Increased Mesolimbic GABA Concentration Blocks Heroin Self-Administration in the Rat

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ABSTRACT

Opiate reinforcement has been hypothesized to be mediated by an inhibition of mesolimbic γ-aminobutyric acid (GABA) release that subsequently disinhibits ventral tegmental area (VTA) dopaminergic neurons. In support of this hypothesis, this study demonstrates that when administered directly into the lateral ventricle, the VTA, or the ventral pallidum, but not the nucleus accumbens, γ-vinyl-GABA (GVG, an irreversible GABA-transaminase inhibitor, 20–50 μg) dose-dependently blocked heroin (0.06 mg/kg) self-administration (SA), as assessed by an increase in heroin SA at low doses of GVG and an initial increase followed 1 to 2 h later by a blockade of heroin SA at higher GVG doses. This effect lasted 3 to 5 days. In drug-naive rats, intra-VTA GVG pretreatment also prevented or delayed acquisition of heroin SA for 2 days. This GVG effect was prevented or reversed by systemic or intra-VTA pretreatment with the GABAA receptor antagonist 2-hydroxysaclofen, but not the GABAA receptor antagonist bicuculline. Similarly, coadministration of heroin with aminooxy-acetic acid (1–4 mg/kg) or ethanolamine-O-sulfate (50–100 mg/kg), two reversible GABA transaminase inhibitors, dose-dependently reduced heroin reinforcement. Co-administration of (±)-nipecotic acid (0.1–5 mg/kg) with heroin, or intra-VTA or -ventral pallidum pretreatment with (±)-nipecotic acid (10 μg) or NO-711 (2 μg), two GABA uptake inhibitors, significantly increased heroin SA behavior, an effect also blocked by systemic 2-hydroxysaclofen, but not bicuculline. Taken together, these experiments, for the first time, demonstrate that pharmacological elevation of mesolimbic GABA concentration blocks heroin reinforcement by activating GABAA receptors, supporting the GABAergic hypothesis of opiate reinforcement and the incorporation of GABA agents in opiate abuse treatment.
(SA) (Smith et al., 1985), systemic or intra-accumbal administration of DA antagonists does not alter i.v. heroin SA (Ettenberg et al., 1982). Destruction of NAcc presynaptic DA terminals selectively attenuates cocaine, but not heroin, SA (Pettit et al., 1984). Opiates, when systemically self-administered (Chang et al., 1997; Lee et al., 1999), or locally administered into the NAcc (Hakan and Henriksen, 1989), significantly inhibit the spontaneous firing of NAcc neurons. Because NAcc efferent projections are mostly GABAergic (Groenewegen and Russchen, 1984; Kalivas et al., 1993) and terminate principally in the ventral pallidum (VP) and the VTA (Mogenson and Nelson, 1983), two critical areas in mediating opiate reinforcement, we hypothesize that, similar to the VTA, opiates also inhibit NAcc GABAergic projection cells that ultimately disinhibit postsynaptic VP neurons and VTA DA neurons. Such a mechanism may, in part, explain the proposed DA-independent opiate reward mechanism.

To test the hypothesis that opiate reinforcement is mediated, at least in part, by inhibiting both VTA and NAcc GABAergic cells, this study examined the effects of elevated endogenous mesolimbic GABA concentration on heroin SA. After being released into the synaptic cleft where it activates specific GABA receptor subtypes, GABA is taken up into presynaptic neuronal terminals and glial cells by GABA uptake carriers (Krogsgaard-Larsen and Johnston, 1975), where it is further catabolized by the enzyme GABA-transaminase (GABAT) (Sabers and Gram, 1992). Thus, the strategy used in this study was to elevate GABA concentration by administering GABAT or GABA uptake inhibitors systemically or directly into the lateral ventricles, VTA, NAcc, or VP either before or during heroin SA. Our results support the GABAergic hypothesis of opiate reinforcement by demonstrating dose- and time-dependent SA inhibition after administration of GABA-enhancing drugs.

**Materials and Methods**

**Surgical Preparation.** Male Sprague-Dawley rats (Sasco, Madison, WI) weighing 250 to 350 g at the time of surgery were individually housed and maintained on a 12-h light/dark cycle (lights on at 8:00 PM) with free access to food and water. One hundred rats were sexually housed and maintained on a 12-h light/dark cycle (lights on at 8:00 PM) with free access to food and water. One hundred rats were allocated, at least in part, by inhibiting both VTA and NAcc GABAergic cells, this study examined the effects of elevated endogenous mesolimbic GABA concentration on heroin SA. After being released into the synaptic cleft where it activates specific GABA receptor subtypes, GABA is taken up into presynaptic neuronal terminals and glial cells by GABA uptake carriers (Krogsgaard-Larsen and Johnston, 1975), where it is further catabolized by the enzyme GABA-transaminase (GABAT) (Sabers and Gram, 1992). Thus, the strategy used in this study was to elevate GABA concentration by administering GABAT or GABA uptake inhibitors systemically or directly into the lateral ventricles, VTA, NAcc, or VP either before or during heroin SA. Our results support the GABAergic hypothesis of opiate reinforcement by demonstrating dose- and time-dependent SA inhibition after administration of GABA-enhancing drugs.

**Effects of GABAT Inhibitors on Heroin SA.** When microinjected into the lateral ventricles (i.c.v.), the irreversible GABAT inhibitor GVG (20 μg, n = 6) significantly increased heroin SA behavior for about 24 h, whereas a higher dose (50 μg, n = 4) first decreased and then completely blocked SA behavior 2 h after GVG administration, an effect that lasted for about 3 days (Fig. 1, A and B). In an identical manner, pretreatment with GVG (20–50 μg) into the VTA also dose dependently inhibited heroin reinforcement. Although a significant increase in heroin SA was seen after low-dose (20 μg) administration, the pattern of operant responding after high-dose GVG (50 μg) was more complex, with an increase in lever pressing in the first 2 h followed by a virtual cessation during the remaining hours of the 4-h session (data not shown). A similar pattern of responding was seen after saline substitution during heroin SA (Fig. 1, A and B). This GVG blockade lasted 3 to 4 days after a single VTA injection (Fig. 2A). Intra-VP administration of the same doses of GVG also altered heroin SA with the same dose- and time-dependent patterns as intra-VTA administration (Fig. 3, A and B). In contrast, the 50-μg dose of GVG injected bilaterally into...
the NAcc had no significant effect on heroin SA (Fig. 4). Likewise, intra-NAcc administration of another GABAT inhibitor, AOAA (2 μg), also had no effect on heroin SA. AOAA did, however, significantly increase heroin SA when microinjected into the VTA (Fig. 4).

To determine whether GVG could also interfere with the acquisition of heroin SA, GVG was administered into the VTA (50 μg, n = 8) before the first day of heroin exposure. A single injection was able to delay heroin SA acquisition for 2 days in heroin-naïve rats (Fig. 2B). Thereafter, these rats gradually began responding at rates comparable with rats receiving heroin alone. After SA was established, a second GVG injection was given (day 7) and induced a marked increase in SA. This large burst in operant behavior mainly occurred within the first 1 to 2 h of the 4-h SA session, followed once again by complete SA blockade the following day.

To determine the receptor mechanisms mediating this GVG effect, the GABA_A and GABA_B receptor antagonists bicuculline and 2-hydroxysaclofen (2-OH-saclofen), respectively, were administered systemically in separate groups of rats. After stable SA behavior, 2-OH-saclofen (2 mg/kg), but not bicuculline (0.1 mg/kg), pretreatment blocked the SA inhibition induced by intra-VTA GVG administration. In contrast, neither 2-OH-saclofen nor bicuculline alone significantly altered heroin SA (days 4 and 6, respectively in Fig. 2C). Consistent with this observation, intra-VTA 2-OH-saclofen (2 μg) administered three days after GVG-induced SA blockade also reversed the SA inhibition (n = 4), with behavioral rates increasing from 1.8 ± 0.2 to 9.5 ± 2.3 SA/4 h (data not shown).

The effects of two reversible GABAT inhibitors, AOAA (1–4 mg/kg) and EOS (50–100 mg/kg), on heroin SA were examined in two additional groups of rats. Coadministration of either drug with heroin dose dependently blocked heroin reinforcement, manifest as a compensatory increase in SA at the lower doses and blockade of SA at the higher doses of each drug (Fig. 5, A and B). Similarly, intra-VTA microinjection of AOAA (2 μg) also significantly increased heroin SA (Fig. 4).

Effects of GABA Uptake Inhibitors on Heroin SA.

When coadministered with heroin, all doses (0.1–5 mg/kg) of NipA, a GABA uptake inhibitor, increased heroin SA (Fig. 6A). Similarly, microinjections of NipA into the VTA (10 μg) or the VP (10 μg) consistently increased operant responding for heroin (Fig. 6B). Another selective GABA uptake inhibitor, NO-711, microinjected into the VTA (2 μg), also significantly increased heroin SA behavior (Fig. 6B). The effect of NipA was selectively attenuated by systemic injection of 2-OH-saclofen (2 mg/kg, n = 8), but not bicuculline (0.1 mg/kg, n = 8) (Fig. 7). Systemic 2-OH-saclofen or bicuculline administered alone did not significantly affect SA behavior.

Discussion

This study demonstrates, for the first time, that elevating synaptic GABA levels by direct i.c. administration of the GABAT inhibitors GVG, AOAA, or EOS, or the GABA uptake inhibitors NipA or NO-711, dose dependently reduces heroin reinforcement (defined as an increase in SA at low doses and a time-dependent SA blockade at higher treatment doses, independent of nonspecific effects on locomotion), and prevents or delays acquisition of heroin SA. This effect was seen with i.c.v., VTA, or VP injections, but not after NAcc administration. The inhibitory effects of intra-VTA GVG or systemic NipA were blocked or reversed by the GABA_B antagonist 2-OH-saclofen, but not the GABA_A antagonist bicuculline, suggesting an effect mediated by GABA_B receptors.

VTA GABAergic Mechanism of Opiate Reinforcement.

Two types of neurons, primary dopaminergic projection neurons and secondary GABAergic inhibitory interneurons, have been identified within the VTA. Both the intrinsic VTA GABAergic interneurons and GABAergic axon terminals from the NAcc synapse onto VTA DA neurons (Johnson and North, 1992b; Kalivas et al., 1993). Systemic or iontophoretic administration of morphine increases the firing rate of the DA neurons by binding to μ-opiate receptors located predominantly on and inhibiting GABAergic interneurons (Matthews and German, 1984; Dilts and Kalivas, 1989; Johnson and North, 1992a).

This VTA GABA microcircuitry is supported by several behavioral and neurochemical studies: 1) opiates inhibit GABA release from the rat midbrain (Renno et al., 1992), 2)
neurons. In contrast, GABA<sub>B</sub> receptors are located mainly on DA neurons (Johnson and North, 1992a) and reduces VTA and NAcc DA release (Kliteneck et al., 1992; Xi and Stein, 1999). Similarly, baclofen alone inhibits firing of VTA DA neurons (Johnson and North, 1992a) and reduces VTA and NAcc DA release (Kliteneck et al., 1992; Xi and Stein, 1998), suggesting that VTA GABA<sub>B</sub> receptors play a critical role in mediating endogenous GABA modulation of VTA DA neurons. In contrast, GABA<sub>A</sub> receptors are located mainly on VTA interneurons (Dilts and Kalivas, 1989), with their activation disinhibiting VTA DA cells, thereby increasing NAcc DA release (Xi and Stein, 1998).

By directly manipulating mesolimbic GABA concentration, both by inhibiting the GABA degradation enzyme GABAT or by inhibiting GABA uptake, the current data support the above opiate reinforcement hypothesis. In addition, intra-VTA GVG pretreatment not only blocked heroin-reinforced SA but also prevented or delayed acquisition of heroin SA in drug-naïve rats, and did so for up to 4 days after a single injection. This effect was blocked or reversed by i.v. or intra-VTA pretreatment with the GABA<sub>B</sub> antagonist 2-OH-saclofen, but not by the GABA<sub>A</sub> antagonist bicuculline, suggesting that the GVG effect was mediated by increased GABA concentration acting on VTA GABA<sub>B</sub> receptors. These data are consistent with our previous reports demonstrating that the selective GABA<sub>B</sub> agonist baclofen, but not the GABA<sub>A</sub> agonist muscimol, blocks heroin reinforcement (Xi and Stein, 1998, 1999). Because administration of GVG, AOAA, and EOS had no significant effects on spontaneous locomotor behavior, these data suggest a specific GABA-induced reinforcement blockade, rather than a nonspecific effect on motor behavior.

The relatively long duration inhibitory effect of GVG administration on heroin SA is consistent with the drug’s irreversible effect on GABA transaminase, requiring de novo synthesis of new enzyme. For example, GVG dose dependently increases brain GABA concentration for more than 48 h (Jung et al., 1977; Qume and Fowler, 1996). It should be noted, however, that GVG also inhibits GABA uptake, which may have contributed to its strong inhibitory effect on heroin reinforcement (Christensen et al., 1991; Jolkkonen et al., 1992).

Consistent with the ability of GVG to reduce heroin reinforcement, GVG also is known to decrease DA release in the NAcc and the striatum (Morgan and Dewey, 1998) and can significantly antagonize cocaine-induced CPP (Morgan et al., 1997) and locomotor behavioral sensitization (Dewey et al., 1997). Taken together, these data suggest that heroin and cocaine share a common mesolimbic GABAergic reinforcement mechanism.

Finally, the reversible GABAT inhibitors, AOAA and EOS, also dose dependently reduced heroin reinforcement. Low doses of both drugs increased, whereas high doses completely blocked heroin SA behavior. However, in contrast to the central effect of GVG, intra-VTA administration of AOAA only increased heroin SA, an effect that lasted for less than 24 h; complete SA blockade was never seen with any dose tested. This latter observation is consistent with the pharmacological properties of AOAA acting as a competitive, reversible GABAT inhibitor (Qume and Fowler, 1996). Similarly, coadministration of heroin with NipA, a GABA uptake inhibitor (Krogsgaard-Larsen and Johnston, 1975), significantly increased SA within a wide dose range, while administration of NipA or NO-711 into either the VTA or VP also significantly antagonized cocaine-induced CPP (Morgan et al., 1997) and locomotor behavioral sensitization (Dewey et al., 1997). Taken together, these data suggest that heroin and cocaine share a common mesolimbic GABAergic reinforcement mechanism.
the VTA, the NAcc is also involved in processing opiate reinforcement. When assessed by either SA or CPP, intra-accumbal injections of opiates exert reinforcing (Van der Kooy et al., 1982; Goeders et al., 1984) effects that can be blocked by intra-NAcc administration of opiate antagonists (Vaccarino et al., 1985). While specific neurochemical mechanisms are still poorly understood, it has been proposed that opiate-induced NAcc DA release plays a critical role, possibly by inhibiting NAcc GABAergic efferent cells (Swerdlow et al., 1990; Bourdelais and Kalivas, 1992). However, several pieces
of evidence conflict with this DA hypothesis. For example, while 6-hydroxydopamine NAcc lesions have been reported to disrupt opiate reward (Smith et al., 1985), systemic or intra-accumbal administration of DA antagonists does not alter heroin SA. Numbers in parentheses represent the number of rats per group. *P < .05, **P < .01, compared with heroin alone group.

Based on the VTA GABAergic hypothesis, we now hypothesize that opiates inhibit NAcc GABAergic projection neurons by acting on both VTA DA projection neurons and directly within the NAcc, the latter comprising a non-DA-dependent reinforcement mechanism. Thus, only interfering with the former may incompletely block opiate reinforcement by leaving the latter non-DA mechanism intact. Because the major NAcc efferents are GABAergic and project both to the VP and back to the VTA (Groenewegen and Russchen, 1984; Kalivas et al., 1993), opiate inhibition of NAcc GABA neurons can disinhibit both the target VP neurons and the VTA DA projection neurons. As such, the former action will produce a DA-independent opiate reinforcement, whereas the latter will potentiate the mesolimbic DA-dependent mechanism in a positive feedback manner. In the present experiment, GVG administered into the VP or VTA, two main NAcc GABAergic projection areas, dose dependently reduced heroin reinforcement, supporting this GABAergic disinhibitory hypothesis.

**VP GABAergic Mechanism of Opiate Reinforcement.** Although the VP serves as a major anatomical target of NAcc efferent projections and provides inputs to the medial prefrontal cortex, amygdala, and lateral hypothalamus, systems subserving drug-taking behavior (Groenewegen et al., 1993), its role in mediating opiate reward is less well understood. Pallidal GABAergic fibers project back to the NAcc and the VTA to form a complex local modulatory circuit (Kalivas et al., 1993; Churchill and Kalivas, 1994). In this study, intra-NAcc administration of GVG or AOAA had no significant effect on heroin SA, consistent with our previous report demonstrating that intra-NAcc injection of the GABAB agonist baclofen had no effect on heroin reinforcement (Xi and Stein, 1999). In addition, ibotenic acid lesions of VP neurons block both heroin and cocaine SA behavior, supporting a role for the VP in drug reinforcement (Hubner and Koob, 1990).

In summary, we have demonstrated that elevation of endogenous GABA concentration by either GABAT inhibitors or GABA uptake inhibitors consistently blocks heroin SA behavior. These data provide, for the first time, direct evi-
dence to support the GABAergic hypothesis of opiate reinforcement and suggest that GABA enhancers, such as GVG and NipA, may be effective in treating opiate addiction.

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References


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