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Effects of oxazepam on methamphetamine-induced conditioned place preference

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Abstract

Our laboratory has been investigating the role for the hypothalamo–pituitary–adrenal (HPA) axis and benzodiazepines in the behavioral effects of cocaine for several years now. The following represents our initial investigation of the influence of benzodiazepines on methamphetamine reward using conditioned place preference. In these experiments, methamphetamine (0.5 mg/kg ip) resulted in a robust conditioned place preference that was attenuated when the rats were pretreated with oxazepam (10 mg/kg ip) on the day of preference testing. These data suggest a potential role for benzodiazepines in the behavioral effects of methamphetamine. Additional research will be necessary to determine if the nature of these effects is similar with what has been observed with cocaine.

Keywords: Oxazepam; Methamphetamine; Conditioned place preference; Benzodiazepine; HPA Axis

1. Introduction

Over the last several years, our laboratory, as well as a number of others, has investigated the role for stress and the subsequent activation of the hypothalamo–pituitary–adrenal (HPA) axis in the behavioral effects of cocaine (Goeders, 2002a,b). One area of investigation involved the relationship between benzodiazepines and cocaine (Goeders, 1997) because benzodiazepines are among the most widely prescribed drugs for the pharmacological management of stress-related anxiety (Baldessarini et al., 1996). Specifically, we found that chronic, daily injections of cocaine decreased benzodiazepine receptor binding in terminal fields for the mesocorticolimbic dopaminergic system, while increasing labeling in terminal fields for the nigrostriatal system (Goeders et al., 1990; Goeders, 1991).

In a very different series of experiments, we found that pretreatment with the benzodiazepine receptor agonist, chlordiazepoxide, significantly decreased cocaine self-administration in rats (Goeders et al., 1989). The effects of chlordiazepoxide on drug intake were attenuated when the unit dose of cocaine was increased, suggesting that chlordiazepoxide decreased, rather than augmented, cocaine reinforcement. In a follow-up experiment, pretreatment with alprazolam also decreased cocaine self-administration without affecting food-maintained responding during the same session (Goeders et al., 1993), suggesting that these effects may have resulted from specific actions on cocaine reinforcement rather than nonspecific effects on the ability of the rats to respond (Goeders, 2002a). We recently reported that alprazolam also reduces the ability of conditioned cues to reinstate extinguished cocaine seeking in rats (Clampitt et al., 2001), suggesting a role for benzodiazepines in the relapse to cocaine use as well.

We have recently initiated investigations into the potential role for the HPA axis in methamphetamine reinforcement to determine if similar mechanisms are involved in the behavioral effects of both cocaine and methamphetamine. The following represents our initial study with benzodiazepines, which was designed to determine the effects of oxazepam on the expression of methamphetamine-induced conditioned place preference in rats (Bardo and Bevins, 2000). Oxazepam has been reported to be much less preferred by addicts when compared with other benzodiazepines such as alprazolam (Iguchi et al., 1989, 1993),

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suggesting that oxazepam is less rewarding than alprazolam is, at least in that population. We therefore chose oxazepam for study in this experiment in the hopes that any inherent rewarding effects would not interfere with the effects of the drug on methamphetamine place preference.

2. Method

2.1. Subjects

Twenty-four adult male Wistar rats (375–399 g) were obtained from Harlan Sprague–Dawley (Indianapolis, IN) and randomly divided into four treatment groups ($n = 6$ per group). The animals were housed individually in plastic containers, with a laminar flow unit and air filter, in a temperature- and humidity-controlled American Association for Accreditation of Laboratory Animal Care accredited facility (lights on from 6 a.m.–6 p.m.). Each rat was allowed a minimum of 4 days to habituate to the colony before testing began and was briefly handled each day during this habituation process. The rats were allowed free access to both food and water throughout the experiment, except during testing. All procedures were approved by the LSUHSC-S Institutional Animal Care and Use Committee and were carried out in accordance with the NIH *Principles of laboratory animal care* (NIH Publication No. 85-23).

2.2. Apparatus

Two identical Plexiglas chambers (78.7 × 21 × 40.6 cm) were placed side by side in a closed room (2.3 × 3.9 m). Each chamber was divided into two compartments of equal size by a sliding partition. This partition could either be used to restrict the rats to one compartment during conditioning or it could be replaced by a partition containing a 7.6-cm-high × 14-cm-wide opening to allow the rats to move between the sides of the chamber on the test days. The two compartments of the chambers were distinguished by color (i.e., white vs. black), floor texture (i.e., wire mesh vs. bars) and by the odor of the chips used in the litter (i.e., pine vs. cedar). A single 75-W 125–130 V light illuminated the apparatus from the ceiling.

2.3. Procedure

The rats' preferences for one compartment of the conditioned place preference chamber or the other were determined before conditioning with methamphetamine was initiated. On the Friday before the conditioning was scheduled to start, the rats were randomly placed into one or the other compartment of the chamber and were allowed to freely explore both compartments for 30 min. The time spent on each side of the apparatus was recorded, as was the number of crosses between the compartments that the rats made. The compartment in which each rat spent the most time was recorded as its preferred side. The rats were then returned to their home cages. At least 2 h after this initial exposure to the apparatus, the rats were injected with either vehicle (5% emulphor in saline, 1 ml/kg ip) or oxazepam (10 mg/kg ip), depending on their treatment groups (see below), and returned to their home cages. This was done to minimize the potential nonspecific effects of acute oxazepam administration during the place preference test that was conducted on the following Friday. Previous research from our laboratory has demonstrated that the first exposure to a new drug can influence behavior simply due to the novelty of that drug. Prior exposure before testing reduces this "novelty" effect (Goeders et al., 1993; Clampitt et al., 2001).

Conditioning began on the Monday following the initial exposure to the chamber (above) and lasted for 4 days. During this time, the rats were injected with either saline (1 ml/kg ip) or methamphetamine (0.5 mg/kg ip) on alternate days and were placed in one compartment of the place preference apparatus or the other, with the partition in place to restrict the movement of the rat to that specific compartment for 30 min each day. The pairing of compartments with methamphetamine or vehicle was randomly assigned among the rats, with half of the rats receiving methamphetamine in the black compartment and the other half receiving methamphetamine in the white compartment. The initial preference for a specific compartment was not taken into account so that the distribution would be unbiased.

On Friday, following four consecutive days of conditioning, the rats were injected with either vehicle (5% emulphor in saline, 1 ml/kg ip) or oxazepam (10 mg/kg ip) 30 min prior to testing. The animals were then placed into the chamber and were allowed to freely roam between the two compartments for 30 min. The time spent in both compartments was recorded, as was the number of crosses between compartments.

2.4. Drugs

Methamphetamine was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC) and was dissolved in bacteriostatic, heparinized 0.9% saline. Methamphetamine was delivered at a dose of 0.5 mg/kg ip, which was shown in pilot studies to produce conditioned place preference. Oxazepam was purchased from Research Biochemicals International (Natick, MA) and was administered intraperitoneally as a suspension in 5% emulphor in a volume of 1 ml/kg. We chose the 10 mg/kg dose of oxazepam in this experiment because this was the lowest dose that effectively reduced intravenous cocaine self-administration in rats (Guerin et al., 2003).

2.5. Statistical analysis

The data collected included the time spent in each compartment of the place preference apparatus and the number of crosses between sides. Data are presented as

the means (± S.E.M.). The significance of the differences between the means was determined using a one-way analysis of variance on the difference scores (i.e., postconditioning score minus preconditioning score), followed by Tukey's all pairwise multiple comparison procedures, with statistical significance set at $P < .05$.

3. Results

A one-way analysis of variance indicated a significant effect of the treatment conditions on the time spent in the two compartments of the place preference apparatus [$F(3,23) = 4.512$, $P = .014$]. Rats that received injections of saline during conditioning did not show a preference for either compartment and spent approximately equal time on each side (Fig. 1). Saline-conditioned rats that were pretreated with oxazepam before the place preference testing did not display a preference either. In contrast, methamphetamine induced a positive place preference, and the time that the methamphetamine-treated rats spent on the drug-paired side of the apparatus during the place preference testing was significantly greater than the postconditioning time spent by the rats injected with saline during conditioning and pretreated with either vehicle ($q = 4.020$) or oxazepam ($q = 4.168$) during testing. Pretreatment with oxazepam prior to the place preference testing completely reversed methamphetamine-induced place preference, and the time spent in the drug-paired compartment of the chamber was significantly less than the time spent by rats injected with methamphetamine during conditioning that were pretreated with vehicle ($q = 4.500$). There were no differences in the time spent in the drug-paired compartment among rats injected with saline during conditioning and pretreated with either vehicle or oxazepam during testing or those injected with methamphetamine during conditioning and oxazepam during testing. There were no significant differences [$F(3,23) = 0.357$, $P = .785$] in the number of crosses between the two compartments of the place preference apparatus among the groups (63 ± 4 pre, 57 ± 8 post, saline/vehicle; 59 ± 8 pre, 48 ± 9 post, saline/oxazepam; 64 ± 6 pre, 68 ± 5 post, methamphetamine/vehicle; 66 ± 6 pre, 61 ± 6 post, methamphetamine/oxazepam).

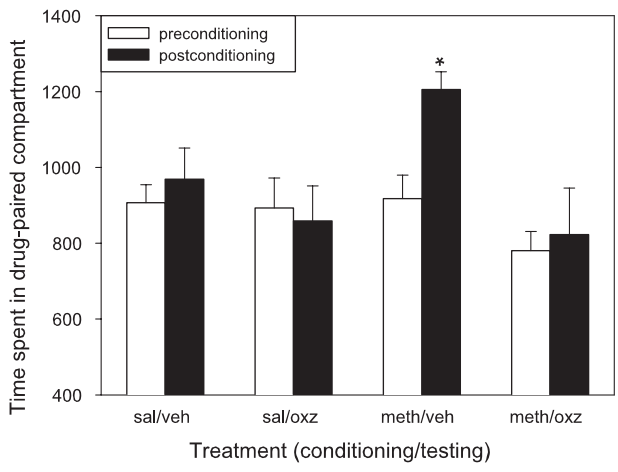

Fig. 1. Effects of oxazepam (10 mg/kg ip) on the expression of a methamphetamine-induced conditioned place preference. Data are the means (± S.E.M.) for $n = 6$ per treatment group and are presented as the total time that was spent in the methamphetamine-paired compartment. * $P < .05$ for the postconditioning scores.

4. Discussion

Injections of saline did not induce either place preference or aversion. These data suggest that although the injections were randomly assigned regardless of the rats' initial preferences for the apparatus, the overall procedure resulted in unbiased results. On the other hand, however, methamphetamine injections (0.5 mg/kg ip) resulted in a significant place preference for the methamphetamine-paired compartment, suggesting that this procedure measured the rewarding effects of methamphetamine. Comparable results have previously been obtained using this procedure with similar doses of methamphetamine in rats and mice (Suzuki and Misawa, 1995; Tokuyama et al., 1996; Shimosato et al., 2001; Li et al., 2001, 2002; Schindler et al., 2002).

Pretreatment with oxazepam (10 mg/kg ip) did not alter the effects of saline injections on place preference or aversion, suggesting that an acute injection of oxazepam did not induce effects of its own on the day of preference testing, which was one of the reasons why we selected oxazepam for this experiment. However, oxazepam attenuated the expression of the methamphetamine-induced place preference, with the amount of time spent in the methamphetamine-paired compartment no different from that observed in rats injected with saline in both compartments. Neither methamphetamine nor oxazepam affected the number of crosses between the two compartments of the testing apparatus, suggesting that the effects of oxazepam on the preference for the methamphetamine-paired compartment were not due to a nonspecific decrease in motor behavior. Other laboratories have previously reported that benzodiazepines (e.g., diazepam, triazolam) also block the expression of amphetamine- (Meririnne et al., 1999; Leri and Franklin, 2000; Pettit et al., 1989) and cocaine-induced (Meririnne et al., 1999) conditioned place preference. These data are in line with our findings and suggest that benzodiazepines and, by inference, the HPA axis may be involved in methamphetamine, as well as in cocaine and amphetamine, reward (Goeders, 2002a,b).

Oxazepam is a benzodiazepine receptor agonist that binds to the benzodiazepine recognition site on the $GABA_A$/benzodiazepine/chloride ion macromolecular complex. The binding of benzodiazepine agonists to this complex results in an increase in GABAergic activity mediated through $GABA_A$ receptors (Ticku, 1983; Saano, 1987; Shephard, 1987), suggesting that the effects that we ob-

served on the actions of methamphetamine in conditioned place preference were also mediated, in part, through $GABA_A$ receptors. Other laboratories have reported that the $GABA_B$ receptor agonist, baclofen, prevents the development and expression of methamphetamine-conditioned place preference (Li et al., 2001) as well as reduces intravenous methamphetamine self-administration in rats (Ranaldi and Poeggel, 2002). Taken together with the results of this investigation, these data further support the hypothesis that the rewarding effects of methamphetamine can be modified through manipulations of the GABAergic neuronal system, possibly through interactions with monoaminergic systems including dopamine.

In summary, we report that the administration of methamphetamine (i.e., 0.5 mg/kg ip) results in reward, as measured using conditioned place preference. Furthermore, this place preference was attenuated following pretreatment with the benzodiazepine agonist oxazepam, suggesting a role for GABA in the behavioral effects of methamphetamine. These results are very similar with those that we have obtained with cocaine and suggest that similar mechanisms may underlie the effects of both types of psychomotor stimulants. Additional research will be necessary to determine if the nature of these effects is similar with what has been observed with cocaine.

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References

Baldessarini RJ. Drugs and the treatment of psychiatric disorders: psychosis and anxiety. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, editors. Goodman and Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill; 1996. p. 399–430.

Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology 2000;153:31–43.

Clampitt DM, Peltier RL, Goeders NE. The effects of alprazolam on the ability of conditioned cues to reinstate extinguished cocaine-seeking behavior in rats. Drug Alcohol Depend 2001;63(Suppl. 1):S28.

Goeders NE. Cocaine differentially affects benzodiazepine receptors in discrete regions of the rat brain: persistence and potential mechanisms mediating these effects. J Pharmacol Exp Ther 1991;259:574–81.

Goeders N.E.. Neuroendocrine role in cocaine reinforcement. Psychoneuroendocrinology 1997;22:237–259.

Goeders NE. The HPA axis and cocaine reinforcement. Psychoneuroendocrinology 2002a;27:13–33.

Goeders NE. Stress and cocaine addiction. J Pharmacol Exp Ther 2002b; 301:785–9.

Goeders NE, McNulty MA, Mirkis S, McAllister KH. Chlordiazepoxide alters intravenous cocaine self-administration in rats. Pharmacol Biochem Behav 1989;33:859–66.

Goeders N, Bell V, Guidroz A, McNulty M. Dopaminergic involvement in the cocaine-induced up-regulation of benzodiazepine receptors in the rat striatum. Brain Res 1990;515:1–8.

Goeders NE, McNulty MA, Guerin GF. Effects of alprazolam on intravenous cocaine self-administration in rats. Pharmacol Biochem Behav 1993;44:471–4.

Guerin GF, Carlson KA, Goeders NE. Oxazepam decreases cocaine self-administration in rats. Abstract Viewer and Itinerary Planner. Washington, DC: Society for Neuroscience; 2003. Online. Prog. No. 854.18.

Iguchi MY, Griffiths RR, Bickel WK, Handelsman L, Childress AR, McLellan AT. Relative abuse liability of benzodiazepines in methadone maintained populations in three cities. NIDA Res Monogr 1989; 95:364–5.

Iguchi MY, Handelsman L, Bickel WK, Griffiths RR. Benzodiazepine and sedative use/abuse by methadone maintenance clients. Drug Alcohol Depend 1993;32:257–66.

Leri F, Franklin KB. Effects of diazepam on conditioned place preference induced by morphine or amphetamine in the rat. Psychopharmacology 2000;150:351–60.

Li SM, Yin LL, Ren YH, Pan LS, Zheng JW. GABA(B) receptor agonist baclofen attenuates the development and expression of D-methamphetamine-induced place preference in rats. Life Sci 2001;70:349–56.

Li SM, Ren YH, Zheng JW. Effect of 7-nitroindazole on drug-priming reinstatement of D-methamphetamine-induced conditioned place preference. Eur J Pharmacol 2002;443:205–6.

Meririnne E, Kankaanpaa A, Lillsunde P, Seppala T. The effects of diazepam and zolpidem on cocaine- and amphetamine-induced place preference. Pharmacol Biochem Behav 1999;62:159–64.

Pettit HO, Batsell WR, Mueller K. Triazolam attenuates amphetamine but not morphine conditioned place preferences. Psychopharmacology 1989;98: 483–6.

Ranaldi R, Poeggel K. Baclofen decreases methamphetamine self-administration in rats. NeuroReport 2002;13:1107–10.

Saano V. GABA-benzodiazepine receptor complex and drug actions. Med Biol 1987;65:167–73.

Schindler CW, Bross JG, Thorndike EB. Gender differences in the behavioral effects of methamphetamine. Eur J Pharmacol 2002;442:231–5.

Shephard RA. Behavioral effects of GABA agonists in relation to anxiety and benzodiazepine action. Life Sci 1987;40:2429–36.

Shimosato K, Watanabe S, Kitayama S. Differential effects of trihexyphenidyl on place preference conditioning and locomotor stimulant activity of cocaine and methamphetamine. Naunyn-Schmiedeberg's Arch Pharmacol 2001;364:74–80.

Suzuki T, Misawa M. Sertindole antagonizes morphine-, cocaine-, and methamphetamine-induced place preference in the rat. Life Sci 1995;57: 1277–84.

Ticku MK. Benzodiazepine–GABA receptor-ionophore complex. Current concepts. Neuropharmacology 1983;22:1459–70.

Tokuyama S, Takahashi M, Kaneto H. The effect of ginseng extract on locomotor sensitization and conditioned place preference induced by methamphetamine and cocaine in mice. Pharmacol Biochem Behav 1996;54:671–6.