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Chronic exercise decreases sensitivity to mu opioids in female rats: Correlation with exercise output

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Abstract

Aerobic exercise stimulates the release of endogenous opioid peptides and increases nociceptive (i.e., pain) threshold in a naloxone-reversible manner. During chronic exercise, sensitivity to the antinociceptive effects of morphine and other mu opioids decreases, leading some investigators to propose that exercise may lead to the development of cross-tolerance to exogenously administered opioid agonists. The purpose of the present study was to examine the effects of chronic exercise on sensitivity to mu opioids, and to determine if changes in opioid sensitivity during chronic exercise are correlated with exercise output. Eight female rats were obtained at weaning and housed in standard laboratory cages that did not permit any exercise beyond normal cage ambulation. Following 6 weeks under these conditions, opioids possessing a range of relative efficacies at the mu receptor (morphine, levorphanol, buprenorphine, butorphanol) were examined in a warm-water, tail-withdrawal procedure. Under sedentary conditions, all opioids produced dose-dependent increases in tail-withdrawal latencies, and high levels of antinociception were observed for all drugs. Following these tests, rats were reassigned to exercise conditions and transferred to cages equipped with running wheels. Under these conditions, rats ran an average of 7154 rev/day (7869 m/day), with a range across rats from 4501 to 10,164 rev/day (4951–11,180 m/day). Sensitivity to all four opioids decreased significantly during the exercise period, resulting in 2- to 5-fold decreases in the potency of morphine, levorphanol and buprenorphine, and decreases in the effectiveness of buprenorphine and butorphanol. When rats were returned to sedentary conditions, sensitivity to all four opioids increased significantly and returned to that observed prior to the exercise period. For all drugs, there was a positive correlation between exercise output and changes in opioid sensitivity between sedentary and exercise conditions. These data suggest that chronic exercise decreases sensitivity to mu opioids in female rats, and that these changes in sensitivity are positively correlated with exercise output.

Keywords: Antinociception; Cross-tolerance; Exercise; Female; Mu opioid; Running wheel; Rat

Acute exercise stimulates the release of endogenous opioid peptides and increases nociceptive (i.e., pain) threshold in a naloxone-reversible manner (Carmody and Cooper, 1987; Shyu et al., 1982; Tierney et al., 1991). Chronic exercise leads to the sustained release of these peptides and subsequent decreases in sensitivity to morphine and other mu opioid agonists (Kanarek et al., 1998; Mathes and Kanarek, 2001; Smith and Yancey, 2003). These findings have prompted some investigators to propose that chronic exercise may lead to the development of cross-tolerance between endogenous opioid peptides released during exercise and exogenously administered mu opioid agonists. In support of this hypothesis, Houghten et al. (1986) reported that rats with free access to running wheels had fewer beta-endorphin binding sites than sedentary rats, which was presumed to reflect a compensatory down-regulation of opioid receptors during exercise. Such reductions in the number of opioid receptors have also been reported during chronic treatment with exogenous opioids (Chen et al., 1997; Diaz et al., 1995; Malatynska et al., 1996, but see Brady et al., 1989; Yoburn et al., 1993), and would account for the decreased sensitivity to morphine and other mu opioid agonists in exercising subjects.

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Studies have shown that the intensity of aerobic exercise is positively correlated with the release of beta-endorphin and other opioid peptides (Goldfarb et al., 1990; Mehl et al., 2000; Mougin et al., 1988). Thus, increasing exercise output may be akin to increasing the maintenance dose of an opioid agonist during chronic treatment. This is a potentially significant issue, given that higher maintenance doses of morphine and other mu opioids produce greater reductions in the number and sensitivity of opioid receptors (Baumhaker et al., 1994; Belcheva et al., 1993; Law and Bergsbaken, 1995), and greater degrees of tolerance and cross-tolerance (Adams and Holtzman, 1990; Smith and Picker, 1998; Yoburn et al., 1993) than lower maintenance doses. Similarly, higher levels of exercise output may produce greater compensatory responses within the opioid receptor system, and greater degrees of cross-tolerance to exogenously administered opioid agonists.

The purpose of the present study was to examine sensitivity to the antinociceptive effects of mu opioids in female rats with free access to running wheels, and to determine whether exercise output is correlated with changes in opioid sensitivity in individual subjects. Most studies examining the effects of exercise on sensitivity to morphine and other mu opioids have used a between-subjects design, comparing subjects with free access to running wheels to sedentary control subjects (e.g., Lett et al., 2002; Mathes and Kanarek, 2001; Smith and Yancey, 2003). Only a few studies have employed a within-subjects design, and those studies have typically employed only one housing reversal (e.g., Kanarek et al., 1998). In contrast, the present study employed a within-subjects ABA design, such that changes in opioid sensitivity could be examined from sedentary to exercise conditions, and from exercise to sedentary conditions. We have previously reported that differences in opioid sensitivity between sedentary and exercising subjects may be reflected as either differences in potency or effectiveness of the test drug, depending on its relative efficacy at the mu receptor (Smith and Yancey, 2003). As such, the present study tested opioids possessing a range of relative efficacies at the mu receptor so that changes in both potency and effectiveness could be examined.

1. Methods

1.1. Animals

Eight female Long–Evans rats were obtained at weaning (21 days) from Charles River Laboratories (Raleigh, NC, USA). Under sedentary conditions, rats were housed individually in standard, polycarbonate cages (50×28×20 cm) that permitted no exercise beyond normal cage ambulation. Under exercise conditions, rats were housed individually in modified cages of equal dimensions, but with a running wheel (35 cm diameter) affixed to the interior of the cage (Harvard Apparatus, Boston, MA, USA). Wheel revolutions were counted continuously by magnetic switches and recorded weekly. All rats were kept in a colony room maintained on a 12 h light/dark cycle (lights on: 0700) with food and drinking water freely available in the home cages. Throughout the study, subjects were tested and maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of Davidson College and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996).

1.2. Testing schedule

The study employed a within-subjects ABA design. Upon arrival, all rats were assigned to sedentary conditions and maintained under these conditions for 6 weeks prior to behavioral testing. Rats were then tested at weekly intervals in the warm-water, tail-withdrawal procedure with the following drugs: morphine, butorphanol, buprenorphine and levorphanol (in that order). Previous studies from our laboratory have shown that a 7-day interval between antinociceptive tests is sufficient to prevent the development of mu-opioid tolerance that could lead to systematic changes in opioid sensitivity (Smith and Gray, 2001; Smith et al., 2005). After completion of these tests, rats were reassigned to exercise conditions and transferred to cages equipped with running wheels. After 6 weeks under these new conditions, the effects of all four opioids were reexamined in the tail-withdrawal procedure. Rats had free access to running wheels throughout this phase, except when they were removed from their cages for testing. At the completion of these tests, rats were returned to sedentary conditions and transferred back to their original cages. After 6 weeks, the antinociceptive effects of all four opioids were reexamined a final time in the tail-withdrawal procedure.

1.3. Antinociceptive testing

Rats were restrained during antinociceptive tests in clear acrylic restraint tubes (PGC Scientific, Frederick, MD, USA). Water was heated and maintained at 50° and 54 °C via thermostat-controlled water baths (Fisher Scientific, Pittsburgh, PA, USA). Tail-withdrawal latencies were measured with a hand-operated stopwatch with a time resolution of 0.01 s. Prior to the first test session, rats were habituated to both the injection procedure and restraint tube confinement.

Antinociceptive tests were conducted according to procedures described previously (Smith and French, 2002; Smith et al., 2003, 2004). Briefly, rats were placed into restraint tubes with their tails hanging freely off the edge of a table. The distal 8–10 cm of the tail was then immersed into a cup containing either 50° or 54 °C water, and the latency to withdraw the tail was measured. Approximately 3 min separated the two temperature presentations, and the order of temperature presentation was counterbalanced across rats. A cut-off latency of 15 s was employed in all tests to prevent tissue damage. Testing commenced only when a rat kept its tail in room temperature water (24 °C) for 15 s in two successive determinations. Previous studies employing the tail-withdrawal procedure have generally reported that a 3-min interval between stimulus presentations is sufficient for baseline tail-withdrawal latencies to return to normal (Barrett et al., 2002; Cook et al., 2000).

All opioids were administered using a cumulative dosing procedure. In this procedure, each rat was removed from its restraint tube, injected with a dose of the test drug, and then

immediately returned to the tube. After a 15-min interval, the latency for each rat to withdraw its tail from the 50° and 54 °C water was determined. Immediately following testing at both temperatures, each rat was administered the next dose of the test drug, such that the dose increased the cumulative amount of drug administered in that session by 0.5 or 1.0 log unit. Each test session consisted of 4–5 components, with increasing doses of the test drug administered at the beginning of each subsequent component. Once a maximal effect was obtained at one temperature, no further tests were conducted at that temperature.

1.4. Drugs

Morphine sulfate and buprenorphine hydrochloride were generously supplied by the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, NC, USA). Levorphanol tartrate and butorphanol tartrate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in sterile saline and injected i.p. in a volume of 1.0 ml/kg of body weight.

1.5. Data analysis

Tail-withdrawal latencies were converted to percent maximal possible effect using the following equation: % maximal possible effect = [(observed − baseline) / (15 s − baseline)] × 100. These data were then used to calculate simple main effects of dose and condition (sedentary I vs. exercise vs. sedentary II) using repeated-measures ANOVAs, with dose and condition both serving as within-subject factors. For each dose–effect curve, the dose estimated to produce 50% of the maximal possible effect (A50) was computed for each rat mathematically (least squares method) by log-linear interpolation using at least three points on the ascending portion of the curve (Procedure 8, Tallarida and Murray, 1987). Under conditions in which a drug failed to produce at least an 80% antinociceptive effect, area under the curve (AUC) estimates were made for each rat using the Trapezoidal Rule (Procedure 25, Tallarida and Murray, 1987).

For drugs yielding A50 values, potency ratios were determined for each rat by dividing individual A50 values obtained under exercise conditions by individual A50 values obtained under each of the sedentary conditions. For drugs yielding AUC estimates, effectiveness ratios were determined for each rat by dividing individual AUC estimates obtained under exercise conditions by individual AUC estimates obtained under each of the sedentary conditions. Separate potency and/or effectiveness ratios were determined for each of four conditions: (1) the initial sedentary condition versus the exercise condition at the low nociceptive intensity, (2) the initial sedentary condition versus the exercise condition at the high nociceptive intensity, (3) the subsequent sedentary condition versus the exercise condition at the low nociceptive intensity, and (4) the subsequent sedentary condition versus the exercise condition at the high nociceptive intensity. In addition, mean potency and effectiveness ratios were determined by averaging the individual potency and effectiveness ratios across the four conditions. These potency and effectiveness ratios were then plotted as a function of exercise output for individual rats to determine the correlation between changes in opioid sensitivity and exercise output. Exercise output was defined in individual rats as the mean number of wheel revolutions per day (rev/day) over the entire testing period. For each drug, a least-squares regression line was drawn and a Pearson correlation coefficient was determined with the aid of commercially available software (SigmaPlot for Windows, v. 8.0; SPSS for Windows, v. 13.0).

2. Results

2.1. Running rates

During the exercise period, rats ran an average of 7154 rev/day (7869 m/day), with a range across rats from 4501 rev/day (4951 m/day) to 10,164 rev/day (11,180 m/day). Running rates steadily increased during the first 4 weeks of exposure to the running wheel before leveling out until antinociceptive testing commenced. Running rates declined slightly after the first antinociceptive test, and remained stable thereafter (data not shown).

2.2. Baseline tail-withdrawal latencies

Baseline tail-withdrawal latencies (i.e., tail-withdrawal latencies in the absence of drug administration) differed between temperatures and across conditions (Table 1). A repeated-measures ANOVA revealed a main effect of temperature ($F[1, 9] = 84.97$, $p < 0.05$) and condition ($F[2, 9] = 12.80$, $p < 0.05$). Under all conditions, tail-withdrawal latencies were greater at the low temperature than at the high temperature. Post-hoc tests revealed that tail-withdrawal latencies under the initial sedentary condition were significantly less than those under both the exercise condition and the subsequent sedentary condition ($p < 0.05$); however, no significant differences existed between the exercise condition and the subsequent sedentary condition.

2.3. Mu-opioid antinociception

Morphine and levorphanol produced dose-dependent increases in tail-withdrawal latencies at both water temperatures and produced maximal effects (i.e., ≥80% maximal possible effect) under all conditions (Fig. 1). Sensitivity to the antinociceptive effects of both drugs decreased significantly

Table 1
Mean (SE) baseline tail-withdrawal latencies (s) during the initial sedentary period (Sedentary I), exercise period (Exercise), and subsequent sedentary period (Sedentary II) when tested at low (50 °C Water) and high (54 °C Water) nociceptive intensities

Condition	50 °C Water	54 °C Water
Sedentary I	3.56 (0.32)	2.29 (0.09)
Exercise	4.45 (0.17)	3.29 (0.08)
Sedentary II	5.45 (0.40)	3.49 (0.31)

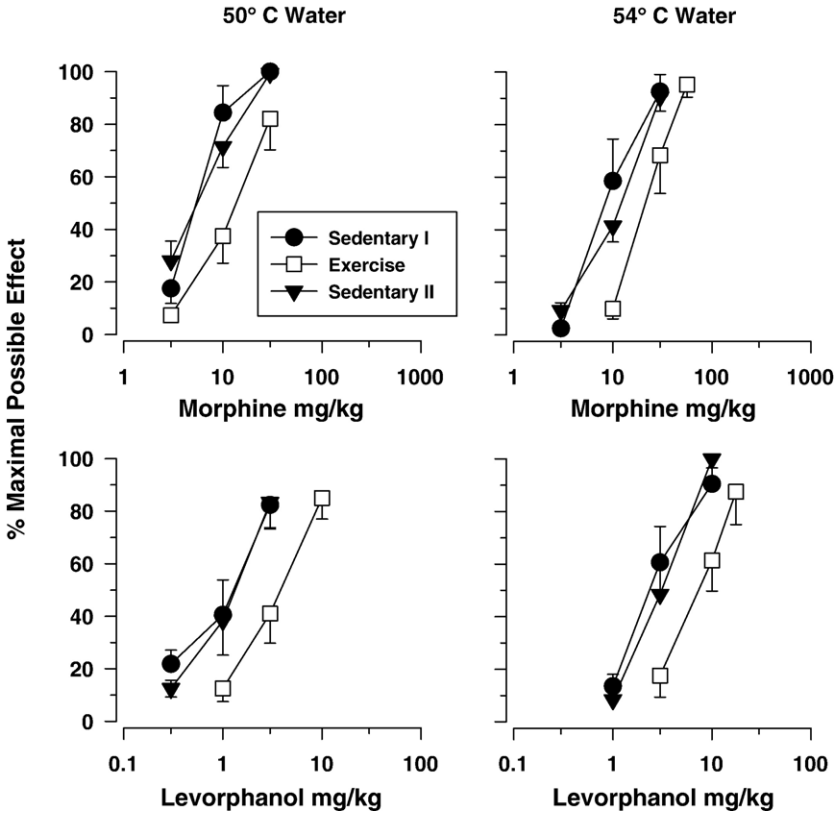

Fig. 1. Effects of morphine and levorphanol in the warm-water, tail-withdrawal procedure. Left panels depict data collected in 50 °C water; right panels depict data collected in 54 °C water. Ordinates reflect tail-withdrawal latencies expressed as a percentage of the maximal possible effect. Abscissas reflect the dose of test drug in mg/kg of body weight. All data points depict the mean (*SE*) of eight rats.

during the exercise condition, as revealed by rightward shifts in the dose–effect curves and increases in A50 values (i.e., decreases in potency) from sedentary to exercise conditions (Table 2). When rats were transferred back to standard laboratory cages at the conclusion of the exercise condition, sensitivity to both opioids increased significantly and returned to that observed prior to the exercise period. Consistent with these observations, repeated-measures ANOVAs revealed significant main effects of dose and condition for both drugs at both water temperatures ($p<0.05$). Analysis of relative potency estimates revealed that morphine was from 2.01 to 2.28 fold more potent, and that levorphanol was from 2.23 to 3.46 fold more potent, under sedentary than exercise conditions (Table 3).

Table 2
A50 values[a] (95% confidence limits) of test drugs in rats during the initial sedentary period (Sedentary I), exercise period (Exercise), and subsequent sedentary period (Sedentary II) when tested at low (50 °C Water) and high (54 °C Water) nociceptive intensities

Test drug	50 °C Water	54 °C Water
Morphine		
Sedentary I	6.00 (4.49–8.00)	9.37 (6.88–12.76)
Exercise	12.27 (8.67–17.37)	21.91 (17.62–27.25)
Sedentary II	5.72 (4.33–7.55)	10.51 (8.33–13.27)
Levorphanol		
Sedentary I	1.03 (0.66–1.61)	2.69 (1.94–3.74)
Exercise	3.51 (2.55–4.83)	7.02 (5.03–9.81)
Sedentary II	1.15 (0.78–1.70)	2.93 (2.30–3.74)
Buprenorphine		
Sedentary I	0.41 (0.32–0.53)	0.63 (0.42–0.96)
Exercise	0.94 (0.63–1.41)	4.37 (1.84–10.41)
Sedentary II	0.37 (0.29–0.47)	0.55 (0.34–0.90)
Butorphanol		
Sedentary I	[b]	[b]
Exercise	[b]	[b]
Sedentary II	[b]	[b]

[a] All data expressed in mg/kg.
[b] Could not be determined (maximal effects were not obtained).

Table 3
Relative potency estimates (95% confidence limits) of test drugs between sedentary and exercise conditions when tested at low (50 °C Water) and high (54 °C Water) nociceptive intensities

Test drug	50 °C Water	54 °C Water
Morphine		
Sedentary I	2.06 (1.38–3.28)	2.28 (1.59–3.23)
Sedentary II	2.15 (1.43–3.46)	2.01 (1.43–2.78)
Levorphanol		
Sedentary I	3.46 (2.08–5.81)	2.55 (1.60–4.06)
Sedentary II	2.57 (1.49–4.31)	2.23 (1.49–3.29)
Buprenorphine		
Sedentary I	2.19 (1.46–3.44)	4.56 (2.29–11.30)
Sedentary II	2.08 (1.33–3.40)	4.79 (2.21–13.75)

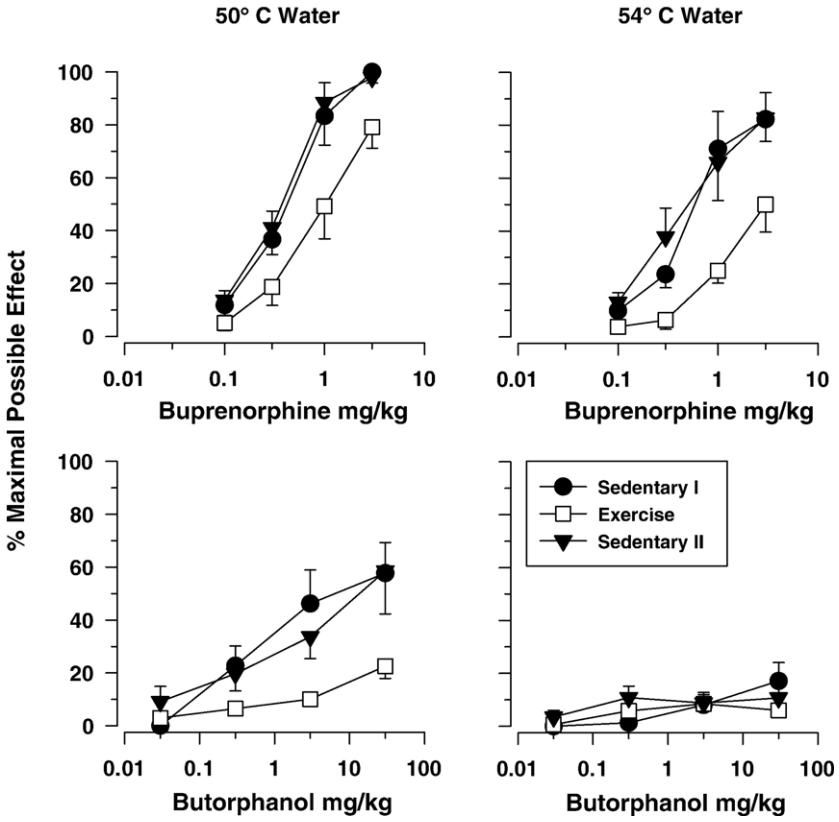

Fig. 2. Effects of buprenorphine and butorphanol in the warm-water, tail-withdrawal procedure. Left panels depict data collected in 50 °C water; right panels depict data collected in 54 °C water. Ordinates reflect tail-withdrawal latencies expressed as a percentage of the maximal possible effect. Abscissas reflect the dose of test drug in mg/kg of body weight. All data points depict the mean (*SE*) of eight rats.

Buprenorphine produced dose-dependent increases in tail-withdrawal latencies, but its maximal effect differed across conditions (Fig. 2). During the initial sedentary condition, buprenorphine produced maximal effects at both water temperatures. Sensitivity to buprenorphine decreased during the exercise period, such that it failed to produce maximal effects at either temperature. These decreases in sensitivity were reflected as both increases in A50 values and decreases in AUC values (Tables 2 and 4, respectively). Sensitivity to buprenorphine increased when rats were transferred back to the sedentary condition, such that it once again produced maximal effects at both temperatures. Repeated-measures ANOVAs revealed significant main effects of dose and condition for buprenorphine at both water temperatures ($p<0.05$). Analysis of relative potency estimates revealed that buprenorphine was from 2.08 to 4.79 fold more potent under sedentary than exercise conditions (Table 3).

Butorphanol produced dose-dependent increases in tail-withdrawal latencies at the low temperature, but failed to produce a maximal effect under all conditions examined (Fig. 2). In contrast to the other test drugs, butorphanol was devoid of antinociceptive activity (<20% maximal possible effect) at the high temperature. Sensitivity to the antinociceptive effects of butorphanol at the low temperature decreased significantly during the exercise condition, as revealed by a downward shift in its dose–effect curve and a decrease in its AUC value (Table 4). When rats were transferred back to standard laboratory cages at the conclusion of the exercise condition, sensitivity to butorphanol increased significantly at the low temperature and approximated that observed prior to the exercise period. Repeated-measures ANOVAs revealed significant main effects of dose and condition for butorphanol at the low temperature ($p<0.05$), but not the high temperature.

2.4. Correlation between changes in opioid sensitivity and exercise output

For data obtained during the exercise period, a simple regression analysis was performed on baseline tail-withdrawal

Table 4
Mean AUC values (SE) of test drugs in rats during the initial sedentary period (Sedentary I), exercise period (Exercise), and subsequent sedentary period (Sedentary II) when tested at low (50 °C Water) and high (54 °C Water) nociceptive intensities

Test Drug	50 °C Water	54 °C Water
Buprenorphine		
Sedentary I	87.94 (8.05)	70.31 (11.74)
Exercise	55.03 (10.66)	29.09 (5.06)
Sedentary II	92.75 (6.99)	75.82 (13.94)
Butorphanol		
Sedentary I	97.64 (22.41)	18.00 (7.96)
Exercise	29.44 (6.47)	17.63 (3.37)
Sedentary II	87.14 (16.32)	26.57 (6.79)

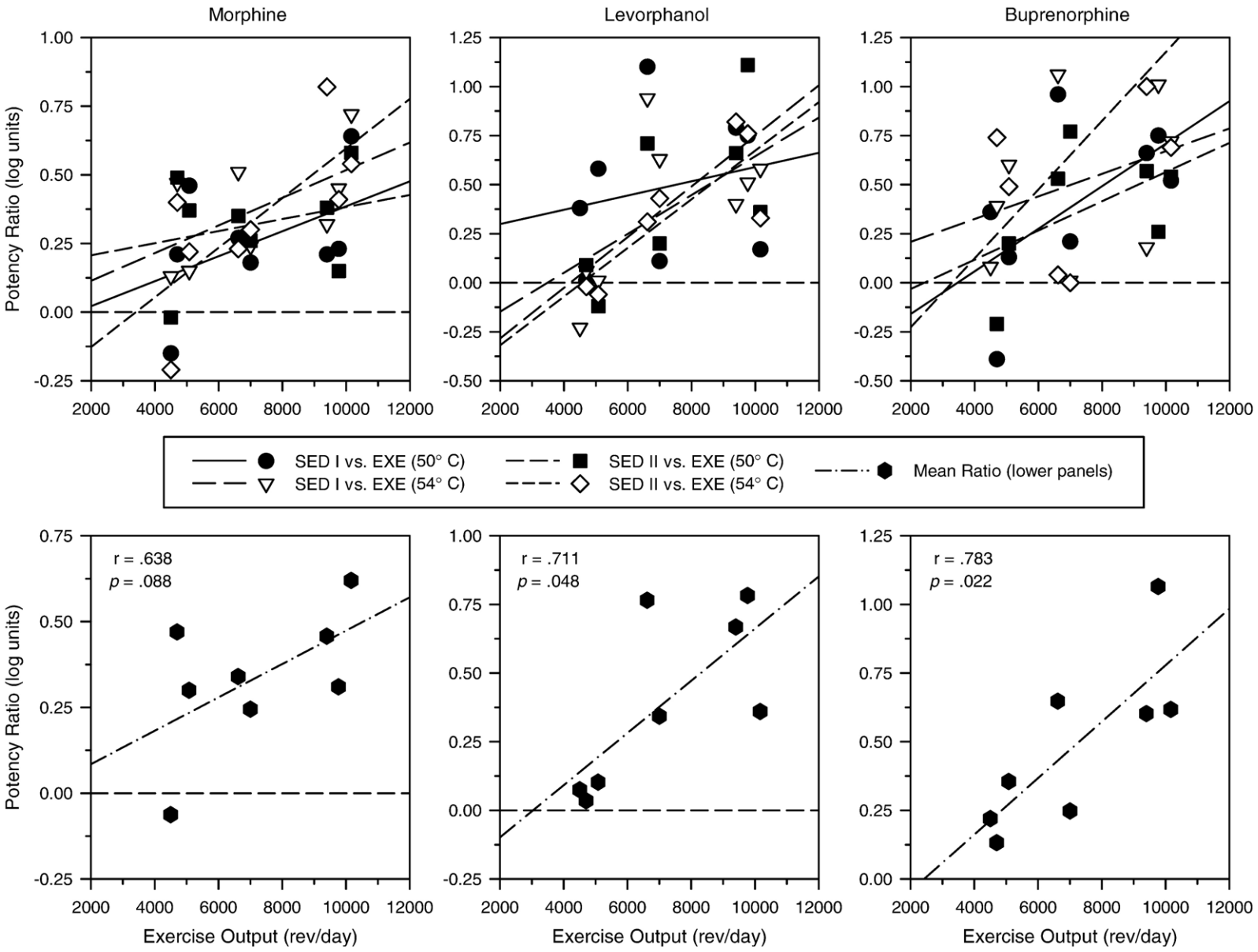

Fig. 3. Correlations between exercise output and changes in potency of morphine, levorphanol and buprenorphine between sedentary and exercise conditions. All data reflect the ratio of A50 values between sedentary and exercise conditions expressed in log units (note differences in ordinates across panels). Upper panels reflect potency ratios between the initial sedentary period and exercise period (SED I vs. EXE) and between the subsequent sedentary period and exercise period (SED II vs. EXE). Upper panels also show potency ratios collected at both low (50 °C) and high (54 °C) nociceptive intensities. Lower panels show mean potency ratios as determined across all four conditions. Exercise output is expressed as the mean number of wheel revolutions per day over the entire testing period.

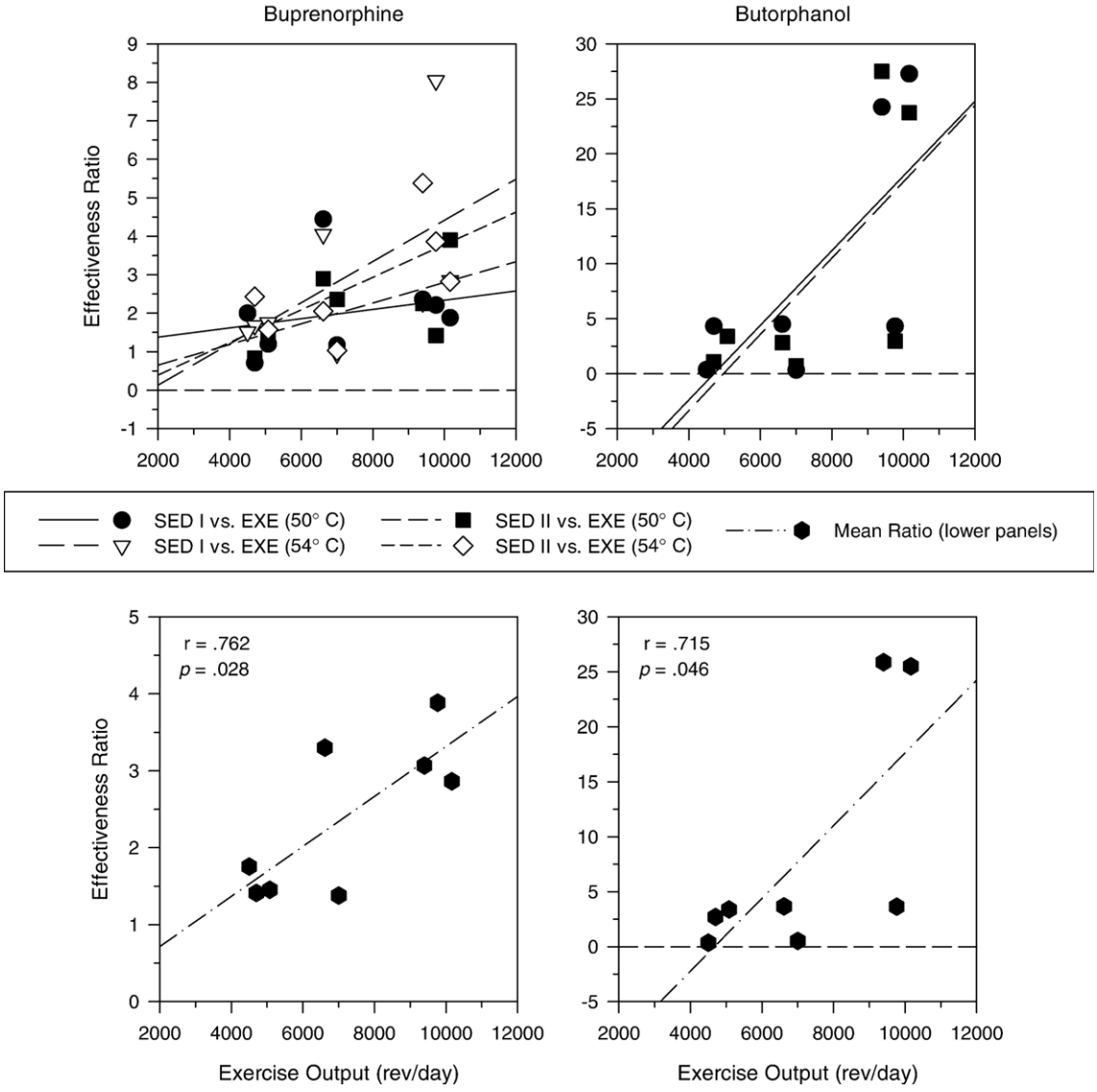

Fig. 4. Correlations between exercise output and changes in effectiveness of buprenorphine and butorphanol between sedentary and exercise conditions. All data reflect the ratio of AUC estimates between sedentary and exercise conditions (note differences in ordinates across panels). Upper panels reflect effectiveness ratios between the initial sedentary period and exercise period (SED I vs. EXE) and between the subsequent sedentary period and exercise period (SED II vs. EXE). Upper panels also show effectiveness ratios collected at both low (50 °C) and high (54 °C) nociceptive intensities. Lower panels show mean effectiveness ratios as determined across all four conditions. Because butorphanol failed to produce antinociceptive effects at the high temperature, only data obtained at the low temperature were used in the correlational analysis. Exercise output is expressed as the mean number of wheel revolutions per day over the entire testing period.

latencies and exercise output (rev/day). Correlation coefficients were low and failed to reach statistical significance at both the low ($r=0.142$; $p=0.736$) and high ($r=0.303$; $p=0.466$) temperature (data not shown). A similar analysis was performed on individual A50 and/or AUC estimates for each of the four drugs tested. No significant correlations were observed for any of the drugs at either nociceptive intensity (data not shown). For all subsequent analyses, changes in opioid sensitivity between sedentary and exercise conditions (as defined by potency and effectiveness ratios) were used as the dependent measure.

For those drugs producing maximal effects under at least one condition (i.e., morphine, levorphanol and buprenorphine), potency ratios between sedentary and exercise conditions (expressed in log units) were plotted as a function of exercise output for individual rats. Positive correlations were observed for all three drugs between each of the sedentary conditions and the exercise condition, and these effects were observed at both the low and high temperatures (Fig. 3, upper panels). Positive correlations were also observed when mean potency ratios were used in the regression analysis (Fig. 3, lower panels). In this analysis, correlation coefficients were statistically significant for levorphanol ($r=0.711$; $p=0.048$) and buprenorphine ($r=0.78$; $p=0.022$), and approached statistical significance for morphine ($r=0.64$; $p=0.088$). For all three drugs, changes in sensitivity were minimal in those rats that ran the least (<6000 rev/day), and greatest in those rats that ran the most (>8000 rev/day).

For those opioids that failed to produce a maximal effect under at least one condition (i.e., buprenorphine and

butorphanol), effectiveness ratios between sedentary and exercise conditions were plotted as a function of exercise output for individual rats. For butorphanol, these ratios could only be determined at the low temperature, as this drug was devoid of antinociceptive activity at the high temperature. Similar to that seen in the potency analysis, positive correlations were observed for both drugs between each of the sedentary conditions and the exercise condition; for buprenorphine, these effects were observed at both the low and high temperature (Fig. 4; upper panels). When mean effectiveness ratios were used in the regression analysis (Fig. 3, lower panels), significant positive correlations were observed for both buprenorphine ($r=0.762$; $p=0.028$) and butorphanol ($r=0.715$; $p=0.046$). For both drugs, changes in sensitivity were typically smallest in those rats that ran the least and greatest in those rats that ran the most.

3. Discussion

We have previously reported that differences in opioid sensitivity between sedentary and exercising rats may be reflected as either differences in potency or effectiveness of the test drug, depending on its relative efficacy at the mu receptor (Smith and Yancey, 2003). Consequently, the present study tested opioids with a range of relative efficacies so that changes in both potency and effectiveness could be examined. Studies employing both in vivo (Adams et al., 1990; Morgan and Picker, 1996; Paronis and Holtzman, 1992; Walker and Young, 2001; Young et al., 1992; Zimmerman et al., 1987) and in vitro (Emmerson et al., 1996; Selley et al., 1998, 2000) techniques report that the four opioids tested differ in their relative efficacy at the mu receptor, with a rank order of morphine ≥ levorphanol > buprenorphine > butorphanol. In the present study, all four opioids produced dose-dependent increases in antinociception during the initial sedentary period (i.e., approximately 6 weeks after arrival and prior to transfer to exercise cages), but differences in effectiveness were observed across drugs and nociceptive intensities. For example, whereas morphine and levorphanol produced maximal levels of antinociception at both temperatures, butorphanol was only partially effective at the low temperature and was devoid of antinociceptive effects at the high temperature. These findings are consistent with a large number of studies reporting that high-efficacy opioids are effective across a wide range of nociceptive intensities, whereas low-efficacy opioids are effective only at low nociceptive intensities (Butelman et al., 1995; Cook et al., 2000; Morgan et al., 1999; Smith et al., 1999; Walker et al., 1993).

When subjects were transferred to cages equipped with running wheels, all rats began running within 24 hours. Running rates gradually increased over the next 4 weeks, before leveling out approximately 2 weeks prior to behavioral testing. During the exercise period, rats ran an average of 7154 rev/day (7869 m/day), with a range across rats from 4501 to 10,164 rev/day (4951–11,180 m/day). These running rates were markedly greater than those reported in a previous study from our laboratory using male rats (Smith and Yancey, 2003). This was to be expected, as female rats run more than male rats when given free access to running wheels (Boakes et al., 1999; Eikelboom and Mills, 1988).

Sensitivity to all four opioids decreased significantly under exercise conditions. Dose–effect curves for morphine, levorphanol and buprenorphine shifted 2 to 3 fold to the right during the exercise period, indicating a loss of potency to their antinociceptive effects. In addition, the maximal effect produced by buprenorphine at the high temperature was markedly reduced, indicating a loss of effectiveness under these conditions. A similar loss of effectiveness was observed for butorphanol at the low temperature, where its maximal effect was reduced from 58% to 22%. Both drugs were tested up to doses that could be safely tested in this procedure, as doses higher than those depicted produce signs of toxicity that are not readily reversed by naloxone (personal observations). The loss of sensitivity observed under exercise conditions was fully reversible. When subjects were transferred back to standard laboratory cages for 6 weeks, the potency and/or effectiveness of each drug increased significantly and returned to that observed prior to the exercise period. Furthermore, these effects were consistent across all four opioids and at both nociceptive intensities.

The finding that buprenorphine and butorphanol were maximally effective under sedentary conditions but failed to produce a maximal effect under exercise conditions suggests that the relative efficacy of these drugs decreased during the exercise period. Drug combination tests have shown that these drugs reliably function as opioid antagonists in this procedure under conditions in which they fail to produce a maximal effect when administered alone (Morgan et al., 1999; Smith and Gray, 2001; Smith et al., 1999). Furthermore, we have collected data showing that butorphanol antagonizes the effects of morphine in exercising rats under conditions in which it fails to produce a maximal effect when administered alone (unpublished observations). Collectively, these data suggest that it is unlikely that buprenorphine and butorphanol would have produced maximal effects in the exercise condition, even if higher doses were tested.

It is important to note that the effects observed during the exercise period cannot be attributed to maturational factors. We have previously reported that sensitivity to the antinociceptive effects of mu opioids in the tail-withdrawal procedure increases as a function of age (Smith and Gray, 2001, but see Crisp et al., 1994; Hamm and Knisely, 1985; Islam et al., 1993). Although the present study took place over the course of 8 months, opioid sensitivity varied as a function of experimental condition (i.e., sedentary vs. exercise), and not as a function of developmental period (e.g., periadolescence vs. adulthood). Similarly, changes in opioid sensitivity could not be attributed to changes in baseline measures of nociception. Whereas baseline tail-withdrawal latencies systematically increased over the course of the study, opioid sensitivity decreased then increased as a function of experimental condition. The finding that tail-withdrawal latencies increased from the initial sedentary to exercise condition was expected, as previous studies have reported that exercise increases nociceptive thresholds in both human and animal subjects (Janal, 1996; Koltyn, 2000; O'Connor and Cook, 1999; Shyu et al., 1982). However, the

finding that tail-withdrawal latencies increased further from the exercise to the second sedentary condition was unexpected, and cannot be readily explained by endogenous opioid activity. Although the exact mechanisms responsible for these increases in tail-withdrawal latencies are not known, it should be noted that previous studies have reported increases in nociceptive threshold across maturational stages (Conway et al., 1998; Falcon et al., 1996; Hu et al., 1997).

Previous studies have failed to find a correlation between simple indices of opioid sensitivity (e.g., A50 values) and exercise output in individual subjects (Kanarek et al., 1998; Smith and Yancey, 2003). In the present study, regression analyses performed on individual A50 and AUC estimates failed to reveal a significant correlation between opioid sensitivity and exercise output. Such correlations fail to take into account individual differences in opioid sensitivity and do not directly address the changes in opioid sensitivity induced by exercise. The within-subjects ABA design of the present study allowed us to determine the correlation between changes in opioid sensitivity across the various experimental manipulations and exercise output in individual rats. As noted above, exercise decreased the potency of morphine, levorphanol and buprenorphine to produce antinociceptive effects at both low and high nociceptive intensities. These changes in potency (as measured by relative potency estimates) were positively correlated with exercise output in individual rats. For all three drugs, similar correlation coefficients were obtained for the changes in potency from sedentary to exercise conditions, and from exercise to sedentary conditions. Although a causal relationship between changes in opioid sensitivity and exercise output cannot be inferred from these data, it is important to note that these effects cannot be attributed to differences in baseline levels of pain sensitivity during the exercise period. Indeed, there was no correlation between baseline tail-withdrawal latencies and exercise output at either the low or high nociceptive intensity under exercise conditions.

Changes in sensitivity to butorphanol were reflected as changes in effectiveness across conditions. Because the maximal effect produced by butorphanol under the exercise condition was less than 50% of the maximal possible effect, relative potency estimates could not be used for the correlational analysis. Instead, AUC estimates were made for each rat and these estimates were used to construct effectiveness ratios, which provided a quantifiable measure of the change in sensitivity to butorphanol between sedentary and exercise conditions. Similar to that observed for the other opioids, a positive correlation was observed between changes in sensitivity to butorphanol and exercise output in individual rats. This analysis was also used to examine the changes in effectiveness of buprenorphine, which exhibited changes in both potency and effectiveness across conditions. The correlation coefficients for buprenorphine were similar regardless of the type of analysis performed, which supports the utility of an effectiveness analysis under conditions in which relative potency estimates cannot be obtained.

It is important to note that no attempt was made to control for the estrous cycle, and thus it is possible that cyclical fluctuations in gonadal hormones confounded the various measures of opioid sensitivity and exercise output. It is unlikely that variations in the hormonal milieu significantly influenced the potency or effectiveness of the opioids examined, as previous studies have reported that differences in opioid sensitivity across the estrous cycle are typically small and not readily apparent in all rat strains (Craft and Bernal, 2001; Mogil et al., 2000; Stoffel et al., 2003). Consistent with this premise, individual A50 and AUC values obtained in the initial sedentary period were similar to those obtained in the subsequent sedentary period, even though it is unlikely that all subjects were in the same phase of the estrous cycle when tested in the two conditions. In contrast, it is likely that the estrous cycle contributed to significant within-subject variability in exercise output. Previous studies have reported marked differences in exercise output across the estrous cycle, with exercise typically reaching a peak during the night of estrous (Eckel et al., 1998, 2000; Wollnik and Turek, 1988). Daily wheel revolutions were not measured in the present study, and thus it cannot be determined whether changes in opioids sensitivity were correlated with local (i.e., daily) rates of exercise output. It is thus recommended that future studies specifically examine the role of the estrous cycle in mediating the relationship between changes in opioid sensitivity and exercise.

The data generated in the present study are consistent with those we reported previously in male rats (Smith and Yancey, 2003), and support the hypothesis that chronic exercise produces cross-tolerance to exogenously administered opioid agonists. It is interesting to note that the differences between sedentary and exercise conditions in the present study were similar to, or in some cases, less than those reported in our previous study. This was unexpected, as female rats in the present study ran significantly more than male rats in our previous study. The reason that females were not more sensitive to the effects of exercise is not known, but a possible explanation may involve sex differences in opioid sensitivity. Females are less sensitive than males to the effects of mu opioid agonists (Barrett et al., 2001; Bartok and Craft, 1997; Cicero et al., 1996; Cook et al., 2000), and females exhibit less tolerance and cross-tolerance than males during chronic treatment with a given dose of morphine (Craft et al., 1999). Interestingly, this latter finding can be explained by the former finding: if females are less sensitive than males to the acute effects of mu opioids, then the chronic dose of morphine in opioid tolerance studies is functionally lower in females than males. Consistent with this hypothesis, when the functional dose of morphine is equated between females and males during chronic treatment, the development of tolerance and cross-tolerance is comparable between the two sexes (Barrett et al., 2001). In a similar fashion, if females are less sensitive to the effects of endogenous opioid peptides, then any compensatory responses induced by the prolonged release of these peptides (as is the case during exercise) will be attenuated in females relative to males. Thus, even though higher levels of exercise output may produce greater concentrations of endogenous opioid peptides in females, these concentrations are likely to be no more than functionally equivalent to those produced in males. Although

speculative, this explanation would account for the attenuated response of females to exercise-induced decreases in opioid sensitivity.

In conclusion, chronic exercise decreased the potency and/or effectiveness of mu opioids to produce antinociceptive effects in a tail-withdrawal assay employing both low and high nociceptive intensities. The effects of exercise on opioid sensitivity were fully reversible, in that sensitivity to each opioid returned to that observed originally when rats were returned to sedentary conditions. Importantly, changes in opioid sensitivity were positively correlated with exercise output in individual rats, with those rats running the most exhibiting the greatest changes in opioid sensitivity across conditions. These data support the hypothesis that chronic exercise produces cross-tolerance to exogenously administered opioid agonists, and suggest that increasing exercise output may be functionally equivalent to increasing the maintenance dose of an opioid agonist during chronic treatment. Although female rats in the present study ran more than male rats from previous studies, exercise-induced changes in opioid sensitivity were similar to or less than those reported previously in males. These results suggest that females may be less responsive than males to exercise-induced decreases in opioid sensitivity.

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