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Aspects of Abstinence After Morphine Ingestion

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RÖNNBÄCK, L., P. S. ERIKSSON, J. ZEUCHNER, L. ROSENGREN AND A. WRONSKI. *Aspects of abstinence after morphine ingestion.* PHARMACOL BIOCHEM BEHAV **28**(1) 87–93, 1987.—Sprague-Dawley male rats were intoxicated with morphine, using an ingestion method where exposed and control rats received equivalent amounts of calories and nutrients. The degree of physical dependence on morphine was demonstrated by studying and quantifying abstinence symptoms after withdrawal or after administration of opiate antagonists. The aims of the study were (1) to further enlighten the specificity and validity of the intoxication method concerning physical dependence, and (2) to determine whether some of the abstinence signs might be of value to facilitate quantitation of the degree of physical dependence on morphine, with diet and fluid intake being maintained under control. Withdrawn rats showed a decreased fluid diet intake and a body weight loss, the latter partly due to anorexia. Other mild abstinence signs were irritation, tremor and some motor excitation. The body weight loss during the first day of morphine withdrawal was proportional to the accumulated drug dose (between 25 and 300 mg morphine PO/kg b.wt.). However, prolonged morphine treatment on one dose (340 mg/kg b.wt.) did not reinforce the body weight changes caused by morphine withdrawal. The succeeding weight gain some days after morphine withdrawal was not entirely dependent on the amount of fluid diet intake. Methadone was shown to partially block the decrease in diet intake and the weight loss seen during morphine withdrawal. The naloxone-precipitated withdrawal symptoms were motor excitation, cholinergic signs, body weight loss, diarrhoea and decreased diet intake. The weight loss 2 hr after naloxone administration to long-term intoxicated rats was proportional to the naloxone dose. It is suggested that the ingestion method produces a high degree of physical dependence and that, among abstinence symptoms after withdrawal from morphine, changes in b.wt. might be a parameter easy to quantitate in order to determine the degree of physical dependence upon morphine in Sprague-Dawley rats.

Abstinence Body weight Fluid diet Ingestion intoxication Methadone Morphine Naloxone Physical dependence Rat

THE initial weight loss during morphine withdrawal is assumed to be one of the more sensitive and reliable measures for withdrawal in the rat [1, 8, 25, 26]. It comprises reduced food and water intake, as well as increased defecation and micturition [8]. The magnitude and duration of the weight loss has been shown previously to be related to the preceding treatment dose [1,25]. Body weight loss becomes evident shortly after withdrawal and rarely lasts longer than a few days [19,25]. Following the initial weight loss an increase in the rate of body weight gain has been reported [20].

In order to evaluate optimal conditions for studies on morphine tolerance and physical dependence, an ingestion method for morphine intoxication was developed [32]. The drug-treated and control rats receive the same amount of fluid diet and, therefore, are in caloric balance. When differences in food intake occur, pair-feeding is used. A number of parameters, such as weight loss, decreased food intake, diarrhoea, tremor and irritability can easily be registered during withdrawal or abstinence precipitated by morphine antagonists or exclusion of morphine from the diet, respectively [32].

The aims of this study were twofold: (1) to further evaluate the validity and specificity of the ingestion method for morphine intoxication concerning physical dependence by evaluating the relation between morphine dose and intoxication time vs. the degree of withdrawal symptoms. Abstinence reactions were measured qualitatively (tremor, irritability, motor excitation, diarrhoea) and quantitatively (changes in body weight and fluid diet consumption). The ability of methadone to ameliorate the morphine withdrawal symptoms was also investigated. (2) Furthermore, whether or not the body weight loss during abstinence is dependent on decreased fluid diet consumption, as previously shown [8], and whether or not body weight loss might be a useful parameter in quantifying the degree of the abstinence reaction, were also evaluated.

METHOD

Animals

One-hundred-seventy-six male Sprague-Dawley rats (ob-

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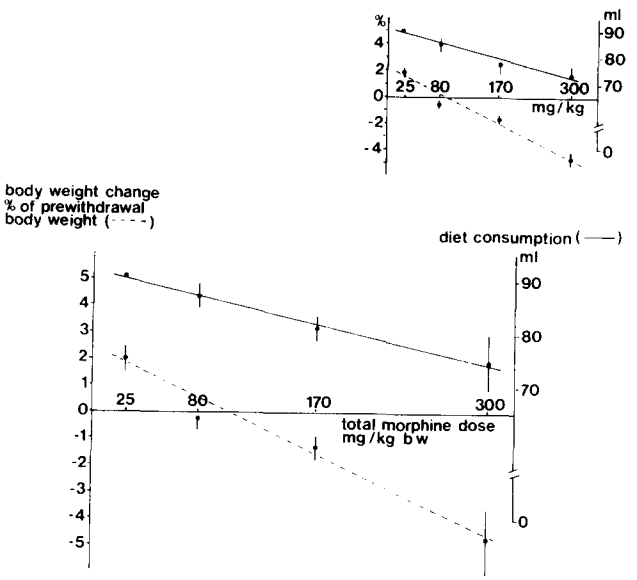

FIG. 1. Four groups of Sprague-Dawley rats with 5 rats in each were intoxicated with morphine for one day (total dose 25 mg/kg b.wt.), 2 days (total dose 25+55 mg/kg b.wt.), 3 days (25+55+90 mg/kg b.wt.) and 4 days (25+55 mg + 90+130 mg/kg b.wt.), respectively. They were started on morphine consecutively so that all animals received their last dose on day 4. On day 5 morphine was withdrawn from all involved rats. Thereafter, the animals were fed control fluid diet. The figure demonstrates b.wt. loss during the first day of withdrawal (expressed as percent of the b.wt. the day prior to withdrawal) as a function of accumulative morphine dose (expressed as mg/kg b.wt.). The b.wt. loss during the first day of withdrawal in the different groups is proportional to the accumulative morphine dose (linear regression analysis; r=0.98). There is also a linear correlation between total morphine dose and fluid diet consumption (r=0.99). Inset figure shows the relations between body weight loss and fluid diet consumption, respectively, versus cumulative morphine dose during a 4-day treatment where all rats had been treated with morphine each day. Similar results are seen (r=0.99 for both curves).

tained from A-lab, Stockholm, Sweden) with an initial body weight of 150 g were used.

Morphine Intoxication Procedure

An ingestion method for long-term morphine intoxication was used as described previously [32]. The animals were fed a fluid diet, with casein as the protein source. The fluid diet included a vitamin mixture, salt, plus cystine, methionine and sugar mixed to a fluid with olive and corn oils as stabilizers. Morphine was dissolved in the diet with the daily dose increasing from 25 mg/kg b.wt. up to 340 mg/kg b.wt. in 8 days. Plasma morphine levels of 2–3 μg/ml were reached during the day [32]. Total fluid diet and body weight were measured at the same time each day or as otherwise indicated.

Drugs

Morphine chloride and methadone hydrochloride were bought from Apoteksbolaget, Sweden. Naloxone was partly obtained from Apoteksbolaget, Sweden, and was in part kindly donated by Winthrop, Sweden.

Determination of Hematocrit

Blood samples taken from rats after morphine withdrawal for one or several days were taken from the tail vein under light ether anesthesia. The samples were collected in heparinized capillaries and centrifuged, after which the hematocrit ratio was calculated.

Statistical Evaluation

Statistical evaluations were done according to Student's *t*-test. S.E.M. values are given.

Experimental Protocol

Relation between morphine dose and time of intoxication to the degree of body weight loss after withdrawal. Forty animals were processed for 2 experiments, each with four groups and 5 rats in each group. After control diet prefeeding, the animals were administered morphine. In experiment 1, morphine was added to the liquid diet consecutively for 4 days in a dose of 25 mg/kg b.wt. on the first day of treatment, increasing up to 55, 90 and 130 mg/kg b.wt. during the next days. Thus one animal group was started on day 4 with 25 mg/kg b.wt., another group was started on day 3 with 25 mg/kg b.wt. and then given 55 mg/kg b.wt. on day 4, etc. Each group not only differed in total dose, but also differed in the length of exposure time. In experiment 2, all animals were given morphine every day, i.e., a total dose of 25 mg/kg b.wt. over 4 days for one group and a total dose of 80 mg/kg b.wt. during 4 days for group 2, etc. On day 5 morphine was withdrawn. The animals were thereafter fed control diet. Body weight and control fluid diet were measured every day.

Relation between time of intoxication and the degree of b.wt. change after withdrawal. Thrity six animals were divided into 6 groups, intoxicated for 4, 7, 11 days, and for 2, 3 and 4 weeks on 340 mg/kg b.wt. In all groups there had been a previous dose increment for eight days as described above. The aim was to study changes in b.wt. and fluid diet consumption during withdrawal after varying preceding intoxication times on 340 mg/kg b.wt.

Relation between different doses of naloxone and b.wt. loss in long-term intoxicated rats. Sixty animals were intoxicated with morphine up to a dose of 340 mg/kg b.wt./day, and were maintained on that dose for another 6 days. Naloxone, in different concentrations (3.5, 6.5 and 7.5 mg/kg b.wt.) was injected IP into 30 rats (each dose in 10 rats). Another 30 animals were injected with saline and used as controls. Injections were given at 7 p.m. (serum morphine level was 2.3±0.5 μg/ml). Body weight was measured at two, four and five hours after injection. In the group of rats receiving 6.5 mg naloxone/kg, the b.wt. was also measured at 30 and 60 min after injection.

The influence of diet intake on b.wt. during withdrawal. In order to study the influence of diet consumption on b.wt. changes during withdrawal, a comparison was made between intoxicated and control animals by pair-feeding. Six rats were intoxicated up to a dose of 340 mg/kg/day and maintained on that dose for three weeks. Another 6 rats served as controls and received equal amounts of control fluid diet by pair-feeding. Withdrawal was precipitated by exclusion of morphine from the diet, b.wt. and fluid diet consumption being measured every 6 hr over 3 days. During this period, the rats were administered fluid diet every 6 hr. By pair-feeding the control animals received equal amounts of diet, as the abstinent animals had consumed 24 hr previously.

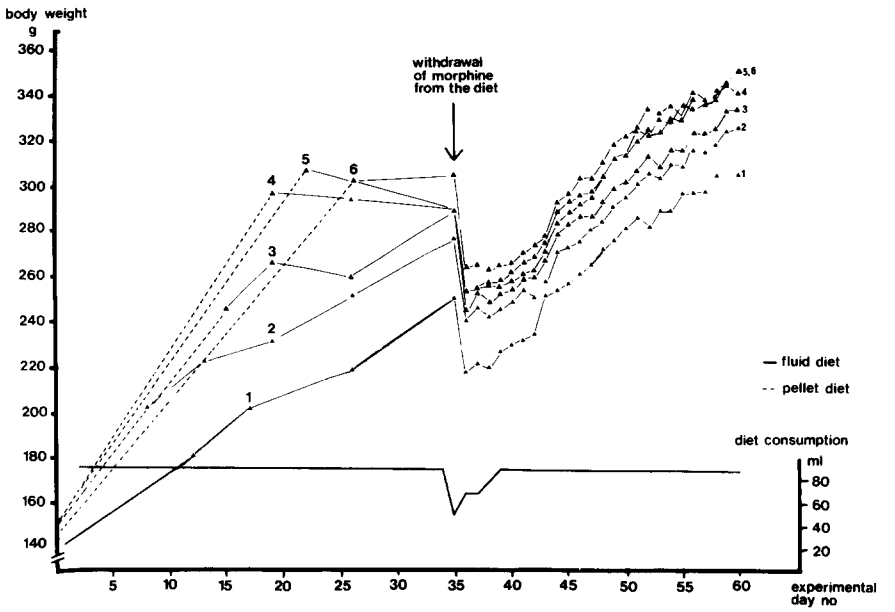

FIG. 2. Six groups of rats with six animals in each group were intoxicated with morphine for 4 weeks (No. 1), 3 weeks (No. 2), 2 weeks (No. 3), 11 days (No. 4), 7 days (No. 5) and 4 days (No. 6), according to the dose increment of 25, 55, 90, 130, 175, 225, 290, 340 mg/kg B.wt. per day. The animals were prefed with pellet diet and water ad lib until intoxication was started, according to the figure. When morphine was withdrawn, all groups showed a weight loss on day 1, ranging from 12.0 to 15.2%. The body weight prior to withdrawal of morphine was reached on days 8 to 11 after withdrawal (=days 42–45 in the figure). By paired Student's *t*-test no statistically significant difference ($p \geq 0.05$) was found between the groups concerning the percentage weight loss or amount of fluid diet consumed during the first day of withdrawal. Neither was there any statistically significant difference in b.wt. gain during days 2 to 8 (day Nos. 36–42 in the figure) after withdrawal. There was a linear weight gain during days 11 to 25 after withdrawal (days 45–59 in the figure) (r=0.95). There was no statistically significant difference in b.wt. gain between the groups during this period.

Relation between morphine treatment and b.wt. changes during withdrawal. Eighteen animals were intoxicated with morphine up to a dose of 340 mg/kg b.wt./day over a period of 8 days after which the dose was maintained for further 2 weeks. Fluid diet intake and b.wt. were measured every day. Morphine was withdrawn from the diet for 16 days. Thereafter morphine (340 mg/kg b.wt.) was given to the animals for 3 days.

Influence of methadone on body weight changes following withdrawal. In order to study the blocking ability of methadone on b.wt. changes during withdrawal (i.e., abstinence) an experiment was performed consisting of ten animals divided into 2 groups of five. The morphine dose was increased up to 340 mg/kg b.wt. in eight days, as described above, and then maintained for two more days on the same dose. On the eleventh day, the morphine was withdrawn from all ten animals. Five rats were substituted with 50 mg methadone/kg b.wt./day for another five days. Food consumption and body weight were measured every 24 hr during the experiment period.

RESULTS

Relation Between Morphine Dose and Time of Intoxication vs. the Degree of Body Weight Loss After Withdrawal

The mean b.wt. change during the first day of morphine withdrawal was expressed as percentage of the b.wt. the day prior to withdrawal, and was plotted versus total dose of morphine in mg/kg during the experiments. The weight loss during the first day of withdrawal in the different groups of experiment 1 was proportional to the cumulative morphine dose, the linear correlation coefficient being (r=0.98). Furthermore, a linear correlation was found between total morphine dose and fluid diet consumption (r=0.99) (Fig. 1). Similar results were obtained from experiment 2 (r=0.99 for both b.wt. loss and fluid diet consumption) (Fig. 1, inset). *Conclusion*: This means that there is a correlation between the cumulative morphine dose and body weight loss after withdrawal.

Relation Between Time of Intoxication and the Degree of b.wt. Changes After Withdrawal

When morphine was withdrawn, all groups showed weight loss on the first day ranging from 12.0–15.2%. On day 5 after morphine withdrawal (day 39 in Fig. 2), all rats started to regain their weight, their b.wt. prior to withdrawal of morphine was reached on day 8 and 11 after withdrawal (days 42–45 in Fig. 2). There was no statistically significant difference between the groups with respect to the percentage weight loss or the amount of fluid diet consumed during the first day of withdrawal. Neither was there any statistically significant difference in weight gain between the groups during days 2 to 8 after withdrawal (days 36–43 in Fig. 2).

During days 11 to 25 after withdrawal, 96% of all the rats

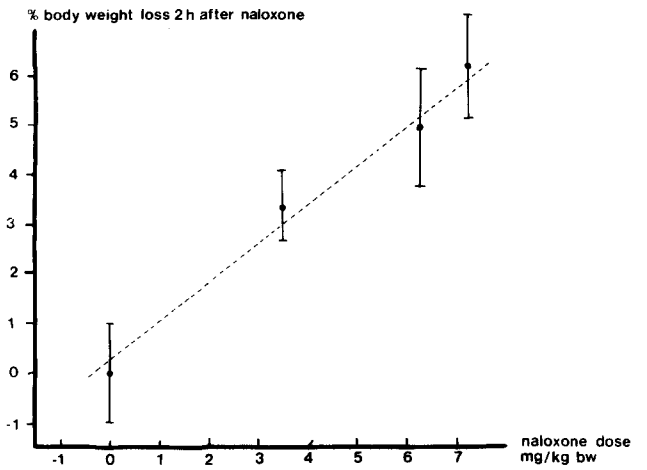

FIG. 3. Sixty animals were intoxicated with morphine up to an end-dose of 340 mg/kg b.wt. per day and were maintained at this dose for 6 days. Naloxone in different concentrations (3.5, 6.5 and 7.5 mg/kg b.wt) was injected IP into 30 rats (10 rats for each dose). Thirty animals were injected with saline and used as controls. B.wt. was measured at different time intervals up to 5 hr after the naloxone injection. Maximal b.wt. loss was seen 2 hr after injection in the groups receiving 3.5 and 6.5 naloxone/kg b.wt., and after 4 hr for the group receiving 7.5 mg/kg b.wt. A linear dose response correlation (r=0.99) was obtained comparing percentage b.wt. loss in the different groups 2 hr after the injection.

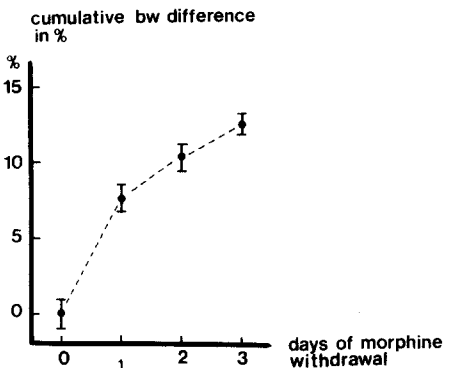

FIG. 4. Six male rats were intoxicated with morphine up to a dose of 340 mg/kg b.wt. per day and maintained on this dose for 3 weeks. Six other rats, serving as controls, received equal amounts of control fluid diet by pair-feeding. Withdrawal was precipitated by exclusion of morphine from the diet and b.wt. and fluid diet consumption was measured every 6 hr during the 3 subsequent days. By pair-feeding, the control rats received equal amounts of diet every 6 hr as the abstinent rats had consumed 24 hr before. The accumulated b.wt. difference between abstinent and control rats during 3 days is shown. The figure demonstrates that, even under strictly controlled, equal diet intake to control and morphine-abstinent animals there is a statistically significant decrease in b.wt. over the 3 days subsequent to withdrawal in the morphine-abstinent animals.

had a linear weight gain (r=0.95). There was no statistically significant difference in b.wt. gain between the groups during this period.

In group 1 (i.e., the animals intoxicated for 4 weeks) 83% of the rats had a linear weight gain prior to withdrawal. In this group, no statistically significant difference was seen in weight gain prior to withdrawal, compared to that seen in the same group after withdrawal during days 11 to 25 (days 45–49 in the figure). *Conclusion*: This means that there is no relation between time of intoxication and the degree of b.wt. changes after withdrawal.

Relation Between Different Doses of Naloxone and b.wt. Loss in Long-Term Intoxicated Rats

Maximal loss in b.wt. was seen 2 hours after the naloxone injection in the groups receiving 3.5 and 6.5 mg naloxone/kg b.wt., and 4 hr after in the group receiving 7.5 mg/kg. A linear dose response correlation (r=0.99) was obtained comparing percentage weight loss in the different groups 2 hr after the injection (Fig. 3). *Conclusion*: This means that there is a correlation between naloxone dose and loss in b.wt. in long-term intoxicated rats.

The Influence of Diet Intake on b.wt. During Withdrawal

Both abstinent and pair-fed control animals lost b.wt. during the first day of withdrawal. However, during the following 2 days controls had a weight gain whereas abstinent rats continued to lose. The cumulative percentage b.wt. difference between abstinent and control rats is shown in Fig. 4. Measurement of hematocrit on the 2nd day of abstinence showed 46.8±0.8% for control rats and 50.5±0.8% for abstinent rats, a statistically significant difference ($p \leq 0.01$). Four days after withdrawal there were no significant differences in hematocrit between the groups. *Conclusion*: The body

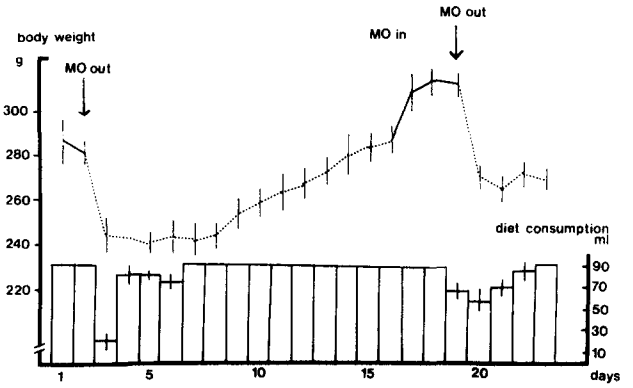

FIG. 5. Eighteen animals were intoxicated with morphine up to a dose of 340 mg/kg b.wt. per day over a period of 8 days. The animals were kept on that dose for another 2 weeks. Fluid diet intake and b.wt. were measured daily. During the first day of morphine withdrawal, the b.wt. decreased 13.7±0.2%. This was followed by an increase in b.wt. 3 days after withdrawal, i.e., day 5 in the figure until day 15. When morphine in a peroral dose of 340 mg/kg b.wt. was given to the animals on day 16, the b.wt. gain increased significantly during the next day compared to the b.wt. gain during the prior 2 days ($p \leq 0.05$). When morphine was withdrawn on day 18 a b.wt. loss of 12.5±0.9% was seen. Thus the b.wt. loss at the second withdrawal is similar to that at the first withdrawal of the experiment.

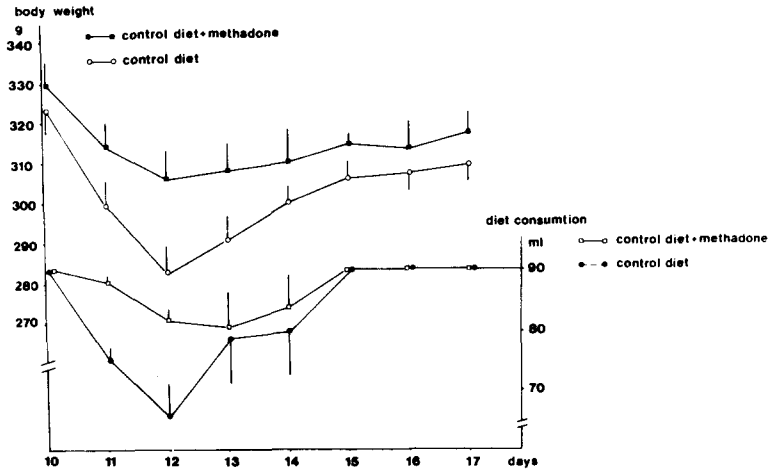

FIG. 6. Ten animals were intoxicated up to a dose of 340 mg/kg b.wt. morphine and then maintained for two more days. Thereafter, morphine was excluded from the diet (day 10). Five rats were substituted with 50 mg methadone/kg b.wt. The maximum weight loss was seen on day 12. Both the body weight loss and the decrease in food intake was markedly reduced in the methadone-treated rats, the body weight loss being reduced by 44% ($p \leq 0.01$).

weight loss after morphine withdrawal is not only due to the decrease in fluid diet intake.

Relation Between Morphine Treatment and b.wt. Changes During Withdrawal

During the first day of morphine withdrawal, the body weight decreased by 13.7±0.2% (Fig. 5). Three days after withdrawal (i.e., day 5 in Fig. 5) and onwards there was an increase in b.wt. until day 15 (Fig. 5) when the b.wt. reached the same level as before withdrawal. When morphine in a peroral dose of 340 mg/kg b.wt. was given to the animals on day 16, the b.wt. gain increased significantly during the next day compared to the b.wt. gain during the prior two days ($p \leq 0.05$). When morphine was withdrawn on day 18, a body weight loss of 12.5±0.9% ($p \leq 0.001$), i.e., similar to that in the beginning of the experiment (Fig. 5).

Conclusion. This means that after morphine has been withdrawn for up to 2 weeks in long-term intoxicated rats, just 1 day of morphine treatment seems to quickly activate the metabolic changes towards a tolerant state, so that the animals can immediately tolerate 340 mg morphine/kg b.wt. After just 3 days of morphine treatment, the physical dependence is restored and withdrawal produces a degree of weight loss similar to that produced during the previous morphine withdrawal.

Influence of Methadone on b.wt. Changes Following Withdrawal

The body weight loss and decrease of fluid diet intake following morphine withdrawal were less prominent after administration of methadone in a dose of 50 mg/kg b.wt./day (Fig. 6). *Conclusion*: This means that methadone partially blocks the loss in body weight during morphine withdrawal.

DISCUSSION

Knowledge of the withdrawal syndrome and methods for its quantification is essential in the study of the physical dependence. When dealing with precipitated withdrawal in mice, stereotyped jumping has generally been considered a suitable sign for its quantification [10, 17, 18]. In other species, point ranking systems have been developed. However, the literature is inconclusive in evaluating the relative importance of the different signs [16, 28, 29]. A possible explanation could be that the various signs of precipitated withdrawal do not all increase concomitantly with increasing dependence [2]. The possible "abstenoid" reactions [22], food and water suppressant effect of naloxone should also be kept in mind [5,23]. Changes in body weight during both precipitated withdrawal and withdrawal due to exclusion of morphine have been suggested to be a simple and reliable index for determining the degree of physical dependence in rat [1, 8, 11, 25].

Morphine in appropriate concentrations decreases food and water intake (see [20]). Thus, erroneous conclusions can be drawn regarding body weight gain during morphine intoxication. As in most intoxication methods, it is difficult to determine whether changes in body weight during morphine treatment are dependent on morphine's reducing effect directly on body weight or indirectly on food intake. Our ingestion method [32], ensuring equivalent diets for both intoxicated and control animals by pair-feeding, seems adequate for studying changes in body weight during morphine intoxication and withdrawal. In accordance with previous studies [19, 24, 27], our data show a reduction in the growth curve during chronic morphine treatment. The reason is not fully understood. Two possible mechanisms are altered levels of anabolic hormones [12] or increased food utilisation [13].

Upon withdrawal from large doses of morphine, the early phase, called "primary abstinence" [19] consists of weight loss. It is manifest within 8 to 16 hours following withdrawal with a maximal weight loss during the first 24 hr, and reaching a minimal body weight in 24 to 48 hr. When precipitating withdrawal with morphine antagonists, weight loss is seen during the first 2 to 4 hr and is accompanied by a decreased fluid diet intake and diarrhoea. Using metabolic cages, we

found that weight losses approximated the sum of decreases in the fluid diet intake and increases in defecation and urination (unpublished), in accordance with Goode [8]. Weight loss, therefore, seems to provide a useful index of the natural morphine withdrawal [11], and is proportional to the morphine dose. When animals are well intoxicated, longer intoxication on the same dose does not affect b.wt. changes upon withdrawal. It might be less useful in assessments of precipitated abstinence, since in that case it seems to be associated mainly with defecation [11,30]. Increased physical activity or other undefined factors might be of some importance in causing body weight loss.

Since methadone is used in the therapy of morphine addicts, and since methadone has been shown to induced physical dependence with several characteristics in common with morphine [15] it was interesting to note that methadone could partially block the abrupt withdrawal reaction after morphine addiction.

Two to four days after withdrawal, b.wt. increases. It occurs more rapidly than in controls despite intake of the same amount of diet. If morphine is offered the rats during this weight gain period, they increase in weight even more rapidly (20 to 30 g in 24 to 48 hr). Thus, the weight gain during this phase is considered to be a loss of tolerance to the weight depressing effect caused by morphine. This was also pointed out by Mucha and Kalant [20]. The basis of the increased gain in b.wt. during this phase is not known, regulation of b.wt. being a complex process that is still not fully understood [21].

One important question is whether the observed weight changes are central or peripheral effects of morphine. Some data in the literature show that, during the chronic morphine treatment, the gut of dogs [3] and of guinea-pigs [7] become tolerant to morphine. In view of the close connection between tolerance and physical dependence (see [9]), one might expect that dependence develops in the gut itself and, as a consequence, withdrawal diarrhoea is of peripheral origin. Laschka *et al.* [14] demonstrated mild diarrhoea in rats even after intraventricular administration of a morphine antagonist. Central components, however, cannot be excluded [14,18]. It has also been suggested that the constipation effect of acute morphine is largely due to a central action, which results in a visceral component (see [4,31]). Possibly, both central and peripheral mechanisms are involved in the withdrawal diarrhoea. Gold *et al.* [6] considered the locus coerulus as one structure from where central abstinence symptoms were precipitated.

In conclusion, we suggest that body weight changes are a reliable index for determining the degree of physical dependence in Sprague-Dawley rats. It is a parameter which is easy to quantify and which shows little variation between animals.

ACKNOWLEDGEMENTS

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