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Prazosin differentially affects extinction of cocaine CPP based on dose and initial preference

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Abstract

Recent work has demonstrated that α_1 -adrenergic receptor blockade impairs extinction in fear conditioning paradigms in rodents. However, studies of the role of α_1 -adrenergic receptors in extinction using other conditioning paradigms, such as those examining the conditioned effects of drug of abuse, have provided inconsistent results. In this article, we reanalyze and extend previously-reported findings of the effect of prazosin, an α_1 -adrenergic receptor antagonist, on the extinction of a cocaine-induced condition place preference in rats, using a median split of performance during the initial test for preference. This new reanalysis, which includes further extinction testing, revealed a paradoxical dose effect. A single post-test administration of a lower dose of prazosin, 0.3 mg/kg IP, impaired extinction in rats that demonstrated a below-median preference during initial testing, but had no effect on extinction in rats that demonstrated an above-median preference during initial testing. In contrast, a single post-test administration of a higher dose of prazosin, 1.0 mg/kg IP, enhanced extinction in rats that demonstrated an above-median preference during initial testing, but had no effect on extinction in rats that demonstrated a below-median preference during initial testing. Consistent with other studies of fear and drug conditioning, these results suggest the involvement of the α_1 -adrenergic receptor in the formation of extinction memories, but also indicate a potentially important differential effect on extinction based on the dose of prazosin and the strength of the initial learning.

Keywords

extinction; drug conditioning; cocaine; conditioned place preference; memory; reconsolidation

Introduction

Emotional arousal can enhance learning and memory, an effect mediated by norepinephrine signaling acting at adrenergic receptors. Preclinically, adrenergic receptors modulate the consolidation of both aversive and appetitive memories following associative learning between conditioned and unconditioned stimuli [1]. Adrenergic receptors have also been demonstrated to play an important role in the modulation of extinction learning [2–6]. Clinically, drugs acting at noradrenergic receptors are being examined as pharmacotherapies to supplement extinction-based therapies for disorders characterized by extremely maladaptive emotional memories, such as post-traumatic stress disorder [3].

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In terms of extinction learning, the involvement of the α_1 -adrenergic receptor has only recently begun to be characterized. For example, in contextual fear conditioning, Bernardi et al. [7] demonstrated that mice administered prazosin, an α_1 -adrenergic receptor antagonist, following nonreinforced exposure to the training context impaired the rate of extinction across trials. This impairment of extinction of contextual fear by systemic prazosin has also been seen in rats, in which it was additionally demonstrated that prazosin microinjected into the medial prefrontal cortex-- a key structure involved in the extinction of conditioned fear-- also impaired extinction [8]. Thus, it is becoming apparent that α_1 -adrenergic receptors are an important contributor to the extinction of contextual fear.

The potential contribution of α_1 -adrenergic receptors to extinction in other conditioning paradigms, such as those involving drugs of abuse, is less clear. Bernardi et al. [7] found that prazosin administered to mice following several nonreinforced tests in the cocaine conditioned place preference (CPP) paradigm failed to affect the rate of extinction of preference for the cocaine-paired environment. However, prazosin-treated mice showed an increased preference relative to vehicle-treated mice during cocaine reconditioning trials, indicative of an effect on the persistence of extinction. In contrast, a single administration of prazosin either systemically or into the basolateral amygdala in rats following a nonreinforced test for cocaine CPP not only failed to impair extinction on a subsequent test, but actually reduced the preference relative to vehicle-treated animals, indicative of an enhanced extinction or reconsolidation-like impairment [9]. Because of these inconsistencies in the effects of prazosin on extinction learning, especially with respect to the CPP paradigm, further examination of the role of α_1 -adrenergic receptor antagonists in this paradigm during extinction is warranted.

In the current study, we sought to clarify the role of prazosin in the extinction of cocaine CPP by reanalyzing data from our aforementioned study [9] with additional data not previously examined. Based on ample evidence indicating that the effects of manipulations on extinction may depend critically on the behavioral impact of the extinction session [10–13], we examined the behavioral response to prazosin with respect to differences in the initial response to nonreinforced presentation of drug-associated cues based on a median split [10–12].

Materials & Methods

Subjects

Forty-eight male Sprague Dawley rats, aged 10–12 weeks at the start of experiments, were used as subjects. Rats were housed two per cage, with food and water available *ad libitum*. Experiments were performed in accordance with the NIH guidelines for the care and use of laboratory animals.

Apparatus & Behavioral Procedures

CPP was assessed using an unbiased design [14] in four automated one-compartment place conditioning chambers (modified from San Diego Instruments, San Diego, CA) and involved the following phases performed on consecutive days: habituation, conditioning and testing (extinction sessions), as described in detail previously [9]. Briefly, on alternate days over eight conditioning sessions (four cocaine/four vehicle), rats received cocaine (Sigma, St. Louis, MO; 20 mg/kg IP) prior to 25-min conditioning trials on either a GRID or HOLE floor and vehicle (saline; 1 ml/kg) prior to 25-min trials on the alternate floor. For extinction sessions, rats received a saline injection immediately prior to placement into the apparatus with half GRID/half HOLE floor for a 15-min (Session 1) or 25-min preference test (Sessions 2–6). Rats received a single injection of prazosin (Sigma; 0.3 mg/kg or 1.0 mg/kg

IP) or vehicle (sterile water; 2ml/kg) immediately following Session 1. For our reanalysis, which consists of data from a subset of rats that received four additional extinction sessions not reported previously (Sessions 3–6), preference is indicated as the percent time on the cocaine-paired floor during the first 15 min of each session. Rats with an initial preference below 50% for the cocaine-paired floor were dropped from subsequent analyses ($n = 10$). To further examine the effect of prazosin, rats in the vehicle and prazosin groups were separated into two subgroups (HIGH or LOW preference) based on a median split of the percent time on the drug-paired floor in each group during Session 1.

Statistical analysis

Data were analyzed using repeated measures ANOVA with Bonferroni *post hoc* tests performed where indicated. Significance was set at $p < .05$.

Results

Rats showed a CPP during the first extinction session that did not differ as a function of group assignment ($F(2,35) = 2.5, p = .11$; Figure 1A). There was a main effect of session [$F(3.7,129.2) = 8.3, p < .001$], a main effect of drug [$F(2,35) = 5.2, p < .05$], and no interaction [$F(7.4,129.2) = 0.6, p = .74$]. Bonferroni *post hoc* tests revealed that the 0.3 and 1.0 mg/kg prazosin-treated animals differed over the course of extinction trials ($p < .01$), while there was no difference between vehicle- and either of the prazosin-treated groups ($ps > .20$). Activity levels (*data not shown*) did not differ across extinction trials between groups, as indicated by a significant main effect of session [$F(3.9,137.3) = 16.0, p < .001$], but no effect of drug [$F(2,35) = 1.5, p = .24$] and no interaction [$F(7.8,137.3) = 0.5, p = .84$]. Thus, prazosin had no residual effects on locomotor activity. To examine the effects of prazosin on strong and weak preferences, animals were divided into HIGH and LOW preference groups (above or below the group's median, respectively).

Rats in the HIGH subgroup showed a CPP during Session 1 that did not differ as a function of group assignment ($F(2,16) = 1.9, p = .19$; Figure 1B). There was a main effect of session [$F(5,80) = 7.6, p < .001$], a main effect of drug [$F(2,16) = 7.7, p < .01$], and no interaction [$F(10,80) = 0.5, p = .90$]. Bonferroni *post hoc* tests revealed that the 1.0 mg/kg prazosin-treated animals differed from both the vehicle and 0.3 mg/kg prazosin groups over the course of extinction trials ($ps < .05$), while there was no difference between vehicle- and 0.3 mg/kg prazosin-treated animals ($p = 1.0$). These results indicate that the higher dose of prazosin enhanced extinction in the HIGH preference subgroup, while the lower dose of prazosin had no effect. Activity levels (*data not shown*) did not differ across extinction trials between groups, as indicated by a significant main effect of session [$F(3.1,49.9) = 11.6, p < .001$], but no effect of drug [$F(2,16) = 0.7, p = .52$] and no interaction [$F(6.2,49.9) = 1.0, p = .46$]. Thus, prazosin had no residual effects on locomotor activity.

Rats in the LOW subgroup showed a CPP during Session 1 that differed as a function of group assignment ($F(2,16) = 6.7, p < .01$; Figure 1C). Bonferroni *post hoc* tests revealed that the 1.0 mg/kg prazosin-treated animals demonstrated a significantly lower initial preference than the other two groups ($ps < .05$); however, the vehicle and 0.3 mg/kg prazosin groups did not differ ($p = 1.0$). There was a main effect of drug [$F(2,16) = 4.8, p < .05$], but no main effect of session [$F(3.1,80) = 2.1, p = .10$] or interaction [$F(6.2,80) = 1.4, p = .24$]. Bonferroni *post hoc* tests revealed that the 0.3 mg/kg prazosin-treated animals differed from the vehicle group over the course of extinction trials ($p < .05$), while there was no difference between vehicle- and 1.0 mg/kg prazosin-treated animals ($p = 1.0$). These results indicate that the lower dose of prazosin impaired extinction in the LOW preference subgroup, while the high dose of prazosin had no effect. Activity levels (*data not shown*) did not differ across extinction trials between groups, as indicated by a significant main effect of session [$F(5,80)$]

= 6.6, $p < .001$], but no effect of drug [$F(2,16) = 0.7$, $p = .51$] and no interaction [$F(10,80) = 1.1$, $p = .37$]. Thus, prazosin had no residual effects on locomotor activity.

Discussion

The current reanalysis of our previous finding [9], which includes examination of additional extinction sessions and a median-based behavioral performance approach, indicates that the α_1 -adrenergic receptor antagonist prazosin can both impair and enhance extinction of a cocaine CPP in rats when administered following an initial test of preference, depending on the dose. Furthermore, these effects of prazosin on CPP were contingent upon the initial preference of the animal, and were maintained over several trials despite the animals receiving only a single administration of prazosin.

The lower dose of prazosin (0.3 mg/kg) impaired extinction only in animals with a lower initial preference, suggesting that weaker learned behaviors may be more vulnerable to the effects of low-dose prazosin. This finding is consistent with recent work demonstrating the ability of α_1 -adrenergic receptor antagonists to impair the rate of extinction of contextual fear conditioning in both mice and rats [7,8] and the persistence of extinction of cocaine CPP in mice [7]. In contrast, the higher dose of prazosin (1.0 mg/kg) enhanced extinction or produced a reconsolidation-like effect, as evidenced by a decrease in preference following a single post-session administration only in animals with a higher initial preference. Interestingly, this effect-- in which prazosin enhanced, rather than impaired, learning-- is consistent with recent findings in the fear literature. Lazzaro et al. [15] demonstrated that terazosin, another α_1 -adrenergic receptor antagonist, administered both systemically and intra-lateral amygdala prior to conditioning trials, enhanced fear learning. Similar mechanisms may result in the enhancement of extinction seen in our study. For example, inasmuch as the extinction of a behavioral response likely represents the balance between the impairment of an excitatory drive and the enhancement of an inhibitory drive [for an extensive discussion on distinctive circuits mediating excitation and inhibition during extinction learning, see [16]], it is possible that the different doses of prazosin have contrasting effects on excitatory and inhibitory neuronal populations and brain regions that mediate these different processes.

Previous studies have demonstrated that several drugs, especially those involved in mediating the stress response and/or arousal, can both enhance and impair memory. The effect of epinephrine follows an inverted-U dose-response function, such that low-to-intermediate doses enhance memory while high doses impair memory [17,18], implicating arousal level, which is correlated with alterations in brain norepinephrine levels [19], as a key determinant of retention [19,20]. Unfortunately, the role of differential levels of antagonists on these effects has thus far been unexplored. However, norepinephrine acts on postsynaptic α_1 -adrenergic receptors to mediate its effects on memory and regulates its own activity via negative feedback involving the activation of presynaptic α_2 -adrenergic receptors [21]. Thus, the two doses of prazosin used here may differentially alter this negative feedback process based on low vs. high arousal and corresponding norepinephrine levels to enhance or impair further norepinephrine release, resulting in differential extinction learning.

We cannot exclude the possibility that one or both doses of prazosin used in the current study may exert nonspecific effects unrelated to α_1 -adrenergic receptor blockade. For example, previous studies have suggested that prazosin at high doses may have affinity for the α_2 -adrenergic receptor [22], and thus may directly regulate norepinephrine release presynaptically, which also could explain the differential effects of dose outlined here. However, prazosin administration at doses similar to those employed in the current study

demonstrates minimal affinity for the α_2 -adrenergic receptor in rat brain [reviewed in 23]. However, several subtypes of the α_1 -adrenergic receptor have been characterized in the brain, with different affinities for prazosin, and these low and high affinity binding sites showed changes in density based on the chronic administration of low and high prazosin doses similar to or higher than those used here [24]. These subtypes mediate different signal transduction mechanisms [25] that may be responsible for the differences in behavior seen here. Although our behavioral effects were achieved after a single administration, additional studies are needed to better characterize how the cellular and molecular effects of prazosin interact with behavior.

Other studies support the idea that the effects of pharmacological manipulations on extinction may depend critically on the behavioral impact of the extinction session [10–12]. These experiments, as well as the current study, encourage analyzing behavioral responses in different ways, including examining extinction at different parts of the behavioral scale and in subpopulations that show different levels of initial conditioning, especially regarding measures of CPP [22]. One critical feature of drug dependence in humans is the magnitude of the associations between the rewarding effects of the drug and the cues that predict drug availability. The findings presented here further implicate the strength of drug associations as a potential indicator of resistance to the pharmacological supplementation of extinction-based therapies.

Conclusion

Our findings further suggest a role for α_1 -adrenergic receptors in drug conditioning paradigms, indicating that prazosin can alter extinction learning based on the dose of the drug and the initial level of learning. These results may have important implications for the use of α_1 -adrenergic receptors as a supplement to therapies designed to extinguish chronic maladaptive memories.

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Figure 1.

Post-session prazosin both impaired and enhanced extinction of a cocaine-induced CPP, based on dose and initial preference. Data represent mean ($\pm$ SEM) percent time on the drug-paired floor during the 15-min Session 1 and the first 15-min of each subsequent session. (A) Overall, rats administered 0.3 mg/kg prazosin ($n=12$) or 1.0 mg/kg prazosin ($n=12$) following Session 1 showed no difference in extinction compared to the vehicle-treated group ($n=14$). (B) Rats in the HIGH initial preference group administered 1.0 mg/kg prazosin ($n=6$) showed an enhanced extinction relative to vehicle- ($n=7$) and 0.3 mg/kg prazosin-treated ($n=6$) animals. (C) Rats in the LOW initial preference group administered 0.3 mg/kg prazosin ($n=6$) showed an impaired extinction relative to vehicle- ($n=7$) and 1.0 mg/kg prazosin-treated ($n=6$) animals.