Dopamine Release in the Nucleus Accumbens during Heroin Self-Administration Is Modulated by Kappa Opioid Receptors: An In Vivo Fast-Cyclic Voltammetry Study

ZHENG-XIONG XI, SCOTT A. FULLER and ELLIOT A. STEIN

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

Accepted for publication September 19, 1997 This paper is available online at http://www.jpet.org

ABSTRACT

Mu and kappa opioid agonists are known to produce different, and sometimes opposite, effects on several pharmacological and behavioral measures. However, whether kappa agonists can be used to antagonize the reinforcing and putative dopamine (DA)-releasing properties of a mu agonist such as heroin is unclear. With the use of the high temporal and spatial resolution of in vivo fast-cyclic voltammetry to measure changes in extracellular DA in the nucleus accumbens (NAcc), we observed (1) dose-dependent increases in DA in the NAcc during heroin self-administration (SA), (2) that coadministration of the kappa agonist U50,488H with heroin or intracerebroventricular dynorphin A pretreatment significantly depressed the heroin-stimulated DA release during SA, where U50,488H alone inhibited the basal DA release in the NAcc, (3) that coadministration of low-dose U50,488H or dynorphin A significantly increased heroin SA behavior, whereas high-dose U50,488H, which alone did not support SA behavior, reduced or completely blocked heroin SA and (4) that nor-binaltorphimine dihydrochloride (a selective kappa receptor antagonist) potentiated DA release in the NAcc and modestly decreased heroin SA. Taken together, these data suggest that endogenous kappa receptor activation can inhibit mu agonist-induced activation of the mesolimbic DA pathway, which may in turn depress heroin-induced reinforcement.

Several lines of evidence suggest that activation of mu and kappa opioid receptors leads to functionally opposite effects. For example, central kappa receptor activation can increase morphine analgesia and antagonize both morphine tolerance and physical dependence in morphine-tolerant animals, as well as antagonize withdrawal responses in human heroin addicts (Aceeto et al., 1982; Friedman et al., 1981; Green and Lee, 1988; Lee and Smith, 1984; Tuluñay et al., 1981; Wen and Ho, 1982). Electrophysiological studies have demonstrated that intravenous or iontophoretic administration of morphine excites DA cells in the rat VTA and in the substantia nigra pars compacta (Gysling and Wang, 1983; Matthews and German, 1984; Yin and Mogenson, 1980), whereas U50, a kappa receptor agonist, inhibits DA cells (Walker et al., 1987). Microdialysis studies have also consistently demonstrated that i.c.v. or VTA microinjections of morphine, β-endorphin, DAMGO or [d-Pen²,d-Pen⁵]enkephalin result in significant increases in extracellular DA and DA metabolite concentrations in the NAcc (Devine et al., 1993; Di Chiara and Imperato, 1988; Leone et al., 1991; Mulder et al., 1984; Narita et al., 1992; Spanagel et al., 1990a, 1990b, 1992; Werling et al., 1988). In contrast, systemic or i.c.v. administration of two kappa agonists, E-2078 (a stable dynorphin analog) or U50, significantly decreases extracellular DA and DA metabolite concentration in the mesolimbic ventral striatum (Devine et al., 1993; Di Chiara and Imperato, 1988; Narita et al., 1992; Spanagel et al., 1990a, 1992; Werling et al., 1988).

A similar dichotomy appears to exist with respect to DA-dependent locomotor behavior. Microinjections of the selective mu agonist DAMGO or the selective delta agonist [d-Pen²,d-Pen⁵]enkephalin i.c.v. (Mickley et al., 1990) or bilaterally into the VTA (Latimer et al., 1987) produce forward locomotion or, after unilateral administration, contraversive circling (Jenck et al., 1988). In contrast, VTA kappa receptors appear not to be involved in opioid-induced locomotion, although both i.c.v. DynA and systemic U50 produce significant decreases in locomotion (Jenck et al., 1988).

Finally, conditioned place preference experiments have further demonstrated that mu and kappa opioid receptor

ABBREVIATIONS: FCV, fast cyclic voltammetry; DAMGO, [d-Ala²,N-MePhe⁷,Gly-oïl]enkephalin; SA, self-administration; VTA, ventral tegmental area; NAcc, nucleus accumbens; DA, dopamine; AA, ascorbic acid; DOPAC, dihydroxyphenylacetic acid; U50, U50,488H; DynA, dynorphin A(1–17); nor-BNI, nor-binaltorphimine dihydrochloride; i.c.v., intracerebroventricular; GABA, γ-aminobutyric acid.

Received for publication March 10, 1997.

†This work was supported in part by United States Public Health Service Grant DA09465 (E.A.S.) and NIDA/INVEST Fellowship N01DA-3-0002 (Z.-X.X.).

The Journal of Pharmacology and Experimental Therapeutics Vol. 284, No. 1 0022-3565/98/2841-0151$03.00/0
agonists produce positive reinforcing or reward (Mucha and Herz, 1986; Suzuki et al., 1991, 1993) and negative reinforcing or aversive (Bals-Kubik et al., 1989; Barr et al., 1994; Tang and Collins, 1985) effects, respectively. Furthermore, systemic administration of kappa agonists U50 and E-2078 (a stable dynorphin analog) also suppress the morphine-induced place preference (Funada et al., 1993).

Based on the above data, we hypothesize that kappa agonists may antagonize heroin (mu agonist)-reinforced SA behavior, one of the most reliable animal models of the reinforcing effects of addictive drugs. To help explain the possible mechanisms of this hypothesis, the interaction of mu and kappa receptor activation on DA release in the NAcc during heroin SA was investigated simultaneously in freely moving, behaving animals. In addition, although a large body of evidence supports the hypothesis that the mesolimbic DA system plays an essential role in the development and maintenance of heroin SA, the exact relationship between this behavior and mesolimbic DA activity remains incomplete. Whether neurochemical modulation of this system can subsequently alter an animal’s drug-taking behavior is also poorly understood. Such data would provide further evidence to support the DA hypothesis of drug abuse and serve as a potential bioassay to explore new agents in the treatment of opiate abuse. As such, in the present study, the high temporal and spatial resolution of in vivo FCV was used to evaluate fluctuations in extracellular DA concentration associated with heroin SA and the role of kappa receptor activation on both heroin SA behavior and DA release in the NAcc.

Materials and Methods

**Surgical preparation.** Twenty-six male Long-Evans rats (Sasco, Madison, WI) weighing 300 to 450 g at the time of surgery were housed individually and maintained on a 12-hr light/dark cycle with free access to food and water (lights on from 8:00 p.m. to 8:00 a.m.). With the animals under sodium pentobarbital anesthesia (60 mg/kg i.p.), rats were implanted with a chronic Silastic jugular catheter that was passed subcutaneously to exit between the shoulders. After a 3-day recovery, rats were trained to self-administer heroin (0.16 mg/kg i.v.) for 5 days. Rats demonstrating stable SA behavior (≥10-min interinjection intervals, ≥20% change in total SAs per session) were again anesthetized with sodium pentobarbital, and a carbon fiber electrode was stereotaxically implanted into the NAcc (coordinates: 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). In some animals, a 30-gauge stainless-steel guide cannula was also implanted into the anterior portion of the ipsilateral lateral ventricle (coordinates: 0.8 mm posterior to bregma, 1.6 mm lateral to midline and 3.2–3.4 mm ventral to the surface of the cortex). To minimize coating of the carbon fiber by tissue fragments during implantation, the dura and pia mater were prepunctured with a 23-gauge syringe needle. Ag/AgCl reference and stainless-steel ground electrodes were implanted into the ipsilateral and contralateral parietal cortex, respectively. Miniature pin connectors soldered to the three electrodes were inserted into a plastic strip connector and secured with acrylic dental cement to four stainless steel screws threaded into the skull. The jugular catheter was passed subcutaneously to terminate on the head assembly.

**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-phenylendiamine (1 g of Epon Resin: 0.14 g of O-phenylendiamine). The electrode assembly was baked at 300°C for ≥3 hr 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 AA and the DA metabolite, DOPAC. Electrodes were dipped into 5% Nafion, air-dried and baked at 85°C for 5 min; the entire procedure was repeated five or six times. Before implantation, electrodes were calibrated for their DA sensitivity and their selectivity to DA and DOPAC in a 0.1 M phosphate-buffered saline solution consisting of 154 mM NaCl, 78 mM Na2HPO4, 7H2O and 18 mM NaH2PO4, pH 7.2 to 7.4.

**FCV.** Electrochemical measurements were performed with a microcomputer-based voltammetric instrument (IVEC10; Medical Systems, Greenvale, NY). The FCV waveform consisted of one cycle of a triangle wave initiated at 0 mV vs. Ag/AgCl and swept between 1.0 and −0.5 mV with a scan rate of 50 V/sec and a repetition rate of 1.0 Hz. For quantification, the electrode oxidation current was integrated between 400 and 900 mV and converted to dopamine concentration using the in vitro calibration data obtained before electrode implantation according to the working hypothesis that signal changes were due entirely to changes in DA concentration. To estimate electrode DA selectivity in vivo, several rats received i.p. injections of apomorphine, a D1/D2 receptor agonist, or nomifensin, a DA reuptake inhibitor, to modulate endogenous extracellular DA concentration changes while the NAcc electrochemical signal was recorded.

In the present study, several lines of evidence suggest that DA was the main contributor to the rapid signal changes recorded; these include (1) the use of multiple coating of the ion-selective polymer Nafion (Capella et al., 1990; Gerhardt et al., 1984). Only electrodes were used that showed a minimum DA sensitivity of 50 nM, a linear response to increasing DA concentrations (r > 0.95) and high selectivity to DA against AA (range, 800–5000:1) and DOPAC (range, 500–2000:1). In addition, (2) because all reduction/oxidation current ratios ranged from 0.6 to 0.95, contributions from AA (reduction/oxidation = 0) or 5-hydroxytryptamine (reduction/oxidation, 0.1–0.2) can be assumed to be minimal, although DA (reduction/oxidation, 0.7–0.8) cannot be completely separated from DOPAC (reduction/oxidation, 0.9–1.0) through the use of this method (Gratton et al., 1989). Also, (3) FCV electrochemical signal changes were recorded with an accuracy of 10 mV, whereas the DA metabolite DOPAC is usually produced 10 to 20 min after DA release and slowly decreases thereafter (Di Chiara and Imperato, 1988; Gratton et al., 1989). Therefore, DOPAC may have made some contribution to the slow tonic signal increases seen during the 5-hr recording sessions but not to the fast phasic signal increases seen within the first 10 min after heroin SA. It should also be noted that DOPAC is found mostly intracellularly and exists as a consequence of changes in DA turnover. A number of factors, including (1) the use of multiple coating of the ion-selective polymer Nafion (Capella et al., 1990; Gerhardt et al., 1984). Only electrodes were used that showed a minimum DA sensitivity of 50 nM, a linear response to increasing DA concentrations (r > 0.95) and high selectivity to DA against AA (range, 800–5000:1) and DOPAC (range, 500–2000:1). In addition, (2) because all reduction/oxidation current ratios ranged from 0.6 to 0.95, contributions from AA (reduction/oxidation = 0) or 5-hydroxytryptamine (reduction/oxidation, 0.1–0.2) can be assumed to be minimal, although DA (reduction/oxidation, 0.7–0.8) cannot be completely separated from DOPAC (reduction/oxidation, 0.9–1.0) through the use of this method (Gratton et al., 1989). Also, (3) FCV electrochemical signal changes were recorded with an accuracy of 10 mV, whereas the DA metabolite DOPAC is usually produced 10 to 20 min after DA release and slowly decreases thereafter (Di Chiara and Imperato, 1988; Gratton et al., 1989). Therefore, DOPAC may have made some contribution to the slow tonic signal increases seen during the 5-hr recording sessions but not to the fast phasic signal increases seen within the first 10 min after heroin SA. It should also be noted that DOPAC is found mostly intracellularly and exists as a consequence of changes in DA turnover. A number of factors, including
saline. A 60-W white light situated above the chamber was simultaneously illuminated with each drug infusion. Usually, the first 30 to 60 min of each recording session consisted of base-line electrochemical measurements with the lever removed.

**Drug treatment.** During the first 2 to 3 days of recording, all 26 rats received heroin (0.06 mg/kg/infusion) on a continuous reinforcement (FR1) schedule. Rats were then divided into three groups: (1) heroin SA dose-response rats received 0.06, 0.1 and 0.2 mg/kg/injection for an additional 3 to 5 days ($n = 14$); some rats received naloxone (10 mg/kg i.p. 5–10 min before SA) on the last day of recording; (2) rats receiving heroin SA (0.06 mg/kg/injection) plus U50 (0.1 or 0.5 mg/kg), a selective kappa agonist, coadministered for 3 to 5 days ($n = 6$) with subsequent DynA (1 μg i.c.v.; 1 injection/hr) pretreatment or saline (3 rats) rats receiving heroin SA (0.06 mg/kg/injection) plus pretreatment with nor-BNI, a selective kappa antagonist (1 μg i.c.v.) or saline at 10 min before testing for 2 days ($n = 6$) with subsequent DynA (1 μg i.c.v.; 1 injection/hr) pretreatment administered during heroin (0.06 mg/kg) SA for an additional 2 to 3 days ($n = 4$). U50, DynA, nor-BNI, apomorphine and nomifensin were purchased from Research Biochemicals (Natick, MA), and a fresh solution dissolved in sterile saline was made each day. Heroin and naloxone were donated by the Resource Technology Branch, National Institute on Drug Abuse. All i.c.v injections were delivered in a volume of 1 to 2 μl over 1 to 2 min.

**Histology.** On completion of each 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 electrode and i.c.v. guide cannulae tips were verified histologically.

**Data reduction and statistical analyses.** Only data obtained during regularly spaced self-injections ($>$10-min interinjection intervals) were analyzed. To increase signal detection threshold, the relative FCV signal changes were averaged across trials with the lever press set to zero (0 nM DA, 0 min) for both time and amplitude in a manner similar to that used to analyze electrical evoked potentials. Signal values were first averaged across the 60 consecutive 1-sec oxidation cycles each minute during each SA trial and then averaged across all trials within each session. Finally, all repeated sessions for each rat and all rats in each group were combined, thereby significantly increasing the signal detection threshold. Data are expressed as mean amplitude ± S.E.M. and expressed in nanomolar concentration of DA based on the in vitro calibration factor of each electrode. Two-way analyses of variance were used to analyze DA release alterations before and after heroin SA and drug treatment effects. Student’s t tests were applied to analyze the effects of drug treatment on number of heroin SAs in each 5-hr session. Significance was set at $P < 0.05$.

**Results**

**Heroin SA behavior.** Typically, all rats rapidly learned the operant task and reliably self-administered heroin within the first week of training. The few rats with unstable SA and poor electrochemical responses were eliminated from the study. Stereotypy developed over the 5 to 10 daily SA sessions, resulting in decreased general locomotor behavior, whereas total SAs increased slightly. Although SA behavior varied across different rats and sessions, there were no significant differences in either the pattern of responses or mean SA rate across sessions (data not shown). The SA rate was maximal in the first hour (4.35 ± 0.20) of a session, followed by a reduced rate of 2.6 to 3.2 injections/hr with a mean interinjection interval of 15 to 25 min at 0.06 mg/kg/injection. Total heroin intake decreased in a dose-dependent manner with increasing heroin doses (see fig. 2B).

**NAcc electrochemical signal changes during heroin SA.** When data from all heroin SA trials in a given rat were averaged, three major electrochemical signal response patterns were seen: a monophasic response increase (8 of 14 rats), a biphasic initial signal increase followed by a decrease (3 of 14) and an initial signal decrease followed by an increase (3 of 14). All three patterns were seen after SA of 0.06 and 0.1 mg/kg/injection of heroin, although only monophasic response increases were seen after high (0.2 mg/kg/injection) heroin dose SA. These three electrochemical response patterns are depicted in figure 1A (from several representative rats during individual heroin SA trials).

Both individually and when averaged together, a dose-dependent increase in NAcc DA release occurred during heroin SA. Figure 1B depicts representative original DA signal changes, and figure 2A depicts mean DA signal changes averaged across all rats and sessions at different heroin doses. Typically, electrochemical signals increased immediately after heroin SA, although in 3 rats an initial small signal decrease followed by the increased response was also observed (fig. 1A). Only the dominant, initial signal increases were analyzed for the present report.

The averaged peak DA concentration changes were $-97 \pm 22$, $-178 \pm 45$ and $-225 \pm 17$ nM after the SA of 0.06, 0.1 and 0.2 mg/kg/injection of heroin, respectively (fig. 2A). Time to peak change occurred at 5, 7 and 3 min with increasing heroin doses, whereas the duration of the effect increased from $\sim 10$ min after the low dose to 20 and 30 min after the two higher doses. A two-way analysis of variance with repeated measures revealed a significant effect of time [F(4,17) = 6.07, $P < 0.001$] on signal changes associated with heroin (0.06 mg/kg; $n = 11$) SA. Pretreatment with 10 mg/kg naloxone, a preferential mu receptor antagonist, administered i.p. 5 to 10 min before heroin SA significantly blocked the heroin-induced increase in DA release in the NAcc (fig. 2A). The SA behavior was also significantly decreased or completed blocked during the 2-hr observation period in these heroin-trained rats. Similar dose-dependent, monophasic increases in DA concentration were also observed after passive administration of heroin (data not shown). No significant signal changes were seen when saline was substituted for heroin during either active or passive administration (fig. 2A). Finally, the number of heroin SAs per session dose-dependently decreased as DA signal increased (fig. 2B).

Although highly variable, slow tonic signal increases were also observed in the majority of SA sessions. As can be seen in figure 1C, the rapid phasic signal changes after each SA were superimposed on a slow, tonic change in signal baseline that developed during the SA session. These tonic signals dramatically increased after the first few drug injections of a session, with each subsequent injection resulting in slower and smaller signal increases. After the last drug injection of a session, the electrochemical signal slowly decreased toward baseline.

**Effects of the selective kappa agonists U50 and DynA on heroin SA behavior and NAcc DA release.** Coadministration of U50 (0.1 mg/kg) with heroin (0.06 mg/kg) significantly reduced and subsequently reversed the heroin-induced increase in NAcc DA (fig. 3A). With a latency of $\sim 3$ min after SA, the electrochemical signal rapidly decreased and reached a maximal reduction of $-184 \pm 75$ nM below base line within $\sim 8$ min and gradually recovered to base-line.
levels within 15 min. Concurrent with the decrease in DA release, SA behavior significantly increased from $16.3 \pm 2.2$ to $28.8 \pm 5.9$ SAs/5-hr session. However, increasing the dose of U50 to 0.5 mg/kg led to a significant decrease in SA behavior over the 5-hr session (fig. 3B), with most SA behavior occurring within the first half hour of the session and virtually ceasing thereafter. At this high dose (0.5 mg/kg) of coadministered U50, the electrochemical signal was irregular and unstable, with an increase in general locomotor behavior (not shown). Consistent with these data, i.c.v. pre-

**Fig. 1.** Representative original oxidation current signal changes (calibrated in nM of DA) during heroin SA. A, Different patterns of electrochemical responses during heroin (0.06 mg/kg) SA in individual rats. B, Dose-dependent responses (saline, 0.06, 0.1 and 0.2 mg/kg/injection heroin) derived from one rat. C, Tonic signal change during a single 1-hr continuous SA session. Arrows (in A and B) indicate heroin SA response time. Plots illustrate the 2 min before and 10 to 15 min after heroin SA. Arrows (in C) indicate heroin SAs within a whole session. OX and RED in A and C represent oxidation and reduction currents, respectively. Numbers in each panel correspond to date, rat and file; for example, 1229-4-1/2/3 means Dec. 29/rat 4/files 1–3.
treatment with 1 μg DynA produced a similar decrease in heroin-induced DA release and likewise significantly increased SA behavior from 16.3 ± 2.2 to 28.1 ± 7.5 SAs/5-hr session (fig. 3). Similar effects were also observed after a higher dose (2 μg) of DynA was administered.

When tested alone, U50 (0.5 mg/kg) failed to support SA behavior in a group of rats (n = 5) previously trained to SA heroin (fig. 4). However, when passively administered, U50 (0.1, 0.25 or 0.5 mg/kg) dose-dependently decreased basal DA release in the NAcc from base-line control (0 nM) to −144.7 ± 43.2, −302.5 ± 66.1 and −417.2 ± 77.7 nM at 9, 11 and 15 min, respectively. The rapid signal decrease occurred with a latency of 3 to 6 min, reached its minimal value within 12 to 20 min and gradually returned to baseline ≈40 min later at the dose of 0.5 mg/kg U50 (fig. 4).

Effects of nor-BNI on heroin SA and DA release during heroin SA. In contrast to that seen with kappa receptor agonists, pretreatment with 1 μg i.c.v. nor-BNI (a selective kappa antagonist) 10 min before the heroin SA session resulted in a biphasic action: a modest decrease in heroin-stimulated DA release within 30 to 60 min, followed by a significant enhancement in DA release during heroin SA that lasted for ≥24 hr. The mean peak signal amplitude increased from 96.9 ± 21.3 nM after heroin alone to 172.8 ± 19.7 nM...
after heroin plus nor-BNI pretreatment (fig. 5). Likewise, DA signal duration increased from ~11 min to 30 min after nor-BNI pretreatment. Nor-BNI (1 μg i.c.v.) alone also elevated the DA signal with a latency of ~30 to 40 min. Heroin SA behavior decreased, although nonsignificantly, after nor-BNI from 16.2 ± 2.2 to 13.0 ± 5.1 SAs/5 hr.

**In vivo electrode calibration.** To estimate relative electrode selectivity to DA in vivo, two drugs known to act on dopaminergic transmission were administered at the end of several experiments. Apomorphine (1.0 mg/kg i.p., n = 6), a D1/D2 receptor agonist (which is assumed to depress impulse-dependent DA release), sharply decreased the electrochemical signal with a maximal (~398.9 ± 98.8 nM) effect seen at ~4 min and lasting ~20 min. In contrast, nomifensine, a DA reuptake inhibitor, augmented basal DA efflux with a maximal signal of 295.1 nM DA observed 5 to 10 min after drug administration and lasting ~30 min (fig. 6).

**Histology.** All electrode tips were located in the NAcc, with 19 located in the medioventral (shell) and 7 located in the laterodorsal (core) part of the nucleus (fig. 7). All the guide cannulae tips were located in the anterior portion of the ipsilateral lateral ventricle (not shown).
The high temporal and spatial resolution of FCV was used in freely behaving rats to measure real-time NAcc DA release and its modulation by opioid kappa receptors during heroin-reinforced SA. The two major findings in this report are (1) heroin SA behavior caused a dose-dependent, naloxone-reversible increase in DA efflux in the NAcc that was inversely proportional to the number of drug SAs (i.e., with increasing heroin dose, DA release increased but SA responses per 5-hr session decreased), and (2) central kappa opioid receptor activation by U50 (a selective kappa agonist) or DynA (an endogenous kappa agonist) significantly decreased basal DA release and antagonized heroin-stimulated DA release during SA. In contrast, blockade of kappa opioid receptors by nor-BNI, a selective kappa receptor antagonist, facilitated SA-induced DA release while nonsignificantly decreasing heroin SA behavior.

**Role of the mesolimbic DA system in mediating heroin reinforcing effects.** A large body of evidence supports the hypothesis that the mesolimbic DA system plays a major role in heroin-reinforced behaviors. First, the NAcc contains significant intrinsic dynorphinergic and enkephalinergic interneurons and is innervated by beta-endorphin fibers originating in the medial basal hypothalamus (Johnson et al., 1980; Watson et al., 1982; Weber et al., 1982). Both D1 and D2 receptors are also well documented in the mesolimbic system (Boyson et al., 1986). Second, unilateral microinjections of morphine into the VTA induces contralateral rotation, which is indicative of an increase in dopaminergic striatal neurotransmission (Holmes et al., 1983).

Third, microinjections of morphine or heroin into the VTA and NAcc induces positive reinforcement in conditioned place preference experiments and can be recognized in drug discrimination experiments (Olds, 1982; Philips and LePiane, 1980; see review by Wise and Hoffman, 1992). Furthermore, morphine, enkephalin and DAMGO have been demonstrated to support SA behavior when injected into the VTA (Bozarth et al., 1981; Devine and Wise, 1994; Goeders et al., 1984). Microinjection of opioid receptor antagonists into the VTA or NAcc (Brett and Wise, 1983; Vaccarino and Corrigal, 1987) or chemical lesion of VTA DA neurons with 6-hydroxydopamine reinforced SA. The two major findings in this report are (1) heroin SA behavior caused a dose-dependent, naloxone-reversible increase in DA efflux in the NAcc that was inversely proportional to the number of drug SAs (i.e., with increasing heroin dose, DA release increased but SA responses per 5-hr session decreased), and (2) central kappa opioid receptor activation by U50 (a selective kappa agonist) or DynA (an endogenous kappa agonist) significantly decreased basal DA release and antagonized heroin-stimulated DA release during SA. In contrast, blockade of kappa opioid receptors by nor-BNI, a selective kappa receptor antagonist, facilitated SA-induced DA release while nonsignificantly decreasing heroin SA behavior.

**Role of the mesolimbic DA system in mediating heroin reinforcing effects.** A large body of evidence supports the hypothesis that the mesolimbic DA system plays a major role in heroin-reinforced behaviors. First, the NAcc contains significant intrinsic dynorphinergic and enkephalinergic interneurons and is innervated by beta-endorphin fibers originating in the medial basal hypothalamus (Johnson et al., 1980; Watson et al., 1982; Weber et al., 1982). Both D1 and D2 receptors are also well documented in the mesolimbic system (Boyson et al., 1986). Second, unilateral microinjections of morphine into the VTA induces contralateral rotation, which is indicative of an increase in dopaminergic striatal neurotransmission (Holmes et al., 1983).
(Bozarth and Wise, 1981; Spryaki, et al., 1982) can attenuate the reinforcing effect of heroin and block the acquisition of heroin SA, suggesting that both the VTA and NAcc play critical roles in opioid self-administration behavior. Intracranial electrical self-stimulation experiments also provide direct evidence supporting the role of the mesolimbic DA system in drug abuse (Fihiger, et al., 1987). For example, intracranial electrical self-stimulation frequency or current-response curves are consistently shifted to the left in the presence of opioids (Esposito and Kornetsky, 1978).

Fourth, opiates activate the mesolimbic DA system. The firing rate of DA containing cells in this region increases after either systemically or microiontophoretically applied morphine (Kelley, et al., 1980). Additional evidence for an increase in DA release after heroin administration has been obtained with in vivo microdialysis and chronooamperometry (Imperato and Di Chiara, 1985; Kiyatkin, et al., 1993, 1994; Rada, et al., 1991; Spanagel, et al., 1990a, 1990b). Fifth, pharmacological modulation of mesolimbic DA receptors predictably regulates opioid-induced behaviors or electrochemical signals (Gilbert, et al., 1995; Shippenberg and Herz, 1988). In the present study, a dose-dependent increase in DA release in the NAcc accompanied a dose-dependent decrease in SA behavior during heroin SA, suggesting that DA levels in the NAcc may be directly related to heroin reinforcement. Naloxone concurrently blocked both heroin-induced DA release and SA behavior, suggesting central mu opioid receptor involvement. Taken together, these data lend further support for the dopamine hypothesis of heroin addiction.

Role of the mesolimbic DA system in mediating aversive effects of opioids. Activation of kappa opioid receptors or blockade of endogenous mu opioid receptors by naloxone or naltrexone produces aversive effects in drug-dependent or drug-naive animals (Barr, et al., 1994; Spanagel, et al., 1994a) and is dysphorigenic in humans (Crowley, et al., 1985; Pfeiffer, et al., 1986). Mesolimbic D1 receptors apparently are involved in both the reinforcing and aversive effects of opioids (Leone and Di Chiara, 1987; Shippenberg and Herz, 1987, 1988). The kappa agonist U50 has been demonstrated to inhibit DA cells in the VTA (Walker, et al., 1987). Microdialysis studies further demonstrate that kappa agonists can inhibit NAcc basal DA release (Devine, et al., 1993; Di Chiara and Imperato, 1988; Narita, et al., 1992; Spanagel, et al., 1990a, 1992; Werling, et al., 1988). A similar inhibition of kappa agonists on calcium-dependent K+-stimulated [3H]DA release was also demonstrated in a rat striatal synaptosomal preparation in vitro (Ronken, et al., 1993). Together, these data suggest that mu and kappa agonists may act at the same dopaminergic substrate but exert opposite effects. However, it was not known whether similar opposite actions would occur immediately after mu or kappa agonist administration alone in freely behaving rats or whether antagonistic interactions would occur when the agonists were coadministered.

The present in vivo FCV results demonstrate that (1) low-dose U50 coadministered with heroin increased SA behavior, whereas high-dose U50 blocked SA behavior, suggesting that kappa-receptor activation competes with the rewarding properties of mu receptor activation during heroin SA; (2) U50 was not self-administered in rats previously trained to SA heroin, which is consistent with experiments demonstrating the aversive actions of kappa compounds; and (3) passively administered U50 or coadministration with heroin significantly decreased both the basal and heroin-stimulated DA release in the NAcc. A similar decrease in DA release was also observed after i.c.v. pretreatment with the endogenous kappa agonist DynA, supporting the hypothesis that mu- and kappa-agonists act at the same dopaminergic substrate, but exert opposite effects. (4) Blockade of endogenous kappa receptors by nor-BNI, a potent and long-lasting kappa receptor antagonist, significantly facilitated heroin-stimulated DA release in the NAcc ~30 to 60 min after nor-BNI i.c.v. pretreatment. A nonsignificant decrease in heroin SA behavior was also observed, suggesting that kappa receptor blockade may increase the positive reinforcing actions of heroin by preventing drug-induced aversive properties secondary to kappa receptor activation. The initial small decrease in SA after nor-BNI may be related to an initial mu opioid receptor blockade (Endoh, et al., 1992; Hornan, et al., 1992, Spanagel, et al., 1994b; Wettstein and Groueli, 1996). This nor-BNI effect may partially explain the nonsignificant change in total heroin SA per session observed because an initial decrease in DA release may compensatorily result in increased SA behavior. (5) High-dose naloxone concurrently blocked both heroin-induced DA release and SA behavior. Taken together, these data support the hypothesis that activation of kappa receptors or inactivation of mu receptors in the mesolimbic DA system may mediate certain aversive actions of opioids.

Neurochemical mechanisms of actions of mu and kappa agonists on DA release. The mechanism of mu agonist-induced increases in DA release in the mesolimbic system has been well characterized. Kelley, et al. (1980) proposed that opioid agonist-induced increases in A10 DA cell firing rates result from an indirect, disinhibitory action in the VTA. In support of this hypothesis, two types of neurons in the rat VTA have been electrophysiologically identified (Johnson and North, 1992). Ventral mesencephalic 6-hydroxydopamine lesions fail to alter VTA [125I]DAMGO binding, but quinolinic acid lesions of this area substantially decrease DAMGO binding, suggesting that a dense population of mu receptors reside on secondary interneurons rather than on the primary VTA dopaminergic cells themselves (Dilts and Kalivas, 1989; Mansour, et al., 1988). Met-enkephalin reduces the GABA components of VTA-evoked synaptic potentials, and both DAMGO and enkephalin hyperpolarize nondopaminergic interneurons in the VTA (Johnson and North, 1992) and rat hippocampus (Madison and Nicoll, 1988). Direct evidence has demonstrated that morphine presynaptically inhibits GABA release from GABAergic interneurons in the rat hippocampus (Cohen, et al., 1992) and midbrain (Renno, et al., 1992), suggesting that opioid-induced actions on DA cells may be mediated by GABAergic interneurons. Furthermore, a direct GABAergic inhibitory modulation on dopaminergic activity has been found. Microiontophoretic application of GABA into the VTA increases DA neuronal spike height while decreasing impulse flow (Matthews and German, 1984), which is thought to be a consequence of DA cell hyperpolarization. Kalivas, et al. (1990) found that VTA microinjections of the GABA_A agonist baclofen antagonized increases in NAcc DA after VTA microinjections of DAMGO. Similarly, superfusion of baclofen produces hyperpolarization and increases potassium conductance in rat substantia nigra neurons, suggesting that the effect of GABA on dopaminergic neurons is mediated
through GABA<sub>B</sub> receptors located on DA cells (Lacey et al., 1988; Pinnock, 1984). In contrast, GABA<sub>A</sub> agonists appear to act presynaptically on GABAergic neurons to produce inhibition of these neurons, yielding a net disinhibition of dopaminergic neurons (Churchill et al., 1992; Grace and Bunney, 1979).

In contrast to mu receptors, kappa receptors in the VTA do not seem to participate in modulating basal mesolimbic dopaminergic activity in that kappa receptor modulation appears to reside in mesolimbic DA terminal fields. In support of this conclusion, few if any kappa receptors (Jomary et al., 1988; Watson et al., 1982) or dynorphin cells (Watson et al., 1982; Weber et al., 1982) have been identified in the VTA, whereas a high density of kappa receptors and dynorphin innervation have been consistently observed in the NAcc and the caudate putamen (Jomary et al., 1988; Mansour et al., 1988; Watson et al., 1982). Microinjections of U50 or U69,593 into the VTA fail to alter extracellular ventral striatal or NAcc DA and DA metabolite concentrations (Devine et al., 1993; Spanagel et al., 1992). Furthermore, Johnson and North (1992) reported that U50 also fails to hyperpolarize nondopaminergic neurons in a VTA slice preparation. However, systemic or i.c.v. (this report) administration of selective kappa agonists results in decreased DA and DA metabolite concentration in the NAcc (Di Chiara and Imperato, 1988) and ventral striatum (Devine et al., 1993; Spanagel et al., 1992). Furthermore, Johnson and North (1992) reported that U50 fails to hyperpolarize nondopaminergic neurons in a VTA slice preparation. However, systemic or i.c.v. administration of selective kappa agonists results in decreased DA and DA metabolite concentration in the NAcc (Di Chiara and Imperato, 1988) and ventral striatum (Devine et al., 1993; Spanagel et al., 1992). Taken together, these data support the hypothesis that kappa agonists presynaptically inhibit DA release from dopaminergic terminals in the NAcc, whereas mu agonists such as heroin induce NAcc DA release mainly indirectly by a disinhibitory mechanism in the VTA.

Comparison of FCV and chronoamperometry. One of the major findings in this study is the dominant monophasic (DA) signal increase observed immediately after heroin SA. Although initial signal decreases or biphasic signal changes were also observed (occasionally) in several rats (≈20%), our major observation is not consistent with electrochemical results obtained using chronoamperometry (Kiyatkin, 1994; Kiyatkin et al., 1993). Several methodological variables may help explain this apparent disparity. Perhaps the most important difference between the present report and those of Kiyatkin et al. is that our rats were pretrained for up to 1 week to SA heroin before electrode implantation and FCV recording. Because Kiyatkin was interested in studying the development rather than the maintenance of SA behavior, rats in those studies were initially drug naive when recordings began. Thus, those biphasic chronoamperometric signals were derived from animals during their first exposures to heroin SA, whereas our FCV signals were from animals well experienced with the procedure and drug. It has been speculated that DA responses to heroin in the NAcc may differ with periods of exposure to heroin, suggesting that some adaptive changes may have occurred within the mesolimbic DA system (Self et al., 1995; Tjon et al., 1994), leading to the differences in electrochemical data.

A second major difference between the studies may be related to the recording techniques used. The single 8-μm carbon fiber probe and high scan speed used in FCV may reveal different temporal and spatial signal changes from those obtained using three 30-μm carbon fiber electrodes in chronoamperometry. In addition, the dose of heroin administered also may be a determinant of the DA response pattern observed because only monophasic mean signal increases were observed in the group of rats receiving the higher heroin dose. The relationship between dose and DA response pattern is being investigated. In any case, both the rapid monophasic and the slow tonic signal increases observed in the present study are consistent with considerable microdialysis data demonstrating basal DA increase in the NAcc after heroin SA (Di Chiara and Imperato, 1988; Spanagel et al., 1990a, 1990b, Rada et al., 1991; Wood, 1983). However, we also note that Hemby et al. (1995) found no significant changes in DA during heroin SA, whereas the destruction of DA in the NAcc selectively attenuated cocaine but not heroin SA in rats (Pettit et al., 1984).

In summary, these data taken together suggest that presynaptic kappa receptors in the NAcc play an important role in modulating mesolimbic DA activity. The antagonism between mu and kappa receptors on DA release observed in the present report is consistent with the hypothesis that activating mu receptors located on GABAergic interneurons in the VTA increases DA release via a disinhibitory mechanism, whereas activating kappa receptors located on DA terminals in the NAcc decreases DA release directly. In view of the ability of central nervous system kappa receptors to increase morphine analgesia, suppress the development of morphine tolerance and antagonize heroin-induced reinforcement, it is proposed that the coadministration of mu and kappa receptor agonists might be an effective pharmacological strategy in the treatment of drug abuse and potentially in the alleviation of pain as well.

References


neurotensin receptors within the mesolimbic dopamine system. *Brain Res* 488: 311–327.


Johnson SW and North RA (1992b) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.


Johnson SW and North RA (1992e) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992f) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992g) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992h) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992i) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.


Johnson SW and North RA (1992k) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.


Johnson SW and North RA (1992m) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992n) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992o) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.


Johnson SW and North RA (1992q) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992r) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992s) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992t) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992u) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.


Johnson SW and North RA (1992w) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.

Johnson SW and North RA (1992x) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.


Johnson SW and North RA (1992z) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.


Johnson SW and North RA (1992b) Two types of neurons in the rat ventral tegmental area and their synaptic input. *J Physiol (Lond)* 440:455–468.


Send reprint requests to: Elliot A. Stein, Ph.D., Department of Psychiatry, 8701 Watertown Plank Road, Milwaukee, WI 53226. E-mail: estein@mcw.edu