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Quantitative Relationships Among Measures of Morphine Tolerance and Physical Dependence in the Rat

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MUCHA, R. F., H. KALANT AND M. A. LINSEMAN. *Quantitative relationships among various measures of morphine tolerance and physical dependence in the rat.* PHARMAC. BIOCHEM. BEHAV. **10**(3) 397–405, 1979.—The influence of treatment dose on a number of characteristics of opiate tolerance was studied in male Sprague-Dawley rats treated with daily intraperitoneal (IP) injections of morphine sulfate. Zero, 7.5, 15, 25 or 45 mg/kg/day was given for 34 consecutive days and the degree of morphine effect on four different tests was periodically assessed. Dose-related effects on tailflick latency (tail immersion test, 1.5 hr post injection), swimming test (2 hr post injection), and body weight gain revealed the development of tolerance; there was a non-dose-related hyperthermia (1.5 hr post injection) to which rapid sensitization occurred. All changes reached asymptote and the rate and extent of change varied with the test. Plots of log response vs test day for tailflick and swimming indicated an early steep component and a later less steep component of decline. Subsequent testing indicated that the log-dose/response (LDR) curves for tailflick latency and time to maximum hyperthermia shifted to the right by an amount dependent on the treatment dose; there was no change in the curve for hyperthermia duration. In high dose groups no further shift occurred, but the tailflick LDR curves became flattened. The tailflick LDR curve changes were replicated in rats treated for 24 days with 0, 8, 24, 48, 96, or 240 mg/kg/day. Subsequently, a constellation of withdrawal signs precipitated by naloxone HCl (1 mg/kg, IP) was measured. On the basis of the relation between treatment dose and the magnitude of the various measures, there was a parallel between analgesia tolerance and some, but not all, signs of physical dependence.

Rat Morphine Dose response Intraperitoneal injection Tolerance Physical dependence Analgesia Temperature Weight change Swimming Naloxone-precipitated

ALTHOUGH opiate tolerance has been studied for decades and a vast literature has grown on possible molecular mechanisms (cf. [5,17]), there is still a lack of certain basic data on its general characteristics. The present paper reports the effect of systematically varying treatment dose on several of the characteristics of tolerance. The precise rationale for the experimental designs stemmed from specific deficiencies associated with definition of tolerance, rate of acquisition, and relation to physical dependence.

Acquired tolerance to the effects of a drug is best defined as the degree of parallel shift of the log-dose/response (LDR) curve along the log-dose axis [10,15]. The findings on LDR curve changes during the development of morphine tolerance are somewhat contradictory. Most investigators (cf. [9, 10, 27]) report only the parallel shift of the LDR curve. Others [8, 14, 25] have observed a combination of shift and reduction of the slope after treatment with morphine. At present, there is no clear explanation for the difference between the various reports. Therefore, a purpose of the present investigation was to replicate and study the flattening of the LDR curve in rats made tolerant to morphine administered by daily IP injection.

Until recently, there have been very few quantitative studies on the development of opiate tolerance. There were two distinct reasons for its study here. First, these data are important for the analysis of possible mechanisms of tolerance. In one of the very few systematic studies of the rate of tolerance onset, Goldstein and Sheehan [11] manipulated the interval between injections of 20 mg/kg of levorphanol and found that tolerance to running fits in mice was a first-order process with a half-life of 16 to 48 hr. They have related these kinetic data to the possible degradation of an enzyme or other functional protein. However, comparable findings have never been reported for other drugs, species or measures of opiate effect. Second, data on the time course of tolerance development are important for interpreting apparent variations in tolerance produced under differing conditions (cf. [18]). The present studies were designed, in part, to measure the extent of tolerance, independently of rate. Different degrees of LDR curve shift are indicative of different extents of tolerance to various opiate effects (cf. [9,10]), but only if comparisons are made after tolerance has clearly reached asymptote. If comparisons are drawn before tolerance is complete and if the opiate effects differ only in

their rate of tolerance development, there will also be different degrees of LDR curve shift. Therefore, conclusions regarding extent of tolerance will be erroneous. Since complete time course data for all measures proposed for the study of extent of tolerance were not previously reported, it was necessary for these to be collected first.

The relation between opiate tolerance and physical dependence is theoretically and clinically important, but the studies have been only semisystematic and the data are incomplete. Most studies, for example, correlate only a single indicator of physical dependence, such as naloxone-precipitated jumping [10,27] or wet-dog shakes [4], with measures of tolerance. The importance of measuring a number of signs of morphine withdrawal is indicated by the finding that intensity of some withdrawal signs bears a biphasic relation to intensity of the treatment [3]. Therefore, we compared the degree of tolerance on an analgesia test with measurements on a variety of separate signs of physical dependence.

METHOD

Animals

Male Sprague-Dawley rats (240–260 g) were purchased from Canadian Breeding Laboratories (Constant, Quebec). They were housed singly in stainless steel cages, with ad lib access to Purina rat chow and water. Room lights were on from 0700 to 1900 hrs. Ambient temperature was 20–24°C. Rats were handled for 5 min every 2 days for at least 12 days before the experiment. All treatments and tolerance tests commenced at 0900 hr and the injections were generally completed by 1030 hr.

Drugs

Doses of morphine sulfate (BDH) and naloxone hydrochloride (Endo) were expressed in terms of the salts. The solutions were made with physiological saline and injected IP at room temperature, except for solutions with concentrations of 63 mg/ml and greater, which were injected at a temperature of 30–32°C.

Tests and Apparatus

Analgesia was measured with a modified tailflick test [24]. It consisted of measuring the latency to the nearest 0.2 sec between immersion of the distal half of the rat's tail perpendicularly into warm water and emergence of the tip from the water. The temperature of the water, 55°C in Experiment 1 and 53.5°C in Experiment 2, produced baseline latencies of 2–4 sec in most naive rats. If the rat did not respond within 15 sec the tail was removed from the water and a score of 15 sec was recorded.

The swimming test was similar to that of Cochin and Kornetsky [6]. The rat was placed at one end of a 20 × 350 cm tank filled to a depth of 23 cm with water at 19°C. The time taken by the rat to swim to a dry 18 × 18 cm platform at the other end was measured to the nearest 0.2 sec. If the rat failed to reach the platform in 60 sec, it was removed from the water and given a score of 60 sec.

For the rectal temperature test, a thermistor probe was inserted 5.5 cm into the rectum. After 30 sec equilibration, the temperature (nearest 0.1°C) was recorded on a Yellow Springs Instrument Co. electrical thermometer. Body weights were measured to the nearest 1 g.

The withdrawal test was a modification [19] of the procedure of Bläsig *et al.* [3]. The weight and rectal temperature were first recorded and then the rat was placed in a clear 17 × 22 × 23.5 cm Plexiglas box. After a 10-min acclimation period, the rat was injected (1 ml/kg) with 1 mg/kg naloxone and returned to the box for 30 min. The presence or absence of the following signs was checked every 10 min: screaming-on-touch, hostility-on-handling, ptosis, salivation, rhinorrhea, lacrimation, diarrhea, and penile ejaculation (penile erection, presence of discharge). The incidence of the following behaviors during the 30-min observation period was counted: circling (complete circles), rearing, jumping (on to the edge of the box), wet dog shakes, episodes of teeth chattering, and writhing movements. At the end of the 30-min observation period the rectal temperature was again measured and the animals were returned to their home cages. Body weights were again recorded the next morning.

Statistical Analyses

A number of measures showed skewed distributions. Therefore, the data were analysed by nonparametric tests [23]. Where applicable, these were two-tailed and a probability of <0.05 was the accepted level of significance. The data are summarized in the figures with standard error of the mean (SE) depicted as a vertical line; when no line is shown, the SE was less than the dimension of the plotted point.

EXPERIMENT 1A: TIME COURSE OF TOLERANCE DEVELOPMENT TO VARIOUS MORPHINE EFFECTS

Procedures

Following a 7-day training and acclimation period, each rat received one daily trial on the swimming task and daily exposure to the rectal temperature and tailflick procedures. On the last training day, six rats that had swimming scores of more than 30 sec were dropped from the study. Commencing 1 day later, each rat received single daily injections for 38 consecutive days. On the odd-numbered days a rectal temperature test, followed immediately by a tailflick test, was taken 90 min after the injection. On alternate days one swimming test was given exactly 2 hr after the injection. The different testing times, determined from pilot data, were employed in order to have the doses produce similar degrees of impairment on the swimming and tailflick tests. On the basis of scores from the first 4 days, during which the injections were saline only, 50 rats were divided into five matched equal-sized groups which received 0, 7.5, 15, 25, or 45 mg/kg of morphine daily. All injections were given in a volume of 1 ml/kg.

Results

The time courses of changes in group mean response on the tailflick and swimming tests are given in Figs. 1 and 2, respectively. On the first morphine test, all scores for the morphine groups, except the 7.5 mg/kg group swimming score, were significantly longer than those of the 0 mg/kg group and the increases were dose-related. Subsequently, the morphine effects on both tests decreased in a roughly exponential fashion. By the fourth test day, all these morphine-produced effects had declined significantly from the level seen on Test Day 1. By the 15th test day, only the

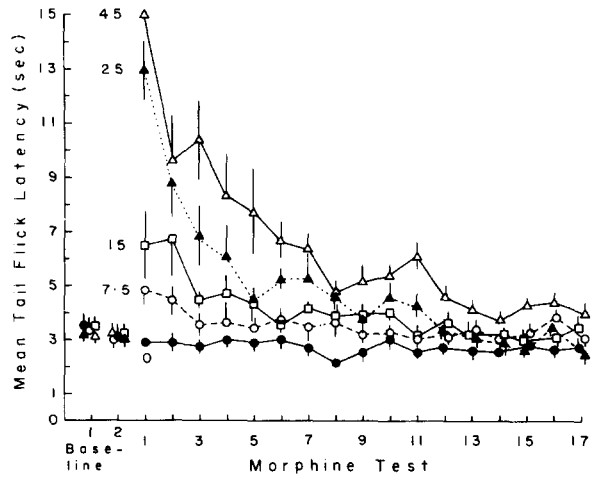

FIG. 1. Mean tailflick latency measured after every second injection for groups of rats (10/group) injected IP daily with 0, 7.5, 15, 25, or 45 mg/kg of morphine, as indicated by number beside each curve.

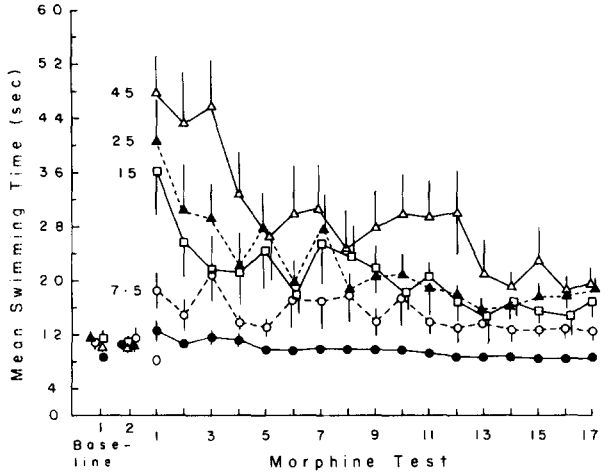

FIG. 2. Mean swimming time for respective groups in Fig. 1 following injections alternate to those preceding the tailflick test. Number by each curve indicates daily morphine dose in mg/kg.

45 mg/kg group still differed significantly from the controls on the tailflick test; on the swimming test all morphine groups differed significantly from the controls. The controls also showed a significant but small fall in swimming time over the treatment period.

A plot of the mean log tailflick latency and log swimming time against test days showed an apparent biexponential decay of morphine effect with an initial (Test Days 1–3) rapid phase and slower second phase. For an individual animal this was typically seen as an initial maximum or near maximum score followed by a precipitous drop to an intermediate level from which the scores declined gradually towards the baseline. The second component for the tailflick data had mean half-lives (± SE) of 27.0 ± 3.9 (n=5), 22.1 ± 2.3 (n=8), and 20.5 ± 2.1 (n=7) days for the 15, 25, and 45 mg/kg groups, respectively. In parentheses are the numbers of animals used to determine the means; those animals were excluded for which fewer than five points were available to describe the second component of decline, and for this reason no value is given for the 7.5 mg/kg group. The difference between the mean half-lives of the 15 and 45 mg/kg group was significant, indicating a dose dependency. The swimming data were too variable to provide a reliable estimate of the regression line for the second component for a sufficient number of individual rats; however, it appeared that the decline was more gradual than for tailflick. There were too few data for accurate measurement of the first component of decline, but it was estimated to have a half-life of 1 to 2 days on both tests.

The body weight data are summarized in Fig. 3. A combination of two morphine effects on body weight resulted in a dose-dependent difference in mean body weight among the various groups at the end of the treatment period. First, morphine caused an obvious initial dose-dependent suppression of weight gain. This was reflected in the mean number of test days required for individual rats of the respective groups to show a weight increase of more than 5 g over the weight on the first test day (see Table 1). Second, once resumption of weight gain occurred, it continued at a steady rate that was significantly reduced by the higher treatment doses (see Table 1). Thus, as for swimming impairment, complete tolerance failed to occur.

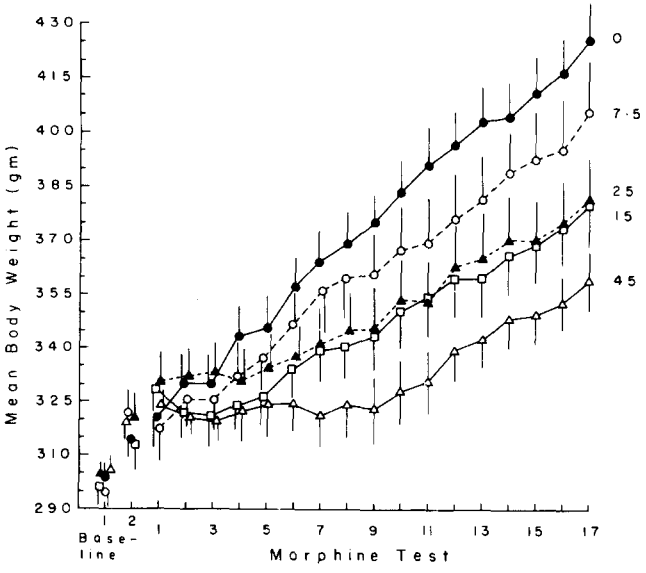

FIG. 3. Mean body weight for groups in Fig. 1 measured on day of tailflick test. Number of each curve indicates daily morphine dose in mg/kg.

The rectal temperature data are summarized in Fig. 4. Morphine produced hyperthermia on all test days with virtually no overlap between data of the control group and those of the morphine groups. The degree of hyperthermia on Test Day 1, however, was not dose-dependent. With chronic treatment, there appeared to be a rapid sensitization of the hyperthermic response in all morphine groups. By the fourth test day the 45 mg/kg group showed a significantly higher temperature than on the first. For the other three groups, the response was significantly greater on the eighth

TABLE 1

MEAN TEST DAYS REQUIRED TO GAIN 5 G AFTER START OF TREATMENT AND MEAN RATE OF WEIGHT GAIN ON FINAL 9 TEST DAYS FOR VARIOUS MORPHINE-TREATED GROUPS

Daily Treatment Dose (mg/kg)†	Mean Tests to Weight Gain of 5 g (± SE)	Mean Final Weight Gain (g) per Test (± SE)
0	2.7 ± 0.2*	5.3 ± 0.4*
7.5	2.4 ± 0.2*	4.3 ± 0.3*
15	4.1 ± 0.5	3.2 ± 0.4
25	6.3 ± 1.4	3.2 ± 0.2
45	9.4 ± 1.3	3.3 ± 0.3

*$p<0.05$ compared to each of 15, 25, and 45 mg/kg groups.
†10 rats/group.

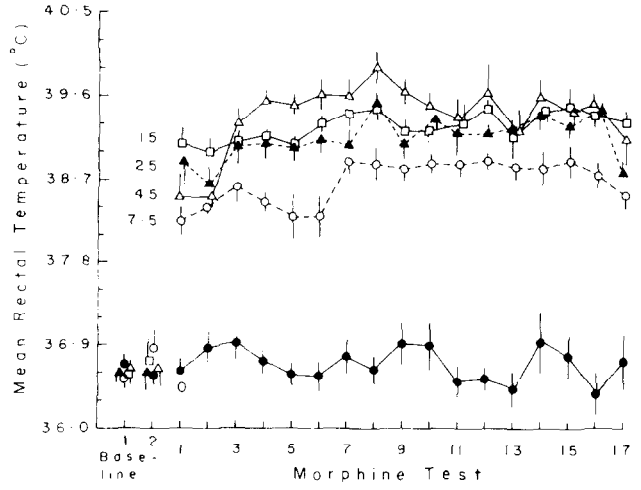

FIG. 4. Mean rectal temperature for groups in Fig. 1 measured immediately prior to administration of tailflick test. Number by each curve indicates daily morphine dose in mg/kg.

than on the first test day. Following this sensitization period the magnitude of hyperthermia became dose-related. The 7.5 mg/kg group mean temperature was consistently lower than those of the other three morphine groups, and there were differences between the 45 and 25 mg/kg groups on Test Days 5 to 9 and between the 45 and 15 mg/kg groups on Test Days 4, 5, 8, and 9. There were, however, no consistent differences among the three groups on the last five test days.

EXPERIMENT 1B: LDR CURVES OF RATS TREATED WITH DIFFERENT DOSES OF MORPHINE

Procedure

The day after completion of Experiment 1A, the same groups of animals were started on Experiment 1B, which lasted 18 days. On Days 6, 9, 12, 15 and 18, tailflick latencies were measured at 1.5 hr and rectal temperatures at 1.5, 3, 4.5, 6, 7.5, and 9 hr after different test doses of morphine (all 5 ml/kg, IP). The test doses of the saline-control animals on these five test days were 2.5, 5, 10, 20, and 40 mg/kg, respectively, given in increasing order, and for the various morphine-treated groups they were 60, 120, 240, 480, and 960% of the respective chronic treatment doses used in Experiment 1A. On days other than test days each rat received the same treatment dose as in Experiment 1A.

Results

The tailflick LDR curves obtained from the five test days are shown in Fig. 5. The major effects of chronic morphine treatment were evident. The first was a dose-related shift of the curves to the right. The shift was quantified by estimating ED_{50}s from probit analyses [12]. The calculated values were 19, 42, 94, 120, and 135 mg/kg for the 0, 7.5, 15, 25, and 45 mg/kg groups, respectively. There was an excellent linear relation between log ED_{50} and the lower treatment doses, but for the upper two doses the results confirmed the visual impression that the shift of the LDR curves was reaching a maximum. The second effect was an apparent reduction in slope of the curve in rats treated with high doses. The change in slope was associated with a reduction in maximum latency. There were no significant differences between the mean latencies at the two highest doses for each of the 25 and 45 mg/kg groups, but there were significant differences between the mean latencies at the two highest test doses and the mean latency of the respective middle test dose.

The rectal temperature tests indicated that all morphine doses produced significant hyperthermia, defined as elevation of temperature above either of two baseline measurements. The baseline values for each rat comprised the two temperature measures taken 9 hr after injection of the two lower test doses. From other work we have found that these values accurately reflect the premorphine temperatures. The duration of the hyperthermia was dose-related and the LDR curves of this measure are in Fig. 6. Clearly, very little tolerance developed. This was in contrast to the tolerance observed for the temperature response consisting of time from injection to maximum hyperthermia (Fig. 7). This response has been reported to reveal tolerance [13,26]. Gunne [13] also indicated that time to maximum hyperthermia reflects the magnitude of the initial hypothermia response to morphine. There was no apparent flattening of these LDR curves for any of the treatment dose groups tested.

EXPERIMENT 2: COMPARISON OF DEVELOPMENT OF TOLERANCE AND PHYSICAL DEPENDENCE

The specific purpose of this experiment was two-fold. First, the LDR-curve changes of the previous experiment

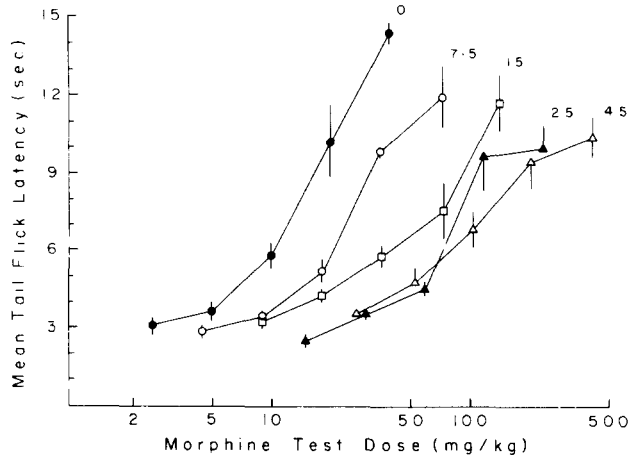

FIG. 5. Tailflick latency LDR curves for groups in Fig. 1 after 34 days of treatment with the daily doses of 0, 7.5, 15, 25, or 45 mg/kg, as indicated by numbers beside respective curves.

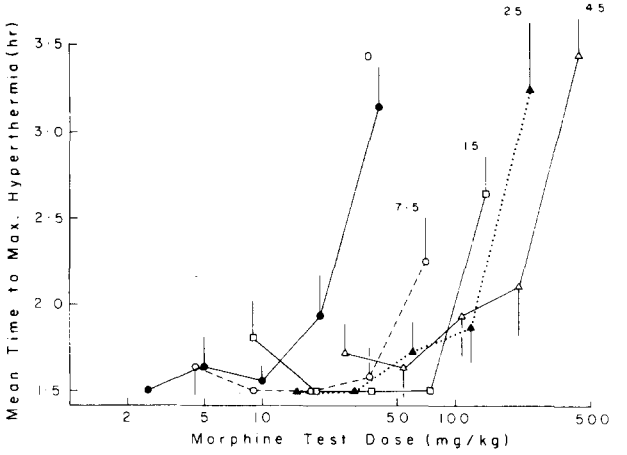

FIG. 7. LDR curves of time to maximum morphine-produced temperature increases for various morphine-treatment groups in Fig. 5. Number by each curve indicates daily morphine dose in mg/kg.

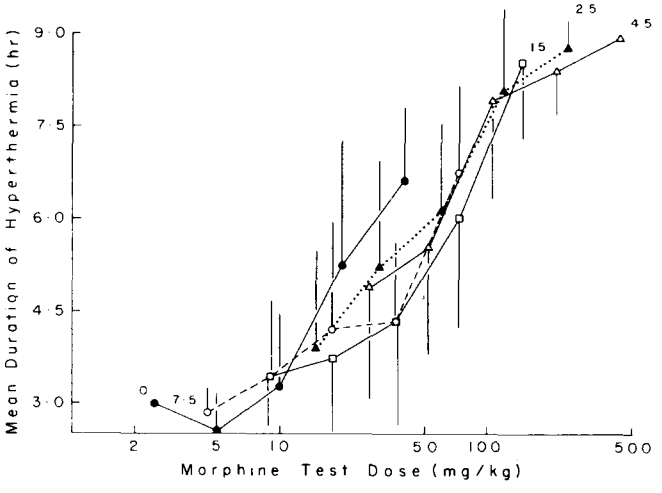

FIG. 6. LDR curves of duration of morphine-produced hyperthermia for various morphine-treatment groups in Fig. 5. Number by each curve indicates daily morphine dose in mg/kg.

were replicated and extended to include higher doses. Second, following the LDR-curve determinations naloxone-precipitated withdrawal signs were measured in these same rats in order to permit inferences about the relation of tolerance to physical dependence.

Procedure

From a group of 82 naive rats, three groups of 12 each were selected and given daily IP injections (5 ml/kg) containing 0, 8, and 24 mg/kg morphine, respectively, for a period of 24 days. The remaining 46 rats were started at 24 mg/kg. However, with a series of dose increments, implemented at 2-day intervals, they were brought to, and maintained at, a daily treatment dose of 48 (n=12), 96 (n=12), or 240 mg/kg (n=22). The first three increments, where appropriate, were each 24 mg/kg and the next three were each 48 mg/kg. The selection procedure at each stage was designed to yield a balanced distribution of body weights among the various dosage groups. Each group remained on its own maintenance dose level for at least 10 days. On Day 24, four rats were dropped from the experiment: one each in the 48 and 96 mg/kg groups because of excessive weight loss, and one each from the 8 and 96 mg/kg groups because of pronounced self-mutilation. On this day the mean body weights (g ± SE) of the 0, 8, 24, 48, 96, and 240 mg/kg groups were 371 ± 12, 350 ± 10, 322 ± 7, 316 ± 8, 307 ± 9, and 299 ± 9 g, respectively.

Between Days 25 and 37, LDR curves for morphine analgesia were determined for the various treatment groups by measurement of tailflick latency 90 min after injection of morphine. Five different test doses were used for each group; each rat within the group received all five doses. These were 4, 10, 25, 63, and 100 mg/kg for the 0 mg/kg group, and 25, 63, 155, 390, and 630 mg/kg for the other groups (all in 10 ml/kg). Tests were conducted at 3-day intervals, and the four lowest test doses for each rat were given in a randomized order. The highest test dose was kept until the fifth test day; this lowered the risk of killing some of the animals before completion of testing with the lower doses. On the intervening days, the respective maintenance dose was given to each rat. A separate group of 10 rats on the 240 mg/kg maintenance dose was tested once only, at a dose of 975 mg/kg (10 ml/kg). Three rats of this group died of overdose before the test could be conducted, so that data were available from only seven. The remaining seven rats were not used for any further testing and were sacrificed.

Withdrawal testing was carried out on the rats remaining in good health after the LDR determination (n= 12 in the 0 and 240; n=10 in the 24, 48, and 96; and n=9 in the 8 mg/kg group). Rats were tested two at a time to a maximum of 12 per day over a 6-day period. Each rat received at least three daily maintenance injections of morphine after the end of the LDR study, the last one being 24–30 hr before the withdrawal test. The order of testing was balanced across treatment groups.

Results

The LDR curves for the tailflick tests are shown in Fig. 8. As in Experiment 1B, the LDR curves showed a shift to the right in the lower treatment dose groups, and a shift to the right and decrease in the slope in the three high treatment dose groups. This flattening was most apparent in the 240 mg/kg group. This curve did, however, have a significant positive slope indicating that the shape of the curve was not due to total loss of response. The data also indicated that the lateral shift in the LDR curves approached a maximum with treatment doses of intermediate magnitude. The only significant differences among the respective means of the highest three treatment groups were between the 48 and 240 mg/kg groups at the 390 and 630 mg/kg test doses. These were in contrast to the lower dose groups where significant differences were found between all the corresponding means of the adjacent groups.

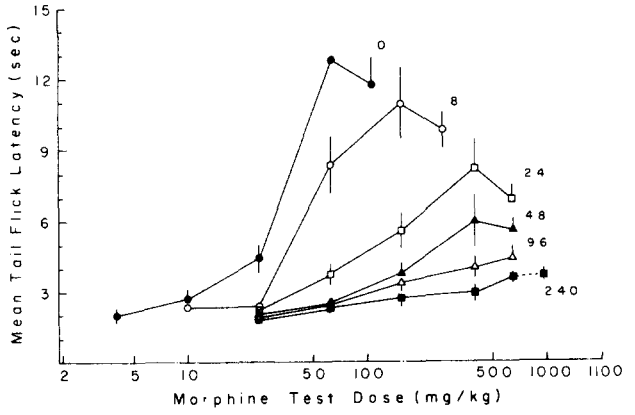

FIG. 8. Tailflick latency LDR curves for rats treated IP with 0 (n=12), 8 (n=11), 24 (n=12), 48 (n=11), 96 (n=10), or 240 (n=12) mg/kg/day for 24 days. First four points of each curve were determined in random order on first four tests; fifth was determined on fifth test. The test doses used for the various groups are given in the text. The response to the highest dose on 240 mg/kg curve came from a separate group (n=7) of rats. Number by each curve indicates daily morphine dose in mg/kg.

The LDR curves of the high treatment dose groups also showed a lowering of the maximum attainable response. In the 240 mg/kg groups there were no significant differences between the mean tailflick latencies at the four highest test doses, and after the heroic dose of 975 mg/kg the mean tailflick latency was only 3.7 sec.

The results of the withdrawal tests are summarized in Figs. 9 (checked signs) and 10 (counted signs). Of the checked signs, scream-on-touch, ptosis, diarrhea, and penile ejaculation were present more frequently in the morphine-treated groups than in the saline. Except for diarrhea which evidenced a near-maximum response even after the 8 mg/kg treatment, the frequency of these signs in the morphine groups was dose-related. Rhinorrhea, salivation, and lacrimation were apparent to a minor degree only in the high dose treatment groups, suggesting that their frequency might increase with higher treatment doses.

With regard to the counted signs (Fig. 10), all except circling were significantly influenced by the chronic morphine

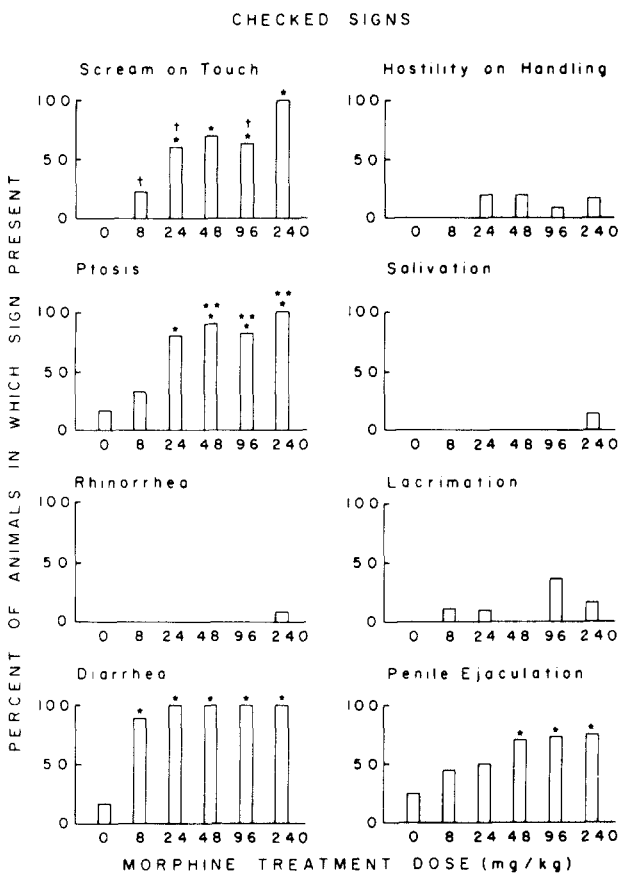

FIG. 9. Percent of rats in 0 (n=12), 8 (n=9), 24 (n=10), 48 (n=10), 96 (n=10), and 240 (n=12) mg/kg groups showing checked withdrawal signs after IP naloxone (1 mg/kg). Symbols denote significant differences from 0 (one star), 8 (two stars), and 240 mg/kg group (cross).

treatment. Clear maximums were reached for rearing, teeth chattering, and weight change. In contrast, the frequency of wet dog shakes and the postnaloxone temperature changes appeared to be still increasing over the range of treatment doses used. The magnitude of writhing and possibly jumping were greatest in the intermediate dose range. This was analogous to the results of Bläsig *et al.* [3] who found this effect for writhing and wet dog shakes. However, a biphasic effect could be verified statistically only in the case of writhing, for which the combined data of the 96 and 240 mg/kg groups were significantly lower than those of the 24 and 48 mg/kg groups.

DISCUSSION

Most of the findings reported here are consistent with those described separately by many other investigators. However, there has been a general lack of systematic comparisons across tests and treatment doses within the same experiment. Such comparisons are necessary to permit clear understanding of the relationships among the various phenomena of tolerance and physical dependence.

A number of characteristics of morphine tolerance were studied in the present experiments. The first is directly concerned with the definition of morphine tolerance. It is gen-

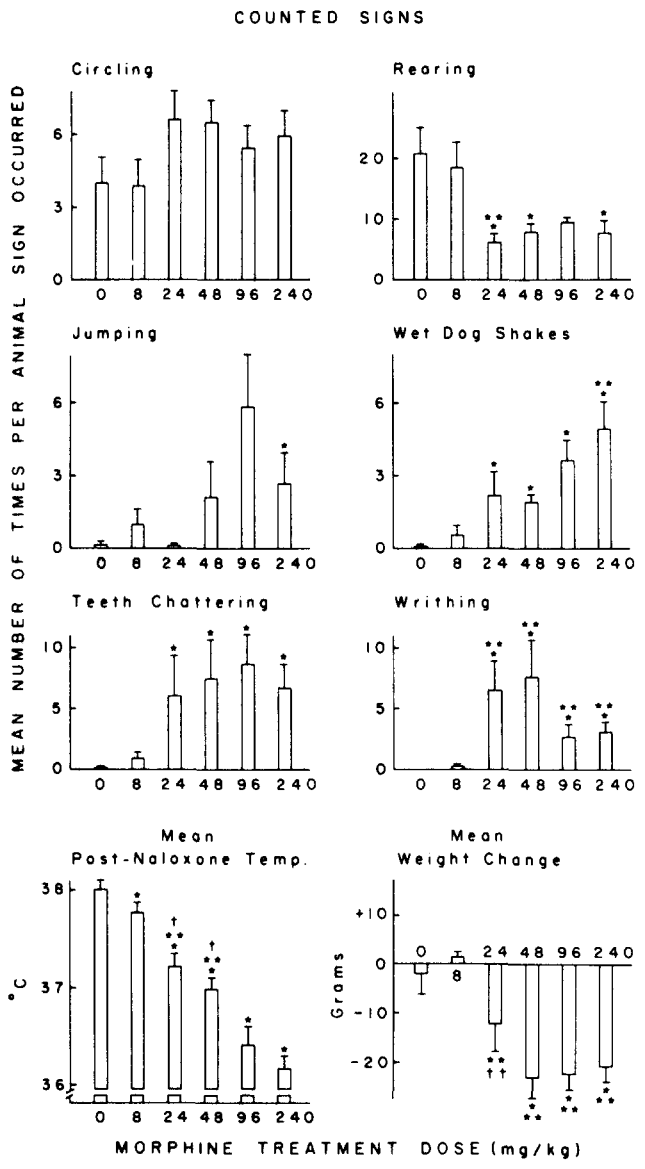

FIG. 10. Mean frequency of counted withdrawal signs in respective groups of Fig. 9. Symbols explained in previous caption.

erally agreed that tolerance is associated with a shift of the LDR curve to the right (cf. [10,15]). In the present study it was clearly demonstrated that treatment with high doses of morphine also caused a flattening of the analgesia LDR curve. These results replicated similar observations reported for rodents implanted with morphine pellets [14,25] and for rats injected twice daily with morphine [8]. In other work using treatment conditions similar to Experiment 2, we have demonstrated this effect with morphine-induced immobility [21] and time to maximum hyperthermia (Mucha and Kalant, unpublished findings), indicating that the flattening of the LDR curve may be a general feature of tolerance to high doses of opiates. These results, therefore, emphasize the importance of measuring tolerance with LDR curves. The use of LDR curves was further supported by comparing the results of Experiment 1A and 1B. Clearly, the LDR curves could distinguish between treatment groups which showed complete and indistinguishable tolerance when it was measured by decreased response to a given dose.

A second related issue concerned the variation in apparent degree of tolerance measured in terms of different effects of morphine. This was demonstrated initially by Cochin and Kornetsky [6] and more recently by Fernandes *et al.* [10], with tolerance expressed as a decreased response to a given dose of morphine. The results of Experiment 1A replicated some of these findings and extended them to include body weight changes and temperature increases. A similar phenomenon for tolerance measured by LDR curves was recently reported [9,10]. These data were confirmed and extended in Experiment 1B by contrasting the LDR curves for duration of hyperthermia with those of time to maximum hyperthermia and latency to tailflick.

The third characteristic of morphine tolerance studied was the rate of its development. Tolerance to tailflick analgesia occurred with two phases, an initial fast one and a second slower one. A similar effect occurred with swimming time. Measurement of the second component indicated an apparent dose-related half-life of several weeks while the value for the first component was estimated to be only a few days. Because of the novelty of these findings we have repeated the tailflick portion of this experiment in naive rats and replicated the finding of two components of tolerance [22]. In an analogous experiment using levorphanol-induced running fits in mice, Goldstein and Sheehan [11] found only the fast initial component. The nature of the difference between the two experiments was not obvious; however, it may be related to the type of response that was measured. Assuming that the development and loss of tolerance follow similar kinetics, the data of Cox *et al.* [8] may help to rule out other factors. They induced tolerance in both rats and mice with several drugs including morphine, methadone, and diamorphine, and found that analgesia tolerance was lost in a biphasic pattern very reminiscent of that of tolerance development in Experiment 1A. In addition, we have measured the development of tolerance to tailflick analgesia and bar test immobility in the same rats and found the biphasic pattern of tolerance only for analgesia [22].

The fourth characteristic of tolerance was its extent, which was found to be a logarithmic function of the treatment dose. LDR curves from both Experiments 1B and 2 indicated dose-related differences between groups with low and intermediate treatment doses and few differences between the LDR curves of groups treated with high doses of morphine. The LDR curve differences were not due to dose-related differences in rate of tolerance development since Experiment 1A indicated that tolerance reached asymptote before measurement of the LDR curves. Fernandes *et al.* [10] reported a similar effect on hypothermia in mice; however, they did not use enough treatment doses to provide similar data for other opiate effects.

The final characteristic of tolerance dealt with its relation to physical dependence. To date there have been relatively few reports dealing specifically with this problem. Way *et al.* [27] and Cicero and Meyer [4] found a positive correlation between analgesia tolerance and sensitization to naloxone-precipitated jumping in mice and wet dog shakes in rats, respectively. Fernandes *et al.* [10], however, found that when hypothermia and lethality instead of analgesia were used to measure tolerance the extent of tolerance development did not parallel that of naloxone-produced jumping. We have done the converse experiment by measuring a number

of indices of physical dependence and relating these to a single measure of tolerance (tailflick analgesia). Tolerance and physical dependence developed in parallel when dependence was measured by ptosis, diarrhea, penile ejaculation, teeth chattering, and weight change. However, other withdrawal signs such as temperature changes, scream-on-touch, salivation, and rhinorrhea did not show close correlation with the degree of tolerance; the intensity of these signs continued to increase as a function of treatment dose after tolerance had reached a maximum.

Thus, it is apparent from the present data and those previously published [10] that the choice of responses to measure tolerance and dependence will determine whether the two phenomena appear to be related or not. Moreover, there is little indication of what sign best reflects the basic mechanism of each phenomenon. The most suitable responses should reflect tolerance and dependence that occur at the same locus. Our experiments indicated that temperature and weight change are two possibilities, since these responses allow measurement of both tolerance and dependence.

The present study also brings up the major issue of whether or not tolerance is a single process or multiple processes. Tolerance, for example, was found to occur with different apparent rates and to different degrees when results on a variety of measures of opiate effects were compared. In addition, there were two components of tolerance development as determined by rate of development and two components of LDR curve change. Similarly, comparison of the dose-related changes in the magnitude of various measures of physical dependence suggested that some of these vary independently of others. Thus, these data are consistent with the notion of two or more components of tolerance, but they do not offer a clear rejection of a hypothesis that tolerance is a unitary process (cf. [7]). There are a number of ways to account for the present data within the single process notion. For example, a single adaptive change in a common opiate receptor may affect various neuronal pathways differently because of interaction with different modulatory influences in each pathway. Alternatively, tolerance may occur separately in each effector system responsible for a particular end-result of morphine action, the degree of tolerance varying with the degree of induced disturbance in each pathway [16]. Finally, the demonstration of different types of receptors associated with the expression of different opiate effects [20] suggests that tolerance might be associated with a receptor change, but that this change can vary in degree for different receptor types.

A final comment relates to the use of daily injections for producing morphine tolerance and physical dependence. First, the method was very effective since both tolerance and dependence were seen with as little as 8 mg/kg/day. In addition, compared to the commonly used pellet-implant method, it has the advantage of an infinitely variable intensity of drug exposure, exact control of the dose, and ease of measuring tolerance at any state of its development. Daily injections produce wide fluctuations in levels of free drug while pellets produce sustained levels; nevertheless, the end results are quite comparable [1,2]. Our withdrawal syndrome data, for example, differed only to a minor extent from those of pellet implanted rats [3]. The present injection procedure also had a low rate of animal attrition. Way *et al.* [27] reported that morphine pellet implantation resulted in loss of up to 14% of animals through morbidity or death.

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