Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 85 (2006) 762–768
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Persistent exercise attenuates nicotine- but not clonidine-induced antinociception in female rats

Wendy Foulds Mathes, Robin B. Kanarek *

Department of Psychology, Tufts University, 490 Boston Avenue, Medford, MA 02155, USA

Received 14 February 2006; received in revised form 3 November 2006; accepted 20 November 2006
Available online 2 January 2007

Abstract

Exercise decreases the antinociceptive effects of opiate drugs. It has been hypothesized that the exercise-induced attenuation of opiate drug action is the result of the development of cross-tolerance between endogenous opioids released during exercise and exogenous opiates. The present study was designed to evaluate the role of exercise on non-opiate antinociception. Female Long–Evans rats were allowed ad lib access to running wheels. After 3 weeks, antinociceptive responses of animals were measured using the tail flick test following the administration of clonidine or nicotine. Nicotine and clonidine both produced dose-dependent increases in antinociceptive responses. Active animals were significantly less sensitive to nicotine-induced antinociception than inactive animals. There was no difference between the two groups in clonidine-induced antinociception. The results of these experiments suggest that exercise does not attenuate non-opioid, clonidine-induced antinociception. However, exercise does attenuate nicotine-induced antinociception. Therefore, the effect of persistent exercise on analgesic drugs is not specific to opiates.

Keywords: Exercise; Opioids; Antinociception; Nicotine; Clonidine; Female rats

1. Introduction

Chronic access to voluntary running wheel activity decreases the antinociceptive effects of opiate drugs. This effect is observed with several μ and κ opioid receptor agonists, as well as with the morphine metabolite and μ receptor agonist, morphine-6 glucuronide (M6G) (Kanarek et al., 1998; D'Anci et al., 2000; Mathes and Kanarek, 2001; Smith and Yancey, 2003; Smith et al., 2004). It is hypothesized that the decrease in antinociceptive potency of opiate agonists in active animals compared to inactive animals is due to the development of cross-tolerance with endogenous opioid peptides released during exercise (Kanarek et al., 1998).

While there exists a large body of evidence linking exercise and opioids (e.g. Carr et al., 1981; Gambert et al., 1981; Blake et al., 1984; Grossman and Sutton, 1985; Radosevich et al., 1989; Pierce et al., 1993), exercise affects many other biological systems (e.g. Ardies et al., 1989; Dishman, 1997; Morishima et al., 2006; McMaster and Carney, 1985; Mollenauer et al., 1992). For example, prolonged running wheel activity protects against norepinephrine (NE) depletion in the locus coeruleus (LC) and attenuates escape deficit after uncontrollable footshock (Dishman, 1997). HPLC analysis of brain monoamines shows that exercising animals exposed to uncontrollable footshock have higher NE levels in the LC than their sedentary counterparts (Dishman, 1997). More recently, Morishima et al. (2006) have demonstrated that rats genetically predisposed to high levels of wheel running have increased extracellular NE levels in the hippocampus, leading to the down regulation of α24-noradrenergic receptors. McMaster and Carney (1985) have reported that chronic treadmill running attenuates the behaviorally disruptive effects of the anticholinergic drugs scopolamine hydrobromide and scopolamine methylbromide in rats. Therefore, it is possible that the attenuating effects of exercise on drug action are a general phenomenon caused by exercise-induced alterations in several physiological systems.

* Corresponding author. Tel.: +1 617 627 5902; fax: +1 617 627 3178.
E-mail address: robin.kanarek@tufts.edu (R.B. Kanarek).

0091-3057/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.11.011

The present experiments were designed to determine the effects of exercise on antinociceptive drugs that act through noradrenergic and cholinergic systems. Active and inactive female rats were evaluated for antinociception following the administration of clonidine or nicotine. Clonidine is an α2-noradrenergic agonist used to control blood pressure in humans (Roehrich and Gold, 1987). Clonidine administration often results in a non-opioid antinociception similar to that seen after cold-water swimming (Roehrich and Gold, 1987; Kiefel and Bodnar, 1991). Nicotine is an agonist of the nicotinic acetylcholine receptor. Acting though the α4β2 subtypes of the nicotinic receptor, nicotine administration leads to profound antinociception in several species (Damaj et al., 2000).

2. Materials and methods

2.1. Animals

Fifty-six female virus and antibody free Long–Evans rats weighing 200–250 g (Charles River Laboratories, Portage, MI) were used. Animals were maintained in a temperature- and humidity-controlled room with a reverse 12:12-h light/dark cycle (lights off: 0800–2000 h).

Rats were housed individually in standard stainless-steel hanging laboratory cages or in Wahmann (Baltimore, MD) LC-34 activity wheels with stainless steel side cages. Active animals had 24 h access to the running wheels for at least 3 weeks prior to nociceptive testing. Wheel turns were monitored by a microswitch that recorded each 360° of rotation. In all experiments, body weights of rats in the active and inactive groups were similar at the start of the experiment.

All animals had ad libitum access to ground Purina laboratory rodent chow (#5001) presented in Wahmann 306A food cups with lids and tap water. Food and water intakes, body weights and wheel rotations were recorded every other day.

2.2. Drugs

Clonidine HCl (Sigma, St. Louis, MO) and nicotine bitartrate (Sigma, St. Louis, MO) were dissolved in 0.9% sterile saline and administered in a volume of 1 ml/kg.

2.3. Nociceptive testing

Rats were tested for antinociception using the tail flick method. Prior to the initiation of nociceptive testing, running wheel doors were closed and remained closed for the duration of the testing period. Animals, held in a clean cloth by the same experimenter, were placed on the tail flick apparatus and their tails gently smoothed into a groove with a photocell. A light heat source focused on the tail was then activated and was automatically turned off when the animal flicked its tail. To prevent tissue damage, if the animal failed to flick its tail the light source automatically turned off after 9 s. The intensity of the light was set so that baseline tail flick latencies averaged between 2 and 4 s. Baseline latencies were defined as the median of 3 tail flicks, each separated by approximately 20 s. All testing was done during the dark portion of the daily diurnal cycle.

2.4. Experiment 1: clonidine antinociception

Eight rats housed in standard laboratory cages and eight rats housed in running wheels were tested. Baseline tail flick latencies were determined immediately prior to drug administration. Clonidine was administered IP in a cumulative dosing paradigm where the animals were injected every 30 min with increasing doses of the drug as described by Duttaroy et al. (1997). The resulting cumulative doses used were 0.05, 0.1, 0.2, 0.4 and 0.8 mg/kg. Decay of the drug's antinociceptive effect was also evaluated. Tail flick latencies were measured 30, 60, 90 and 120 min after the last clonidine injection.

2.5. Experiment 2: clonidine antinociception

To increase the maximum dose of clonidine being examined, antinociception was evaluated following the administration of 0.18, 0.32, 0.56, 1.0 and 1.8 mg/kg clonidine, IP, in drug-naïve active and inactive rats. Experimental procedures were carried out as described above.

2.6. Nicotine antinociception

Ten drug-naïve rats housed in standard laboratory cages and ten drug-naïve rats housed in running wheels were used. Baseline tail flick latencies were determined immediately prior to drug administration. Nicotine was administered SC in a cumulative dosing paradigm. As previous work had demonstrated that nicotine's antinociceptive actions occurred more rapidly than those of clonidine (Kiefel and Bodnar, 1991; Mandillo and Kanarek, 2001), the rats were injected with increasing doses of nicotine every 5 min. The resulting cumulative doses were 0.03, 0.1, 0.3, 1.0 and 3.0 mg/kg nicotine base. To evaluate the decay of the drug's antinociceptive actions, tail flick latencies were measured every 5 min for 40 min after the last nicotine injection.

Table 1
Average daily caloric intakes, body weights and wheel rotations in active and inactive rats

	Body weight (g)	Caloric intake (kcal)	Wheel rotations
Experiment 1			
Active	261.25±6.92	89.83±3.97**	10569.4±869.3
Inactive	274.50±9.60	67.55±2.94	
Experiment 2			
Active	248.70±7.30	84.97±2.21**	12878.6±1639.6
Inactive	265.22±10.18	67.53±4.29	
Experiment 3: nicotine			
Active	225.33±5.07*	75.83±4.38	7556.4±749.0
Inactive	244.80±4.94	65.01±3.13	

All values expressed as mean±SEM.
Significant difference between active and inactive animals *$p<0.05$ **$p<0.01$.

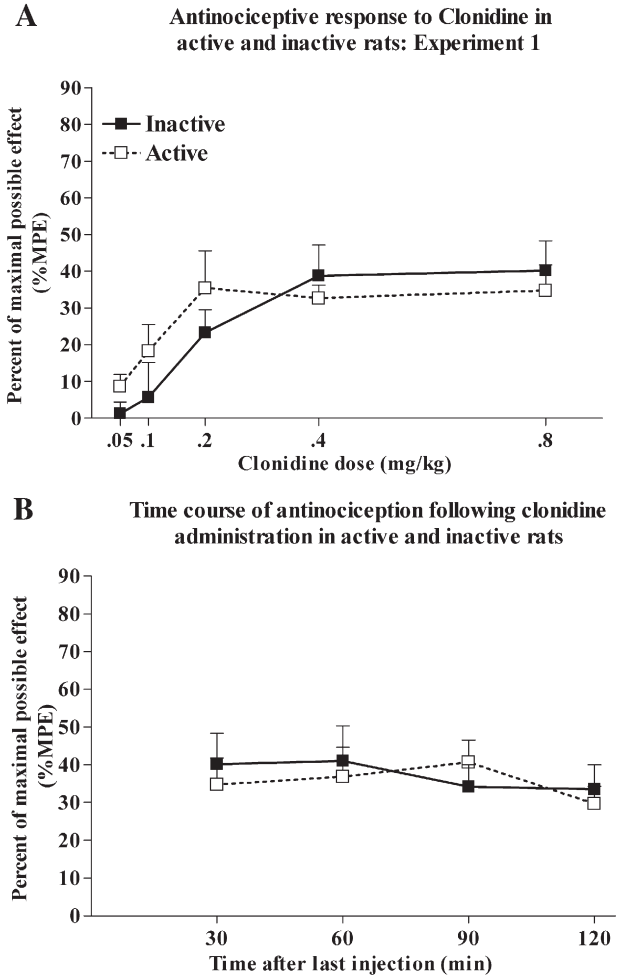

Fig. 1. There were no differences between active and inactive rats in the magnitude (A) or duration (B) of clonidine-induced antinociception at low doses.

2.7. Data analysis

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

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

Data were analyzed using SPSS (SPSS Inc., Chicago, IL). Main effects within each experiment were determined by repeated measures ANOVA. Independent samples *t*-tests were used to analyze daily food and water intakes and body weight data.

2.8. Ethical statement

All pharmacological and experimental procedures were approved by the Tufts University Institutional Animal Care and Use Committee prior to the onset of these experiments.

3. Results

3.1. Food and fluid intakes and body weights

In all three experiments, daily caloric intakes of rats running in activity wheels were greater than those of inactive rats (Table 1). Similarly, in all three experiments water intakes were significantly greater in the active animals than in inactive animals. In both studies in which clonidine was used, although active animals weighed less than inactive animals at the time of nociceptive testing, these differences in body weight were not significant (Table 1). In the nicotine study, active animals weighed significantly less than active animals at the time of nociceptive testing. In all three studies, running wheel activity increased gradually over the 3-week period prior to testing. For the week immediately preceding antinociceptive testing, active animals averaged 10,569.4±869.3 rotations per day in experiment 1, 12,878.6±1639.6 rotations per day in experiment 2 and 7556.4±749.0 in experiment 3.

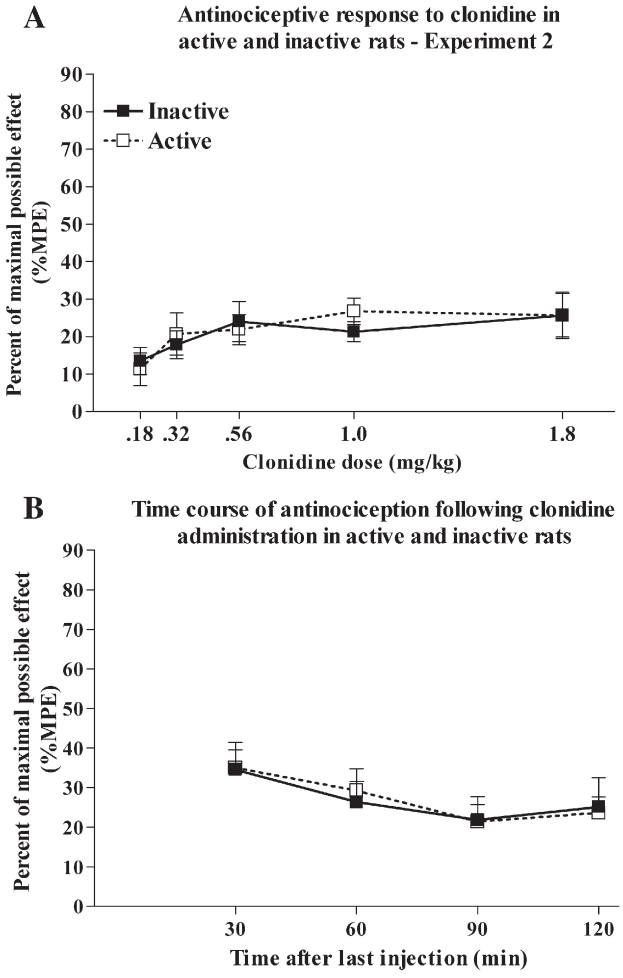

Fig. 2. There were no differences between active and inactive rats in the magnitude of clonidine-induced antinociception at higher doses (A). There was a significant decrease in clonidine-induced antinociception over the 120-min recovery period ($p=0.020$), but the duration of antinociception did not differ between active and inactive animals (B).

3.2. Experiment 1: clonidine-induced antinociception

Repeated measures ANOVA revealed a significant dose-dependent effect of clonidine administration on tail flick latencies ($F(4,56)=15.138$, $p<0.001$) such that higher doses of the drug induced greater levels of antinociception. There was no effect of activity ($F(1,14)=0.304$, $p=0.590$) (Fig. 1) on clonidine-induced antinociception. Likewise, there was no difference between active and inactive animals in the time of recovery from clonidine-induced antinociception ($F(1,14)=0.004$, $p=0.950$). In fact, antinociceptive responding did not decrease over the time period tested in either of the two groups ($F(2,28)=1.547$, $p=0.231$) (Fig. 1).

3.3. Experiment 2: Clonidine-induced antinociception

Similar to the results of the first clonidine experiment, repeated measures ANOVA revealed a significant dose-dependent effect of clonidine administration on tail flick latencies ($F(4,68)=4.027$, $p<0.01$) but no effect of activity ($F(1,17)=0.046$, $p=0.832$) (Fig. 2). Antinociceptive responding significantly decreased as a function of time since the last injection ($F(2,28)=3.580$, $p=0.020$); however, there were no differences between active and inactive animals in recovery time from clonidine-induced antinociception ($F(1,17)=0.003$, $p=0.995$). Antinociceptive responding in both the active and inactive animals was significantly higher 30 min after the last clonidine injection than it was at 120 min after the last injection ($t=-2.183$, $p<0.05$).

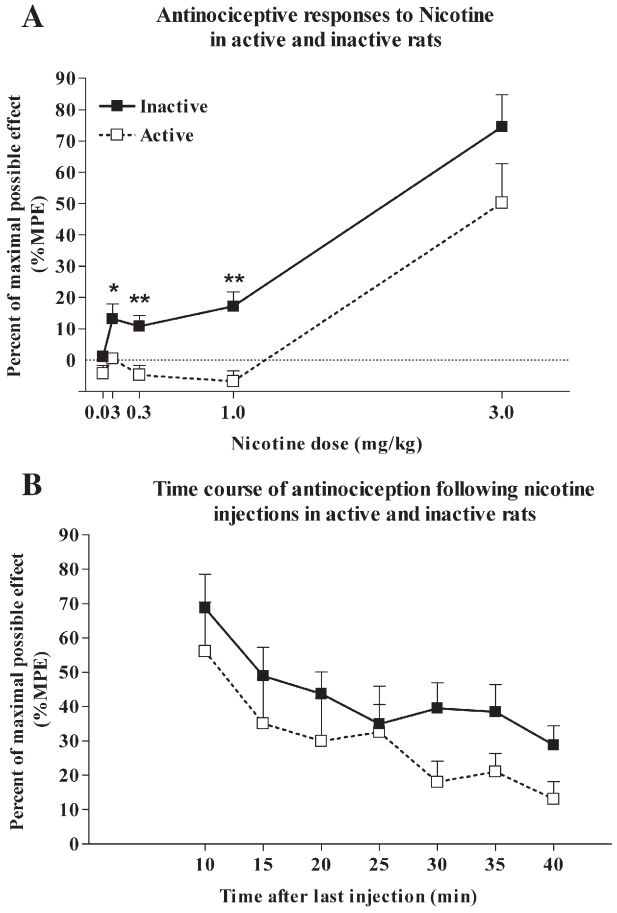

Fig. 3. Overall, active rats were significantly less sensitive to the antinociceptive effects of nicotine administration than their inactive counterparts ($p<0.001$) (A). *$p<0.05$, significant difference between active and inactive rats at each dose of nicotine. Antinociceptive responding declined over time in both groups. However, the duration of antinociception was shorter in active animals than in inactive animals (B).

3.4. Nicotine-induced antinociception

Repeated measures ANOVA revealed a significant dose-dependent effect of nicotine administration on tail flick latencies ($F(4,68)=43.732$, $p<0.001$) such that antinociceptive responding increased as a function of increasing drug dose (Fig. 3). Additionally, active animals were significantly less sensitive to the antinociceptive properties of nicotine than inactive animals ($F(1,17)=14.747$, $p=0.001$) (Fig. 3). Because a number of active rats failed to obtain MPEs of 50%, IED_{25s} instead of IED_{50s} were compared in active and inactive rats. IED_{50s} of active rats (2.12±0.23 mg/kg) were significantly greater than those of inactive animals (1.32±0.22 mg/kg) ($F(1, 17)=6.32$, $p<0.05$).

There was no difference between active and inactive animals in the time of recovery from nicotine-induced antinociception ($F(1,17)=1.665$, $p=0.214$). However, antinociceptive responding did decrease as a function of time in both groups ($F(6,102)=12.326$, $p<0.001$) (Fig. 3).

4. Discussion

Animals allowed access to running wheels for 3 weeks showed significantly less antinociception following nicotine administration than their inactive counterparts. However, there was no difference in the antinociceptive potency of clonidine between the active and inactive rats or in the rate of recovery from nicotine- or clonidine-induced antinociception between active animals and inactive animals. The results of these experiments show that the decrease in sensitivity to antinociceptive drugs seen in active animals is not specific to opiate agonists. However, the attenuating effect of running wheel activity does not generalize to all antinociceptive drugs.

Research has demonstrated that running wheel activity attenuates the antinociceptive potency of opioid drugs (Kanarek et al., 1998; D'Anci et al., 2000; Mathes and Kanarek, 2001; Smith and Yancey, 2003; Smith et al., 2004). The experiments described within this manuscript are the first to describe the effects of running wheel activity on non-opioid analgesics. Although interactions between both nicotine and clonidine with the endogenous opioid system have been previously described (e.g. Tanda and Di Chiara, 1998; Mathieu-Kai and Besson, 1998; Wewers et al., 1999; Hahn et al., 2000; George et al., 2000; Berrendero et al., 2002; for a review, see Millan, 2002; Martin and Eisenach, 2001; Furst, 1999), these two analgesic agents exert their effects primarily through cholinergic and noradrenergic receptor activation, respectively.

Spinally mediated nicotine-induced antinociception results from the activation of the α4β2 nicotinic AchR subtype located

throughout the peripheral and central nervous systems (Bitner et al., 1998; Damaj et al., 2000). This activation leads to the increased release of several neurotransmitters, including NE (Li and Eisenach, 2002) and Ach. Although a large body of evidence exists examining the effects of exercise on cholinergic mechanisms within the cardiovascular and musculoskeletal systems, little data are available examining the effects of exercise on acetylcholine within the CNS. It is possible that exercise-induced alterations in cholinergic and noradrenergic mechanisms contributed to the decrease in nicotine-induced antinociception in exercising rats.

On the other hand, several studies have suggested that nicotine exerts some of its effects through interactions with the endogenous opioid system (e.g. Tanda and Di Chiara, 1998; Mathieu-Kai and Besson, 1998; Wewers et al., 1999; Hahn et al., 2000; George et al., 2000; Berrendero et al., 2002). For instance, the κ opioid agonists U50,488, U69,593 and IC-977 block the locomotor stimulating effects of nicotine administration (Hahn et al., 2000). This effect can be reversed with κ opioid receptor antagonists, indicating the involvement of the κ opioid receptor in nicotine-induced locomotor activity. Similarly, chronic nicotine administration leads to an up-regulation of μ opioid receptors in the striatum of male and female rats while, at the same time, decreasing striatal met-enkephalin levels (Wewers et al., 1999). Likewise, pretreatment with the κ opioid agonists U50488 and TRK-820 attenuates mecamylamine precipitated withdrawal symptoms in nicotine-dependent rats (Ise et al., 2002). Davenport et al. (1990) reported that nicotine administration prior to the central administration of β-funaltrexamine (β-FNA), an irreversible μ opioid receptor antagonist, attenuated β-FNA antagonism of morphine-induced antinociception. The authors hypothesized that nicotine administration led to endogenous opioid peptide release, which activated the μ opioid receptor and blocked β-FNA's effect (Davenport et al., 1990). Similarly, μ opioid receptor knock-out mice show a decreased response to the antinociceptive, rewarding and locomotor effects of nicotine administration compared to their wild-type counterparts (Berrendero et al., 2002). Moreover, naloxone attenuates nicotine-induced antinociception in mice (Zarrindast et al., 1997). Cross-tolerance to the antinociceptive and hypothermic effects of nicotine and morphine has also been demonstrated (Zarrindast et al., 1999, 2001). Thus, the decreased antinociceptive potency of nicotine in active rats compared with inactive controls may be due to nicotinic interactions with the endogenous opioid system.

Likewise, there is an abundance of data that shows that clonidine and opiates work synergistically to attenuate pain. In fact, clonidine is used clinically as an adjuvant to opiate analgesia. One of the benefits of using clonidine and other noradrenergic agonists in addition to opiate treatment is that noradrenergic agonists continue to elicit antinociception in subjects that have developed tolerance to opiate treatment (Millan, 2002; Martin and Eisenach, 2001; Furst, 1999). Running wheel activity leads to decreased opiate-induced antinociception, presumably the result of the development of cross-tolerance between endogenous opioids and exogenous opiates (Kanarek et al., 1998; Mathes and Kanarek, 2001; Smith and Yancey, 2003; Smith et al., 2004). The results of the present study show that clonidine is equipotent in inducing antinociception in active and inactive animals. Therefore, consistent with the clinical data, animals that demonstrate an apparent tolerance to the antinociceptive effects of opiates are still sensitive to the antinociceptive effects of clonidine.

The studies discussed herein were performed using female rats. Females were chosen because in previous studies in our laboratory, it was observed that females were more sensitive to the pain relieving action of nicotine than males (Mandillo and Kanarek, 2001) and run more in activity wheels than males (Kanarek et al., 1998). However, it is recognized that sex differences in pain sensitivity and responses to analgesic drugs, including nicotine and clonidine, are not uncommon findings (e.g. Kiefel and Bodnar, 1991; Craft and Milholland, 1998; Carstens et al., 2001; Mandillo and Kanarek, 2001). For example, both Craft and Milholland (1998) and Mandillo and Kanarek (2001) reported that the antinociceptive effects of nicotine were greater in female rats than in males. In contrast to these findings, Carstens et al. (2001) found that depending on the testing procedure, there were either no differences in nicotine-induced antinociception in male and female rats, or that males were more sensitive to the antinociceptive properties of nicotine than females. With respect to clonidine, few studies have address the role of sex in determining the pain relieving properties of the drug. However, as with nicotine, the results of studies which have addressed this issue are not consistent, with some pointing to sex differences in clonidine-induced antinociception (Kiefel and Bodnar, 1991; Nag and Mokha, in press) and others indicating no sex differences in pain sensitivity following drug administration (Kroin et al., 2003). A number of variables may contribute to the inconsistent findings with respect to the effects of sex on drug-induced analgesia. These factors included genetic background, the type of pain test employed, the intensity of the painful stimulus and the efficacy of the drug being tested (Craft, 2003). Detailed studies using these variables, as well as sex, will be needed for a more complete understanding of the role of activity in determining the antinociceptive potency of nicotine and clonidine.

Because female rats were used, it is important to address the possible effects that ovarian hormones may have had on the results. Alterations of ovarian hormones across the estrous cycle have been linked to changes in pain sensitivity (e.g. Cruz et al., 1996; Frye et al., 1993; Martinez-Gomez et al., 1994). Previous research from this laboratory has shown that voluntary running wheel activity does not alter the duration of the estrous cycle as measured using vaginal swabs (Mathes and Kanarek, 2001). The duration of estrous cycle in both active and inactive rats ranged from 3 to 6 days with the majority of the animals having cycles that were 4 or 5 days long. All phases of the estrus cycle were represented on any given day. In light of these findings, it is unlikely that any differences in pain sensitivity between active and inactive animals are due to exercise-induced alterations in ovarian hormones.

In summary, running wheel activity attenuates the antinociceptive effects of nicotine without altering the effects of clonidine. The fact that exercise attenuates the antinociceptive

effects of nicotine brings into question whether the effects of exercise on opiate-induced antinociception are due to cross-tolerance or to alterations in other physiological mechanisms. These results thus prompt the need for more investigation to uncover the mechanism by which long-term exercise alters the antinociceptive potency of drugs.

Acknowledgments

The authors would like to thank Rinah Yamamoto for her assistance on this project. This research was funded by NIDA grant number RO1 DA 04132.

References

Ardies CM, Morris GS, Erickson CK, Farrar RP. Both acute and chronic exercise enhance in vivo ethanol clearance in rats. J Appl Physiol 1989;66: 555–60.

Berrendero F, Kieffer BL, Maldonado R. Attenuation of nicotine-induced antinociception, rewarding effects, and dependence in mu-opioid receptor knock-out mice. J Neurosci 2002;22:10935–40.

Bitner RS, Nikkel AL, Curzon P, Arneric SP, Bannon AW, Decker MW. Role of the nucleus raphe magnus in antinociception produced by ABT-594: immediate early gene responses possibly linked to neuronal nicotinic acetylcholine receptors on serotonergic neurons. J Neurosci 1998;18: 5426–32.

Blake MJ, Stein EA, Vomachka AJ. Effects of exercise training on brain opioid peptides and serum LH in female rats. Peptides 1984;5:953–8.

Carr DB, Bullen BA, Skrinar GS, Arnold MA, Rosenblatt M, Beitins IZ, et al. Physical conditioning facilitates the exercise-induced secretions of β-endorphin and β-lipotropin in women. New Engl J Med 1981;305: 560–3.

Carstens E, Anderson KA, Simons CT, Carstens MI, Jinks SL. Analgesia induced by chronic nicotine infusion in rats, differences by gender and pain test. Psychopharmacology 2001;157:40–5.

Craft RM. Sex differences in drug-and non-drug-induced analgesia. Life Sci 2003;72:2675–88.

Craft RM, Milholland RB. Sex differences in cocaine- and nicotine-induced antinociception in the rat. Brain Res 1998;809:137–40.

Cruz Y, Martinez-Gomez M, Manzo J, Hudson R, Pacheco P. Changes in pain threshold during the reproductive cycle of the female rat. Physiol Behav 1996;59:543–7.

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.

Damaj MI, Meyer EM, Martin BR. The antinociceptive effects of α7 nicotinic agonists in an acute pain model. Neuropharmacology 2000;39:2785–91.

Davenport KE, Houdi AA, Van Loon GR. Nicotine protects against υ-opioid receptor antagonism by β-funaltrexamine: evidence for nicotine-induced release of endogenous opioids in brain. Neurosci Lett 1990;113:40–6.

Dewey WL, Harris LS. The tail flick test. In: Ehrenpreis S, Neidle A, editors. Methods in narcotics research. New York: Marcel Dekker; 1975. p. 101–9.

Dishman RK. Brain monoamines, exercise, and behavioral stress: animal models. Med Sci Sports Exerc 1997;29:63–74.

Duttaroy A, Kirtman R, Farrell F, Phillips M, Philippe J, Monderson T, et al. The effects of cumulative dosing on the analgesic potency of morphine. Pharmacol Biochem Behav 1997;58:67–71.

Frye CA, Cuevas CA, Kanarek RB. Diet and estrous cycle influence pain sensitivity in rats. Pharmacol Biochem Behav 1993;45:255–60.

Furst S. Transmitters involved in antinociception in the spinal cord. Brain Res Bull 1999;48:129–41.

Gambert SR, Garthwaite TL, Pontzer CH, Cook EE, Tristani FE, Duthie EH, et al. Running elevates plasma β-endorphin immunoreactivity and ACTH in untrained human subjects. Proc Soc Exp Biol Med 1981;168: 1–4.

George TP, Verrico CD, Xu L, Roth RH. Effects of repeated nicotine administration and footshock stress on rat mesoprefrontal dopamine systems: Evidence for opioid mechanisms. Neuropsychopharmacology 2000;23: 79–88.

Grossman A, Sutton JR. Endorphins: What are they? How are they measured? What is their role in exercise? Med Sci Sports Exerc 1985;17: 74–81.

Hahn B, Stolerman IP, Shoaib M. Kappa-opioid modulation of nicotine-induced behavior. Neuropharmacology 2000;29:2848–55.

Ise Y, Narita M, Nagase H, Suzuki T. Modulation of κ-opioidergic systems on mecamylamine-precipitated nicotine-withdrawal aversion in rats. Neurosci Lett 2002;323:164–6.

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;77: 1–9.

Kiefel JM, Bodnar RJ. Roles of gender and gonadectomy in pilocarpine and clonidine analgesia in rats. Pharmacol Biochem Behav 1991;41:153–8.

Kroin JS, Buvanendran A, Nagalla SKS, Tuman KJ. Postoperative pain and analgesic responses are similar in male and female Sprague–Dawley rats. Can J Anesth 2003;50:904–8.

Li XH, Eisenach JC. Nicotinic acetylcholine receptor regulation of spinal norepinephrine release. Anesthesiology 2002;96:1450–6.

Mandillo S, Kanarek RB. Chronic sucrose intake enhances nicotine-induced antinociception in female but not male Long–Evans rats. Pharmacol Biochem Behav 2001;68:211–9.

Martin TJ, Eisenach JC. Pharmacology of opioid and nonopioid analgesics in chronic pain states. J Pharmacol Exp Ther 2001;299:811–7.

Martinez-Gomez M, Cruz Y, Salas M, Hudson R, Pacheco P. Assessing pain threshold in the rat: changes with estrus and time of day. Physiol Behav 1994;55: 651–7.

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.

Mathieu-Kai A-M, Besson M-J. Repeated administration of cocaine, nicotine and ethanol: effects on preprodynorphin, preprotachykinin A and preproenkephalin mRNA expression in the dorsal and the ventral striatum of the rat. Mol Brain Res 1998;54:141–51.

McMaster SB, Carney JM. Changes in drug sensitivity following acute and chronic exercise. Pharmacol Biochem Behav 1985;23:191–4.

Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355–474.

Mollenauer S, Bryson R, Speck C, Chamberlin JR. Effects of exercise on ethanol-induced hypothermia and loss of righting response in C57BL/6J mice. Pharmacol Biochem Behav 1992;43:285–90.

Morishima M, Harada N, Hara S, Sano A, Seno H, Takahashi A, et al. Monoamine Oxidase A activity and norepinephrine level in hippocampus determine hyperwheel running in SPORTS rats. Neuropsychopharm 2006;31:2627–38.

Nag S, Mokha SS. Activation of $\alpha_2$adrenoceptors in the trigeminal region produced sex-specific modulation of nociception in the rat. Neuroscience, in press.

Pierce EF, Eastman NW, Tripathi HI, Olson KG, Dewey WL. Plasma β-endorphin immunoreactivity: response to resistance exercise. J Sports Sci 1993;11:499–502.

Radosevich PM, Nash JA, Lacy DB, Odonovan C, Willians PE, Abumrat NN. Effects of low-and high intensity exercise on plasma and cerebrospinal fluid levels of ir-β-endorphin, ACTH, cortisol, norepinephrin and glucose in the conscious dog. Brain Res 1989;498:89–98.

Roehrich H, Gold MS. Clonidine. Adv Alcohol Subst Abuse 1987;7:1-16.

Smith MA, Yancey DL. Sensitivity to the effects of opioids in rats with free access to exercise wheels: mu-opioid tolerance and physical dependence. Psychopharmacology 2003;168:426–34.

Smith MA, McClean JM, Bryant PA. Sensitivity to the effects of a kappa opioid in rats with free access to exercise wheels: differential effects across behavioral measures. Pharmacol Biochem Behav 2004;77:49–57.

Tanda G, Di Chiara G. A dopamine-mu1 link in the rat ventral tegmentum shared by palatable food (fonzies) and non-psychostimulant drugs of abuse. Eur J Neurosci 1998;10:1179–87.

Wewers ME, Dhatt RV, Snively TA, Tejwani GA. The effect of chronic nicotine on antinociception, opioid receptor binding and met-enkephalin levels in rats. Brain Res 1999;822:107–13.

Zarrindast MR, Pazouki M, Nassir-Rad S. Involvement of cholinergic and opioid receptor mechanisms in nicotine-induced antinociception. Pharmacol Toxicol 1997;81:209–13.

Zarrindast MR, Khoshayand MR, Shafaghi B. The development of cross-tolerance between morphine and nicotine in mice. Eur Neuropsychopharmacol 1999;9:227–33.

Zarrindast MR, Barghi-Lashkari S, Shafizadeh M. The possible cross-tolerance between morphine-and nicotine-induced hypothermia in mice. Pharmacol Biochem Behav 2001;68:283–9.