Baclofen Inhibits Heroin Self-Administration Behavior and Mesolimbic Dopamine Release

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ABSTRACT
An emerging hypothesis to explain the mechanism of heroin-induced positive reinforcement states that opiates inhibit γ-aminobutyric acid (GABA)-ergic interneurons within the mesocorticolimbic dopamine (DA) system to disinhibit DA neurons. In support of this hypothesis, we report that the development of heroin self-administration (SA) behavior in drug-naïve rats and the maintenance of SA behavior in heroin-trained rats were both suppressed when the GABA\_A receptor agonist baclofen was coadministered with heroin. Microinjections of baclofen into the ventral tegmental area (VTA), but not the nucleus accumbens, decreased heroin reinforcement as indicated by a compensatory increase in SA behavior. Additionally, baclofen administered alone or along with heroin dose-dependently reduced heroin-induced DA release. This effect was blocked partially by intra-VTA infusion of the GABA\_B antagonist 2-hydroxyaslofen, suggesting an additional, perhaps GABA\_A receptor-mediated, disinhibitory effect. Taken together, these experiments, for the first time, demonstrate that heroin-reinforced SA behavior and nucleus accumbens DA release are mediated predominantly by GABA\_B receptors in the VTA and suggest that baclofen may be an effective agent in the treatment of opiate abuse.

A large body of experimental evidence supports the hypothesis that the mesocorticolimbic dopamine (DA) system, which originates in the ventral tegmental area (VTA) and projects rostrally to the nucleus accumbens (NAcc) and the medial prefrontal cortex, plays a critical role in mediating opiate reinforcement. For example, μ and δ receptor agonists can be self-administered directly into the VTA (Devine and Wise, 1994) or the NAcc (Goeders et al., 1984), whereas chemical lesion of VTA DA neurons with 6-hydroxydopamine (6-OHDA) (Sypriaki et al., 1982) or opioid receptor antagonists microinjected into the VTA (Britt and Wise, 1983) block heroin SA behavior. Similarly, microinjections of morphine or heroin into the VTA or NAcc induces positive reinforcement as assessed by conditioned place preference (Phillips and LePiane, 1980). Despite this converging evidence, a full description of the mechanisms of opioid-induced reinforcement are still lacking.

Opiate reinforcement is proposed to be mediated by a disinhibitory mechanism, i.e., opiates inhibit VTA γ-aminobutyric acid (GABA)-ergic interneurons to decrease GABA release, which subsequently disinhibits VTA DA neurons, leading to an increase in NAcc DA release (Kelley et al., 1980). Several lines of evidence support this hypothesis. First, systemic or microiontophoretic-applied morphine into the VTA increases the firing rate of dopaminergic neurons and inhibits the firing rate of inhibitory interneurons (Kelley et al., 1980; Gysling and Wang, 1983; Mathews and German, 1984; Johnson and North, 1992a). Second, microdialysis and electrochemical studies demonstrate an increased NAcc DA release after heroin administration (Spanagel et al., 1990; Rada et al., 1991; Xi et al., 1998). Third, anatomic evidence suggests that opioid μ receptors are located predominantly on nondopaminergic interneurons in the VTA (Mansour et al., 1987; Dilts and Kalivas, 1989). Finally, morphine presynaptically inhibits GABA release from GABAergic interneurons in the rat midbrain (Renno et al., 1992). Together, these data suggest that opioid-induced excitatory effects on VTA DA cells are mediated, at least in part, by inhibiting GABAergic interneurons.

Based on the above, we hypothesize that GABAmimetic agents that increase GABAergic transmission in the mesolimbic DA system will reduce or block heroin reinforcement, manifest as a decrease in SA behavior and reduced NAcc DA release. However, because we reported previously that the GABA\_B receptor agonist muscimol neither substituted for heroin during heroin SA behavior nor reduced heroin-induced DA release (Xi and Stein, 1998), we investigated the effects of the GABA\_B receptor agonist baclofen on heroin-
reinforced SA behavior and NAcc DA release. The results demonstrate that baclofen significantly reduces heroin-reinforced SA behavior and NAcc DA release, suggesting a potential therapeutic role in the treatment of opiate dependence.

**Materials and Methods**

**Surgical Preparation.** Forty-six male Sprague-Dawley rats (Sasco, Madison, WI), weighing 250 to 350 g at the time of surgery, were housed and maintained individually on a 12-h light/12-h dark cycle (lights on at 8:00 PM) with free access to food and water. Under sodium pentobarbital anesthesia (60 mg/kg i.p.), rats were implanted with a chronic silicone rubber jugular catheter that passed s.c. to terminate on a head assembly. Rats were divided into two groups, one for SA alone (n = 39), and the other for electrochemical recordings (n = 7). To observe the effects of central receptor modulation on heroin SA, 12 rats in the SA alone group also were implanted with unilateral 30-gauge stainless steel guide cannula into the VTA (4.8 mm posterior to bregma, 2.4 mm lateral to midline, and 7.7 mm ventral to the surface of the cortex with 10° lateral angle) and the NAcc (1.7 mm anterior to bregma, 1.6 mm lateral to midline, and 7.2–7.4 mm ventral to the surface of the cortex). A carbon-fiber electrode was implanted stereotaxically into the NAcc in the electrochemical recording group. This second surgery was performed after stable heroin SA behavior was established, usually 1 week after training initiation. To minimize coating of the carbon fiber by tissue fragments during implantation, the dura and pia mata were punctured by a 23-gauge syringe needle. Ag/AgCl reference and miniature pin connectors soldered to the three electrodes were inserted into a plastic strip connector and secured, together with the i.v. catheter and intracranial cannula, with acrylic dental cement to four stainless steel screws threaded into the skull. Three days were allowed for recovery from surgery before DA measurement and/or SA training.

**Heroin-SA Procedure.** Operant boxes (30 × 40 × 60 cm) equipped with a lever mounted on one side wall 5 cm above the cage floor were placed in sound- and light-attenuated chambers. The i.v. catheter was connected to a syringe pump (Razel, Stamford, CT) through polyethylene tubing and a liquid commutator. Each lever press delivered an infusion of heroin (approximately 100 μl) over a 10-s period. Depending on the experiment, heroin (0.06 mg/kg) or heroin plus baclofen (0.5 or 1 mg/kg) dissolved in sterile saline was administered per lever press. A 60-W white light located above the chamber was illuminated simultaneously with each drug infusion. Each SA session lasted for 4 h, and each rat was tested for 5 to 9 days on a continuous reinforcement (FR1) schedule.

GABA agents (baclofen, 2-hydroxyxasclfen, and muscimol) were purchased from Research Biochemical International (Natick, MA) and dissolved fresh each day in sterile saline. Heroin was donated by the Resource Technology Branch, National Institute on Drug Abuse. All injections into the VTA and NAcc were delivered in a volume of 1 or 2 μl over 1 or 2 min, respectively.

**Microelectrode Fabrication and In Vitro Calibration.** Electrodes were fabricated from a single 8-μm diameter carbon fiber that extended 250 μm beyond the tip of a pulled glass capillary and fixed in the capillary by a drop of Epon Resin mixed with O-phenylenediamine (1 g Epon Resin to 0.14 g O-phenylenediamine). The electrode assembly was baked at 300°C for at least 3 h until the melted Epon Resin reached the capillary tip. Electrodes were coated with a 5% Nafion solution (Aldrich Chemical, Milwaukee, WI), an ion-selective polymer that promotes the passage of cations such as DA and impedes the passage of anions, primarily ascorbic acid (AA) and the DA metabolite, dihydroxyphenylacetic acid (DOPAC). Electrodes were dipped into 5% Nafion, air-dried, and baked at 85°C for 5 min; the entire procedure was repeated five to six times. Before implantation, electrodes were calibrated for their DA sensitivity and their selectivity to DA against AA and DOPAC in a 0.1 M PBS solution (154 mM NaCl, 78 mM Na₂HPO₄·7H₂O, 18 mM NaH₂PO₄·H₂O, pH 7.2–7.4). Only electrodes that showed a minimum DA sensitivity of 50 nM and a linear response to increasing DA concentrations (r > 0.995), and high selectivity to DA against AA (range, 800–5000:1) and DOPAC (range, 500–2000:1) were used for DA recordings in vivo.

**Fast-Cyclic Voltammetry (FCV).** Electrochemical measurements were performed with a microcomputer-based voltammetric instrument (IVEC10; Medical Systems, Greenvane, NY). The FCV wave form consisted of one cycle of a triangle wave initiated at 0 mV versus Ag/AgCl and swept between 1.0 mV and −0.5 mV at a scan rate of 50 mV and a repetition rate of 1.0 Hz. The electrode oxidation current was integrated between 400 and 900 mV and converted to DA concentration by using each electrode’s in vitro calibration data obtained before implantation according to the working hypothesis that signal changes were due entirely to changes in DA concentration.

**Data Reduction and Statistical Analyses.** To increase signal detection, FCV signal changes were averaged across trials with the lever press set to zero for both time and amplitude. Signal values first were averaged across the 60 consecutive, 1-s oxidation cycles during each drug injection and then averaged across all injections within each 3-h recording session. Finally, all repeated sessions for each rat and all rats in each drug treatment group were combined. Data are expressed as mean amplitude ± S.E.M. and expressed in micromolar DA. Two-way ANOVAs (time × treatment) were used to analyze changes in DA release before and after drug injection. Student’s t tests were used to analyze the effect of baclofen on heroin SA. Significance was set to p ≤ .05 throughout.

**Histology.** Upon completion of each experiment, rats were anesthetized deeply with pentobarbital and transcardially perfused with PBS followed by 10% formaldehyde solution. Brains were sectioned at 40 μm, and electrode and cannula tips were verified histologically.

**Results**

**Heroin SA Behavior and NAcc DA Release.** Typically, rats rapidly learned the operant task and reliably self-administered heroin after 2 to 3 days of training. Rats with unstable SA behavior or poor electrochemical responses were eliminated from further study. Although SA behavior varied across rats and sessions, the pattern of responses and the mean SA rate across sessions were very stable over time. Electrochemical recordings were initiated after 1 week of SA training. Consistent with the observed behavioral patterns, the electrochemical signal also varied somewhat across trials, sessions, and rats. However, when averaged together, an increased DA signal change was observed consistently in five of the seven rats tested. The effects of baclofen on heroin-induced DA release were analyzed only for these five rats. Figure 1A (a) depicts a representative original DA signal change after heroin (0.06 mg/kg) SA. Both DA-dependent oxidation and reduction currents increased after i.v. heroin administration. In some instances, a small signal decrease also was observed for a few seconds before the signal increase. When averaged across all rats and all days, the DA-dependent electrochemical signal increased significantly after heroin administration, reached a mean peak amplitude of 0.24 ± 0.10 μM after 4 min (p < .05, compared with baseline levels), and returned to baseline at about 12 min (Fig. 1B).

**Effects of Baclofen on Heroin SA.** When coadministered with heroin during the initiation of SA training, ba-
clofen dose dependently decreased heroin SA behavior (Fig. 2). At the low dose of baclofen used (0.5 mg/kg, \( n = 8 \)), heroin SA increased significantly from 1.8 ± 0.4 to 2.8 ± 0.5 responses within the first half-hour (\( p < .05 \)). SA behavior decreased rapidly thereafter and remained below heroin-alone values for the rest of the session (Fig. 2B). When switched to heroin alone on days 6 and 7, SA behavior increased rapidly to approximately the values of the heroin-
alone control group (18.3 ± 2.7 SA/4 h, n = 7). When baclofen again was coadministered with heroin on days 8 and 9, rats completely ceased SA behavior (Fig. 2A). A similar suppression of SA also was observed in the heroin-alone group when baclofen was coadministered with heroin on day 8 (Fig. 2A). Furthermore, the high dose of baclofen (1.0 mg/kg, n = 11) completely prevented the acquisition of heroin SA behavior. Even when switched to heroin alone on days 8 and 9, no SA behavior was observed in this group (Fig. 2A). In contrast, coadministration of the GABA_A agonist muscimol (0.1 mg/kg) with heroin had no significant effect on SA behavior in four rats tested (data not shown).

When baclofen (2 μg) was infused into the VTA of well trained animals, heroin SA increased significantly (Fig. 3A; p < .001). The same effect also was observed in three rats when baclofen was microinfused to the VTA and NAcc, and all microinjection guide cannulae were located within the VTA and NAcc.

**Effects of Baclofen on Heroin-Induced DA Release in the NAcc.** As illustrated in Fig. 1, A and B, systemic coadministration of baclofen dose-dependently decreased heroin-induced DA release in the NAcc (F(3, 19) = 9.54; p < .05). This effect was blocked by ipsilateral VTA injections of 1 μg of the GABAB receptor antagonist 2-hydroxy saclofen [Fig. 1, A (d) and B]. Systemic baclofen administered alone (1.0 mg/kg, n = 4) significantly decreased basal NAcc DA release, with a peak amplitude of -0.67 ± 0.19 μM reached at 12 min; DA signal recovered at about 34 min after drug administration.

**Electrode Localization.** Histological examination demonstrated that all seven electrode tips were located within the shell portion of the NAcc, and all microinjection guide cannulae were located within the VTA and NAcc.

**Discussion.** The present study demonstrates that when administered i.v., baclofen dose-dependently reduces heroin-reinforced SA behavior. This effect appears to be mediated by GABAB receptors in the VTA because baclofen decreased heroin-induced NAcc DA release and increased heroin SA behavior only when injected directly into the VTA (but not NAcc), and the GABAB antagonist 2-hydroxy saclofen injected into the VTA blocked the systemic baclofen effects.

Several independent lines of evidence suggest that activation of the mesolimbic DA system is necessary for expression of heroin’s rewarding effects. For example, high-density opioid μ receptors and intrinsic enkephalinergic innervation have been identified within the VTA (Dilts and Kalivas, 1989), and microinjections of morphine or heroin into the VTA produce positive reinforcement as assessed by conditioned place preference; the latter can be prevented by destruction of intrinsic DA neurons with 6-OHDA (Phillips and LePiane, 1980). In addition, opioids can also be self-administered into the VTA (Devine and Wise, 1994), and the opioid receptor antagonist naloxone administered into the VTA significantly attenuates opiate reinforcement and blocks the acquisition of heroin SA behavior (Britt and Wise, 1983). Microdialysis and voltammetry studies demonstrate an increased DA release in the NAcc after heroin administration (Di Chiara and Imperato, 1988; Spanagel et al., 1990; Xi et al., 1998), although conflicting reports exist (Kiyatkin et al., 1993).

Consistent with our previous report (Xi et al., 1998), an increased DA release after heroin administration was observed in the majority of rats studied (five of seven) after 1 week of SA training. The remaining two rats demonstrated a significant decrease in DA after heroin. The mechanism of these different heroin effects in individual rats is unclear. A
similar DA decrease response has been observed in drug-naive rats (Kiyatkin et al., 1993), suggesting that chronic or repeated heroin exposure may induce an adaptive mesolimbic DA system response that leads to increased NAcc DA release (Sself et al., 1995).

In contrast to the VTA DA hypothesis, evidence also exists that opiates can be self-administered directly into the NAcc (Goeders et al., 1984) and opiate antagonists administered into the NAcc attenuate i.v. heroin SA (Vaccarino et al., 1985). Systemic or intra-NAcc administration of DA antagonists does not alter i.v. heroin SA (Ettenberg et al., 1982). Destruction of presynaptic DA terminals in the NAcc, using the neurotoxin 6-OHDA, selectively attenuates cocaine but not heroin SA (Pettit et al., 1984). These data suggest the existence of a DA-independent mechanism in the NAcc. However, the precise neurochemical mechanisms of opiate reinforcement in the NAcc are not clear. Morphological evidence demonstrates that the majority of NAcc neurons are GABAergic and comprise the final common-output neurons of the NAcc (Nagai et al., 1983; Bowery et al., 1987). These medium, spiny GABAergic neurons receive multiple inputs, including DA from the VTA, glutamate from the PFC, and enkephalin from local interneurons, and project primarily to the ventral pallidum (Sesack and Pickel, 1990). It is believed that DA can directly inhibit these GABAergic output neurons (De France et al., 1985), which may be the final mediator for the DA-dependent reinforcing mechanism (Koob and Bloom, 1988). In this schema, opiate-induced direct inhibition of these NAcc GABAergic output neurons will produce a similar but DA-independent rewarding effect. That is, opiate reinforcement may be mediated by an indirect disinhibition of DA neurons in the VTA and a direct inhibition of GABAergic output neurons in the NAcc.

Two types of neurons, primary dopaminergic projection neurons and secondary GABAergic inhibitory interneurons, have been identified within the VTA (Nagai et al., 1983; Johnson and North, 1992b), with the latter shown to synapse onto the projection neurons (Nagai et al., 1983). Autoradiographic evidence demonstrates that opioid µ receptors are located mainly on the interneurons and not the DA neurons (Dilts and Kalivas, 1989). Systemic or iontophoretic administration of morphine increases the firing rate of VTA DA neurons (Mathews and German, 1984) and causes an increase in NAcc DA release (Di Chiara and Imperato, 1988; Xi et al., 1998), effects that may be mediated by inhibition of GABAergic interneurons (Johnson and North, 1992a). Opiates inhibit GABA release in the dorsal portion of the midbrain (Renno et al., 1992). Together, these data suggest that opiate activation of and within the mesolimbic DA system is mediated by inhibiting GABAergic interneurons.

Although both GABA_A and GABA_B receptor subtypes have been identified within the VTA (Nagai et al., 1983; Bowery et al., 1987), the role of each in mediating heroin reinforcement is still unclear. Mounting evidence suggests that the reinforcing effects of opiates principally are mediated by GABA_B receptors located on dopaminergic neurons. In support of this hypothesis, activation of these receptors inhibits DA cells (Johnson and North, 1992a), decreases impulse generation (Olpe et al., 1977), and reduces DA release in the VTA and NAcc (Kliteneck et al., 1992; Xi and Stein, 1998). Furthermore, VTA microinjections of baclofen reverse or block morphine-induced VTA DA release (Kliteneck et al., 1992).

DAMGO ([d-Ala²,N-Me-Phe⁴,Gly-d⁵]-enkephalin)-induced elevation of DA metabolites in the NAcc (Kalivas et al., 1990), and morphine-induced conditioned place preference (Tsuji et al., 1996).

The present study demonstrates that systemic coadministration of baclofen with heroin dose-dependently decreases heroin-reinforced SA behavior. Low doses of baclofen significantly increased SA behavior in the first half-hour of each SA session, suggesting a compensatory-enhanced behavioral response because of partial heroin reinforcement blockade. With increasing doses, baclofen completely blocked SA behavior. This effect could not have been due to nonspecific, drug-induced locomotor suppression because a slight increase in food SA was observed in a separate group of six rats receiving 1 mg/kg baclofen i.p. (unpublished observation), which is consistent with a previous report (Roberts et al., 1996). Similarly, when microinjected either unilaterally or bilaterally into the VTA at doses between 2 and 6 µg, baclofen significantly increased heroin SA behavior, suggesting that not only was locomotor behavior not impeded, but also blockade of VTA GABA_B receptors only partially reduces heroin reinforcement. In contrast, microinjection of baclofen into the NAcc had no significant effects on heroin SA behavior and is consistent with the distribution of GABA_B receptors in the NAcc (Bowery et al., 1987). To exclude the possibility of a false-negative effect, heroin microinjected into the NAcc significantly decreased systemic heroin SA, consistent with previous reports (Pettit et al., 1984; Vaccarino et al., 1985). In vivo electrochemical recordings of NAcc DA release demonstrated that baclofen also dose-dependently decreased heroin-induced DA release, an effect blocked by intra-VTA microinjection of the GABA_B receptor antagonist 2-hydroxysclafen. Taken together, these data support the hypothesis that VTA GABA_B receptors play a sufficient role in mediating heroin reinforcement.

In contrast to the above, the role of GABA_A receptors in mediating heroin reinforcement is much more complex. Systemic administration of GABA_A agonists often appears to excite VTA DA neurons (Waszczak and Walters, 1980; Kalivas et al., 1990), whereas intracellular recordings demonstrate that GABA_A agonists directly hyperpolarize VTA DA cells (Olpe et al., 1977; Johnson and North, 1992b), suggesting that GABA_A receptors may be located on both GABAergic cells and DA neurons; activation of GABA_A receptors on GABAergic cells will disinhibit DA neurons. In support of this hypothesis, autoradiographic evidence demonstrates that GABA_A receptors are located mainly on GABAergic cells in the VTA (Churchill et al., 1992) and systemic administration of GABA_A agonists significantly inhibit VTA GABAergic cells (O'Brien and White, 1987). Our previous electrochemical study suggested further that GABA_A receptors are located on both VTA DA neurons and GABAergic interneurons (Xi and Stein, 1998). In the present experiment, heroin-induced a significant increase in NAcc DA release after VTA GABA_A receptor blockade, suggesting a disinhibitory effect mediated by GABA_A receptors on VTA DA neurons. Taken together, activation of GABA_A receptors on GABAergic cells will produce a disinhibitory effect, whereas activation of GABA_A or GABA_B receptors on DA cells, by elevating endogenous, synaptic GABA concentration (such as with GABA transaminase inhibitors or GABA uptake inhibitors), will...

Several recent studies have demonstrated that baclofen reduces intracranial self-stimulation reward threshold (Wilklick and Kokkinidis, 1995), attenuates cocaine reinforcement (Roberts et al., 1996), and suppresses cocaine craving in humans (Ling and Majewska, 1998). Because cocaine activates the mesocorticolimbic DA pathway by inhibiting DA reuptake into presynaptic terminals, the present experimental results, combined with these cocaine experiments, support the hypothesis that baclofen may serve as a promising agent to treat drug abuse. The proposed GABAergic hypothesis of heroin reinforcement provides a theoretical rationale for evaluating baclofen as a potential heroin pharmacotherapy.

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