

Stimulation of cortical acetylcholine release following blockade of ionotropic glutamate receptors in nucleus accumbens

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Abstract

In vivo microdialysis techniques were used to determine the ability of glutamate receptors within the nucleus accumbens to trans-synaptically modulate the basal forebrain cortical cholinergic system. Rats were implanted with a dialysis probe in the medial prefrontal cortex to measure changes in cortical acetylcholine efflux and in the ipsilateral nucleus accumbens to locally manipulate glutamate receptor activity. Intra-accumbens perfusion of the broad spectrum ionotropic glutamate receptor antagonist kynurenate (1.0, 5.0 mM) led to a dose-dependent increase (maximum of 200%) in cortical acetylcholine efflux. This stimulated efflux was reproduced with the intra-accumbens perfusion of the AMPA/kainate antagonist DNQX (0.1, 0.25, 2.5 mM; maximum increase of 200%) or the NMDA antagonist D-CPP (10.0, 100.0, 200 μ M; maximum increase of 400%). These results reveal a significant glutamatergic tone within the accumbens of awake rats and support the hypothesis that accumbens efferents to basal forebrain modulate the excitability of the basal forebrain cortical cholinergic system.

Introduction

A number of clinical (e.g. Parasuraman & Haxby, 1993) and animal (Muir *et al.*, 1992; Voytko *et al.*, 1994; Chiba *et al.*, 1995) studies demonstrate that the integrity of cortical cholinergic transmission is crucial for normal attentional processing. Using operant tasks designed to assess attention in animals, this laboratory and others have demonstrated the *necessity* of basal forebrain activation and cortical cholinergic transmission for attentional processing (Muir *et al.*, 1994, 1995; McGaughy *et al.*, 1996; McGaughy & Sarter, 1998; Turchi & Sarter, 2001).

Several recent studies, using *in vivo* microdialysis techniques in behaving animals have demonstrated that performance in tasks that explicitly assess attentional processing is also *sufficient* to stimulate the basal forebrain cortical cholinergic system. Cortical ACh efflux is enhanced in rats performing in the five-choice serial reaction time task (Dalley *et al.*, 2001; Passetti *et al.*, 2000) and during performance in an operant task validated to assess and explicitly tax sustained attention (Himmelheber *et al.*, 2000, 2001; Arnold *et al.*, 2002). Moreover, presentation of a visual distractor to rats in this sustained attention task yielded further increases in cortical ACh efflux than seen in animals exhibiting normal task performance (Himmelheber *et al.*, 2000).

Given the critical role of the basal forebrain cortical cholinergic system to attentional processing and the contribution of attentional impairments to the cognitive deficits seen in a variety of neuropsychiatric disorders (Sarter & Bruno, 1997, 1999), several studies have focused on characterizing the distributed neural system that modulates the excitability of the basal forebrain. The nucleus

accumbens is an important link in this system. The relevant efferent pathway of the accumbens, for this modulation, is the GABAergic projection to the basal forebrain (Zaborszky & Cullinan, 1992; Zahm & Heimer, 1993). Manipulations of neurotransmission within the nucleus accumbens have been shown to modulate the ability of behavioural (Arnold *et al.*, 2000) and pharmacological (Moore *et al.*, 1999) stimuli to enhance cortical ACh efflux.

The accumbens GABAergic projections to basal forebrain are contacted by glutamatergic afferents from prefrontal cortex, hippocampus and amygdala to the accumbens (Groenewegen *et al.*, 1991; Pennartz *et al.*, 1994; Meredith, 1999) and contain NMDA, AMPA/kainate, and metabotropic glutamate receptor (mGluR) groups, mGluR1, 3, 4 and 5 (Lu *et al.*, 1998; Testa *et al.*, 1994). This chemoanatomical organization suggests that changes in glutamate receptor activity in nucleus accumbens, might affect the excitability of the basal forebrain cortical cholinergic system and hence, cortical ACh release, via changes in activity of accumbens efferents to the basal forebrain. The test of this hypothesis was initiated in the current experiment. Rats were equipped with two microdialysis probes, one in the nucleus accumbens and another in the ipsilateral prefrontal cortex. Cortical ACh efflux was determined, in separate groups of rats, following the intra-accumbens perfusion of kynurenate (a broad spectrum ionotropic glutamate receptor antagonist), DNQX (an AMPA/kainate antagonist), or D-CPP (an NMDA receptor antagonist).

Materials and methods

Subjects

Adult male Fisher-344/Brown Norway F1 hybrid rats (Harlan Sprague-Dawley, Indianapolis, IN, USA), weighing between 250 and 350 g, served as subjects in this experiment. Animals were

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maintained in a temperature-controlled environment on a 12 h light : 12 h dark cycle (lights on at 06.30 h) with food and water available *ad libitum*. Before surgery, animals were housed in pairs in stainless steel hanging cages. One day prior to surgery, animals were moved to individual plastic cages with corn-cob bedding (Harlan Teklad, Madison, WI, USA) where they were housed for the duration of the experiment. Animal care and experimentation were performed in accordance with protocols approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee and consistent with the NIH Guide for the Care and Use of Laboratory Animals.

Guide cannula surgery

Following 1 week of handling and 4 days of habituation to the microdialysis testing environment (see below), animals were anaesthetized with inhalant isoflurane (2%, 0.6 L/min) and two thermoplastic resin microdialysis guide cannula [0.72 mm o.d., Bioanalytical Systems (BAS), W. Lafayette, IN, USA] were stereotactically implanted using coordinates calculated according to the atlas of Paxinos & Watson (1986). The microdialysis cannula for the mPFC was positioned such that when the microdialysis probe was inserted into the guide the coordinates for the probe tip were: A 3.7 mm, and L 1.0 mm from Bregma, and V 3.0 mm from dura mater at 10° rostral. The second microdialysis cannula, targeted for the ipsilateral shell of the nucleus accumbens, was positioned such that when the microdialysis probe was inserted into the guide the coordinates for the probe tip were: A 0.70 mm, and L 1.1 mm, from Bregma; V 7.3 mm from dura mater at 15° caudal. The positions of the guide cannulae were fixed with dental cement and skull screws. At the conclusion of surgery animals received a prophylactic dose of the antibiotic amoxicillin (30 000 units, subcutaneously). The hemisphere of guide cannulae placement was counterbalanced across animals. Following surgery, animals were allowed to recover during three additional days of habituation to the microdialysis testing environment.

Microdialysis sessions

Animals were habituated to clear plastic concentric dialysis bowls [35 cm high by 38 cm deep; Carnegie Medicin (CMA), Stockholm, Sweden] in the testing room for 5–7 h each day. The first microdialysis session was performed on the fourth postsurgical day. Microdialysis was conducted using a repeated perfusion paradigm in which the rat received a different pharmacological treatment on each microdialysis session, with an 'off day' between each session. This repeated perfusion paradigm allows the assessment of the effects of multiple drug treatments, including control conditions, in the same animal and has been validated previously for measurements of cortical ACh (Moore *et al.*, 1995b; Bruno *et al.*, 1999), striatal ACh (Johnson & Bruno, 1995) and striatonigral GABA (Byrnes *et al.*, 1997) efflux by demonstrating that neither basal release nor drug effects interact significantly with the order of the dialysis sessions (Moore *et al.*, 1995b). The validity of repeated perfusion designs is again addressed in the present manuscript.

On each dialysis day, animals were allowed to acclimatize to the testing chamber for at least 30 min prior to the insertion of the concentric dialysis probes (0.32 mm o.d., 2.0 mm exposed membrane tip; BAS) through the guide cannula. Probes were perfused at a flow rate of 1.25 µL/min with an artificial cerebrospinal fluid (aCSF; pH = 7.2) containing (in mM): NaCl, 166.5; NaHCO₃, 27.5; KCl, 2.4; Na₂SO₄, 0.5; KH₂PO₄, 0.5; CaCl₂, 1.2; MgCl₂, 0.8; glucose, 1.0. The aCSF perfused through the probe in the mPFC also contained a low concentration of the acetylcholinesterase inhibitor neostigmine

bromide (0.025 µM) in order to reliably detect basal cortical ACh efflux. The probes were attached to a dual channel liquid swivel (Instech, Plymouth Meeting, PA) and perfused for 3 h before the collection of dialysates began – an interval that results in a stable basal ACh efflux that is highly (> 95%) dependent on voltage-gated Na⁺ channels (Moore *et al.*, 1992). Following the discard period, microdialysis samples were collected every 15 min

Typically, four collections were taken before drug administration in order to establish a stable level of basal efflux, and the inlet line to the accumbens probe was switched to a syringe containing the drug or vehicle solution immediately following the baseline period. A 15-min discard period was observed prior to additional dialysate collections to account for dead volume in the tubing. Drug perfusion was 90 min in duration followed by the return of the accumbens perfusate to aCSF, and three additional collections were taken following a 15-min discard period. At the end of each session, probes were removed, stylets were replaced, and the animals were returned to their home cages. The order of drug administration was randomized within each treatment group. All treatment regimes included a vehicle session (aCSF).

Drug treatments

This experiment was divided into three treatment groups as a function of the type of ionotropic glutamate receptor antagonist that was perfused into the shell region of the accumbens. In the first treatment group (*n* = 8), each subject underwent three microdialysis sessions during which the broad spectrum ionotropic glutamate receptor antagonist kynurenic acid (kynurenate, 4-hydroxyquinoline-2-carboxylic acid; RBI, Natick, MA, USA) was perfused locally into the shell of the accumbens. Kynurenate was dissolved in 200.0 µL of 0.1 N NaOH, and after adding aCSF, the solution was adjusted for pH (7.2–7.4) with 0.1 N HCl. In order to maintain consistency between vehicle and drug solutions, 200.0 µL 0.1 N NaOH was added to the aCSF vehicle solution and pH was adjusted accordingly. Kynurenate was administered at two different doses (1.0 and 5.0 mM) that were identical to doses that have previously been shown to attenuate stimulated ACh efflux when perfused through a microdialysis probe terminating in the basal forebrain (Fadel *et al.*, 2001).

In the second treatment group (*n* = 7), each subject underwent four microdialysis sessions during which the competitive non-NMDA ionotropic glutamate receptor antagonist DNQX (6,7-dinitroquinoline-2,3(1H,4H)-dione; RBI, Natick, MA, USA) was perfused through the probe located in the shell of the accumbens. DNQX was dissolved in 100.0 µL 0.1 N NaOH, and after adding aCSF, the solution was adjusted for pH (7.2–7.4) with 0.1 N HCl. In order to maintain consistency between vehicle and drug solutions, 100.0 µL 0.1 N NaOH was added to the aCSF vehicle solution and the pH was adjusted accordingly. DNQX was administered at three different doses (0.01, 0.25, and 2.5 mM), which are similar to doses that attenuate stimulated ACh efflux when administered through the microdialysis probe into the basal forebrain (Fadel *et al.*, 2001).

In the third treatment group (*n* = 7), each subject underwent four microdialysis sessions during which the competitive NMDA antagonist D-CPP (3-[2-carboxypiperazin-4-yl] propanephosphonic acid; RBI, Natick, MA, USA) was perfused through the probe located in the shell of the accumbens. D-CPP was dissolved in HPLC-grade water before dilution with aCSF. D-CPP was administered at three different doses (10.0, 100.0, and 200 µM) that are similar to doses that have been shown to effectively stimulate hippocampal ACh efflux when administered through a microdialysis probe in the septum (Giovannini *et al.*, 1997).

At the conclusion of the final dialysis session, a subset of the subjects received a 15-min perfusion through the dialysis probes of the fluoroprobe Fluoro-Gold (0.1% solution; FluoroChrome, Englewood, CO) to further visualize probe placements and to provide an approximation of the extent of the perfusion zone. Rats were perfused transcardially for subsequent histological processing (see below).

Verification of probe placement

Within 3 days of the last microdialysis session, animals were given an overdose of sodium pentobarbital and transcardially perfused with 0.2% heparin in 0.9% saline followed by 10.0% formalin. The brains were removed and stored in 10.0% formalin at 4 °C. To cryoprotect the brains they were transferred to 30.0% sucrose phosphate buffer at least 3 days prior to sectioning. To verify probe placement, several sections (45 µm) encompassing the dialysis probe placement underwent Nissl staining. Only those subjects that had both microdialysis probes placed within the target regions (mPFC and shell region of the accumbens) were included in the subsequent analyses.

Neurochemical analysis of acetylcholine

ACh in dialysates collected from the mPFC was determined by high-performance liquid chromatography with electrochemical detection. Briefly, 12 µL from each collection were injected, and ACh and choline were separated by a C-18 carbon polymer column (250 × 3 mm; ESA Inc., Chelmsford, MA, USA) using a sodium phosphate mobile phase (in mM: Na₂HPO₄, 100; TMACl, 0.5; octane-sulphonic acid, 2.0 mM; microbiocide, 0.005%; pH 8.00). To reduce the electrochemical activity generated by the solvent front and choline in order to allow the operation of the detector at a higher gain, an enzyme reactor containing choline oxidase and catalase (ESA) was installed prior to the analytical column. ACh was hydrolysed on a postcolumn enzyme reactor and converted to hydrogen peroxide (Potter *et al.*, 1983), which was detected using a peroxidase-wired glassy carbon electrode (Huang *et al.*, 1995). The detection limit for ACh under these conditions was ~5 fmol/12 µL injection.

Statistical analysis

Levels of basal ACh efflux for each subject in each dialysis session were defined as the mean of the baseline collections. In order to determine whether basal ACh efflux differed during the baseline period for each drug group, two-factor repeated measures ANOVAs (DOSE × TIME and SESSION × TIME) were conducted. Significant differences in basal collections were further analysed using paired *t*-tests.

The remainder of the statistical analyses was performed on data expressed as a percentage change from the mean baseline. A similar series of statistical analyses were conducted for each drug group. To determine the stability of basal efflux and the effects of drug administration and drug removal on ACh efflux, a two-way repeated measures ANOVA, was conducted using factors of DOSE and TIME (all collections during the microdialysis session). If Mauchly's Test of Sphericity revealed a significant difference in heterogeneity of variance, the Huynh-Feldt correction was used to adjust the degrees of freedom (Keppel, 1991). In the event of a significant effect of DOSE and/or TIME, *post-hoc* analyses (Tukey's test) were conducted to test for the significance of multiple comparisons. Finally, in order to gain further insight into the potential impact of repeated dialysis sampling on stimulated ACh efflux, means, for maximal drug doses, from animals tested on Sessions 1 or 2 were compared with means from animals similarly tested on Sessions 3 or 4 using *t*-tests for

dependent measures. In all cases, statistical significance was defined as $P = 0.05$.

Results

Microdialysis probe placements

Histological examination of microdialysis probe placements revealed that all cortical probes were in the mPFC and encompassed the shell region of the nucleus accumbens. In some cases, the accumbens probes extended into the core region, but the extent of this overlap yielded no systematic differences in the effects on basal or stimulated ACh efflux. Figure 1 depicts representative probe placements.

Effects of kynurenate on cortical ACh efflux

An initial analysis of absolute cortical ACh efflux, prior to drug perfusion, revealed a stable baseline (TIME, $F_{2,14} = 2.057$, $P = 0.165$) that did not differ as a function of the eventual dose of drug received (SESSION, $F_{2,14} = 0.746$, $P = 0.492$). Thus, subsequent expressions of the effects of the nonselective ionotropic glutamate antagonist kynurenate are expressed as a percent change from the mean (\pm SEM) basal efflux value of 0.032 ± 0.002 pmol/12 µL.

The effects of intra-accumbens perfusion of kynurenate on cortical ACh efflux, relative to the aCSF vehicle, are depicted in Fig. 2. Perfusion with aCSF had no overall effect on ACh efflux ($P > 0.1$). Kynurenate dose-dependently stimulated ACh efflux beyond basal levels as evidenced by significant effects of DOSE ($F_{2,14} = 9.840$, $P = 0.002$), TIME ($F_{12,84} = 4.298$, $P = 0.001$) and a DOSE-TIME interaction ($F_{24,168} = 5.055$, $P = 0.001$). While there was no significant overall difference in ACh efflux between the 1.0 mM kynurenate and vehicle condition ($P > 0.5$), ACh efflux, following the 5.0 mM dose of kynurenate, was increased relative to both the lower dose and the vehicle condition (both $P < 0.05$). *Post-hoc* comparisons revealed significantly greater efflux, following the higher dose (relative to the lower dose or vehicle), at collections 5–10 (all $P < 0.05$). Following termination of the kynurenate perfusion, there were no differences in cortical ACh efflux among the three groups at collections 12 and 13 (both $P > 0.5$).

Effects of the AMPA/kainate antagonist DNQX on cortical ACh efflux

An initial analysis of basal cortical ACh efflux revealed that baseline values were similar among the four doses ($P > 0.1$). There were also no significant differences among the four dialysis sessions ($P > 0.1$). There was, however, a marginally significant difference among the four baseline collections (TIME, $F_{3,18} = 4.437$, $P = 0.047$) due to a spurious increase in basal efflux during the fourth collection period. Overall, mean (\pm SEM) efflux (pmol/12 µL) for the four baseline collections was 0.035 ± 0.006 , 0.031 ± 0.003 , 0.036 ± 0.004 , and 0.042 ± 0.005 . While we do not have an explanation for this spurious increase, extracellular levels of ACh were generally stable (stability of baselines was clearly demonstrated in the present kynurenate and CPP experiments) and impulse dependent 3 h after probe insertion (Johnson & Bruno, 1995; Moore *et al.*, 1995a, b).

The effects of intra-accumbens perfusion of the AMPA/Kainate antagonist DNQX on cortical ACh efflux, relative to the aCSF vehicle, are depicted in Fig. 3. Perfusion of aCSF had no overall effect on ACh efflux ($P > 0.05$). Perfusion of DNQX resulted in an overall dose-dependent increase in ACh efflux (DOSE, $F_{3,18} = 5.046$, $P = 0.01$) and the magnitude of this effect changed over time (TIME, $F_{12,72} = 9.605$, $P = 0.001$; TIME × DOSE, $F_{36,216} = 5.875$, $P = 0.001$). Pairwise comparisons of overall drug effects revealed

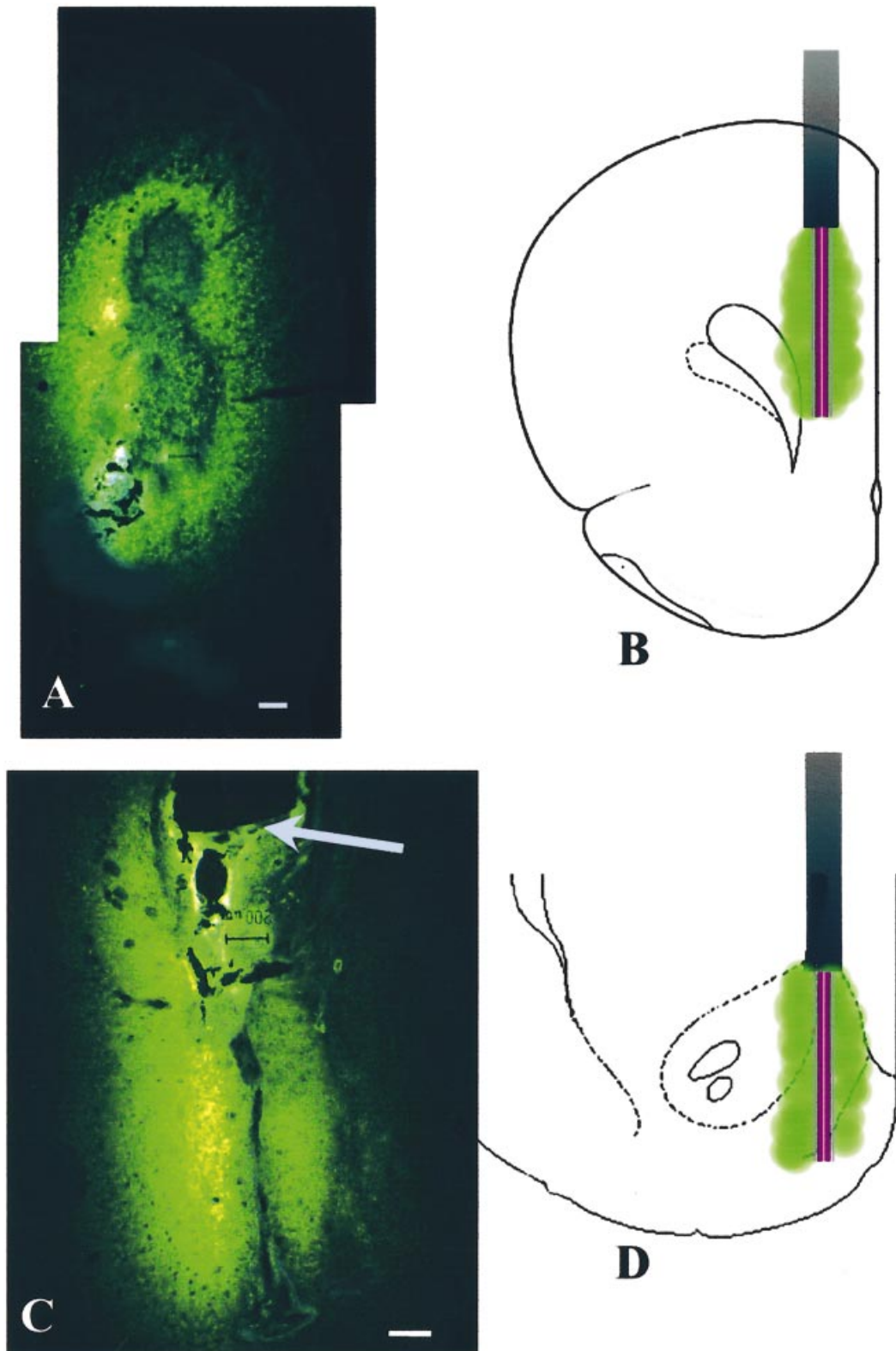


FIG. 1. Spread of Fluoro-Gold (FL) perfused through the probe at the end of the experiments (see Materials and methods for details). The spread of this fluorophore is considered to support verification of the placement of the probe and to provide an approximate estimate of the region that was dialysed. (A and C) Photomicrographs of the spread of FL in the mPFC (A) and the accumbens (C; 200- μ m scales inserted). (B and D) Schematic illustrations of the placement of the guide and probe and of the spread of FL. In the mPFC, the spread of FL was mostly restricted to the pre- and infra-limbic areas, and to the ventral parts of the cingulate cortex. In the accumbens, FL was mostly concentrated in the shell and extended into the medial third of the core. In panel C, the tract produced by the probe is clearly visible, as is the tip of the guide (see arrow).

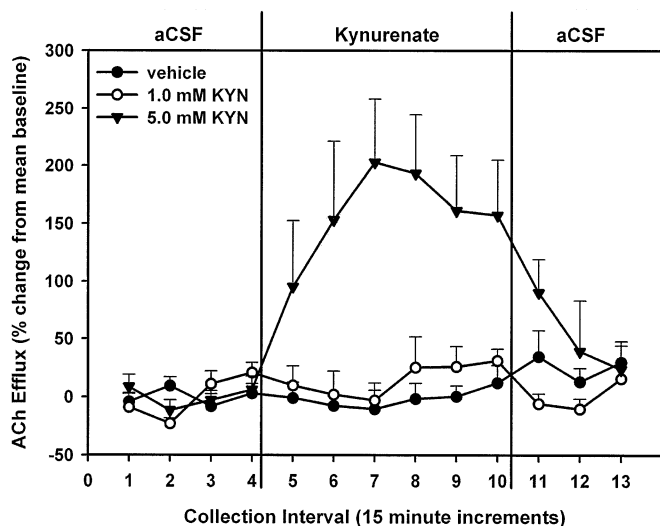


FIG. 2. ACh efflux from medial prefrontal cortex following the perfusion of vehicle (aCSF) or the nonselective ionotropic glutamate antagonist kynurenatate into the shell region of the nucleus accumbens. The order of the three doses was counterbalanced across animals ($n = 8$). ACh efflux (mean \pm SEM) is expressed as a percent change from the mean baseline and was collected in 15-min intervals with the first four collections representing baseline efflux. Immediately following the fourth baseline collection, the intra-NAC perfusion of kynurenatate (KYN) began. A 15-min discard period was observed to account for dead volume in the lines and then collections were resumed. Following 90 min of drug perfusion, immediately after the tenth collection, the control perfusion of aCSF was resumed. Again, a 15-min discard period was observed prior to the final 45 min of collection. ACh efflux was significantly elevated during the perfusion of the higher dose (5.0 nM) relative to both the lower dose (1.0 nM) and the vehicle session.

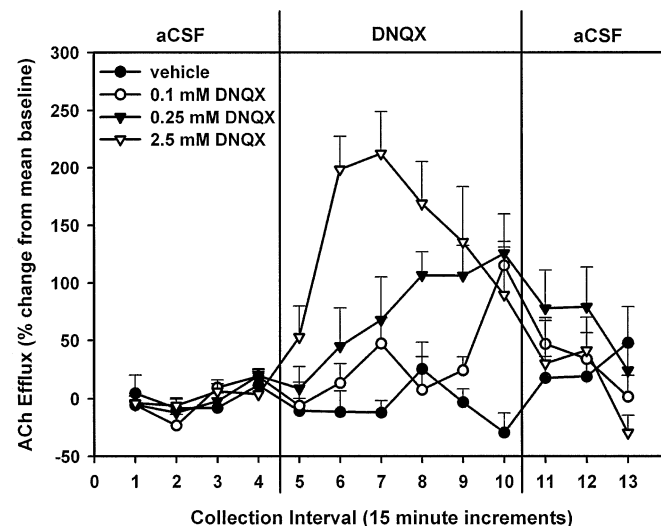


FIG. 3. ACh efflux from medial prefrontal cortex following the perfusion of vehicle (aCSF) or the AMPA/kainate antagonist DNQX into the shell region of the nucleus accumbens. The order of the four doses was counterbalanced across animals ($n = 7$). ACh efflux (mean \pm SEM) is expressed as a percent change from the mean baseline and was collected in 15-min intervals with the first four collections representing baseline efflux. Immediately following the fourth baseline collection, the intra-NAC perfusion of DNQX began. A 15-min discard period was observed to account for dead volume in the lines and then collections were resumed. Following 90 min of drug perfusion, immediately after the tenth collection, the control perfusion of aCSF was resumed. Again, a 15-min discard period was observed prior to the final 45 min of collection. Stimulation of ACh efflux differed following perfusion of the 0.25 and 2.5 mM doses of DNQX and the effects following either dose were greater than that seen following the lowest dose (0.1 mM) and the vehicle session.

that the highest dose of DNQX (2.5 mM) was significantly greater than the vehicle condition ($P < 0.05$) with all other comparisons not being significant.

Post-hoc analyses revealed that ACh efflux following 2.5 mM DNQX was significantly higher than all other doses beginning ~30 min after perfusion of the drug (collection 6) and continuing through collection 8 (all $P < 0.01$). By collection 10, ACh efflux following each dose of DNQX was significantly greater than that seen during perfusion of the aCSF vehicle (all $P < 0.01$) although they were not different amongst themselves. Finally, upon termination of drug perfusion (collection 11), there were no significant group differences in cortical ACh efflux as they returned back to baseline values.

Effects of the NMDA antagonist D-CPP on cortical ACh efflux

There were no differences in overall basal cortical ACh efflux across the four baseline collection intervals ($P > 0.05$). Baseline efflux did, however, differ among the four dialysis sessions ($F_{3,18} = 7.506$, $P = 0.007$). Mean (\pm SEM) basal efflux (pmol/12 μ L) for the four sessions was 0.053 ± 0.0043 , 0.0035 ± 0.00373 , 0.0023 ± 0.0043 , and 0.0023 ± 0.0040 . *Post-hoc* analyses revealed that the significant effect of the session was due to the relatively high basal efflux in the first session as compared to each of the subsequent three sessions (all $P < 0.05$). As the assignments of D-CPP doses (0, 10, 100, and 200 μ M) were completely counterbalanced across dialysis sessions, it is extremely unlikely that differences in basal ACh efflux in one out of the four sessions could significantly contribute to the systematic, dose-related effects described below.

The effects of intra-accumbens perfusion of D-CPP or its vehicle on cortical ACh efflux are illustrated in Fig. 4. Perfusion of aCSF had no effect on ACh efflux ($P > 0.05$). In contrast, perfusion of D-CPP resulted in a marked stimulation of ACh efflux beyond basal levels as evidenced by significant overall effects of DOSE ($F_{3,18} = 11.121$, $P = 0.001$), TIME ($F_{12,72} = 29.398$, $P = 0.001$) and a DOSE-TIME interaction ($F_{36,216} = 4.986$, $P = 0.01$). Subsequent comparisons revealed that overall ACh efflux following perfusion with the highest dose of D-CPP (200 mM) resulted in higher values than either of the other two doses or aCSF vehicle (all $P < 0.05$). *Post-hoc* analyses of dose effects at various collection intervals revealed that ACh efflux following 200 mM D-CPP was higher than all other conditions on collections 6–13 (all $P < 0.05$). ACh efflux following the intermediate dose (100 mM) was greater than that seen in the vehicle control group during collections 9–10 (both $P < 0.05$). Interestingly, cortical ACh efflux in the group that received the 200 mM dose of D-CPP remained elevated, relative to all other groups, even after the drug was discontinued immediately after collection 10 (all $P < 0.05$).

The effects of repeated microdialysis perfusions on stimulated ACh efflux

Finally, in an effort to further validate the use of the repeated microdialysis perfusion testing used in these experiments, we conducted two *post-hoc* analyses on the impact of this procedure on the ability of the AMPA/kainate and NMDA antagonists to stimulate cortical ACh efflux. In order to increase the statistical power of these analyses the stimulated efflux data from dialysis sessions 1 and 2 were combined and compared, using a *t*-test for dependent means, with values of stimulated efflux from dialysis

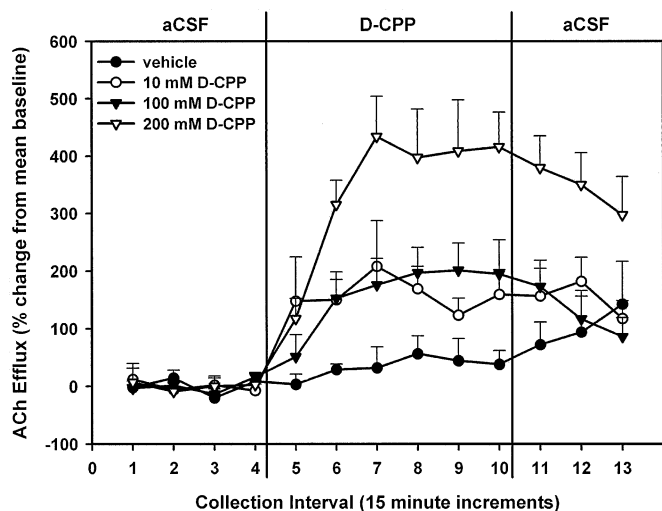


FIG. 4. ACh efflux from medial prefrontal cortex following the perfusion of vehicle (aCSF) or the NMDA antagonist D-CPP into the shell region of the nucleus accumbens. The order of the four doses was counterbalanced across animals ($n = 7$). ACh efflux (mean \pm SEM) is expressed as a percent change from the mean baseline and was collected in 15-min intervals with the first four collections representing baseline efflux. Immediately following the fourth baseline collection, the intra-NAC perfusion of DNQX began. A 15-min discard period was observed to account for dead volume in the lines and then collections were resumed. Following 90 min of drug perfusion, immediately after the tenth collection, the control perfusion of aCSF was resumed. Again, a 15-min discard period was observed prior to the final 45 min of collection. ACh efflux was significantly elevated, relative to vehicle, during the perfusion of the two highest doses of D-CPP. ACh efflux was higher following 200 μ M than following 100 μ M.

sessions 3 and 4. We limited this analysis to the highest doses of each antagonist. With respect to the AMPA/Kainate antagonist DNQX, the mean increase of $225 \pm 125\%$ from ACh baseline from animals treated with 2.5 mM on either session 1 or 2 did not differ significantly from the $180 \pm 72\%$ increase seen in animals similarly treated on either session 3 or 4 ($t_5 = 1.01$, $P = 0.359$). Likewise, with respect to the NMDA antagonist D-CPP, the mean increase of $411 \pm 189\%$ from ACh baseline from animals treated with 200 μ M on either session 1 or 2 did not differ significantly from the $450 \pm 210\%$ increase seen in animals similarly treated on either session 3 or 4 ($t_5 = -0.258$, $P = 0.807$).

Discussion

The results of these experiments demonstrate that antagonism of ionotropic glutamate receptors in the shell region of the nucleus accumbens results in a marked and long-lasting increase in ACh release in the ipsilateral mPFC. This effect was dose-dependent and was seen following blockade of either AMPA/kainate or NMDA receptors, although the magnitude and duration of the stimulated ACh efflux appeared to be larger following the NMDA antagonist. The following discussion will address two issues related to these findings, firstly, the mechanisms that might underlie the trans-synaptic activation of the basal forebrain cholinergic system following antagonism of ionotropic glutamate receptors in the accumbens and secondly, the potential relationships between changes in accumbens ionotropic glutamate receptor activity, cortical ACh release and attentional processes.

Medium spiny GABAergic projections from the accumbens shell to the basal forebrain are contacted by glutamatergic afferents

(Meredith, 1999) and contain ionotropic NMDA and AMPA/kainate glutamate receptors (Lu *et al.*, 1999) as well as metabotropic glutamate receptor types mGluR 1,3,4,5 (Testa *et al.*, 1994; Lu *et al.*, 1999). The working model used here for the trans-synaptic regulation of the basal forebrain cortical cholinergic system, contends that GABAergic projections from the shell region of the accumbens to the basal forebrain (Zaborszky & Cullinan, 1992; Zahm & Heimer, 1993) play a key role in modulating the excitability of corticopetal cholinergic projections. Whether this contact is directly onto corticopetal cholinergic neurons or is indirect via interneurons is still unresolved (see Zahm *et al.*, 1999). However, several observations reveal that the level of GABA_A receptor activity in basal forebrain can markedly influence cortical ACh efflux in awake rats. Administration of benzodiazepine receptor ligands, that either positively or negatively modulate GABA_A receptors, into the basal forebrain either attenuate or potentiate, respectively, cortical ACh efflux following presentation of an activating environmental stimulus (Moore *et al.*, 1995a). Similarly, intrabasalis perfusion of the BZR agonist CDP, a positive modulator of GABA_A receptors, attenuates the increases in cortical ACh efflux seen following systemic administration of amphetamine (Arnold *et al.*, 2000).

Collectively, these results demonstrate a role for the accumbens in modulating the activity of the basal forebrain cortical cholinergic system. The authors are currently in the process of more directly testing the hypothesis that decreases in basal forebrain GABA release mediate the effects of intra-accumbens ionotropic receptor blockade by administering NMDA and/or AMPA antagonists into the accumbens shell and determining whether there is a reduction in GABA efflux in basal forebrain. An additional test of this hypothesis predicts that the ability of ionotropic glutamate receptor antagonists to stimulate cortical ACh efflux will be antagonized or blocked with intrabasalis perfusion of GABA_A receptor agonists or positive modulators. Finally, it should be acknowledged that NAC efferents may ultimately impact on cortical cholinergic transmission via circuits involving ventral pallidum-thalamo-cortical projections. The present experiment did not attempt to identify the relative contributions between this pathway and that of the more direct basal forebrain corticopetal cholinergic projections.

The fact that competitive antagonists to ionotropic glutamate receptors in the accumbens were so effective in stimulating basal cortical ACh efflux in animals that were relatively inactive (i.e. lying still) is unexpected. This observation suggests activity within excitatory afferents to the accumbens and the resultant glutamate receptor activity is significant even under these apparently restful conditions.

Another somewhat unexpected aspect of these data is the time-course of the stimulated ACh efflux. In the case of each antagonist, cortical ACh efflux did not rise beyond basal values until the drugs had been perfused into the accumbens for at least 30 mins. This delay may simply reflect the time necessary for antagonists to reach threshold concentrations and to diffuse and come into contact with sufficient numbers of glutamate receptors. At the same time, this delayed onset may provide some preliminary insights into the signalling mechanisms underlying the effect, suggesting a role for transduction events that occur more slowly than simply changes in the activity of ionotropic glutamatergic (in accumbens) and GABAergic (in basal forebrain) receptors. The authors are currently testing the hypothesis that changes in metabotropic glutamate receptor activity within the accumbens plays a role in the ability of ionotropic glutamate receptor antagonists to stimulate cortical ACh efflux. There are suggestions, at the neuronal level, of reciprocal functional relations between ionotropic and metabotropic glutamate

receptors in the accumbens (Hu & White, 1996; Martin *et al.*, 1997). It is conceivable that in the current study the perfusion of ionotropic antagonists resulted in a shift in balance toward a slower evolving, more enduring metabotropic-mediated inhibition of GABA release in basal forebrain and that this led to an increase in the excitability of the basal forebrain cortical cholinergic system and, hence, in levels of extracellular ACh.

It is also noteworthy that cortical ACh efflux remained markedly elevated following perfusion with the highest dose of D-CPP (200 μ M) even 45 min after the drug perfusion was terminated. While the mechanism underlying this unanticipated finding remains unknown, it suggests that there may be long-lasting, multisynaptic modulatory changes as a result of the perfusion of this dose of the NMDA antagonist.

The present experiments did not address the source of the glutamatergic inputs into the accumbens shell that were antagonized following the perfusion of kynurenate, D-CPP, and DNQX. The accumbens receives glutamatergic afferents from the cortex (Groenewegen *et al.*, 1982; Sesack *et al.*, 1989), ventral hippocampus (Kelley & Domesick, 1982; Groenewegen *et al.*, 1987) and the basolateral amygdala (Robinson & Beart, 1988; Kita & Kitai, 1990). While these experiments produced a rather global reduction in excitatory transmission within NAC, the marked and persistent effects of ionotropic glutamate receptor antagonists indicate a significant degree of excitatory accumbens afferent activity even in animals that had been well-habituated to the testing room and consequent handling associated with the dialysis session.

The ability of ionotropic glutamate receptor antagonists to stimulate cortical ACh efflux raises the issue of the role of glutamatergic transmission in the accumbens under behavioural conditions known to activate the basal forebrain cortical cholinergic system. Activation of this system, as evidenced by an increase in cortical ACh efflux, accompanies performance in a sustained attention task (Himmelheber *et al.*, 2000, 2001; Arnold *et al.*, 2002) and in the five-choice serial reaction time task that measures visual attention (Dalley *et al.*, 2001; Passetti *et al.*, 2000), during the early stages of acquisition of a conditioned discrimination (Orsetti *et al.*, 1996), and following exposure to novelty (Giovannini *et al.*, 2001; Neigh *et al.*, 2001). Unfortunately, there are no available data on the release of glutamate within the accumbens during exposure to any of these situations. While there are numerous studies implicating a crucial role for accumbens glutamatergic transmission in conditioned responding (Burns *et al.*, 1994; Smith-Roe & Kelley, 2000), reinstatement of self-administration of drugs (Cornish & Kalivas, 2000), stimulant-induced motility (Kim & Vezina, 1997) and novelty-induced changes in accumbens DA efflux (Legault & Wise, 2001), speculations about whether excitatory afferents into the accumbens are activated or not, and the source of these inputs (i.e. cortical, hippocampal, amygdalar), during conditions known to activate the basal forebrain cortical cholinergic system, await the results of additional experiments.

In conclusion, the results of the present studies indicate that antagonism of AMPA/kainate or NMDA receptors within the nucleus accumbens results in an activation of the basal forebrain cortical cholinergic system as evidenced by marked and persistent elevations of cortical ACh efflux. While it is premature to speculate that the local administration of ionotropic glutamate receptor antagonists models more transient reductions in glutamatergic afferent activity that may occur under conditions of active processing of behaviourally relevant stimuli, these data are consistent with previous demonstrations of a role for the accumbens in the trans-synaptic modulation of the basal forebrain cortical cholinergic system (see Sarter & Bruno,

1999; Sarter *et al.*, 2001; for reviews). This modulation may provide a neuronal mechanism for motivational variables to bias arousal and attention for the processing of biologically meaningful stimuli. Furthermore, pathological regulation of this modulation, for example in drug addiction or schizophrenia, may abnormally bias the subject to allocate attentional resources to selected stimuli thereby reducing the subject's potential to engage in alternative behavioural or cognitive activities. Thus, abnormal regulation of cortical cholinergic inputs, through alterations in accumbens efferent activity, may explain, in part, the cognitive processes underlying compulsive drug use or schizophrenic symptoms.

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Abbreviations

ACh, acetylcholine; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; BZR, benzodiazepine receptor; DA, dopamine; mPFC, medial prefrontal cortex; NAC, nucleus accumbens; nBM, nucleus basalis Meynert; SIO, substantia innominata.

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