Pharmacology Biochemistry & Behavior, Vol. 11, pp. 197–201. Printed in the U.S.A.

Increased Weight Gain as a Morphine Withdrawal Response in Rats

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Received 6 April 1979

MUCHA, R. F. AND H. KALANT. *Increased weight gain as a morphine withdrawal response in rats.* PHARMAC. BIOCHEM. BEHAV. 11(2) 197–201, 1979.—Adult male Wistar rats injected daily with 20 or 200 mg/kg morphine-SO_4 for 35 days suffered a dose-dependent weight loss over the first 3 days of morphine withdrawal. However, during the next 28 days they gained weight more rapidly than controls, the rates being related to the previous morphine dosage. These findings were replicated in Sprague-Dawley rats treated for 26 days with 60 mg/kg morphine. Food-restricted controls suffering weight losses equal to those of the morphine-treated or morphine-withdrawn groups did not subsequently gain weight as rapidly as the latter groups. Therefore the rapid post-withdrawal weight gain may be a true adaptive response to the weight suppressing effects of morphine. Also, comparisons of weight changes during treatment in the two experiments indicated possible strain differences for tolerance to morphine's direct weight-reducing effect.

Morphine Weight changes Physical dependence Protracted dependence Strain differences

WITHDRAWAL of morphine after a period of chronic exposure produces two apparent effects on body weight. The first is weight loss, one of the more sensitive and reliable features of physical dependence on morphine [1, 7, 26]. It comprises reduced food and water intake, and increased defecation and urination [7]; and the magnitude and duration of weight loss is related to the preceding treatment dose [1,26]. Body weight loss is evident shortly after withdrawal and rarely lasts longer than a few days [20,26].

The second effect is an increase in the rate of body weight gain following recovery from the weight loss. This phenomenon has received almost no attention and data supporting its existence are largely incidental. Fluid intake, known to affect body weight [4], was markedly increased in abstinent rats [20]. In addition, the data in several reports [1, 9, 20, 26] suggest the presence of higher rates of weight gain for previously morphine-treated rats compared to control rats. However, only recently was any formal mention of this body weight effect made. Thornhill *et al.* [27] pointed out that the rate of growth of heroin-withdrawn rats was much higher than that of saline-treated rats.

The probable reason why the increase in body weight gain has not received systematic study is the assumption that the increase is a compensatory response to the weight losses produced by the morphine treatment and withdrawal. Indeed, it was argued by Thornhill *et al.* [27] that heroin interfered with normal growth. It was, therefore, not suprising that heroin withdrawn rats showed higher rates of gain than control rats. An alternative hypothesis is that the increased rate of weight gain is a long term effect of morphine on body weight control mechanism.

In view of the importance of long term effects of chronic drug administration for understanding the mechanisms of tolerance and dependence [6], the present study tested these two hypotheses. Body weights were measured in morphine-withdrawn rats and in rats with body weights decreased by restricted feeding.

METHOD

Animals

Adult male rats (Wistar in Experiment 1 and Sprague-Dawley in Experiment 2) weighing 240–260 g at the time of purchase from Canadian Breeding Laboratories (Constant, Quebec) were used. They were housed singly in stainless steel cages, in temperature controlled rooms with lights on from 0800–2000 hr. Prior to the experiments food was ad lib and the rats were weighed weekly. Water was always ad lib.

Drugs

Doses of morphine sulfate (BDH), expressed in terms of the salt, were dissolved in physiological saline and injected IP. Treatment doses, in volumes of 4 ml/kg, were given in single daily injections.

Procedure

Body weight was manipulated by means of weight pairing. From a group of 120 rats available for Experiment 1, 60 rats were selected on the basis of body weight and divided into 10 groups of 6 each, with individual body weights differing less than 5 g. In Experiment 2, 40 rats were selected from an initial pool of 75 and similarly divided into 10 groups of 4

each. One rat from each of the matched groups was assigned randomly to each treatment condition. Some conditions involved ad lib food presentation, while others involved food restriction designed to yield body weights matched with those of specified ad lib feeding groups. The daily weight change of a rat in the ad lib group governed the amount of food given next day to its matching rat in the paired group. The weights of the rats were recorded at the time of the daily injection, and if the weight of the ad lib rat stayed the same as the day before, the weight of the weight-paired rat was held constant by putting 25 g of food into the hopper. If the weight of the ad lib match went up (or down), 5 g more (or less) food was given to the paired rat. Because of differences in body size, the amount of food required to maintain body weight was 20 g a day for some rats and 30 g for others.

Experiment 1. Of six experimental groups (10/group), three groups received 0, 20, or 200 mg/kg of morphine and ad lib feeding. The dose of the 200 mg/kg rats was increased over the first five injections from an initial 40 mg/kg, by four successive equal-sized daily increments. There were three weight-paired groups, of which two received saline and weight-pairing to the 20 and 200 mg/kg ad lib groups, respectively. The third group received 20 mg/kg morphine, but was weight-paired to the 200 mg/kg ad lib group. The maintenance injections were continued for 35 days, except for Days 22 and 29 when tests for analgesia were given. The data from these tests will be described elsewhere. Commencing with Day 36 all injections were stopped and for 28 days each rat received ad lib feeding and daily weighing.

Experiment 2. There were four groups, three receiving daily injections of saline and one 60 mg/kg morphine. The treatment lasted 26 days and rats of the morphine and one saline group were fed ad lib; the other two saline groups were weight-paired to the morphine rats. Final injections were on Day 26 but body weights were recorded periodically for 87 days. One group of weight-paired rats commenced ad lib feeding; the other received only 5 g of food in their hoppers on Days 27 to 29, to simulate the weight loss produced by morphine withdrawal, and then received mild food restriction, as previously described, for 1 day, and ad lib feeding thereafter.

Statistical Analyses

The statistical tests were two-tailed and employed the procedures of Kirk [14]. *A priori* statistical tests were used throughout the study except for analyses of the withdrawal-related body weight data in Experiment 1. In these cases, since no *a priori* hypotheses were advanced, Tukey "A" tests followed significant analyses of variance. All tests employed Dunn's adjustment when more than one comparison of individual means was made. Throughout the text results are presented as mean ± SE.

RESULTS

Experiment 1

The body weights of the various treatment groups are summarized in Fig. 1. The means were derived from data from 10 rats each, except for the 20 mg/kg-treated group weight-paired to the 200 mg/kg group. In this group there were 10 rats at each point until Day 29 and eight at each subsequent point, since two rats died during tests of analgesia on Day 29.

It is clear from the figure that the regimen of morphine administration produced a treatment dose-related suppression of body weight gain, as previously reported [22,23]. The data prior to Day 22 were analyzed. The overall mean of the

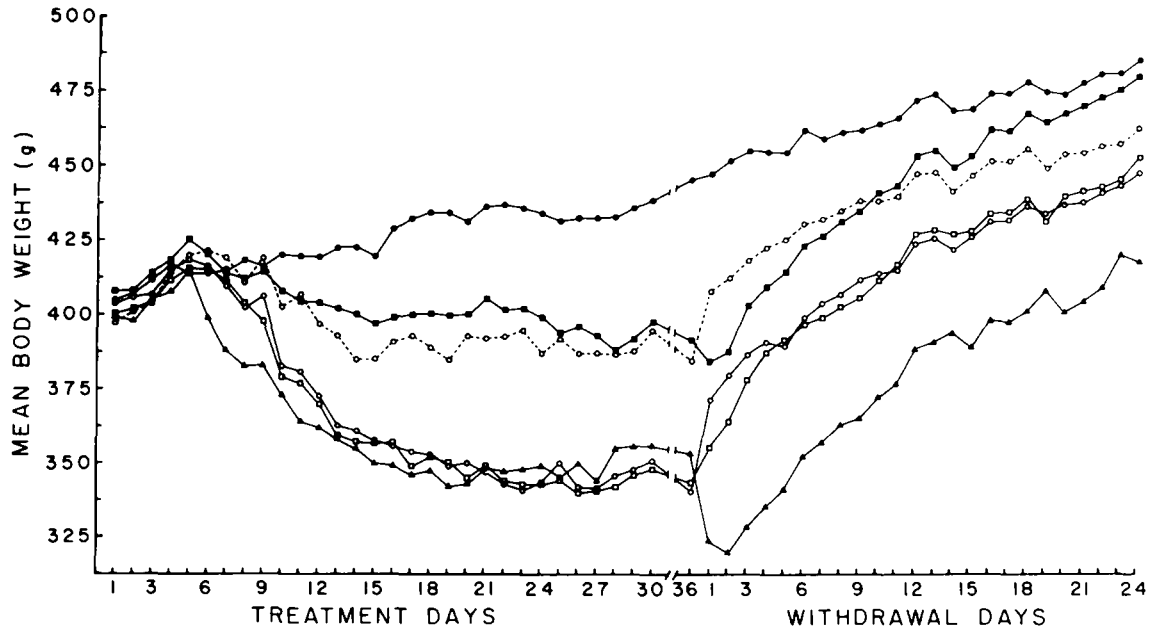

FIG. 1. Body weights of Wistar rats in Experiment 1 on various regimens of daily morphine and weight manipulation: (●——) saline, fed ad lib; (■——) morphine 20 mg/kg, fed ad lib; (▲——) morphine 200 mg/kg, fed ad lib; (○----) saline, weight-paired to 20 mg/kg morphine group; (○——) saline, weight-paired to 200 mg/kg morphine group; and (□——) morphine 20 mg/kg, weight-paired to 200 mg/kg group. The SE values of the various points were all less than 11.6.

ad lib fed saline group was significantly greater than the mean of the 20 mg/kg ad lib fed group, $F(1,1566)=24.5$, $p<0.05/3$, and the latter was significantly greater than the ad lib 200 mg/kg mean, $F(1,1566)=46.7$, $p<0.05/3$. It is also apparent from the figure that the body weight pairing procedure produced body weights comparable to those produced by morphine.

Withdrawal from morphine treatment on Day 36 produced a clear treatment-dose-related drop in body weight of the ad lib-fed rats (see Fig. 1). Analysis of variance of the weight change between Day 36 of treatment and the first day of withdrawal was highly significant, $F(5,52)=119$, $p<0.0001$. A *posteriori* tests of data from the ad lib-fed rats indicated significant mean differences between the saline and the 200, $q(6,52)=15.0$, $p<0.05/4$, the saline and the 20, $q(6,52)=5.2$, $p<0.05/4$, and the 200 and the 20 mg/kg groups, $q(6,52)=10.4$, $p<0.05/3$. The duration of the weight loss for the 20 mg/kg ad lib-fed rats was 1 day and for the 200 mg/kg rats it was between 1 and 4 days (mean=2.5 ± 0.3). The average day of the maximum weight loss in the 200 mg/kg group was 1.8 ± 0.2. Not surprisingly, the switch to ad lib feeding for the weight-paired rats on Day 36 produced a sharp gain in weight. However, it was interesting that this also occurred for each of the 20 mg/kg treated rats weight-paired to the 200 mg/kg group. The weight gain in this group between Day 36 of treatment and the first day of withdrawal was significantly higher than that of the ad lib saline group, $q(6,52)=6.5$, $p<0.05/3$, but significantly lower than that of the saline group paired to the 200 mg/kg rats, $q(6,52)=8.2$, $p<0.05/3$, and of the saline group paired to the 20 mg/kg group, $q(6,52)=5.9$, $p<0.05/3$. This fact suggests that some effects related to morphine withdrawal were present.

From the 4th day on, all individual subjects exhibited a steady body weight increase. Using slopes of best-fitting lines drawn through the graphed data, mean daily rates of weight gain were determined for successive 7-day blocks between Days 4 and 24, inclusive. From a summary of these data in Fig. 2, it is apparent that the morphine treatment caused a dose-dependent increase in the rate of weight gain during withdrawal. Following a highly significant group effect on an overall analysis of variance, $F(5,52)=36.4$, $p<0.001$, separate analyses of the overall means were carried out. The overall mean of the ad lib 20 mg/kg group was significantly greater than that of its weight-paired saline control, $q(6,52)=8.15$, $p<0.05$. Similarly, the overall mean of the 200 mg/kg group significantly exceeded those of its weight-paired control groups, one treated with saline, $q(6,52)=10.0$, $p<0.05/3$, and one with 20 mg/kg morphine, $q(6,52)=6.5$, $p<0.05/2$. There was, however, for saline rats weight-paired to 200 mg/kg rats, an overall mean rate significantly higher than for ad lib saline rats, $q(6,52)=9.9$, $p<0.05/2$. On the last 7-day block the mean rate of the ad lib 200 mg/kg morphine group was significantly greater than that of the saline group weight-paired to the 200 mg/kg group, $q(6,156)=5.6$, $p<0.05$, suggesting a long-term effect of the 200 mg/kg morphine.

Experiment 2

A summary of the body weights measured during this experiment is presented in Fig. 3. On the last day of treatment, the 60 mg/kg mean differed significantly from the saline mean, $t(18)=21.4$, $p<0.04$, but the means of the morphine and two weight-paired control groups were very similar. The data obtained during withdrawal replicated the previous results. As of Day 5, all rats began a stable weight gain. The gain over Days 5–11 for the weight-paired group placed on ad lib feeding at the start of withdrawal was significantly less than the gain of the morphine group, $t(18)=5.1$, $p<0.05/2$. The same was true of the group that was food-restricted until Day 4 of withdrawal to mimic the weight loss caused by withdrawal, $t(18)=3.8$, $p<0.05/2$. The rate of gain in the morphine rats declined over the remaining period after withdrawal, but their mean gain between Days 12 and 38 was still significantly higher than those of the two weight-paired groups, $t(18)=4.8$, $p<0.05/2$; $t(18)=2.5$, $p<0.05/2$, respectively. There were no significant differences in weight gain following this period so that the growth curves became parallel. However, an analysis of variance followed by a comparison of the overall mean body weights of the four groups between Days 42 and 87 indicated that the mean weight of the morphine rats was significantly larger than the combined means of the weight-paired controls, $F(1,36)=4.25$, $p<0.05$.

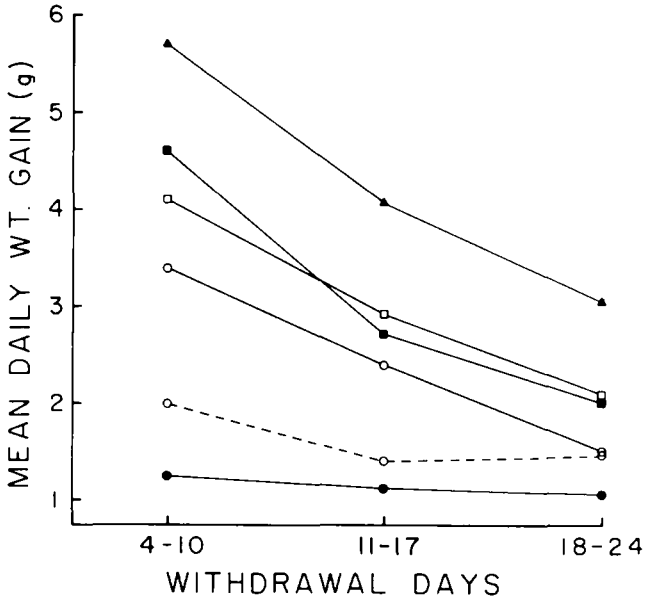

FIG. 2. Daily weight gain during the three 7-day blocks between withdrawal Day 4 and 28, inclusive, in Experiment 1, for rats identified in Fig. 1 caption. The SE values of the various points were all less than 0.5.

Additional analyses of the data during treatment indicated that there was an apparent development of tolerance to the weight-reducing effect of morphine. There was a significant increase in body weight for the morphine group between Days 12 and 21 of treatment, $t(8)=2.4$, $p<0.05$. Only 9 rats were used for this comparison since one rat chipped its tooth during the 10-day period and showed irregular body weight changes; however, its weight also showed an upward trend.

DISCUSSION

The results of the present study indicated a clear increase in body weight gain in morphine-withdrawn rats. This effect was treatment-dose-dependent and long term, with greater weight gains compared to control still apparent several weeks after the recovery from withdrawal-related weight

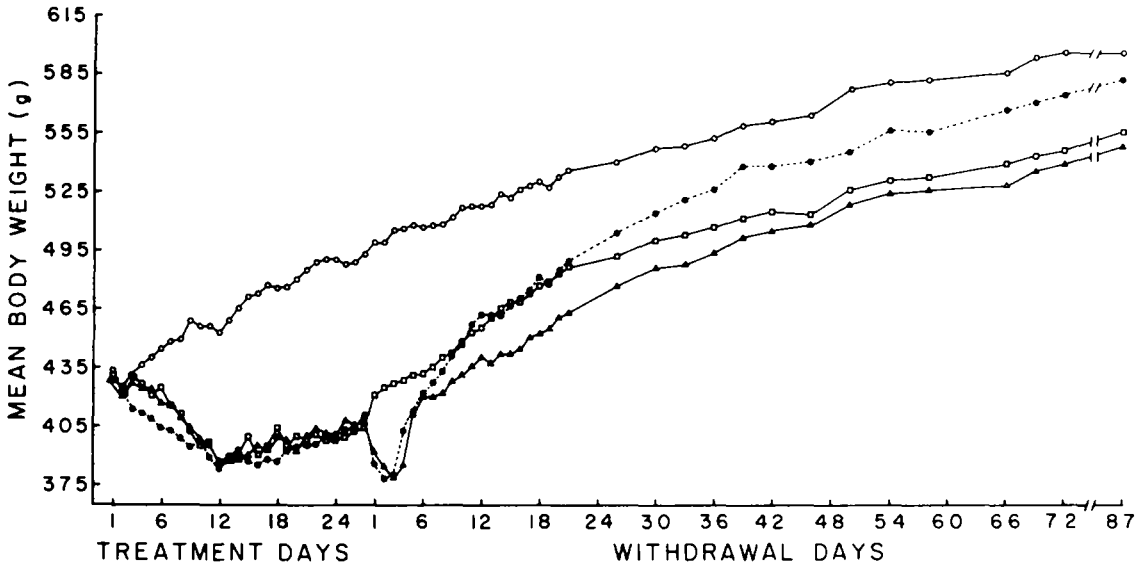

FIG. 3. Body weights of Sprague-Dawley rats in Experiment 2 on various regimens of daily morphine and weight manipulation: (○——) saline, fed ad lib; (●----) morphine 60 mg/kg, fed ad lib; (□——) saline, weight-paired to morphine group until start of withdrawal; and (△——) saline, weight-paired to morphine group until the latter started gaining weight after morphine withdrawal. The SE values of the various points were all less than 15.5.

loss. It is known that weight-reduced animals show compensatory increases when ad lib feeding is resumed [21,29]. However, this alone does not explain the effect in morphine-withdrawn animals, since equally weight-reduced control groups [17,25] did not gain as rapidly as the morphine-withdrawn rats. The characteristics of the increased weight gain strongly suggested that it is a true sign of physical dependence. Consistent with this idea are the data of Bhargava and Matwyshyn [2], collected during a 3-day withdrawal period. Naloxone produced a drop in body weight followed by a weight gain, whereas only the body weight drop occurred without naloxone.

It has long been puzzling that weight loss occurs both during morphine administration and after morphine withdrawal. The homeostatic theory of morphine tolerance [10] predicts that the acute effects and the changes typical of physical dependence should be opposite, as seen for analgesia [28]. Our present findings are consistent with this prediction, and suggest that the later weight-gain increase in morphine withdrawal represents the rebound from the acute weight-gain-suppressing effects of morphine. The duration of this rebound is several weeks (Fig. 3), which is comparable to the time in which naloxone can elicit retrograde taste aversion to saccharine in previously morphine-withdrawn mice [18] and the time that it takes for rats and mice to show a reversal of opiate tolerance [5]. The early weight loss in morphine withdrawal, on the other hand, may primarily reflect the counterpart of tolerance to the direct intestinal effect of morphine, as suggested by Burks *et al.* [3]. Thus, these two different withdrawal effects may reflect two mechanisms of tolerance, differing in their rates of offset [6] and the characteristics of their log-dose/response curve changes [22,23].

The basis of the increased body weight gain is not known. Weight reduction produced by morphine may arise through decreases in food and water intake [16] (though vasopressin release occurs, promoting water retention [13]), altered levels of anabolic hormones [15], increased food utilization due to large increases in motor activity [16] and inhibition of RNA and protein synthesis [12]. Conceivably, the increased body weight gain during withdrawal may arise from effects exactly opposite to these treatment-related effects. Body weight regulation however, is a complex process that is still not fully understood [24] and there are a number of other ways that the preceding morphine treatment could have resulted in increased body gain. For example, diet palatability influences body weight regulation [24] and it is not certain whether the present phenomenon would occur with foods of varying palatability. If such effects occur, another question is whether they arise from direct or conditioned effects [8]. In addition, the greater body weight gain is only clear when the morphine groups are compared to the groups experiencing body weight decreases produced by food restriction, since body weight reduction by itself results in compensatory increases in weight gain. It will be interesting to see whether other types of controls, using quinine adulteration or heavy work schedules [24] to produce body weight reduction, allow quantitatively similar effects to be detected. Body weight changes may be secondary to specific molecular changes produced by morphine administration. For example, recent evidence by Margules *et al.* [19] indicated a relation between overeating and high plasma and pituitary β-endorphin levels in genetically obese rats and mice, and changes in endogenous opioid activity have been noted during the development of morphine tolerance [11].

The results of the present study bring data to bear on another issue in the opiate literature. A comparison of the body weight data from the treatment period in Experiments 1 and 2 suggests strain differences for tolerance development to morphine's suppression of body weight. In Experiment 1, Wistar rats showed no development of tolerance to this effect during the 21-day treatment period, even to a dose as

low as 20 mg/kg, despite clear tolerance to the analgesic action. Sprague-Dawley rats, in Experiment 2, showed clear tolerance to 60 mg/kg after 16 days of treatment, an onset time comparable to that seen previously in this strain of rat [22]. Whether the effect reflects a difference in the capacity to develop tolerance, or only in the rate of its acquisition, remains to be determined.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the able assistance of L. Currin, D. Lindholm, K. Potter and K. Voshart.

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