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Noradrenergic antagonism enhances the conditioned aversive effects of cocaine

Kevin B. Freeman [a,*], Andrey Verendeev [b], Anthony L. Riley [b]

[a] Division of Neurobiology and Behavior Research, Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS 39216, United States
[b] Psychopharmacology Laboratory, Department of Psychology, American University, Washington, DC 20016, United States

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Abstract

The propensity to self-administer cocaine may be a function of both its positively reinforcing and aversive effects, with the latter acting as a limiting factor on overall drug taking. However, relative to what is known about the physiological underpinnings of cocaine's positively reinforcing effects, little is known about its aversive effects. There is some evidence that cocaine's aversive effects, as indexed in the conditioned taste aversion (CTA) preparation, are catecholaminergically mediated, i.e., through cocaine's actions on the dopaminergic and noradrenergic neurotransmitter systems. Although limited evidence suggests a role for dopamine, there has yet to be a direct assessment of noradrenergic involvement. To better characterize a role for this system, cocaine-induced CTAs (10, 18 and 32 mg/kg) were conducted under conditions of antagonism at the norepinephrine α_1 and β receptors using prazosin (0.3 mg/kg; Experiment 2) and propranolol (10 mg/kg; Experiment 3), respectively, at doses that were determined to be non-aversive (Experiment 1). In each case of noradrenergic antagonism, CTAs with cocaine were not attenuated, suggesting that this drug's conditioned aversive effects are mediated by non-noradrenergic NT activity. Furthermore, prazosin and propranolol administration appeared to facilitate the conditioned aversive effects of cocaine. The implications of these findings in regards to other neurochemical processes are discussed.

Keywords: Conditioned taste aversion; Cocaine; Norepinephrine; Prazosin; Propanolol

1. General introduction

The propensity to self-administer cocaine (or any other drug of abuse, e.g., alcohol or heroin) may be a function of a complex motivational array that depends not only on its rewarding effects but on its aversive properties, as well (Riley and Simpson, 2001; Stolerman and D'Mello, 1981). According to this logic, cocaine use may be more likely to occur under conditions where its rewarding effects assume a greater proportion of its net stimulus valence than its aversive effects. This notion gives specific importance to the understanding of cocaine's aversive effects (both behaviorally and biochemically) as they may factor into the initial use of the drug as well as its subsequent escalation and maintenance.

Various behavioral methodologies have been developed that are thought to selectively index a drug's aversive effects (see Ettenberg, 2004; Koob et al., 1997; Riley and Simpson, 2001; Spealman, 1979). One such preparation is the conditioned taste aversion (CTA) design, a model in which an animal learns to avoid flavored solutions previously paired with the administration of an aversive drug stimulus (e.g., LiCl; see Riley and Freeman, 2004). Initially, the CTA phenomenon was characterized with emetic stimuli (e.g., LiCl, radiation; see Freeman and Riley, in press; Revusky and Garcia, 1970), but subsequent assessments have extended the range of effective agents to include an array of self-administered (SA) compounds, one of these being cocaine (see Cappell and Leblanc, 1973; Foltin and Schuster, 1982; Goudie et al., 1978; Hunt and Amit, 1987). Thus, the CTA design is well-suited for the characterization of cocaine's aversive effects which are not readily apparent in preparations that index positive reinforcement (e.g., SA,

* Corresponding author. Tel.: +601 815 9203; fax: +601 984 5998.
E-mail address: kfreeman@psychiatry.umsmed.edu (K.B. Freeman).

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conditioned place preference [CPP]; see Cappell and LeBlanc, 1977; Hunt and Amit, 1987; Riley et al., in press; although see Grigson, 1997 for an alternative interpretation of this issue). Moreover, the assay's utility is strengthened by the fact that aversions can be detected at doses of psychostimulants and opiates that are positively reinforcing in other preparations (Cappell and Leblanc, 1973; Cappell et al., 1973; see Hunt and Amit, 1987) or under identical parametric conditions (see Reicher and Holman, 1977; Simpson and Riley, 2005; White et al., 1977; Wise et al., 1976).

Although cocaine's efficacy in the CTA preparation has been demonstrated repeatedly, little is known about the neurochemical mediation of its aversive effects. The identification of the mechanisms involved is complicated by its diverse pharmacology. In addition to its function as a local anesthetic (i.e., its ability to block neuronal membrane sodium channels; Matthews and Collins, 1983), cocaine is a non-selective systemic inhibitor of the transporters for the three monoamine neurotransmitters (NT) dopamine (DA), norepinephrine (NE) and serotonin (5-HT; Taylor and Ho, 1978; Woolverton and Johnson, 1992). Therefore, when administered, it causes transient increases in the extracellular levels of all three monoamine NTs (Taylor and Ho, 1978). Subsequent to this extracellular NT increase, each of the monoamine NTs interacts with its cognate synaptic receptors, which themselves are represented by numerous subtypes within each NT system (for reviews of monoamine pharmacology, see Cooper et al., 2003; Nestler et al., 2001). Thus, the biological actions resulting from cocaine administration could be mediated by monoamine NT activity at any number of receptor subtypes and in any combination within and between the monoamine NT systems.

However, there is some evidence suggesting that cocaine's aversive effects are catecholaminergically mediated (i.e., mediated by DA and/or NE activity). For example, Goudie et al. (1975) demonstrated that the acquisition of CTAs with amphetamine, a psychostimulant with a catecholaminergic pharmacological profile similar to cocaine (Riddle et al., 2005), could be attenuated by pretreatment with alpa-methylparatyrosine (AMPT), an inhibitor of the enzyme, tyrosine hydroxylase (TH). Inhibition of TH reduces the levels of both DA and NE by inhibiting their synthesis (see Cooper et al., 2003). Because catecholamine depletion attenuated the acquisition of a CTA with amphetamine, it was suggested that DA and/or NE played some role in the induction of amphetamine's aversive effects. Consistent with this position, Roberts and Fibiger (1975) demonstrated a similar attenuation of an amphetamine-induced CTA by central catecholamine depletion with intraventricular injections of the catecholamine neuronal toxin 6-hydroxydopamine (6-OHDA). Interestingly, this same treatment had no effect on a LiCl-induced CTA, suggesting that the attenuation of amphetamine-induced CTA with central 6-OHDA treatment was not due to a generalized disruption of an associative process governing all forms of CTA learning (Roberts and Fibiger, 1975; Stricker and Zigmond, 1974).

Although these studies suggested a role for catecholamines in the induction of amphetamine-induced CTA, they did not address the relative contributions of dopaminergic and noradrenergic systems in these aversions. Assessing a role for DA specifically, Grupp (1977) pretreated rats with the dopamine receptor antagonist, pimozide, before a saccharin–amphetamine pairing. Pimozide attenuated the acquisition of a CTA with a low dose of amphetamine (1.0 mg/kg), but not with a higher one (2.0 mg/kg), suggesting that DA played only a partial role in amphetamine's aversive effects. The results with amphetamine were relevant to the mediation of cocaine's aversive effects because they suggested that increased catecholamine activity, an effect induced by cocaine and amphetamine alike, may have aversive stimulus properties. Thus, in the first investigation of a catecholamine influence in cocaine-induced CTA, Hunt et al. (1985) pretreated rats with pimozide before a saccharin–cocaine pairing. Similar to the results with amphetamine (Grupp, 1977), pimozide pretreatment attenuated the acquisition of a cocaine-induced CTA, but had no effect on LiCl-induced aversions. Thus, as with amphetamine, there appeared to be a dopaminergic component in the induction of cocaine's aversive effects. However, a role for noradrenergic activity in cocaine's aversive effects remained to be determined.

A recent study by Freeman et al. (2005) compared cocaine to three transporter inhibitors in the CTA design, each with relative specificity for the monoamine transporters for DA, 5-HT and NE. Although each inhibitor conditioned some degree of aversion, the NET inhibitor, desipramine, approximated the acquisition function of cocaine more closely than the DAT and SERT inhibitors, GBR 12909 and clomipramine, respectively, with clomipramine inducing only marginal aversions at all doses tested. Thus, it was suggested that NE may play a role, possibly a prominent one, in the induction of cocaine's aversive effects.

To investigate this possibility, the current series of experiments assessed the contribution of cocaine's noradrenergic actions to its aversive effects by administering NE receptor antagonists before cocaine in the CTA design. In Experiment 1, dose–response analyses were conducted for the α_1 and β antagonists, prazosin and propranolol, respectively. Once non-aversive doses were determined for each compound, prazosin (0.3 mg/kg; Experiment 2) and propranolol (10 mg/kg; Experiment 3) were administered before cocaine (10, 18 and 32 mg/kg) in the CTA preparation to examine the effects of NE receptor antagonism on the acquisition of cocaine-induced CTA.

2. Experiment 1

2.1. Introduction

Selective receptor antagonists are useful tools in the characterization of the neurochemical mediation of drug effects (Woolverton and Kleven, 1988). By removing a receptor's functional activity with a selective antagonist, its role in an effect of interest can be inferred from the impact that is made on that effect. However, when used in this way, consideration of the direct effects of receptor antagonists must be taken into account, as they may induce effects on their own that are independent of the test drug's effects. For instance, antagonizing receptor X to assess its role in the aversive effects of drug Y would reveal little about the pharmacological underpinnings of drug Y's aversive effects if the antagonist for

receptor X were itself aversive. Thus, before an antagonist drug can be used to investigate another drug's role in an effect of interest, it must first be screened to ensure that it has no impact on the measured variable when administered alone.

Most classes of psychoactive compounds are capable of inducing taste aversions (Cappell and LeBlanc, 1977; Hunt and Amit, 1987). This poses a problem for the use of antagonists in the study of the aversive effects of other drugs because of the risk that the antagonist itself may condition aversions. One way around this issue is to give the antagonist *before* saccharin is offered, and then to administer the primary drug of interest *after* saccharin access. Given that backwards conditioning (i.e., the US preceding the CS) induces weak aversions or no aversions at all (see Barker et al., 1977; Garcia and Kimeldorf, 1957), administering the antagonist before saccharin ensures that the taste will be more strongly associated with the primary drug's effects (administered after saccharin access) rather than the antagonist's effects. However, this design has a potential flaw in that the antagonist drug, when administered before saccharin presentation, may cause a generalized suppression of fluid intake (see Hunt et al., 1985) which makes comparisons with control (vehicle-pretreated) animals problematic.

Another possibility is to administer both the antagonist and the test drug after saccharin access, but to use a dose for the antagonist that is not aversive by itself, thus, ensuring that the expression of the aversion is not due to the direct effects of the antagonist compound. However, this imposes an upper limit on the possible dose range for the antagonist compound, which may, at lower doses, be insufficient to influence the test drug's effects. As such, dose–response analyses should be performed in the CTA preparation for candidate antagonists to determine the highest, non-aversive dose possible to maximize the high-dose option for that compound.

To identify doses for prazosin and propranolol that met these conditions, three doses of the α_1 antagonist, prazosin, and the β antagonist, propranolol, were tested for their ability to condition aversions. Specifically, rats were given access to a novel saccharin solution and injected intraperitoneally (IP) with either prazosin (0.3, 1.0 and 3.0 mg/kg) or propranolol (1.0, 3.0 and 10 mg/kg). The doses and route of administration were chosen based on work demonstrating these parameters to be effective in modulating other cocaine-induced behaviors (Harris et al., 1996; Spealman, 1995; Wellman et al., 2002; Zhang and Kosten, 2005).

2.2. Method

2.2.1. Apparatus

Subjects were housed in individual stainless-steel, wire-mesh cages on the front of which graduated Nalgene tubes could be placed for the presentation of either water or saccharin. Subjects were maintained on a 12 L:12 D cycle, with lights on at 0800 h, and at an ambient temperature of 23 °C. Food was available *ad libitum*.

2.2.2. Subjects

The subjects were 58 (8–9 per group) male Sprague–Dawley rats, approximately 120 day s of age and 300–400 g in weight at the beginning of each experiment. The study described was approved by the Institutional Animal Care and Use Committee (IACUC) at American University and was conducted under the procedures recommended by the Guide for the Care and Use of Laboratory Animals (1996) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003). Food and water consumption were monitored daily to assess the health of the subjects.

2.2.3. Drugs and solutions

Prazosin–HCl and propranolol–HCl (Sigma) were prepared as 1 mg/ml and 10 mg/ml solutions, respectively, in distilled water. Full crystal solubility for prazosin required gentle heating and stirring. All drug doses are expressed as the salt. Saccharin (0.1% sodium saccharin, Sigma) was prepared as a 1 g/l solution in tap water.

2.2.4. Procedure

2.2.4.1. Phase I: Habituation. Following 23-h water deprivation, subjects were given 20-min access to water. This procedure was repeated daily until all subjects were approaching and drinking from the tube within 2 s of its presentation.

2.2.4.2. Phase II: Conditioning. Immediately following access to saccharin, the subjects were ranked according to saccharin consumption and assigned to seven groups (n=8–9 per group) such that each group was comparable in consumption. Approximately 30 min after saccharin access, the animals were removed from their home cages and injected IP in an adjacent room with prazosin (0.3, 1.0 or 3.0 mg/kg) or propranolol (1.0, 3.0 or 10.0 mg/kg). A final group of animals was injected with the drug vehicle (distilled water) equivolume to the highest propranolol dose. This treatment resulted in the following groups: Groups Pz-0.3, Pz-1, Pz-3, Pp-1, Pp-3, Pp-10 and Veh. The first variable in each group designation refers to the drug administered, i.e., Pz (prazosin) and Pp (propranolol). The second variable refers to the dose, i.e., 0.3 (0.3 mg/kg), 1 (1.0 mg/kg), 3 (3.0 mg/kg) and 10 (10 mg/kg). Animals injected with the drug vehicle were designated as Group Veh. On the following 3 water-recovery days, all animals were given 20-min access to water. No injections were given following water access on these days. This alternating procedure of conditioning/water recovery was repeated until all subjects received four complete cycles. On the day following the final water-recovery session, all subjects were given 20-min access to saccharin in a one-bottle test of the aversion to saccharin (Aversion Test). No injections were given following the test.

2.2.5. Statistical analysis

Because there were no between-drug comparisons (i.e., prazosin vs. propranolol), the prazosin and propranolol dose–response functions were examined with separate analyses, but done so with the same control group for each analysis (Group Veh). Differences in mean saccharin consumption on the Aversion Tests were analyzed using an Analysis of Variance (ANOVA) test with the between subjects variable of Dose (0, 0.3, 1.0 and

3.0 mg/kg for prazosin; 0, 1.0, 3.0 and 10.0 mg/kg for propranolol). Each analysis included three planned comparisons: Groups Pz-0.3, Pz-1 and Pz-3 compared to Group Veh and Groups Pp-1, Pp-3 and Pp-10 compared to Group Veh. Fisher's LSD post-hoc analyses were used to compare group means.

2.3. Results

Fig. 1a illustrates mean saccharin consumption for subjects receiving prazosin (0.3, 1.0 or 3.0 mg/kg) or its vehicle (distilled water; 0 mg/kg) on the Aversion Test. An ANOVA test revealed a significant main effect for Group ($F(3,28)=5.748$; $p=.003$). Fisher's LSD post-hoc analyses were used to compare treatment group means (i.e., Groups Pz-0.3, Pz-1 and Pz-3) to the consumption average of vehicle-injected controls (i.e., Group Veh). Groups Veh and Pz-0.3 did not differ significantly in saccharin consumption ($p=.131$), but Groups Pz-1 and Pz-3 each drank significantly less than Group Veh (all p's $\leq .004$).

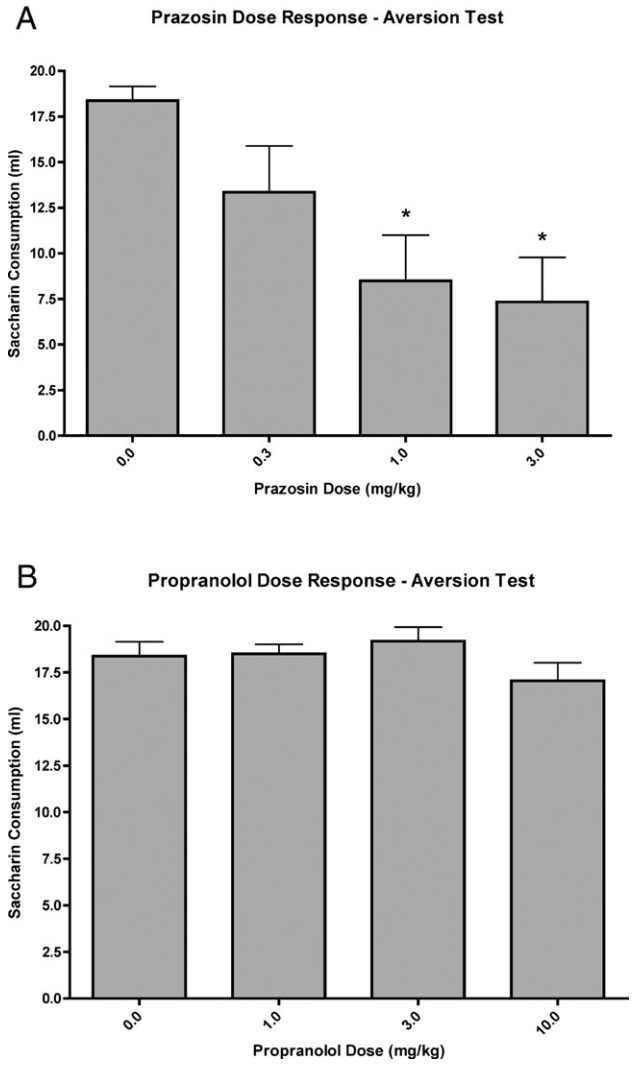

Fig. 1. Panel A illustrates mean saccharin consumption for subjects receiving prazosin (0.3, 1.0 or 3.0 mg/kg; Groups Pz-0.3, Pz-1 and Pz-3, respectively) or its vehicle (0 mg/kg; Group Veh) on the Aversion Test. Panel B illustrates saccharin consumption for subjects receiving propranolol (1.0, 3.0 or 10.0 mg/kg; Groups Pp-1, Pp-3 and Pp-10, respectively) or its vehicle (0 mg/kg; Group Veh) on the Aversion Test. Bars above and below each point represent S.E.M. *Significantly different from the 0 mg/kg condition.

Fig. 1b illustrates mean saccharin consumption for subjects receiving propranolol (1.0, 3.0 or 10.0 mg/kg) or its vehicle (distilled water; 0 mg/kg) on the Aversion Test. A one-way ANOVA revealed no significant main effect of Group ($F(3,29)=1.325$; $p=.285$), indicating that none of the tested doses of propranolol was sufficient to condition an aversion.

2.4. Discussion

Three doses of prazosin (0.3, 1.0 and 3.0 mg/kg) and propranolol (1.0, 3.0 and 10 mg/kg) demonstrated to be effective in modulating cocaine-induced behaviors (Harris et al., 1996; Wellman et al., 2002; Zhang and Kosten, 2005) were tested for their ability to condition aversions. In the case of prazosin, all doses tested with the exception of the lowest dose (0.3 mg/kg) induced significant aversions. As such, for the subsequent analysis examining the effects of α_1 antagonism on cocaine-induced CTA (see Experiment 2), 0.3 mg/kg of prazosin was used. This dose is effective in attenuating the discriminative stimulus effects of cocaine (Spealman, 1995) and reinstatement of cocaine-seeking following a cocaine prime (Zhang and Kosten, 2005). Thus, there is a basis for assuming that 0.3 mg/kg of prazosin might be sufficient to modulate the expression of a cocaine-induced CTA if α_1 receptor activation mediates cocaine's aversive effects. In the case of propranolol, none of the doses tested conditioned aversions. Therefore, a 10 mg/kg dose (the highest dose tested) was used in the assessment of β antagonism on cocaine-induced CTA (see Experiment 3). This dose of propranolol modulates cocaine's locomotor effects and decreases the rate of responding for self-administered cocaine in a pharmacologically specific manner, i.e., the effect does not appear to be due to general response suppression (see Harris et al., 1996). As such, 10 mg/kg of propranolol appears to be both non-aversive and within an effective range for the modulation of cocaine-induced effects.

3. Experminent 2

3.1. Introduction

Actions at the α_1 receptor have been implicated in a number of cocaine's effects. For example, Wellman et al. (2002) pretreated animals with prazosin before administering cocaine and found that antagonizing the α_1 receptor attenuated cocaine-induced increases in locomotor activity as well as cocaine-induced hypophagia (appetite suppression), suggesting a role for NE activity at the α_1 receptor in the mediation of these cocaine-induced behaviors. Furthermore, prazosin pretreatment antagonizes the discriminative stimulus effects of cocaine in a drug discrimination (DD) procedure, necessitating the administration of higher doses of cocaine to engender cocaine-appropriate responding relative to a cocaine-alone baseline (Spealman, 1995). In regards to cocaine's toxic effects, prazosin pretreatment attenuates cocaine-induced increases in arterial pressure (Mo et al., 1999) and raises the dose threshold for

cocaine's convulsive and lethal effects (Tella et al., 1992), suggesting that NE's action at the α_1 receptor plays some role in the mediation of cocaine's sympathomimetic and toxic effects. Thus, NE activity at the α_1 receptor appears to mediate a number of cocaine's effects as evidenced by the attenuation of these effects with α_1 receptor blockade.

To extend these findings to the aversive effects of cocaine, Experiment 2 tested prazosin with cocaine in a CTA procedure. Specifically, rats were given access to a novel saccharin solution and injected 30 min later with either prazosin (IP; 0.3 mg/kg; see Experiment 1) or vehicle. Then, following a 30 min interval, rats were injected SC with one of three doses of cocaine (10, 18 and 32 mg/kg). The dose of prazosin (0.3 mg/kg) was chosen primarily because it was below the threshold needed to condition an aversion (see Experiment 1). However, it should be noted that this dose has been shown to be efficacious in the modulation of other cocaine-mediated behaviors (Spealman, 1995; Zhang and Kosten, 2005). The prazosin–cocaine interval was chosen based on work showing this temporal parameter to be effective in the antagonism of cocaine's effects (Spealman, 1995; Zhang and Kosten, 2005). If the aversive effects of cocaine are mediated by NE activity at the α_1 receptor, then prazosin treatment should attenuate the effect. However, given that the effects of α_1 antagonism on cocaine aversions have never been examined, there remained the possibility that prazosin would have no effect or could possibly enhance cocaine's aversive effects. Therefore, a dose range for cocaine was chosen that included a non-aversive dose (10 mg/kg; Busse et al., 2005) to allow for the detection of enhancement (by facilitating an aversion to a sub-threshold dose of cocaine, 10 mg/kg) or attenuation (by antagonizing the aversion to 18 and/or 32 mg/kg cocaine).

3.2. Method

3.2.1. Apparatus

All housing and testing equipment were identical to those described in the Method section of Experiment 1.

3.2.2. Subjects

The subjects were 74 male Sprague–Dawley rats, approximately 120 day s of age and 300–400 g at the beginning of the experiment.

3.2.3. Drugs and solutions

Prazosin–HCl (Sigma) was prepared as a 0.5 mg/ml solution in distilled water. Full crystal solubility required gentle heating and stirring. Cocaine–HCl was prepared as a 10 mg/ml solution in sterile saline. Cocaine was generously provided by NIDA. All drug doses are expressed as the salt. Saccharin (0.1% sodium saccharin, Sigma) was prepared as a 1 g/l solution in tap water.

3.2.4. Procedure

3.2.4.1. Phase I: Habituation. The habituation procedure was identical to the one described in the Method section of Experiment 1.

3.2.4.2. Phase II: Conditioning. Immediately following access to saccharin, the subjects were ranked according to saccharin consumption and assigned to eight groups (n=9–10 per group) such that each group was comparable in consumption. Approximately 30 min after saccharin access, the animals were removed from their home cages and injected IP in an adjacent room with prazosin (0.3 mg/kg) or its vehicle and replaced in their home cages. After another 30 min, the same animals were removed from their home cages again and injected SC with cocaine (10, 18 or 32 mg/kg) or its vehicle (equivolume to the highest cocaine dose). Although the injections for cocaine were extended to a 1-h CS–US interval in this experiment, this has been demonstrated to have no impact on the magnitude of cocaine-induced CTAs relative to a 10-min interval (Freeman and Riley, 2005). The described treatment resulted in the following groups: Groups V-V, V-10, V-18, V-32, Pz-V, Pz-10, Pz-18 and Pz-32. The first variable in each group designation refers to the drug antagonist condition, i.e., Pz (prazosin) and V (prazosin vehicle). The second variable refers to the dose of cocaine, i.e., V (0 mg/kg; cocaine vehicle), 10 (10 mg/kg), 18 (18 mg/kg) and 32 (32 mg/kg). All remaining components of the conditioning procedure were identical to those described in the Method section of Experiment 1.

3.2.5. Statistical analysis

Differences in mean saccharin consumption on the Aversion Test were analyzed using a 2×4 ANOVA with the between subjects factors of pretreatment (prazosin vs. vehicle) and cocaine dose (0, 10, 18 and 32 mg/kg). Fisher's post-hoc analyses were used to make ten planned comparisons: Groups V-V to Pz-V; Groups V-10, V-18 and V-32 to Group V-V; Groups Pz-10, Pz-18 and Pz-32 to Group Pz-V; and finally comparisons between each of the within-dose cocaine conditions (e.g., comparing Group V-10 to Pz-10).

3.3. Results

Fig. 2 illustrates the mean saccharin consumption for subjects receiving either prazosin or its vehicle followed by either cocaine at one of three doses (10, 18 or 32 mg/kg) or its vehicle. Each possible combination of conditions is designated a group assignment according to the nomenclature specified in the Method section of the current experiment (see above). A 2×4 ANOVA revealed main effects of pretreatment ($F(1,66)$= 20.738; $p \leq .0009$) and cocaine dose ($F(3,66)$=41.956; $p \leq .0009$) as well as a significant pretreatment×cocaine dose interaction ($F(3,66)$=3.09; p=.033). There was no significant difference in saccharin consumption between Groups V-V and P-V (p=.475), indicating that prazosin (0.3 mg/kg) was not aversive. Furthermore, Group V-10 did not significantly differ in saccharin consumption from Group V-V (p=.397), indicating that the lowest dose of cocaine (10 mg/kg) was not sufficient to condition an aversion. However, the two highest doses of cocaine were effective in conditioning aversions with V-18 and V-32 drinking significantly less saccharin than Group V-V (all p's ≤ .0009). To test the effects of α_1 antagonism on cocaine's aversive effects, prazosin was administered before cocaine.

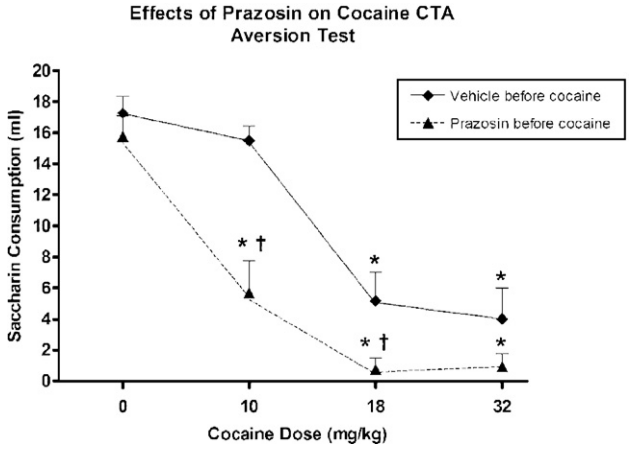

Fig. 2. Illustrates mean saccharin consumption for subjects receiving prazosin (0.3 mg/kg) or its vehicle followed by cocaine at one of four doses (0, 10, 18 or 32 mg/kg) on the Aversion Test. Bars above and below each point represent S.E.M. *Significantly different from matched prazosin condition (non-cocaine control). †Significantly different from matched cocaine condition of same dose (non-prazosin control).

Prazosin appeared to enhance the aversive effects of cocaine as Group Pz-10 drank significantly less than Group Pz-V ($p \leq .0009$), an effect opposite of expectation if α_1 activity were a mediator of cocaine's aversive effects. When comparing the cocaine-treated subjects within each dose, prazosin pretreatment appeared to enhance the aversive effects of cocaine as Groups Pz-10 and Pz-18 consumed significantly less than Groups V-10 and V-18, respectively (all p's $\leq .039$). There was no significant difference in saccharin consumption between Groups V-32 and Pz-32 ($p = .148$).

3.4. Discussion

To examine a role of α_1 receptor activation in the induction of cocaine's aversive effects, prazosin was administered prior to cocaine in a CTA procedure. At the two lowest doses of cocaine tested (10 and 18 mg/kg), prazosin pretreatment appeared to enhance the aversive effects of cocaine. Specifically, animals treated with prazosin prior to the injection of cocaine drank significantly less saccharin than vehicle-treated and cocaine-treated animals on the Aversion Test. At the lowest dose, this was a robust effect given that neither cocaine (10 mg/kg) nor prazosin (0.3 mg/kg), when administered alone, induced an aversion. At the highest dose of cocaine tested (32 mg/kg), prazosin administration did not significantly modulate (i.e., increase or decrease) the aversive effects of cocaine relative to cocaine-alone controls, although a trend suggested enhancement.

The current results indicate that α_1 receptor activation is not likely mediating cocaine's aversive effects. This does not rule out a role for NE receptor activation, however, as the aversive effects of cocaine could still be mediated by activity at β receptors (assessed in Experiment 3). However, the fact that prazosin administration enhanced cocaine's aversive effects implies more than the α_1 receptor's absence in these effects; it indicates that NE activation may actually serve a mitigating role in the expression of cocaine's aversive effects (see General discussion).

4. Experiment 3

4.1. Introduction

The results of Experiment 2 suggest that α_1 receptor activation does not play a role in the induction of cocaine's aversive effects. However, there remains the possibility that cocaine's aversive effects are noradernergically-mediated through β receptor activity. In the following experiment, rats were treated with the non-selective β antagonist, propranolol, prior to aversion conditioning with cocaine to examine the effects of β antagonism on the expression of cocaine-induced CTA. Specifically, rats were given access to a novel saccharin solution and injected 40 min later with either propranolol (IP; 10 mg/kg; see Experiment 1) or vehicle. Then, following a 20 min interval, rats were injected SC with one of three doses of cocaine (10, 18 and 32 mg/kg). The dose of propranolol (10 mg/kg) as well as the propranolol–cocaine injection interval used in this assessment (20 min) were chosen based on work showing these parameters to be effective in the behavioral and neurochemical modulation of other cocaine-induced effects (Harris et al., 1996). If the aversive effects of cocaine are mediated by NE activity at the β receptor, then it is expected that propranolol treatment will attenuate the effect.

4.2. Method

4.2.1. Apparatus

All housing and testing equipment were identical to those described in the Method section of Experiment 1.

4.2.2. Subjects

The subjects were 74 male Sprague–Dawley rats, approximately 120 days of age and 300–400 g at the beginning of the experiment.

4.2.3. Drugs and solutions

Propranolol–HCl (Sigma) was prepared as a 10 mg/ml solution in distilled water. Cocaine–HCl was prepared as a 10 mg/ml solution in sterile saline. Cocaine was generously provided by NIDA. All drug doses are expressed as the salt. Saccharin (0.1% sodium saccharin, Sigma) was prepared as a 1 g/l solution in tap water.

4.2.4. Procedure

4.2.4.1. Phase I: Habituation. The habituation procedure was identical to the one described in the Method section of Experiment 1.

4.2.4.2. Phase II: Conditioning. Immediately following access to saccharin, the subjects were ranked according to saccharin consumption and assigned to eight groups ($n = 9$–10 per group) such that each group was comparable in consumption. Approximately 40 min after saccharin access, the animals were removed from their home cages and injected IP in an adjacent room with propranolol (10 mg/kg) or its vehicle and replaced in their home cages. After another 20 min, they were removed from

their home cages again and injected SC with cocaine (10, 18 or 32 mg/kg) or its vehicle (equivolume to the highest cocaine dose). The temporal spacing for the propranolol and cocaine injections (20 min) was chosen based on a previous assessment showing this spacing to be effective in modulating cocaine-induced behaviors (Harris et al., 1996). The described treatment resulted in the following groups: Groups V-V, V-10, V-18, V-32, Pp-V, Pp-10, Pp-18 and Pp-32. The first variable in each group designation refers to the drug antagonist condition, i.e., Pp (propranolol) and V (propranolol vehicle). The second variable refers to the dose of cocaine, i.e., V (0 mg/kg; cocaine vehicle), 10 (10 mg/kg), 18 (18 mg/kg) and 32 (32 mg/kg). All remaining components of the conditioning procedure were identical to those described in the Method section of Experiment 1.

4.2.5. Statistical analysis

Differences in mean saccharin consumption on the Aversion Test were analyzed using a 2×4 ANOVA with the between subjects factors of pretreatment (propranolol vs. vehicle) and cocaine dose (0, 10, 18 and 32 mg/kg). Fisher's post-hoc analyses were used to make ten planned comparisons: Groups V-V to Pp-V; Groups V-10, V-18 and V-32 to Group V-V; Groups Pp-10, Pp-18 and Pp-32 to Group Pp-V; and finally comparisons between each of the within-dose cocaine conditions (e.g., comparing Group V-10 to Pp-10).

4.3. Results

Fig. 3 illustrates the mean saccharin consumption for subjects receiving either propranolol or its vehicle followed by either cocaine at one of three doses (10, 18 or 32 mg/kg) or its vehicle. Each possible combination of conditions is designated a group assignment according to the nomenclature specified in the Method section of the current experiment (see above). A 2×4 ANOVA revealed a main effect of cocaine dose ($F(3,66)=117.22$; $p\leq.0009$) but no main effect of pretreatment ($F(1,66)=1.69$; $p=.198$) nor a significant pretreatment×cocaine dose interaction ($F(3,66)=2.334$; $p=.082$). There was no difference in saccharin consumption between Groups V-V and Pp-V ($p=.716$), indicating that propranolol (10 mg/kg) was not aversive. However, cocaine, when given alone, was aversive at all doses tested (10, 18 and 32 mg/kg) as evidenced by the significantly lower consumption of saccharin by Groups V-10, V-18 and V-32 relative to Group V-V (all p's$\leq.006$). Notably, unlike the results of Experiment 2, the lowest dose of cocaine in this assessment (10 mg/kg) conditioned an aversion. To assess the effects of β antagonism on cocaine's aversive effects, propranolol (10 mg/kg) was given before cocaine at the same range of doses. Similar to the cocaine-alone groups, Groups Pp-10, Pp-18 and Pp-32 each drank significantly less saccharin than their respective control, Group Pp-V (all p's$\leq.0009$). However, subjects in Group Pp-10 drank significantly less than those in Group V-10 ($p=.012$), suggesting that propranolol may have enhanced the conditioned aversive effects of cocaine. There were no differences in consumption between Groups V-18 and Pp-18 or Groups V-32 and Pp-32 (all p's$\geq.266$).

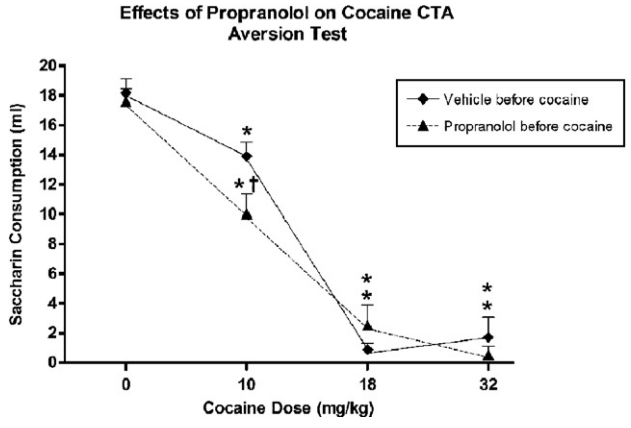

Fig. 3. Illustrates mean saccharin consumption for subjects receiving propranolol (10 mg/kg) or its vehicle followed by cocaine at one of four doses (0, 10, 18 or 32 mg/kg) on the Aversion Test. Bars above and below each point represent S.E.M. *Significantly different from matched propranolol condition (non-cocaine control). †Significantly different from matched cocaine condition of same dose (non-propranolol control).

4.4. Discussion

To examine a role for β receptor activation in the induction of cocaine's aversive effects, propranolol was administered prior to cocaine (but after saccharin access) in a CTA procedure. Unlike the results in Experiment 2, the lowest dose of cocaine (10 mg/kg) produced weak aversions. Propranolol treatment slightly enhanced the aversive effects of the lowest dose of cocaine (10 mg/kg). At the two highest doses of cocaine (18 and 32 mg/kg), there were no significant differences in mean consumption between cocaine-injected animals treated with propranolol or vehicle. Thus, as with the α_1 receptor, β receptor activity does not appear to be a contributing factor in the induction of cocaine's aversive effects. Taken together with the results of Experiment 2, it appears that NE activity does not mediate cocaine's conditioned aversive effects.

5. General discussion

In the current analysis, prazosin (0.3 mg/kg) and propranolol (10 mg/kg), respectively, were administered before cocaine (10, 18 and 32 mg/kg) to assess the role of NE receptor activity in cocaine's aversive effects. Neither antagonist attenuated the aversions induced by cocaine. Rather, each antagonist (at specific doses) when combined with cocaine resulted in greater suppression of saccharin intake than the administration of cocaine alone. These data suggest that cocaine's aversive effects are not mediated by noradrenergic activity, which leaves DA as the most likely catecholaminergic mediator of cocaine's aversive effects. Consistent with this notion is the demonstration that pimozide, a DA receptor antagonist, attenuates the acquisition of a cocaine-induced CTA (Hunt et al., 1985) while prazosin and propranolol, each antagonists for NE receptors, do not.

That prazosin and propranolol appeared to enhance cocaine's aversive effects suggests that NE receptor activation, stemming from the extracellular build-up of NE following cocaine

administration, may play an ameliorating role in the expression of cocaine's conditioned aversive effects. However, it is unknown how prazosin and propranolol could effect this outcome. Given that DA antagonism attenuates cocaine-induced CTA (Hunt et al., 1985) and that propranolol enhances the extracellular increases in DA associated with cocaine administration (Harris et al., 1996), propranolol (i.e., β antagonism) may enhance cocaine's aversive effects by increasing its associated dopaminergic activity. This would not explain prazosin's enhancement, however, especially since α_1 antagonism has been shown to attenuate dopaminergic cell activity and many cocaine-mediated behaviors related to DA activation (e.g., locomotor, discriminative stimulus effects; see above). Therefore, the best information that can be gleaned from these data regarding the enhancement seen with prazosin and propranolol is that antagonism at the α_1 and β receptors can enhance cocaine's aversive effects but may do so through different processes.

The fact that prazosin and propranolol each enhanced the aversive effects of cocaine is also interesting because these compounds often modulate cocaine-mediated behaviors in opposite manners. For instance, prazosin antagonizes the discriminative stimulus effects of cocaine (Spealman et al., 1995) while propranolol enhances them (Kleven and Koek, 1998). Furthermore, cocaine-induced locomotor activity is attenuated with prazosin (Wellman et al., 2002) but enhanced by propranolol administration (Harris et al., 1996). Also, prazosin serves to protect against cocaine-toxicity by raising the lethal and convulsive doses of cocaine while propranolol enhances these effects by lowering the effective doses for cocaine (Tella et al., 1992). At the neurochemical level, propranolol has been shown to produce robust enhancement of cocaine-induced DA accumulation in the extracellular space of the nucleus accumbens (Harris et al., 1996), an effect consistent with its ability to enhance cocaine's locomotor and discriminative stimulus effects. Alternatively, prazosin administration decreases dopaminergic tone in this area when administered alone (Sommermeyer et al., 1995) and may also diminish cocaine-related increases in extracellular DA, although this has yet to be tested. Why these various effects related to cocaine administration are modulated differentially within the noradrenergic system, while CTA is not, is unknown, but it does reinforce the notion that the induction of cocaine's conditioned aversive effects occur independently of NE receptor activation, perhaps even more so than the dopaminergically-characterized locomotor and discriminative stimulus effects (which themselves can both be enhanced and attenuated by manipulations of the noradrenergic system; see above).

The suggestion that DA mediates the conditioned aversive effects of cocaine raises the possibility that a single NT system contributes to its positively reinforcing, discriminative stimulus and aversive effects. Assuming that a drug's aversive effects act to limit its overall consumption, it may seem counterintuitive that the very system responsible for its aversive effects is also critical for its positively reinforcing effects (i.e., the dopaminergic system). However, one possibility that could explain this apparent paradox is that cocaine's conditioned aversive and positively reinforcing effects may have a common neurochemical mediator but different substrates of origin. Consistent with this position, Isaac et al. (1989) demonstrated that place preferences with cocaine were ablated with lesions to the mesocortical DA target areas in the prefrontal cortex while CTAs were not affected. Furthermore, rats show CPP but not CTA with microinfusions of amphetamine into the nucleus accumbens with the opposite anatomical dissociation occurring with infusions into the area postrema (Carr and White, 1986). What remains to be seen is if lesions to other reward circuitry (e.g., nucleus accumbens, ventral tegmental area) will disrupt cocaine-induced CTA in a manner similar to self-administration (see Koob et al., 1987). Such assessments will help to disentangle the substrates (or prove their commonality; see Grigson, 1997) mediating cocaine's positively reinforcing and conditioned aversive effects and allow for finer resolution in the determination of a possible dopaminergic origin to cocaine CTA.

The enhancement of cocaine's aversive effects by noradrenergic receptor antagonism has thus far been interpreted as a pharmacologically specific effect involving an interaction between the pharmacological actions of cocaine and the NE receptor antagonists, prazosin and propranolol. However, as previously noted, most classes of psychoactive compounds are capable of inducing CTAs (Cappell and LeBlanc, 1977; Hunt and Amit, 1987). Thus, one could argue that the apparent enhancement produced by prazosin and propranolol are merely additive effects between two psychoactive drugs that are working through different mechanisms to affect a single measured variable (i.e., saccharin consumption). Furthermore, in the absence of any antagonism of cocaine's aversive effects in the current series of studies, there is no evidence that the design used here, one in which both the antagonist compounds and cocaine are given after saccharin access, can be used to pharmacologically characterize cocaine's aversive effects through receptor antagonism. However, there are reasons to believe that these potential concerns did not impact the data. First, the doses of prazosin and propranolol used in Experiments 2 and 3 were not effective in inducing aversions on their own. And even if some conditioning occurred below a hypothetical threshold required for producing suppression of saccharin consumption, the effects of additivity should have been modest given that the low dose of cocaine (10 mg/kg) was either weakly aversive or not aversive at all (see Experiments 2 and 3). However, in the case of prazosin, the combination of antagonist and cocaine produced robust suppression of saccharin consumption well beyond what would be expected of simple additivity (see Experiment 2), which is highly suggestive of a pharmacologically-specific interaction between cocaine's actions and α_1 receptor antagonism. As for the effectiveness of the current design in detecting antagonism of a drug's aversive effects, others have reported attenuation of aversions with other psychoactive compounds when antagonists were given after saccharin access along with the test drug. For instance, nicotine-induced CTAs are attenuated when nicotine is co-administered after saccharin access with the nicotinic receptor antagonist, dihydro-β-erythroidine (Gommans et al., 2000; Shoaib et al., 2000). Similar results have been reported with morphine-induced CTA and antagonism with naloxone (Le Blanc and Cappell, 1975;

van der Kooy and Phillips, 1977). Thus, use of a CTA design wherein an antagonist and a test drug are administered after saccharin access can reveal pharmacological antagonism of a drug's effects.

The focus of the current analysis has operated under the assumption that reduced consumption of a flavor paired with the administration of cocaine reflects the development of an association between the taste CS and cocaine's aversive effects. However, independent of the pharmacological determinants mediating these effects, there are various interpretations regarding the stimulus qualities that are most relevant to cocaine's effectiveness in inducing CTAs. Some have argued that the novelty of the drug-state mediates the effect, positing that a homeostatic shift from baseline induces a form of conditioned fear to the taste CS, thus resulting in its subsequent avoidance (Amit and Baum, 1977; Gamzu, 1977; Hunt and Amit, 1987; Parker, 2003). However, given that CTAs with cocaine can be acquired in animals with a history of non-reinforced cocaine exposure (Riley and Simpson, 1999), it is unlikely that the novelty of the drug state is the sole factor (if one at all) in the mediation of cocaine's suppressive effects in the CTA preparation. Others have interpreted CTAs with drugs of abuse such as cocaine to be a reflection of their positively-reinforcing effects, arguing that the less rewarding taste CS is rejected due to the anticipation of the more rewarding drug state previously associated with the taste (see reward comparison hypothesis, Grigson, 1997). It is unlikely that this model can account for cocaine's conditioned effects in the current study, however, because cocaine was administered SC. Interestingly, this route of administration induces *greater* taste aversions and *weaker* place preferences than the same dose of cocaine given IP (see Busse et al., 2005; Ferrari et al., 1991; Mayer and Parker, 1993). The reason for this may be due to differences in drug absorption. In rats, cocaine (15 mg/kg) administered IP reaches peak plasma levels at 15 min while the same dose administered SC peaks at 180 min (Lau et al., 1991). And given that cocaine's efficacy as a positive reinforcer is directly related to its rate of delivery (Abreu et al., 2001; Liu et al., 2005; Woolverton and Wang, 2004), it is not surprising that the IP route is more effective in conditioning place preferences than the SC route. In relation to the current data, if greater reward is produced by IP cocaine (as indexed by stronger CPP), it would be predicted from the reward comparison hypothesis that this route would induce greater taste aversions than the SC route. Accordingly, we argue that such a model does not mediate the conditioned suppressive effects of cocaine reported here. It should be noted, however, that there is no consensus as to the basis of aversion learning with cocaine in the CTA preparation. Thus, interpretations as to what specific stimulus effects mediate cocaine-induced suppression or are impacted by the pharmacological probes used in the current study must be made with caution. Outside of the CTA preparation, cocaine has been demonstrated to induce aversive stimulus effects through anxiogenesis (Ettenberg, 2004; Knackstedt et al., 2002) and withdrawal (Koob et al., 1997), effects that have yet to be tested as mediators in the induction of conditioned aversions with cocaine. Further characterization of cocaine's neurochemical and anatomical substrates relevant to its conditioned aversive effects should help to delineate roles for these various stimulus effects in the induction of cocaine CTA, as this will help to determine degrees of overlap between the substrates governing cocaine's conditioned aversive effects and its other stimulus properties (e.g., anxiogenesis, reward).

In conclusion, the current results suggest that DA is the sole catecholaminergic mediator of cocaine's conditioned aversive effects by demonstrating that NE receptor antagonism does not attenuate cocaine-induced CTA in a manner similar to DA receptor antagonism (see Hunt et al., 1985). To better characterize a role for the dopaminergic system at the molecular level, antagonists selective for its receptor subtypes (i.e., D_1–D_5) should be examined for their ability to modulate the acquisition of cocaine-induced CTA. At the anatomical level, selective neurotoxic lesions of dopaminergic substrates known to be involved in the induction of cocaine's behavioral effects (e.g., ventral tegmental area, nucleus accumbens, striatum, medial prefrontal cortex) should be conducted to better characterize the substrates and circuits involved in the induction of its aversive effects. Assessments such as these will facilitate the characterization of the systems and sites of action mediating the aversive effects of cocaine and will ultimately lead to a better understanding of the physiological origins of a major factor affecting the acceptability of this drug of abuse.

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