Pharmacology, Biochemistry and Behavior 91 (2009) 295–302

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The α_1 adrenergic receptor antagonist prazosin reduces heroin self-administration in rats with extended access to heroin administration

Thomas N. Greenwell *[,1], Brendan M. Walker [2], Pietro Cottone, Eric P. Zorrilla, George F. Koob

Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, 10550 North Torrey Pines Road, SP30-2400, La Jolla, CA 92037, United States

ARTICLE INFO

Article history:
Received 3 May 2007
Received in revised form 27 June 2008
Accepted 1 July 2008
Available online 23 July 2008

Keywords:
Drug abuse
Escalation
Opiate
Norepinephrine
alpha-1 receptor

ABSTRACT

Previous studies have reported that noradrenergic antagonists alleviate some of the symptoms of opiate withdrawal and dependence. Clinical studies also have shown that modification of the noradrenergic system may help protect patients from relapse. The present study tested the hypothesis that a dysregulated noradrenergic system has motivational significance in heroin self-administration of dependent rats. Prazosin, an α_1-adrenergic antagonist (0.5, 1.0, 1.5 and 2.0 mg/kg, i.p.), was administered to adult male Wistar rats with a history of limited (1 h/day; short access) or extended (12 h/day; long access) access to intravenous heroin self-administration. Prazosin dose-dependently reduced heroin self-administration in long-access rats but not short-access rats, with 2 mg/kg of systemic prazosin significantly decreasing 1 h and 2 h heroin intake. Prazosin also reversed some changes in meal pattern associated with extended heroin access, including the taking of smaller and briefer meals (at 3 h), while also increasing total food intake and slowing the eating rate within meals (both 3 h and 12 h). Thus, prazosin appears to stimulate food intake in extended access rats by restoring meals to the normal size and duration. The data suggest that the α_1 adrenergic system may contribute to mechanisms that promote dependence in rats with extended access.

© 2008 Elsevier Inc. All rights reserved.

1. Introduction

To understand better the mechanisms underlying heroin addiction, animal models relevant to components of heroin dependence have been sought. Such models of dependence have involved opiate exposure/withdrawal paradigms (e.g., chronic morphine pellet implantation and multiple morphine injections), reinstatement of heroin-seeking, and operant self-administration in limited access sessions (Young et al., 1977; Bozarth and Wise, 1985; Shaham et al., 1998; Carrera et al., 1999; Erb and Stewart, 1999; Hutcheson et al., 2001; Azar et al., 2003). However, only recently have models studying extended drug access in rats been modified to incorporate the excessive and increasing drug intake associated with human addiction.

Heroin and cocaine are both self-administered in increasing quantities when animals are allowed extended, as opposed to limited, access, a finding termed "escalation" (Ahmed and Koob, 1998; Ahmed et al., 2000). Extended access to heroin consumption (11 h/day) increased intake and persistently increased the motivation to take heroin, reflected by the increased responding for heroin after footshock stress, more lever responding for heroin, and slower extinction of heroin-seeking behavior compared with short-access controls (Ahmed et al., 2000). Rats allowed 23 h access to a fixed unit dose of heroin showed even more dramatic, spontaneous escalation in intravenous self-administration of heroin (Chen et al., 2006). These "escalation" models of heroin self-administration have face and predictive validity for modeling the compulsive drug intake associated with heroin dependence in humans (Ahmed and Koob, 1998; Ahmed et al., 2000; Koob et al., 2004; Chen et al., 2006). Short-access rats, in contrast, limit consumption to lower, more stable levels, and show significantly decreased extinction responding, a faster extinction rate, and are less prone to footshock-induced reinstatement than long-access rats (Ahmed et al., 2000). Thus, the short- *vs.* long-access models of intake offer further predictive validity via the inclusion of controls that, while having experience with opioid self-administration, differ in heroin intake, helping to discern the effects of treatments directed toward opioid use *vs.* excessive use.

Additionally, rat models of extended heroin access have been useful particularly when comparing the behavioral profiles associated with different stages in the development of heroin dependence, such as circadian and meal pattern measures of food intake (Chen et al., 2006). Previous research has shown that changes in the amount and

* Corresponding author. Division of Neuroscience and Behavior, National Institute on Alcohol Abuse and Alcoholism, 5635 Fishers Lane, Room 2052, MSC 9304, Bethesda, MD 20892, United States. Tel.: +1 301 443 1192; fax: +1 301 443 1650.
E-mail address: greenwellt@mail.nih.gov (T.N. Greenwell).
[1] Present address: Division of Neuroscience and Behavior, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852, United States.
[2] Present adress: Department of Psychology, Washington State University, Pullman, WA 99164, United States.

0091-3057/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2008.07.012

pattern of food intake may be valuable as a sensitive indicator of the effects of heroin (Thornhill et al., 1976) and the development of heroin dependence (Chen et al., 2006). Meal pattern analysis revealed that smaller and briefer, but more, meals of food were taken within 7 days of daily extended (23 h) access to heroin (Chen et al., 2006). Thus, neuroadaptive mechanisms contributing to dependence also may be reflected not only in increased heroin self-administration, but also in changes in homeostatic processes measured by meal pattern analysis.

The challenge now is to identify the neuroadaptive mechanisms that mediate the change in motivation for heroin that occurs during the transition to dependence. Neurochemical systems implicated include γ-aminobutyric acid (GABA), dopamine, corticotropin-releasing factor (CRF), neuropeptide Y, and norepinephrine (Koob, 1992). An interaction between opioidergic and noradrenergic systems has been proposed (Aghajanian, 1978), and α_2- and β-adrenergic receptors have been targeted for alleviating opioid withdrawal (Redmond and Huang, 1982; Funada et al., 1994). Clonidine, an α_2-adrenergic receptor agonist, has been reported to be effective in reducing opiate withdrawal symptoms in humans and animals (Gold et al., 1978; Katz, 1986). Recently, studies have noted the importance of noradrenergic signaling in mediating not only opiate withdrawal (for review, see Maldonado 1997), but also opiate reward (Olson et al., 2006).

Furthermore, data suggest that α_1 receptor modulation of the effects of opioids may be more important than previously hypothesized. Prazosin, an α_1 adrenergic receptor antagonist, binds all three α_1 receptor subtypes with high affinity (Nicholas et al., 1996). Prazosin was found to block acquisition of morphine-induced conditioned place preference in mice (Zarrindast et al., 2002). Mice lacking α_{1b} receptors had decreased locomotor hyperactivity and an attenuated conditioned place preference in response to morphine administration (Drouin et al., 2002). Prazosin also reversed tolerance to morphine analgesia and attenuated morphine withdrawal-induced weight loss in mice (Ozdogan et al., 2003; Zarrindast et al., 2002; Drouin et al., 2002). Therefore, the α_1 receptor appears to have a role in opiate reward, tolerance, and withdrawal, and thus was hypothesized to have a role in the increased heroin self-administration that develops with prolonged drug access.

The present study tested the hypothesis that administration of prazosin would decrease drug self-administration in long-access (12 h) rats compared with limited access (1 h) control rats and determined whether prazosin would reverse some additional measures associated with dependence, such as the meal pattern changes observed in rats with extended access to heroin (Chen et al., 2006).

2. Materials and methods

2.1. Animals

Adult male Wistar rats ($n = 14$; Charles River, Raleigh, NC) weighing between 200 and 250 g at the beginning of the experiments were housed in groups of three in a humidity- and temperature-controlled (22 °C) vivarium on a 12 h light/dark cycle with *ad libitum* access to food and water. The animals were allowed to acclimate to these conditions for at least 7 days. All procedures adhered to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

2.2. Surgery

Rats were anesthetized with an isoflurane/oxygen vapor mixture (2.0–2.5%) and prepared with chronic intravenous catheters as previously described (Caine et al., 1993). Briefly, the catheters consisted of a 14 cm length of silastic tubing fitted to a guide cannula (Plastics One, Roanoke, VA) bent at a right angle. The skull was exposed and cleaned, and four skull screws were implanted, one in each quadrant. The bent guide cannula was secured rostral-caudally to the center of the skull using cranioplastic cement. The catheter tubing was passed subcutaneously from the animal's skull to the right jugular vein, which was punctured with an 18-gauge needle. Then, 3.7 cm of the silastic tubing were inserted into the vein and tied gently with suture thread. Surgery was conducted under sterile conditions, and all connections involving the catheter were kept as sterile as possible. All animals were allowed to recover for a minimum of 1 week before receiving access to heroin self-administration. Catheters were flushed daily with 0.2 ml of sterile physiological saline containing heparin (30 USP units/ml) and the antibiotic Timentin (SmithKline Beecham Pharmaceuticals, Philadelphia, PA) given in the same volume to all rats.

2.3. Catheter patency

Catheter patency was tested whenever an animal not receiving drug pretreatments displayed behavior outside baseline parameters. In these cases, 0.1 ml of the ultra short-acting barbiturate anesthetic Brevital sodium (1% methohexital sodium, Eli Lilly, Indianapolis, IN) was administered through the catheter. Animals exhibiting prominent signs of anesthesia (pronounced loss of muscle tone) within 3 s of intravenous injection and recovering within 5 min after the injection were assumed to have patent catheters. Animals that lost catheter patency per this assay during the course of the experiment were excluded from the experiment and data analysis.

2.4. Self-administration chambers

For each session, the animals were placed into operant cages located inside ventilated, sound-attenuating chambers equipped with a 1.1 W miniature light bulb synchronized to the 6 AM (lights on)/6 PM (light off) light/dark cycle. The catheter fittings on the animals' skulls were connected to polyethylene tubing contained inside a protective metal spring (tether) that was suspended into the operant chamber from a liquid swivel attached to a balance arm. Heroin was delivered by a syringe pump (Razel) as described in Caine et al. (1993). Modifications from the Caine et al. (1993) procedure were made to use a two-rotation-per-minute syringe pump motor to push on a 30 ml syringe for 4.5 s to deliver a 0.1 ml infusion. The 30 ml syringe size was used to ensure that sufficient drug was available for the full 12 h session without having to replace syringes. Each operant session was performed using one active and one inactive retractable lever that extended approximately 1 in. into the chamber. Following completion of each fixed-ratio 1 (FR1) requirement, a 28 V white stimulus light located above the active lever signaled the delivery of a drug and remained on for a 20 s timeout (TO) period, during which responses were recorded but had no scheduled consequences.

2.5. Drugs

Prazosin hydrochloride (the hydrochloride salt of 1-[4-amino-6,7-dimethoxy-2-quinazolinyl]-4-[2-furoyl] piperazine; Sigma) was administered intraperitoneally (2 ml/kg) in sterile water. Heroin (3,6-diacetylmorphine; International Union of Pure and Applied Chemistry: [5α,6α]-7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol diacetate [ester]) was generously provided by the National Institute on Drug Abuse (Bethesda, MD) and dissolved in 0.9% normal sterile saline.

2.6. Intravenous heroin self-administration

Rats were allowed to nosepoke for food (Cat# 5TUM, 45 mg pellets, TestDiet, Richmond, IN) or water (100 µl per nosepoke) for 23 h/day for 5 days prior to surgery. After 7 days of recovery from surgery,

animals were allowed to lever press for intravenous heroin (0.06 mg/kg/0.1 ml infusion/4.5 s; FR1; TO 20 s, 1 h per day). No food restriction was used to establish operant responding. During the acquisition of heroin self-administration (8 days of 1 h sessions), food and water were not available to the rats while in the test chambers. After each training session, rats were returned to their home cages with food and water freely available until the next day's session. Following the 8 days of acquisition, rats were separated into short (1 h) and long (12 h) heroin access groups. Long- and short-access sessions began at the onset of the rats' dark cycle. Short-access rats had no access to food or water during the 1 h limited access sessions. Long-access rats had concurrent access to food and water during the 12-hour heroin session. Rats in both access conditions had free access to food and water in the home cage between daily self-administration sessions (Table 1). During sessions for the long-access rats, lever presses for drug infusion and nosepokes for food and water were recorded with 10 ms resolution as described previously (Chen et al., 2006; O'Dell et al., 2007).

2.7. Prazosin testing

Long-access rats were tested for 12 h/day for 6 days/week, and short-access rats were tested 1 h/day for 6 days/week (n=7/group). Long-access rats were allowed to remain in the boxes for an additional 3 h, during which food and water, but not drug, were available (drug levers were retracted) and responses were recorded until the experimenter arrived to remove the animals from the boxes and return them to their home cages. Three animals that eventually were used in the prazosin experiments had a 21 day history of 23 h access but then were changed to 12 h access for 30 days before prazosin testing. As with the 12 h access rats, the 23 h access rats had no prior drug testing before prazosin. Because no difference in baseline 1 h or 12 h heroin responding in these three animals were observed, compared with rats with a history of continuous 12 h access, the two groups were combined to form the 12 h group used in the present experiment. Prazosin testing was done in a Latin-square design, with every other day as a testing day (one heroin day with no drug treatment to wash-out the prazosin), with one dose per day (Table 2). Prazosin testing did not begin until within-subjects ANOVA indicated that the LgA and ShA groups each did not differ in the mean response rate across 3 consecutive days of testing.

Prazosin was prepared in sterile water, sonicated for 15 min, and injected intraperitoneally. Injections in a volume of 2 ml/kg at doses of 0, 0.5, 1, 1.5, or 2 mg/kg were given 30 min prior to the start of operant sessions for heroin, based on an onset of action between 5 and 40 min in rats (Menkes et al., 1981). Operant responding for food and water was measured in the long-access rats for the duration that they were in the boxes. Food intake was corrected for body weight and metabolism using Kleiber's law [(gram food)/(body weight in kg)$^{0.75}$] (Sidhu, 1992). One rat's data were omitted from all analyses because of significant weight loss (>5% of body weight) from vehicle day 1 to test day 3 that occurred because of problems with the food hopper system.

Table 1
Prazosin testing schedule

12 h group	
5:30 PM	Prazosin or vehicle injection
6:00 PM	Food, water, and heroin available; rats in boxes
6:00 AM	Heroin levers retract; food and water still available
9:00 AM	Rats removed from boxes and placed in home cages with *ad libitum* access to food and water
1 h group	
10:30 AM	Prazosin or vehicle injection
11:00 AM	Heroin available; rats in boxes
12:00 PM	Short-access rats removed from boxes and placed in home cages with *ad libitum* access to food and water

Table 2
Prazosin testing schedule

Heroin day 53	Vehicle day 1
Heroin day 54	
Heroin day 55	Test day 1
Heroin day 56	
Heroin day 57	Test day 2
Heroin day 58	
Heroin day 59	Test day 3
Heroin day 60	
Heroin day 61	Test day 4
Heroin day 62	
Heroin day 63	Test day 5
Heroin day 64	
Heroin day 65	Vehicle day 2
Heroin day 66	

2.8. Meal pattern analysis

Meal pattern analysis was performed using a drinking-inclusive meal definition validated previously (Zorrilla et al., 2005). A meal was

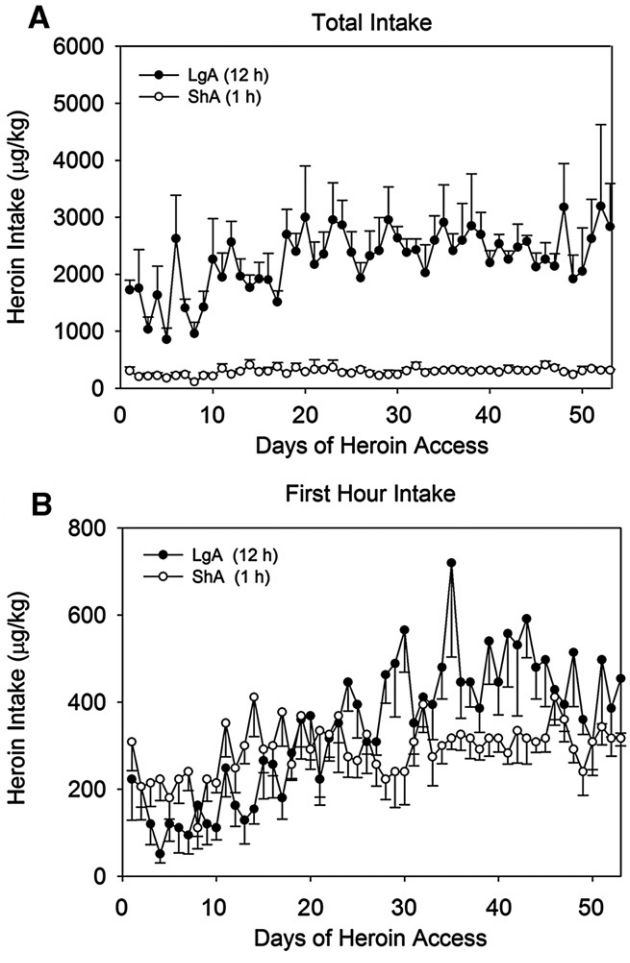

Fig. 1. Increased heroin intake in long-access (12 h) rats. Mean±SEM of heroin intake in µg/kg. 12 h rats (n=4) increased intake from ~1500 µg/kg over the first 3 days to 3200 µg/kg of heroin/day by day 52 (A). These data did not include rats given 23 h access to heroin because they did not have 53 days of 12 h heroin access. Comparison of first hour heroin intake of long- and short-access rats reveal higher heroin intake in the long-access (12 h) rats after day 28 (B), but no significant differences in first hour heroin intake were observed.

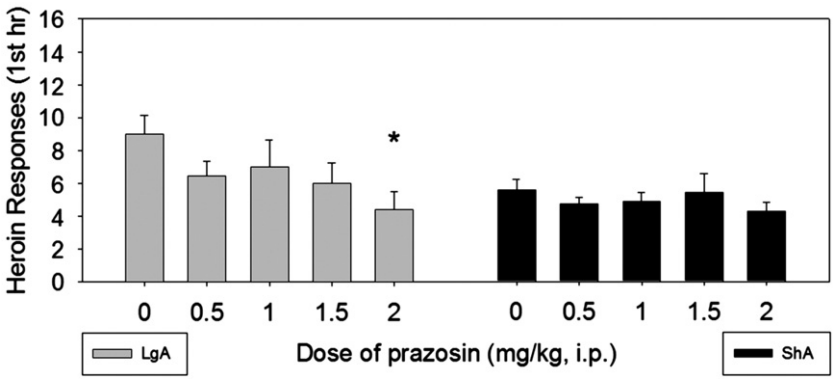

Fig. 2. Prazosin decreased first hour heroin responding in long-access (12 h) rats but not in short-access (1 h) rats. Prazosin at a dose of 2 mg/kg significantly (*$p<0.05$) reduced heroin intake (mean ± SEM) in the first hour in 12 h access rats (long-access, $n=7$, left panel). No effect was observed in short-access rats ($n=7$, right panel) at any of the doses tested. *$p<0.05$, overall dose effect and a specific effect at the 2 mg/kg dose compared with vehicle.

defined as a burst of responding for either food or water that contained at least five food-directed responses or 0.225 g (Demaria-Pesce and Nicolaidis, 1998; Zorrilla et al., 2005), and the maximum interval (inter-response interval) between ingestive responses that was considered to continue the ongoing meal was set as 5 min between either food or water responses, based on previous observations (Zorrilla et al., 2005). This threshold criterion was selected to provide the most stable estimate of meal structure under the current experimental conditions (Zorrilla et al., 2005). Under this definition, perceived meal termination was uniformly and immediately followed by the behavioral satiety sequence (Zorrilla et al., 2005). Also following perceived meal termination, rats initially exhibited an extremely low probability of subsequent meal initiation that monotonically increased with time, findings consistent with predictions of satiety (Zorrilla et al., 2005).

The following parameters for nocturnal meal structure then were calculated: (i) total quantity of prandial food intake, (ii) total duration of prandial intake, (iii) meal frequency (number of meals), (iv) average meal size, (v) average meal duration, and (vi) response rate (eating and drinking rate) during meals. Meal duration was calculated as the total time from the first to the last response of a meal, and duration of eating within the meal was calculated as the duration of consecutive responses for food. Thus, transitions between eating and drinking were included in the total meal duration but not in the duration of eating. Meal sizes for eating were calculated as the average number of food-directed responses during a meal. Rates of eating were calculated by dividing each meal size by the duration of eating within meals. In the absence of experimental treatments, rats normally exhibit high stability in these measures of meal patterns (calculated as an intraclass correlation of absolute agreement; Shrout and Fleiss, 1979; average $r=0.77$ across 3 weeks of testing; Zorrilla et al., 2005).

Table 3
Effects of prazosin on heroin responding

Time-point	Heroin responding				
	Prazosin dose (i.p.)				
	Vehicle	0.5 mg/kg	1.0 mg/kg	1.5 mg/kg	2 mg/kg
1 h	9.0±1.2	6.7±1.1	6.7±1.6	5.4±1.2	5.0±1.3*
2 h	12.6±2.0	11.0±1.8	10.6±2.4	9.3±2.7	9.1±1.8*
3 h	14.9±2.6	14.4±2.4	15.6±3.7	13.0±3.3	13.6±2.6
Total (12 h)	53.4±9.5	47.6±6.6	47.3±8.6	54.6±13.4	47.9±9.5

Effects of prazosin on heroin responding. Mean ± SEM of heroin responses in long-access rats ($n=7$) over 1, 2, 3, and 12 h. Prazosin had a significant effect on heroin responding at the 1 h and 2 h time-points (*$p<0.05$), but not at the 3 h or 12 h time-points.

2.9. Statistical analysis

Heroin self-administration data (1 h, 2 h, 12 h) were analyzed using one-way or two-way ANOVA as dictated by the experimental design. Meal pattern data were analyzed using repeated measures ANOVAs, except a Student's *t*-test was performed to identify meal pattern changes induced by heroin from day 0 to the average of days 51 and 54 (before and after the first vehicle day, respectively, for a more representative measure). When the assumption of sphericity was not met, the Greenhouse–Geisser correction was used. In addition to ANOVAs, we performed a dose-by-access linear trend contrast analysis. We subsequently identified the source of any interaction with dose using simple main effects (within-subjects) and individual means comparisons with Dunnett's test. With dose–response functions, a powerful way of using ANOVA is to perform linear trend analysis (Bewick et al., 2004; Bretz et al., 2004; Rosner, 1995; Sheskin, 2004). With a significant dose × access linear trend contrast, a simple main effect of dose on heroin intake within each group was performed using the error term $MS_{B\times subjw\cdot groups}$ (means squares of the within-subjects dose factor [*B*] × subjects within-groups), and the *F* ratio = $[(MS_{b\ at\ a1})/(MS_{B\times subjw\cdot groups})]$, where *b* = a given dose, and *a* = a given access condition (Winer, 1962). Statistical analyses were conducted

Table 4
Effects of extended heroin access on meal microstructure

	Day 0	Day 51 and 54 average
3 h time-point		
Total food intake (g/kg$^{0.75}$)	8.7±0.8	9.2±1.0
Total duration of prandial intake (food only) (min)	11.8±1.2	19.5±1.6**
Meal frequency	2.6±0.4	4.6±0.4**
Average meal size (g/kg$^{0.75}$)	3.7±0.4	2.2±0.4*
Average meal duration (food only) (min)	5.0±0.6	4.9±0.8
Eating rate (mg/s)	6.5±0.3	5.2±1.0
12 h intake		
Total food intake (g/kg$^{0.75}$)	33.8±1.9	31.0±2.8
Total duration of prandial intake (food only) (min)	47.0±3.5	61.2±4.1*
Meal frequency	10.3±1.0	16.3±1.2**
Average meal size (g/kg$^{0.75}$)	3.5±0.3	1.9±0.1**
Average meal duration (food only) (min)	4.9±0.4	3.8±0.3*
Eating rate (mg/s)	6.4±0.2	5.5±0.7

Effects of extended heroin access on meal microstructure. Mean ± SEM of meal pattern measures at 3 h and 12 h from day 0 (pre-heroin) to days 51 and 54 (the average days near prazosin testing, which began on day 55) in long-access rats ($n=7$). At 3 h, extended heroin access significantly increased the total duration of prandial intake and meal frequency and decreased average meal size. At 12 h, extended heroin access significantly increased total duration of prandial intake and meal frequency and decreased average meal size and average meal duration. *$p<0.05$, **$p<0.001$.

Table 5
Prazosin effect on food intake in long-access rats

Food Intake (g/kg$^{0.75}$)					
Time-point	Prazosin dose (i.p.)				
	Vehicle	0.5 mg/kg	1.0 mg/kg	1.5 mg/kg	2.0 mg/kg
1 h	2.6±1.3	4.3±1.1	3.8±0.8	2.3±0.5	4.5±1.2
2 h	5.7±0.9	9.1±1.5*	8.1±1.2	5.4±0.7	7.8±0.9
3 h	8.5±1.4	13.2±1.2*	12.9±1.2	11.2±0.7	12.5±1.1*
12 h	30.6±2.9	39.7±1.5*	39.2±3.2	35.3±0.8	36.1±2.3

Effect of prazosin on food intake in long-access rats. Mean±SEM of g/kg food intake in long-access rats (n=7), corrected for body weight using Kleiber's equation (Sidhu, 1992). Food intake was increased by prazosin compared with vehicle at 2, 3, and 12 h, but not at 1 h. *p<0.05.

using SPSS v.12.0 (Chicago, IL) and Microsoft Excel 2003 (Redmond, WA). The level of significance was set at p<0.05.

3. Results

3.1. Heroin escalation

For all figures, the error bars in the figures reflect between-subject variability, whereas the statistical test included each animal as its own control. Rats allowed access to 12 h heroin/day for 40 days (n=4) increased their heroin intake to 3.2 mg/kg by day 24 (Fig. 1). These data did not include rats given 23 h access to heroin because they did not have 53 days of 12 h heroin access. A two-way, mixed design ANOVA from day 1 to day 53 of heroin access with time as a repeated measure and access condition (long or short) as a between-subjects factor also revealed an effect of Time on 1 h heroin intake [overall ANOVA: F(52,624)=3.837, p<0.001; linear contrast: F(1,12)=26.531, p<0.001]. A linear contrast Time×Access interaction was observed [F(1,12)=13.263, p<0.01], indicating that the long-access rats significantly increased their first hour intake to a greater degree than the short-access rats. A one-way repeated measures ANOVA on 1 h heroin intake in long-access rats from day 1 to day 53 revealed a significant increase of heroin intake over time [overall ANOVA: F(52,312)=4.835, p<0.001; linear contrast: F(1,6)=24.592, p<0.01], whereas a one-way repeated measures ANOVA on heroin intake in short-access rats yielded no significant increase in heroin intake over time (p>0.05).

3.2. Effects of prazosin on heroin self-administration

A two-way mixed ANOVA on the first hour of heroin self-administration (Dose(repeated measure)×Access(between-subjects factor)) revealed a significant effect of dose (overall ANOVA: F(4,48)=3.357; p<0.05; linear contrast: F(1,12)=14.237; p<0.01) but no main effect of drug access (p=0.168). Although no significant Dose×Access interaction was observed on 1 h heroin self-administration in the overall ANOVA, a significant linear contrast Dose×Access interaction on 1 h heroin self-administration was observed between the 1 h and 12 h groups [F(1,12)=6.328; p<0.05). Follow-up analysis showed a significant linear dose trend analysis [F(1,6)=12.688; p<0.05) in the long-access, but not short-access (p=0.229) group. Pairwise comparisons showed a significant effect in heroin self-administration between the vehicle and 2 mg/kg dose (p<0.05) in long-access rats (Fig. 2).

A one-way repeated measures ANOVA on 2 h heroin responding in the long-access group revealed no significant effect of prazosin on heroin self-administration in the overall ANOVA, but a significant dose-dependent linear contrast effect was observed [F(1,6)=17.763; p<0.01). Pairwise comparisons showed that the highest dose of prazosin (2 mg/kg) significantly reduced heroin responding compared with the vehicle condition (p<0.05) (Table 3). No effect of prazosin was observed on 3 h or 12 h heroin responding (Table 3).

3.3. Food and water responding

For both 3 h and 12 h time-points, no change was found in the weight-normalized cumulative intake of food from day 0 to days 51 and 54 of heroin access (Table 4). Prazosin did not reliably alter 30 min (data not shown) or 1 h (Table 5) cumulative food intake determined by repeated measures ANOVA (p>0.05 for all measures). However, 2 h cumulative food intake was increased by prazosin [F(4,24)=3.023; p<0.05], with a significant increase from vehicle at the 0.5 mg/kg dose (p<0.05; Table 5). The 0.5 mg/kg (p<0.05) and 2.0 mg/kg (p<0.01) doses of prazosin increased 3 h cumulative food intake significantly compared with vehicle treatment [ANOVA: F(4,24)=3.661; p<0.05; linear contrast: F(1,6)=13.236; p<0.05] (Table 5). Prazosin also increased 12 h cumulative food intake at the 0.5 mg/kg dose [ANOVA: F(4,24)=2.735; p<0.05, pairwise comparison between vehicle and 0.5 mg/kg dose: p<0.01; no significant linear contrast] (Table 5). Prazosin did not cause any significant changes in water intake at any time-point (data not shown).

3.4. Meal pattern analysis

Similar to previous reports (Chen et al., 2006), the long-access rats showed significant changes in meal pattern from day 0 (pre-heroin) to day 54 (Table 4). In the 12 h access group, rats ate significantly more frequently (number of meals; p<0.01), but also smaller (average meal size; p<0.01) and briefer meals (average meal duration; p<0.05) by day 54 as determined by repeated-measures ANOVAs (Table 4). However, the overall time spent eating was significantly increased (total meal duration; p<0.05) following 54 days of extended access to heroin self-administration.

Similar to what was observed with the 12 h analysis, rats also exhibited more, but smaller, meals within the first 3 h of the dark (active) cycle and an increase in total eating duration by day 54 compared with day 0, as shown by event recordings from a representative rat (Fig. 3). However, the average meal duration was not briefer within the first 3 h, unlike the observation in the 12 h analysis (Table 4).

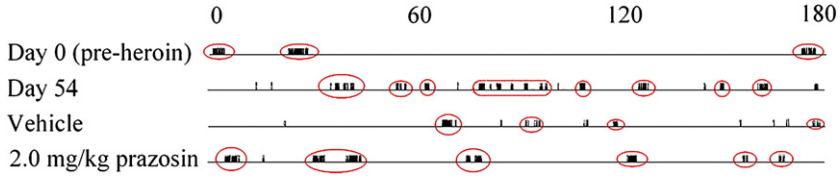

Fig. 3. Prazosin reverses heroin-induced meal changes over the first 3 h of heroin access. The event recordings from a representative rat showing food (up-ticks) responses day 0 and day 54 of heroin extended access days (Day 0, Day 54), vehicle and the highest dose (2.0 mg/kg) of prazosin. Meals are the red ovals, and were defined as a burst of responding for food and water that contained at least five food-directed responses (Demaria-Pesce and Nicolaidis 1998; Zorrilla et al., 2005) The rat eats about three large meals before being exposed to heroin (Day 0), but then increases the amount of meals but decreases the size of the meals (Day 54; second trace). Prazosin increases the duration, frequency and size of meals compared to Day 54 and the vehicle, but the rat still has shorter and more meals than Day 0, indicating that there is not a full reversal on eating pattern by prazosin.

Table 6
Effects of prazosin on meal microstructure

	Prazosin dose (i.p.) (days 55–64)				
	Vehicle	0.5 mg/kg	1.0 mg/kg	1.5 mg/kg	2.0 mg/kg
3 h intake					
Total food intake ($g/kg^{0.75}$)	8.4±1.6	13.2±1.2*	11.7±1.2	10.1±1.2	12.2±1.0*
Total duration of prandial intake (food only) (min)	18.5±3.8	32.1±5.3	23.0±4.0	33.2±3.8*	32.3±4.6
Meal frequency	5.3±0.6	6.3±0.8	5.7±0.6	6.0±0.5	5.0±0.5
Average meal size ($g/kg^{0.75}$)	1.5±0.2	2.3±0.3*	2.2±0.3	1.7±0.2	2.6±0.2**
Average meal duration (food only) (min)	3.4±0.5	5.2±0.6	4.4±1.1	5.5±0.4*	6.7±1.0*
Eating rate (mg/s)	5.5±1.3	4.7±0.6	5.9±0.8	3.5±0.7	4.6±0.8
12 h intake					
Total food intake ($g/kg^{0.75}$)	30.4±2.7	39.7±1.3*	35.6±2.1	34.1±1.1	35.8±2.6*
Total duration of prandial intake (food only) (min)	67.1±15.4	76.0±7.7	58.1±4.5	91.9±10.9	75.3±7.6
Meal frequency	17.7±1.2	19.1±1.8	17.3±1.4	18.7±1.2	16.3±2.1
Average meal size ($g/kg^{0.75}$)	1.7±0.1	2.2±0.2	2.1±0.2	1.9±0.2	2.4±0.3
Average meal duration (food only) (min)	3.7±0.7	4.0±0.3	3.5±0.4	4.9±0.4	4.8±0.4
Eating rate (mg/s)	5.8±1.1	5.8±0.6	6.5±0.5	4.4±0.7	5.3±0.6

Effects on prazosin on meal microstructure. Mean±SEM of meal pattern measures over different prazosin doses (0, 0.5, 1.0, 1.5, and 2.0 mg/kg) in long-access rats ($n=7$). At 3 h, prazosin significantly increased total food intake, total duration of prandial intake, average meal size, and average meal duration. At 12 h, prazosin significantly increased total food intake. *$p<0.05$, **$p<0.001$.

3.5. Effect of prazosin on meal pattern analysis

Table 6 shows a summary of prazosin's effect on meal pattern analysis results. Within the first 3 h, prazosin significantly reversed the effects of extended heroin access by increasing the average meal size [overall ANOVA: $F(4,24)=3.602$; $p<0.05$; linear contrast: $F(1,6)=7.947$, $p<0.05$] and the duration of meals [overall ANOVA: $F(1,6)=2.881$; $p<0.05$; linear contrast: $F(1,6)=5.991$; $p<0.05$). For an example of a representative rat, see Fig. 3. Prazosin also increased the total quantity [overall ANOVA: $F(4,24)=3.046$; $p<0.05$; linear contrast: $F(1,6)=6.284$, $p<0.05$] and duration [overall ANOVA: $F(4,24)=3.369$; $p<0.05$; linear contrast: $F(1,6)=22.343$, $p<0.01$] of prandial food intake (Table 6). Pairwise comparisons showed that the 0.5 mg/kg and 2 mg/kg doses of prazosin significantly increased the average size of meals and total food intake (Table 6). The 1.5 mg/kg and 2 mg/kg doses of prazosin increased the total duration of eating, with the former reliably increasing the average meal duration compared with vehicle treatment. Prazosin did not reliably alter the frequency of meals or the eating rate within meals during the first 3 h. Thus, prazosin appeared to reverse, at least partially, the effect of heroin escalation on some meal pattern measures (Fig. 3, and see also Tables 4 and 6).

During the entire 12 h access period, prazosin significantly increased cumulative food intake [$F(1,6)=3.434$; $p<0.05$] that pairwise comparisons revealed to be higher than vehicle at the 0.5 mg/kg ($p<0.01$) and 2 mg/kg ($p<0.05$) doses (Table 6). Prazosin did not significantly alter other 12 h meal measures (i.e., total duration of food intake, meal frequency, average meal size and duration; $ps>0.05$; Table 6).

4. Discussion

The present study demonstrated that rats given extended access to heroin increased their heroin intake, while rats given limited (1 h) access did not increase their intake across 53 days of 12 h/day access, similar to previous results (Ahmed et al., 2000; Chen et al., 2006). This study also showed that prazosin, an α_1-adrenergic receptor antagonist, reduced heroin self-administration in the first 1 h of access in long-access rats, but not in short-access rats. Prazosin also significantly reduced 2 h heroin responding in long-access rats. Similar results have been observed with increase self-administration of ethanol and cocaine associated with dependence (Wee et al., 2008; Walker and Koob, 2007). In addition, prazosin reversed several meal pattern changes induced by prolonged heroin access, including the taking of smaller and briefer meals (at 3 h) associated with opioid dependence. Prazosin also increased food intake.

In this experiment, heroin was available 7 days/week for 14 consecutive days during extended access and thereafter 6 days/week, which suggests that any variability in the pattern of heroin intake can not be attributed to off-days or withdrawal because the variability remains throughout the experiment. In other experiments, we have seen a small "deprivation effect" after the day off, reflected by an increase in heroin intake (similar to the alcohol deprivation effect, only not as pronounced). However, on the days after this deprivation-induced increase in intake, a decrease in intake occurred, and this might account for some of the variability after day 14. During prazosin testing, rats had one intervening day off between test day 2 and test day 3, and the overall reduction of heroin intake by prazosin might have even been attenuated by a slight increase in heroin intake for rats tested on day 3 due to the break between day 2 and day 3, but the Latin-square design (mixed doses each day) would control for any day effects of heroin intake. Thus, the effect we observed with prazosin administration was attributed to a true drug effect.

Many studies have shown that the adrenergic system is important in modulating aspects of drug dependence, but few studies have investigated the role of the α_1-adrenergic receptor in these changes. The present results show that blockade of α_1-receptors selectively reduces heroin self-administration during the first hour of access in rats with a history of long access to heroin, providing support for the hypothesis that overactivation of noradrenergic systems may play a role in the motivational aspects of dependence via α_1 receptors. Prazosin at 2 mg/kg reduced cumulative heroin responding for up to 2 h (Table 3), suggesting that the time-course of intraperitoneal administration of prazosin at this dose lasts for at least 2 h. Heroin responding returned to baseline levels at the high dose after 6 h of heroin access, which is in agreement with a duration of action of prazosin of 2–3 h.

The onset of action of prazosin administered intravenously is 5–40 min in rats (Menkes et al., 1981), but to our knowledge, no animal studies have directly assessed prazosin's duration of action. In humans, the half-life of prazosin is 2.2–3.7 h (Bateman et al., 1979), suggesting that prazosin may have stopped working within 3.5 h of injection in this experiment. We analyzed the results of the experiment by hour (data not shown) and found that the high dose of prazosin resulted in compensation of heroin intake after 6 h, whereby higher heroin intake was seen after 6 h so that no differences were observed in 12 h heroin intake. The effect of prazosin on heroin administration was the most pronounced during the first two h of lever pressing.

Numerous studies have shown that noradrenergic brain signaling is involved in opiate withdrawal (Redmond and Huang, 1982; Taylor et al., 1991; Maldonado, 1997; Aston-Jones et al., 1999). Adrenergic antagonists alleviate opiate withdrawal, and blockade of α_1 receptors appear to have a role in reducing some opiate withdrawal symptoms (van der Laan, 1987; Ozdogan et al., 2003). The α_1-adrenergic receptor is expressed in a variety of regions of the central nervous system, including the olfactory system, cerebral cortex, cingulate cortex, amygdala, reticular nucleus of the thalamus, hippocampus, dentate gyrus, and trigeminal nucleus (McCune et al., 1993; Day et al., 1997).

Noradrenergic systems in the brain also have a key role in mediating behavioral and autonomic responses to stress, and a known link exists between stress and drug addiction (Koob, 1992;

Shaham et al., 2000; Morilak et al., 2003). Clinical studies suggest that prazosin reduces the symptoms of posttraumatic stress disorder, especially night awakening and nightmares, in combat veterans (Raskind et al., 2000; Raskind et al., 2002; Raskind et al., 2003). Thus, prazosin may reduce heroin responding by alleviating negative emotional or arousal states that are hypothesized to drive the increased heroin responding associated with extended access. The bed nucleus of the stria terminalis may be important for the noradrenergic influence in driving motivational effects of drug dependence based on studies of adrenergic agent microinfusion into specific brain regions. The α_2-adrenergic agonist clonidine and β-adrenergic antagonists reduced opiate withdrawal when microinjected into the bed nucleus of the stria terminalis (Aston-Jones et al., 1999; Delfs et al., 2000). A subtype of the α_1-adrenergic receptor (α_{1a}) has been localized to the bed nucleus of the stria terminalis as well as various feeding-related hypothalamic nuclei (including the paraventricular and ventromedial hypothalamus) (Day et al., 1997). One hypothesis is that the bed nucleus of the stria terminalis also may be important for α_1-adrenergic receptor-mediated effects, especially negative emotional effects that may drive opioid self-administration, whereas the feeding effects may be mediated through the paraventricular nucleus of the hypothalamus.

Alternative mechanisms to explain the α_1 antagonist-mediated decrease of heroin responding in long-access rats include weakened positive reinforcement (Dworkin et al., 1988), attenuation of incentive-learning (Hutcheson et al., 2001), and blunting of the incentive salience of the drug reinforcer (Pecina et al., 2006). Prazosin attenuated morphine-induced acquisition of conditioned place preference and opiate withdrawal in mice (van der Laan, 1987; Ozdogan et al., 2003), suggesting that blockade of reward effects or alleviation of negative reinforcement processes occurs. However, based on the present study, because prazosin did not alter heroin self-administration in short-access rats, the results suggest that the α_1-adrenergic system is differentially recruited only after extended access to heroin. Also, prazosin itself does not have rewarding effects when measured by conditioned place preference (Zarrindast et al., 2002).

Prazosin decreased heroin self-administration and increased total cumulative food intake, indicating that the drug did not non-specifically impair appetitive behavior. Prazosin reversed reductions in meal size and meal duration that resulted from prolonged access to heroin self-administration, while increasing the duration of prandial intake even further beyond heroin-naive levels (Tables 5 and 6). Prazosin increased food intake by increasing the size of meals, which also became longer individually and in aggregate. The literature suggests that prazosin also can reverse anti-appetitive effects (hypophagia) of acute cocaine exposure (Wellman et al., 2002). Human clinical data suggest that α_1 antagonists increase appetite, and, conversely, the α_1 agonist phenylpropanolamine has been used as an appetite suppressant in weight loss efforts (Bray, 2000), in part because it suppresses food intake via an α_1-adrenergic receptor-dependent mechanism (Wellman and Davies, 1992; Wellman et al., 1997). Thus, the observed orexigenic effects of prazosin may be attributable to not only normalization of the effects of extended heroin access but also to direct stimulatory actions on appetite.

The paraventricular nucleus (PVN) appears to be a very important site of action for the α_1-ligand-mediated effects on feeding. Prazosin at 2 mg/kg (i.p.) reversed the feeding-suppressive effects of α_1 agonists injected into the PVN but had no effect on feeding when administered alone (Wellman and Davies, 1992). In addition to α_1 receptors, dopamine D_1 receptors also are important for anorectic actions in the PVN (Cheng and Kuo, 2003). Thus, the PVN might be a major brain area where systemic prazosin exerts the reversal of heroin-induced changes in feeding patterns described in the present study.

An alternative hypothesis to explain the effects of prazosin on increased meal size is that prazosin reversed a highly sensitive index of opioid dependence (Chen et al., 2006). According to this hypothesis, the meal pattern changes would anticipate and possibly precede other motivational effects in the development of dependence. Indeed, prazosin decreased heroin intake at the 1 h and 2 h time-points but did not affect feeding measures until the 2 h time-point. Also, lower doses of prazosin appeared to more potently influence the meal pattern measure, whereas only the highest prazosin dose decreased heroin self-administration in long-access rats. We observed similar responding for food and water after prazosin testing as before prazosin. Thus, we concluded that the prazosin may have caused a transient reversal in heroin-induced meal pattern changes. However, the increase in food and water responding during prazosin treatment additionally served as a control for any non-specific effects on appetitive responding in the long-access rats. The data show that prazosin selectively decreases heroin intake through the measure of operant heroin responses on an active lever while there are slight increases in food intake, arguing against a non-specific effect of the drug on appetitive responding or that there were any effects on locomotor responding. However, one caveat is that food and water are necessary commodities, and future studies may utilize saccharin responses as a non-drug reinforcing control rather than food and water. A final consideration is that the concurrent access to food and water in the long-access rats may have potentiated the effect of prazosin on heroin responding in the long-access rats. Buprenorphine produced greater decreases in cocaine self-administration when rats had concurrent access to food and water than in rats with no food or water (Comer et al., 1996). However, prazosin reduced cocaine intake in only long-access rats and not in short-access rats given no concurrent access to food or water (Wee et al., 2008), suggesting again that prazosin selectively decreased heroin self-administration independent of increases in food intake in the current study. Determining whether separate α_1-adrenergic brain circuits control feeding and compulsive drug self-administration will be challenges for future research.

In summary, these data provide evidence for a role of the noradrenergic system in driving the increased heroin self-administration observed in rats with extended access. The data presented here, combined with previous studies, suggest that the α_1-adrenergic receptor may be a key mediator of opioid dependence mechanisms and that activation of noradrenergic systems may help drive elevated opioid self-administration in dependence. Prazosin also may reverse heroin-induced meal pattern changes and/or act on separate systems that control feeding, thus showing some potential overlap of noradrenergic mechanisms in stress, feeding, and compulsive drug intake.

Acknowledgements

Supported by National Institutes of Health grants DA019295 (F32 to TNG) and DA004043 (GFK) from the National Institute on Drug Abuse and the Pearson Center for Alcoholism and Addiction Research. The authors thank Mr. Robert Lintz for excellent technical assistance and Mr. Michael Arends for editorial assistance.

References

Aghajanian GK. Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine. Nature 1978;276:186–8.
Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. Science 1998;282:298–300.
Ahmed SH, Walker JR, Koob GF. Persistent increase in the motivation to take heroin in rats with a history of drug escalation. Neuropsychopharmacology 2000;22:413–21.
Aston-Jones G, Delfs JM, Druhan J, Zhu Y. The bed nucleus of the stria terminalis. A target site for noradrenergic actions in opiate withdrawal. Ann NY Acad Sci 1999;877:486–98.
Azar MR, Jones BC, Schulteis G. Conditioned place aversion is a highly sensitive index of acute opioid dependence and withdrawal. Psychopharmacology (Berl) 2003;170:42–50.
Bateman DN, Hobbs DC, Twomey TM, Stevens EA, Rawlins MD. Prazosin, pharmacokinetics and concentration effect. Eur J Clin Pharmacol 1979;16:177–81.

Bewick V, Cheek L, Ball J. Statistics review 9: one-way analysis of variance. Crit Care 2004;8:130–6.
Bozarth MA, Wise RA. Toxicity associated with long-term intravenous heroin and cocaine self-administration in the rat. JAMA 1985;254:81–3.
Bray GA. A concise review on the therapeutics of obesity. Nutrition 2000;16:953–60.
Bretz F, Pinheiro JC, Branson M. On a hybrid method in dose finding studies. Methods Inf Med 2004;43:457–60.
Caine SB, Lintz R, Koob GF. Intravenous drug self-administration techniques in animals. In: Sahgal A, editor. Behavioural Neuroscience: A Practical Approach, vol. 2. New York: Oxford University Press; 1993. p. 117–43.
Carrera MR, Schulteis G, Koob GF. Heroin self-administration in dependent Wistar rats: increased sensitivity to naloxone. Psychopharmacology (Berl) 1999;144:111–20.
Chen SA, O'Dell LE, Hoefer ME, Greenwell TN, Zorrilla EP, Koob GF. Unlimited access to heroin self-administration: independent motivational markers of opiate dependence. Neuropsychopharmacology 2006;31:2692–707.
Cheng JT, Kuo DY. Both (1-adrenergic and D1-dopaminergic neurotransmissions are involved in phenylpropanolamine-mediated feeding suppression in mice. Neurosci Lett 2003;347:136–8.
Comer SA, Lac ST, Wyvell CL, Carroll ME. Combined effects of buprenorphine and a nondrug alternative reinforcer on IV cocaine self-administration in rats maintained under FR schedules. Psychopharmacology (Berl) 1996;125:355–60.
Day HE, Campeau S, Watson Jr SJ, Akil H. Distribution of alpha 1a-, alpha 1b- and alpha 1d-adrenergic receptor mRNA in the rat brain and spinal cord. J Chem Neuroanat 1997;13:115–39.
Delfs JM, Zhu Y, Druhan JP, Aston-Jones G. Noradrenaline in the ventral forebrain is critical for opiate withdrawal-induced aversion. Nature 2000;403:430–4.
Demaria-Pesce VH, Nicolaidis S. Mathematical determination of feeding patterns and its consequence on correlational studies. Physiol Behav. 1998;65:157–70.
Drouin C, Darracq L, Trovero F, Blanc G, Glowinski J, Cotecchia S, et al. Alpha1b-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. J Neurosci 2002;22:2873–84.
Dworkin SI, Guerin GF, Goeders NE, Smith JE. Kainic acid lesions of the nucleus accumbens selectively attenuate morphine self-administration. Pharmacol Biochem Behav 1988;29:175–81.
Erb S, Stewart J. A role for the bed nucleus of the stria terminalis, but not the amygdala, in the effects of corticotropin-releasing factor on stress-induced reinstatement of cocaine seeking. J Neurosci 1999;19:RC35.
Funada M, Suzuki T, Sugano Y, Tsubai M, Misawa M, Ueda H, et al. Role of beta-adrenoceptors in the expression of morphine withdrawal signs. Life Sci 1994;54: PL113–8.
Gold MS, Redmond Jr DE, Kleber HD. Clonidine blocks acute opiate-withdrawal symptoms. Lancet 1978;2:599–602.
Hutcheson DM, Everitt BJ, Robbins TW, Dickinson A. The role of withdrawal in heroin addiction: enhances reward or promotes avoidance? Nat. Neurosci. 2001;4:943–7.
Katz JL. Effects of clonidine and morphine on opioid withdrawal in rhesus monkeys. Psychopharmacology (Berl) 1986;88:392–7.
Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 1992;13:177–84.
Koob GF, Ahmed SH, Boutrel B, Chen SA, Kenny PJ, Markou A, et al. Neurobiological mechanisms in the transition from drug use to drug dependence. Neurosci Biobehav Rev 2004;27:739–49.
Maldonado R. Participation of noradrenergic pathways in the expression of opiate withdrawal: biochemical and pharmacological evidence. Neurosci Biobehav Rev 1997;21:91–104.
McCune SK, Voigt MM, Hill JM. Expression of multiple alpha adrenergic receptor subtype messenger RNAs in the adult rat brain. Neuroscience 1993;57:143–51.
Menkes DB, Baraban JM, Aghajanian GK. Prazosin selectively antagonizes neuronal responses mediated by alpha1-adrenoceptors in brain. Naunyn Schmiedebergs Arch Pharmacol 1981;317:273–5.
Morilak DA, Cecchi M, Khoshbouei H. Interactions of norepinephrine and galanin in the central amygdala and lateral bed nucleus of the stria terminalis modulate the behavioral response to acute stress. Life Sci 2003;73:715–26.
Nicholas AP, Hokfelt T, Pieribone VA. The distribution and significance of CNS adrenoceptors examined with in situ hybridization. Trends Pharmacol Sci 1996;17:245–55.
O' Dell LE, Chen SA, Smith RT, Specio SE, Balster RL, Paterson NE, et al. Extended access to nicotine self-administration leads to dependence: circadian measures, withdrawal measures, and extinction behavior in rats. J Pharmacol Exp Ther 2007;320:180–93.
Olson VG, Heusner CL, Bland RJ, During MJ, Weinshenker D, Palmiter RD. Role of noradrenergic signaling by the nucleus tractus solitarius in mediating opiate reward. Science 2006;311:1017–20.
Ozdogan UK, Lahdesmaki J, Scheinin M. Influence of prazosin and clonidine on morphine analgesia, tolerance and withdrawal in mice. Eur J Pharmacol 2003;460:127–34.
Pecina S, Schulkin J, Berridge KC. Nucleus accumbens corticotropin-releasing factor increases cue-triggered motivation for sucrose reward: paradoxical positive incentive effects in stress? BMC Biol 2006;4:8.
Raskind MA, Dobie DJ, Kanter ED, Petrie EC, Thompson CE, Peskind ER. The alpha1-adrenergic antagonist prazosin ameliorates combat trauma nightmares in veterans with posttraumatic stress disorder: a report of 4 cases. J Clin Psychiatry 2000;61:129–33.
Raskind MA, Thompson C, Petrie EC, Dobie DJ, Rein RJ, Hoff DJ, et al. Prazosin reduces nightmares in combat veterans with posttraumatic stress disorder. J Clin Psychiatry 2002;63:565–8.
Raskind MA, Peskind ER, Kanter ED, Petrie EC, Radant A, Thompson CE, et al. Reduction of nightmares and other PTSD symptoms in combat veterans by prazosin: a placebo-controlled study. Am J Psychiatry 2003;160:371–3.
Redmond DE, Huang YH. The primate locus coeruleus and effects of clonidine on opiate withdrawal. J Clin Psychiatry 1982;43:25–29.
Rosner B. Fundamentals of biostatistics. Boston: Duxbury; 1995.
Shaham Y, Erb S, Leung S, Buczek Y, Stewart J. CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor1 receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. Psychopharmacology (Berl) 1998;137:184–90.
Shaham Y, Erb S, Stewart J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Brain Res Rev 2000;33:13–33.
Sheskin DJ. Handbook of parametric and nonparametric statistical procedures. Boca Raton: Chapman and Hall; 2004. p. 678–9.
Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychol Bull 1979;86:420–8.
Sidhu KS. Basis for body weight exponent (0.75) as a scaling factor in energy metabolism and risk assessment. J Appl Toxicol 1992;12:309–10.
Taylor JR, Lewis VO, Elsworth JD, Pivirotto P, Roth RH, Redmond Jr DE. Yohimbine co-treatment during chronic morphine administration attenuates naloxone-precipitated withdrawal without diminishing tail-flick analgesia in rats. Psychopharmacology (Berl) 1991;103:407–14.
Thornhill JA, Hirst M, Gowdey CW. Disruption of diurnal feeding patterns of rats by heroin. Pharmacol Biochem Behav 1976;4:129–35.
van der Laan JW. Dopaminergic and alpha 1-adrenergic properties of B-HT920 revealed in morphine-dependent rats. Pharmacol Biochem Behav 1987;26:265–9.
Walker BM, Koob GF. The γ-aminobutyric acid-B receptor agonist baclofen attenuates responding for ethanol in ethanol-dependence rats. Alcohol Clin Exp Res 2007;31:11–8.
Wee S, Mandyam CD, Lekic DM, Koob GF. Alpha 1-noradrenergic system role in increased motivation for cocaine intake in rats with prolonged access. Eur Neuropsychopharmacol 2008;18:303–11.
Wellman P, Ho D, Cepeda-Benito A, Bellinger L, Nation J. Cocaine-induced hypophagia and hyperlocomotion in rats are attenuated by prazosin. Eur J Pharmacol 2002;455:117–26.
Wellman PJ, Davies BT. Reversal of cirazoline- and phenylpropanolamine-induced anorexia by the alpha 1-receptor antagonist prazosin. Pharmacol Biochem Behav 1992;42:97–100.
Wellman PJ, McMahon LR, Green T, Tole A. Effects of the alpha 1a-adrenoceptor antagonist RS-17053 on phenylpropanolamine-induced anorexia in rats. Pharmacol Biochem Behav 1997;57:281–4.
Winer BJ. Statistical principles in experimental design. New York: McGraw-Hill; 1962.
Young GA, Moreton JE, Meltzer LT, Khazan N. 1-alpha-acetylmethadol (LAAM), methadone and morphine abstinence in dependent rats: EEG and behavioral correlates. Drug Alcohol Depend 1977;2:141–8.
Zarrindast MR, Bahreini T, Adl M. Effect of imipramine on the expression and acquisition of morphine-induced conditioned place preference in mice. Pharmacol Biochem Behav 2002;73:941–9.
Zorrilla EP, Inoue K, Fekete EM, Tabarin A, Valdez GR, Koob GF. Measuring meals: structure of prandial food and water intake of rats. Am J Physiol Regul Integr Comp Physiol 2005;288:R1450–67.