2004

Serotonin1B receptors in the ventral tegmental area modulate cocaine-induced elevations of dopamine release in the nucleus accumbens

Laura O'Dell, *University of Texas at El Paso*

0022-3565/04/3112-711–719$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 311:711–719, 2004

Vol. 311, No. 2
69278/1173870
Printed in U.S.A.

Serotonin$_{1B}$ Receptors in the Ventral Tegmental Area Modulate Cocaine-Induced Increases in Nucleus Accumbens Dopamine Levels

L. E. O'Dell and L. H. Parsons

Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California

Received March 30, 2004; accepted June 28, 2004

ABSTRACT

Previous work has demonstrated that peripheral serotonin$_{1B}$ (5-HT$_{1B}$) receptor agonist administration facilitates the behavioral and neurochemical effects of cocaine. This study used dual probe microdialysis to investigate whether activation of serotonin$_{1B}$ (5-HT$_{1B}$) receptors in the ventral tegmental area (VTA) alters the ability of peripherally administered cocaine to elevate dopamine (DA) levels in the ipsilateral nucleus accumbens (NAcc) of drug-naive Wistar rats. Intra-VTA administration of the selective 5-HT$_{1B}$ agonist 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo [3,2-*b*]pyridin-5-one dihydrochloride (CP 93,129) by reverse dialysis produced a dose-dependent (30 and 100 μM) potentiation of cocaine-induced (10 mg/kg i.p.) increases in NAcc DA efflux and concurrent cocaine-induced decreases in VTA GABA efflux. There was no effect of either local CP 93,129 or peripheral cocaine on VTA glutamate efflux. Intra-VTA administration of the 5-HT$_{1A/7}$ receptor agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT; 100 μM) did not alter cocaine-induced alterations in NAcc DA or VTA GABA, suggesting that the effects of CP 93,129 were not mediated through 5-HT$_{1A}$ receptors. Moreover, the effects of intra-VTA CP 93,129 (100 μM) on both cocaine-induced increases in NAcc DA levels and cocaine-induced decreases in VTA GABA levels were reversed by coadministration of the selective 5-HT$_{1B}$ receptor antagonist 3-[3-(dimethylamine)propyl]-4-hydroxy-*N*-[4-(4-pyridinyl] phenyl] benzamide dihydrochloride (GR 55562; 300 μM). In the absence of cocaine, intra-VTA CP 93,139 produced an increase in NAcc DA and decrease in VTA GABA levels. However, intra-VTA GR 55562 alone had no effect on any of our neurochemical measures. These findings indicate that activation of VTA 5-HT$_{1B}$ receptors potentiates cocaine-induced increases in NAcc DA levels by enhancing the ability of cocaine to decrease VTA GABA efflux.

The reinforcing effects of cocaine are mediated, in large part, by the mesolimbic dopamine (DA) pathway that originates in the ventral tegmental area (VTA) and terminates in several forebrain structures, including the nucleus accumbens (NAcc; Koob et al., 1994; Wise 1996). Several lines of evidence indicate that 5-hydroxytryptamine (serotonin) (5-HT) systems heavily modulate mesolimbic DA activity. Indeed, the VTA receives a dense serotonergic innervation, and serotonergic manipulations in the VTA modulate the behavioral and neurochemical effects produced by cocaine (for review, see Walsh and Cunningham, 1997).

A growing body of evidence indicates that 5-HT$_{1B}$ receptors play an important role in modulating the behavioral, neurochemical, and cellular effects of cocaine (Callahan and Cunningham, 1995, 1997; Parsons et al., 1998, 1999; Rocha et al., 1998; Castanon et al., 2000; Przegalinski et al., 2001). Although the neural mechanisms involved in these processes have not been elucidated, several recent studies implicate the VTA as an important locus for the modulatory influence of 5-HT$_{1B}$ receptors on cocaine-induced behaviors. For example, neurochemical and electrophysiological investigations have shown that 5-HT$_{1B}$ receptors in the VTA exert an excitatory influence on mesolimbic DA cell activity (Cameron and Williams, 1994, 1995; Yan and Yan, 2001a). Moreover, intra-VTA administration of the selective 5-HT$_{1B}$ receptor agonist CP 93,129 dose dependently potentiates the discriminative stimulus properties of cocaine, whereas intra-VTA administration of the selective 5-HT$_{1B}$ receptor antagonist GR 55562 attenuates cocaine discrimination (Filip et al., 2003). Further support for an involvement of VTA 5-HT$_{1B}$ receptors in the

Financial support for this research was provided by National Institute on Drug Abuse Grant DA-11004. This manuscript is publication 16483-NP from The Scripps Research Institute.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.104.069278.

ABBREVIATIONS: DA, dopamine; VTA, ventral tegmental area; NAcc, nucleus accumbens; 5-HT, 5-hydroxytryptamine (serotonin); GLU, glutamatel; aCSF, artificial cerebral spinal fluid; 8-OH-DPAT, 8-hydroxy-2-dipropylaminotetralin; CP 93,129, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo [3,2-*b*]pyridin-5-one dihydrochloride; GR 55562, 3-[3-(dimethylamine)propyl]-4-hydroxy-*N*-[4-(4-pyridinyl] phenyl] benzamide dihydrochloride.

modulation of cocaine-induced behaviors comes from a recent study that used viral-mediated gene transfer to overexpress 5-HT_{1B} receptors in NAcc projection neurons (Neumaier et al., 2002). Microinjections of viral vectors containing the 5-HT_{1B} receptor gene into the NAcc shell resulted in increased 5-HT_{1B} receptor expression in the VTA, and animals treated in this manner displayed increased cocaine-induced motor activation and cocaine-induced conditioned place preference relative to control animals receiving blank vector injections.

Although the neurochemical mechanisms involved in the modulation of cocaine-induced behaviors by VTA 5-HT_{1B} receptors are not known, it is likely that this process involves a modulation of cocaine-induced increases in mesolimbic DA neurotransmission. The VTA receives GABA afferents from the NAcc, and this feedback system provides tonic inhibitory regulation of midbrain DA cell activity. 5-HT_{1B} receptor mRNA is translocated to axon terminals (Bruinvels et al., 1994), and because 5-HT_{1B} mRNA is densely expressed in the NAcc (Bruinvels et al., 1994) and there is a high density of 5-HT_{1B} binding sites in the VTA (Bruinvels et al., 1993, 1994), it is possible that 5-HT_{1B} receptors are expressed on GABAergic terminals in the VTA. 5-HT_{1B} receptors exert an inhibitory control over neuronal activity through a negative coupling with adenylate cyclase, and thus activation of VTA 5-HT_{1B} receptors may reduce local GABA release, thereby disinhibiting the mesolimbic DA projection as supported by recent electrophysiological experiments (Johnson et al., 1992a; Cameron and Williams, 1994, 1995).

Based on this construct, it may be hypothesized that VTA 5-HT_{1B} receptors modulate the behavioral effects of cocaine by altering cocaine-induced increases in NAcc DA levels. The present study was designed to directly test this hypothesis by characterizing the effects produced by pharmacological manipulation of VTA 5-HT_{1B} receptors on cocaine-induced increases in NAcc DA levels in Wistar rats. An in vivo microdialysis probe implanted in the VTA was used to locally administer various doses of a 5-HT_{1B} agonist, a selective 5-HT_{1B} antagonist, or an agonist/antagonist combination. A second microdialysis probe implanted in the ipsilateral NAcc was used to monitor DA efflux after a peripheral cocaine injection. To investigate a possible GABAergic link in this process, amino acid content in the VTA dialysates were also monitored. Glutamate (GLU) levels in the VTA were also monitored based on evidence that 5-HT_{1B} heteroreceptors can modulate release of this excitatory amino acid (Muramatsu et al., 1998).

Materials and Methods

Animals and Surgery. All procedures were conducted in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Wistar rats (Charles River, Hollister, CA) weighing 325 to 400 g were stereotaxically implanted unilaterally with two microdialysis guide cannulae (Plastics One, Roanoke, VA): one lowered to 2 mm above the ventral surface of the NAcc (from bregma AP, +1.7; ML, ±1.4; from dura V, −6.1) and the other lowered to 1 mm above the ventral surface of the ipsilateral VTA (AP, −4.8; ML, ±0.8; V, −7.4) using the Paxinos and Watson (1988) atlas. The animals were allowed to recover for 4 to 5 days before experimentation.

Microdialysis Testing. At least 12 h before dialysis testing, microdialysis probes were lowered into each brain region (active membrane length was 2 mm for NAcc and 1 mm for VTA) as described in Parsons et al. (1999) and were perfused overnight with artificial cerebrospinal fluid (aCSF) at a rate of 0.2 μl/min. The next day, the perfusate flow rate was increased to 0.6 μl/min for the NAcc probes and to 1.1 μl/min for the VTA, and the probes were allowed to equilibrate for at least 1 h before the experiment. Dialysate samples were then collected at 10-min intervals from both probes throughout each phase of the experiment and were immediately frozen and stored for subsequent analysis. After a 30-min baseline sampling period, animals received intra-VTA perfusions of aCSF containing either drug-free aCSF ($N = 6$), the selective 5-HT_{1B} agonist CP 93,129 (30 μM; $N = 5$, or 100 μM; $N = 6$), the selective 5-HT_{1B} antagonist GR 55562 (100 or 300 μM; $N = 4$ in each group), or a combination of CP 93,129 and GR 55562 (100 μM CP 93,129 and 300 μM GR 55562; $N = 6$). The latter group received GR 55562 alone for 20 min before the solution containing the agonist/antagonist combination. To control for a possible effect of CP 93,129 on VTA 5-HT_{1A} receptors, an additional group was perfused with the 5-HT_{1A} agonist 8-OH-DPAT (100 μM; $N = 6$). Twenty minutes later, all animals in the groups described above received an intraperitoneal injection of cocaine (10 mg/kg), followed by an additional 60 min of dialysate collection. To evaluate neurochemical effects produced by activation or blockade of VTA 5-HT_{1B} receptors in the absence of cocaine, two additional groups of animals received either CP 93,129 (100 μM; $N = 6$) or GR 55562 (300 μM; $N = 5$), followed 20 min later by a systemic injection of saline. Dialysate neurotransmitter levels were not adjusted by in vitro recovery values. The doses of 5-HT_{1B} compounds were chosen based on the reported affinity of these compounds for 5-HT_{1B} receptors (Macor et al., 1990) and on previous reports that have observed significant neurochemical effects of these compounds when delivered locally by reverse dialysis (Yan and Yan, 2001a).

Analytical Procedures. Dopamine was quantified from 5-μl volumes of dialysate injected onto a microbore high-performance liquid chromatography system equipped with a 1 × 100-mm column (3-μm BetaBasic packing material, C18 stationary phase; Keystone, Bellefonte, PA) and eluted using a mobile phase composed of a 50 mM NaH_2PO_4 (monohydrate) buffer (pH 3.92) with 17% (v/v) acetonitrile, 0.27 mM Na_2-EDTA, 0.4% (v/v) triethylamine, and 3.27 mM decane sulfonic acid delivered at 30 μl/min by a model 500D syringe pump (Isco, Lincoln, NE). Dopamine was detected with an amperometric detector (model MP 1304; Princeton Applied Research, Princeton, NJ) using dual glassy carbon working electrodes (BAS Bioanalytical Systems Inc., West Lafayette, IN), set at 700 and −10 mV. Dopamine concentrations were determined using an external calibration curve.

Dialysate amino acid content was determined using capillary electrophoresis with laser-induced fluorescence detection. Derivatization of the amino acids was achieved by mixing 6 μl of microdialysate with 9 μl of 40 mM borate buffer (pH 10.5) containing 3.8 mM KCN and 1 μl of 5 mM naphthalene-2,3-dicarboxaldehyde in MeOH. This mixture was allowed to react at room temperature in the dark for 30 min before placing the samples in the refrigerated (10°C) sample tray of the capillary electrophoresis instrument (Agilent Technologies, Wilmington, DE). The derivatized dialysate was subsequently loaded onto a 90-cm fused silica capillary (30 μm inner diameter; sample loading by 50 mbar pressure for 10 s), and the amino acids were separated using +15 kV and a background electrolyte solution consisting of 100 mM borate buffer (pH 9.2) containing 30 mM SDS and 2 mM hydroxypropyl-β-cyclodextrin. The amino acids were detected using a laser-induced fluorescence detector (Zetalif; Picometrics, Ramon Ville, France) equipped with a 442-nm HeCd laser (30 mW; Melles Griot, Carlsbad, CA). External calibration standards were run in duplicate and were interspersed throughout the sample run. The limits of quantitation were approximately 1 nM for each of the analytes. All reagents and amino acid standards were from Sigma-Aldrich (St. Louis, MO).

Probe Placement. To provide functional verification of correct probe placement (Rahman and McBride, 2002), the $GABA_A$ antagonist bicuculline (100 μM) was perfused through the VTA probe upon

completion of the 5-HT$_{1B}$/cocaine manipulations. Only rats that exhibited at least a 2-fold bicuculline-induced increase in NAcc DA levels were included in our analyses. Probe placements were also verified histologically under a microscope by comparing 60-μm brain slices using the Paxinos and Watson (1988) atlas.

Statistical Analyses. Alterations in dialysate concentrations of DA, GABA, and GLU were analyzed using repeated measures analysis of variance with intra-VTA drug treatment as a between-subjects factor and time as a repeated measure. Significant interaction effects were further analyzed using post hoc analyses to compare treatment groups using Fisher's protected least significant difference test, $p < 0.05$.

Results

The average baseline DA concentration in NAcc dialysates was 1.3 ± 0.14 nM (mean ± S.E.M.), and baseline concentrations of GABA and GLU in VTA dialysates were 24.5 ± 1.4 and 274.7 ± 17.5 nM, respectively. Statistical evaluations of baseline dialysate concentrations revealed no significant differences in NAcc DA [$F(6,32) = 1.1$; N.S.], VTA GABA [$F(5,27) = 1.0$, N.S.], or VTA GLU [$F(5,27) = 2.0$, N.S.] between the different treatment groups. Therefore, all data were subsequently transformed to the percentage of change from baseline levels for further comparisons.

The effects of intra-VTA CP 93,129 administration on NAcc DA levels after peripherally administered cocaine are shown in Fig. 1. In control rats receiving drug-free aCSF through the VTA probe, peripheral cocaine administration significantly ($p < 0.01$) increased NAcc dialysate DA levels to approximately 375% of pre-cocaine baseline levels and significantly ($p < 0.05$) decreased VTA GABA levels to approximately 81% of pre-cocaine baseline levels. There was no significant effect of cocaine on VTA dialysate GLU levels. Intra-VTA CP 93,129 administration produced a significant and dose-dependent [$F(20,140) = 4.6$; $p < 0.0001$] facilitation of cocaine-induced increases in NAcc DA levels to peak levels of 508 and 882% of pre-cocaine baseline levels for 30 and 100 μM perfusate CP 93,129 concentrations, respectively. Concurrently, intra-VTA CP 93,129 administration produced a significant and dose-dependent [$F(20,140) = 1.9$; $p < 0.01$] facilitation of cocaine-induced decreases in VTA GABA levels to 68 and 54% of pre-cocaine baseline levels for 30 and 100 μM perfusate CP 93,129 concentrations, respectively. The results for NAcc DA and VTA GABA were consistent with subsequent post hoc analyses on the area under the curve, which were calculated by subtracting basal values for each time point after cocaine administration (i.e., each dialysate value for samples 6–11; $p < 0.05$). There was no significant effect of any CP 93,129 concentration on VTA GLU levels after peripheral cocaine administration.

As shown in Fig. 2, the potentiation of cocaine-induced increases in NAcc DA produced by intra-VTA CP 93,129 (100 μM) was completely reversed by co-perfusion with the selective 5-HT$_{1B}$ receptor antagonist GR 55562 (300 μM) [$F(20,150) = 9.03$; $p < 0.0001$]. Concurrent measures of VTA GABA revealed that the potentiation of cocaine-induced decreases in VTA GABA produced by CP 93,129 administration was also significantly blocked by coperfusion with GR 55562 [$F(20,150) = 2.2$; $p < 0.003$]. The statistical findings for NAcc DA and VTA GABA were consistent with subsequent post hoc analyses on the area under the curve ($p < 0.05$).

As shown in Fig. 3, there was no significant effect of GR 55562 (300 μM) on either cocaine-induced increases in NAcc DA levels [$F(10, 80) = 0.2$; N.S.] or cocaine-induced decreases in VTA GABA levels [$F(10, 80) = 1.6$; N.S.], and no significant effect of GR 55562 on VTA GLU levels [$F(10, 70) = 1.5$; N.S.]. The statistical findings for NAcc DA and VTA GABA and GLU were consistent with subsequent post hoc analyses on the area under the curve ($p < 0.05$).

Two control groups were included that received intra-VTA administration of CP 93,129 or GR 55562 followed by peripheral saline administration. The control group receiving intra-VTA administration of 100 μM CP 93,129 displayed a significant [$F(5,10) = 3.2$; $p < 0.005$] increase in NAcc DA levels with a maximal increase of approximately 143% of baseline (Fig. 4). Intra-VTA administration of 100 μM CP 93,129 also produced a significant decrease [$F(5,10) = 2.3$; $p < 0.05$] in VTA GABA levels relative to baseline. This manipulation did not significantly alter VTA dialysate levels of GLU. The ability of intra-VTA CP 93,129 administration to facilitate NAcc DA levels is consistent with the findings of Yan and Yan (2001a). The control group receiving intra-VTA administration of 300 μM GR 55562 followed by peripheral saline did not display any significant alterations in NAcc DA [$F(4,10) = 0.56$; N.S.], VTA GABA [$F(4,10) = 1.1$; N.S.], or VTA GLU [$F(4,10) = 1.4$; N.S.] levels (Fig. 5).

The possible involvement of 5-HT$_{1A}$ receptors in the effect of intra-VTA CP 93,129 was examined by testing the effect of intra-VTA administration of the 5-HT$_{1A/7}$ agonist 8-OH-DPAT on cocaine-induced alterations in NAcc dialysate DA levels. There was no significant effect of intra-VTA 8-OH-DPAT (100 μM) on cocaine-induced alterations in NAcc DA [$F(10,100) = 1.0$; N.S.] levels (data not shown).

Discussion

The present findings support previous work demonstrating that activation of 5-HT$_{1B}$ receptors facilitates cocaine-induced increases in NAcc DA levels (Parsons et al., 1999). The present study also extends these observations by demonstrating that the VTA is one locus in the brain where 5-HT$_{1B}$ receptors can modulate the neurochemical effects produced by peripherally administered cocaine. The selective involvement of 5-HT$_{1B}$ receptors in the effects produced by CP 93,129 is indicated by the reversal of the 5-HT$_{1B}$ agonist effect by coadministration of the selective and structurally dissimilar 5-HT$_{1B}$ receptor antagonist GR 55562. Moreover, the effect of intra-VTA CP 93,129 on cocaine-induced increases in NAcc DA was not mimicked by the intra-VTA administration of the 5-HT$_{1A/7}$ agonist 8-OH-DPAT. This observation coupled with the very low affinity of CP 93,129 and GR 55562 for other 5-HT receptor subtypes (Koe et al., 1992; Lamothe et al., 1997) suggests a selective role for VTA 5-HT$_{1B}$ receptors in the present observations.

Previous work from our laboratory also demonstrated that systemic administration of a 5-HT$_{1B}$ agonist facilitates cocaine-induced decreases in VTA GABA levels (Parsons et al., 1999). The present findings suggest that these effects are mediated at least in part via activation of VTA 5-HT$_{1B}$ receptors, because direct activation of 5-HT$_{1B}$ receptors in this region also facilitates cocaine-induced decreases in VTA GABA levels. Consistent with previous work by others (Yan and Yan, 2001a,b), we also observed that intra-VTA 5-HT$_{1B}$ agonist administration modestly decreased VTA GABA lev-

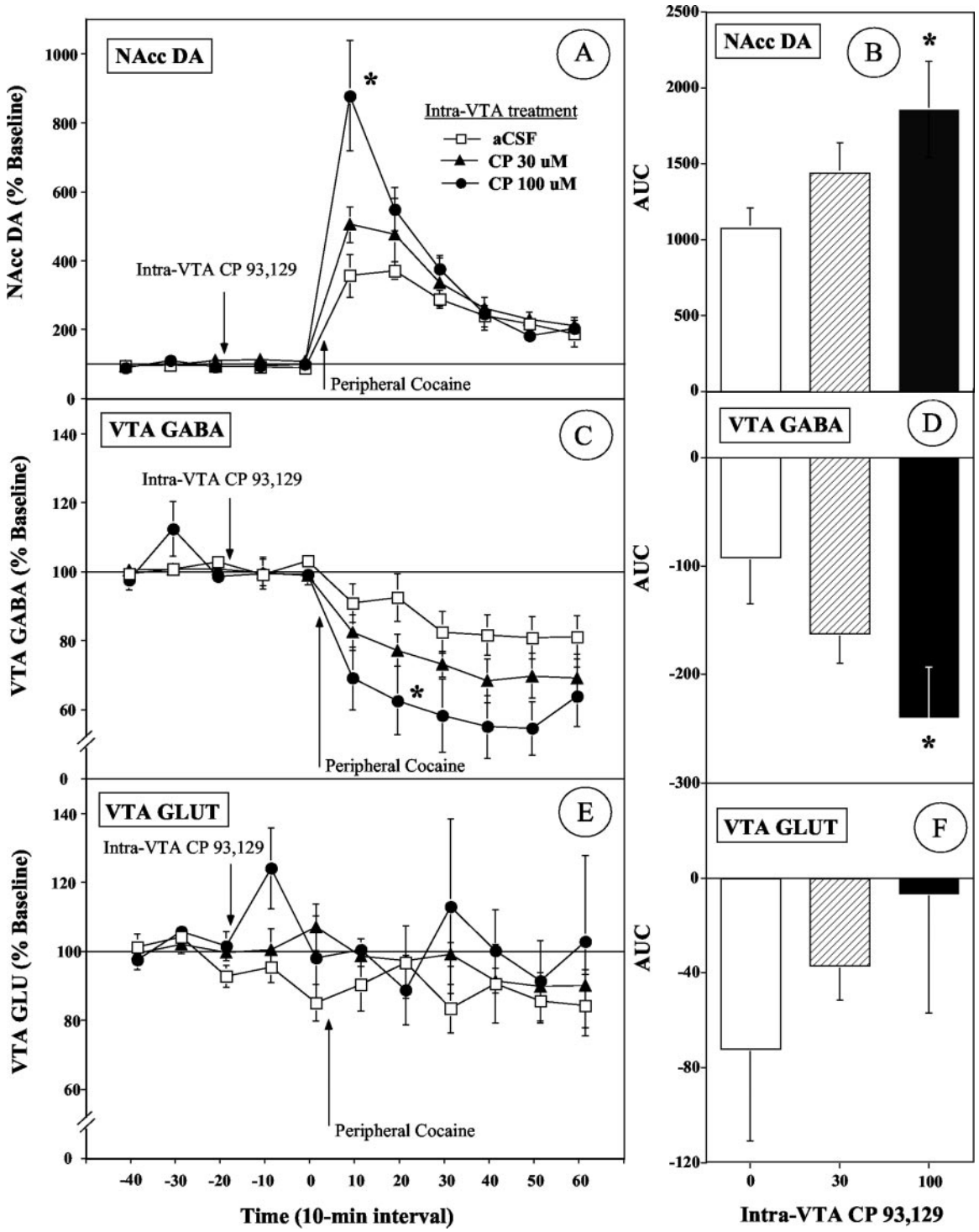

Fig. 1. Intra-VTA 5-HT_{1B} agonist administration potentiates the effects of peripherally administered cocaine on NAcc DA and VTA GABA efflux. The temporal profile of changes in NAcc DA, VTA GABA, and VTA GLU are shown in A, C, and E, respectively (data presented as the percentage of change from drug-free baseline). Intra-VTA drug administration began at $t = -20$ min, and the treatment groups are denoted by open squares (aCSF; $N = 6$), filled triangles (30 μM CP 93,129; $N = 5$), and filled circles (100 μM CP 93,129; $N = 6$). All animals received 10 mg/kg cocaine intraperitoneally at time $t = 0$ min. Area under the curve (AUC) measures for comparison of drug treatment condition on cocaine-induced changes in NAcc DA, VTA GABA, and VTA GLU are shown in B, D, and F, respectively. The AUC was calculated for each animal by subtracting 100 from the percentage of baseline value for each data point and subsequently summing all data points collected during the postcocaine period of dialysate sampling (t = 0–60 min). Asterisks (*) denote significant differences relative to aCSF controls (see text for details). Intra-VTA administration of CP 93,129 facilitated both cocaine-induced decreases in VTA GABA levels and cocaine-induced increases in NAcc DA levels. None of the manipulations significantly altered VTA GLU levels.

els and increased NAcc DA efflux in the absence of cocaine. Together, our findings suggest that 5-HT_{1B} receptors in the VTA modulate NAcc DA transmission indirectly via alterations in GABA transmission. Because mesolimbic DA neurons originating in the VTA are under tonic inhibitory control from a GABA efferent pathway from the NAcc, the reduction

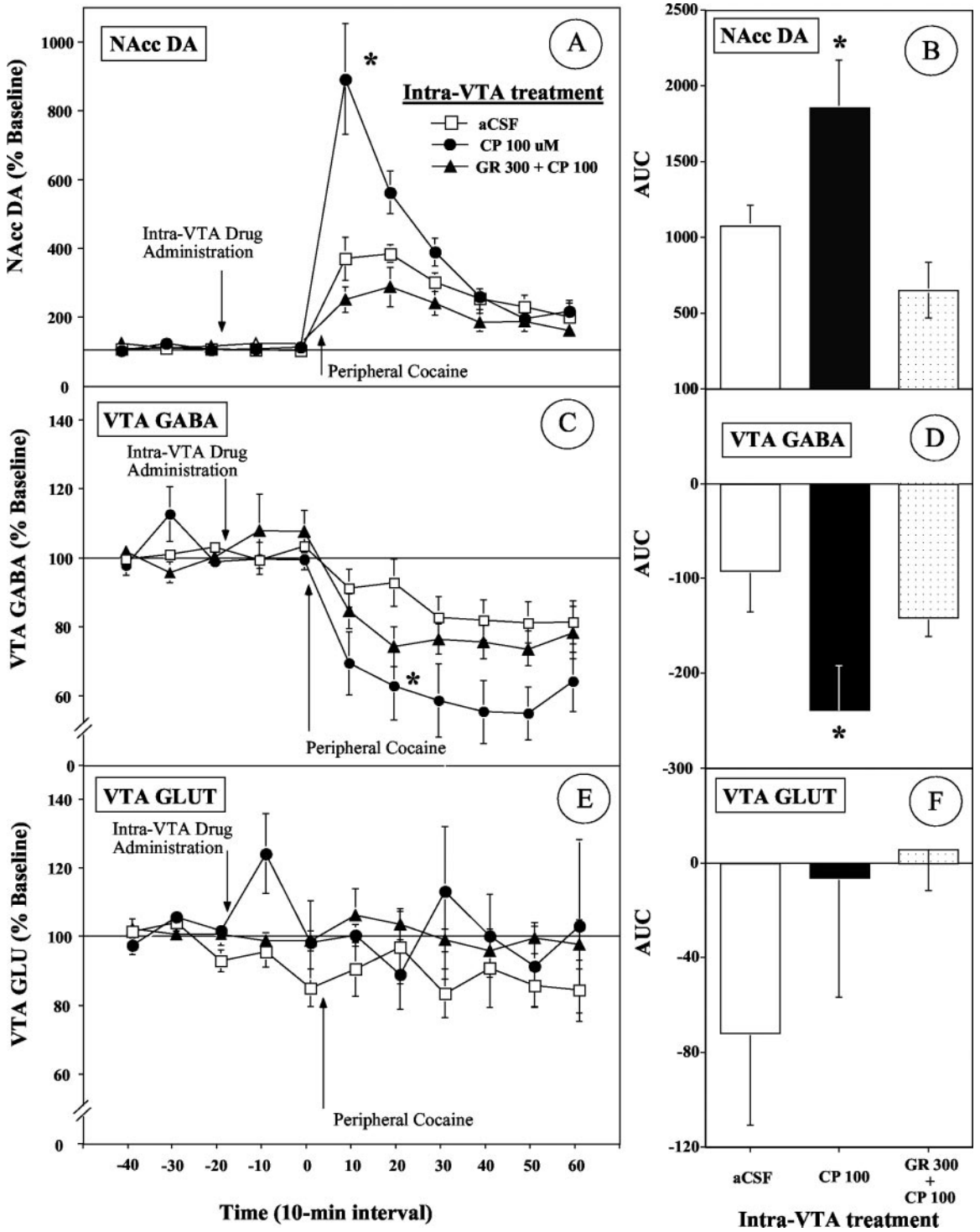

Fig. 2. Reversal of the effect of intra-VTA CP 93,129 administration on cocaine-induced alterations in NAcc DA and VTA GABA by coadministration of the selective 5-HT$_{1B}$ receptor antagonist GR 55562. The temporal profile of changes in NAcc DA, VTA GABA, and VTA GLU are shown in A, C, and E, respectively (data presented as the percentage of change from drug-free baseline). Intra-VTA drug administration began at $t = -20$ min, and the treatment groups are denoted by open squares (aCSF; $N = 6$), filled circles (100 μM CP 93,129; $N = 6$), and filled triangles (100 μM CP 93,129 and 300 μM GR 55562; $N = 6$). All animals received 10 mg/kg cocaine intraperitoneally at time $t = 0$ min. Area under the curve (AUC) measures for comparison of drug treatment condition on cocaine-induced changes in NAcc DA, VTA GABA, and VTA GLU are shown in B, D, and F, respectively (see Fig. 1 legend for details). Some of the same groups are presented in Fig. 1. Asterisks (*) denote a significant difference relative to aCSF controls. The facilitation of both cocaine-induced decreases in VTA GABA levels and cocaine-induced increases in NAcc DA levels induced by intra-VTA CP 93,129 was significantly reversed by the coadministration of GR 55562. None of the manipulations significantly altered VTA GLU levels.

in VTA GABA levels produced by activation of 5-HT$_{1B}$ sites produces an overall disinhibition of the mesolimbic DA projection. This disinhibition in turn likely produces the enhanced dopaminergic response to peripheral cocaine administration observed in the present experiments.

Previous work by others has also led to the hypothesis that

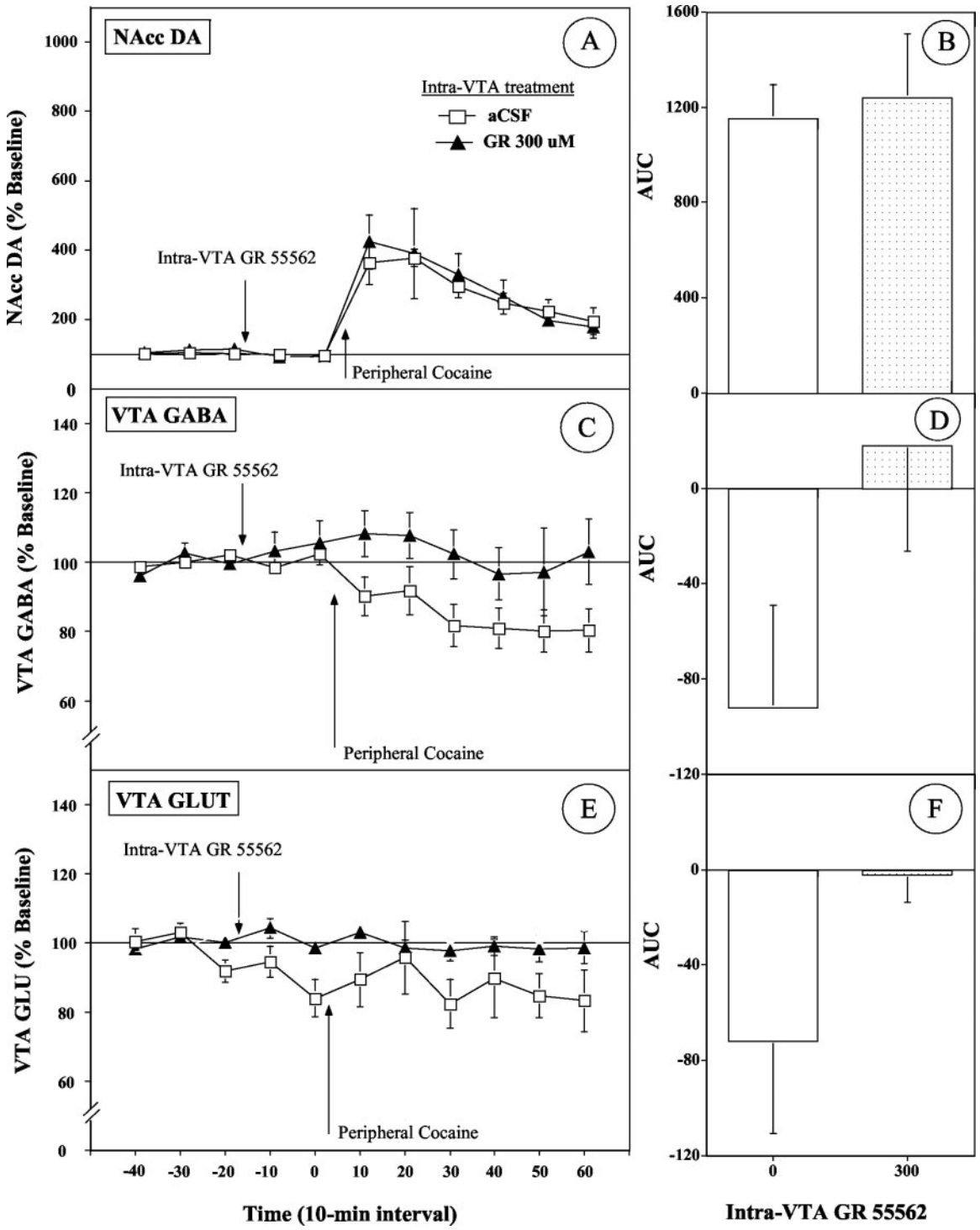

Fig. 3. Intra-VTA administration of the selective 5-HT_{1B} receptor antagonist GR 55562 does not alter the neurochemical response to cocaine. The temporal profile of changes in NAcc DA, VTA GABA, and VTA GLU are shown in A, C, and E, respectively (data presented as the percentage of change from drug-free baseline). Intra-VTA drug administration began at t = −20 min, and the treatment groups are denoted by open squares (aCSF; N = 6) and filled triangles (300 μM GR 55562; N = 4). All animals received 10 mg/kg cocaine intraperitoneally at time t = 0 min. Area under the curve (AUC) measures for comparison of drug treatment condition on cocaine-induced changes in NAcc DA, VTA GABA, and VTA GLU are shown in B, D, and F, respectively (see Fig. 1 legend for details). Some of the same groups are presented in Fig. 1. Intra-VTA administration of GR 55562 tended to attenuate the ability of cocaine to decrease VTA GABA levels; however, this effect was not significant. This manipulation did not significantly alter our measures of NAcc DA or VTA GLU levels.

mesolimbic DA activity is modulated by 5-HT in the VTA (Guan and McBride, 1989), perhaps through a 5-HT_{1B} receptor-mediated modulation of VTA GABA release. For example, using intracellular recordings of DA neurons in rat midbrain slices Johnson et al. (1992a) demonstrated that 5-HT_{1B} receptor activation inhibits GABA release onto $GABA_B$ but not $GABA_A$ receptors. In accordance with these findings [^{3}H]GABA release from isolated VTA slices was found to be

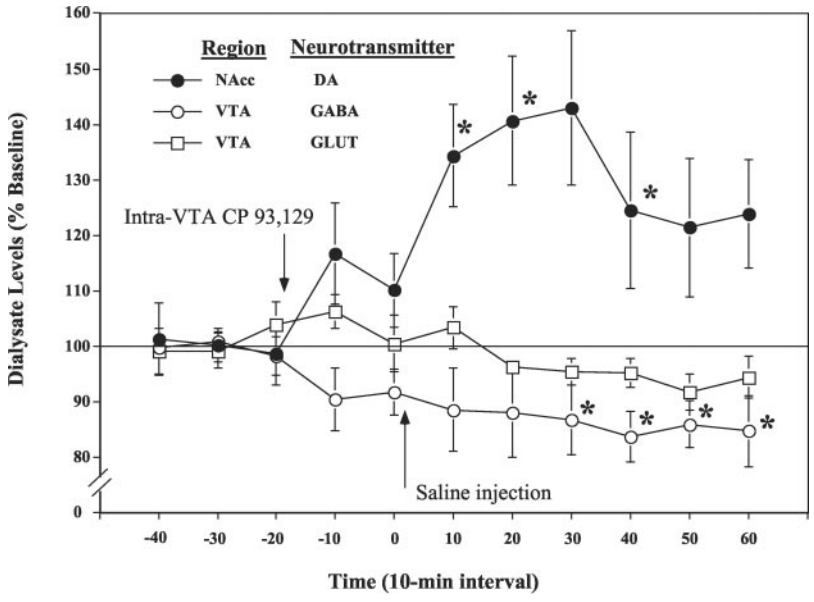

Fig. 4. Intra-VTA administration of the 5-HT$_{1B}$ agonist increases NAcc DA efflux and decreases VTA GABA efflux in the absence of cocaine. The data are presented as the percentage of change from drug-free baseline. Intra-VTA CP 93,129 administration (100 μM; $N = 6$) began at t = −20 min, and the data presented are NAcc DA (closed circles), VTA GABA (open circles), and VTA GLU (open squares). All animals received saline intraperitoneally at time t = 0 min. Asterisks (*) denote a significant difference relative to baseline. Intra-VTA CP 93,129 administration significantly increased NAcc DA levels ($p < 0.005$) and significantly decreased VTA GABA levels ($p < 0.05$; see text for details). There was no significant effect of this manipulation on VTA GLU levels.

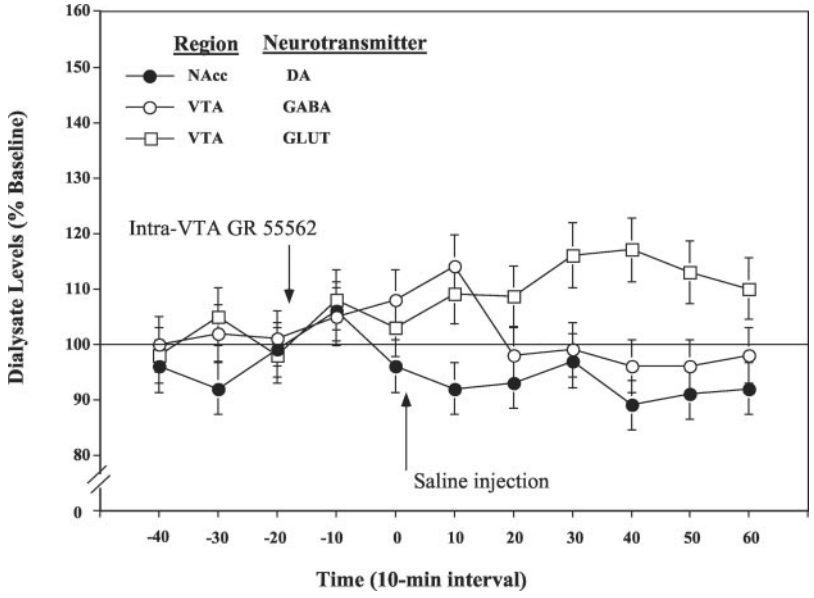

Fig. 5. Intra-VTA administration of the 5-HT$_{1B}$ antagonist GR 55562 does not alter NAcc DA efflux or VTA GABA or GLU efflux. The data are presented as the percentage of change from drug-free baseline. Intra-VTA GR 55562 administration (300 μM; $N = 5$) began at $t = -20$ min, and the data presented are NAcc DA (closed circles), VTA GABA (open circles), and VTA GLU (open squares). All animals received saline intraperitoneally at time $t = 0$ min. Intra-VTA administration of GR 55562 did not significantly alter any of our neurochemical measures.

reduced by 5-HT$_{1B}$ agonist application (Yan and Yan, 2001b), and 5-HT$_{1B}$ receptor stimulation was found to reduce electrically evoked GABA$_B$-mediated inhibitory postsynaptic potentials in DA neurons of rat VTA slices in a manner similar to that produced by cocaine (Cameron and Williams, 1995). Moreover, cocaine-induced reductions in electrically evoked GABA$_B$ inhibitory postsynaptic potentials in DA neurons of rat VTA slices were found to be mediated through 5-HT$_{1B}$ receptor activation (Cameron and Williams, 1994). The present observations extend these findings by using a within-animal in vivo preparation to demonstrate that activation of VTA 5-HT$_{1B}$ receptors facilitates cocaine-induced decreases in VTA GABA efflux and thereby potentiates the effects of peripherally administered cocaine on NAcc DA levels. It is noted, however, that the present data do not rule out the possibility that activation of 5-HT$_{1B}$ autoreceptors in the VTA blunts cocaine-induced increases in VTA 5-HT levels, thereby reducing the influence of 5-HT receptors that exert an inhibitory modulation of mesolimbic DA activity.

Several lines of evidence indicate that in addition to regulating GABA release, 5-HT$_{1B}$ heteroreceptors also provide an inhibitory modulation of GLU release (Muramatsu et al., 1998). Glutamatergic afferents to the VTA are thought to play an important role in regulating the activity of DA neurons in this region (Johnson et al., 1992b; Bonci and Malenka, 1999), and increased glutamatergic tone in the VTA has been reported to produce both excitatory and inhibitory effects on DA cell activity (Fiorillo and Williams, 1998; Takahata and Moghaddam, 2000). Thus, it is conceivable that the presently observed potentiation of cocaine-induced increases in NAcc DA produced by intra-VTA CP 93,129 administration results partly from a 5-HT$_{1B}$ receptor-mediated decrease in VTA GLU efflux. However, the lack of alteration in VTA dialysate GLU levels after any of the manipulations explored in the present experiments argues against this possibility. Although increases in VTA GLU levels have been reported after repeated cocaine administration (Kalivas and Duffy, 1998; Bell et al., 2000), our present findings are consistent with reports that acute cocaine administration

does not alter VTA GLU efflux (Kalivas and Duffy, 1998; Bell et al., 2000; but see Kalivas and Duffy, 1995).

CP 93,129 is one of the most selective 5-HT_{1B} agonists presently available (Macor et al., 1990; Koe et al., 1992). However, it was important to rule out a potential contribution of VTA 5-HT_{1A} receptors in light of observations that DA cell activity in the VTA is increased by 5-HT_{1A} receptor activation (Arborelius et al., 1993; Lejeune and Millan, 1998). Under the presently used conditions there was no significant effect of intra-VTA 8-OH-DPAT administration on either cocaine-induced increases in NAcc DA levels, or cocaine-induced decreases in VTA GABA levels using perfusate 8-OH-DPAT concentrations found to produce significant neurochemical effects in other studies (Kreiss and Lucki, 1994; Tao et al., 2000). Moreover, 5-HT_{1B} agonist-induced alterations in the behavioral effects of cocaine are not mimicked by 5-HT_{1A} agonists and are not reversed by 5-HT_{1A} antagonists (Parsons et al., 1998). Collectively these findings indicate that 5-HT_{1A} receptors do not contribute to the potentiation of cocaine reward or cocaine-induced neurochemical effects produced by 5-HT_{1B} agonist administration.

Growing evidence suggests that 5-HT_{1B} receptors play an important role in modulating the behavioral and neurochemical effects of cocaine. For example, 5-HT_{1B} receptor activation has been shown to enhance the motor stimulant (Przegalinski et al., 2001, 2002; Neumaier et al., 2002), interoreceptive (Callahan and Cunningham, 1995, 1997; Filip et al., 2001, 2002, 2003), conditioning (Cervo et al., 2002; Neumaier et al., 2002), reinforcing (Parsons et al., 1998), and neurochemical (Parsons et al., 1999) effects of cocaine. It is important to note that although 5-HT_{1B} agonist administration produces behavioral effects similar to some aspects of cocaine administration [e.g., increased locomotion (Chaouloff et al., 1999; O'Neill et al., 2000) and partial substitution for cocaine in discrimination tests (Callahan and Cunningham, 1995, 1997; Filip et al., 2001, 2003)], these agonists themselves produce a conditioned place aversion (Cervo et al., 2002) and do not support operant drug self-administration behavior in animals previously trained to self-administer cocaine (Parsons et al., 1998). Thus, the potentiating effects of 5-HT_{1B} agonists on some aspects of cocaine-induced behavior (e.g., place conditioning and operant reinforcement) seem to reflect a facilitation of the behavioral effects of cocaine rather than an additive effect of behaviors produced by the 5-HT_{1B} agonists and cocaine individually.

It is also noteworthy that although the potentiating effects of 5-HT_{1B} agonists on cocaine-induced behaviors are dose dependently reversible by selective 5-HT_{1B} antagonists (Parsons et al., 1998; Filip et al., 2001, 2002, 2003; Przegalinski et al., 2002), the behavioral effects of cocaine are not significantly altered by the 5-HT_{1B} antagonists themselves (Parsons et al., 1998; Filip et al., 2001, 2002; Przegalinski et al., 2001; but see Przegalinski et al., 2002; Filip et al., 2003). The lack of behavioral effects produced by 5-HT_{1B} blockade is consistent with the present finding that intra-VTA administration of a 5-HT_{1B} antagonist did not alter cocaine-induced neurochemical effects. These findings suggest that although overt 5-HT_{1B} receptor stimulation can enhance the behavioral effects of cocaine, 5-HT_{1B} receptors do not normally contribute significantly to the behavioral effects of cocaine. Although the reasons for this discrepancy are not presently understood, it is conceivable that under normal circumstances the facilitory influence of 5-HT_{1B} receptor activation produced by the indirect 5-HT agonist properties of cocaine is masked by the simultaneous activation of 5-HT receptors that exert an inhibitory influence on the behavioral effects of cocaine (e.g., 5-HT_{2C} receptors; Callahan and Cunningham, 1995; Fletcher et al., 2002; Rocha et al., 2002). An interesting point in this regard is that 5-HT_{1B} receptor binding in the NAcc, VTA, substantia nigra, and subiculum (Przegalinski et al., 2003), 5-HT_{1B} mRNA expression in the NAcc and dorsal striatum, and 5-HT_{1B} agonist-induced motor activation (L. H. Parsons, unpublished observations) are each increased during abstinence from repeated cocaine administration. Although potential alterations in the expression and/or function of 5-HT_{2C} receptors during cocaine abstinence have not been explored, it is possible that the relative balance between 5-HT receptor subtypes that provide facilitory (5-HT_{1B}) and inhibitory (5-HT_{2C}) influences on cocaine-induced behaviors is altered after repeated cocaine exposure.

The present finding that activation of VTA 5-HT_{1B} receptors potentiates the effect of cocaine on NAcc DA levels supports previous findings by others implicating these receptors in the regulation of mesolimbic DA activity (Cameron and Williams, 1994, 1995; Yan and Yan, 2001a). Moreover, these findings suggest that VTA 5-HT_{1B} receptors may participate in modulating the behavioral effects produced by cocaine. This hypothesis is supported by the recent observations that VTA 5-HT_{1B} receptors play a role in mediating the discriminative stimulus properties of cocaine (Filip et al., 2003) and that overexpression of 5-HT_{1B} receptors in efferents of the NAcc (including the VTA) increases the locomotor activating and place conditioning effects of cocaine (Neumaier et al., 2002). In addition to the VTA, a number of recent studies also identify 5-HT_{1B} receptors in the NAcc as possibly involved in the modulation of the behavioral effects of cocaine (Hållbus et al., 1997; Boulenguez et al., 1998; Yan and Yan, 2001a; Filip et al., 2002; Przegalinski et al., 2002). The relative influence of 5-HT_{1B} receptors in these regions on the behavioral effects produced by cocaine is presently under investigation.

In summary, the present in vivo experiments demonstrate that activation of VTA 5-HT_{1B} receptors enhances cocaine-induced decreases in VTA GABA efflux, thereby disinhibiting the mesolimbic DA projection and potentiating the ability of peripherally administered cocaine to increase NAcc DA levels. Because of the importance of increased NAcc DA levels in the mediation of the reinforcing effects of cocaine, these findings suggest that 5-HT_{1B} receptors in the VTA contribute to the facilitation of cocaine reward produced by the peripheral administration of 5-HT_{1B} receptor agonists.

Acknowledgments

We acknowledge the technical assistance of Ilham Polis, David Stouffer, and Tony Kerr, and we also appreciate the excellent editorial assistance of Michael Arends. CP 93,129 was a generous gift from Pfizer, Inc.

References

Arborelius L, Chergui K, Murase S, Nomikos GG, Hook BB, Chouvet G, Hacksell U, and Svensson TH (1993) The 5-HT_{1A} receptor selective ligands, (R)-8-OH-DPAT and (S)-UH-301, differentially affect the activity of midbrain dopamine neurons. *Naunyn-Schmiedeberg's Arch Pharmacol* **347:**353–362.

Bell K, Duffy P, and Kalivas PW (2000) Context-specific enhancement of glutamate transmission by cocaine. *Neuropsychopharmacology* **23:**335–344.

Bonci A and Malenka RC (1999) Properties and plasticity of excitatory synapses on

dopaminergic and GABAergic cells in the ventral tegmental area. *J Neurosci* **19:**3723–3730.
Boulenguez P, Peters SL, Mitchell SN, Chauveau J, Gray JA, and Joseph MH (1998) Dopamine release in the nucleus accumbens and latent inhibition in the rat following microinjections of a 5-HT1B agonist into the dorsal subiculum: implications for schizophrenia. *J Psychopharmacol* **12:**258–267.
Bruinvels AT, Landwehrmeyer B, Gustafson EL, Durkin MM, Mengod G, Branchek TA, Hoyer D, and Palacios JM (1994) Localization of 5-HT1B, 5-HT1D alpha, 5-HT1E and 5-HT1F receptor messenger RNA in rodent and primate brain. *Neuropsychopharmacology* **33:**367–386.
Bruinvels AT, Palacios JM, and Hoyer D (1993) Autoradiographic characterisation and localisation of 5-HT1D compared to 5-HT1B binding sites in rat brain. *Naunyn-Schmiedeberg's Arch Pharmacol* **347:**569–582.
Callahan PM and Cunningham KA (1995) Modulation of the discriminative stimulus properties of cocaine by 5-HT1B and 5-HT2C receptors. *J Pharmacol Exp Ther* **274:**1414–1424.
Callahan PM and Cunningham KA (1997) Modulation of the discriminative stimulus properties of cocaine: comparison of the effects of fluoxetine with 5-HT1A and 5-HT1B receptor agonists. *Neuropsychopharmacology* **36:**373–381.
Cameron DL and Williams JT (1994) Cocaine inhibits GABA release in the VTA through endogenous 5-HT. *J Neurosci* **14:**6763–6767.
Cameron DL and Williams JT (1995) Opposing roles for dopamine and serotonin at presynaptic receptors in the ventral tegmental area. *Clin Exp Pharmacol Physiol* **22:**841–845.
Castanon N, Scearce-Levie K, Lucas JJ, Rocha B, and Hen R (2000) Modulation of the effects of cocaine by 5-HT1B receptors: a comparison of knockouts and antagonists. *Pharmacol Biochem Behav* **67:**559–566.
Cervo L, Rozio M, Ekalle-Soppo CB, Carnovali F, Santangelo E, and Samanin R (2002) Stimulation of serotonin1B receptors induces conditioned place aversion and facilitates cocaine place conditioning in rats. *Psychopharmacology* **163:**142–150.
Chaouloff F, Courvoisier H, Moisan MP, and Mormede P (1999) GR 127935 reduces basal locomotor activity and prevents RU 24969-, but not D-amphetamine-induced hyperlocomotion, in the Wistar-Kyoto hyperactive (WKHA) rat. *Psychopharmacology* **141:**326–331.
Filip M, Nowak E, Papla I, and Przegalinski E (2001) Role of 5-hydroxytryptamine1B receptors and 5-hydroxytryptamine uptake inhibition in the cocaine-evoked discriminative stimulus effects in rats. *J Physiol Pharmacol* **52:**249–263.
Filip M, Papla I, Nowak E, Czepiel K, and Przegalinski E (2003) Effects of 5-HT1B receptor ligands microinjected into the ventral tegmental area on cocaine discrimination in rats. *Eur J Pharmacol* **459:**239–245.
Filip M, Papla I, Nowak E, Jungersmith K, and Przegalinski E (2002) Effects of serotonin (5-HT)(1B) receptor ligands, microinjected into accumbens subregions, on cocaine discrimination in rats. *Naunyn-Schmiedeberg's Arch Pharmacol* **366:**226–234.
Fiorillo CD and Williams JT (1998) Glutamate mediates an inhibitory postsynaptic potential in dopamine neurons. *Nature (Lond)* **394:**78–82.
Fletcher PJ, Grottick AJ, and Higgins GA (2002) Differential effects of the 5-HT(2A) receptor antagonist M100907 and the 5-HT(2C) receptor antagonist SB242084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology* **27:**576–586.
Guan XM and McBride WJ (1989) Serotonin microinfusion into the ventral tegmental area increases accumbens dopamine release. *Brain Res Bull* **23:**541–547.
Hållbus M, Magnusson T, and Magnusson O (1997) Influence of 5-HT1B/1D receptors on dopamine release in the guinea pig nucleus accumbens: a microdialysis study. *Neurosci Lett* **225:**57–60.
Johnson SW, Mercuri NB, and North RA (1992a) 5-Hydroxytryptamine1B receptors block the GABAB synaptic potential in rat dopamine neurons. *J Neurosci* **12:**2000–2006.
Johnson SW, Seutin V, and North RA (1992b) Burst firing in dopamine neurons induced by N-methyl-D-aspartate: role of electrogenic sodium pump. *Science (Wash DC)* **258:**665–667.
Kalivas PW and Duffy P (1995) D1 receptors modulate glutamate transmission in the ventral tegmental area. *J Neurosci* **15:**5379–5388.
Kalivas PW and Duffy P (1998) Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. *J Neurochem* **70:**1497–1502.
Koe BK, Neilson JA, Macor JE, and Heym J (1992) Biochemical and behavioral studies of the 5-HT$_{1B}$ receptor agonist CP 94,253. *Drug Dev Res* **26:**241–250.
Koob GF, Caine B, Markou A, Pulverenti L, and Weiss F (1994) Role for the mesocortical dopamine system in the motivating effects of cocaine. *NIDA Res Monogr* **145:**1–18.
Kreiss DS and Lucki I (1994) Differential regulation of serotonin (5-HT) release in the striatum and hippocampus by 5-HT1A autoreceptors of the dorsal and median raphe nuclei. *J Pharmacol Exp Ther* **269:**1268–1279.
Lamothe M, Pauwels PJ, Belliard K, Schambel P, and Halazy S (1997) Differentiation between partial agonists and neutral 5-HT1B antagonists by chemical modulation of 3-[3-(N,N-dimethylamino)propyl]-4-hydroxy-N-[4-(pyridin-4-yl)phenyl-]benzamide (GR 55562). *J Med Chem* **40:**3542–3550.
Lejeune F and Millan MJ (1998) Induction of burst firing in ventral tegmental area dopaminergic neurons by activation of serotonin (5-HT)1A receptors: WAY 100,635-reversible actions of the highly selective ligands, flesinoxan and S 15535. *Synapse* **30:**172–180.
Macor JE, Burkhart CA, Heym JH, Ives JL, Lebel LA, Newman ME, Nielsen JA, Ryan K, Shultz DW, Torgersen LK, et al. (1990) 3-(1,2,5,6-Tetrahydropyrid-4-yl)pyrolo[3,2-b]pyrid-5-one: a potent and selective serotonin (5-HT1B) agonist and rotationally restricted phenolic analogue of 5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. *J Med Chem* **33:**2087–2093.
Muramatsu M, Lapiz MD, Tanaka E, and Grenhoff J (1998) Serotonin inhibits synaptic glutamate currents in rat nucleus accumbens neurons via presynaptic 5-HT1B receptors. *Eur J Neurosci* **10:**2371–2379.
Neumaier JF, Vincow ES, Arvanitogiannis A, Wise RA, and Carlezon WA Jr (2002) Elevated expression of 5-HT1B receptors in nucleus accumbens efferents sensitizes animals to cocaine. *J Neurosci* **22:**10856–10863.
O'Neill MF, Dobson DR, and Sanger GJ (2000) 5-HT(1B/D) receptor agonist, SKF99101H, induces locomotor hyperactivity in the guinea pig. *Eur J Pharmacol* **399:**49–55.
Parsons LH, Koob GF, and Weiss F (1999) RU 24969, a 5-HT1B/1A receptor agonist, potentiates cocaine-induced increases in nucleus accumbens dopamine. *Synapse* **32:**132–135.
Parsons LH, Weiss F, and Koob GF (1998) Serotonin1B receptor stimulation enhances cocaine reinforcement. *J Neurosci* **18:**10078–10089.
Paxinos G and Watson C (1988) *The Rat Brain in Stereotaxic Coordinates*, 2nd ed. Academic Press, New York.
Przegalinski E, Czepiel K, Nowak E, Dlaboga D, and Filip M (2003) Withdrawal from chronic cocaine up-regulates 5-HT1B receptors in the rat brain. *Neurosci Lett* **35:**169–172.
Przegalinski E, Filip M, Papla I, and Czepiel K (2002) Effects of 5-HT1B receptor ligands microinjected into the accumbal shell or core on the cocaine-induced locomotor hyperactivity in rats. *Physiol Pharmacol* **53:**383–394.
Przegalinski E, Filip M, Papla I, and Siwanowicz J (2001) Effect of serotonin (5-HT)1B receptor ligands on cocaine sensitization in rats. *Behav Pharmacol* **12:**109–116.
Rahman S and McBride WJ (2002) Involvement of GABA and cholinergic receptors in the nucleus accumbens on feedback control of somatodendritic dopamine release in the ventral tegmental area. *J Neurochem* **80:**646–654.
Rocha BA, Goulding EH, O'Dell LE, Mead AN, Coufal NG, Parsons LH, and Tecott LH (2002) Enhanced locomotor, reinforcing and neurochemical effects of cocaine in serotonin 5-hydroxytryptamine 2C receptor mutant mice. *J Neurosci* **22:**10039–10045.
Rocha BA, Scearce-Levie K, Lucas JJ, Hiroi N, Castanon N, Crabbe JC, Nestler EJ, and Hen R (1998) Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. *Nature (Lond)* **393:**175–178.
Takahata R and Moghaddam B (2000) Target-specific glutamatergic regulation of dopamine neurons in the ventral tegmental area. *J Neurochem* **75:**1775–1778.
Tao R, Ma Z, and Auerbach SB (2000) Differential effect of local infusion of serotonin reuptake inhibitors in the raphe versus forebrain and the role of depolarization-induced release of extracellular serotonin. *J Pharmacol Exp Ther* **294:**571–579.
Walsh SL and Cunningham KA (1997) Serotonergic mechanisms involved in the discriminative stimulus, reinforcing and subjective effects of cocaine. *Psychopharmacology* **130:**41–58.
Wise RA (1996) Neurobiology of addiction. *Curr Opin Neurobiol* **6:**243–251.
Yan QS and Yan SE (2001a) Activation of 5-HT(1B/1D) receptors in the mesolimbic dopamine system increases dopamine release from the nucleus accumbens: a microdialysis study. *Eur J Pharmacol* **418:**55–64.
Yan QS and Yan SE (2001b) Serotonin-1B receptor-mediated inhibition of [(3)H]GABA release from rat ventral tegmental area slices. *J Neurochem* **79:**914–922.

Address correspondence to: Dr. Loren H. Parsons, The Scripps Research Institute, Department of Neuropharmacology, CVN-7, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. E-mail: lparsons@scripps.edu