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Electrical Stimulation of the Prefrontal Cortex Increases Dopamine Release in the Nucleus Accumbens of the Rat: Modulation by Metabotropic Glutamate Receptors

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In vivo **microdialysis was used to assess the effects of electrical stimulation of the prefrontal cortex (PFC) on dopamine (DA) release in the nucleus accumbens (NAC) of awake, unrestrained rats. The PFC was stimulated bilaterally for 20 min at parameters previously shown to support intracranial self-stimulation in this structure. Stimulation at 50 μA evoked a 38% increase in DA release while 100 μA produced a 69% increase. Thus, phasic activation of the PFC increases DA release in the NAC. Additional experiments were performed to establish whether glutamate receptors in the NAC mediated these effects. The noncompetitive NMDA antagonist dizocilpine maleate (MK-801) and the broad spectrum competitive antagonist kynurenic acid were each applied locally to the NAC via reverse dialysis alone or in combination with electrical stimulation of the PFC (100 μA). Both MK-801 (10 μM) and kynurenic acid (5 mM) increased DA release when administered alone. When a "subthreshold" concentration (i.e., the highest concentration employed that did not itself increase DA release) of either compound was administered together with PFC stimulation, neither kynurenic acid (1 mM) nor MK-801 (1 μM) attenuated the effect of stimulation on DA release, thereby indicating that this effect is not mediated by ionotropic glutamate receptors located within the NAC.**

To examine the possible role of metabotropic glutamate receptors in regulating DA release, the metabotropic glutamate agonist *trans*(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) was employed. When applied locally to the NAC, ACPD had a dose-dependent effect on DA release with a high concentration (1 mM) causing an increase and a lower concentration (100 μM) causing a small decrease. The latter concentration blocked PFC stimulation (100 μA)-induced increases in DA release in the NAC. To determine whether this finding reflected a general inhibitory effect of ACPD on stimulated DA release, ACPD was also applied during electrical stimulation (20 μA) of the ventral tegmental area, the origin of the mesoaccumbens DA projection. Again, ACPD (100 μM) blocked the stimulation-induced increase in DA release. These findings demonstrate that (1) phasic activation of the PFC increases DA release in the NAC, (2) this effect is not mediated by ionotropic glutamate receptors in the NAC, and (3) local metabotropic receptors serve to inhibit stimulation-induced increases in transmitter release from mesoaccumbens DA terminals. These results provide further evidence for functional interactions between metabotropic glutamate receptors and DA in the limbic striatum.

[Key words: ACPD, dialysis, dopamine release, kynurenate, glutamate receptors, MK-801, nucleus accumbens, prefrontal cortex]

Electrical and chemical stimulation of the sensorimotor or prefrontal cortex (PFC) have been shown to enhance dopamine (DA) release in the dorsal striatum and nucleus accumbens (Nieoullon et al., 1978; Murase et al., 1993a; Taber and Fibiger, 1993). The neurotransmitter glutamate (GLU) may be implicated in the mechanism underlying DA release evoked by PFC stimulation. The PFC projects heavily to the dorsal and ventral striatum in a topographically organized manner (Sesack et al., 1989; Berendse et al., 1993). This projection is thought to use GLU or aspartate (ASP) as a neurotransmitter, since lesions of the PFC have been shown to reduce GLU uptake in the dorsal striatum (Divac et al., 1977; McGeer et al., 1977), and ^{3}H-ASP injected into the nucleus accumbens (NAC) is retrogradely transported to the prelimbic areas of the PFC (Christie et al., 1990). *In vivo* studies have demonstrated that electrical or chemical stimulation of the frontal cortex increases the release of both ASP and GLU in the dorsal striatum (Godukhin et al., 1980; Young and Bradford, 1986; Palmer et al., 1989; Perschak and Cuenod, 1990). Although these findings have not been extended to the NAC, the general parallel nature of the cortical projections to these areas of the striatal complex are suggestive of a similar organization. Taken together, these findings suggest that stimulation of the PFC may activate the corticostriatal projection and increase GLU release in the NAC which may, in turn, enhance DA release from mesoaccumbens nerve terminals.

The extent to which GLU may modulate DA release via direct presynaptic actions within the striatum or NAC remains a critical question to this hypothesis. Axoaxonic synapses between cortical and midbrain afferents in the NAC or striatum rarely, if ever, occur (Bouyer et al., 1984; Sesack and Pickel, 1990). Recent *in vivo* microdialysis studies have examined this issue by applying GLU or GLU receptor agonists locally to the NAC via a dialysis probe while concurrently monitoring DA concentrations in the same area (Imperato et al., 1990a,b; Youngren et al., 1993). One

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group of researchers found that the specific GLU receptor agonist NMDA (1 mM) failed to evoke DA release (Imperato et al., 1990b), while the agonists quisqualate and kainate at similar concentrations increased DA release (Imperato et al., 1990a). Others have reported that high concentrations of either GLU or ASP (1–10 mM) evokes DA release, with ASP causing the larger increase (Youngren et al., 1993). Additionally, the NMDA receptor antagonist AP5 was found to be more effective than the non-NMDA antagonist CNQX at reducing the effect of ASP, whereas the reverse held true for the effects of GLU (Youngren et al., 1993). Utilizing electrochemistry to measure DA concentrations, Svensson et al. (1994) found that local administration of either NMDA or AMPA could increase DA release in the NAC, but only at high concentrations that also evoked physiological events consistent with spreading depression. These reports indicate that DA release can be evoked by GLU acting at either NMDA or non-NMDA ionotropic receptors, but that the effect may not be physiological. A survey of the literature addressing the same question in the dorsal striatum points to a similar, inconclusive picture (Moghaddam et al., 1990; Keefe et al., 1992; Westerink et al., 1992; Morari et al., 1993).

The present study was performed to establish whether electrical stimulation of the PFC can increase DA release in the NAC and if so, the extent to which this effect is mediated by GLU receptors in the NAC. To this end, the noncompetitive NMDA antagonist dizocilpine maleate (MK-801) or the broad spectrum ionotropic GLU receptor antagonist kynurenic acid (KYN) was applied locally to the NAC alone or in combination with electrical stimulation of the PFC. Additionally, because the NAC contains relatively high concentrations of metabotropic GLU receptors (Albin et al., 1991; Shigemoto et al., 1992; Testa et al., 1994), we sought to investigate the possible role of these receptors in modulating DA release. Activation of metabotropic GLU receptors mediates a variety of physiological effects in striatal slice preparations (Lovinger et al., 1993; Calabresi et al., 1993), and produces behavioral effects in the intact rodent (Sacaan et al., 1991, 1992; Klitgaard and Laudrup, 1993; Laudrup and Klitgaard, 1993). Furthermore, a recent study has implicated interactions of the GLU metabotropic and dopaminergic systems in regulating motor activity (Sacaan et al., 1992). To address whether GLU metabotropic receptors may play a role in regulation of DA release, the effects of the specific metabotropic agonist *trans*(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) on basal and stimulation-evoked DA release were examined.

Materials and Methods

Surgery. Male Wistar rats, weighing 280–350 gm, were anesthetized with 50–60 mg/kg pentobarbital (i.p.) and placed in a stereotaxic apparatus. A vertical, concentric type dialysis probe with 2.0 mm of active membrane (AN69, Hospal, MW cutoff = 60,000 Da) was implanted in the NAC. Coordinates relative to bregma were A +3.6 mm, L +1.5 mm, and V −7.8 mm (at the ventral extent of the active membrane) according to the atlas of Pellegrino et al. (1979). Bilateral, bipolar stimulating electrodes (MS303/2, Plastic Products, Roanoke VA) separated at the tip (<100 μm) were implanted in the prelimbic area of the PFC. Electrodes were implanted bilaterally because the NAC receives afferents from the PFC of both hemispheres (Sesack et al., 1989; present results). Electrode coordinates were A +5.0 mm, L ±2.4 mm, and V −4.2 mm from bregma. The electrodes were implanted at an angle 20° lateral to the dorsal/ventral axis for ease of implantation. Another group of rats had electrodes implanted in the ventral tegmental area (VTA) in combination with the NAC dialysis probes. VTA electrodes were implanted unilaterally at A +3.5 mm, L +2.4 mm, and V +1.8 mm relative to interaural zero, according to the atlas of Paxinos and Watson (1986). Probe outlets and electrodes were anchored to the skull with two or three stainless steel screws and dental acrylic. Following surgery, animals were housed individually in Plexiglas cages and were given 48 hr to recover prior to initiation of dialysis. Rats were maintained on a 12 L:12 D schedule and had ad libitum access to food and water.

Microdialysis. Dialysis probes were perfused with 1.0 mM aqueous phosphate buffer containing, in mM, NaCl 147, KCl 3.0, CaCl 1.3, and MgCl 1.0 (pH = 7.4) at a rate of 5.0 μl/min. The resulting dialysate passed through outlet tubing (PE10, Clay Adams) into the 100 μl sample loop of an injector (C10W, Valco). Samples were then automatically injected at 10 min intervals into a high performance liquid chromatography (HPLC) system. DA was isolated with reverse phase liquid chromatography (Nucleosil, 5 μm, C18, Chrompack) and detected electrochemically. The mobile phase was delivered at 1.2–1.35 ml/min using an HPLC pump (1350, Bio-Rad) and contained 0.5 mM octane sulfonic acid, 0.01 mM ethylenediamine-tetraacetic acid (EDTA), and 12% methanol in sodium acetate buffer (pH = 4.1). The concentration of DA was determined by sequential oxidation and reduction using a coulometric detector (Coulochem II, ESA) and a flow-through analytical cell (5011, ESA) with the voltage set at +0.4 and −0.35 V, respectively. A pulse dampener (SSI) and guard cell (ESA) were placed between the HPLC pump and the injector. A DA peak on the chromatogram representing a signal-to-noise ratio of 2:1 was deemed the smallest peak measurable. This corresponded to a detection limit of 5 fmol/sample.

In some animals, locomotor activity was measured during the experiment using an automated locomotion detector (Digiscan, Omnitech, Columbus, OH). This apparatus gave a count of horizontal activity measured as the number of interruptions of an infrared light beam during each 10 min dialysis sample.

Treatment was initiated after establishment of a baseline, defined as three consecutive samples with <10% variation in DA levels, and involved either local drug administration or electrical stimulation. The stimulation period started 20 min after initiation of local administration of drug, except in animals receiving TTX that had a 40 min interval before the start of stimulation. Stimulation consisted of a 60 Hz sine wave applied for 20 min in all stimulation treatments, although parameters varied for PFC and VTA stimulation. PFC electrodes were stimulated at 50 and 100 μA, with pulse trains of 0.5 sec duration and 5.1 sec intertrain interval. VTA electrodes were stimulated at 20 μA with 0.2 sec pulse train duration and 1.1 sec intertrain interval. The PFC stimulation parameters were chosen on the basis of a previous study, which found that rats will self-stimulate at these currents (Phillips and Fibiger, 1978). The VTA stimulation parameters were chosen not only because they support self-stimulation in rats, but also because the DA release evoked by this amount of VTA stimulation approximates that found with PFC stimulation in the present study (Fiorino et al., 1993). DA was measured after stimulation to determine the time course of return to baseline.

A within-subjects, counterbalanced design was used in the experimental groups that received both 50 and 100 μA currents to the PFC and in the group that received stimulation of the VTA alone and in combination with ACPD. Rats in these groups had an interval of at least 90 min between treatments for recovery of baseline. All other analyses employed a between-groups design in which rats received only one treatment.

Drugs. All drugs were administered locally to the NAC via reverse dialysis through the same probe used to measure DA. Compounds delivered in this way were dissolved in the perfusion medium and pH was adjusted to 7.1–7.4 with dilute acetic acid or NaOH. MK-801, KYN, and ACPD were obtained from Research biochemical Inc. (Natick, MA). TTX was purchased from Sigma Chemical Co. (St. Louis, MO). All drugs were diluted from frozen aliquots immediately prior to use.

Histology. To confirm that the area of the NAC perfused by the dialysis probe received a projection from the area of the PFC containing the stimulating electrodes, the retrograde label Fluorogold (FG; Fluorochrome Inc.) was employed. A solution of 3% FG in distilled water was infused through the dialysis probes of four rats at the end of the experiment and again 12 hr later. After an additional 24 hr, the dialysis probe was rinsed with distilled water to wash out excess FG. Seven days later animals were given an overdose of chloral hydrate and were perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed, postfixed for 2 hr, and transferred to 0.05 M phosphate buffer for 12 hr. The following day brains were cut in 30 μm coronal slices, mounted on slides, and observed under ultraviolet light.

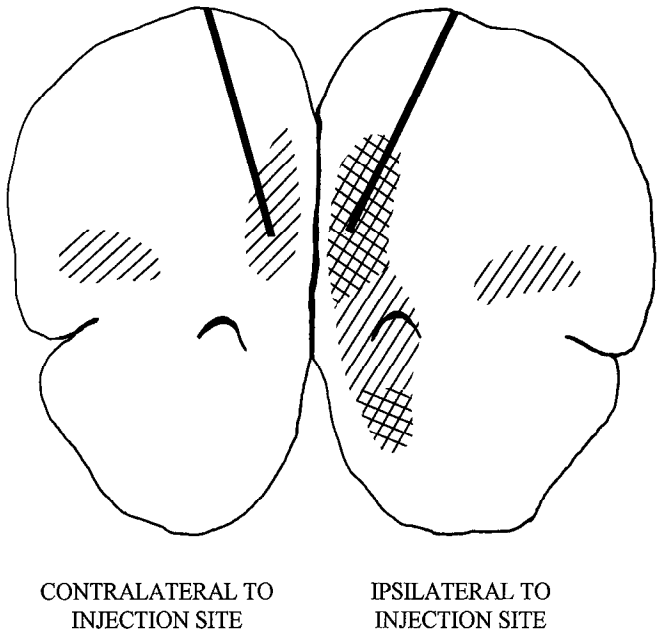

Figure 1. Camera lucida drawing of a coronal section through the PFC showing electrode location. *Hatching* represents areas of retrograde labeling following infusion of Fluorogold through the dialysis probe in the NAC. *Cross-hatching* represents dense labeling, and *rightward hatching* represents sparse labeling.

The remaining rats were overdosed with chloral hydrate and the brains were removed and fixed in 4% paraformaldehyde. At least 3 d later brains were cut in 40 μm sections, mounted on slides, and stained with cresyl violet to confirm electrode and probe locations. All rats used in the present study were confirmed to have dialysis probes located in the NAC and electrodes in the PFC and VTA as appropriate.

Data analysis. For statistical analysis, all data except DA in the presence of TTX were represented as percent baseline, calculated as the three samples preceding experimental manipulation. A one-way ANOVA with repeated measures across time was employed to test each individual treatment. Two-way ANOVAs were used to compare the stimulation effects with and without drug. In all cases, Huynh-Feldt corrections for degrees of freedom were used to account for time as a repeated measure. Post hoc analysis was performed with Tukey's test. To test the effects of TTX on DA release elicited by PFC stimulation, absolute values of DA across time were tested with a one-way ANOVA.

Results

The mean dialysate DA concentration for baseline samples in the group of rats receiving 50 or 100 μA was 51.0 ± 3.9 fmol/sample. No differences in the absolute baseline values were observed between experimental groups [$F(12,60) = 1.80$, $p > 0.05$].

Histology

Figure 1 shows a camera lucida drawing of a coronal section through the PFC showing a typical electrode placement; hatching has been added to show the approximate area of fluorescent labeling following infusion of FG through the dialysis probe. Cross-hatching represents higher levels of retrograde labeling, and rightward hatching represents lower levels. Bilateral labeling of the PFC was obtained following unilateral perfusion in the NAC, although ipsilateral labeling was stronger; this agrees with previous work, which shows substantial crossing of corticostriatal fibers (Sesack and Pickel, 1990). Fluorescent labeling was found in the prelimbic, infralimbic, insular, and piriform cortices as well as the claustrum, amygdala, and olfactory bulbs. All electrode tips were located in the ventral half of the prelimbic area of the PFC.

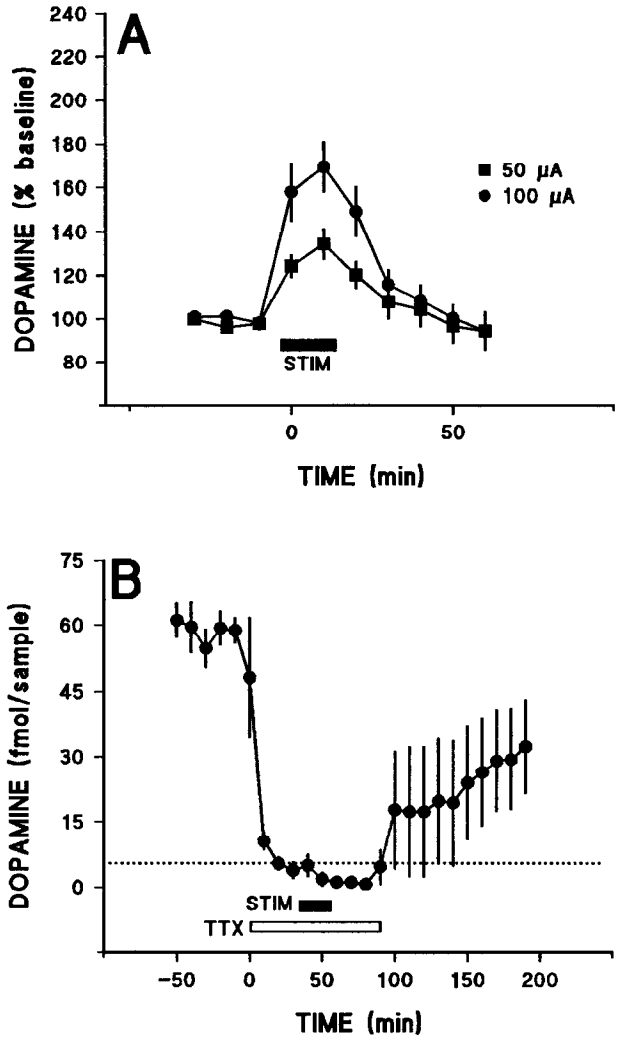

Figure 2. *A*, Dialysate values of DA in the NAC ($n = 6$) during stimulation of the PFC at 50 μA (*squares*) and 100 μA (*circles*; 60 Hz, pulse duration = 0.5 sec, interstimulus interval = 5 sec). *B*, Absolute values of dialysate DA in the NAC during PFC stimulation (100 μA) and concurrent administration of TTX (1 μM) locally via reverse dialysis ($n = 4$). In this and all subsequent figures, data points are represented as the mean ± SEM.

Effects of PFC stimulation on DA in the NAC

During the 20 min periods of 50 or 100 μA stimulation, DA concentrations rose significantly above baseline [$F(12,120) = 22.8$, $p < 0.01$] (Fig. 2*A*). The higher current caused a significantly larger increase than the lower current [$F(12,120) = 2.53$, $p < 0.05$], with 100 μA causing a peak increase of 69% above baseline, and 50 μA causing a peak increase of 37%. Extracellular DA concentrations peaked in the second stimulation sample and returned to baseline 20 min following termination of the stimulation. A group of rats receiving only the 100 μA (Fig. 3*C*) stimulation current showed an increase in DA from stimulation (71 % above baseline) almost identical to that seen in the first experiment (Fig. 2*A*).

Stimulation had an activating effect on locomotor activity at both currents in all rats, although the variation was substantial. Measured as the difference between the sample preceding stim-

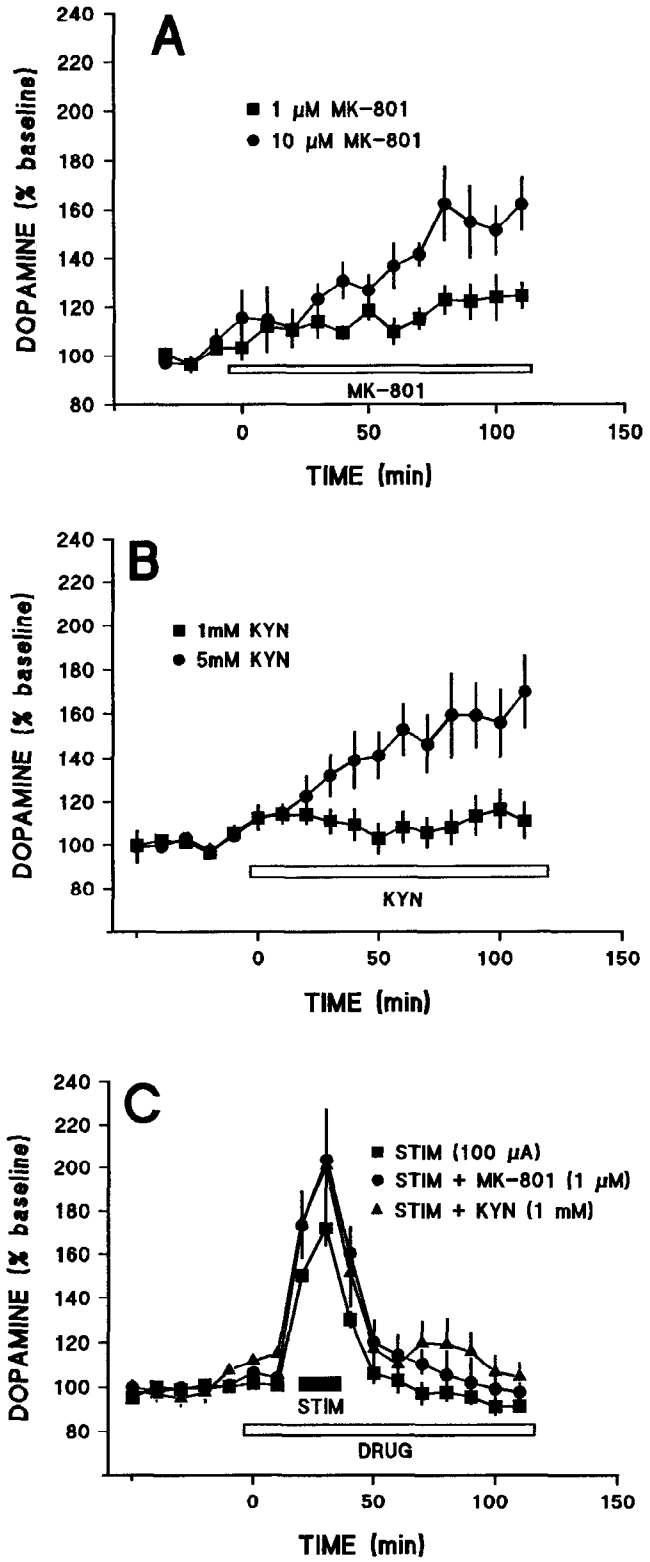

Figure 3. Dialysate values of DA in the NAC during administration of (*A*) MK-801 (1 μM; *squares*, 10 μM; *circles*; $n = 5$, each group) and (*B*) KYN (1 mM; *squares*, 5 mM; *circles*) through the dialysis probe ($n = 5$, each group). *C*, Dialysate values of DA during PFC stimulation (100 μA) in combination with local perfusion of MK-801 (1 μM; *circles*; $n = 6$), KYN (1 μM; *triangles*; $n = 6$), or vehicle solution (*squares*; $n = 5$).

ulation and the sample with peak locomotor activity, locomotor counts ranged from 432 to 3043. Rats displayed normal behaviors such as sniffing, rearing, grooming, eating, and drinking during stimulation. No signs of seizure or stereotypy were observed in any rat.

Effects of TTX on DA release evoked by PFC stimulation

TTX (1 μM) applied locally to the NAC caused a substantial drop in extracellular DA concentrations (Fig. 2*B*). Within 30 min, DA was no longer detectable in three out of the four rats, and the fourth rat's DA levels were undetectable 20 min thereafter. When the PFC was stimulated (100 μA) in this condition, no increase in absolute amounts of extracellular DA was observed [$F(5,15) = 2.339$, $p > 0.05$]. In a group of rats that received PFC stimulation without TTX treatment (Fig. 2*A*), extracellular DA rose an average of 35 fmol/sample.

Effects of locally applied GLU receptor antagonists on DA release

Figure 3 shows the effects of MK-801 and KYN at various concentrations on DA release from the NAC. Both drugs caused dose-dependent increases in DA release. MK-801 (Fig. 3*A*) had a significant effect at a moderate concentration [10 μM [$F(12,48) = 16.14$, $p < 0.01$], whereas a lower concentration (1 μM) had no effect [$F(12,48) = 1.913$, $p > 0.05$]. A high concentration of KYN (5 mM; Fig. 3*B*) caused a significant increase in extracellular DA [$F(12,48) = 5.04$, $p < 0.05$], while 1 mM had no effect [$F(12,48) = 0.681$, $p > 0.05$]. Both drugs required administration for at least 30 min before changes in DA concentrations were clearly seen. For both MK-801 and KYN, the lower concentration was deemed subthreshold on DA release, and was subsequently used in combination with stimulation of the PFC.

Effects of MK-801 and KYN applied locally to the NAC on DA release evoked by electrical stimulation of the PFC

When MK-801 (1 μM) or KYN (1 mM) was applied locally to the NAC for 2 hr beginning 20 min before the initiation of PFC stimulation at 100 μA, the time course of DA release was not different from that observed during stimulation alone (Fig. 3*C*). Stimulation still evoked a significant increase in extracellular DA during treatment with either MK-801 [$F(10,50) = 16.14$, $p < 0.01$] or KYN [$F(10,50) = 12.552$, $p < 0.01$]. When the effect of stimulation coadministered with KYN or MK-801 was compared to DA release induced by stimulation alone, there was no significant effect of drug treatment [$F(10,140) = 0.504$, $p > 0.05$]. Not only did the antagonists fail to block the stimulation-induced increase in DA release, but the tendency was for release to be slightly potentiated. This finding is consistent with the ability of each drug to increase DA release on its own. Higher concentrations of the antagonists were not used because they had significant effects on their own, thereby confounding interpretation.

Effects of ACPD on DA release

Figure 4*A* shows the effects on DA release of two concentrations of ACPD. The higher concentration of ACPD (1 mM) caused a biphasic increase in DA release peaking at 52%, falling back to baseline, then rising gradually over a much longer period [$F(12,48) = 3.721$, $p < 0.05$]. A lower dose of ACPD (100 μM) caused a small decrease in DA levels compared to baseline [$F(12,48) = 7.524$, $p < 0.01$] or compared to a control group

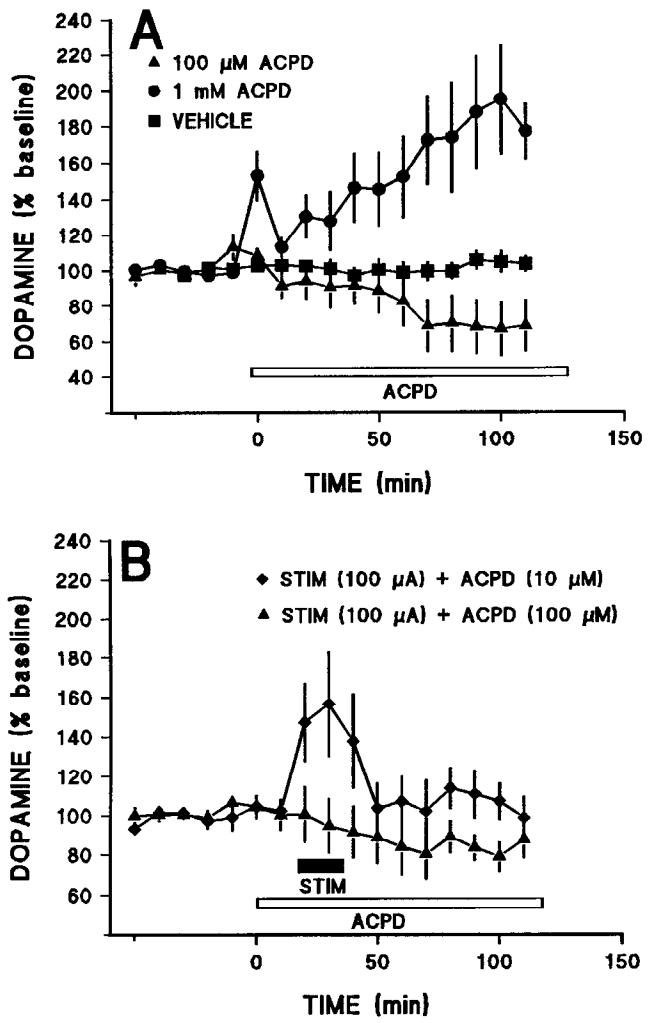

Figure 4. Dialysis values of DA in the NAC during (*A*) administration of ACPD (100 μM; *triangles*, 1 mM; *circles*) or vehicle solution (*squares*; $n = 5$, all groups), or (*B*) PFC stimulation (100 μA) and concurrent local administration of ACPD (10 μM; *diamonds*, 100 μM; *triangles*; $n = 5$).

that only received the standard perfusion solution [$F(12,84) = 4.703$, $p < 0.01$]. This effect was quite small and delayed, however, as the decrease was not statistically significant until an hour after the beginning of local drug application. On the basis of these results, the 100 μM concentration was judged to be subthreshold with respect to DA release and was therefore used in the subsequent experiments.

Effects of ACPD on DA release evoked by PFC stimulation

Figure 4*B* shows the effect of two concentrations of ACPD administered locally to the NAC on DA release evoked by PFC stimulation (100 μA). At the higher concentration, ACPD completely blocked the effect of PFC stimulation on DA release. Accordingly, the stimulation did not cause a significant deviation from baseline across time [$F(11,44) = 1.043$, $p > 0.05$]. The lower concentration of ACPD (10 μM) failed to attenuate the stimulation-induced effect; following administration of this concentration, DA release was not different from that of stimulation alone [$F(10,90) = 0.462$, $p > 0.05$].

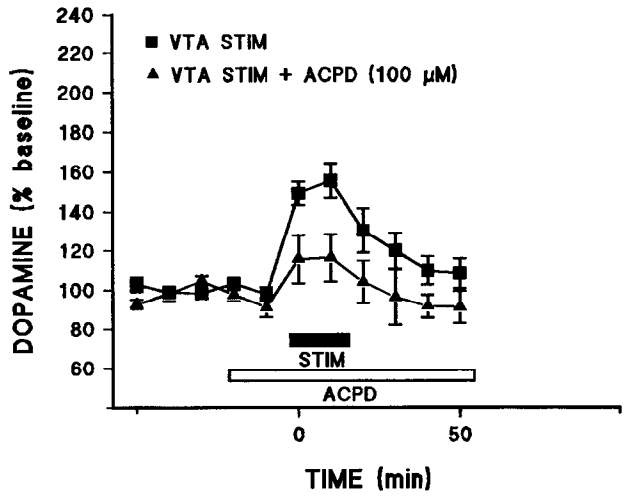

Figure 5. Dialysis values of DA in the NAC ($n = 5$) during VTA stimulation (20 μA, pulse duration = 0.2 sec, interstimulus interval = 1 sec) and concurrent local administration of ACPD (100 μM; *triangles*) or vehicle (*squares*).

Effects of ACPD on DA release elicited by stimulation of the VTA

To establish whether the effect of ACPD on stimulated DA release was specific to cortical stimulation or was representative of a more general phenomenon, extracellular DA was monitored during electrical stimulation of the VTA with and without concurrent local administration of ACPD (100 μM). VTA stimulation (20 μA) caused a significant increase in DA in the NAC to 55% above baseline [$F(10,50) = 11.05$, $p < 0.01$] (see Fig. 5). During local application of ACPD, stimulation of the VTA failed to significantly raise extracellular DA concentrations in the NAC [$F(10,50) = 2.55$, $p > 0.05$].

Discussion

The present results demonstrate that phasic activation of the medial PFC increases DA release in the NAC. The stimulation-evoked increase was TTX sensitive and varied directly as a function of current intensity. The peak increase in extracellular DA obtained during the 100 μA stimulation period was 69% above baseline. In a previous study, identical stimulation currents applied to the dorsal PFC caused an increase of 38% in the dorsal striatum (Taber and Fibiger, 1993). This finding is reminiscent of previous studies showing that a variety of stimuli increase extracellular DA to a greater extent in the NAC than in the dorsal striatum (Abercrombie et al., 1989; Damsma et al., 1992). When applied locally via reverse dialysis, the ionotropic GLU receptor antagonists MK-801 and KYN failed to attenuate the increase in DA release produced by PFC stimulation. This suggests that ionotropic GLU receptors intrinsic to the NAC do not mediate this effect of PFC stimulation. The metabotropic GLU receptor agonist ACPD, also applied locally, blocked the increase in DA release. ACPD also effectively blocked the increase in DA release produced by electrical stimulation of the VTA. These findings indicate that activation of metabotropic GLU receptors has an inhibitory effect on stimulated DA release in the NAC.

Stimulation currents were chosen on the basis of previous studies that showed that rats will self-stimulate the PFC and VTA at approximately the same intensities and train frequencies as those used in the present study (Phillips and Fibiger, 1978;

Fiorino et al., 1993). During PFC stimulation the rats became active, but showed no behavioral abnormalities such as seizures or specific motor responses. They demonstrated nonstereotyped sniffing, locomotion, and some rearing, although the variation between animals was substantial. These observations suggest that the stimulation currents used in the present study can be considered behaviorally relevant. Circling behavior was not apparent during bilateral PFC stimulation. In contrast, during unilateral VTA stimulation, animals often rotated contralateral to the stimulation, consistent with a unilateral increase in DA release (Szostak et al., 1986).

Due to the fact that the stimulating electrodes in the ventral PFC were 2–3 mm from the NAC dialysis probe, it was important to establish that the effect of stimulation was not due to direct depolarization of the DA terminals as a result of current spread. When the Na^+ channel blocker TTX was applied locally to the NAC, DA was no longer detectable in the dialysate, and PFC stimulation failed to increase DA release to detectable levels. Given that the detection limit of our assay was 5 fmol/sample, the stimulation could not have increased DA release by this amount. In contrast, an increase of 35 fmol/sample was observed in rats stimulated without concurrent TTX treatment. These results indicate that the effect of PFC stimulation was impulse dependent and not due to current spread.

Local administration of the specific GLU antagonists MK-801 and KYN through the dialysis probe produced dose-dependent increases in extracellular DA in the NAC. This confirms the findings of previous studies (Imperato et al., 1990b; Keefe et al., 1992). While the increase in DA release was small and required considerable time to appear, it was consistent across animals. The increases were produced by high concentrations of the antagonists and may have involved nonspecific (i.e., glutamate receptor independent) actions of the compounds. It is noteworthy in this regard that MK-801 potently activates DA neurons in the VTA (French et al., 1993; Murase et al., 1993b), and French et al. (1993) concluded that this effect was due to effects of MK-801 not involving NMDA receptors because competitive NMDA antagonists failed to mimic the response. Kynurenic acid was employed in the present study because it is one of the few GLU analogs with antagonist properties at all of the ionotropic receptor subtypes (Perkins and Stone, 1982). When concentrations of MK-801 or KYN that did not by themselves affect DA release were applied to the NAC in combination with electrical stimulation to the PFC, the increase in DA release did not differ from that evoked by stimulation alone. Indeed, there was a tendency for DA release to be slightly potentiated in this circumstance, although this effect did not reach statistical significance. Following the stimulation period, extracellular DA returned to baseline levels within 30 min in both treated and control rats. This provides further evidence that neither of these drug treatments interacted with the stimulation-induced increases in DA release. It is unlikely that the lack of effect could be due to insufficient drug concentrations being applied through the dialysis probe. Previous studies of dialysis probe recoveries suggest that approximately 5–10% of a solute crosses the dialysis membrane (Kendrick, 1988). This indicates that in the present experiments during PFC stimulation extracellular concentrations at the probe surface would reach at least 50–100 nM and 50–100 μM for MK-801 and KYN, respectively. MK-801, at these concentrations, should effectively block NMDA receptors in the vicinity of the dialysis probe since the K_d for this compound in the striatum is 9.0 nM (Wong et al., 1988). This concentration of MK-801 has also been shown to attenuate the effect of local NMDA (1 mM) on DA release in the striatum (Morari et al., 1993). Electrophysiological studies have found that KYN in this dose range antagonizes the postsynaptic effects of GLU at both NMDA and non-NMDA GLU receptors in the striatum (Herrling, 1985). Kynurenic acid (100 μM) has also been found to attenuate NMDA (50 μM)-induced DA release in a slice superfusion preparation (Krebs et al., 1991). Additionally, a microdialysis study has shown that the related compound, 7-chloro-kynurenic acid, when applied locally at 100 μM blocks the effect of local NMDA (300 μM) on DA release in the striatum (Martinez-Fong et al., 1992). We conclude that the increase in DA release produced by stimulation of the PFC occurs independently of ionotropic GLU receptors in the NAC.

This conclusion stands in apparent contrast to a body of research that has examined the effect of locally administered GLU on DA release in both dorsal and ventral striatum. These studies indicate that locally administered GLU, ASP, NMDA, and kainate increase extracellular DA, and that this can be blocked by antagonists specific to either NMDA or non-NMDA receptors (Keefe et al., 1992; Westerink et al., 1992; Morari et al., 1993; Youngren et al., 1993). These findings have raised the possibility that synaptically released GLU has direct depolarizing actions on DA terminals. However, the physiological significance of these results can be questioned on a number of grounds. (1) GLU receptor antagonists do not reduce basal concentrations of DA when administered locally (Imperato et al., 1990b; Moghaddam and Gruen, 1991; Keefe et al., 1992). (2) GLU receptor antagonists fail to block stress-induced increases in striatal DA release (Keefe et al., 1993). (3) GLU receptor agonist-induced increases in extracellular DA concentrations are accompanied by changes in K^+ concentration and a large negative shift in field potential indicative of a pathological state such as spreading depression (Moghaddam et al., 1990; Svensson et al., 1994). (4) The effects of GLU or ASP are only observed at high concentrations (1–10 mM), and are not dose dependent (Moghaddam et al., 1990; Youngren et al., 1993; Svensson et al., 1994).

Studies showing that GLU receptor antagonists applied to the striatum fail to decrease DA release preclude a tonic role for GLU in maintaining basal DA release (Imperato et al., 1990b; Moghaddam and Gruen, 1991; Keefe et al., 1992). The present results confirm and extend these findings and suggest that ionotropic GLU receptors in the NAC do not contribute to the increased DA release that is evoked by a phasic stimulus that is presumed to increase GLU release in the NAC. This finding agrees with the results of a recent study showing that ionotropic GLU receptor antagonists applied to the dorsal striatum fail to block the increase in DA release induced by pharmacological stimulation of the PFC (Karreman and Moghaddam, 1994). Similarly, Keefe et al. (1993) have demonstrated that stress-induced DA release in the dorsal striatum also occurs independently of local GLU receptors. Taken together, these results suggest that neither basal nor stimulated DA release is regulated by ionotropic GLU receptors in the striatum.

The present results indicate that GLU may have inhibitory effects on basal and stimulated DA release via activation of metabotropic receptors within the NAC. Thus, when PFC stimulation was applied in the presence of ACPD in the NAC, no increase in DA release was observed. This appeared to be a general phenomenon, as ACPD also blocked the increases in DA release produced by electrical stimulation of the VTA. The percent increase in DA release produced by stimulation in the

present study (55%) is similar to that found previously in rats self-stimulating or receiving experimenter delivered stimulation at the same currents (Fiorino et al., 1993). The effects of ACPD on stimulation-induced increases in DA release could be either direct or indirect. With respect to direct effects, it is noteworthy that in mesencephalic brain slices ACPD has been observed to depolarize and increase the firing rates of presumed DA neurons (Mercuri et al., 1993). These neurons have recently been demonstrated to express mRNA for one of the metabotropic GLU receptor subtypes (mGLUr1: Martin et al., 1992; Shigemoto et al., 1992; Testa et al., 1994). In addition, the VTA and the NAC contain metabotropic GLU receptor binding sites (Albin et al., 1991). These findings, together with the present results, raise the possibility that DA neurons synthesize and then axonally transport mGLUr1 receptors to their terminal regions where they serve to regulate DA release. With respect to possible indirect effects, Lovinger (1991) and Lovinger et al. (1993) have provided electrophysiological evidence in striatal slice preparations that activation of metabotropic GLU receptors inhibits GLU release at corticostriatal synapses. It remains possible that the effects of ACPD observed in the present experiments were mediated indirectly via actions on metabotropic GLU receptors located on nondopaminergic elements.

Application of ACPD produced biphasic, dose-related effects on DA release, with the lower concentration (100 μM) causing a delayed decrease and the higher concentration (1 mM) causing a gradual increase (Fig. 4*A*). Inasmuch as subtypes of metabotropic GLU receptor have different affinities for ACPD (Schoepp and Conn, 1993), the biphasic response raises the possibility that metabotropic GLU receptor subtypes may have opposing actions on DA release in the NAC. The increase in DA release produced by the higher concentration of ACPD is consistent with two behavioral studies that have reported that locally applied ACPD increases contralateral turning behavior in rats (Sacaan et al., 1991, 1992). In the later report, it was demonstrated that this behavioral response to ACPD could be blocked with α-methyl-para-tyrosine–induced depletions of DA or by the DA receptor antagonist haloperidol. In addition, ACPD increased tissue concentrations of DOPAC and HVA in the striatum (Sacaan et al., 1992).

The anatomical substrate through which stimulation of the PFC enhances DA release remains to be determined. The present results suggest that the corticostriatal GLU projection is not directly involved. Antidromic stimulation of mesocortical DA axons leading to activation of mesoaccumbens DA collaterals cannot account for the present findings, as few, if any, DA-containing neurons project to both PFC and NAC (Lindvall and Bjorklund, 1983). A more plausible mechanism involves the projection from the PFC to the VTA (Sesack and Pickel, 1990). Gariano and Groves (1988) have shown that electrical stimulation of the PFC produces bursts of action potentials in mesencephalic dopaminergic neurons of anesthetized rats. This pattern of electrophysiological activity is associated with increases in DA release from the terminals of these neurons (Nissbrandt et al., 1994). In a study that combined electrophysiological and voltammetric techniques in anesthetized rats, Murase et al. (1993a) demonstrated that activation of the PFC by intracerebral injections of GLU increased burst firing of single dopaminergic neurons in the VTA and enhanced DA release in the NAC. Finally, GLU receptor antagonists applied to the VTA have been shown to block the increase in striatal DA release produced by pharmacological stimulation of the PFC (Karreman and Moghaddam, 1994). The present results obtained in awake, freely moving animals are entirely consistent with these observations and suggest that the PFC stimulation-induced increases in accumbal DA release may have been due to the activation of the cortico-VTA projection which, in turn, increased burst firing of the dopaminergic neurons in the VTA.

On the basis of behavioral studies, the PFC has long been thought to inhibit subcortical DA mechanisms. For example, DA-mediated behaviors such as amphetamine-induced stereotypy and locomotor activity are enhanced by PFC lesions (Adler, 1961; Lynch et al., 1969; Iversen et al., 1971; Jaskiw et al., 1990). On the other hand, while lesions of the PFC have been reported to produce transient (2 week) increases in measures of subcortical DA turnover (Jaskiw et al., 1990), most studies have failed to observe such effects (Scatton et al., 1982; Christie et al., 1986). Notably, a recent microdialysis study found that PFC lesions did not affect amphetamine-induced DA release 5 or 15 d after a PFC lesion, times at which the same study showed amphetamine-induced locomotor activity to be enhanced (Whishaw et al., 1992). The present findings point to an excitatory influence of the PFC on subcortical DA and are consistent with the results of other stimulation studies that have examined this issue (Nieoullon et al., 1978; Murase et al., 1993a; Taber and Fibiger, 1993). It is unlikely therefore that PFC lesions enhance DA-mediated behaviors by "disinhibiting" subcortical DA systems.

In conclusion, the increase in DA release in the NAC evoked by activation of the PFC does not appear to be mediated by ionotropic GLU receptors intrinsic to the NAC. On the other hand, the metabotropic GLU receptor agonist ACPD attenuates the effect of PFC stimulation on DA release in the NAC. A general inhibitory effect of ACPD on evoked DA release is indicated as this compound also antagonized increases in DA release produced by stimulation of the VTA. These data implicate metabotropic GLU receptors in mediating GLU/DA interactions in limbic striatum. Recent hypotheses concerning the pathophysiology of schizophrenia have suggested that the corticostriatal projection and its effect on DA metabolism may be implicated in this disorder (Weinberger, 1987; Robbins, 1990; Davis et al., 1991). The present results provide new information on how these systems interact *in vivo*, and could therefore aid the further development of these models.

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