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

ZHENG-XIONG XI and ELLIOT A. STEIN

Departments of Cellular Biology, Neurobiology and Anatomy (Z.-X.X., E.A.S.), Psychiatry and Behavioral Medicine (E.A.S.), Pharmacology and Toxicology (E.A.S.), and The Biophysics Research Institute (E.A.S.), Medical College of Wisconsin, Milwaukee, Wisconsin

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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) dopamine 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-aminobutyric acid (GABA)-transaminase, dose dependently reduced 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-naïve rats, intra-VTA GVG pretreatment also prevented or delayed acquisition of heroin SA behavior, an effect also blocked by systemic 2-hydroxy-saclofen, but not the GABA_A 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 (±)-nipeptoc acid (0.1–5 mg/kg) with heroin, or intra-VTA or ventral pallidum pretreatment with (±)-nipeptoc acid (10 μg) or NO-711 (2 μg), two GABA uptake inhibitors, significantly increased heroin SA behavior, an effect also blocked by systemic 2-hydroxy-saclofen, but not bicuculline. Taken together, these experiments, for the first time, demonstrate that pharmacological elevation of mesolimbic GABA concentration blocks heroin reinforcement by activating GABA_A receptors, supporting the GABAergic hypothesis of opiate reinforcement and the incorporation of GABA agents in opiate abuse treatment.

Heroin is the most rapidly acting and most abused of the opiates. Unfortunately, its high abuse liability is not matched by effective pharmacological liability. Currently, the most effective treatment strategy is opiate replacement therapy with methadone or its derivative, l-α-acetylmethadone. However, these drugs are accompanied by their own dependence liability, emphasizing the need for new treatment strategies based on reducing opiate reinforcement.

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, has been consistently demonstrated to play a critical role in mediating opiate reinforcement (Bardo, 1998). Although the mechanisms underlying this reinforcement are still incompletely understood, the current hypothesis is that opiates inhibit GABAergic cells to disinhibit mesocorticolimbic system DA neurons (Johnson and North, 1992a). Several lines of evidence support this hypothesis. For example, systemic or VTA microiontophoretic morphine increases the firing rate of dopaminergic neurons and inhibits the firing rate of inhibitory interneurons (Gysling and Wang, 1983; Johnson and North, 1992a). Microdialysis and electrochemical studies demonstrate an increase in NAcc DA release after heroin administration (Spanagel et al., 1990; Xi et al., 1998). Furthermore, opiate μ-receptors are predominantly located on VTA GABAergic interneurons (Dilts and Kalivas, 1989), and morphine presynaptically inhibits γ-aminobutyric acid (GABA) release within the medial portion of the rat midbrain (Renno et al., 1992). We (Xi and Stein, 1998, 1999) have previously demonstrated that pharmacological down-regulation of VTA DA neuronal activity and NAcc DA release after administration of κ-opiate agonists or the GABA_A agonist halafen reduces heroin reinforcement.

Thus, although the mechanisms of opiate action within the VTA are emerging, it is as yet unclear whether similar actions of opiates also occur in the NAcc. Experimental evidence has suggested both a DA-dependent and DA-independent mechanism processing opiate reinforcement within the NAcc. For example, whereas 6-hydroxypoline lesions within the NAcc can disrupt morphine self-administration.

ABBREVIATIONS: DA, dopamine; GABA, γ-aminobutyric acid; GABAT, GABA transaminase; GVG, γ-vinyl-GABA; 2-OH-saclofen, 2-hydroxy-saclofen; SA, self-administration; CPP, conditioned place preference; VP, ventral pallidum; VTA, ventral tegmental area; NAcc, nucleus accumbens; AOAA, aminooxy-acetic acid; EOS, ethanolamine-O-sulfate; NipA, (±)-nipeptoc acid; i.c., intracranial.
(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 Nielson, 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 subdivided into five heroin SA groups: γ-vinyl-GABA (GVG; n = 44), aminoxy-acetic acid (AOAA; n = 16), ethanalamine-O-sulfate (EOS; n = 8), (z)-nipecoic acid (NipA; n = 28), and a saline substitution group (n = 4). Based on the specific experiment, some rats received more than one drug, and their numbers are represented in more than one of the above groups. Under sodium pentobarbital anesthesia (60 mg/kg i.p.), heroin SA rats were implanted with a chronic silicone rubber jugular catheter that passed s.c. to terminate on a head assembly. To observe the effects of central receptor modulation on heroin SA, 96 rats were also implanted with 30-gauge stainless steel guide cannula bilaterally into the lateral ventricles (coordinates: 0.8 mm posterior to bregma, 1.6 mm lateral to midline, and 4.2 mm ventral to the surface of the cortex), the VTA (4.8 mm posterior to bregma, 1.0 mm lateral to midline, and 7.7 mm ventral to the surface of the cortex), the NAcc (1.6 mm anterior to bregma, 1.6 mm lateral to midline, and 7.2–7.4 mm ventral to the surface of the cortex), or the VP (0.3 mm anterior to bregma, 2.5 mm lateral to midline, and 7.8 mm ventral to the surface of the cortex). Three days were allowed for recovery from surgery before 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 housed 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 µg) over a 10-s period. Depending on the experiment, heroin (0.06 mg/kg) or heroin plus a GABA agent dissolved in sterile saline was administered per lever press. A 60-W light located above the chamber was simultaneously illuminated with each drug infusion. Each SA session lasted 4 h, and each rat was tested for 5 to 9 days on a fixed ratio 1 schedule.

The effects of the GABA agents on SA behavior (VG, 20–50 µg i.c.v., VTA, NAcc, or VP; AOAA, 1–4 mg/kg i.v.; EOS, 50–100 mg/kg i.v.; NipA, 0.1–5 mg/kg i.v. or 10–20 µg bilaterally directly into the VTA or VP) were assessed after stable SA behavior was established (within ±5% of the mean responses for 3 days of heroin alone training). Additionally, to observe the effects of GVG on heroin SA acquisition, a group of drug-naive rats received GVG before heroin exposure. All GABA agents were purchased from Research Biochemicals International (Natick, MA) and dissolved fresh each day in sterile saline. Heroin was donated by the Resource Technology Branch, National Institute on Drug Abuse (Bethesda, MD). All injections into the lateral ventricles, the VTA, the NAcc, or the VP were delivered in a volume of 1 or 2 µl over 1 or 2 min, respectively. Intracranial (i.c.) drug injection doses were divided evenly into each hemisphere and are reported as total amount administered.

**Statistical Analyses.** All data are presented as mean ± S.E. Student’s t-test and/or two-way ANOVAs were used to assess drug effect significance. A P < .05 was used throughout.

**Histology.** On completion of each SA experiment, rats were deeply anesthetized with pentobarbital and transcardially perfused with phosphate-buffered saline followed by 10% formalin. Brains were sectioned at 40 µm, and cannula tips verified histologically. Only rats with properly located cannula were included in subsequent behavioral data analyses.

**Results**

**Heroin SA Behavior.** Typically, rats rapidly learned the operant task and reliably self-administered heroin after 2 to 3 days of training. Four rats with unstable SA behavior or misplaced or blocked i.c. cannula were eliminated from the study and excluded from data analysis. Although SA rates and interinjection intervals varied somewhat across rats and sessions, the pattern of responses and the mean SA rate across sessions were very stable over time (Fig. 1, heroin control curve).

**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 1 to 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
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 GABAB antagonist 2-OH-saclofen, but not the GABAA antagonist bicuculline, suggesting an effect mediated by GABAB 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)
intra-VTA microinjections of the GABA\(_B\) agonist baclofen block opiate-induced DA release in the VTA (Kliteneck et al., 1992) and the NAcc (Kalivas et al., 1990), and 3) intra-VTA baclofen blocks morphine-induced conditioned place preference (CPP; Tsuji et al., 1996) and heroin SA behavior (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\(_B\) receptors play a critical role in mediating endogenous GABA modulation of VTA DA neurons. In contrast, GABA\(_A\) 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\(_B\) antagonist 2-OH-saclofen, but not by the GABA\(_A\) antagonist bicuculline, suggesting that the GVG effect was mediated by increased GABA concentration acting on VTA GABA\(_B\) receptors. These data are consistent with our previous reports demonstrating that the selective GABA\(_B\) agonist baclofen, but not the GABA\(_A\) 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 only increased heroin SA, suggesting partial heroin reinforcement reduction. Incomplete blockade of heroin reinforcement by NipA, even at a dose 50 times higher than its minimal effective dose, may suggest that GABA uptake mechanisms play a secondary role to GABAT degradation in synaptic GABA removal. Taken together, however, these data strongly support a role for both VTA and NAcc GABA in heroin reinforcement.

**NAcc GABAergic Mechanism of Opiate Reinforcement.** Several lines of evidence suggest that, in addition to
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 suggest that GABA inhibition in the NAcc contributes to the reinforcing effects of opiates. For example, pretreatment with GABA antagonists, such as GVG (GVG), into the VP dose dependently blocked heroin SA behavior. A, time-dependent GVG effect across experimental days. After 3 days of heroin SA training, GVG was administered on day 4 (arrow). The low GVG dose (●, 20 μg, n = 5) significantly increased heroin SA, suggesting a partial reinforcement blockade, whereas the high dose of GVG (○, 50 μg, n = 3) almost completely blocked SA behavior. Both dose effects lasted at least 3 days. B, time course of day 4 GVG administration on heroin SA plotted as a function of time within session. The high dose caused a biphasic increase in responding that, after 2 h, completely suppressed responding. The solid curve (■, n = 30) shows the heroin SA control group. **P < .01, compared with heroin alone group.

Fig. 4. Pretreatment with the GABAT inhibitors GVG and AOAA into the NAcc had no effect on heroin SA. A, time course of GVG (●, 50 μg, n = 9) and AOAA (○, 2 μg, n = 4) on heroin SA plotted during daily 4-h training sessions. B, total SA responding within 4-h sessions after NAcc GVG or AOAA administration had no effect on heroin SA. In contrast, intra-VTA administration of AOAA (2 μg, n = 3) significantly increased heroin SA, suggesting partial reinforcement blockade. The solid curve (■, n = 30) shows the heroin SA control group. Numbers in parentheses represent the number of rats in each group. *P < .05, compared with heroin alone group.

Fig. 5. Dose-dependent effects of AOAA (A) and EOS (B) on heroin SA. A, time course of AOAA effects during 4-h SA sessions plotted as time within session. At 1 mg/kg (●, n = 8), AOAA significantly increased heroin SA behavior, whereas 2 mg/kg (○, n = 8) induced a compensatory increase in SA within the first half-hour that progressed to a time-dependent decrease in responding; 4 mg/kg (●, n = 8) AOAA almost completely blocked heroin SA during the entire session. All dose effects were significant after 2 h when compared with heroin control group. B, time course of EOS effects during 4-h SA sessions. The low dose of EOS (○, 50 mg/kg, n = 6) increased SA, whereas the high dose (●, 100 mg/kg, n = 6) significantly reduced SA behavior. The solid curve (■) shows the heroin alone control.
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 (Ettenberg et al., 1982), and destruction of NAcc presynaptic DA terminals selectively attenuates cocaine but not heroin SA (Pettit et al., 1984). Electrophysiological studies have shown that local microiontophoretic application of morphine into the NAcc (Hakan and Henriksen, 1989), or i.v. SA of heroin (Chang et al., 1997; Lee et al., 1999), markedly suppresses NAcc neuronal activity. These data suggest that both a DA-dependent and a DA-independent mechanism may exist within the NAcc to mediate opiate reinforcement. However, the precise circuitry of such a proposed DA-independent reinforcement mechanism is still unclear.

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-VTA administration of GVG, AOAA, or NipA significantly reduced heroin reinforcement, suggesting that the VP projections to VTA DA neurons play an important role in mediating opiate reinforcement. In contrast, 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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Send reprint requests to: Elliot A. Stein, Ph.D., Department of Psychiatry, 8701 Watertown Plank Rd., Milwaukee, WI 53226. E-mail: estein@mcw.edu