Pharmacology Biochemistry and Behavior, Vol. 61, No. 1, pp. 19–27, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(98)00059-8

Chronic Running-Wheel Activity Decreases Sensitivity to Morphine-Induced Analgesia in Male and Female Rats

ROBIN B. KANAREK, AMY V. GERSTEIN, RACHEL P. WILDMAN, WENDY FOULDS MATHES AND KRISTEN E. D'ANCI

Tufts University Department of Psychology, Research Building, 490 Boston Ave., Medford, MA 02155

Received 7 February 1997; Revised 12 January 1998; Accepted 8 February 1998

KANAREK, R. B., A. V. GERSTEIN, R. P. WILDMAN, W. F. MATHES AND K. E. D'ANCI. *Chronic running-wheel activity decreases sensitivity to morphine-induced analgesia in male and female rats.* PHARMACOL BIOCHEM BEHAV **61**(1) 19–27, 1998.—The effects of exercise on morphine-induced analgesia were examined in male and female Long–Evans rats. In Experiment 1, 10 male rats were housed in standard laboratory cages, and 10 in activity wheels for 20 days prior to nociceptive testing. Pain thresholds were assessed using a tail-flick (TF) procedure. Morphine sulfate was administered using a cumulative dosing procedure (2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 mg/kg). TF latencies were measured immediately prior to and 30 min following each injection. In Experiment 2, morphine-induced analgesia was examined in females in an identical manner to that of Experiment 1. Additionally, to determine if the attenuation of morphine-induced analgesia was permanent or reversible, after the initial test nociceptive test, previously active female rats were placed in standard cages, and previously inactive females placed in running wheels for 17 days prior to a second nociceptive test. Baseline TF latencies were significantly shorter in active male rats than in inactive animals. Additionally, both active male and female rats displayed decreased morphine-induced analgesia relative to inactive controls. Moreover, females that had been inactive and then were permitted to run showed a suppression in morphine-induced analgesia relative to presently inactive rats, and to their own nociceptive responses when sedentary. In contrast, morphine-induced analgesia in initially active females who were housed in standard cages during part 2 of Experiment 2 was enhanced relative to their first nociceptive test and to presently active rats. Experiment 3 examined the effects of short-term (24 h) running on antinociception. Baseline TF latencies were shorter in active rats than inactive rats. However, no differences in morphine-induced analgesia were observed as a function of short-term exposure to exercise. Experiment 4 investigated whether differences in body weight contributed to the differences in morphine-induced analgesia between chronically active and inactive animals. %MPEs did not vary among male rats maintained at 100, 85, or 77% of their free-feeding body weight. These results indicate that chronic activity can decrease morphine's analgesic properties. These effects may be due to crosstolerance between endogenous opioid peptides released during exercise and exogenous opioids. © 1998 Elsevier Science Inc.

Morphine Activity Opioids Analgesia Pain Tail flick Tolerance Female Exercise Running wheels Long–Evans Body weight Food restriction

ENDURANCE exercise is associated with increases in plasma levels of β-endorphin in humans and animals [e.g., (1,4,8,12, 14–16,19,21,37–39,45)]. This increase in β-endorphin occurs across a variety of forms of exercise such as running, cycling, and aerobic workouts, and in untrained, as well as trained males and females (4,8,14–16,19,37–39,45). Although some investigators have reported that elevations in β-endorphin are directly related to intensity of the exercise [e.g. (8, 37,38)], others have not found evidence for this relation (15,39).

Requests for reprints should be addressed to Robin B. Kanarek, Tufts University Department of Psychology, Research Building, 490 Boston Ave., Medford, MA 02155.

It has been hypothesized that the increase in plasma β-endorphin that occurs following exercise leads to changes in mood and pain sensitivity (11,22,23,39–42). More specifically, it has been proposed that elevations in β-endorphin produce an opiate-like euphoria leading to positive mood states including the so-called "runner's high" (26,32). Additionally, given that opioid peptides have an analgesic action, it has been suggested that exercise-induced enhancement of β-endorphin leads to an increase in pain tolerance. In support of this suggestion, several researchers have reported that human subjects are less sensitive to a painful stimulus after exercise than before (22, 23,29,36). However, other investigators (35) have argued that this result may be confounded by the fact that in most studies assessing the effects of exercise on pain sensitivity, subjects' pain tolerance was measured both preceding and following exercise. As pain itself can induce an analgesic response, it is proposed that results taken as evidence for exercise-induced analgesia may be due to the analgesic actions of the pain pretest, rather than to exercise, per se. Indeed, when both the effects of exercise and prior pain testing were controlled, posttests measures of pain on a cold pressor test demonstrated that preexposure to pain testing, but not exercise had a significant analgesic action (35).

To better understand the role of exercise in mediating pain responses, the present experiments compared baseline pain responses and morphine-induced analgesia in rats housed in running wheels (active), and rats housed in standard laboratory cages (inactive). As previous work had demonstrated gender differences in patterns of nutrient intake in response to voluntary exercise (24), and in sensitivity to morphine's antinociceptive properties (6), the effects of exercise were examined in male and female animals. In the first two experiments, body weights of active animals were less than those of inactive animals. Thus, an additional experiment was conducted to determine whether exercise-induced differences in pain sensitivity and morphine-induced analgesia might be due to differences in body weight.

EXPERIMENT 1

METHOD

Subjects

Twenty adult male Long–Evans VAF rats (Charles River, Portage, MI) weighing between 215 and 265 g at the beginning of the experiment were used. Ten rats were housed individually in standard stainless steel hanging cages, and 10 rats were housed individually in Wahman (Timonium, MD) LC-34 activity wheels with adjoining cages. Wheel turns were monitored by a microswitch such that only complete 360° turns were recorded. Animals were maintained in a temperature-controlled room (21 ± 2° C) maintained on a 12:12 h reverse light–dark cycle (lights on: 2000–0800 h). All experimental procedures were conducted under red lights during the middle of the dark cycle (1000–1600 h).

All rats were given ad lib access to ground Purina chow (#5001) and water. The chow was presented in Wahman LC-306A stainless steel food cups with lids. The food cups were clipped to the cage floors to prevent spillage. Water was available in glass bottles fitted with drip-proof stainless steel stoppers. Food and water intakes, body weights, and wheel revolutions were measured every other day. Rats were given 20 days to acclimate to handling procedures and to the running wheels prior to onset of nociceptive testing.

Drugs

Morphine sulfate (generously provided by the National Institute on Drug Abuse) was dissolved in physiological saline at a concentration of 2.5 mg/ml. Injections were administered subcutaneously in a volume of 1.0 ml/kg.

Nociceptive Testing

Pain thresholds were determined using the radiant heat tail-flick assay (9). Rats were gently held in a clean cloth by the same experimenter. Animals were placed on the tail-flick apparatus (Endie Instrument Co., Montpelier, VT) with their tails smoothed into a groove that contained a photocell. A light source was activated and the light remained focused on the tail until the rat moved its tail, thus switching the light off, or until 10 s had elapsed. The intensity of the light was adjusted to obtain baseline tail-flick latencies of 2–4 s. As suggested by previous researchers (9), a cutoff time, approximately three times greater than the mean baseline latency (i.e., 10 s) was chosen to prevent tissue damage to the tail.

Immediately before nociceptive testing, the doors to the running wheels were closed for the duration of the test. Baseline measures were determined by using the median of three tail-flick tests, separated by approximately 15 s. Immediately after determining baseline latencies, rats were injected with 2.5 mg/kg morphine. Thirty minutes later, for each rat, a single measure of tail-flick latency was determined, and the rat again injected with morphine. This procedure was repeated until a cumulative dose of 15 mg/kg was obtained.

All procedures were approved by the Tufts University Institutional Animal Care and Use Committee.

Statistical Analysis

Data on daily food intake and body weight gain across the experiment were analyzed using *t*-tests.

Prior to statistical analyses, antinociceptive data were converted to the percent maximal possible effect (%MPE), which was calculated as follows:

$$\%\text{MPE} = \frac{(\text{test latency} - \text{baseline latency})}{(\text{maximal latency} - \text{baseline latency})} \times 100$$

where the maximal latency is the cut off time of 10 s (13). The data then were analyzed with two-way ANOVAs (running condition by dose) with dose as a repeated measure. Post hoc comparisons were done with Bonferroni-Dunn *t*-tests to determine specific differences at different doses (31).

Relationships between activity and antinociceptive responses were determined by calculating Pearson product-moment correlations between the number of wheel turns made by individual animals during the 24 h preceding antinociceptive tests and their %MPEs at each dose of morphine.

RESULTS

Food Intake and Body Weights

Mean daily food intake of active males (26.8 ± 2.1 g/day) was significantly, $t(1, 18) = 2.30$, $p < 0.05$, greater than intake of inactive males (24.9 ± 1.6 g/day). However, this increase in daily food intake was insufficient to overcome the increase in energy output. Across the study, active rats gained signifi-

cantly, $t(1, 18) = 4.04$, $p < 0.001$, less weight (72.4 ± 22.4 g) than inactive rats (110.9 ± 20.2 g). At the time of nociceptive testing, mean body wight of active rats (300.9 ± 21.0 g) was significantly, $t(1, 18) = 10.60$, $p < 0.001$, less than that of inactive rats (360.8 ± 26.1 g).

Wheel Activity

Mean daily wheel revolutions (± SD) for active rats was 4416 ± 3491 over the 20-day activity period. However, this number is not representative of activity at the time of analgesic testing as running levels increased with time. During the 24 h preceding nociceptive testing, wheel turns across the group averaged 9100 ± 4045. There was substantial between subject variability in number of daily wheel turns. However, across the experiment, individual rats were consistent in the amount of activity in which they engaged.

Antinociceptive Responses

Baseline tail-flick latencies were significantly $F(1, 18) = 7.32$, $p < 0.001$, shorter in active rats (2.11 ± 0.48 s) than in inactive rats (3.32 ± 0.79 s).

Following morphine administration, %MPEs increased significantly, $F(5, 90) = 4.28$, $p < 0.005$, as a function of drug in both activity conditions. However, across drug doses, %MPEs were significantly, $F(1, 18) = 6.40$, $p < 0.02$, lower in active rats than in inactive animals (Fig. 1). Post hoc comparisons revealed that %MPEs of active rats were significantly (ps < 0.05) less than those of inactive rats at doses of 2.5, 7.5, and 12.5 mg/kg morphine.

Numbers of wheel turns were not significantly correlated with %MPEs at any dose of morphine (Table 1).

TABLE 1

PEARSON PRODUCT-MOMENT CORRELATIONS BETWEEN NUMBER OF WHEEL TURNS AND %MAXIMAL POSSIBLE EFFECT ON A TAIL-FLICK TEST FOLLOWING MORPHINE ADMINISTRATION FOR MALE RATS

Dose of Morphine (mg/kg)	Correlation	Significance
2.5	0.03	NS
5.0	0.41	NS
7.5	0.30	NS
10.0	0.28	NS
12.5	0.44	NS
15.0	0.59	NS

NS = not significant.

EXPERIMENT 2

In the previous study, active male rats gained significantly less weight than nonactive males. Therefore, it is possible that the decrease in %MPEs in these animals was related to their reduced body weight. In contrast to males, active female rats are reported to increase food intake and gain similar amounts of weight as nonactive controls (24). Additionally, recent work has indicated that male rats are more sensitive to morphine's antiociceptive actions than females (6). Thus, to separate the effects of activity from those of weight, and to determine if exercise differentially affected morphine-induced analgesia in males and females, nociceptive tests were conducted in female rats. Additionally, to investigate if the effects of exercise on morphine-induced analgesia were permanent or reversible, in part 2 of the experiment, previously active rats were housed in standard cages, and inactive rats in activity wheels for an additional 17 days and then retested for morphine-induced analgesia.

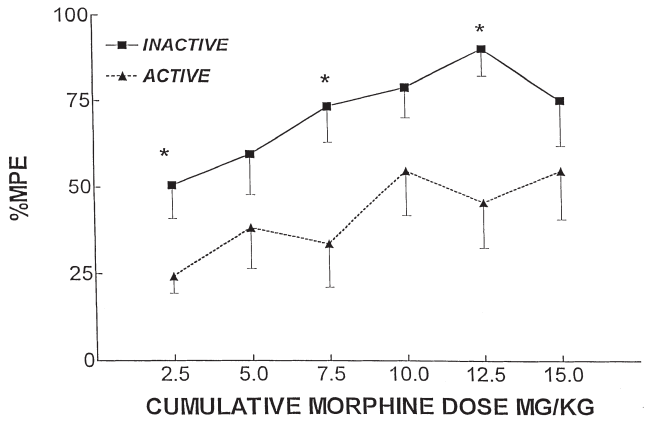

FIG. 1. Mean ± SEM % maximal possible effects (%MPEs) following cumulative morphine administration in active (▲) and inactive (■) male rats. %MPEs of rats that ran in activity wheels for 20 days prior to nociceptive tests were significantly ($p < 0.05$) lower than those of inactive animals.

METHOD

Subjects

Twenty adult female Long–Evans VAF rats (Charles River, Portage, MI), weighing between 130 and 145 g at the start of the experiment, were used. Housing, feeding, and handling procedures were identical to those in Experiment 1.

Running Conditions

For the first part of the experiment, 10 rats were housed in the running wheels and 10 in standard laboratory cages. Rats in the running condition were permitted free access to the running wheels for 17 days prior to the first nociceptive test.

During the second part of the experiment, rats that had been housed in the wheels were moved to standard hanging cages, and the previously inactive rats were moved to the running wheels. The rats were given free access to the running wheels for 17 days when the second nociceptive test ensued.

Nociceptive Testing

On both days of nociceptive testing, the doors to the running wheels were closed for the duration of the test. Nociceptive testing was conducted in the same manner as described in Experiment 1. However, because baseline tail-flick latencies were shorter in the females than in the males, 9 s rather than

10 s was used as the cutoff time for the light source on the tail-flick apparatus.

RESULTS

Food Intake and Body Weights

During part 1 of the experiment, active females consumed significantly, $t(1, 18) = 5.24$, $p < 0.001$, more food per day (21.4 ± 2.0 g) than inactive rats (17.0 ± 1.7 g). Although the active females ate more food, they weighed significantly, $t(1, 18) = 2.27$, $p < 0.05$, less (202 ± 18.5 g) than inactive rats (218 ± 10.1 g) on the first test day.

In part 2, mean daily food intake of active females (previously inactive) did not differ from that of inactive rats (previously active) (active = 20.6 ± 1.2 g; inactive = 20.1 ± 1.2 g).

Additionally, body weight did not differ between the two groups on the second test day (active = 241 ± 13.0 g; inactive = 252 ± 23.5 g).

Wheel Activity

Mean wheel turns (± SD) for active rats averaged 6391 ± 5307 a day in part 1. However, as observed in males, wheel turns increased over time, and during the 24 h preceding the first test averaged 11544 ± 9352.

During part 2, rats made an average of 7990 ± 7140 wheel turns per day; during the 24 h preceding the second nociceptive test, wheel turns averaged 10322 ± 5033.

As observed in males, there was substantial between subject variability in daily wheel turns for females. However, again, individual females were relatively consistent in the number of wheel turns made from day to day.

Nociceptive Responses

During part 1, baseline tail-flick latencies did not differ as a function of running condition (active rats = 2.29 ± 0.42 s; inactive rats = 2.56 ± 0.51 s). On the first test day, %MPEs increased as a function of drug dose, $F(5, 90) = 6.14$, $p < 0.005$. Additionally, at all except the 2.5 mg/kg dose, %MPEs of active rats were decreased relative to those of inactive animals, $F(1, 18) = 3.23$, $p < 0.09$.

Baseline tail-flick latencies also did not differ as a function of activity during part 2 of the study (active rats = 2.49 ± 0.51 s; inactive rats = 2.95 ± 0.60 s). On the second test day, nociceptive responses increased significantly as a function of drug dose, $F(5, 90) = 2.58$; $p < 0.05$. Across drug doses, %MPEs of active rats were significantly lower, $F(1, 18) = 30.36$, $p < 0.005$, than those of inactive rats.

Comparison between parts 1 and 2 of the experiment revealed significant interactions between running condition and phase of experiment, $F(1, 18) = 35.87$; $p < 0.001$, and among dose, running condition, and phase of experiment, $F(5, 90) = 2.52$, $p < 0.05$. Further analysis indicated that %MPEs for females that were inactive and then active were significantly lower in part 2 of the experiment than in part 1, $F(1, 9) = 15.00$, $p < 0.005$. In contrast, for females that were active and then nonactive, %MPEs were significantly greater during part 2 of the experiment than in part 1, $F(1, 9) = 21.06$, $p < 0.005$ (Fig. 2).

Pearson product-moment correlations between number of wheel turns made during the 24 h preceding and %MPEs for both parts of the experiment, with one exception (part 1: 15.0 mg/kg) were not significant (Table 2).

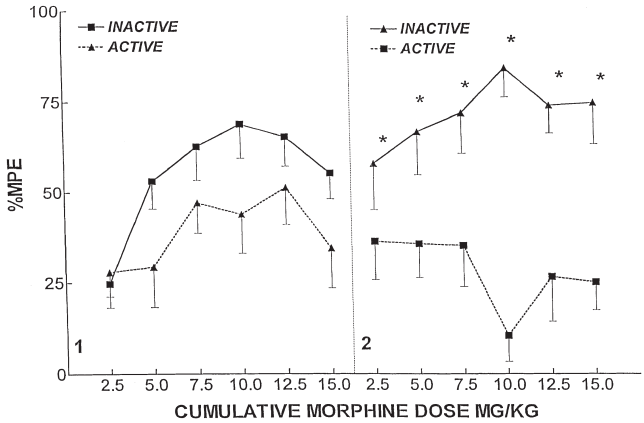

FIG. 2. Mean ± SEM % maximal possible effects (%MPEs) following cumulative morphine administration in active and inactive female rats. %MPEs of rats that ran in activity wheels for 17 days prior to nociceptive tests were lower than those of inactive females (left panel). Upon reversal of activity conditions, %MPEs of rats which were previously inactive and then were permitted to run for 17 days were significantly ($p < 0.005$) lower than those of previously active rats (right panel). Additionally, comparisons across nociceptive tests revealed that %MPEs of initially active females were significantly ($p < 0.005$) greater, and %MPEs of initially inactive females, significantly ($p < 0.005$) lower on the second test day.

EXPERIMENT 3

The results of Experiments 1 and 2 indicated that running for an extended period of time led to a reduction in morphine-induced analgesia. One question raised by these findings is how rapidly does running alter morphine-induced analgesia. To begin to answer this question, Experiment 3 examined pain sensitivity and morphine-induced analgesia in rats that ran for only 24 h preceding nociceptive testing.

METHOD

Subjects and Procedure

Eleven male Long–Evans rats weighing between 250 and 300 g at the beginning of the experiment were used. Rats were

TABLE 2

PEARSON PRODUCT-MOMENT CORRELATIONS BETWEEN NUMBER OF WHEEL TURNS AND %MAXIMAL POSSIBLE EFFECT ON A TAIL-FLICK TEST FOLLOWING MORPHINE ADMINISTRATION FOR FEMALE RATS

Dose of Morphine (mg/kg)	Part 1	Part 2
2.5	0.21 NS	−0.04 NS
5.0	0.15 NS	−0.16 NS
7.5	−0.13 NS	−0.27 NS
10.0	0.01 NS	−0.27 NS
12.5	0.08 NS	−0.51 NS
15.0	0.63 $p < 0.05$	0.27 NS

NS = not significant.

given ad lib access to ground Purina chow and water. To adapt animals to the laboratory, five rats were housed individually in standard cages, and six rats were housed in the running wheels with the door between the wheel and adjoining cage closed for 6 days. On the seventh day, the doors between the wheels and the cages were opened and active rats were allowed to run for 24 h. The doors to the wheels then were closed and nociceptive tests conducted as in the preceding experiments with the exception that morphine was administered in a manner to achieve cumulative doses of 0.625, 1.25, 2.5, 5.0, and 10.0 mg/kg. This regime was used to determine the effects of acute exercise on a wider range of morphine doses than had been used in the previous studies.

RESULTS

Food Intake, Body Weight, and Wheel Turns

No differences in food intake were observed between active and inactive rats during the 24 h preceding nociceptive testing (active rats = 22.6 ± 4.4 g; inactive rats = 23.0 ± 1.1 g). Although active rats weighed less (264 ± 11.7 g) than inactive rats (280 ± 13.7 g) at the time of nociceptive tests, this difference was not significant. Active rats made an average of 1617 ± 497.8 wheel turns in 24 h.

Nociceptive Responses

There was a trend, $t(9) = 2.19$, $p < 0.06$, for baseline tail-flick latencies to be shorter in active (3.3 ± 0.5 s) than in inactive rats (3.9 ± 0.3 s). %MPEs increased significantly, $F(4, 36) = 67.27$, $p < 0.001$, as a function of morphine dose. However, %MPEs did not differ as a function of activity condition (Fig. 3).

EXPERIMENT 4

In Experiment 1 and part 1 of Experiment 2, active rats weighed significantly less than inactive ones. Hence, it was not possible to distinguish between the effects of activity level and body weight on pain sensitivity and morphine-induced analgesia. Experiment 4 was conducted to determine whether differences in body weight alone were sufficient to induce changes in pain sensitivity and %MPEs following morphine administration.

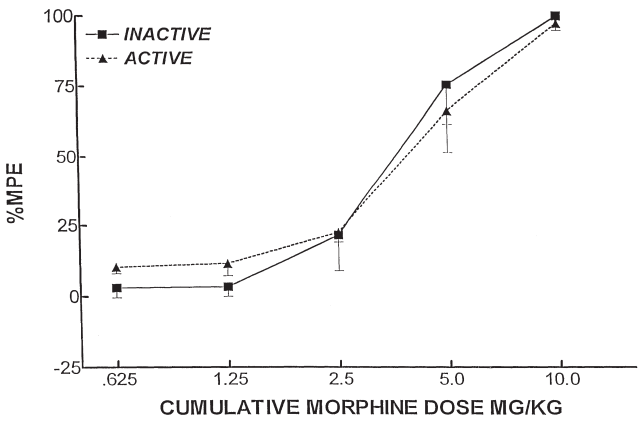

FIG. 3. Mean ± SEM % maximal possible effects (%MPEs) following cumulative morphine administration in active (▲) and inactive (■) male rats. %MPEs did not differ as a function of activity condition in rats that ran for only 24 h preceding nociceptive tests.

METHOD

Subjects and Feeding Conditions

Twenty-seven adult male Long–Evans rats (Charles River, Portage, MI) weighing between 225–250 g were individually housed in standard stainless steel hanging cages. Lighting and temperature conditions were identical to those of the prior experiments.

Nine rats were given ad lib access to Purina laboratory rodent chow #5001 and tap water at all times. The remaining animals were divided into two groups that were food restricted for 22 days until their mean body weights were either 85% ($n = 9$) or 77% ($n = 9$) of the mean free feeding body weight of the 100% group. Food and fluid intakes and body weights were measured daily.

At the termination of the experiment, animals were anesthetized with sodium pentobarbital (100 mg/kg) and epididymal fat pads were removed and weighed.

Nociceptive Testing

Pain thresholds were determined in an identical manner as in Experiment 1 and 2. Morphine was administered according to a cumulative dose procedure similar to that of the preceding experiments (2.5, 5.0, 7.5, 10.0, or 12.5 mg/kg).

Statistical Analysis

Prior to statistical analysis, antinocicpetive data were converted to %MPEs. Data from one animal were removed from the analysis because of missing data, and data from another animal were removed because its baseline tail-flick latency was two standard deviations from the mean. The data were then analyzed with two-way ANOVAs (feeding condition by dose) with dose as a repeated measure. Pearson product moment correlations were conducted between both body weights and fat pad weights of animals and %MPEs at each dose of morphine.

RESULTS

Body Weight and Epididymal Fat Pad Weights

Body weights at the time of nociceptive testing were 371.3 ± 21.9 g for the free-feeding group, 314.9 ± 9.0 g for the 85% group, and 287 ± 5.3 g for the 77% group, $F(2, 22) = 167.9$; $p < 0.001$. Restricting food intake was associated with significant reductions in both absolute epididymal fat pad weights, $F(2, 22) = 16.7$, $p < 0.001$, and fat pad weights per 100 g of body weight, $F(2, 22) = 7.0$, $p < 0.01$ (Table 3).

Nociceptive Responses

Baseline TF latencies did not vary as a function of body weight (100% group = 3.7 ± 1.2 s; 85% group = 3.4 ± 0.6 s; 77% group = 3.5 ± 0.5 s). %MPEs increased directly as a function of drug dose, $F(4, 88) = 10.77$, $p < 0.001$. However, %MPEs did not vary as a function of dietary condition, $F(2, 22) = 0.731$, NS. Additionally, the interaction between dose and dietary condition was not significant, $F(8, 88) = 1.92$, NS (Fig. 4).

Neither body weights nor fat pad weights were significantly correlated with %MPEs for any dose of morphine (Table 4).

TABLE 3

MEAN ± SD DATA FOR DAILY CALORIC INTAKE, FINAL BODY WEIGHT, AND ABSOLUTE AND RELATIVE EPIDIDYMAL FAT PAD WEIGHTS FOR RATS MAINTAINED AT 100, 85, OR 77% OF FREE-FEEDING BODY WEIGHT

% Free Feeding Weight	Caloric Intake (kcal)	Final Body Weight (g)	Absolute Fat Pads (g)	Relative Fat Pads (g/100 g b.wt)
100%	93.7 ± 5.02	371.3 ± 21.9a	3.1 ± 0.8a	0.9 ± 0.2a
85%	68.8 ± 1.4	314.9 ± 9.0b	2.4 ± 0.4b	0.8 ± 0.1ab
77%	63.7 ± 1.1	287.0 ± 5.3c	1.8 ± 0.3c	0.6 ± 0.1b

Numbers within a column not sharing a common letter are significantly ($p < 0.05$) different from each other.

TABLE 4

PEARSON PRODUCT-MOMENT CORRELATIONS BETWEEN BODY WEIGHTS (b. wt) AND ABSOLUTE AND RELATIVE EPIDIDYMAL FAT PAD WEIGHTS AND %MAXIMAL POSSIBLE EFFECT ON A TAIL-FLICK TEST FOLLOWING MORPHINE ADMINISTRATION

Morphine Dose (mg/kg)	b. wt. Correlations	Absolute Fat Pads Correlations	Fat Pads/100 g b. wt. Correlations
2.5 ($n = 25$)	0.01 NS	−0.08 NS	−0.15 NS
5.0 ($n = 25$)	−0.17 NS	−0.22 NS	−0.15 NS
7.5 ($n = 25$)	−0.01 NS	−0.03 NS	−0.05 NS
10.0 ($n = 25$)	0.06 NS	−0.15 NS	−0.21 NS
12.5 ($n = 25$)	0.26 NS	0.19 NS	0.17 NS

NS = not significant.

DISCUSSION

Both chronically active male and female rats were less sensitive to morphine's analgesic properties than inactive animals. For chronically active males, this difference was observed for all drug doses, and for females, on the initial test day, with all but the lowest dose of morphine. When activity conditions were reversed for females, on the second test day, nociceptive responses of presently active rats were significantly lower than those of inactive animals at all drug doses. In contrast to rats that ran for 17–20 days, rats that ran for only 24 h were not less sensitive to morphine-induced analgesia than inactive rats.

On the initial test day, body weights of both chronically active males and females were significantly lower than those of their inactive counterparts. Viewed alone, this finding suggests that differences in body weight contributed to the reduction in sensitivity to morphine's antinociceptive actions in active animals. However, the females in part 2 of Experiment 2 did not differ in body weight, and in Experiment 4, there were no differences in %MPEs as a function of body weight. Additionally, correlational analyses of data from Experiment 4 revealed no significant relations between %MPEs and either body weight or fat pad weights at the time of nociceptive testing. Thus, it can be concluded that the reduction in morphine-induced analgesia in active rats is not simply the result of decreased body weight.

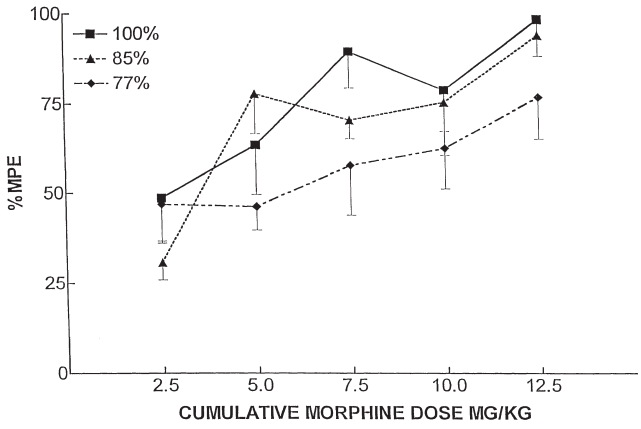

FIG. 4. Mean ± SEM % maximal possible effects (%MPEs) following cumulative morphine administration in male rats maintained at 100, 85, or 77% of free-feeding body weight.

Chronic activity decreased morphine-induced analgesia in both male and female rats. However, comparisons between Experiment 1, and part 1 of Experiment 2 suggest that under similar test conditions, male are more sensitive to morphine's antinociceptive properties than females. This finding is in agreement with recent work by Cicero and colleagues (6) demonstrating that male rats are more sensitive to the antinociceptive actions of morphine on a number of measures of pain mediated by both spinal and supraspinal mechanisms. Additionally, as serum levels of morphine did not vary in males and females in the study by Cicero and coworkers (6), it was hypothesized that gender differences in morphine-induced analgesia reflected enhanced central nervous system sensitivity to morphine in males compared to females rather than simply variations in blood opioid levels.

Alterations in both baseline pain sensitivity and morphine-induced analgesia have been observed across the estrous cycle in female rats (17,30,33). Thus, the data obtained in Experiment 2 could be confounded by the phase of the estrous cycle in which the female rats were tested. However, for several reasons this seems unlikely. First, all or the majority of the active rats would have to have been in one phase of the estrous cycle when tested for morphine-induced analgesia while the majority of inactive rats would have to have been in a different phase of the cycle. Also, although studies have shown variations in pain thresholds and morphine-induced analgesia as a function of the estrous cycle, the results of these studies have not been consistent. For example, Martinez-Gomez and colleagues (33) reported that tail-flick latencies were significantly shorter during estrus and metestrus than during diestrus or proestrus, while Frye and co-workers (17) found that tail-flick latencies were significantly shorter during proestrus than during metestrus. Finally, recent studies have shown that external factors, such as diet, override variations in pain sensitivity and morphine-induced analgesia across the estrous cycle (17,18). Future studies will investigate whether exercise also eliminates estrous-related alterations in pain thresholds and analgesic responses to opiate drugs.

Previous work has led to the proposal that exercise is associated with an increase in pain thresholds. For example, a number of researchers have demonstrated that pain tolerance is greater following than preceding exercise [e.g., (22,23,26,29, 36,40)]. The results of the present experiment, however, do

not support this proposal. In Experiment 1, baseline tail-flick latencies of active male rats were significantly shorter than those of inactive animals indicating a decrease in pain tolerance in active animals, while in Experiment 2 and 3, there were no differences in baseline tail-flick latencies. Several factors could explain the differences in the effects of exercise on pain thresholds between the present experiments and previous ones. First, the majority of prior studies on pain thresholds used human subjects (22,23,29,36) rather than rats. Thus, species differences could have contributed to the discrepancies between this and previous work. Also, previous work with human subjects suggests that the effects of exercise on pain thresholds vary as a function of 1) the type of pain being measured; 2) the time delay between exercise and the nociceptive test; and 3) the intensity of the exercise (26,29). For example, Janal and colleagues (26) found that 20 min after a long-distance run, trained male runners reported less pain on an ischemic pain test than preceding the run. In contrast, subjects tested 30 min after the run report more pain on the ischemic pain test than preceding the run (26). Additionally, in the same subjects, no differences in pain sensitivity were found as a function of exercise on a cold pressor test (26). With respect to intensity of exercise, two studies demonstrated that dental pain thresholds increase directly as a function of intensity of exercise on a bicycle ergometer (29,36). Finally, as mentioned in the introduction, it is possible that preexercise exposure to the pain test could play a role in postexercise responses (35). It is clear from these experiments with humans that the effects of exercise on pain thresholds are influenced by a number of factors. It is assumed that similar variables may play a role in mediating the interaction between exercise and pain thresholds in experimental animals.

In the one previous study on the effects of exercise on pain sensitivity that was done with rats, Shyu and co-workers (40) reported that animals exercising in running wheels displayed an increase in the "squeak" threshold to tail shocks relative to inactive rats. Moreover, in this experiment, the "squeak" threshold was directly related to the amount of running activity, and declined following 6 h of inactivity (40). These findings again do not agree with those of the present studies. Differences in the type of pain stimulus or the degree of activity could explain these discrepancies. Earlier studies have suggested that plasma β-endorphin levels increase directly with the intensity of exercise (39). It is possible that the amount of exercise performed by rats in the present experiments was insufficient to raise plasma β-endorphin levels enough to increase pain tolerance. However, previous studies examining the effects of running reported increases in plasma β-endorphin levels when rats made approximately 4,400 wheel turns in the preceding 24 h (1). This number of wheel turns is considerably less than that observed in either Experiment 1 or 2. Thus, although no definite conclusions can be drawn without direct measurements of plasma β-endorphin levels, it appears that the level of running in the present experiments was sufficient to raise plasma β-endorphin levels.

Direct comparisons revealed that levels of running were greater in Experiments 1 and 2, averaging between 9–11 kilometers/24 h on the night preceding nociceptive tests, than in the study by Shyu et al. (40) in which rats ran an average of 7 kilometers/24 h. It should be noted with respect to the effects of intensity of exercise on pain sensitivity, in the present experiments, substantial variability was observed between animals in the number of wheel turns made per day. For example, in Experiments 1 and 2, there were rats who made approximately 1000 wheel turns a day, and others that made more than 10,000 wheel turns a day. To determine if daily number of wheel turns influences baseline tail-flick latencies and/or morphine-induced analgesia, correlation coefficients were calculated between these variables. In contrast to the results of the study by Shyu, no significant correlations between daily wheel turns and baseline tail-flick latencies or %MPEs following morphine administration were found in either Experiment 1 or 2. These results suggest that in this situation, intensity of exercise is not directly related to pain thresholds or morphine-induced analgesia.

Although intensity of exercise was not directly related to morphine-induced analgesia in the present experiments, duration of activity did influence animal's responses to morphine's antinociceptive properties. In contrast to the diminished sensitivity to morphine-induced analgesia observed in rats that ran for an extended period of time, morphine-induced analgesia was not altered in rats that ran for only 24 h when compared to their inactive counterparts. In another experiment that specifically assessed the effect of duration of activity on morphine-induced analgesia, similar results were obtained. No differences in morphine-induced analgesia were observed between inactive rats and rats allowed to run for either 24 h or 7 days preceding nociceptive tests. However, following 14 days of access to the running wheels, active rats were less sensitive to morphine's analgesic actions than inactive animals (Kanarek D'Anci, and Gerstein, unpublished findings). As discussed below, chronic running could lead to the release of β-endorphin and subsequent crosstolerance to morphine. It may be that for crosstolerance to occur, receptors must be exposed to the endogenous ligand for an extended period of time.

In Experiment 2, antinociceptive responses of female rats that were initially inactive and then allowed to run were significantly lower during the second nociceptive test than during the first. Although this reduction in antinociceptive responses may be in part due to an increase in activity prior to the second test, it also may be simply the result of the development of tolerance to morphine's analgesic properties. Tolerance to morphine-induced analgesia develops rapidly and persists for long periods of time (7,10,25,43,44). One way to test if activity accentuates the development of tolerance to morphine's analgesic actions would be to compare morphine-induced analgesia between rats that remained inactive during two nociceptive tests and rats that were initially inactive and then allowed to run. If morphine-induced analgesia decreased to a greater degree in the latter group, it could be concluded that activity contributes to the development of tolerance in rats.

In contrast to antinociceptive responses of rats that were inactive and then allowed to run, %MPEs of rats that were housed in activity wheels prior to the first nociceptive test and in standard laboratory cages prior to the second test, were significantly greater during the second than the first test. These results suggest that cessation of activity leads to sensitization to morphine's antinociceptive actions.

From the results of the present experiments it could be hypothesized that chronic exercise stimulates the release of β-endorphin, which subsequently results in crosstolerance to morphine. In support of the first part of this hypothesis, as previously discussed, running-wheel activity leads to increases in β-endorphin levels in rats (1). With respect to the second part, a number of researchers have provided evidence of crosstolerance between endogenous and exogenous opioids [e.g., (2,5)]. For example, Christie and colleagues (5) found diminished responsiveness to morphine-induced analgesia in mice that had been exposed to a chronic schedule of warm water swimming. These researchers proposed that chronic

swim stress exposed opiate receptors to their endogenous ligands in manner similar to chronic administration of exogenous opioids. They further indicate that this exposure resulted in crosstolerance to morphine (5).

The present results have both theoretical and practical implications. On a theoretical level, they provide further evidence that environmental factors play an important role in mediating the actions of opioid drugs. Previous work has shown that a number of factors including housing conditions, diet, and stress alter the behavioral consequences of opioid agents (3,10,18,20,27,28,34). The present experiments add exercise to the list of variables that moderate the effects of opioid drugs. On a practical level, these results call into the question the idea that prolonged exercise has analgesic properties.

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