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Physical Dependence and Tolerance Development after Chronic Exposure to Low Levels of Morphine in the Rat

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KOSERSKY, D. S., M. D. KOWOLENKO AND J. F. HOWES. *Physical dependence and tolerance development after chronic exposure to low levels of morphine in the rat.* PHARMAC. BIOCHEM. BEHAV. 12(4) 625–628, 1980.—A sustained-release delivery system containing ^{14}C-morphine was implanted subcutaneously in rats. Measurement of urinary excretion of ^{14}C suggested a steady state release of approximately 640 μg ^{14}C morphine/day during a 10-day test period. Tolerance developed rapidly to the analgetic effects produced by an injected ED_{95} dose of morphine sulfate in implanted rats tested on the hot-plate. Physical dependence, determined by naloxone-precipitated abstinence behavior, was evident in rats at 24 hr. Morphine dosage was estimated to be as low as 2.5 mg/kg/day. Peak abstinence behavior was observed on Day 4. However, naloxone-precipitated withdrawal signs were markedly diminished by Day 6 and essentially absent by Day 8. These results are discussed with reference to the suggestion that metabolic changes, occurring during chronic exposure to morphine, may explain the lack of abstinence behavior during a time when maximal concentrations of urinary morphine were observed and a high degree of tolerance was manifest.

Rats Morphine Tolerance Physical dependence Naloxone-precipitated withdrawal

THE subcutaneous implantation of morphine pellets is a widely used method for the rapid induction of physical dependence and tolerance to morphine in rats and mice [2, 15, 16]. Investigators who have adopted this procedure, however, have utilized pellets containing relatively large quantities of morphine, usually 75–100 mg morphine alkaloid/pellet. We have examined the development of physical dependence and tolerance to relatively low concentrations of morphine in rats, using a sustained-release preparation of morphine reported previously in mice [7,9].

METHOD

Male, Charles River CD rats (Charles River Laboratories, Wilmington, MA), 200–250 g, were made tolerant to and dependent on morphine by the subcutaneous implantation of 1–2 small morphine rods in the dorsal scapular region. Each rod contained 12.5 mg of (^{14}C)morphine alkaloid (New England Nuclear Corp., Boston, MA; specific activity, 1.4 μCi / g) prepared in a polylactic polyglycolic acid copolymer matrix (Dynatech R/D Co., Cambridge, MA) [14].

The daily urinary excretion of (^{14}C)morphine was determined in 10 rats, each implanted with 2 rods and maintained individually in separate metabolism cages. Urine samples were collected at 24 hr 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.

Tolerance development to the analgesic properties of morphine was assessed at 24 hr intervals for 10 consecutive days after morphine rod implantation. Analgesia, measured by the hot-plate procedure of Eddy and Leimbach [4], was defined as a 100% increase in the response latency to a hot-plate maintained at 56°C. At this temperature, non-treated control rats responded usually within 9–12 sec. Separate groups (n=6, each group) of non-implanted and morphine rod-implanted rats (2 rods, each rat) were challenged daily with an ED_{95} dose of morphine sulfate (10 mg/kg, IP). Non-implanted control group rats injected with this dose of morphine responded usually within 20–26 sec. Rats were tested twice on the hot-plate at 30 min before and after morphine injection. Rods were not removed prior to testing. 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 by the incidence of various withdrawal signs precipitated by graded doses of naloxone hydrochloride (1.0–5.6 mg/kg, IP) administered to separate groups (n=6, each group), of implanted rats (1–2 rods) on successive days after rod implantation. The morphine rods were not removed prior to naloxone injection. Withdrawal signs including teeth chatter, wet-dog shakes, irritability to

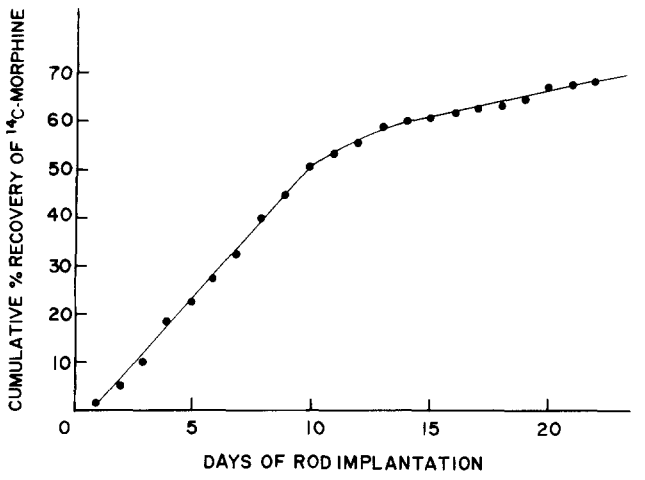

FIG. 1. Urinary excretion of ^{14}C derived from implanted 50% w/w (^{14}C)morphine base-loaded rods of 50/50 poly(L(+)-lactic-co-glycolic acid).

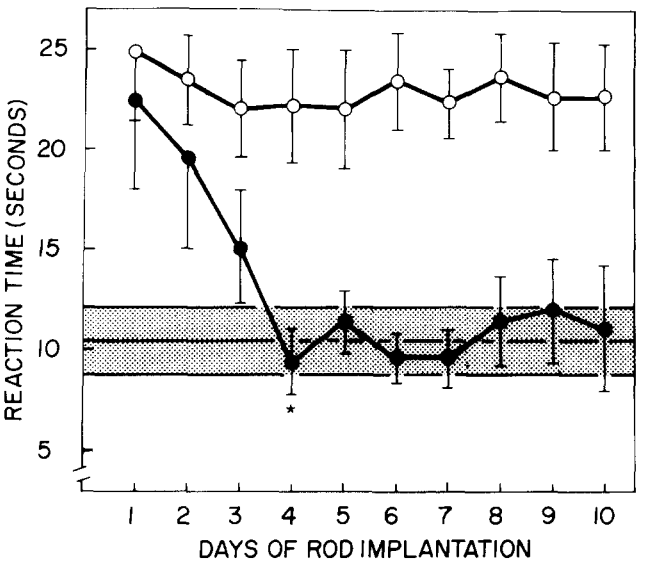

FIG. 2. Development of tolerance to the analgesic effects of morphine in rats tested on the hot-plate. Open circles represent the mean response latency of non-implanted control rats 30 min after injection of morphine sulfate (10 mg/kg, IP). Closed circles represent the mean response latency of rod-implanted rats 30 min after morphine sulfate injection (10 mg/kg, IP). Each point represents the mean response latency (±SEM) of groups of 6 rats. The shaded area represents the mean ± the range of standard errors of the daily response latencies of separate groups of normal, non-injected control rats. *$p<0.05$ (minimum) differs from non-implanted control rats, Days 4–10.

handling (vocalization) and stereotyped withdrawal jumping from an elevated platform (74 cm) were recorded as quantal events in each group for a 20 min period after naloxone administration [11]. Separate groups of rats were used on each successive day of rod implantation for the assessment of physical dependence.

RESULTS

The results of the daily urinary excretion study (Fig. 1) were consistent with previous findings with mice and rats [8,9]. 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 51% of the implanted ^{14}C had been measured in the urine. (^{14}C)morphine rods were essentially depleted of morphine after 22 days of implantation. The mean daily excretion rate (Days 1–10) was estimated to be approximately 640 μg/rod per day.

Tolerance developed rapidly to the analgesic effects produced by the implanted morphine rods. Increased reaction times (11.4 ± 1.60 sec mean ± SEM) on the hot-plate were evident in groups of rats 24 hr after rod implantation. However, the response latencies of implanted and non-implanted rats were essentially identical by 48 hr and for the remainder of the 10-day test period.

A significant increase in response latency was measured in morphine-injected rats 24 hr after rod implantation (reaction time = 22.4 ± 4.52 sec). However, on subsequent days the reaction times of implanted animals injected with an ED_{95} dose of morphine sulfate (10 mg/kg, IP) decreased rapidly. By the fourth day, the reaction times of morphine-injected, implanted rats 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 ED_{95} dose of morphine is shown in Fig. 2.

Table 1 summarizes the acute effects produced by graded doses of naloxone administered to groups of rats implanted with 1 or 2 morphine rods. Characteristic signs of precipitated withdrawal behavior including wet-dog shakes, teeth chatter, jumping and vocalization were elicited by effective doses of naloxone 24 hr after morphine implantation. Peak withdrawal activity was generally observed on Day 4. By Day 6, responses to an acute dose of naloxone were markedly diminished and were essentially absent by Day 8. In general, rats implanted with 2 morphine rods and injected with the higher doses of naloxone exhibited a greater degree of abstinence behavior.

DISCUSSION

The results of this study clearly show that a significant degree of tolerance and physical dependence can be produced in the rat by continuous exposure to relatively low levels of morphine. Rats implanted with a sustained-release preparation, estimated to deliver a dose of morphine as low as 2.5 or 5.0 mg/kg per day, exhibited typical acute abstinence behavior following the administration of moderate doses of naloxone.

We have found the time course for the development of tolerance and physical dependence in morphine rod-implanted rats to be in agreement with that reported by Cicero and Meyer [2] and other investigators using pellets containing relatively large quantities 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 [1] 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 dissolved [5]. However, Patrick *et al.* [12] and Lesher and Spratto [10] have shown that encapsulation of

TABLE 1

NALOXONE-PRECIPITATED ABSTINENCE SIGNS IN RATS IMPLANTED WITH SUSTAINED RELEASE MORPHINE RODS. VALUES ARE EXPRESSED AS THE PROPORTION OF RATS IN EACH GROUP (n=6) EXHIBITING DESIGNATED ABSTINENCE SIGNS WITHIN 20 MIN AFTER ADMINISTRATION OF NALOXONE

	Rats implanted with 1 rod (12.5 mg morphine)			Rats implanted with 2 rods (25.0 mg morphine)		
	Naloxone dose (mg/kg IP)					
Days after implant	1	3.2	5.6	1	3.2	5.6
Wet-dog shakes						
1	0/6	3/6	4/6	1/6	3/6	4/6
2	0/6	4/6	3/6	1/6	5/6	2/6
3	1/6	4/6	4/6	2/6	5/6	4/6
4	2/6	3/6	3/6	3/6	5/6	4/6
5	1/6	2/6	2/6	0/6	3/6	4/6
6	0/6	1/6	0/6	0/6	1/6	1/6
7	0/6	0/6	0/6	0/6	0/6	0/6
Teeth chatter						
1	0/6	1/6	0/6	1/6	3/6	4/6
2	0/6	2/6	3/6	0/6	3/6	3/6
3	0/6	3/6	3/6	1/6	2/6	3/6
4	2/6	3/6	4/6	2/6	3/6	2/6
5	1/6	2/6	2/6	1/6	1/6	0/6
6	0/6	0/6	1/6	0/6	0/6	0/6
7	0/6	0/6	0/6	0/6	0/6	0/6
Jumping						
1	0/6	0/6	2/6	0/6	2/6	2/6
2	0/6	0/6	3/6	0/6	1/6	2/6
3	1/6	0/6	1/6	0/6	3/6	3/6
4	0/6	2/6	1/6	2/6	2/6	3/6
5	0/6	1/6	0/6	1/6	3/6	2/6
6	0/6	0/6	0/6	0/6	1/6	1/6
7	0/6	0/6	0/6	0/6	0/6	1/6
Vocalization						
1	0/6	2/6	1/6	0/6	3/6	4/6
2	0/6	2/6	3/6	0/6	3/6	3/6
3	2/6	2/6	3/6	1/6	2/6	3/6
4	2/6	2/6	4/6	2/6	1/6	2/6
5	0/6	0/6	2/6	0/6	1/6	0/6
6	0/6	0/6	0/6	1/6	0/6	1/6
7	0/6	0/6	0/6	0/6	0/6	0/6

morphine pellets in rats and mice does not cause an appreciable decrease in morphine absorption. Moreover, we have found that naloxone-precipitated withdrawal activity diminished rapidly during a time when maximal concentrations of urinary morphine were observed and a high degree of tolerance was manifest. This would suggest that naloxone-precipitated abstinence is time dependent and a function of both morphine and naloxone concentrations.

In this regard, Patrick *et al.* [13] have reported a dispositional tolerance to occur in rats infused chronically with high levels of morphine. Brain morphine concentrations increased during the first 3 days of a 6-day continuous IP infusion regimen. However, after reaching peak magnitude on Day 3, brain morphine levels declined steadily and significantly during the remaining days of infusion. It was suggested that increased conjugation of morphine was responsible for the dispositional changes observed.

Preliminary experiments in our laboratory support this suggestion. We have noted increased morphine glucuronide levels in the urine of rats on successive days after morphine rod implantation. Furthermore, increased conjugation has been cited as a factor in the metabolic tolerance to levorphanol in the mouse [6]. Although mechanisms mediating particular abstinence signs may be selectively altered in rats after a certain period of time, a decrease in brain morphine concentrations after chronic exposure to effective levels of morphine may account for the disappearance of precipitated abstinence signs noted in the present study.

In this regard, decreased brain concentrations of morphine may become ineffective in maintaining a state of physical dependence. Spontaneous abstinence signs may be masked by the continued presence of morphine but readily precipitated by naloxone through the 5th day of morphine-rod implantation. The continuing tolerance to the analgesic actions of morphine is in agreement with the work of Cochin *et al.* [3], Patrick *et al.* [13] and others.

In view of these considerations, further investigation of the dispositional and metabolic changes brought about by chronic morphine treatment appear warranted.

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