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The GABA-B antagonist 2-hydroxysaclofen reverses the effects of baclofen on the discriminative stimulus effects of D-amphetamine in the conditioned taste aversion procedure

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ABSTRACT

Some of the behavioral effects of D-amphetamine (D-AMPH) are mediated by an increase in dopamine neurotransmission in the nucleus accumbens. However, there is evidence that gamma-amino-butyric-acid-B (GABA-B) receptors are involved in some behavioral effects of D-AMPH and cocaine. Here, we examined the effects of baclofen on the discriminative stimulus properties of D-AMPH, using conditioned taste aversion (CTA) as the drug discrimination procedure. Male Wistar rats were deprived of water and trained in the CTA procedure. They received D-AMPH (1 mg/kg, i.p.) before gaining access to saccharin, which was followed by an injection of LiCl. On alternate days, the subjects received saline before and after the access to saccharin. After the rats learned the D-AMPH-saline discrimination, the standard dose of D-AMPH was replaced by different doses of D-AMPH, baclofen (a GABA-B receptor agonist), 2-hydroxysaclofen (a GABA-B receptor antagonist), a combination of baclofen + D-AMPH, or a combination of 2-hydroxysaclofen + baclofen + D-AMPH. Baclofen did not substitute for D-AMPH, but, when combined with D-AMPH, it produced a small but significant decrease in the discriminative stimulus effects of D-AMPH. This effect was reversed by administration of 2-hydroxysaclofen. These data suggest that GABA-B receptors play a regulatory role in the discriminative stimulus effects of D-AMPH.

1. Introduction

The abuse of psychostimulants, such as D-amphetamine (D-AMPH) and cocaine, causes multiple psychiatric disorders, including drug addiction. Therefore, it is important to understand the neurobiology of psychostimulant-related behaviors. Cocaine and D-AMPH are indirect monoamine agonists that exhibit an affinity for the dopamine (DA), norepinephrine (NE), and serotonin (5-HT) transporters involved in neurotransmitter reuptake and vesicular storage systems (Rothman and Baumann, 2003). Cocaine inhibits the reuptake of DA, NE, and 5-HT, thereby increasing the synaptic levels of these neurotransmitters. D-AMPH blocks the uptake of DA, NE, and 5-HT into synaptic vesicles, thereby promoting an increase in the cytoplasmic concentration of these monoamines. Subsequently, as the levels of cytoplasmic monoamines increase, those monoamines exit the neuron via a reversal in the direction of plasma membrane transporters, which leads to an increase in synaptic DA, NE and 5-HT levels (Amara and Sonders, 1998; Elliot and Beveridge, 2005; Kahlig and Galli, 2003; Rothman and Baumann, 2003).

The mesolimbic DAergic system, particularly the projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc), is an important locus for the production of the locomotor, reinforcing, rewarding and discriminative stimulus effects of psychostimulants such as cocaine and D-AMPH (Di Chiara, 1995; Filip and Cunningham, 2002; Koob, 1992; Pontieri et al., 1995). The administration of D-AMPH or cocaine rapidly increases DAergic neurotransmission by interfering with DA transporter function as described above. As a consequence, it produces an increase in DAergic signaling in limbic areas (Koob, 1992; Koob and Bloom, 1988).

Recent evidence also suggests a potential role for gamma-amino-butyric-acid (GABA) neurotransmission in modulating some of the behavioral effects of psychostimulants. Thus, it has been reported that GABA-B receptor agonists are effective in attenuating some behavioral effects of psychostimulants that may be related to the abuse of these drugs. For example, the selective GABA-B receptor agonist baclofen reduces the reinforcing effects of cocaine (Roberts and Andrews, 1997), nicotine (Fattore et al., 2002), methAMPH (Ranaldi and Poeggel, 2002) and D-AMPH (Brebner et al., 2005). Furthermore, baclofen administration decreases the conditioned locomotion elicited by cues associated with cocaine (Hotsenpiller and Wolf, 2003). Other GABA-B receptor agonists also reduce psychostimulant-related

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behaviors. Brebner et al. (1999) reported that the selective GABA-B receptor agonist CGP44532 was effective in attenuating cocaine self-administration in rats subjected to a progressive ratio schedule.

All drugs of abuse have the ability to produce powerful interoceptive stimuli, which may be regarded as an indirect measure of the potential abuse of a drug. The drug discrimination paradigm is a useful behavioral tool to assess the interoceptive properties of drugs of abuse. D-AMPH is able to produce discriminative stimulus effects that are modulated by DA-related ligands (Druhan et al., 1991; Herrera and Velázquez-Martínez, 1997). Therefore, drug discrimination procedures that use D-AMPH as the training drug may be useful for studying DA-GABA interactions. The present study was designed to examine the effects of the GABA-B receptor agonist baclofen on D-AMPH discrimination in rats using conditioned taste aversion (CTA) as the drug discrimination procedure. In order to determine if these effects result from a specific action of baclofen on GABA-B receptors, we examined the effects of the selective GABA-B receptor antagonist 2-hydroxysaclofen on baclofen's effects on the discriminative stimulus effects of D-AMPH. In a drug discrimination procedure using CTA, rats receive the administration of the training drug before their access to saccharin and followed by an injection of LiCl. On alternate days, the rats receive the training drug vehicle before and after their access to saccharin. Once the effects of the training drug have been established as a discriminative stimulus, on substitution and combination tests other dose of the training drug, other drugs, or various drug combinations are administered to assess whether these drugs can substitute for or antagonize the training drug cue. This procedure has been used to study the neurobiological mechanisms of different drugs, such as 5-HTergic agonists (Lucki and Marcoccia, 1991), morphine (Martin et al., 1990; Stevenson et al., 2000), the delta agonist SNC80 (Stevenson et al., 2002), ethanol (Quertemont, 2003) and naloxone (Davis et al., 2008). Drug discrimination training using CTA is more rapid than two-lever operant training, which can be an advantage when drugs that produce toxic effects or induce neuroadaptations with chronic use are utilized as training drugs.

2. Materials and methods

2.1. Animals

Ten male Wistar rats, 120 days old and weighing 200–250 g at the start of experiments, were obtained from the breeding colony of the FES Iztacala-UNAM, México. They were housed individually in stainless steel cages with freely available food (Teklad LM485 Rat Diet by Harlan) and were maintained under a 12 h light/dark cycle with the lights on at 08:00 h and a temperature of 21 (±1) °C. The rats had access to liquid solutions through one or two inverted graduated cylinders placed in the front wall of the cage. Animal care and handling procedures were conducted in accordance with the Official Mexican Norm (NOM-062-ZOO-1999), which is entitled "Technical Specifications for the Production, Care, and Use of Laboratory Animals" and these procedures were approved by the local bioethics committee.

2.2. Drugs

The drugs used in this study were D-amphetamine sulfate, (±)-baclofen (Sigma-Aldrich, St. Louis, MO, USA), 2-hydroxysaclofen (Tocris, Ballwin, MO, USA) and LiCl (Sigma-Aldrich, México). (±)-baclofen and D-AMPH were dissolved in water, and 2-hydroxysaclofen was dissolved in an isotonic (5%) glucose solution. All drugs were administered i.p. (1 ml/kg). LiCl was administered at a dose of 0.34 mEq (2.0 ml/kg of a 0.177 M solution). Saccharin (Sigma-Aldrich, México) was dissolved in distilled water to a concentration of 0.15% (w/v). The drugs and saccharin solutions were prepared fresh daily.

2.3. Procedure

2.3.1. Discrimination training

Subjects were water deprived for 23.5 h and trained for seven days to drink water daily within a 20-min period. Thereafter, they were trained for two days to drink a saccharin solution in 10-min sessions. For training in the CTA procedure, the subjects underwent drug or saline trials as follows.

2.3.1.1. Drug trials. After D-AMPH administration (1.0 mg/kg, i.p.), the subjects were placed in the experimental cages, where 30 min later they were given 10 min of access to an inverted graduated cylinder that contained the saccharin solution. Immediately thereafter, the subjects received an i.p. injection of LiCl, and they were returned to their home cages.

2.3.1.2. Saline trials. After the administration of isotonic saline (1.0 ml/kg, i.p.), the subjects were placed in the experimental cages, where 30 min later they were given access to the saccharin solution for 10 min. Immediately thereafter, the rats again received isotonic saline and were returned to their home cages.

The subjects underwent a total of nine drug and nine saline trials. Drug and saline trials were separated by two days. On those days, the rats remained in their home cages and had access to tap water for 30 min a day. Drug and saline trials alternated randomly, with the restriction that the drug trials did not occur on more than two consecutive occasions.

2.3.2. Generalization and combination tests with D-AMPH and GABA-B receptor ligands

The tests were carried out on a 4-day cycle. On the first day, the subjects participated in a drug trial as described above. On the second day, the subjects remained in their home cages and had a 30-min period of free access to tap water. On the third day, the rats participated in a saline trial as described above. On the fourth day, the subjects received a particular test dose of D-AMPH, a dose of a different drug or a combination of two or three drugs. Subsequently, they had a two-bottle test for 10 min, with one bottle containing tap water and the other containing the saccharin solution. No saline or LiCl was administered on these occasions. The dose and time intervals between the administration and testing for each drug were selected according to the literature: D-AMPH, 0.1–1.0 mg/kg, 30 min (Herrera and Velázquez-Martínez, 1997); baclofen, 1.0–5.6 mg/kg, 30 min (Brebner et al., 2005); and 2-hydroxysaclofen, 0.3–3.0 mg/kg, 30 min (Balerio and Rubio, 2002). The dose to be tested was chosen randomly, and the cycle was repeated until all doses of the substitution drug had been evaluated. The order of testing of the drugs was also randomized. The training dose of D-AMPH and saline (see figures) was evaluated immediately after the training period, using the full four-day cycle that ended in the two-bottle test. It was evaluated again before the assessment of the various doses of each drug. These values were used to create a dose–response curve of each drug. To examine the effects of GABA-B receptor ligands on the discriminative stimulus properties of D-AMPH, doses of baclofen (1.0–5.6 mg/kg, 30 min), 2-hydroxysaclofen (0.3–3.0 mg/kg, 30 min) or 2-hydroxysaclofen (0.3–3.0 mg/kg, 30 min) + baclofen (5.6 mg/kg, 30 min) were administered in combination with D-AMPH (1.0 mg/kg, 30 min).

2.4. Data analysis

During acquisition, saccharin intake during the drug and saline trials was recorded and compared using two-way ANOVA, with treatment condition (drug vs. saline) serving as the first factor and trial number as the second factor. For the second factor, only the last three trials of each condition were analyzed. During the two-bottle generalization tests, water and saccharin intakes were recorded, and

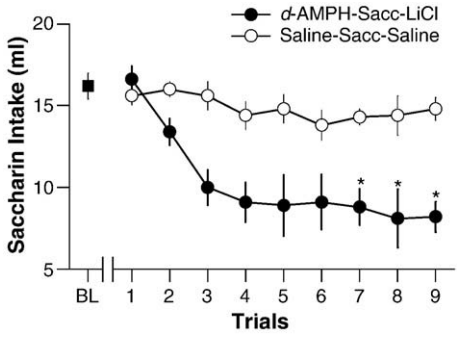

Fig. 1. Acquisition of D-AMPH-saline discrimination using CTA procedure. Points are means ± SEM of 10 rats, and asterisks indicate significant differences (Newman–Keuls test, $p<0.05$) between saccharin intake on the D-AMPH and saline trials. BL: baseline of saccharin intake. Sacc: saccharin.

saccharin preference was calculated according to the formula $A/(A+B)$, where A is saccharin intake and B is water intake. In this formula, an index of 0.0 indicates a strong aversion to saccharin, while 1.0 indicates strong preference for saccharin. Preference data were analyzed using one-way ANOVA. When a significant effect of treatment was obtained, the Newman–Keuls test ($p<0.05$) was used for *a posteriori* comparisons. In the generalization tests, the following criteria were used: full substitution, ≥80%; partial substitution, >30% and <80%; and no substitution, ≤30%.

3. Results

3.1. Acquisition of the discriminative stimulus properties of D-AMPH

Rats learned to discriminate between the D-AMPH and saline solution (Fig. 1). There were no significant differences ($F[2,27]=0.474$, $p>0.05$) between saccharin intake in the baseline sessions, the first drug trial and the first saline trial. When D-AMPH was followed by saccharin-LiCl pairings, a reduction of saccharin intake was observed; and two-way ANOVA revealed significant differences between the last three drug trials and the last three saline trials (treatment condition, $F[1,54]=49.63$, $p=0.0001$). The effects of trial number ($F[2,54]=0.119$, $p>0.05$) and the interaction ($F[2,54]=0.085$, $p>0.05$) were not significant.

3.2. Generalization tests with D-AMPH

The administration of the different doses of D-AMPH that were evaluated during the two-bottle test induced a dose-dependent stimulus control (Fig. 2). The evaluation of the 1.0 mg/kg dose that was administered during the evaluation of the dose–response curve replicated the stimulus control that was exerted by D-AMPH during the first determination of the training dose (97% substitution). One-way ANOVA revealed a significant effect of treatment condition ($F[4,45]=10.704$, $p=0.0001$). The Newman–Keuls test revealed that treatment with saline, 0.1 mg/kg D-AMPH and 0.3 mg/kg D-AMPH yielded results that were significantly different from the training dose of D-AMPH.

3.3. Generalization tests with GABA-B receptor ligands

Different doses of baclofen or 2-hydroxysaclofen did not substitute for D-AMPH (Fig. 2). In fact, a higher dose of baclofen or 2-hydroxysaclofen resulted in 22% and 9% substitution, respectively. One-way ANOVA revealed a significant difference for baclofen ($F[4,45]=11.659$, $p=0.0001$) and 2-hydroxysaclofen ($F[4,45]=8.540$, $p=0.0001$). The Newman–Keuls tests revealed that saccharin preference at all doses of baclofen and 2-hydroxysaclofen was significantly different from that observed during the determination of the training dose of D-AMPH.

3.4. Combination tests with GABA-B receptor ligands + D-AMPH

The results of the combination tests with baclofen + D-AMPH, 2-hydroxysaclofen + D-AMPH and 2-hydroxysaclofen + baclofen + D-AMPH are shown in Fig. 3. We observed that administration of various doses of baclofen with a fixed dose of D-AMPH (1.0 mg/kg) produced a decrease in the discriminative stimulus properties of D-AMPH. For example, the D-AMPH cue decreased from 97% to 39.8% when the highest dose of baclofen was combined with D-AMPH. One-way ANOVA revealed a significant effect of treatment ($F[4,45]=15.554$, $p=0.0001$). Post-hoc analysis revealed that this effect was due to the combination of 5.6 mg/kg of baclofen and 1.0 mg/kg of D-AMPH (Newman–Keuls test). In contrast, when 2-hydroxysaclofen (0.3–3.0 mg/kg) was administered with a fixed dose of D-AMPH, it did not affect the discriminative cue that was produced by D-AMPH ($F[4,45]=27.298$, $p=0.0001$). However, as also shown in Fig. 3, 2-hydroxysaclofen reduced the effects of baclofen on D-AMPH-induced discriminative cue ($F[4,45]=8.928$, $p=0.001$). The Newman–Keuls test revealed that a condition in which 0.3 or 1.0 mg/kg of 2-hydroxysaclofen was administered in combination with

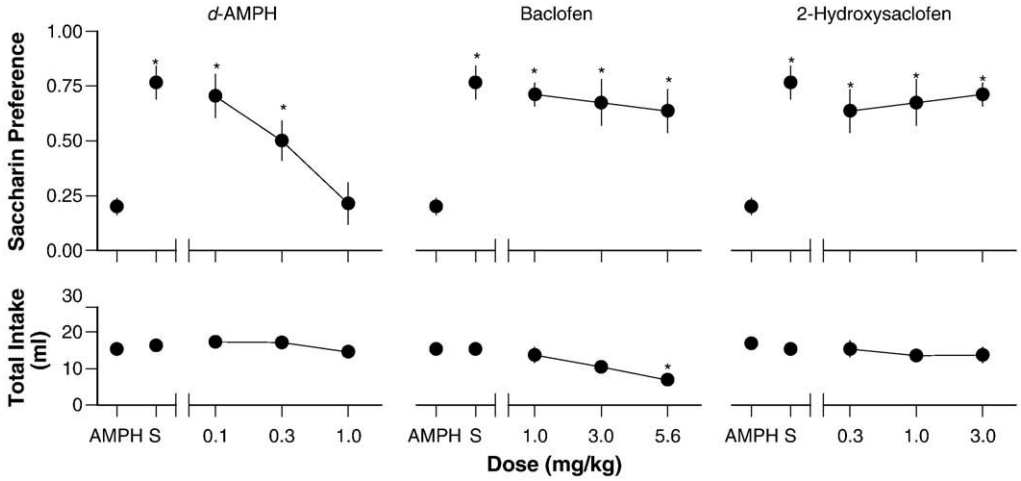

Fig. 2. Top. Saccharin preference (discrimination index) during generalization tests with D-AMPH (left-hand graph), baclofen (middle graph), and 2-hydroxysaclofen (right-hand graph). Points are means ± SEM for 10 rats. Unconnected points in each graph show the data for the training dose of D-AMPH (1.0 mg/kg) (AMPH) and saline (S), which was derived before the determination of the full dose–response curve for each compound. Asterisks indicate significant differences (Newman–Keuls test, $p<0.05$) between saccharin preference observed after the training dose of D-AMPH and that observed after the different doses of D-AMPH, baclofen, or 2-hydroxysaclofen. Bottom. Total fluid intake during the corresponding sessions.

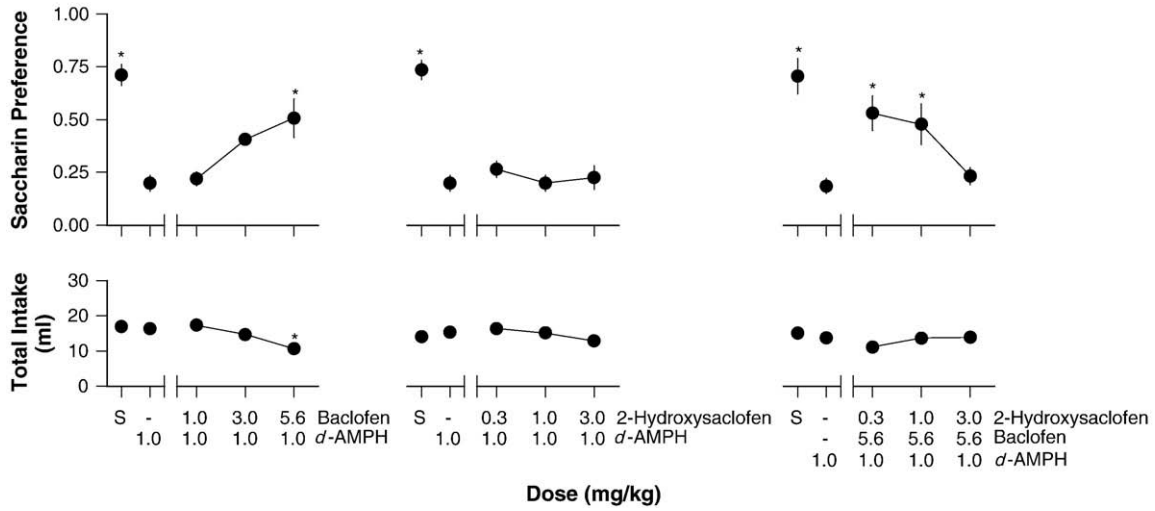

Fig. 3. Top. Saccharin preference during combination tests with the GABA-B receptor ligands + D-AMPH in rats trained to discriminate D-AMPH (1.0 mg/kg) from saline. Points are the means ± SEM of 10 rats. Asterisks indicate significant differences (Newman–Keuls test, $p<0.05$) between saccharin preference observed after GABA-B receptor ligands + D-AMPH or saline (S), and that observed after the training dose of D-AMPH (1.0 mg/kg). Bottom. Total fluid intake during the corresponding sessions.

baclofen and D-AMPH differed significantly from the condition in which only D-AMPH (1.0 mg/kg) was administered.

3.5. Liquid intake in generalization tests

The total intake of liquids was not disrupted by D-AMPH ($F[4,45]=0.769$, $p>0.05$), 2-hydroxysaclofen ($F[4,45]=0.505$, $p>0.05$), 2-hydroxysaclofen + D-AMPH ($F[4,45]=0.455$, $p>0.05$) or 2-hydroxysaclofen + baclofen + D-AMPH ($F[4,45]=2.476$, $p>0.05$). In contrast, total liquid intake was disrupted by baclofen ($F[4,45]=15.554$, $p=0.001$). A Newman–Keuls test revealed that the condition where 5.6 mg/kg of baclofen was administered was significantly different from saline and from the D-AMPH training dose. Total liquid intake was also disrupted during administration of baclofen + D-AMPH ($F[4,45]=4.111$, $p=0.006$). Newman–Keuls tests revealed that the condition where 5.6 mg/kg of baclofen + D-AMPH were administered was significantly different from saline and from the D-AMPH training dose.

4. Discussion

The purpose of the present study was to examine the effects of the GABA-B receptor agonist baclofen on D-AMPH discrimination in rats using CTA as the drug discrimination procedure. We found that rats were able to discriminate D-AMPH from saline after nine drug and nine saline trials. These results are similar to those obtained in previous studies using D-AMPH as the training drug in a CTA paradigm (Herrera and Velázquez-Martínez, 1997; Miranda et al., 2007). We also found, in both the generalization and combination tests that baclofen and 2-hydroxysaclofen did not substitute for D-AMPH. However, administration of baclofen in combination with D-AMPH produced a decrease in the D-AMPH-induced discriminative cue; this effect was small but significant. Baclofen is a selective GABA-B receptor agonist that has been shown to be active in animal behavior models of drug addiction (Brebner et al., 2002; Roberts, 2005).

In the present study, the systemic administration of 2-hydroxysaclofen, which is a GABA-B receptor antagonist, reduced the effects of baclofen on the D-AMPH-induced discriminative cue, which provided additional evidence that baclofen's effect in this experiment is mediated by GABA-B receptors. It should be noted that the systemic administration of 2-hydroxysaclofen did not alter the D-AMPH-induced discriminative cue. These observations suggest that the GABA-B receptor may be involved in the modulation of the discriminative cue of D-AMPH.

Although there was no control group in the present study to assess the unconditioned effects of D-AMPH on saccharin consumption that could mask the discriminative control of D-AMPH on discrimination training, this possibility can be excluded for several reasons. First, several doses of D-AMPH were tested during the substitution and combination tests, including the training dose, and there was no effect on total intake. Second, we previously reported that a control group of rats under a D-AMPH-saccharin-saline condition did not reduce saccharin intake after nine trials (Herrera and Velázquez-Martínez, 1997). Third, a dose–response curve for the discrimination of D-AMPH was established in the present study in order to ensure that the rats responded on the basis of the D-AMPH discriminative cue.

The above behavioral results are consistent with previous reports that demonstrated that the GABA-B receptor agonist baclofen attenuates psychostimulant-induced behaviors that are related to drug addiction. For example, it has been reported that baclofen pretreatment reduces cocaine self-administration in rats that respond under fixed ratio schedules (Brebner et al., 2000a), progressive ratio schedules (Arnold and Roberts, 1997; Roberts et al., 1996; Roberts and Brebner, 2000), discrete trial schedules of reinforcement (Brebner et al., 1999), and second-order schedules (Di Ciano and Everitt, 2003). It has also been reported that baclofen decreases D-AMPH self-administration under fixed ratio or progressive ratio schedules (Brebner et al., 2005) and attenuates conditioned locomotion to cues that are associated with cocaine administration (Hotsenpiller and Wolf, 2003). Furthermore, baclofen reduces the behavioral effects of alcohol (Colombo et al., 2002), nicotine (Paterson et al., 2004) and heroin (Di Ciano and Everitt, 2003).

In contrast to the results of the present experiment and the findings reviewed above, some studies have reported that baclofen does not alter the discriminative stimulus properties of cocaine (Munzar et al., 2000; Negus et al., 2000) and methAMPH (Munzar et al., 2000). The reason for this discrepancy is not clear. The present experiment used D-AMPH as the training drug, whereas the studies that reported negative results used cocaine or methAMPH. Although all of these drugs increase DAergic neurotransmission via interactions with the DA transporter (Howell and Kimmel, 2008), their mechanisms of action are slightly different (see Introduction). Another potentially important factor is the procedure that is used to establish drug discrimination. The CTA procedure was used in the present study, whereas a two-lever procedure with food reinforcement was used in those studies that reported negative results.

It is important to note that, in the present study the highest dose of baclofen significantly decreased total liquid intake from 15 ml to 6 ml. Although the rats significantly reduced their total intake of liquids on the preference test, the rats were still able to choose between saccharin and water. This could reflect not only a general disruption of rat behavior due to baclofen-mediated sedation and motor impairment (Ong and Kerr, 2005) but also the possibility that baclofen specifically inhibits the discriminative stimulus effects of D-AMPH. Other studies have reported that baclofen doses of 5.0 mg/kg decrease cocaine self-administration without affecting food self-administration (Roberts et al., 1996). It has also been reported that 3.0 mg/kg of baclofen decreased ethanol-reinforced responses without affecting water-reinforced responses (Liang et al., 2006). In general, it can be suggested that GABA-B agonists, such as baclofen, reduce some behavioral effects of the drug of abuse. At high doses, baclofen also reduces motor activity, and, as a consequence, total liquid intake is reduced, as was observed in the present experiment.

The mechanism underlying baclofen's effects on the discriminative stimulus effects of D-AMPH that were observed in the present study may involve the GABAergic modulation of DAergic transmission within the VTA. Several lines of evidence support this suggestion. First, the VTA contains DAergic neurons and GABA interneurons, which represent an important locus for the production of some abuse-related behavioral effects of psychostimulants (Kalivas, 1993; Koob, 1992). Second, anatomical evidence suggests that GABA-B receptors are located within the VTA (Bowery et al., 1987; Johnson and North, 1992; Kalivas, 1993; Nagai et al., 1983; Wirtshafter and Sheppard, 2001). Third, biochemical and behavioral studies have reported that the infusion of baclofen into the VTA decreases DA release in the NAcc (Westerink et al., 1996; Yoshida et al., 1994), which suggests that activation of GABA-B receptors located on the cell bodies of mesolimbic DAergic neurons is involved in the biochemical effects of baclofen. Furthermore, microinjections of baclofen into the VTA reduced cocaine self-administration under fixed ratio (Shoaib et al., 1998) and progressive ratio schedules (Brebner et al., 2000b), and microinjections also reduced heroin self-administration (Xi and Stein, 1999) and D-AMPH-induced motor activity (Kalivas et al., 1990). Taken together, these data suggest that baclofen may inhibit the enhancement of the mesolimbic DAergic neurotransmission produced by drugs of abuse.

Although the above evidence suggests that activation of GABA-B receptors in the VTA may be involved in the D-AMPH-induced discriminative cue, activation of GABA-B receptors in different mesolimbic structures, such as the NAcc, can not be excluded. Indeed, this may explain the results of the present study, since microinjections of baclofen into the NAcc also reduce cocaine self-administration (Shoaib et al., 1998).

However, our results could also be explained by perceptual masking effects, this possibility can be excluded because some studies have reported that the co-administration of baclofen with cocaine or methAMPH, which are D-AMPH-like stimulants, did not attenuate the discriminative signal of these drugs (Munzar et al., 2000; Negus et al., 2000). If baclofen's effects were due to perceptual masking in drug discrimination studies, it might be expected that baclofen would also affect the discriminative stimulus properties of cocaine or methAMPH.

With regard to the CTA as a behavioral baseline for drug discrimination learning, the present results and others suggest that this behavioral baseline is a useful tool to research the neurobiological mechanisms that are involved in drug addiction. Furthermore, it has been demonstrated that pharmacological agents that function as training drugs or as substitution drugs in a standard two-lever operant procedure can also function as training drugs or as substitution drugs in the CTA procedure (Orr et al., 2008). However, there may be some exceptions (Quertemont, 2003), or at least strong differences, in the dosage and the time that is required for training (Davis et al., 2008).

In conclusion, the present results indicate that the GABA-B receptor agonist baclofen decreases the discriminative stimulus effects of D-AMPH. This effect is reversed by the GABA-B receptor antagonist 2-hydroxysaclofen. These data provide further evidence for the role of GABA-B receptors in the modulation of some of the behavioral effects of psychostimulants.

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