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Chronic running wheel activity attenuates the antinociceptive actions of morphine and morphine-6-glucouronide administration into the periaqueductal gray in rats

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Abstract

Chronic exercise in a running wheel increases baseline pain sensitivity while attenuating the antinociceptive effects of peripherally administered opiate agonists in laboratory rodents. To determine if these effects are due to exercise-induced changes in the central nervous system (CNS) or an artifact of exercise-induced alterations in peripheral physiology, the present study evaluated the antinociceptive actions of centrally administered opiate agonists in active and inactive female rats. Rats were implanted with cannula into the right periaqueductal gray (PAG) area of the midbrain. After the completion of the surgery, the animals were allowed ad libitum access to running wheels or housed in standard cages for three weeks. Pain sensitivity was measured on the tail flick test before and immediately following microinjections of either morphine (0, 2.5, 5.0, 10.0, 20.0 μg/rat) or the more potent morphine metabolite, morphine-6-glucuronide (M6G) (0, 0.03, 0.1, 0.3, 1.0 μg/rat). Baseline tail flick latencies were significantly shorter in active than in inactive rats. Additionally, active animals were less sensitive to the antinociceptive effects of morphine and M6G than inactive rats. These findings provide evidence for the involvement of the CNS in exercise-mediated alterations in pain sensitivity and opiate drug actions.

Keywords: Periaqueductal gray; Opioids; Morphine; Morphine-6-gluronide; Exercise; Wheel running; Antinociception; Pain

1. Introduction

In rodents, chronic running wheel activity increases baseline pain sensitivity while decreasing the antinociceptive potency of opiate drugs. It is hypothesized that these effects are due to exercise-induced alterations in the endogenous opioid system. More specifically, it is proposed that chronic running wheel activity stimulates the endogenous opioid system leading to the development of opioid-induced hyperalgesia and cross-tolerance between endogenous opioids released during exercise and exogenously administered opioid analgesics (Kanarek et al., 1998; D'Anci et al., 2000; Mathes and Kanarek, 2001; Smith and Yancey, 2003).

It is possible, however, that exercise reduces the pain relieving actions of opioid drugs by altering peripheral physiology. In both humans and laboratory animals, exercise leads to an increase in lean body mass and a decrease in fat deposition, as well as modifications in cardiovascular functioning and metabolism. These exercise-induced physiological adaptations could in turn modify the behavioral effects of opiate drugs. For instance, exercise typically decreases the amount of neutral free fat within the blood, which could lead to a reduction in the storage of fat soluble drugs, such as morphine. In addition, exercise may increase the concentration of plasma proteins that typically bind with drugs. If binding is increased by exercise, drugs would be less available to be distributed to tissue in active than inactive rats. Hence, exercise-induced alterations in plasma proteins and neutral free fats could result in decreased drug efficacy (van Baak, 1990). Furthermore, changes in blood flow and metabolism that occur with exercise can alter the clearance rate of drugs after systemic administration. For instance, if an injection is given in a site where blood flow is increased by exercise, then absorption of the drug will be faster in active than inactive individuals. Moreover, exercise-induced increases in blood flow to the liver can increase the rate of drug metabolism,

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which could make less morphine available to bind to nerve tissue to cause a behavioral effect (van Baak, 1990).

One method to circumvent the potential peripheral physiological effects of exercise on opioid-induced antinociception is to administer opioid agonists directly into the central nervous system. Thus, in the present experiments, the antinociceptive effects of morphine and its more potent metabolite, morphine-6-glucuronide (M6G), after direct injection into the periaqueductal gray (PAG) area of the midbrain central nervous system were compared in active and inactive animals. The PAG contains high concentrations of endogenous opioid peptides and receptors, and is a primary locus for opioid-induced antinociception within the CNS. Injections of mu opioid agonists into the PAG increases antinociceptive responses in laboratory rodents, while inactivation of this area of the brain reduces the pain relieving properties of peripherally and centrally administered opioid analgesics (Watkins and Mayer, 1982; Jensen and Yaksh, 1986; Gutstein et al., 1998; Helmstetter et al., 1998; Krzanowska and Bodnar, 1999; Tershner et al., 2000; Kanarek et al., 2001; Oliveira and Prado, 2001; Almeida et al., 2004; Fields, 2004). If running wheel activity increases the activity of the endogenous opioid system, and thus, leads to cross-tolerance between endogenous and exogenous opioids, then direct injection of morphine and M6G into the PAG should result in a lower antinociceptive response in active compared with inactive animals.

Previous research indicates that pain sensitivity and responses to opioid analgesics vary across the estrous cycle in laboratory rodents. However, the results of studies assessing the effects of variations in gonadal hormones in modulating responsiveness to pain and opioid drugs are inconsistent. For example, while results of some experiments indicate that female rats are more sensitive to pain during estrus and least sensitive during proestrus, results of other studies have not confirmed these findings (Frye et al., 1993; Martinez-Gomez et al., 1994; Mogil et al., 2000; Stoffel et al., 2003; Craft, 2003; Shekunova and Bespalov, 2004). The inconsistencies in experimental findings most probably reflect differences among studies with respect to the type of antinociceptive test used, the strain of rodent, and age of the animal. Additionally, in studies examining the effects of opioid analgesics on pain sensitivity, both the dose and efficacy of the drug may interact with hormonal status to influence results (e.g. Mogil et al., 2000; Tershner et al., 2000; Craft, 2003; Stoffel et al., 2003). To determine if stages of the estrous cycle interacted with the effects of running on pain sensitivity and opioid-induced antinociception, estrous cycles were monitored in the present experiments.

2. Materials and methods

2.1. Animals and housing

Adult female Long–Evans rats (Charles River Laboratories) weighing 200–250 g at the beginning of the experiments, were separated into two groups. One group of rats (active) was housed individually in Wahmann running wheels (circumference of the wheel = 1.13 m) with stainless steel side cages. Wheel turns were monitored by a microswitch such that only complete 360° turns were recorded. Wheel turns were measured over each 24-h period. The remaining rats (inactive) were housed individually in standard stainless steel hanging laboratory cages. Inactive and active rats were maintained in the same temperature-controlled vivarium (21 ± 1 °C) with a 12–12 h reverse light–dark cycle (lights on: 2000–0800 h). All animals had ad libitum access to water and ground Purina chow.

2.2. Cannula implantation

Animals were anesthetized with 100 mg/kg ketamine and 6 mg/kg xylazine, (IP). A stainless steel outer guide cannula (22 gauge, Plastics One, Roanoke, VA) was implanted unilaterally into the right periaqueductal gray according to the atlas of Paxinos and Watson (coordinates AP −5.8; DV −4.7; ML −0.8). The cannula was secured into place with three skull screws partially imbedded in the bone, and fixed with dental acrylic. A 30 gauge dummy cannula was placed in each cannula to keep it patent. Rats were injected with antibiotics (Cephazolin 20 mg/kg, IM, Henry Schein, Melville, NY) immediately following and for three days after surgery.

2.3. Drugs

Morphine sulfate (National Institute on Drug Abuse, Bethesda, MD) and morphine-6-glucuronide (National Institute on Drug Abuse) were dissolved in 0.9% sterile saline and administered in a volume of 2 μl/rat.

2.4. Microinjections and antinociceptive testing

Following surgery, rats were allowed three weeks to adapt to the experimental housing conditions before the initiation of antinociceptive testing. The time between surgery and testing was based on previous research, which had demonstrated that a minimum of three weeks is required to observed activity-induced alterations in antinociceptive responses following peripheral administration of opioid analgesics (Kanarek et al., 1998).

Immediately prior to testing, running wheels were closed and remained closed for the duration of the testing period. One group of active and inactive rats received morphine sulfate (0.0, 2.5, 5.0, 10.0, 20.0 μg/2μl/rat) using an injection cannula (28 gauge, Plastics One) attached to a 25 μl gas tight Hamilton syringe via silastic tubing. Administration was performed using a syringe pump (Harvard Apparatus, Natick, MA) at a rate of 1 ul/min. The injection cannula remained in place for one minute after the termination of the drug injection. A separate group of drug-naive active and inactive rats received M6G (0.0, 0.03, 0.1, 0.3, 1.0 μg/2μl/rat) using the same procedures as those described for morphine.

Pain sensitivity was measured using the radiant heat tail flick test (D'Amour and Smith, 1941; Tjolsen and Hole, 1998; Hogan, 2002). Testing was initiated 5 h after the onset of the dark cycle. All rats were gently held with a clean cloth by the same experimenter. Animals were placed on the tail flick

apparatus (Endie Instrument Co., Montpelier, VT) with their tails gently smoothed into a groove containing a photocell. A light source was then activated and focused on the rat's tail. The light automatically turned off either when the animal moved its tail or 9 s had elapsed. The cut off time of 9 s was chosen to prevent tissue damage to the animal's tail. Baseline latencies were defined as the median of 3 tail flicks, each separated by approximately 15 s. Each determination of tail flick latency was performed on a different portion of the tail. Tail flick latencies were determined to the nearest 0.1 s.

Antinociceptive responses were measured using a single tail flick determination 10, 20, 40, and 60 min following drug administration. Within each drug condition, active and inactive rats were given each of the five doses of morphine or M6G in a counterbalanced order. Drugs were administered such that each dose was represented on each test day. At least two days intervened between test days.

2.5. Determination of estrous cycle

To determine the day of the estrous cycle, samples of vaginal epithelium were collected from all rats by lavage at 10:00 am each day during the two weeks preceding nociceptive testing. Vaginal epithelial cells, stained with Cresyl violet, were examined using low-power light microscopy, and classified as being characteristic of diestrus, estrus, metestrus, or proestrus on the basis of the relative proportion of cornified, nucleated, and leukocytic cells.

2.6. Histological analysis of cannula placement

Upon completion of the experiment, rats were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine (IP) and perfused transcardially with heparinized saline followed by 4% paraformaldehyde. Brains were extracted, sliced into 40 μm sections using a freezing microtome and examined under light microscopy for cannula placement.

All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Tufts University Institutional Animal Care and Use Committee.

2.7. Data analyses

Antinociceptive responses were expressed as percent maximal possible effect (%MPE) as defined by Dewey and Harris (1975), which was calculated as follows:

$$\%\mathrm{MPE} = [(\text{ test latency} - \text{baseline latency})/ (\text{ maximal latency} - \text{baseline latency})]*100$$

On the basis of the results of previous experiments (Kanarek et al., 1998; D'Anci et al., 2000; Mathes and Kanarek, 2001), it was predicted that active rats would be less sensitive to the pain relieving actions of morphine and M6G than inactive rats. To test this prediction, main effects of group and dose within each experiment were determined by repeated measures ANOVA using SPSS (SPSS Inc., Chicago, IL). Multiple post hoc *t*-tests were performed where appropriate. Group differences in baseline tail flick latencies were analyzed using Student's *t*-tests.

3. Results

3.1. Cannula placement

Histological examination showed that cannulae were placed in the lateral PAG but not within the dorsal or ventral tracts.

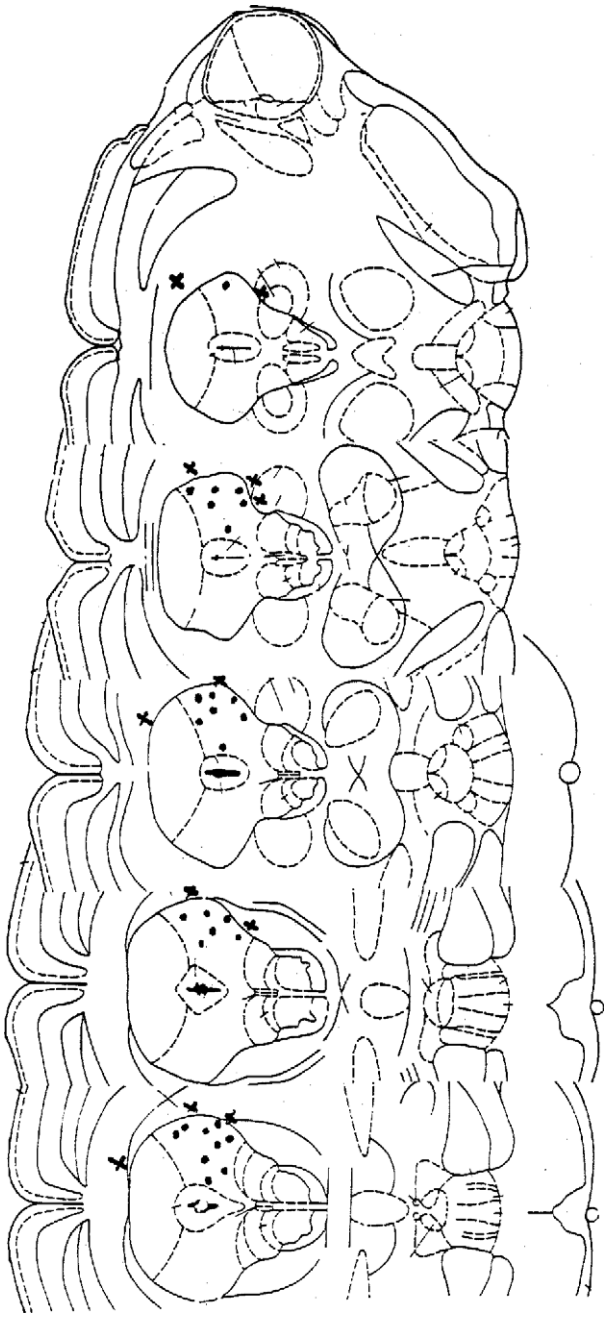

Fig. 1. Cannula placements within the PAG. Figures depict sections 5.8–6.8 mm caudal to bregma according to the atlas of Paxinos and Watson (1986). Enclosed dots represent correct placements, X represents incorrect placements, and one animal's cannula was in the aqueduct depicted by the white circle in the aqueduct in the diagram to the far left.

Animals whose cannulae were not within the PAG were eliminated from data analyses. (Fig. 1).

3.2. Morphine antinociception

Following histological confirmation of cannula placement, 16 rats were used in the analyses evaluating the effect of exercise on morphine-induced antinociception ($n=8$). Active rats tested for morphine antinociception averaged 5253±1236 wheel rotations per day over the last two weeks of the experiment. Although there were no differences in initial body weights of rats housed in activity wheels or standard cages, at the time of testing for morphine-induced antinociception, active rats weighed less than inactive rats (active=249±10.0 g; inactive rats=271.3±7.1 g).

No differences in baseline tail flick latencies were observed across nociceptive tests. However, baseline tail flick latencies were significantly lower in active rats compared with inactive rats. When collapsed across test days, mean baseline latencies were 2.42±0.57 and 3.09±0.60 s for active and inactive animals, respectively ($t=5.67$, $p<0.001$).

Antinociceptive responding, expressed as %MPE, increased directly as a function of the dose of morphine ($F_{3,42}=12.64$, $p<0.01$) (Fig. 2-A). %MPEs did not differ between active and inactive animals when analyzed across all drug doses and time periods using repeated measures ANOVA ($F_{1,14}=1.41$, $p=0.25$). However, at the highest dose of morphine, active rats displayed less antinociceptive behavior than inactive rats at all time points. Due to a high degree of variability among the animals, this statistic failed to reach significance ($F_{1,13}=3.43$, $p=0.087$).

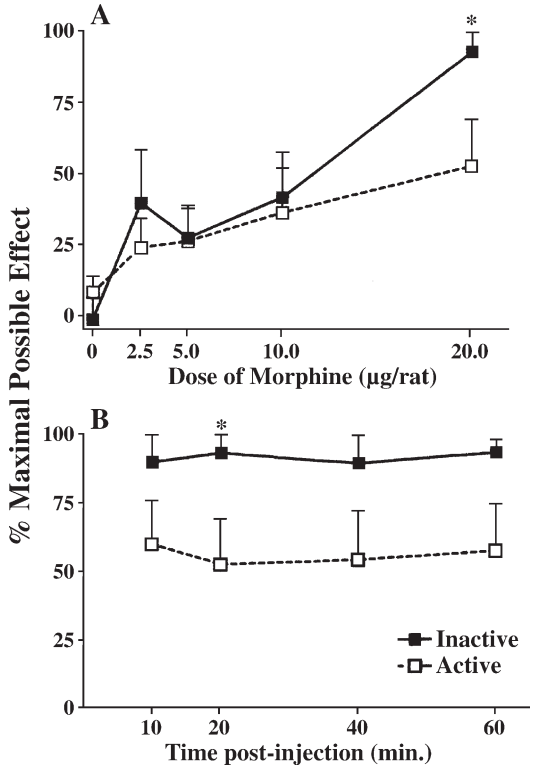

Fig. 2. A. Antinociceptive responses 20 min following intra-PAG administration of varying doses of morphine in active ($n=8$) and inactive rats ($n=8$). %MPEs were significantly lower in active rats than in inactive rats following the administration of 20.0 µg morphine. B. Time course of morphine-induced antinociception following the administration of 20 µg morphine. Across time, active rats ($n=7$) were less sensitive to the antinociceptive effects of morphine than inactive rats ($n=6$) ($F_{1,13}=3.43$, $p=0.087$). *$p<0.05$.

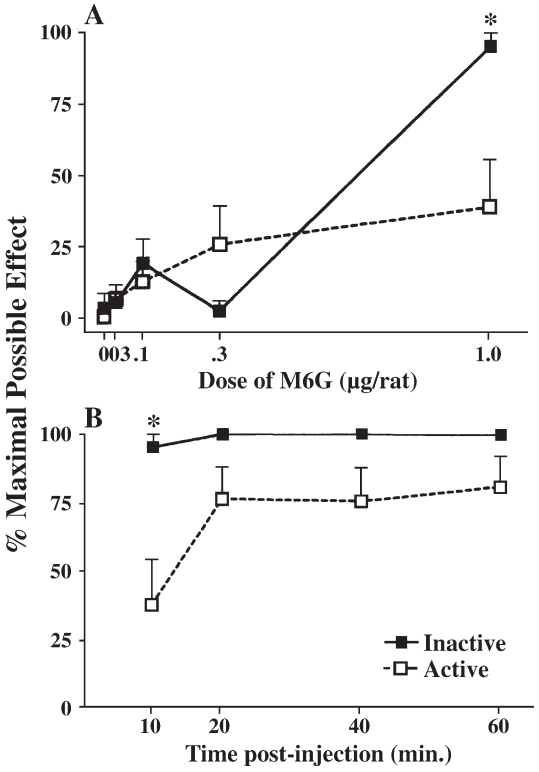

Fig. 3. A. Antinociceptive responses 10 min following intra-PAG administration of varying doses of M6G in active ($n=7$) and inactive rats ($n=6$). %MPEs were significantly lower in active rats than in inactive rats following the administration of 1.0 µg M6G. B. Time course of M6G-induced antinociception following the administration of 1.0 µg morphine. Across time, active rats ($n=7$) were significantly less sensitive to the antinociceptive effects of M6G than inactive rats ($n=6$) ($F_{1,11}=9.86$, $p<0.01$). *$p<0.05$.

3.3. Morphine-6-glucuronide antinociception

Following histological confirmation of cannula placement, 13 rats were used in the analyses evaluating the effect of exercise on M6G-induced antinociception ($n=7$, $n=6$ for active and inactive rats, respectively). Active rats tested for M6G antinociception were averaging 7330±424 wheel rotations per day over the last two weeks of the experiment. While there were no differences in initial body weights, after three weeks of housing, active rats weighed significantly less than inactive rats (active rats=218±2.5 g; inactive rats=239±5.8 g; $t=3.59$, $p<0.01$).

No differences in baseline tail flick latencies were observed across nociceptive tests. Baseline tail flick latencies preceding M6G injections were significantly lower in active animals compared with inactive controls. Mean baseline latencies were 2.19±0.44 and 2.48±0.54 s for active and inactive rats, respectively ($t=2.95$, $p<0.01$).

%MPEs in both active and inactive rats increased as a function of the dose of M6G ($F_{1,17}=49.45$, $p<0.01$) (Fig. 3-A). When analyzed across all doses and time points using repeated measures ANOVA, antinociceptive responses of active and inactive animals did not differ ($F_{1,11}=0.63$, $p=0.44$). However, active rats were significantly less sensitive to the antinociceptive effects of 1.0 μg M6G than inactive rats ($F_{1,11}=9.86$, $p<0.01$). Post hoc *t*-tests revealed that antinociceptive responses in active rats were significantly lower than those in inactive animals at 10 min post injection ($t=3.27$, $p<0.05$) and remained lower for the duration of the test period (Fig. 3-B).

3.4. Estrous cycle

Analysis of vaginal smears revealed that there were no differences in length of the estrous cycle between active and inactive rats preceding injections of either morphine or M6G. In both the inactive and active conditions rats displayed estrous cycles of 4 to 5 days. As a result of the relatively small number of rats in each stage of the estrous cycle on each test day, it was not possible to statistically determine if there were differences in pain sensitivity or responsiveness to the opioid analgesics as a function of stage of the estrous cycle at which they were tested. However, when data were examined across test days, no differences in pain sensitivity or opioid-induced analgesia were noted as a function of stage of the estrous cycle.

4. Discussion

Chronic exercise increased baseline pain sensitivity, while decreasing the antinociceptive effects of centrally administered opiate drugs. These findings extend and corroborate previous studies, which demonstrated that chronic exercise increases pain sensitivity (i.e. produces hyperalgesia) while attenuating the antinociceptive effects of peripherally administered opioid drugs (i.e. produces tolerance) (Kanarek et al., 1998; D'Anci et al., 2000; Mathes and Kanarek, 2001; Smith and Yancey, 2003; Smith et al., 2004).

The presence of increased pain sensitivity in active rats can be compared to the hyperalgesia reported after administration of morphine and other opioid analgesics. Opioid-induced hyperalgesia is revealed in laboratory animals who have been rendered tolerant to opioids either through repeated injections or chronic drug administration, and display increased sensitivity to pain when tested in the absence of the opioid (Vanderah et al., 2000, 2001; Celerier et al., 2001; Crain and Shen, 2001; Mao, 2002; Mao et al., 2002; Ossipov et al., 2003). It has been suggested that increased activation within the dynorphin pathway contributes to hyperalgesia in morphine tolerant animals as well as neuropathic pain and chronic pain states in humans and laboratory animals (Vanderah et al., 2000, 2001; Mao, 2002; Mao et al., 2002; Ossipov et al., 2003).

Exercise can also increase dynorphin expression (Aravich et al., 1993; Werme et al., 2000) suggesting that exercise-induced activation of the descending dynorphin pronociceptive pain pathway may contribute to hyperalgesia in active animals. The concordance between exercise- and opioid-induced increases in pain sensitivity indicate that alterations in pain sensitivity in active rats may be a function of exercise-induced release of endogenous opioid peptides, which then produce neuroplastic changes similar to those resulting from the administration of exogenous opioids.

Although baseline tail flick latencies were significantly lower in active than inactive rats preceding injections of either morphine or M6G, baseline latencies, for both active and inactive rats, were longer in those given morphine than those given M6G. Several reasons are offered to explain the differences in baseline latencies between animals in the two drug conditions. Rats were tested at different times for morphine- and M6G-induced antinociception. Thus, the rats in the two drug conditions came from different litters, and may have been subjected to different handling and shipping conditions prior to arrival in the laboratory. Additionally, at the time of surgery, rats given M6G weighed less than those given morphine. This weight difference may also have contributed to the variation in baseline tail flick latencies between rats given morphine or M6G. Despite the differences in baseline tail flick latencies between rats subsequently given morphine or M6G, as in other studies (Kanarek et al., 1998; D'Anci et al., 2000; Mathes and Kanarek, 2001), active rats in each drug condition were more sensitive to pain than inactive rats indicating that the effects of exercise on pain sensitivity override differences in baselines due to age, body weight, or handling conditions.

With respect to exercise-induced alterations in drug-mediated antinociceptive responses, exercise decreased the pain relieving properties of both morphine and its metabolite, M6G. However, as previously reported, M6G was a more potent analgesic than morphine (Abbott and Palmour, 1988; Paul et al., 1989; Easterling and Holtzman, 1998). For example, 20 μg of morphine was needed to produce similar antinociceptive responses to 1 μg of M6G. Despite this difference in potency, exercise had a similar effect on the behavioral consequences of the two drugs. This finding supports the idea that exercise-induced alterations in drug action are not merely due to changes in drug metabolism as M6G is not altered further by metabolic processes (Abbott and Palmour, 1988; Paul et al., 1989; Easterling and Holtzman, 1998).

With systemic drug administration, it is possible that running wheel activity decreased the efficacy of opioid drugs by altering drug absorption, distribution to tissues, and/or drug elimination (van Baak, 1990). By administering psychoactive drugs directly onto the neuronal cells with which they interact, central administration eliminates the possibility that alterations in peripheral metabolism are of importance in determining the effects of exercise on the pain relieving properties of opioid analgesics. Thus, the present results demonstrate that the effects of exercise on opioid-induced antinociception cannot primarily be attributed to pharmacokinetic variables introduced through exercise. The fact that active animals are less sensitive to the antinociceptive properties of morphine and M6G after both central and peripheral administration indicates that running wheel activity changes not only the peripheral physiology of animals, but also modifies activity within the central nervous system.

Following peripheral drug administration, active animals not only display a decrease in opioid-induced antinociception, but also recover more rapidly from the antinociceptive effects of morphine and M6G than inactive animals (Mathes and Kanarek, 2001). The results of the present experiment showed that the antinociceptive effects of centrally administered morphine and M6G did not decline over the 60 min testing period in either active or inactive animals. These findings suggest that the decline in antinociception following peripheral drug administration may be the result of exercise-induced changes in the peripheral physiology of the active animals. Thus, the duration of drug action may be the result of exercise induced changes in drug pharmacokinetics but these changes cannot account for exercise-induced changes in drug efficacy.

The present experiments were carried out using female rats. Estrous cycles were monitored daily but the results of vaginal cytology did not reveal any differences in estrous cycle duration between active and inactive animals. There was no evidence of phase synchrony as each phase of the estrous cycle was identified on any given day. Although ovarian hormones have been identified as contributing to alterations in pain sensitivity and morphine-induced antinociception (Frye et al., 1992, 1993; Cruz et al., 1996; Bartok and Craft, 1997; Fillingim and Ness, 2000; Mogil et al., 2000), it is unlikely that the differences in pain sensitivity between active and inactive animals can be attributed to ovarian hormones since there were no differences in estrous cycles between the two groups.

The results of the present experiments support the hypothesis that exercise-induced alterations in pain sensitivity and responsiveness to opioid drugs are consequences of increases in the activity of the endogenous opioid system. Further evidence for this hypothesis comes from studies demonstrating that exercise results in elevations in β-endorphin and dynorphin levels in both rodents and humans (e.g. Christie and Chesher, 1982; Janal et al., 1984; Schwarz and Kindermann, 1992; Aravich et al., 1993; Angelopoulos, 2001; Asahina et al., 2003). In addition to altering the pain relieving actions of opioid analgesics, exercise-induced increases in endogenous opioid levels may lead to physical dependence similar to that observed following chronic administration of opioid drugs. In support of this possibility, it has been reported that when chronically exercising rats are prohibited from running (Hoffmann et al., 1987) or are injected with an opioid antagonist (Smith and Yancey, 2003) they display behaviors similar to those observed during opioid withdrawal (e.g wet dog shakes; diarrhea; abnormal postures; and increased aggression).

Wheel running behavior, like opioid drugs, can serve as a potent reinforcer. Rats will perform operant responses to obtain access to a wheel (Collier and Hirsch, 1971; Sherwin, 1998; Belke, 2004). Moreover, running wheel behavior can lead to the development of a conditioned place preference for the location of the wheel. This preference can be blocked by the administration of naloxone. Additionally, cross-tolerance develops between the rewarding effects of morphine and wheel running behavior (Lett et al., 2000, 2001, 2002). Additional evidence that exercise and opioids may be acting on the same neural substrates to produce their rewarding effects comes from research demonstrating that exercise can significantly reduce opioid self-administration in laboratory rodents (McLachlan et al., 1994).

In conclusion, the results of the present experiment in conjunction with studies demonstrating 1) exercise-induced increases in endogenous opioid peptides; and 2) similarities in the rewarding effects of opioid drugs and excessive exercise lend support for the hypothesis that wheel running alters the activity of the endogenous opioid system. If exercise works on the same neurochemical systems as opioid drugs, then modifying activity levels could have significant consequences for mediating the pain relieving or rewarding properties of these drugs.

Acknowledgements

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