) containing 12·5 mg of [^{14}C]morphine alkaloid (New England Nuclear Corp., Boston, Mass., U.S.A.; specific activity 1·4 μCi g^{-1}) in a polylactic/polyglycolic acid copolymer matrix.

The daily urinary excretion of [^{14}C]morphine was determined in 10 implanted mice maintained individually in separate metabolism cages. Urine samples were collected at 24 h intervals. The cages were rinsed with distilled water and the washings added to the urine. This combination was further diluted to 10 ml with distilled water. A sample (1·0 ml) was taken and added to 15 ml of Aquasol liquid scintillation cocktail (New England Nuclear Corp.). The radioactivity was measured using a Beckman LS-230 liquid scintillation counter. A quench curve, constructed from a series of ^{14}C-quench standards, was used to normalize the counts of each urine sample. Urine samples were collected daily until urinary radioactivity levels fell to background values.

The development of tolerance to the analgesic properties of morphine was assessed at 24-h intervals for 10 consecutive days after morphine rod implantation. Analgesia, measured by the hot-plate procedure of Eddy & Leimbach (1953), was defined as a 100% increase in the response latency to a hot-plate maintained at 56 °C. At this temperature, non-treated control mice responded within 6–8 s. Separate groups of non-implanted and morphine rod-implanted mice (n = 10, each group) were challenged daily with an ED95 dose of morphine sulphate (10 mg kg^{-1} i.p.). Non-implanted controls injected with this dose of morphine responded usually within 19–22 s. Mice were tested twice on the hot-plate at 30 min before and after morphine injection. Each group was tested once only on a given day and not used again. Significant differences in response latencies were determined by Student's *t*-test for unpaired observations.

The development and degree of physical dependence on morphine were assessed in groups of implanted mice (n = 10, each group) by estimating the median effective dose (ED50) of naloxone hydrochloride required to precipitate withdrawal jumping from an elevated platform (Way et al 1969). Naloxone ED50 values were determined daily for 10 consecutive days after morphine rod implantation by the method of Dixon (1965). A separate group of mice was used on each successive day of rod implantation for the assessment of physical dependence. Although not quantitated, the occurrence of other naloxone-precipitated withdrawal signs, such as ear blanching, paw tremor, increased motor activity, diarrhoea and abnormal posturing, was recorded.

The results of the daily urinary excretion study were consistent with previous reports on mice and rats (Howes & Schwope 1976; Kosersky & Ryan 1978; Kosersky & Howes 1978). The urinary excretion of ^{14}C, derived from implanted [^{14}C]morphine rods, approximated zero-order kinetics over the first 10 days during which time 62% of the implanted ^{14}C had been measured

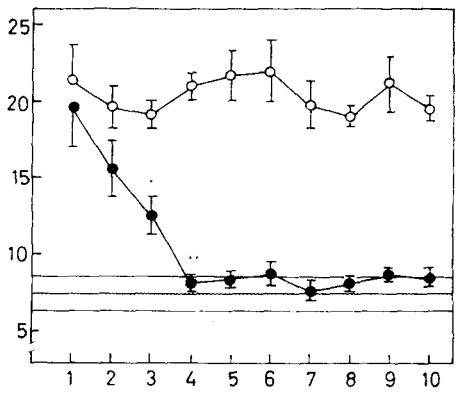

FIG. 1. Development of tolerance to the analgesic effects of morphine in mice tested on the hot-plate. Open circles represent the mean response latency of non-implanted control mice 30 min after injection of morphine sulphate (10 mg kg^{-1}, i.p.). Closed circles represent the mean response latency of rod-implanted mice 30 min after morphine sulphate injection (10 mg kg^{-1}, i.p.). Each point represents the mean response latency (± s.e.m.) of groups of 10 mice. The shaded area represents the mean ± the range of standard errors of the daily response latencies of separate groups of normal, non-injected control mice. * $P < 0.05$ differs from non-implanted control mice. ** $P < 0.01$ differs from non-implanted control mice, days 4–10. Ordinate: reaction time(s). Abscissa: days of rod implantation.

* Correspondence.

in the urine. [^{14}C]Morphine rods were essentially depleted of morphine after 21 days of implantation. The mean daily excretion rate (days 1–10) was estimated to be approximately 570 μg day^{-1}.

Tolerance developed rapidly to the analgesic effects produced by the implanted morphine rods alone. Increased reaction times (12·5 $\pm$ 0·91 s; mean $\pm$ s.e.m., n = 10) on the hot-plate were evident 24 h after rod implantation. However, the response latencies were essentially identical to those of non-implanted mice at 48 h and for the remainder of the 10-day test period.

Additional groups of implanted and non-implanted control mice were injected with an ED95 dose of morphine sulphate (10 mg kg^{-1}, i.p.) on successive days after morphine rod implantation. In non-implanted controls, this dose consistently increased reaction times on the hot-plate to approximately 19–22 s. A significant increase in response latency was also measured in morphine-injected mice 24 h after rod implantation (reaction time = 19·4 $\pm$ 2·52 s). However, on subsequent days the reaction times of implanted animals decreased rapidly and by the fourth day, the reaction times of morphine-injected, implanted mice were not significantly different from those of non-injected controls, indicating a complete tolerance to the effects of the injected morphine (days 4–10). The time course of tolerance development to the ED95 dose of morphine is shown in Fig. 1.

Naloxone-induced withdrawal jumping was observed within 24 h (naloxone ED50 = 4·20 mg kg^{-1}) and progressively reached its peak at 96 h after rod implantation (naloxone ED50 = 1·78 mg kg^{-1}). Thereafter (days 5–10), naloxone-induced jumping activity diminished rapidly. A 10-fold increase in the naloxone ED50 was noted on day 5 (17·8 mg kg^{-1}). On subsequent days, doses of naloxone up to 100 mg kg^{-1} i.p., failed to elicit the jumping response. However in the absence of jumping activity, other signs of precipitated withdrawal, including ear blanching, paw tremor, increased motor activity, diarrhoea and abnormal posturing, were observed. These abstinence signs were present over the 10-day test period.

The stereotyped jumping behaviour induced by naloxone has found increasing use as an index of physical dependence in mice treated chronically with opiates (Way et al 1969; Cheney & Goldstein 1971; Saelens et al 1971). Moreover, an inverse relationship has been shown to exist between the degree of physical dependence and the dose of naloxone required to elicit jumping behaviour (Way et al 1969).

We have found the time course for the development of tolerance and physical dependence in morphine rod-implanted mice to be in agreement with that reported by Way et al (1969) and other investigators using pellets containing relatively large amounts of morphine. Maximal tolerance and dependence occurred at 3–4 days after implantation. It has been assumed that dependence diminishes rapidly after this period because the implanted pellet becomes encapsulated by fibrous tissue (Blasig et al 1973), or the cellulose, used in the formulation of the pellets, blocks further absorption of morphine after the morphine at the surface of the pellet is absorbed (Gibson & Tingstad 1970). However, Lesher & Spratto (1976) and Patrick et al (1975) have shown that encapsulation of morphine pellets in mice and rats does not cause an appreciable decrease in morphine absorption. Moreover we have found that naloxone-induced withdrawal jumping diminished rapidly during a time when maximal concentrations of urinary morphine were observed, a high degree of tolerance was manifest, and other naloxone-induced withdrawal signs were present.

Naloxone-induced jumping activity is only one expression of morphine dependence. Mechanisms mediating this particular response may be selectively altered after a period of time by constant exposure to effective levels of morphine. The results here reported suggest that naloxone-induced jumping is time-dependent and a function of both morphine and naloxone concentrations. In addition, these results further emphasize the need for caution when using one abstinence sign to measure morphine dependence.

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