Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 77 (2004) 49–57
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Sensitivity to the effects of a kappa opioid in rats with free access to exercise wheels: differential effects across behavioral measures

Mark A. Smith[a,b,c,*], Jacob M. McClean[b,c], Paul A. Bryant[a]

[a]Department of Psychology, Davidson College, Davidson, NC 28035, USA
[b]Program in Neuroscience, Davidson College, Davidson, NC 28035, USA
[c]Center for Interdisciplinary Studies, Davidson College, Davidson, NC 28035, USA

Received 15 June 2003; received in revised form 16 September 2003; accepted 17 September 2003

Abstract

It is well established that chronic exercise decreases sensitivity to mu opioid agonists; however, it is less clear what effects it has on kappa opioids. The purpose of the present study was to examine sensitivity to the effects of the selective, kappa opioid spiradoline in rats with free access to exercise wheels. Rats were obtained at weaning and randomly assigned to either standard polycarbonate cages (sedentary) or modified cages equipped with exercise wheels (exercise). After approximately 7 weeks under these conditions, sensitivity to the effects of spiradoline on tests of antinociception, locomotor activity, conditioned place preference, and diuresis were examined in both groups of rats. Sedentary rats were more sensitive than exercising rats to the antinociceptive effects of spiradoline, and this effect was observed at both low and high nociceptive intensities. In contrast, exercising rats were more sensitive than sedentary rats to the diuretic effects of spiradoline, and slightly more sensitive to spiradoline's effects in the conditioned place preference procedure. No differences in sensitivity were observed to the effects of spiradoline on locomotor activity. Sensitivity to the antinociceptive effects of spiradoline nonsignificantly increased in exercising rats that were reassigned to sedentary housing conditions, and changes in spiradoline sensitivity were correlated with exercise output in individual subjects. Collectively, these data suggest that exercise alters sensitivity to the behavioral effects of kappa opioids, but that the direction and magnitude of this effect depends on the behavioral measure examined.

Keywords: Antinociception; Conditioned place preference; Diuresis; Exercise; Locomotor activity; Kappa; Opioid; Rat; Spiradoline

1. Introduction

Acute exercise stimulates the release of endogenous opioid peptides and produces analgesia that can be reversed by the opioid antagonist naloxone (Janal, 1996; O'Connor and Cook, 1999; Koltyn, 2000). Chronic exercise leads to the sustained release of these peptides and to subsequent decreases in sensitivity to morphine and other mu opioid agonists (Kanarek et al., 1998; Mathes and Kanarek, 2001; Lett et al., 2002). It has been proposed that these decreases in sensitivity may reflect the development of cross-tolerance between endogenous opioid peptides released during exercise and exogenously administered mu agonists (Mathes and Kanarek, 2001; Lett et al., 2002). In support of this hypothesis, Houghten et al. (1986) reported that rats with free access to exercise wheels had fewer beta-endorphin binding sites than sedentary rats, an effect that was presumed to reflect a compensatory down-regulation of opioid receptors during exercise. Such reductions in opioid receptor density have been reported in opioid-treated rats (Rogers and el-Fakahany, 1986; Tao et al., 1987; Bhargava and Gulati, 1990; Diaz et al., 1995), and are believed to contribute to the reductions in opioid sensitivity (i.e., tolerance) observed during chronic opioid administration (Paronis and Holtzman, 1992; Smith and Picker, 1998; Stafford et al., 2001).

The effects of exercise on the kappa opioid receptor system have not been as well characterized as its effects on the mu receptor system. Previous studies (e.g., Aravich et al., 1993; Fontana et al., 1994) have reported that exercise stimulates the release of dynorphin, the principle endogenous ligand for the kappa receptor, but measures of kappa receptor density and sensitivity have not been taken. A recent study

* Corresponding author. Department of Psychology, Davidson College, Davidson, NC 28035-7037, USA. Tel.: +1-704-894-2470; fax: +1-704-894-2512.
E-mail address: masmith@davidson.edu (M.A. Smith).

0091-3057/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2003.09.021

reported that rats with free access to exercise wheels were less sensitive to the antinociceptive effects of the kappa agonist U50,488; however, this effect was not apparent at all doses and time points tested (D'Anci et al., 2000). In comparison, studies conducted with mu agonists have generally reported that exercise produces parallel, rightward shifts in the dose–effect curve for morphine that is apparent across a range of time points (see Kanarek et al., 1998; Mathes and Kanarek, 2001; Smith and Yancey, 2003). Such findings raise the interesting possibility that mu and kappa receptors may be differentially modulated by exercise, and may undergo different compensatory responses during chronic activation by endogenous ligands.

The purpose of the present study was to examine the effects of chronic exercise on sensitivity to spiradoline, a highly selective, kappa opioid agonist. To this end, rats were obtained at weaning and randomly assigned to either standard polycarbonate cages (sedentary) or modified cages equipped with exercise wheels (exercise). After approximately 7 weeks under these conditions, the effects of spiradoline were examined on tests of antinociception, locomotor activity, conditioned place preference, and diuresis. For some exercising rats, housing conditions were reversed (i.e., they were transferred to standard polycarbonate cages) and the antinociceptive effects of spiradoline were reexamined 14 days later under sedentary conditions. In this latter group, the relationship between exercise output (before housing reassignment) and changes in spiradoline sensitivity (after housing reassignment) was also examined.

2. Method

2.1. Animals

Male, Fisher 344 rats were obtained from Charles River Laboratories (Raleigh, NC, USA) at weaning (21 days) and randomly assigned to either sedentary or exercising conditions upon arrival. Sedentary rats ($n = 16$) were housed individually in standard, polycarbonate cages ($50 \times 28 \times 20$ cm) that permitted no exercise beyond ordinary cage ambulation. Exercising rats ($n = 16$) were housed in modified cages of equal dimensions, but with an exercise wheel (35 cm diameter) affixed to the interior of the cage (Harvard Apparatus, Boston, MA, USA). All wheel revolutions were counted by magnetic reed switches and recorded daily. Rats in both groups were kept in a colony room maintained on a 12-h light/dark cycle (lights on: 0700) with food and drinking water available ad libitum in the home cage. All behavioral tests were conducted at approximately the same time each day, usually between the hours of 1000 and 1400.

A total of 16 rats (8 sedentary rats; 8 exercising rats) were tested in the warm-water, tail-withdrawal procedure. These rats were housed under sedentary and exercising conditions for 7 weeks prior to antinociceptive testing with spiradoline. Following behavioral testing, housing conditions were reversed such that sedentary rats were transferred to cages equipped with exercise wheels, and exercising rats were transferred to standard polycarbonate cages. After 14 days under these new conditions, the antinociceptive effects of spiradoline were reexamined.

A second group of 16 rats (8 sedentary rats; 8 exercising rats) were tested in procedures measuring conditioned place preference, diuresis and locomotor activity (in that order). Similar to the other group, these rats were housed under sedentary or exercising conditions for 7 weeks prior to behavioral testing. All 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).

2.2. Antinociception

Subjects were restrained during antinociceptive tests in clear acrylic restraint tubes (PGC Scientific, Frederick, MD, USA). Water was heated and maintained at 50 or 55 °C during these tests via thermostat-controlled water baths (Fisher Scientific, Pittsburgh, PA, USA). Tail-withdrawal latencies were recorded with a hand-operated stopwatch with a time resolution of 0.01 s. Prior to the first scheduled test session, rats were habituated to both the injection procedure and restraint tube confinement.

During test sessions, rats were removed from their home cage and placed into restraint tubes with their tails hanging freely off the edge of a table. The distal 10–12 cm of the tail was immersed in a cup containing either 50 or 55 °C water, and the latency to withdraw the tail was recorded. Approximately 3 min separated the two stimulus presentations, and the order of stimulus presentation was counterbalanced across rats. A cutoff latency of 15 s was employed in all tests to prevent tissue damage.

Spiradoline was administered using a cumulative dosing procedure. In this procedure, each rat was removed from its restraint tube, injected intraperitoneally with the lowest dose of spiradoline, and then immediately returned to the tube. After a 15-min pretreatment interval, the latency for each rat to withdraw its tail from the 50 and 55 °C water was determined. Immediately following testing at both temperatures, each rat was administered the next dose of spiradoline, such that the dose increased the total amount of spiradoline received in that session by 0.5 log unit. Each test session continued for two additional components, with increasing doses of spiradoline administered at the beginning of each subsequent component. Cumulative doses of 1.0, 3.0, 10 and 30 Mg/kg were tested.

2.3. Diuresis

Diuresis testing was conducted in sound-attenuating, operant-conditioning chambers (Med Associates, St. Albans,

VT, USA). One day prior to testing, all rats were habituated to the chambers for 2 h. Drug and saline (control) sessions were conducted over the next two consecutive days, with the order of drug and saline administration counterbalanced across rats. During these sessions, each rat was administered either 10 mg/kg spiradoline or saline, and urine was collected over 2 h in steel pans located beneath the grid floor of the chamber. All rats were normally hydrated at the beginning of the session, but no food or water was available during the session.

2.4. Conditioned place preference

The conditioned place preference procedure employed a three-compartment place preference chamber (Med Associates). The chamber consisted of two choice compartments ($25 \times 20 \times 20$ cm) separated by a neutral center compartment ($13 \times 20 \times 20$ cm). One choice compartment was painted white and had a wire-mesh floor covering pine bedding; the other choice compartment was painted black and had a steel-rod floor covering corncob bedding. The center compartment was painted gray and had a solid PVC floor with no underlying bedding. Each choice compartment was separated from the center compartment by a guillotine door. All behavioral activity was recorded by a video camera mounted 1.5 m above the chamber.

On the day prior to the first conditioning trial, each rat was given a 15-min habituation session during which it had free access to the entire chamber. The habituation session began by placing the rat in the center compartment and opening the guillotine doors leading to the two choice compartments. Entrance into a compartment was recorded when the head and both forepaws passed completely through a doorway. The amount of time spent in each of the three compartments was measured over the 15-min session. Neither group showed a significant bias toward one choice compartment over the other during this session.

Conditioning took place over the next eight consecutive days. During conditioning, rats were injected with either 10 mg/kg spiradoline or saline and placed into one of the two choice compartments for 30 min. Rats were confined to the appropriate compartment for the duration of the trial by closing the guillotine door leading to the center compartment. Drug and saline administration alternated daily such that each rat received four conditioning trials with the saline-paired compartment and four conditioning trials with the drug-paired compartment. For half of the rats, the black compartment served as the drug-paired compartment; for the other half, the white compartment served as the drug-paired compartment.

Place preference was assessed in each rat on the day immediately following the last conditioning trial. Similar to the initial habituation session, this session began by placing the rat in the center compartment and opening both guillotine doors. Rats were given free access to the entire chamber for 15 min, and the amount of time spent in each of the three compartments was measured.

2.5. Locomotor activity

Locomotor activity was measured in an open-field, locomotor-activity chamber measuring $50 \times 50 \times 40$ cm. The interior of the chamber was made of plywood and painted with white latex paint. The lid of the chamber was made of transparent Plexiglas, which allowed all activity to be recorded from a video camera suspended 1.5 m above the chamber. Thick black lines were drawn on the lower surface of the chamber with black ink that could easily be observed from the camera mounted above. These lines divided the lower surface of the chamber into a grid of 25 squares, each measuring 10×10 cm. A wire-mesh screen was suspended 2 cm above the lower surface, and served as the floor of the chamber during behavioral testing.

One day immediately prior to testing, rats were habituated to the testing environment by being placing into the activity chamber for 10 min. Drug and saline (control) tests were conducted over the next two consecutive days, with the order of drug and saline administration counterbalanced across rats. During drug tests, each rat was initially injected with the lowest dose of spiradoline and then returned to its home cage. Following a 15-min pretreatment interval, the rat was placed into the activity chamber for 130 s, and the number of locomotor activity counts was recorded (see Data Analysis) The first 10 s of this interval served as an acclimation period, and only data from the final 120 s were used in the statistical analysis. Immediately following the observation period, the rat was removed from the chamber, injected with the next dose of spiradoline, and returned to its home cage. Fifteen minutes later, the rat was again placed into the chamber and locomotor activity was measured. Each test session continued for two additional components, with increasing doses of spiradoline administered at the beginning of each subsequent component. Cumulative doses of 0.3, 1.0, 3.0 and 10 Mg/kg were tested. Saline control tests were conducted in an identical manner, with the exception that saline was administered at the beginning of each component in lieu of spiradoline.

2.6. Drugs

Spiradoline mesylate was obtained from Sigma Chemical (St. Louis, MO, USA) and dissolved in distilled water. In all tests, spiradoline was administered intraperitoneally in a volume of 1.0–2.0 ml/kg of body weight.

2.7. Data analysis

In antinociceptive tests, tail-withdrawal latencies were converted to percent antinociceptive effect using the following equation: % antinociceptive effect=[(observed − base-baseline)/(15 s − baseline)] × 100. For each dose–effect curve, the dose of spiradoline required to produce 50% of the maximal possible effect (A50) was computed for each rat mathematically (least squares method) by log-linear interpo-

Table 1
Baseline tail-withdrawal latencies[a] of sedentary rats, exercising rats, and exercising rats following housing reassignment (i.e., reversal) when tested at low (50 °C water) and high (55 °C water) temperatures

Group	50 °C water	55 °C water
Sedentary	11.31 (0.77)	7.81 (1.11)
Exercise	11.64 (1.17)	9.73 (1.06)
Reversal	12.08 (0.94)	8.56 (0.92)

[a] All data reflect the mean (S.E.M) of eight rats as measured in seconds.

lation (Procedure 8, Tallarida and Murray, 1987). These A50 values were then analyzed via repeated measures analysis of variance (ANOVA), with group serving as the between-subjects factor and temperature serving as the within-subjects factor. Relative potency estimates comparing the potency of spiradoline between groups and across conditions were also determined (Procedure 11, Tallarida and Murray, 1987).

The correlation between exercise output and changes in kappa opioid sensitivity was examined in exercising rats that were transferred to sedentary housing conditions. Relative potency estimates were obtained for spiradoline at both the 50 and 55 °C temperatures in individual rats by dividing each rat's A50 values obtained under exercise conditions by its A50 values obtained after housing reassignment (i.e., under sedentary conditions). These relative potency estimates were then used to construct a least-squares regression line of the relationship between exercise output (i.e., revolutions per day) and changes in sensitivity to spiradoline in this group of rats. A Pearson correlation coefficient was determined with the aid of commercially available statistical software (SigmaPlot for Windows, v. 8.0).

In the locomotor activity test, activity counts were measured by counting the number of instances in which a rat entered a new 10×10-cm square during each 120-s observation period. Entrances were counted only if the rat crossed the grid line delineating the perimeter of the square with both forepaws. Locomotor activity data were expressed as percent saline control values by dividing the number of activity counts observed during each component of the test session by that obtained during each component of the saline control session, and then multiplying by 100. These locomotor activity data were then analyzed via repeated measures ANOVA, with group serving as the between-subjects factor and dose serving as the within-subjects factor.

In the conditioned place preference procedure, difference scores were obtained for each rat by subtracting the time spent in the drug-paired compartment before conditioning (i.e., during the free-access habituation session) from the amount of time spent in the drug-paired compartment after conditioning (i.e., during the free-access place preference test). Using these difference scores, group effects were determined via a two-tailed, independent-sample *t* test.

In the diuresis test, the amount of urine collected over a 2-h period following spiradoline administration was compared between groups via a two-tailed, independent-sample *t* test.

An alpha level of .05 was used for all statistical tests.

3. Results

3.1. Running rates

In exercising rats, running rates averaged 2809 rev/day (3174 m/day), with a range across rats of 1061 to 6316 rev/day (1199 to 7137 m/day). Running rates were initially low in these rats, but gradually increased over the course of 4 weeks. In eight sedentary rats that were transferred to cages equipped with exercise wheels, running rates averaged only 367 rev/day (415 m/day) after 28 days of wheel exposure.

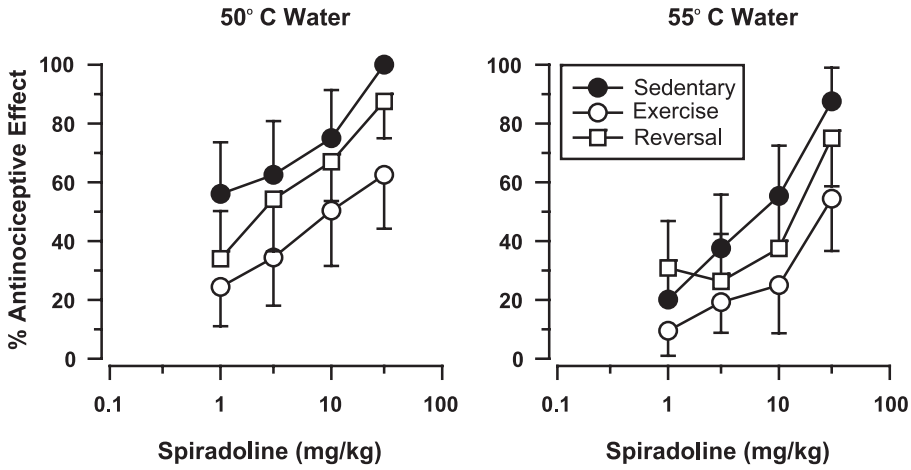

Fig. 1. Effects of cumulative doses of spiradoline in the warm-water, tail-withdrawal procedure. Left panel depicts data collected in 50 °C water; right panel depicts data collected in 55 °C water. Vertical axes reflect tail-withdrawal latencies and are expressed as a percentage of the maximal possible effect. Horizontal axes reflect doses in milligram per kilogram of body weight. Vertical lines on data points represent the S.E.M.; where not indicated, the S.E.M. fell within the data point. Data are shown from sedentary rats, exercising rats, and exercising rats 14 days after housing reassignment (reversal).

Running rates were consistently low in this group and no increasing trends were readily apparent. By the way of comparison, rats in the original exercising group were running an average of 2680 rev/day (3028 m/day) after 28 days. Due to their very low running rates, no further tests were conducted in this group.

3.2. Antinociception

Baseline tail-withdrawal latencies (i.e., tail-withdrawal latencies in the absence of drug administration) were similar between sedentary and exercising rats, and were similar in exercising rats before and after housing reassignment (Table 1). In all cases, tail-withdrawal latencies were greater at the low temperature than at the high temperature.

Spiradoline produced maximal levels of antinociception ($\geq$80% antinociceptive effect) in sedentary rats at both temperatures. In contrast, spiradoline failed to produce maximal responses in exercising rats at either temperature (Fig. 1). Analysis of relative potency estimates (Table 2) revealed that spiradoline was approximately 14-fold more potent in sedentary rats at the low temperature, and approximately 4-fold more potent in sedentary rats at the high temperature. In neither case did the 95% confidence limits overlap 1.0. A repeated measures ANOVA comparing the potency of spiradoline in sedentary and exercising rats revealed a trend in favor of increased sensitivity in sedentary rats [$F(1,14)=3.448$, $P=.084$].

After testing with spiradoline, housing conditions were reversed such that exercising rats were transferred to sedentary housing conditions. After 14 days under these new conditions, sensitivity to the effects of spiradoline increased slightly in this group of rats (Fig. 1). Analysis of relative potency estimates revealed that the potency of spiradoline increased approximately threefold at both temperatures; however, 95% confidence limits overlapped 1.0 in both cases (Table 2). A repeated measures ANOVA comparing the potency of spiradoline in exercising rats before and after housing reassignment revealed a significant effect of temperature [$F(1,14)=7.323$, $P=.017$], but the effect of reassignment was not significant.

The correlation between exercise output and changes in kappa opioid sensitivity was examined in exercising rats that were transferred to sedentary housing conditions. There was a significant positive correlation between running rates prior to housing reassignment and changes in sensitivity to spiradoline after reassignment ($r=.96$, $P=.002$). For instance, the two rats that ran the most were the two rats that exhibited the greatest increase in sensitivity; whereas the two rats that ran the least were the only rats that exhibited decreases in sensitivity. For all rats, changes in sensitivity to spiradoline were similar across the two temperatures (Fig. 2).

Table 2
Potency ratios (95% confidence limits) of spiradoline between conditions when tested at low (50 °C water) and high (55 °C water) temperatures

Condition	50 °C water	55 °C water
Sedentary vs. exercise	14.67 (2.43–180.74)	4.47 (1.22–46.13)
Exercise vs. reversal	3.30 (0.75–47.37)	3.46 (0.61–143.83)
Sedentary vs. reversal	2.82 (0.76–33.38)	1.61 (0.38–9.94)

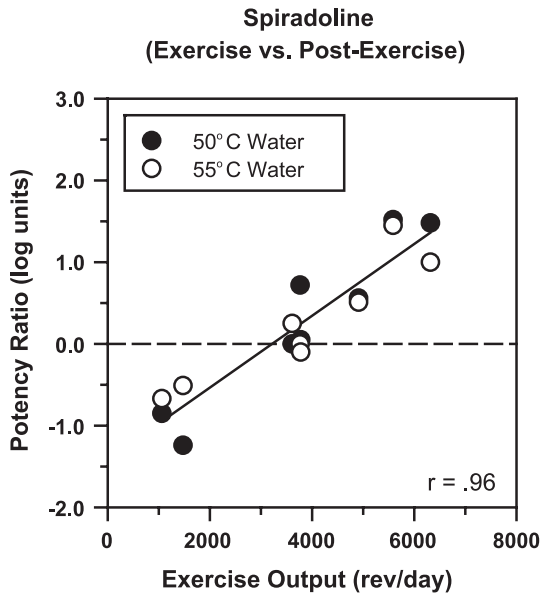

Fig. 2. Relationship between exercise output and changes in sensitivity to spiradoline in exercising rats that were reassigned to sedentary housing conditions. Filled symbols reflect relative potency estimates obtained in 50 °C water; open symbols reflect relative potency estimates obtained in 55 °C water. Data are expressed as the ratio of A50 values obtained before vs. after housing reassignment and are expressed in log units. Exercise output is expressed as the mean number of wheel revolutions per day.

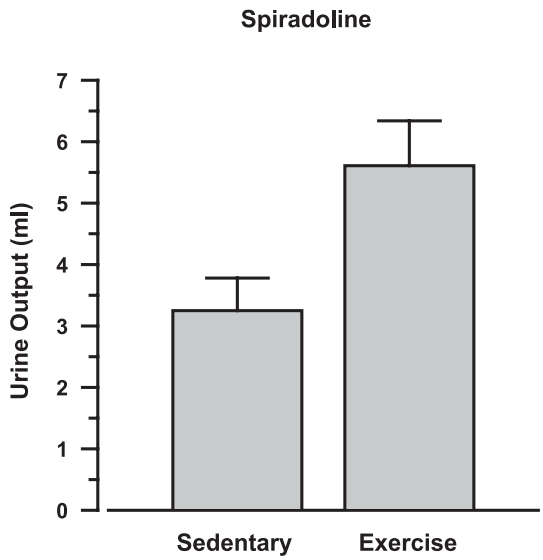

Fig. 3. Effects of 10 mg/kg spiradoline on urine output. Data reflect volume (ml) of urine collected over a 2-h session. Vertical lines represent the S.E.M.

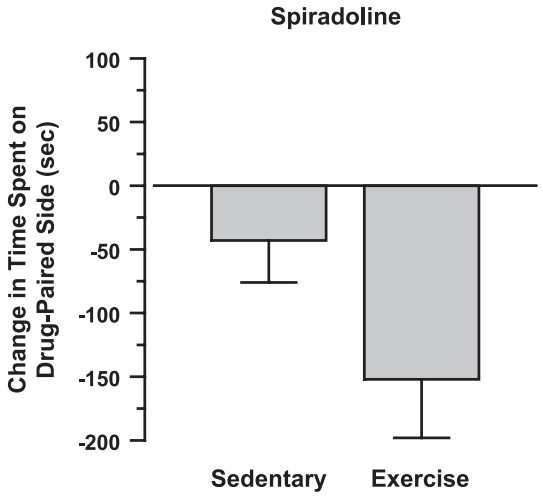

Fig. 4. Effects of 10 mg/kg spiradoline in the conditioned place preference procedure. Data reflect time (s) spent in drug-paired compartment after conditioning minus time (s) spent in drug-paired compartment before conditioning. Vertical lines represent the S.E.M.

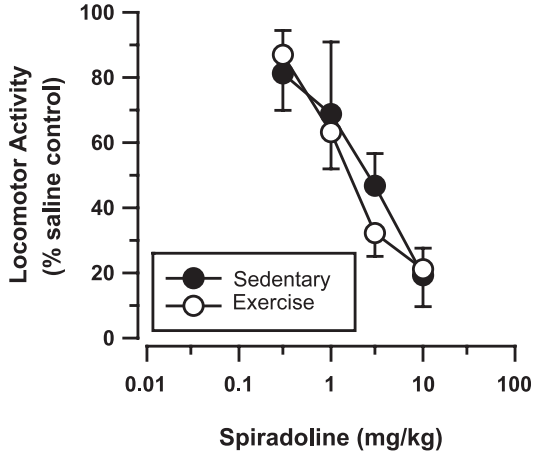

Fig. 5. Effects of cumulative doses of spiradoline in the open-field, locomotor-activity test. Vertical axis reflects locomotor activity counts expressed as a percentage of saline control values. Horizontal axis reflects doses in mg/kg of body weight. Vertical lines on data points represent the S.E.M.; where not indicated, the S.E.M. fell within the data point.

3.3. Diuresis

Urine output was minimal in both groups under saline-control conditions: a mean (S.E.M.) of 0.16 (0.07) and 0.33 (0.07) ml of urine was collected in the sedentary and exercising rats, respectively. Large increases in urine output were seen in both groups following administration of 10 mg/kg spiradoline (Fig. 3); with this effect significantly greater in the exercising group [$t(14) = 2.572$, $P = .022$].

3.4. Conditioned place preference

Prior to conditioning, sedentary and exercising rats spent a mean (SEM) of 277 (45) and 266 (50) sec in the drug-designated compartment, respectively. After conditioning, these values decreased to 233 (11) and 116 (23) sec, respectively (Fig. 4). A two-tailed, independent sample t test revealed a trend in favor of increased sensitivity in exercising rats [$t(14) = 1.860$, $P = .084$]. Analysis of individual data revealed that 8 of 8 exercising rats spent less time in the drug-paired compartment after conditioning, whereas only 4 of 8 sedentary rats spent less time in the drug-paired compartment after conditioning.

Table 3
Mean (S.E.M.) locomotor-activity counts for sedentary and exercising rats during saline control sessions

Component	Sedentary	Exercise
1	54.63 (4.00)	63.00 (4.37)
2	31.50 (6.66)	51.63 (4.68)
3	28.38 (5.59)	41.25 (5.90)
4	21.75 (4.26)	27.38 (4.49)

3.5. Locomotor activity

Under saline-control conditions, locomotor activity counts were greater in exercising rats than sedentary rats in all components (Table 3). A repeated measures ANOVA comparing locomotor activity counts in the saline control test revealed main effects for both group [$F(3,42) = 28.401$, $P < .001$] and component [$F(1,14) = 4.671$, $P = .048$]. Due to these differences between groups and across components, data collected during tests with spiradoline were converted to percent saline control. Spiradoline dose-dependently decreased locomotor activity in both groups of rats, but no differences in sensitivity were observed between the two groups (Fig. 5). Consistent with these observations, a repeated measures ANOVA revealed a significant main effect of dose [$F(3,42) = 18.346$, $P < .001$], but the effect of group was not significant.

4. Discussion

Exercising rats began running within 24 h of wheel exposure. Consistent with earlier reports (D'Anci et al., 2000; Lett et al., 2002), running rates were initially slow, but gradually increased over 4 weeks. Sedentary rats that were transferred to cages equipped with exercise wheels after antinociceptive testing with spiradoline ran at extremely low levels, even after 28 days of wheel exposure. Previous studies have reported that early wheel deprivation retards the later establishment running (Looy and Eikelboom, 1989; Smith and Yancey, 2003), which may explain the low running rates in this group. It is important to note that running rates were very stable throughout this 28-day period, and there was no evidence that running rates would

have increased with further wheel exposure. As a consequence, no further tests were conducted in this group.

Analysis of relative potency estimates indicated that exercising rats were less sensitive than sedentary rats to the antinociceptive effects of spiradoline at both nociceptive intensities. A previous study reported that exercising rats were less sensitive to the antinociceptive effects of U50,488; however, that effect was not observed at all doses and time points tested (D'Anci et al., 2000). In the present study, differences in sensitivity to the antinociceptive effects of spiradoline were modest, with only a fourfold difference observed at the high temperature. Previous studies have frequently reported decreases in sensitivity to the antinociceptive effects of kappa opioids during their chronic administration, an effect generally attributed to the development of tolerance (Horan and Porreca, 1993; Bhargava, 1995; Cao and Bhargava, 1997; Nemmani and Ramarao, 2003). Similarly, it has been suggested that decreases in kappa-opioid sensitivity in exercising animals may reflect the development of cross tolerance between endogenous opioid peptides released during exercise and exogenously administered opioid agonists (D'Anci et al., 2000). The present findings support this possibility, and suggest parallels between chronic exercise and chronic opioid administration on measures of antinociception.

Sensitivity to the effects of spiradoline increased in exercising rats transferred to sedentary housing conditions, suggesting that the effects of exercise were partly reversible. This increase in sensitivity was modest, and only reflected a 3-fold shift to the left in the dose-effect curves. In a previous study, we reported that the dose-effect curve for the mu-opioid buprenorphine shifted more than 10 fold to the left in exercising rats transferred to sedentary housing conditions (Smith and Yancey, 2003). In that study, buprenorphine was tested 42 days after housing reassignment, considerably longer than the 14 days employed in the present study. It is not known whether sensitivity to spiradoline would have continued to increase with longer durations of wheel deprivation, but it is worth noting that sensitivity to the antinociceptive effects of opioid agonists generally returns to normal within 10 days following termination of chronic administration (Garzon et al., 2002). If the differences in sensitivity observed between sedentary and exercising rats were indeed due to the development of tolerance in exercising rats, then one would expect that sensitivity would be fully restored by the time testing was conducted 14 days after their transfer to sedentary housing conditions.

Consistent with previous studies (e.g., Kanarek et al., 1998, Smith and Yancey, 2003), simple correlations between spiradoline sensitivity and exercise output were not significant for any measure examined (data not shown). This is not surprising, given that such correlations do not take into account baseline (i.e., non-exercise) differences in sensitivity. By reassigning exercising rats to standard laboratory cages, we were able to compare changes in sensitivity to spiradoline and exercise output in individual animals. A significant positive correlation was observed between the two measures, with those rats running the most exhibiting the greatest increase in sensitivity to spiradoline. It is interesting to note that the two rats that ran the least exhibited decreases, not increases, in sensitivity to spiradoline after reassignment. This latter finding contrasts with data we reported previously with buprenorphine: in that case, all exercising rats exhibited increases in sensitivity when transferred to sedentary housing conditions, regardless of their initial exercise output (Smith and Yancey, 2003).

A dose of 10 mg/kg spiradoline produced large increases in urine output in both groups of rats, with this effect being most pronounced in exercising rats. Although only a single dose was tested, it is unlikely that the effects of this dose were atypical, as kappa agonists generally produce linear dose effects on this measure (Leander, 1983; Salas et al., 1992). As described previously, studies conducted with both mu and kappa opioids have generally reported only decreases in opioid sensitivity in exercising animals, and to our knowledge, this is the first demonstration that sensitivity to an opioid agonist increases during chronic exercise. It's previously been reported that tolerance develops rapidly to kappa-induced diuresis in rats treated with U50,488 (Vaupel et al., 1990), and thus the present findings do not support the hypothesis that exercise produces effects that are analogous to those seen during chronic drug administration. Rather, these data suggest that tolerance may not be a universal phenomenon of chronic exercise, and that different receptor populations may respond differently during chronic activation by endogenous ligands.

In agreement with previous studies reporting that kappa agonists produce aversive stimulus effects in the conditioned placed preference procedure (Shippenberg and Herz, 1987; Bals-Kubik et al., 1993; del Rosario Capriles and Cancela, 2002), both groups of subjects avoided the compartment paired with 10 mg/kg spiradoline. Spiradoline and other kappa opioids generally produce linear effects in this procedure (Shippenberg and Herz, 1988; Suzuki et al., 1992; Funada et al., 1993), so it is unlikely that the effects produced by this dose were out of the ordinary. Exercising rats were more sensitive than sedentary rats to spiradoline's aversive effects, with all eight exercising rats exhibiting a conditioned avoidance of the spiradoline-paired compartment. These findings contrast with those from a recent study with morphine, where it was reported that exercising rats were less sensitive than sedentary rats to morphine's rewarding effects (Lett et al., 2002). These findings also contrast with a previous study reporting that chronic administration of the kappa-opioid U69,593 produces tolerance to its aversive effects in this procedure (Shippenberg et al., 1988). Whether the present data reflect the development of sensitization to the aversive effects of spiradoline in exercising animals are not known, but they do suggest that the effects of chronic exercise are

not completely analogous to those produced by chronic drug administration.

Consistent with its sedative and muscle-relaxant properties (Butelman et al., 1999), spiradoline decreased locomotor activity in a dose-dependent manner. Sedentary and exercising rats were equally sensitive to the effects of spiradoline on this measure, and no significant differences were observed at any dose tested. Studies examining the effects of kappa opioids on locomotor activity during chronic treatment have produced mixed results. Although one study reported sensitization to the locomotor effects of the kappa-agonist ethylketocyclazocine (Gwynn and Domino, 1984), other studies have reported that the locomotor effects of the kappa-opioids U50,488 and RU-51599 are not altered during chronic administration (Collins et al., 1998; Marinelli et al., 1998). Thus, the observation that exercise did not alter sensitivity to spiradoline's locomotor effects is not atypical given the existing literature. It should be noted that locomotor activity differed markedly between the two groups under baseline (i.e., non-drug) conditions, indicating that the sedentary and exercising manipulations were indeed sufficient to influence the underlying biological systems mediating this behavior.

In a recent study (Smith and Yancey, 2003), we reported that decreases in sensitivity to the effects of mu opioids in exercising animals could be attributed to compensatory responses in the opioid receptor system induced by chronic release of endogenous opioid peptides. These compensatory responses were believed to be similar to those induced by chronic morphine administration, and to include the down-regulation and desensitization of mu receptors. In the present study, the decreased sensitivity to the antinociceptive effects of spiradoline in exercising rats is consistent with the hypothesis that chronic exercise produces functional alterations in the kappa opioid receptor system that are akin to those produced by chronic drug administration. However, the observation that exercising rats were more sensitive to spiradoline's diuretic effects and to its effects in the conditioned place preference procedure conflicts with that previously reported during chronic treatment with kappa agonists (Shippenberg et al., 1988; Vaupel et al., 1990). Unfortunately, the present data do not offer many clues as to why the effects of exercise differed across the behavioral assays examined. Differences in lean body mass and other factors may have contributed to differences in drug bioavailability between the two groups, but it is unlikely that pharmacokinetic variables could have resulted in both increases and decreases in drug sensitivity in exercising subjects. It is possible that kappa receptors in different biological systems are regulated differentially by chronic exercise. In such a scenario, exercise may result in kappa receptors being up-regulated and sensitized in some biological systems, down-regulated and desensitized in other systems, while only being minimally affected in still other systems. Alternatively, there may be concomitant changes occurring within other neurotransmitter systems during exercise that serve to selectively augment or diminish changes occurring within the kappa system. For instance, exercise stimulates the releases of dopamine (de Castro and Duncan, 1985), norepinephrine (Pagliari and Peyrin, 1995), and serotonin (Meeusen et al., 1996), and chronic exercise can lead to compensatory changes in monoamine receptor populations (Gilliam et al., 1984; MacRae et al., 1987). If these neurotransmitter systems interact differently with the kappa receptor system across behavioral endpoints, then their effects could selectively increase or decrease the effects of a kappa agonist depending upon the neurotransmitter and behavioral endpoint involved. These are all issues that will need to be resolved in subsequent studies.

In conclusion, exercising rats were less sensitive than sedentary rats to the antinociceptive effects of spiradoline. When exercising rats were transferred to sedentary housing conditions, sensitivity to the effects of spiradoline increased in some rats, and these increases in sensitivity were positively correlated with exercise output. In contrast to that observed in the antinociceptive procedure, exercising rats were more sensitive than sedentary rats to the effects of spiradoline on urine output, and were slightly more sensitive to spiradoline's effects in the conditioned place preference procedure. Sensitivity to the effects of spiradoline on locomotor activity did not differ between the two groups. These findings suggest that the effects of exercise on kappa opioid receptors may differ across biological systems, or, that changes in other neurotransmitter systems serve to selectively enhance or attenuate changes occurring within the kappa receptor system.

Acknowledgements

This study was supported by Davidson College and US Public Service Grants DA13461 and DA14255 from the National Institute on Drug Abuse. The authors thank Dr. Drake Morgan for helpful comments made on an earlier version of this manuscript, and Amy Becton for expert technical assistance.

References

Aravich PF, Rieg TS, Lauterio TJ, Doerries LE. Beta-endorphin and dynorphin abnormalities in rats subjected to exercise and restricted feeding: relationship to anorexia nervosa? Brain Res 1993;622:1–8.

Bals-Kubik R, Ableitner A, Herz A, Shippenberg TS. Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. J Pharmacol Exp Ther 1993;264:489–95.

Bhargava HN. Non-competitive antagonism of N-methyl-D-aspartate receptor inhibits tolerance to the analgesic action of U-50,488H, a kappa-opioid receptor agonist in the rat. Gen Pharmacol 1995;26:1055–60.

Bhargava HN, Gulati A. Down-regulation of brain and spinal cord mu-opiate receptors in morphine tolerant–dependent rats. Eur J Pharmacol 1990;190:305–11.

Butelman ER, Harris TJ, Kreek MJ. Effects of E-2078, a stable dynorphin A(1–8) analog, on sedation and serum prolactin levels in rhesus monkeys. Psychopharmacology 1999;147:73–80.

Cao YI, Bhargava HN. Effects of ibogaine on the development of tolerance to antinociceptive action of mu-, delta- and kappa-opioid receptor agonists in mice. Brain Res 1997;752:250–4.

Collins RI, Zavala AR, Ingersoll VY, Duke MA, Crawford CA, McDougall SA. Kappa opioid-mediated behavioral sensitization in the preweanling rat: relationship to Fos immunoreactivity. Psychopharmacology 1998; 137:282–91.

D'Anci KE, Gerstein AV, Kanarek RB. Long-term voluntary access to running wheels decreases kappa-opioid antinociception. Pharmacol. Biochem Behav 2000;66:343–6.

de Castro JM, Duncan G. Operantly conditioned running: effects on brain catecholamine concentrations and receptor densities in the rat. Pharmacol. Biochem Behav 1985;23:495–500.

del Rosario Capriles N, Cancela LM. Motivational effects mu- and kappa-opioid agonists following acute and chronic restraint stress: involvement of dopamine D(1) and D(2) receptors. Behav Brain Res 2002;132: 159–69.

Diaz A, Ruiz F, Florez J, Hurle MA, Pazos A. Mu-opioid receptor regulation during opioid tolerance and supersensitivity in rat central nervous system. J Pharmacol Exp Ther 1995;274:1545–51.

Fontana F, Bernardi P, Merlo Pich E, Boschi S, De Iasio R, Capelli M, et al. Endogenous opioid system and atrial natriuretic factor in normotensive offspring of hypertensive parents at rest and during exercise test. J Hypertens 1994;12:1285–90.

Funada M, Suzuki T, Narita M, Misawa M, Nagase H. Blockade of morphine reward through the activation of kappa-opioid receptors in mice. Neuropharmacology 1993;32:1315–23.

Garzon J, Rodriguez-Diaz M, Lopez-Fando A, Garcia-Espana A, Sanchez-Blazquez P. Glycosylated phonducin-like protein long regulates opioid receptor function in mouse brain. Neuropharmacology 2002; 42:813–28.

Gilliam PE, Spirduso WW, Martin TP, Walters TJ, Wilcox RE, Farrar RP. The effects of exercise training on [3H]-spiperone binding in rat striatum. Pharmacol Biochem Behav 1984;20:863–7.

Gwynn GJ, Domino EF. Genotype-dependent behavioral sensitivity to mu vs. kappa opiate agonists. I. Acute and chronic effects on mouse locomotor activity. J Pharmacol Exp Ther 1984;231:306–11.

Horan PJ, Porreca P. Lack of cross-tolerance between U69,593 and bromazocine suggests kappa-opioid receptor multiplicity in mice. Eur J Pharmacol 1993;239:93–8.

Houghten RA, Pratt SM, Young EA, Brown H, Spann DR. Effect of chronic exercise on beta-endorphin receptor levels in rats. NIDA Res Monogr 1986;75:505–8.

Institute of Laboratory Animal Resources. Guide for the care and use of laboratory animals. Washington, DC: National Academy Press, 1996.

Janal MN. Pain sensitivity, exercise and stoicism. J R Soc Med 1996;89: 376–81.

Kanarek RB, Gerstein AV, Wildman RP, Mathes WF, D'Anci KE. Chronic running-wheel activity decreases sensitivity to morphine-induced analgesia in male and female rats. Pharmacol Biochem Behav 1998;61:19–27.

Koltyn KF. Analgesia following exercise: a review. Sports Med 2000;29: 85–98.

Leander JD. A kappa opioid effect: increased urination in the rat. J Pharmacol Exp Ther 1983;224:89–94.

Lett BT, Grant VL, Koh MT, Flynn G. Prior experience with wheel running produces cross-tolerance to the rewarding effect of morphine. Pharmacol Biochem Behav 2002;72:101–5.

Looy H, Eikelboom R. Wheel running, food intake, and body weight in male rats. Physiol Behav 1989;45:403–5.

MacRae PG, Spirduso WW, Cartee GD, Farrar RP, Wilcox RE. Endurance training effects on striatal D2 dopamine receptor binding and striatal dopamine metabolite levels. Neurosci Lett 1987;79:138–44.

Marinelli M, Le Meal M, Piazza PV. Sensitization to the motor effects of contingent infusions of heroin but not of kappa agonist RU-51599. Psychopharmacology 1998;139:281–5.

Mathes WF, Kanarek RB. Wheel running attenuates the antinociceptive properties of morphine and its metabolite, morphine-6-glucuronide, in rats. Physiol Behav 2001;74:245–51.

Meeusen R, Thorre K, Chaouloff F, Sarre S, De Meirleir K, Ebinger G, et al. Effects of tryptophan and/or acute running on extracellular 5-HT and 5-HIAA levels in the hippocampus of food-deprived rats. Brain Res 1996;740:245–52.

Nemmani KV, Ramarao P, Giasenoside KE. Potentiates U-50488H-induced analgesia and inhibits tolerance to its analgesic in mice. Life Sci 2003;72:759–68.

O'Connor PJ, Cook DB. Exercise and pain: the neurobiology, measurement, and laboratory study of pain in relation to exercise in humans. Exerc Sport Sci Rev 1999;27:119–66.

Pagliari R, Peyrin L. Norepinephrine release in the rat frontal cortex under treadmill exercise: a study with microdialysis. J Appl Physiol 1995;78: 2121–30.

Paronis CA, Holtzman SG. Development of tolerance to the analgesic activity of mu agonists after continuous infusion of morphine, meperidine or fentanyl in rats. J Pharmacol Exp Ther 1992;262:1–9.

Rogers NF, el-Fakahany E. Morphine-induced opioid receptor down-regulation detected in intact adult rat brain cells. Eur J Pharmacol 1986;124: 221–30.

Salas SP, Rebleto JS, Lopez LF, Tachibata S, Huldebro-Tore JF. [N-methyl-Tyrl,N-methyl-Arg7-D-Leu8]-dynorphin-A-(1-8) ethylamide, a stable dymorphin analog-produces diuresis by kappa aptale receptor activation in the rat. J Pharmacol Exp Ther 1992;262:979–86.

Shippenberg TS, Herz A. Place preference conditioning reveals the involvement of D1-dopamine receptors in the motivational properties of mu- and kappa-opioid agonists. Brain Res 1987;436:169–72.

Shippenberg TS, Herz A. Motivational effects of opioids: influence of D-1 versais D-2 receptor antagonists. Eur J Pharmacol 1998;151:233–42.

Shippenberg TS, Bennett-Oglesby MW, Ayesta N, Flerr A. Tolerance and selective cross-tolerance to the motivational effects of opioids. Psychopharmacology 1988;96:110–5.

Smith MA, Picker MJ. Tolerance and cross-tolerance to the rate-suppressing effects of opioids in butorphanol-treated rats: influence of maintenance dose and relative efficacy at the mu receptor. Psychopharmacology 1988;140:57–68.

Smith MA, Yancey DL. Sensitivity to the effects of opioids in rats with free access to running wheels: mu opioid tolerance and physical dependence. Psychopharmacology 2003;167:426–34.

Stafford K, Gomes AB, Shen J, Yoburn BC. mu-Opioid receptor down-regulation contributes to opioid tolerance in vivo. Pharmacol Biochem Behav 2001;69:233–7.

Suzuki T, Shiozaki Y, Masukawa Y, Misawa M, Naguse J. The role of mu- and kappa-opioid receptors in cocaine-induced conditioned place preference. Jpn J Pharmacol 1992;58:435–42.

Tallarida RJ, Murray RB. Manual of pharmacologic calculations with computer programs. Berlin: Springer; 1987.

Tao PL, Law PY, Loh HH. Decrease in delta and mu opioid receptor binding capacity in rat brain after chronic etorphine treatment. J Pharmacol Exp Ther 1987;240:809–16.

Vaupel DB, Cone EJ, Johnson RE, Su TP. Kappa opioid partial agonists activity of the enkepahlalin-like pentapeptide BW9242C based on urination and in vitro studies in humans and animals. J Pharmacol Exp Ther 1990;252:225–34.