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Motility Response of Rats to Chronic Constant-dose Treatment with Narcotics

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DAVIS, W. M., K. L. HEMNANI AND H. B. PACE. *Motility response of rats to chronic constant-dose treatment with narcotics. PHARMAC. BIOCHEM. BEHAV.* **17**(3) 489–494, 1982.—The changes in effects on motor activity of rats upon repeated (48 day) dosing with four narcotic analgesics were determined. The following were administered IP once daily in a.m.: morphine sulfate (MOR), 20 mg/kg; dl-methadone HCl (MET), 5 mg/kg; meperidine HCl (MEP), 10 mg/kg; and pentazocine lactate (PEN), 20 mg/kg. Motility was measured in photocell actometers every 4 days for 6 hr after dosing. Activity was elevated after the initial dose as follows: for MOR at hours 3–5, for MET at hours 2–5, for MEP and PEN at hours 2–3. Time of peak response showed no systematic change over days. For all 4 drugs there occurred, upon repeated dosing, a considerable increase in motility over the initial acute response. For MOR the greatest increment occurred between days 12 and 16, but regression analysis showed a strong linear trend of increasing activity from day 1 through day 48. For MET and MEP, activity rose considerably between days 4 and 12 to a maximum, after which the activity trended downward for MET, but showed no continuing fall or climb for MEP. For PEN the greatest increases were from days 4 to 8 and 44 to 48, with an intervening period of relative stability. These results seem to be more readily explainable in terms of increasing sensitivity to the motor excitatory actions of these agents than merely by a development of tolerance to motor-inhibitory actions.

Morphine Methadone Meperidine Pentazocine Narcotics Motor activity Supersensitivity

THE effects of morphine and related narcotic analgesics on general behavior are recognized to be species dependent. That is, some species (e.g., cat, horse, pig) after low to moderate dosages of morphine show behavioral excitation, while others (e.g., rabbit, guinea pig, mouse) respond with signs of CNS depression [22]. Although the laboratory rat had long been categorized with species for which morphine is "almost purely depressant" [22], it has been demonstrated previously that excitation, manifested in hypermotility, was the predominant acute response to lower doses, i.e., 1 to 10 mg/kg IP of morphine sulfate [1, 2, 8, 24], whereas doses in the range of 16 to 40 mg/kg produced a phase of delayed hypermotility following an initial depressant phase [2, 8, 24]. Moreover, the hypermotility induced in rats by morphine under these dose/time conditions was not reduced by tolerance development. Rather, for doses of 10 or 20 mg/kg a definite increase in the motor excitatory response occurs during 30 days of once-daily chronic treatment [2].

Other opioid analgesics (methadone and levorphanol) and one antagonist-analgesic (pentazocine), when tested acutely under the same conditions, were found to cause effects on motility similar to those of morphine [10]. However, alterations of motility by the same agents during repeated dosing were not examined. The present study was performed to test for possible changes in the motility response of rats after repeated doses of narcotic analgesics other than morphine. Included in this comparison to morphine itself were meperidine, methadone and pentazocine. The latter two were chosen because they both elevated motility comparably to morphine in an acute study [10]; meperidine was tested because of the near-absence of such activity in that study, so as to determine if it might show greater likeness to morphine upon chronic administration. Most chronic studies of narcotics have employed stepwise-increasing dosage schedule in order to maximize the development of tolerance and/or physical dependence. To parallel the earlier study of morphine, the present work employed a constant-dosage treatment schedule for which tolerance and dependence might be expected to be less pronounced than in the case of ascending dosage. Thus, the present data may not be equated with activity measures taken during or after administration of a narcotic on an ascending schedule of narcotic dosage [14, 17, 24].

METHOD

The subjects were adult male Wistar rats from a colony maintained by this institution. They were about 380–400 g (18–20 weeks old) at beginning of experimentation. Housing between periods of actometric measurement was in large cages suitable for groups of five rats. Ambient temperature of 22–24°C and a 12-hr daily period of artificial lighting (0600–1800 hr) were observed. Food (Purina Lab Chow) and water were available at all times in both home cages and actometers.

The drugs, sources and dosages used were: morphine sulfate (Merck), 20 mg/kg; methadone hydrochloride (Lilly), 5 mg/kg; meperidine hydrochloride (Winthrop), 10 mg/kg; and

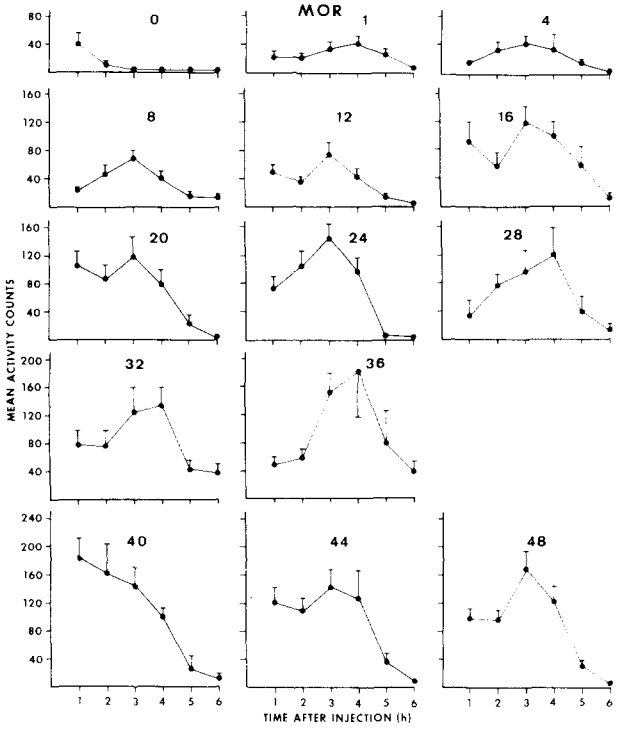

FIG. 1. Time effect curves for motility of rats after IP doses of morphine sulfate (20 mg/kg) daily on days 1–48 or saline on day 0. Vertical bars represent the SEM of means for 10 rats per group, and numbers indicate the days of treatment on which activity was recorded. Acute effects (Day 1) differed significantly ($p<0.05$) from Day 0 at 3, 4 and 5 hr.

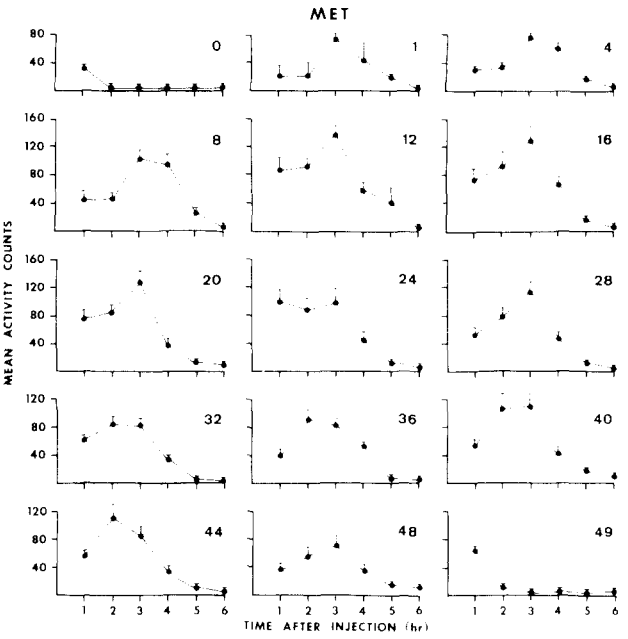

FIG. 2. Time-effect curves for motility of rats after IP doses of methadone hydrochloride (5 mg/kg) daily on days 1–48 or saline on days 0 and 49. Acute effects (Day 1) differed significantly ($p<0.05$) from Day 0 at 2, 3, 4 and 5 hr.

pentazocine lactate (Winthrop), 20 mg/kg. All dosages are expressed as the weight of salt not the free base. Each drug was dissolved in or diluted with distilled water and injected intraperitoneally (IP). All injections were made at 0800 hr.

Motor activity was recorded in a battery of 10 photocell actometers [20] consisting of a circular track 3 in. wide and 6 in. high with an external diameter of 15 in. The outer wall is of sheet metal and the inner one is of wire mesh allowing placement of a food container and water bottle, which were available during experiments. A 6 W (110 V) bulb located at the center of each actometer provides dim illumination to the subject and activates 4 photocells placed at 90° intervals behind holes in the outer sall. Counts from all 4 photocells automatically totalled on the same digital counter. The counting circuitry does not permit repeated counts from the same photocell, as one cell does not reset until a beam to an adjacent cell is interrupted.

Four groups of 10 rats each received daily IP injections of one of the 4 drugs studied throughout a 48-day period. The drugs were tested in two sequences; i.e., morphine and pentazocine were run simultaneously, followed thereafter by methadone and meperidine. Each pair of treatments had the two agents scheduled to begin 2 days apart so that actometric testing days did not coincide. Before initial drug treatment, the rats were given 2 days in the actometers for 6 hr without treatment, then were tested once on day 0 after an IP dose of 0.9% saline solution. These steps were to promote a low initial level of activity as drug treatments began, to favor the display of excitatory effects.

The dosage of morphine was chosen to replicate the condition of an earlier 48-day study [2]; dosages of the other 3 drugs were chosen to be as comparable to this dose of morphine in activity as possible, based on observation of acute responses.

The Student t-test for paired comparisons was used to compare data from the first drug day to the preceding saline test, and from later drug days to day 1. Comparisons across drug treatments employed one-way analysis of variance followed by Duncan's multiple range test, accomplished via appropriate computer programs (Statistical Package for the Social Sciences). Polynomial regression analysis using the BMD-P5R program (rev. August 1976) was applied to the 6-hr totals for each drug over the full course of the study.

RESULTS

The time-effect curves for hourly motility totals on day 0 through 48 for each of the 4 test drugs are shown in Figs. 1 to 4. The acute effects (day 1) of morphine included a mean first-hour activity about one-half that of the saline groups (22.2 vs 40.1), but the difference did not react statistical significance. The counts for hours 3–5 were significantly elevated above control values. The methadone group also had a 1st hour response below that with saline, again not reaching significance, plus increased ($p<0.05$) activity for hours 2–5. In contrast, both pentazocine and meperidine groups had activity equal to their saline (day 0) levels at the first hour followed by significant increases at hours 2 and 3. The time-effect relationships for the four analgesics averaged over the full course of the 48-day treatment and expressed as the hourly means are shown in Fig. 5. The similarity in time-course effects between morphine and methadone and between meperidine and pentazocine is noteworthy.

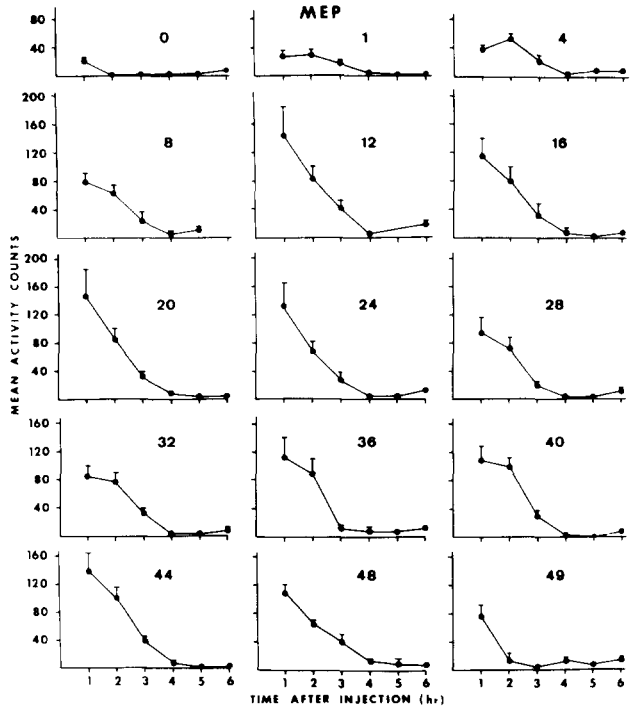

FIG. 3. Time-effect curves for motility of rats after IP doses of meperidine hydrochloride (10 mg/kg) daily on days 1–48 or saline on days 0 and 49. Acute effects (Day 1) differed significantly ($p<0.05$) from Day 0 at 2 and 3 hr.

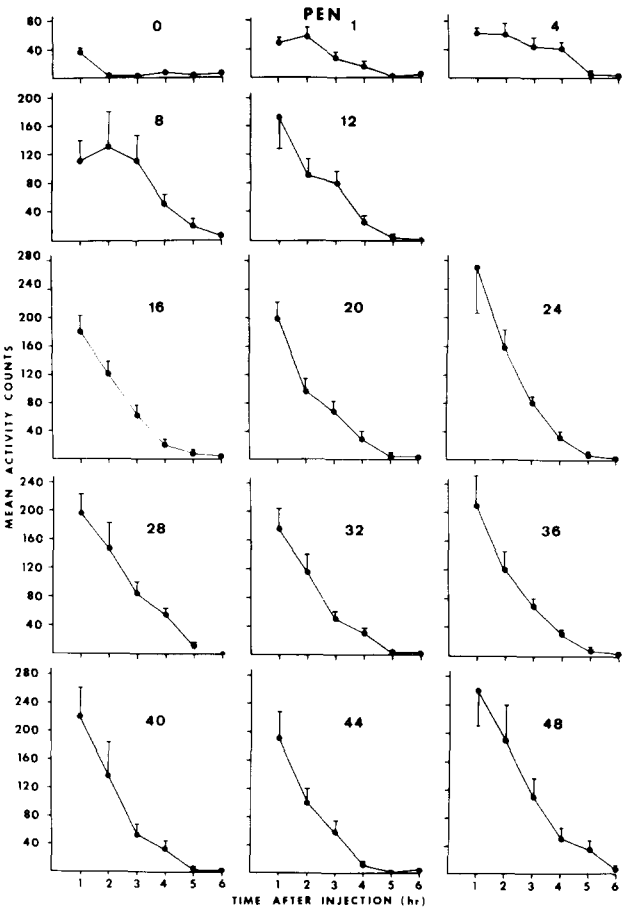

FIG. 4. Time-effect curves for motility of rats after IP doses of pentazocine lactate (20 mg/kg) daily on days 1–48 or saline on day 0. Acute effects (Day 1) differed significantly ($p<0.05$) from Day 0 at 2 and 3 hr.

After saline on day 0 there was no significant variation in 6-hr totals among the 4 groups (Fig. 6). Neither on day 1 were the 6-hr drug responses (mean±SEM) significantly different from one another: methadone (181.5±23.4), pentazocine (153.4±27.3), morphine (143.8±34.6), meperidine (91.9±18.0); $F(3,26)=2.11$, $p>0.05$; however, all except morphine showed a statistically significant elevation over their respective day 0 values. Despite the equality of activity among the four groups on the initial drug day, when the averages for the 6-hr totals across the 13 drug measures were compared, there was a highly significant difference, $F=19.7$, $p<0.0001$. The mean (±SEM) values ranked as follows: pentazocine (381.2±23.2) = morphine (364.6±29.7) > methadone (270.9±41.3) > meperidine (208.0±11.5).

By day 8 all 3 groups except the morphine-treated rats increased significantly over their respective day 1 levels (Fig. 6); i.e., they had shown a significant change from their first drug response. For morphine the greatest changes with repeated dosing was between days 12 and 16, when the elevation above day 1 did become significant; a very similar degree of change occurred for pentazocine between days 4 and 8, and for methadone and meperidine between days 4 and 12.

The computer program for polynomial regression developed equations to describe correctly the linear and/or nonlinear trends of the 48-day activity data. In case of more than one possible fit by different degrees of polynomials, we chose the simplest expression, i.e., that equation with the least number of terms, to use in depicting the data as presented in Fig. 6. For the morphine data, there was clearly a linear trend, with activity increasing throughout the duration of the experiment. For pentazocine there was a 3rd degree (cubic) trend, with strong upward trend early and late in the experiment and a period without appreciable change in the middle. Unfortunately, the apparent late increase was substantiated only by the final (day 48) data point. Meperidine and methadone both required a 4th degree (quartic) function to provide a good fit, as each showed a strong early increase (i.e., through day 12). This was followed by oscillation that for methadone trended downward, but for meperidine showed neither a clearly continuing upward nor downward trend.

In the case of methadone and meperidine, for which activity was recorded following saline injection on day 49, the mean (±SEM) 6-hr counts on days 48, 49 and 0 were as follows: methadone—221.1(±33.9)>89.3(±7.8)>42.7(±4.6); meperidine—245.1(±32.3)>128.1(±26.4)>44.1(±5.5). Thus, motility on day 49 fell significantly (methadone, $p<0.05$; meperidine, $p<0.01$) from the levels of day 48, but was still elevated significantly ($p<0.01$ and $p<0.001$) above the initial saline levels of day 0.

DISCUSSION

The increasing degree of hypermotility after repeated

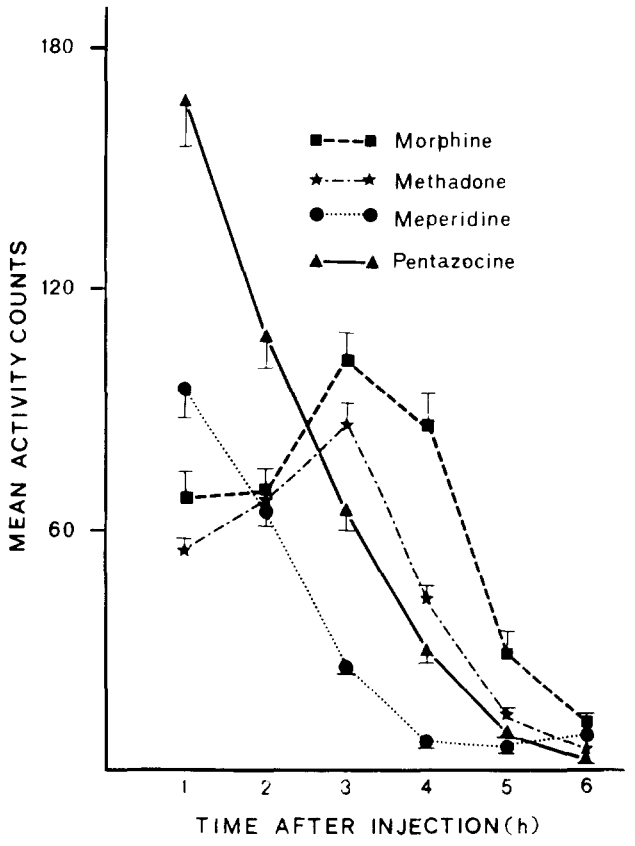

FIG. 5. Time-effect curves for motility after morphine, methadone meperidine or pentazocine averaged across the 13 test sessions on days 1 through 48. Vertical bars represent SEM.

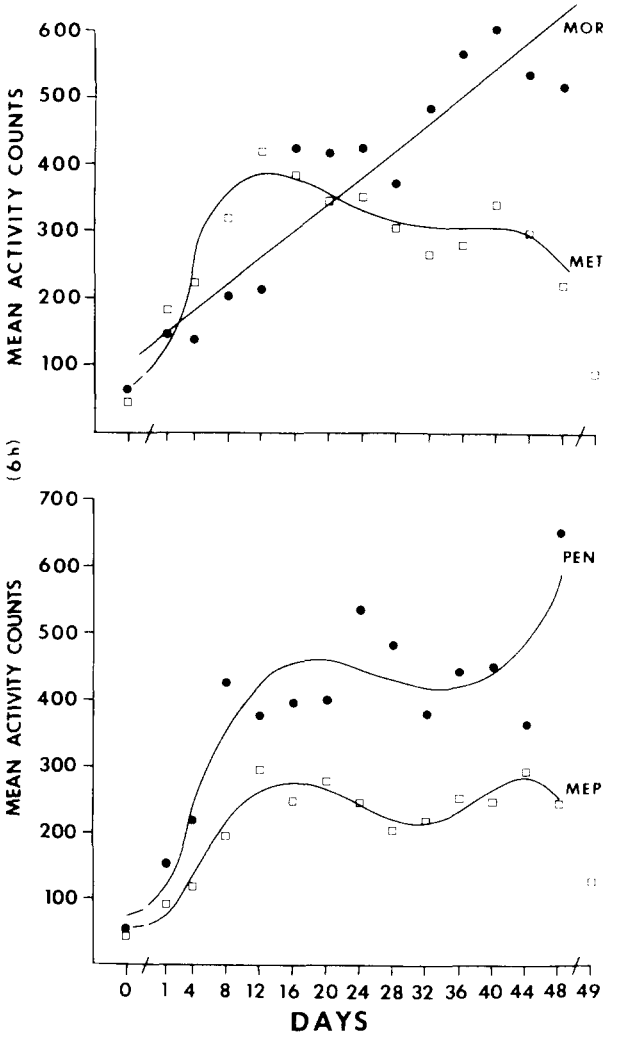

FIG. 6. Total 6-hr motility response after morphine, methadone, meperidine and pentazocine for test sessions on days 1 through 48. Sessions on Days 0 and 49 followed injection of saline. Curves were fitted by means of a polynomial regression analysis. Six hour totals became significantly elevated from the respective Day 0 levels on Day 8 for methadone, meperidine and pentazocine; on Day 16 for morphine.

constant-dose treatment with morphine described here is similar to results of earlier works [2–4] that used Sprague-Dawley rather than Wistar rats, and so the generality of the phenomenon is extended within this species. Moreover, our results with the other analgesics confirms that the amplification of excitatory effects on motility during repeated daily dosing is a phenomenon not confined to morphine, occurring also with two classical narcotic agonists (methadone, meperidine) and a partial agonist (pentazocine). This is in accord with reports of Babbini and coworkers, which showed that the motor excitatory activity of not only morphine, but also methadone, meperidine and pentazocine are considerably augmented in chronically morphine-pretreated rats over drug-naive rats [3, 6, 7].

In this study as before [2], the increase in response to morphine was still occurring at or near the end of a 48-day period, but in another laboratory, such enhancement was found to reach a maximum variously after 13, 26 or 35 days [3, 4, 6]. The absence of a long-continuing amplification of excitatory responsiveness, with the possible exception of pentazocine, is an apparent distinction between morphine and the other analgesics tested.

The Wistar rats used in this study showed a generally lower acute activity response to the analgesics than has been seen in a Sprague-Dawley derived strain [2,10] A difference from earlier chronic studies of morphine [2, 3, 6] was that the delayed hyperactivity phase after morphine or methadone did not show a consistent reduction in latency of onset, nor did the peak of activity occur earlier in the course of repeated treatment. In the case of meperidine and pentazocine, maximal activity always was in the first hour after injection. Although there was a difference in latency to maximal excitation between the two pairs of analgesics, this does not apply generally, as morphine and methadone at lower doses also have shown peak activity in the first hour [10]. A suggestion of an initial, early (first hour) depression of motility by morphine and methadone was seen, but there was not a significant decrease below saline controls as was previously found for morphine [2–3]. However, it must be reiterated that the procedure of this research and the type of actometer employed, do not favor the detection of such initial inhibition of activity, as has been better demonstrated for similar doses in acute studies by others [8, 17, 24]. Meperidine and pentazocine at no time showed any sign of such initial depression.

Although the present data showed little or no direct evidence for a motor-inhibitory component, it must be assumed present to some degree. Marcais *et al.* [15] found that naloxone treatment at 2 hr after a SC dose of morphine HCl (10 mg/kg as free base) caused not only suppression of hypermotility, but also a reversal so that the rats were significantly less active (44%) than saline-treated controls. This result suggests a selective antagonism of naloxone toward the action responsible for the initial akinetic phase, and that this mechanism was still operative, although insufficiently so to predominate over the hyperkinetic action, at two hours after morphine injection. On the contrary, Oka and Hosoya [19] found naloxone to be active against both depressant and excitatory actions of morphine. The observation of Marcais *et al.* [15] might be attributable to naloxone inducing a withdrawal state after acute physical dependence development.

The mechanism for the amplification of the hypermotility caused by the four analgesics upon their repeated administration to rats cannot be clearly deduced from the present data. However, the work of Smee and Overstreet [21] with morphine indicated significant roles both for development of tolerance to behavioral inhibition and for increased sensitivity of central dopaminergic mechanisms in the increased motor activity of rats after repeated daily doses. They found that tolerance developed to the early (30 min) morphine-induced inhibition of activity by the 8th daily 20 mg/kg dose, and that such tolerance was essentially complete by 15 days. Similarly, Babbini *et al.* [3] found that first-hour depression of activity changed to enhancement by day 9 of daily morphin. In the present study, a major increase in the excitatory response to morphine occurred between days 4 and 16.

Furthermore, after three or more weeks of morphine treatment Smee and Overstreet [21] observed that there had developed a supersensitivity to *d*-amphetamine or apomorphine, and a subsensitivity to pimozide, which was attributed to changes in CNS dopaminergic receptors. However, in a rather similar study [5], no supersensitivity to production of stereotypies or hypermotility by amphetamine was observed in chronically morphine-treated rats. Despite this unresolved discrepancy, it still must be considered favorably that supersensitivity of a dopaminergic mechanism could attribute to explaining our data. Further indirect support for the importance of postsynaptic dopaminergic receptor supersensitivity might be inferred from a demonstration of supersensitivity to the acute motor-excitatory effect of morphine after repeated (18-day) treatment with an inhibitor of both dopamine and norepinephrine synthesis. No such effect occurred after like treatment with a selective inhibitor of norepinephrine synthesis [13].

Although results of several studies in rats [9,11] have implied that a noradrenergic mechanism may be most important in the hyperkinetic response to narcotics, there also is evidence that the noradrenergic mechanism operates only in conjunction with an important contribution of dopaminergic function(s) [1, 13, 23]. It has been demonstrated directly that an IP dose of 20 mg/kg morphine to rats increased the firing rate of brain dopaminergic neurons (in substantia nigra) [18]. Moreover, local micro-injection of morphine (5 μg) directly into the ventral tegmentum of rats elicited hyperkinesia that could be blocked by a peripheral injection of either naloxone or haloperidol [12]. There was a progressive augmentation of the motility response upon repeated daily dosing through five days, just as we have seen for IP administration. The authors postulate that the increase in responsiveness to morphine might arise from increased sensitivity of morphine receptors on dopaminergic cell bodies [12].

Assuming the possible occurrence of a similar development of supersensitivity of receptors following other narcotic agonists besides morphine [21], such development might explain in part also our results with methadone, meperidine and pentazocine. In fact it would provide a more apt rationalization than tolerance to inhibitory effects of meperidine and pentazocine, for which there was no suggestion of such inhibition in the present data. However, some caution must be exercised in accepting such an assumption, because, for example, the neurochemical and motility effects of pentazocine may have a different basis from those of morphine, in view of a failure of naloxone to antagonize the motor effects of pentazocine [11].

If a degree of physical dependence were induced by any of the drug treatments in the present study, this should have been reflected in a lowering of activity on the day after cessation of treatment, i.e., during the period of potential early abstinence syndrome [16]. However, the activity of the two groups (methadone and meperidine) tested after saline injection on the first day following cessation of treatment was in each case significantly higher than their respective initial (day 0) saline baseline value. Therefore, the level of motility counts seen in these two drug groups on day 49 gives no evidence for the occurrence of a withdrawal state at this interval and with the prior conditions of this study. This has also been the case for morphine in previous studies [2,6].

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