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Research report

Amphetamine-stimulated cortical acetylcholine release: role of the basal forebrain

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Abstract

Systemic administration of amphetamine results in increases in the release of acetylcholine in the cortex. Basal forebrain mediation of this effect was examined in three experiments using microdialysis in freely-moving rats. Experiment 1 examined whether dopamine receptor activity within the basal forebrain was necessary for amphetamine-induced increase in cortical acetylcholine by examining whether intra-basalis perfusion of dopamine antagonists attenuates this increase. Systemic administration of 2.0 mg/kg amphetamine increased dopamine efflux within the basal forebrain nearly 700% above basal levels. However, the increase in cortical acetylcholine efflux following amphetamine administration was unaffected by intra-basalis perfusions of high concentrations of D1- (100 μM SCH 23390) or D2-like (100 μM sulpiride) dopamine receptor antagonists. Experiments 2 and 3 determined whether glutamatergic or GABAergic local modulation of the excitability of the basal forebrain cholinergic neurons influences the ability of systemic amphetamine to increase cortical acetylcholine efflux. In Experiment 2, perfusion of kynurenate (1.0 mM), a non-selective glutamate receptor antagonist, into the basal forebrain attenuated the increase in cortical acetylcholine produced by amphetamine. Experiment 3 revealed that positive modulation of GABAergic transmission by bilateral intra-basalis infusion of the benzodiazepine receptor agonist chlordiazepoxide (40 μg/hemisphere) also attenuated the amphetamine-stimulated increase in cortical acetylcholine efflux. These data suggest that amphetamine increases cortical acetylcholine release via a complex neuronal network rather than simply increasing basal forebrain D1 or D2 receptor activity.

Theme: Neurotransmitters, modulators, transporters, and receptors

Topic: Interactions between neurotransmitters

Keywords: Acetylcholine; Dopamine; Basal forebrain; Microdialysis; Prefrontal cortex

1. Introduction

Cholinergic neurons located in the basal forebrain project to the cortex and mediate fundamental aspects of cognitive functions. Specifically, these neurons are involved in the mediation of attentional functions such as the detection, selection, and processing of environmental stimuli (for reviews see [9,44]). Consequently, aberrations in the functioning of these basal forebrain corticopetal cholinergic neurons, and in the afferent regulation of their excitability, have been implicated in the manifestation of the cognitive symptoms of several major neuropsychiatric disorders, including schizophrenia, dementia, and compulsive drug use [42].

The cell bodies of the corticopetal cholinergic neurons are diffusely located in the basal forebrain and appear to receive a substantial innervation by GABAergic projections from the shell territory of the nucleus accumbens (NAC) [51]. These basal forebrain cholinergic neurons are believed to be trans-synaptically regulated (at least in part) by this projection from the NAC [50]. Anatomical and neuropharmacological evidence has given rise to the hypothesis that NAC dopamine (DA) receptor stimulation reduces GABAergic inhibition of basal forebrain neurons, including the corticopetal cholinergic projections [2,11,46,50,51]. Consistent with this hypothesis, increases in cortical acetylcholine (ACh) release produced by the systemic administration of a benzodiazepine receptor

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(BZR) partial inverse agonist, FG 7142 (a negative GABA modulator), are attenuated by the blockade of D2 receptors in the NAC [30].

Several studies have shown that systemically-administered psychostimulants activate basal forebrain corticopetal cholinergic neurons, reflected by an increase in ACh release in the cortex [1,5,7,18]. Several lines of evidence indirectly suggest that the increase in cortical ACh release produced by amphetamine is mediated via dopaminergic projections to the NAC. First, application of amphetamine directly into the cortex at low to moderate doses is not sufficient to increase cortical ACh release, suggesting that a local effect within the cortex is insufficient to account for the drug's actions when systemically administered [7,17]. Second, forebrain dopamine (DA)-depleting lesions, including, but not limited to the NAC, partially attenuate the ability of systemically administered amphetamine to increase cortical ACh release [8]. However, recent experiments designed to directly test the involvement of NAC DA receptor activity on the trans-synaptic modulation of amphetamine-induced cortical ACh release reveal that increased DA receptor activity in the NAC per se is neither necessary, nor sufficient, to account for the increase in cortical ACh release produced by systemic amphetamine.

The goal of the present series of experiments was to further characterize the neural mechanisms involved in the increase in cortical ACh release following systemic amphetamine administration. Systemic amphetamine may modulate the excitability of cortical cholinergic neurons more directly, possibly through dopaminergic projections from the ventral tegmental area to the ventral pallidum and substantia innominata [26,35]. Although the precise localization of DA receptors in the basal forebrain is still unclear, their presence has been suggested both anatomically [23,45,52,53] and electrophysiologically [22,28,34,35] and several recent studies have suggested that the basal forebrain plays a role in psychostimulant-induced locomotion and reward [14,19,21,41].

Thus, the aim of the first experiment was to examine whether extracellular DA levels in the basal forebrain increase following systemic amphetamine administration and if so, whether DA receptor activity within the basal forebrain is necessary for the drug-induced increase in cortical ACh release. In this experiment we utilized two microdialysis probes, one to perfuse either a D1 or D2 receptor antagonist directly into the nucleus basalis magnocellularis and substantia innominata (nBM/SI), the region of the basal forebrain that is believed to contain both DA receptors and cholinergic cell bodies [35,52,53]. The other microdialysis probe was used to measure cortical ACh release in the dorsal medial prefrontal cortex (mPFC), an area linked to attentional processing [9] and a terminal region of corticopetal cholinergic neurons [55]. If DA receptor activity in the nBM/SI is necessary for systemic amphetamine's effects on cortical ACh release, then a reduction in receptor activity by administration of DA antagonists prior to amphetamine administration should result in an attenuation of the increase in cortical ACh release produce by amphetamine. We have shown previously that a complex behavioral stimulus (sudden exposure to darkness coupled with the opportunity to consume a palatable food) results in a transient increase in cortical ACh release [1,29,31,32]. To determine if amphetamine has differential effects on cortical ACh release depending on the level of afferent stimulation in the basal forebrain, this stimulus was employed to characterize the potential of amphetamine to modulate behaviorally-stimulated cortical ACh release.

It has been proposed that the role of DA within the basal forebrain is to modulate the signal-to-noise ratio of fast acting excitatory and inhibitory amino acid neurotransmitters [22]. Such a view of the role of DA in the basal forebrain implies that in order for systemic amphetamine to result in an increase in cortical ACh release there must be an increase in excitatory input and/or a reduction of inhibitory input to the basal forebrain. Recently, Fadel et al. [10] demonstrated that behaviorally-stimulated increases in cortical ACh release can be blocked by intra-basalis administration of the non-selective ionotropic glutamate receptor antagonist, kynurenate. Similarly, bilateral intra-basalis infusion of chlordiazepoxide (CDP), a benzodiazepine receptor agonist that facilitates GABAergic inhibition, also was shown to attenuate behaviorally-induced increase in cortical ACh release [29,31,32].

The aim of Experiments 2 and 3 was to determine whether local modulation of the excitability of the nBM/SI neurons could influence the ability of systemic amphetamine to increase cortical ACh release. The excitability of corticopetal cholinergic neurons in the nBM/SI was attenuated in two ways prior to the administration of systemic amphetamine. First, in Experiment 2, using dual microdialysis probes, the effect of unilateral intra-basalis application of kynurenate on the amphetamine-stimulated increase in cortical ACh release was assessed. In Experiment 3, GABAergic transmission in the basal forebrain was amplified through bilateral intra-basalis infusions of CDP prior to systemic administration of amphetamine.

2. Materials and methods

2.1. Subjects

Adult male Fisher-344/Brown Norway F1 hybrid rats (Harlan Sprague–Dawley, Indianapolis, IN), weighing between 250 and 350 g, served as subjects in this experiment. Animals were allowed access to food and water ad libitum and were housed in a temperature- and humidity-controlled colony room kept on a 12:12 light-:dark cycle (lights on at 6:30 a.m.). Prior to guide cannula implantation, animals were housed in pairs in stainless steel hanging cages. On the day prior to surgery, animals

were moved to individual plastic cages (50×23×20 cm; l×w×h) with pine shavings where they were housed for the duration of the experiment. All animal care and experiments were performed in accordance with protocols approved by the University Institutional Laboratory Animal Care and Use Committee of Ohio State University and were consistent with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Guide cannula surgery

Animals were anesthetized with ketamine (100.0 mg/kg, i.p.) and xylazine (3.0 mg/kg, i.p.) prior to stereotaxic surgery. Two stainless steel microdialysis guide cannula were implanted (0.72 mm o.d.), one into the mPFC and the other directed towards the ipsilateral nBM/SI. To place the guide cannula into the mPFC the carrier arm of the stereotaxic apparatus was angled 12° away from vertical towards posterior and the cannula was positioned 3.5 mm anterior to bregma, 0.8 mm lateral to the midline, and 1.0 mm below dura mater (all coordinates according to the atlas of Paxinos and Watson [37]). For placement into the ipsilateral nBM/SI, the carrier arm was angled 15° away from vertical towards anterior and the cannula was positioned 2.1 mm posterior to bregma and 2.6 mm lateral of the midline, and 6.0 mm below dura. In Experiment 3, rather than a microdialysis probe in the nBM/SI, bilateral infusion guide cannula were implanted. These cannula were positioned 0.5 mm posterior to bregma and 3.2 mm lateral to the midline. A hole was drilled in the skull and the cannula were lowered 6.0 mm below dura at a 4° angle towards medial. Following surgery animals were allowed to recover in their home cages for 3 days prior to the first dialysis session.

2.3. Habituation and microdialysis

For Experiments 1 and 2, on each of the 10 days immediately prior to implantation of guide cannula, animals were habituated to concentric dialysis bowls (35×38 cm; h×d; CMA, Stockholm, Sweden) for 6–7 h each day. Between the fourth and fifth hour in the bowl, the animals were exposed to sudden darkness by turning off the room lights (the experimenter was provided illumination with a 60 W red light bulb). Immediately after the lights were extinguished, rats were presented with a single piece of sweetened cereal (Fruit Loop, Kellogg's, Inc., Battle Creek, MI). After several days of this training (referred to as the 'darkness/cereal' stimulus) animals became active immediately after lights were extinguished and rapidly approached and consumed the cereal, typically within 30–60 s. This stimulus has been repeatedly shown to result in a transient (5–10 min) activation of cortical ACh release that quickly returns to basal levels [1,29,31,32].

Microdialysis was conducted using a repeated perfusion paradigm in which rats received a different pharmacological treatment each session, with an 'off day' between each microdialysis session. This repeated perfusion paradigm allows the assessment of the effects of multiple treatments, including control conditions, in the same animal. This procedure has been validated for measurement of cortical ACh efflux [33] as well as for striatal ACh efflux [22] and striato-nigral GABA efflux [3] by showing that neither basal release nor drug effects interact significantly with the order of the dialysis sessions. On each microdialysis day, animals were placed in the testing chambers 30 min prior to the insertion of concentric dialysis probes (0.35 mm o.d., 2.0 mm membrane length; Bioanalytical Systems, W. Lafayette, IN) through the guide cannula. Each probe was perfused at 1.25 μl/min with artificial CSF (aCSF; pH=7.0) containing the following (in mM): NaCl, 166.5; $NaHCO_3$, 27.5; KCl, 2.4; Na_2SO_4, 0.5; KH_2PO_4, 0.5; $CaCl_2$, 1.2; $MgCl_2$, 0.8 and glucose, 1.0. The probes were attached to a dual channel liquid swivel (Instech, Plymouth Meeting, PA) and perfused for 3 h before collection of dialysate began — an interval that results in stable basal ACh efflux that is highly (>95%) dependent on axonal depolarization [29]. The aCSF perfused through the probe implanted in the mPFC contained the acetylcholinesterase inhibitor neostigmine bromide (0.05 μM) to promote recovery of detectable basal levels of ACh. The vials collecting dialysate from the probe in the nBM/SI contained 5.0 μl of a perchloric acid (0.05 N) solution containing sodium bisulfite (200 μM) and EDTA (1.0 mM) as an antioxidant to minimize the degradation of dopamine in these collections. Data were not corrected for individual probe recovery; probe recovery values averaged 15% for ACh and 8% for dopamine.

2.4. Drugs

Systemic injections of the indirect dopaminergic agonist D-amphetamine sulfate (2.0 mg/kg, i.p.; Sigma, St Louis, MO) were delivered in 0.9% sterile saline. This same dose of amphetamine has been shown to reliably increase cortical ACh efflux [1,7,8] as well as to increase behavioral activity [2,24]. In Experiment 1, the D1-like antagonist SCH 23390 (100 μM) or the D2-like antagonist l-sulpiride (100 μM) (RBI, Natick, MA, Sigma Chemical, St Louis, MO, respectively) were administered in aCSF vehicle by perfusion through the microdialysis probe located in the nBM/SI. At this dose, each antagonist delivered into the shell region of the NAC, has been shown to attenuate the increase in cortical ACh efflux produced by systemic administration of a BZR partial inverse agonist [30]. In Experiment 2, kynurenate (1.0 mM), the non-selective ionotropic glutamate receptor antagonist (Sigma Chemical), was perfused through the dialysis probe in aCSF vehicle. This dose of kynurenate, perfused within the basal forebrain, has been shown to attenuate the increase in cortical ACh efflux resulting from stimulation of the pedunculopontine tegmentum (PPT) in anesthetized

rats [39] as well as increases in cortical ACh produced by a behavioral stimulus [10]. In Experiment 3, chlordiazepoxide (CDP, Sigma Chemical, St Louis, MO) was dissolved in 0.9% sterile saline and delivered bilaterally (40 μg/hemisphere) through internal infusion cannula (30-gauge stainless steel; Plastics One, Roanoke, VA) placed in the nBM/SI. Intra-basalis injections were administered in 0.5 μl delivered over 60 s followed by another 60 s with the infusion cannula in place to allow for diffusion away from the infusion site. This dose of CDP (40 μg/hemisphere) has previously been demonstrated to reduce behaviorally-stimulated cortical ACh release while having minimal effects on basal release of ACh [32] (see Section 2.5.3. for justification of infusions).

2.5. Experimental procedure

2.5.1. Experiment 1: Intra-basalis perfusion of D1 or D2 dopamine antagonists

Each subject ($n=6$) received three counterbalanced microdialysis sessions in which drugs were perfused through the probe located in the nBM/SI. In one session subjects were presented with only aCSF as a vehicle control. Another session consisted of intra-basalis administration of the D1 antagonist SCH 23390 (100 μM) and a third session consisted of intra-basalis administration of the D2 antagonist sulpiride (100 μM). During each session, a systemic injection (i.p.) of amphetamine (2.0 mg/kg) was administered 30 min following the introduction of the intra-basalis drug perfusions. To introduce drug solutions to the probe, the inlet line to the probe was switched to a syringe containing the drug (or aCSF) following the baseline period where it remained for the duration of the session. Dialysates were collected for an additional 30 min after the systemic injection of amphetamine. At the end of this 30 min period, room lights were extinguished and the darkness/cereal stimulus was presented. Dialysates were collected for an additional 60 min while the room lights remained off.

2.5.2. Experiment 2: Intra-basalis perfusion of the glutamate antagonist kynurenate

In this experiment, subjects ($n=8$) participated in four counterbalanced microdialysis sessions. The results of two of these sessions are reported elsewhere [10]. These rats had experienced the darkness/cereal stimulus on previous days and microdialysis sessions, however, the stimulus was not employed in the two sessions reported here. To maintain consistency with the other experiments reported by Fadel et al. [10] the current experiment employed a higher level of neostigmine (0.50 μM) than that used in either Experiment 1 or 3 of the current manuscript (0.05 μM). Following the last baseline collection in each of the two sessions reported here, the inlet to the nBM/SI probe was switched to either a syringe containing aCSF or a syringe containing kynurenate (1.0 mM). After the first 45 min (three collections) animals were given a systemic injection (i.p.) of 2.0 mg/kg amphetamine and another three collections were taken.

2.5.3. Experiment 3: Intra-basalis infusion of the benzodiazepine receptor agonist chlordiazepoxide

In the final experiment, subjects ($n=7$) that were implanted with a microdialysis probe in the cortex and bilateral infusion guide cannula in the nBM/SI participated in three counterbalanced microdialysis sessions. Bilateral infusion cannula, rather than a unilateral microdialysis probe, were used in this experiment in order to deliver CDP to the basal forebrain. While this represents a departure from the drug delivery method used for Experiments 1 and 2, this approach was selected because CDP infused as a bolus dose of 40 μg/hemisphere is known to attenuate behaviorally-stimulated cortical ACh release without affecting basal release of ACh [32]. This repeated intra-basalis drug infusion method has been frequently used in behavioral experiments and it does not result in any significant degeneration of neurons within the infusion sphere [47,48].

Following the last baseline collection in each session, internal infusion cannula were fixed into the bilateral guide cannula in the nBM/SI. After the internal cannula were inserted and attached to infusion tubing, collections from the cortical probe were taken for 30 min (two collections). Immediately following these two collections, animals were infused with 0.5 μl over 60 s with either 0.9% saline (one session) or 40 μg/hemisphere CDP (two sessions) at a flow rate of 0.5 μl per min. Internal infusion cannula were then removed, stylets were replaced and animals were immediately given a systemic injection. In one session following the infusion of saline, and in one session following the infusion of CDP, animals were given an injection (i.p.) of 2.0 mg/kg amphetamine. In a third session, following CDP infusion, animals were given an injection of 0.9% saline (1 ml/kg, i.p.) in order to ascertain the effects of CDP on basal ACh release. Four collections were taken following the systemic injection.

2.6. Neurochemical analyses

Acetylcholine levels in dialysates collected from the mPFC were determined by high-performance liquid chromatography with electrochemical detection (HPLC–ED). 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) using a sodium diphosphate mobile phase (100 mM Na_2HPO_4, 5 mM TMACI, 2.0 mM 1-octanesulfonic acid, pH=8.0). ACh was hydrolyzed on a post-column enzyme reactor (ESA, Inc.) and converted to hydrogen peroxide [38] that was detected using a 'peroxidase-wired' [20] ceramic glassy carbon electrode (ESA, Inc.) with the potential set

at −200 mV. The detection limit for ACh under these conditions was approximately 10 fmol/10 μl injection.

Dopamine levels in dialysate collected from the nBM/SI were also determined using HPLC–ED. From each sample 15 μl was injected and DA was detected via a dual electrode coulometric detector (ESA, Inc.) with the potential for the first electrode set at −175.0 mV and for the second electrode set at 175.0 mV. Dopamine was separated on a C-18 carbon polymer column (80×4.6 mm; HR-80, ESA, Inc.) using a sodium phosphate mobile phase (75 mM NaH_2PO_4, 2.0 mM octanesulfonic acid, 25 μM EDTA, 100 μl TEA, and 18.0% methanol, pH 5.6). The detection limit for DA was approximately 0.5 fmol/10 μl injection.

2.7. Histology

Following 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% formalin. Brains were stored in 10% formalin at 4°C for at least 24 h and then transferred to 30% sucrose phosphate buffer until sectioning at least 3 days later. Histological verification of dialysis probe and infusion cannula placement was made using 50 μm Cresyl Violet-stained sections. Fig. 1 depicts the placement of microdialysis probes (or infusion cannula) in mPFC and the nBM/SI from animals in Experiment 1 and Experiment 3 (placements for Experiment 2 were comparable to those of Experiment 1). For microdialysis probes, at least two-thirds of the dialysis membrane had to be located in the mPFC or nBM/SI for the placement to be accepted. Animals with unacceptable placements were dropped from further analysis.

2.8. Statistical analyses

To determine the stability of basal efflux, as well as whether basal ACh or DA efflux changed over repeated dialysis sessions, baseline collections for each session were compared using two-factor repeated measures ANOVA (Session×Time), collapsed across subsequent drug treatments. Baseline collections were also compared by collapsing across session order to confirm that basal efflux did not differ by drug treatment (Drug×Time). All of these data were expressed in picomoles (pmol)/12 μl sample for ACh and as femtomoles (fmol)/15 μl sample for DA. Demonstrating that the basal efflux for each treatment does not differ across drug treatment sessions permits expression of the subsequent data as a percent change from basal levels. For each subject, the mean of the four baseline collections was calculated for each session and the remainder of the statistical analyses were performed on data expressed as a percent change from the mean baseline.

In Experiment 3, two-way repeated measures ANOVA were used to determine if there were differences across session and drug treatments by comparing three collections: the final baseline collection, the collection after infusion cannula were installed, and the collection preceding the actual infusion and systemic injection.

In Experiments 1 and 2, in order to determine whether the intra-basalis perfusion of antagonists affected basal ACh or DA efflux a repeated-measures ANOVA was conducted with Drug and Time (last baseline and two or

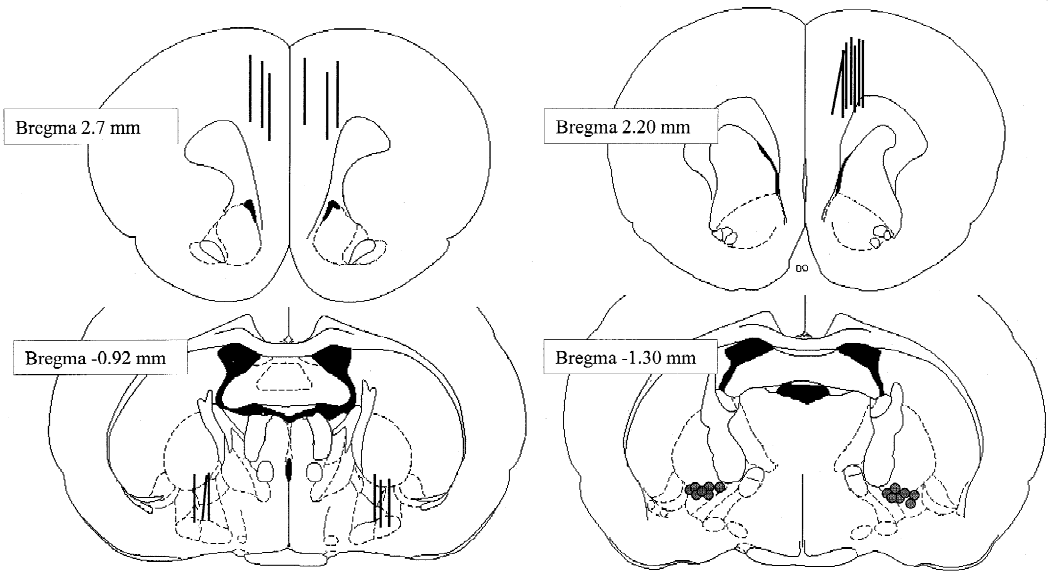

Fig. 1. The drawings of frontal sections on the left indicate the microdialysis probe placements in the mPFC (top left) and nBM/SI (bottom left) for Experiment 1. Placements for microdialysis probes in Experiment 2 were similar to those represented here. The drawings on the right indicate the microdialysis probe placements in the mPFC (top right) and the ventral most aspect of the bilateral infusion cannula in the nBM/SI (shaded circles, bottom right) for Experiment 3.

three collections prior to amphetamine injection) as factors. In order to determine whether intra-accumbens antagonists attenuated the increase in cortical ACh efflux produced by systemic amphetamine, a repeated measures ANOVA (Drug×Time) was conducted over the last baseline and all subsequent collection intervals.

In order to determine whether bilateral infusion of CDP influenced basal efflux of cortical ACh, a one-way repeated measures ANOVA was conducted on the seven collections during the session in which animals were given an infusion of CDP followed by saline injection (CDP+saline). To determine whether infusion of CDP influenced amphetamine-stimulated cortical ACh efflux a repeated measures ANOVA (Drug×Time) was conducted on the two Drug sessions (saline+amphetamine vs. CDP+amphetamine) over the last collection prior to amphetamine injection, and the four collections following the injection.

For all experiments, pair-wise comparisons following a statistically significant main effect or interaction were performed. Based on Keppel's suggestion [25] that the error term for follow-up comparisons in repeated measure designs should be the error of the two conditions in question, means were compared using paired *t*-tests. In recognition of the potential for increases of familywise error, the number of such comparisons was minimized and only used to probe the source of statistically significant main effects or interactions revealed by ANOVAs. When follow-up comparisons exceeded the 'natural limits' (the degrees of freedom in the numerator of the ANOVA), α was subjected to a modified Bonferroni correction (α_{MB} = α* degrees of freedom for the numerator/actual number of comparisons) [25]. When this correction is used the modified significance level (α_{MB}) is reported. All statistical analyses were completed using SPSS (Version 10.0.5, SPSS, Inc., Chicago, IL). The level of significance for all ANOVAs was defined as $P<0.05$ using a Huynh–Feldt correction for heterogeneity of variance.

3. Results

3.1. Experiment 1: Intra-basalis perfusion of D1 or D2 antagonists

3.1.1. Basal levels of dopamine efflux

Basal DA levels in the nBM/SI for the three microdialysis sessions (regardless of subsequent drug treatment during that session) was lower during Session 1 compared to Sessions 2 and 3. The two-factor ANOVA revealed a main effect of Session ($F_{2,10}=9.90$, $P=0.004$), but no effect of Time nor, importantly, an interaction between these factors. The mean (±S.E.M.) values (fmol/15 μl) for Sessions 1–3 collapsed over the four baseline collections were: 1.11±0.23, 6.19±1.12, 5.61±1.69, respectively. Comparisons of these sessions confirmed that basal levels were lower in Session 1 relative to Session 2 ($t_5=-4.89$, $P=0.004$) and Session 3 ($t_5=-2.82$, $P=0.037$), while the latter two sessions did not differ from each other ($t_5=-0.56$, $P=0.598$). This effect of session was likely due to an unfortunate coincidence of a significantly lower mean percent in vitro recovery from the microdialysis probes ($F_{2,10}=8.753$, $P=0.029$) during Session 1 (5.33%±0.99) than the recovery for Session 2 (12.33%±1.56) and Session 3 (9.67%±1.23).

Despite the tendency for the first session to yield lower basal levels of DA, the basal values (mean±S.E.M.) for the three Drug treatments did not differ from one another when collapsed across session order (fmol/15 μl): aCSF (4.73±1.89), SCH23390 (4.21±1.42), and sulpiride (3.88±1.22). The two-factor ANOVA did not yield any effects of Drug ($F_{2,10}=0.08$, $P=0.923$) or Time ($F_{3,15}=0.991$, $P=0.40$) nor a significant interaction ($F_{6,30}=0.738$, $P=0.505$). These data, expressed as a percent change from the average of all four baseline collections are shown in Fig. 2 (−45 to 0 min).

3.1.2. Effects of dopamine antagonists on basal forebrain dopamine efflux

Fig. 2 depicts the effects of DA antagonists on basal efflux of DA. While an ANOVA comparing the aCSF, SCH 23390, and sulpiride sessions over the last baseline and first 30 min of antagonist perfusion did not reveal an effect of Drug ($F_{2,10}=2.690$, $P=0.146$), there was a significant effect of Time ($F_{2,10}=5.32$, $P=0.027$), and more importantly, a Drug×Time interaction ($F_{4,20}=7.04$, $P=0.001$). This interaction was the result of an increase in DA levels in the SCH 23390 session at the 30 min time-point relative to aCSF control ($t_5=-3.87$, $P=0.014$). The sulpiride session did not differ from the aCSF session at any point.

3.1.3. Effects of dopamine antagonists on amphetamine-stimulated basal forebrain dopamine efflux

Systemic administration of 2.0 mg/kg amphetamine just after the collection at 30 min produced a marked increase in nBM/SI DA efflux over the course of the session (Time, $F_{8,40}=30.53$, $P<0.001$). Intra-basalis perfusion of SCH 23390 (100 μM) or sulpiride (100 μM) had no effect on amphetamine-stimulated increases in DA levels in the nBM/SI (Drug, $F_{2,10}=0.25$, $P=0.784$; Drug×Time, $F_{16,80}=0.33$, $P=0.834$). To further describe the effect of systemic amphetamine, follow-up comparisons on the effect of Time were carried out on the aCSF control session. These comparisons revealed that DA efflux was significantly increased over the baseline by the collection at 60 min ($t_5=-2.81$, $P=0.038$), and remained elevated from baseline at the end of the session at 120 min ($t_5=-2.67$, $P=0.045$).

3.1.4. Basal levels of cortical acetylcholine efflux

Basal efflux of cortical ACh was similar across all three microdialysis sessions as a two-factor ANOVA did not reveal an effect of Session ($F_{2,10}=0.98$, $P=0.409$) or an interaction between Session and Time ($F_{6,30}=0.80$, $P=$

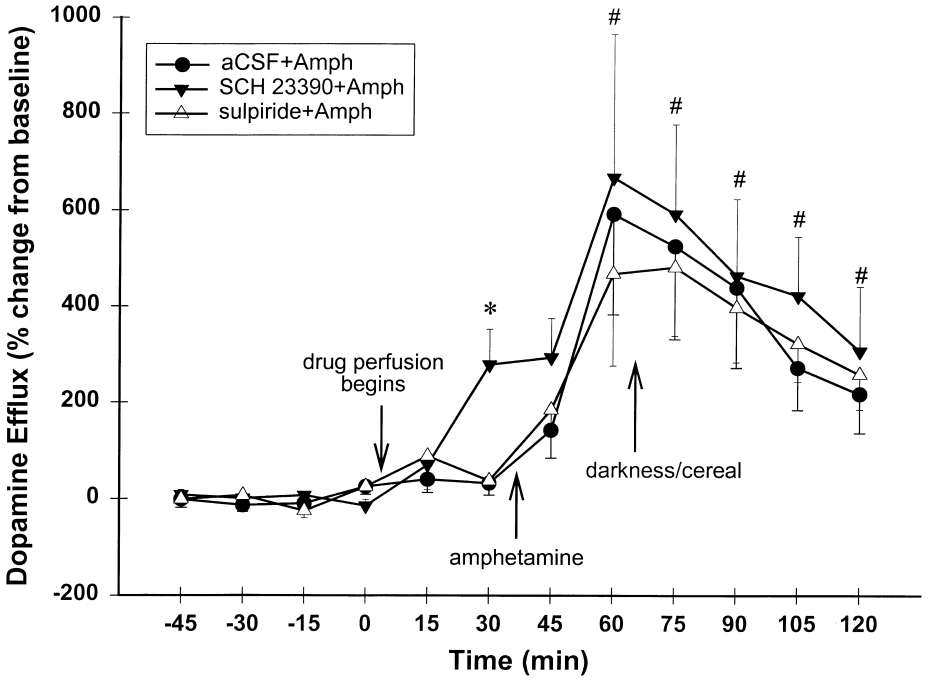

Fig. 2. The effects of systemic amphetamine and local perfusion of DA antagonists on nBM/SI dopamine efflux in nBM/SI under basal and amphetamine-stimulated conditions. All data, including the baseline period (collections from −45 to 0 min) are expressed as mean (±S.E.M.) percent change from the average of the four baseline collections. The animals (n=6) received intra-basalis perfusions indicated by the down arrow of aCSF (a control condition), the D1 antagonist SCH 23390 (100 μM), or the D2 antagonist sulpiride (100 μM). Drug treatment was counterbalanced across dialysis sessions. Systemic administration of amphetamine (2.0 mg/kg, i.p.), indicated by the up arrow, markedly stimulated nBM/SI DA efflux both before and after the darkness/cereal stimulus (given just after the collection at 60 min). Neither antagonist attenuated the ability of systemic amphetamine to stimulate cortical ACh efflux. (* indicates significant difference compared to aCSF control, $P<0.05$; # indicates amphetamine significantly increased dopamine efflux over last baseline (time 0) in aCSF+amphetamine session, $P<0.05$).

0.555). The mean (±S.E.M.) basal values (pmole/12 μl) for each session were: 0.11±0.02, 0.08±0.02, 0.09±0.02, Sessions 1–3, respectively. There was an effect of Time ($F_{10,15}$=15.836, $P<0.001$); pairwise comparisons (α_{MB}= 0.025) confirmed that the collection at 0 min was elevated relative to the collections at −45 min (t_5=−5.29, P= 0.003), −30 min (t_5=−4.60, P=0.006), and −15 min (t_5=−4.30, P=0.008), while no other collections differed from each other (all Ps>0.04). Likewise, basal efflux of cortical ACh was similar when the data were grouped by the three subsequent drug treatments; there was no effect of Drug ($F_{2,10}$=0.042, P=0.959) nor a Drug by Time interaction ($F_{6,30}$=1.401, P=0.247). These data, expressed as a percent change from the mean of all four baseline collections, aCSF (0.09±0.02), SCH23390 (0.09±0.02), and sulpiride (0.10±0.03), are shown in Fig. 3 (−45 to 0 min).

3.1.5. Effects of dopamine antagonists on cortical acetylcholine efflux

Basal efflux of cortical ACh was not changed by the perfusion of either the D1 or D2 antagonist into the nBM/SI (Fig. 3). An ANOVA comparing the aCSF (control), SCH 23390 and sulpiride sessions over the last baseline (collection at 0 min on Fig. 3) and first 30 min of drug perfusion did not yield any significant effects (Drug, $F_{2,10}$=0.231, P=0.798; Time, $F_{2,10}$=2.739, P=0.119; and Drug×Time, $F_{4,20}$=1.68, P=0.216).

3.1.6. Effects of dopamine antagonists on amphetamine-stimulated cortical acetylcholine efflux

Analysis across the entire aCSF session revealed that systemic administration of amphetamine induced a marked increase in cortical ACh efflux relative to the last baseline as evidenced by an effect of Time ($F_{8,40}$=103.67, $P<$ 0.001). Intra-basalis perfusion of SCH 23390 (100 μM) or sulpiride (100 μM) had no effect on amphetamine-stimulated increases of cortical ACh efflux (Drug, $F_{2,10}$=0.001, P=0.982; Drug×Time, $F_{16,80}$=0.542, P=0.619).

3.2. Experiment 2: Intra-basalis perfusion of the glutamate antagonist kynurenate

3.2.1. Basal levels of cortical acetylcholine efflux

Basal efflux of cortical ACh was stable across both Session ($F_{1,7}$=0.007, P=0.937) and Time ($F_{3,21}$=2.95, P=0.056), and there was no interaction between these factors (Session×Time, $F_{3,21}$=0.784, P=0.510). The basal ACh efflux values (pmol/12 μl; mean±S.E.M.) for the two Sessions were: Session 1, 0.28±0.06 and Session 2,

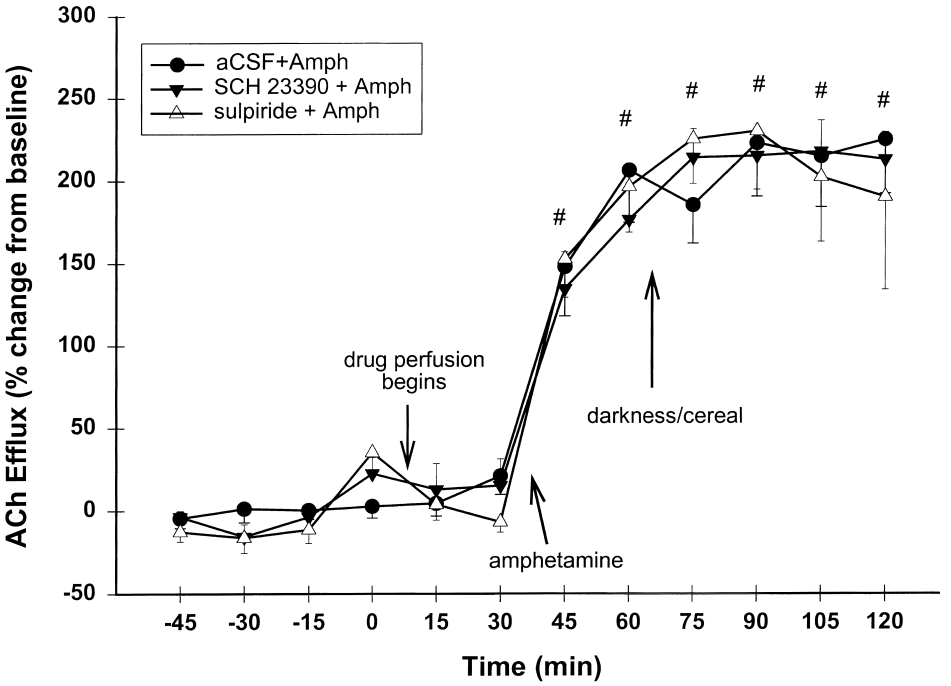

Fig. 3. The effects of systemic amphetamine and local perfusion of DA antagonists on ACh efflux in mPFC under basal and amphetamine-stimulated conditions. All data, including the baseline period (collections from −45 to 0 min) are expressed as mean (±S.E.M.) percent change from the average of the four baseline collections. The animals (n=6) received intra-basalis perfusions indicated by the down arrow of aCSF (a control condition), the D1 antagonist SCH 23390 (100 μM), or the D2 antagonist sulpiride (100 μM). Systemic administration of amphetamine (2.0 mg/kg, i.p.), indicated by the up arrow, markedly stimulated cortical ACh efflux both before and after the darkness/cereal stimulus (given just after the collection at 60 min). Local nBM/SI perfusion of the D1, or D2 antagonists had no affect on basal ACh efflux and did not attenuate the ability of systemic amphetamine to stimulate cortical ACh efflux. (# indicates amphetamine significantly increased ACh efflux over last baseline (time 0) in aCSF+amphetamine session, $P<0.05$).

0.27±0.05. Likewise, basal efflux of ACh was stable across the baseline period when data were analyzed by drug treatment; the two-factor ANOVA did not reveal effects of Drug ($F_{1,7}$=0.708, P=0.428), Time, ($F_{3,21}$= 2.95, P=0.056) or Drug×Time ($F_{3,21}$=1.444, P=0.260). The basal ACh efflux values (mean±S.E.M.) for the two Drug treatments were: aCSF control, 0.25±0.05 and 1.0 mM kynurenate 0.30±0.06. Due to the higher level of neostigmine used in this experiment (0.50 μM), basal values are higher than in those reported in Experiments 1 and 3 using a lower level (0.05 μM) of the acetylcholinesterase inhibitor. These baseline data, expressed as a percent change from the average of all four baseline collections are shown in Fig. 4 (−45 to 0 min).

3.2.2. Effects of kynurenate on cortical acetylcholine efflux

Basal efflux of cortical ACh was not changed by the intra-basalis perfusion of the glutamate antagonist kynurenate into the nBM/SI (Fig. 4). An ANOVA comparing the aCSF (control) session to the kynurenate session over the last baseline (0 min) and first 45 min of drug perfusion did not yield significant effects of Drug ($F_{1,7}$=0.373, P= 0.561), Time ($F_{3,21}$=1.208, P=0.331), and Drug×Time ($F_{3,21}$=1.22, P=0.327).

3.2.3. Effects of kynurenate on amphetamine-induced cortical acetylcholine efflux

Systemic administration of amphetamine (also shown in Fig. 4) induced a marked increase in cortical ACh efflux relative to the collection at 45 min (the last collection prior to amphetamine) as evidenced by an overall effect of Time ($F_{3,21}$=60.22, $P<0.001$). Intra-basalis perfusion of kynurenate (1.0 mM) attenuated the amphetamine-stimulated increase of cortical ACh efflux as evidenced by an effect of Drug ($F_{1,7}$=9.81, P=0.017) and a Drug×Time interaction ($F_{3,21}$=4.99, P=0.018). One-way ANOVAs on each session revealed that amphetamine increased cortical ACh efflux in the aCSF session ($F_{3,21}$=40.53, $P<0.001$). This increase was immediate and long lasting as ACh levels, compared to the collection at 45 min, were elevated at 60 min (t_7=5.71, P=0.001), 75 min (t_7=5.92, P=0.001), and 90 min (t_7=9.18, $P<0.001$). Amphetamine administration during the perfusion of kynurenate also increased cortical ACh efflux ($F_{3,21}$=32.97, $P<0.001$) beginning at 60 min (t_7=2.51, P=0.041), and continuing through the end of the session at 75 min (t_7=5.39, P=0.001) and 90 min (t_7= 12.16, $P<0.001$). The interaction between these two sessions was the result of a significant attenuation of amphetamine-stimulated cortical ACh efflux during the kynurenate session relative to the aCSF session during the

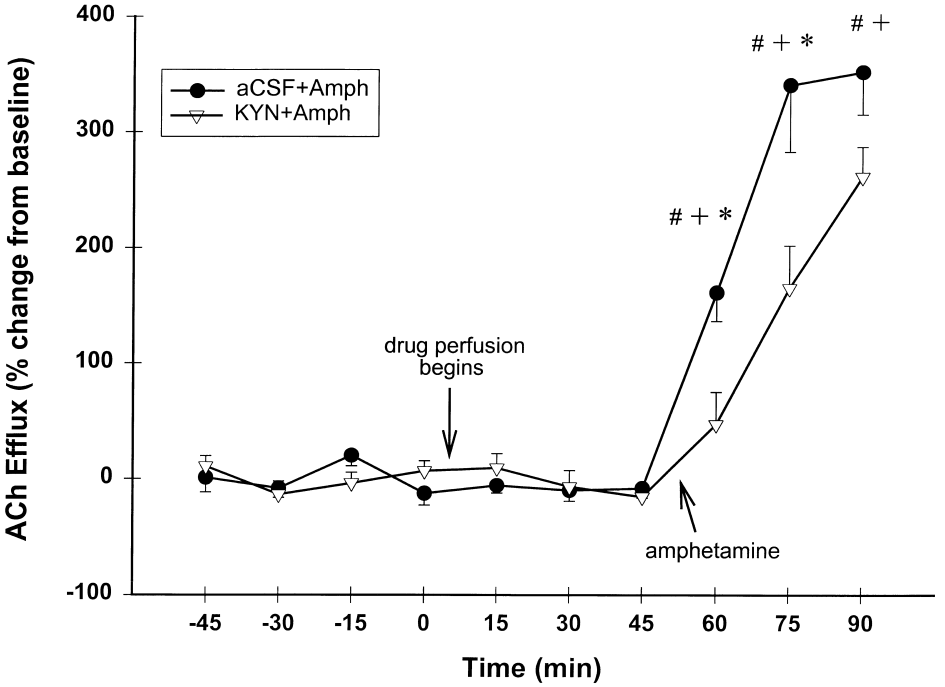

Fig. 4. The effects of systemic amphetamine and local perfusion of kynurenate on ACh efflux in mPFC under basal and amphetamine-stimulated conditions. All data points, including the baseline period (collections from −45 to 0 min) are expressed as mean (±S.E.M.) percent change from the average of the four baseline collections. Session order was counterbalanced across animals (n=8). Systemic administration of amphetamine (2.0 mg/kg, i.p.), indicated by the up arrow, stimulated cortical ACh efflux in both sessions. Local nBM/SI perfusion of kynurenate had no affect on basal cortical ACh efflux; however, the antagonist did attenuate the ability of systemic amphetamine to stimulate cortical ACh efflux. ($^{\#}$ indicates amphetamine significantly increased ACh efflux over last baseline (time 0) in aCSF+amphetamine session, $P<0.05$; $^{+}$ indicates amphetamine significantly increased ACh efflux over last baseline (time 0) in kynurenate+amphetamine session, $P<0.05$; * indicates significant difference between kynurenate+amphetamine and aCSF+amphetamine sessions at that time-point, $P<0.05$).

60 min (t_7=3.74, P=0.007) and 75 min (t_7=2.97, P=0.021) collections. However, by the 90 min collection, stimulated levels of ACh were no longer significantly different between the two treatments (t_7=1.95, P=0.093).

3.3. Experiment 3: Intra-basalis infusion of the benzodiazepine receptor agonist chlordiazepoxide

3.3.1. Basal levels of cortical acetylcholine efflux

Basal cortical ACh efflux did not differ across the three sessions (Session, $F_{2,12}$=0.443, P=0.637; Session×Time, $F_{4,24}$=1.382, P=0.280). The mean basal ACh efflux values (pmol/12 μl; mean±S.E.M.) for the three Sessions (collapsed across time) were: Session 1, 0.25±0.05; Session 2, 0.30±0.06; and Session 3, 0.25±0.05. Basal cortical ACh efflux was also stable when analyzed by the three drug treatments (Drug, $F_{2,12}$=2.524, P=0.122; Drug×Time, $F_{4,24}$=2.697, P=0.089). The mean (±S.E.M.) basal ACh efflux levels for the three drug treatments, collapsed across session were: saline+amphetamine, 0.09±0.01; CDP+amphetamine, 0.14±0.03; CDP+saline, 0.11±0.02. In these analyses, there was an effect of Time ($F_{2,12}$=6.187, P=0.014), confirmed by follow-up comparisons (α_{MB}=0.033) to result from an increase in cortical ACh efflux in the collection following the installation of the infusion cannula (15 min) relative to the last baseline (t_6=−3.26, P=0.017), this collection at 15 min was not significantly different from the subsequent collection at 30 min (t_6=2.47, P=0.048), nor did the last baseline differ from the collection at 30 min (t_6=−0.921, P=0.393).

3.3.2. Effects of chlordiazepoxide on cortical acetylcholine efflux

A one-way ANOVA on the entire CDP+saline session was conducted to examine the effects of CDP infusion and saline injection on basal efflux of cortical ACh (Fig. 5). This analysis resulted in a significant effect of Time ($F_{6,36}$=5.330, P=0.005). Follow-up comparisons (α_{MB}=0.043) revealed that cortical ACh decreased below the last baseline after the installation of the infusion cannula during the collection just prior to the infusion at 30 min (t_6=2.94, P=0.026). In the collection immediately following the infusion of CDP (40 μg/hemisphere) and the injection of saline (at 45 min) ACh levels in the cortex increased (t_6=−3.56, P=0.012). However, by the second collection after saline injection (60 min) ACh levels were

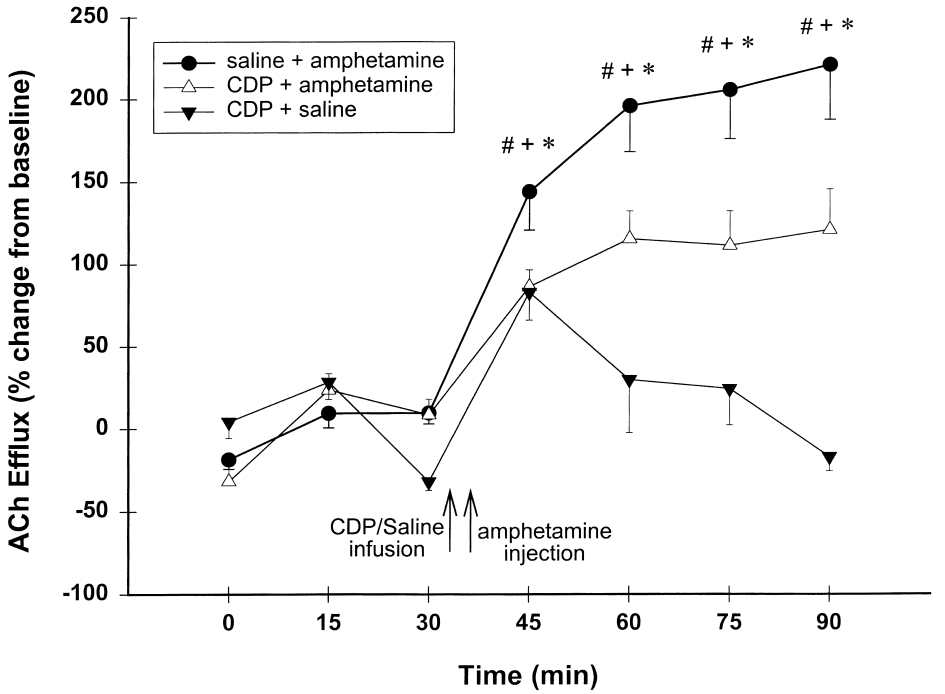

Fig. 5. The effects of systemic amphetamine and nBM/SI infusion of a BZR agonist on ACh efflux in mPFC under basal and amphetamine-stimulated conditions. All data are expressed as mean (±S.E.M.) percent change from the average of the last baseline (0 min), and the collections at 15 and 30 min. The animals ($n=7$) received bilateral intra-basalis infusions of CDP (40 μg/hemisphere) or saline at the end of the collection at 30 min (the left arrow). One minute after the infusion ended stylets were replaced and animals were injected (right arrow) with amphetamine (2.0 mg/kg, i.p.) or saline (1.0 ml/kg, i.p.). Drug treatment was counterbalanced between the dialysis sessions. The sessions consisted of saline infusion followed by amphetamine (saline+amphetamine), CDP infusion followed by amphetamine (CDP+amphetamine), and CDP infusion followed by saline (CDP+saline). CDP+saline produced a transient increase in cortical ACh efflux, while systemic amphetamine (saline+amphetamine) markedly stimulated cortical ACh efflux. Local nBM/SI perfusion of CDP had no affect on basal ACh efflux, but did attenuate amphetamine-stimulated cortical ACh efflux (CDP+amphetamine). ($^{\#}$ indicates amphetamine significantly increased ACh efflux over last baseline (time 0) in saline+amphetamine session, $P<0.05$; $^{+}$ indicates amphetamine significantly increased ACh efflux over last baseline (time 0) in CDP+amphetamine session, $P<0.05$; * indicates significant difference between saline+amphetamine and CDP+amphetamine sessions at that time point, $P<0.05$).

no longer above baseline and did not differ from baseline at any other time point (all Ps>0.07).

3.3.3. Effects of chloridazepoxide on amphetamine-stimulated cortical acetylcholine efflux

Infusion of CDP into the nBM/SI resulted in a significant attenuation of amphetamine-stimulated cortical ACh efflux as evidenced by an ANOVA on the two Drug treatments that included the last collection prior to amphetamine (at 30 min; see Fig. 5) and the four post-amphetamine collections. This ANOVA yielded significant effects of Drug ($F_{1,6}=13.08$, $P=0.012$) and Time ($F_{4,24}=37.16$, $P<0.001$) as well as a Drug×Time interaction ($F_{4,24}=5.79$, $P=0.038$). Follow-up comparisons ($\alpha_{MB}=0.028$) revealed that cortical ACh levels did increase over levels at 30 min by the collection at 45 min in both the saline+amphetamine session ($t_6=-6.19$, $P=0.001$) and the CDP+amphetamine session ($t_6=-4.69$, $P=0.003$). These two comparisons, and the significant effect of Time, indicate that amphetamine did increase cortical ACh in both sessions. During the CDP+amphetamine session there was a significant attenuation in the increase of cortical ACh following the amphetamine injection relative to the saline+amphetamine session. Although these two sessions did not differ at 30 min, the last collection prior to amphetamine administration ($t_6=0.09$, $P=0.931$), they were different from each other at all time-points following the amphetamine injection: 45 min ($t_6=2.94$, $P=0.026$), 60 min ($t_6=3.42$, $P=0.014$), 75 min ($t_6=3.47$, $P=0.014$), and 90 min ($t_6=3.30$, $P=0.017$) collections.

4. Discussion

All three of the experiments reported here are consistent with the results of previously published studies indicating that the systemic injection of amphetamine produces robust, long-lasting increases in cortical ACh release [1,5,7,8]. In addition to this increase in cortical ACh release, systemic administration of amphetamine produced increases in DA levels within the area of the nBM/SI. The amphetamine-stimulated release of ACh was unaffected by local perfusions of either a D1 (SCH 23390) or a D2 (sulpiride) antagonist into the nBM/SI, suggesting that stimulation of these receptors in the nBM/SI is not a

necessary condition for the systemic amphetamine-induced increase in cortical ACh release.

Two additional experiments demonstrated that local modulation of the excitability of nBM/SI cholinergic neurons is capable of attenuating the amphetamine-stimulated increase of ACh release in the cortex. Specifically, perfusion of kynurenate, a non-selective ionotropic glutamate receptor antagonist, or infusion of CDP a BZR agonist which acts as a positive modulator of the $GABA_A$ receptor complex, both served to attenuate the increase in cortical ACh release produced by systemic administration of amphetamine. The remainder of the discussion will focus on specific issues raised by each of these experiments.

4.1. Dopamine antagonists and amphetamine-stimulated cortical acetylcholine and basal forebrain dopamine release

Systemic administration of amphetamine produced increases in cortical ACh efflux approximately 200–250% above basal levels. The darkness/cereal stimulus (Experiment 1) did not appear to influence cortical ACh efflux (or NAC DA) beyond this amphetamine-stimulated increase, indeed the increase following amphetamine appears very similar in the subsequent experiments (Experiments 2 and 3) in which no darkness/cereal stimulus was employed. This is consistent with previous studies [1] that have demonstrated that this stimulus produces a modest, transient increase in cortical ACh efflux.

Neither basal nor amphetamine-stimulated ACh release were affected by intra-basalis perfusions of either the D1 antagonist SCH 23390 or the D2 antagonist sulpiride. This suggests that D1 or D2 receptor activity within the nBM/SI is not necessary for the full effects of systemic amphetamine on cortical ACh release. Although the current study utilized only a single dose of each antagonist, this dose of sulpiride was demonstrated to be sufficient, when perfused into the NAC, to block the increase in cortical ACh release produced by systemic administration of FG 7142, a BZR partial inverse agonist [30], and SCH 23390 (100 μM) showed a similar trend. In addition, a lower dose of SCH 23390 (10 μM) was shown to block AMPA-stimulated GABA efflux in the striatum [3]. However, the possibility that the antagonists may have produced some attenuation at additional doses cannot be ruled out.

The possibility that co-administration of the D1 and D2 antagonists into the nBM/SI may have been more effective than either antagonist alone remains to be tested. However, activity at D1 and D2 receptors may not positively interact in the basal forebrain [15]. Specifically, Gong et al. [15] have shown that a D1 agonist (SKF 38393) infused into the ventral pallidum resulted in an increase in locomotor activity, while a D2 agonist (quinpirole) suppressed locomotion. Moreover, no synergistic effects on locomotion were observed when these two agonists were infused simultaneously into the ventral pallidum.

The neuronal mechanisms responsible for the amphetamine-stimulated increase in cortical ACh release remains unsettled. Day and Fibiger [7] have reported that the effects of systemic amphetamine were completely attenuated with systemic administration of D1 antagonists and markedly decreased with D2 antagonists, confirming that changes in DA receptor activity are likely responsible for the amphetamine-stimulated increase in cortical ACh release. Moreover, a specific role of DA, rather than norepinephrine (NE), was further confirmed by 6-hydroxydopamine lesions of the median forebrain bundle which attenuated amphetamine-induced increases in cortical ACh release while lesions of the dorsal noradrenergic bundle did not [8]. Recent studies have shown that DA receptor activity within the NAC shell is neither necessary nor sufficient to account for the effects of systemic amphetamine on cortical ACh release [1]. In those experiments antagonism of D1, D2, or D1+D2 receptors in the NAC did not attenuate the increase in cortical ACh release produced by systemic amphetamine. While DA receptor activity within the nBM/SI region of the basal forebrain represented the potentially most direct, but largely unexplored substrate for the effects of systemic amphetamine on cortical ACh release, the intra-basalis administration of D1 and D2 receptor antagonists (Experiment 1) did not attenuate amphetamine-stimulated increases in cortical ACh release.

Until recently, application of amphetamine directly into the cortex was also believed to be insufficient to increase cortical ACh release, suggesting that a local effect within the cortex does not account for the actions of systemically administered amphetamine [7]. However, a recent report [17] has demonstrated that different divisions of the mPFC are differentially responsive to local application of amphetamine. Hedou et al. [17] demonstrated that previously tested levels of amphetamine (10 μM) in the perfusion medium [7] up to 1000 μM did not produce changes in dorsal mPFC ACh release, the same region of the cortex examined in the current studies, yet in the ventral mPFC, very high levels of amphetamine in the perfusate (1000 μM) did stimulate release of cortical ACh. However, the 1000 μM dose of amphetamine used by Hedou et al. [17] has been shown to increase DA efflux in the NAC to 25,000 percent of baseline [6], with lower doses of 100 and 250 μM amphetamine producing increases in basal DA levels in the NAC to approximately 2000 and 5000 percent of baseline, respectively. Systemic administration of 2.0 mg/kg amphetamine produced increases in NAC DA of only 150 to 400 percent of baseline. This suggests that while the potential local effects of intra-cortical amphetamine cannot be entirely excluded, the increased levels in mPFC DA (and other catecholamines) likely

produced by systemically administered amphetamine are probably not locally modulating the increased release of ACh in the cortex.

Thus, a precise location for the critical DA receptors mediating the effects of systemic amphetamine on cortical ACh has yet to be determined. The effects of amphetamine on cortical ACh release, rather than producing changes in DA receptor activity in a single critical site, more likely involves simultaneous changes in DA receptor activity in multiple sites in a complex neuronal network interacting with multiple transmitter systems (see [1] for further discussion).

4.2. Kynurenate and amphetamine-stimulated cortical acetylcholine release

As expected, systemic administration of amphetamine (2.0 mg/kg), increased cortical ACh efflux in this experiment over 300% above basal levels. Intra-basalis perfusion of kynurenate (1.0 mM), while having no affect on basal levels of cortical ACh, significantly attenuated amphetamine-stimulated cortical ACh release.

The effects of systemic amphetamine on glutamate levels in the basal forebrain remain largely unexplored. Amphetamine has been shown to increase glutamate levels in the nucleus accumbens, ventral tegmentum and prefrontal cortex [27,40,49], suggesting that amphetamine could increase basal forebrain glutamate levels as well. The circuits mediating such effects of amphetamine on basal forebrain glutamatergic afferents, however, are unclear. Basal forebrain glutamatergic afferents primarily arise from cortical and amygdaloid areas, although brainstem areas such as the PPT may also send glutamatergic projections to the basal forebrain [4,12,16,54]. However, the projections from the PPT are less well documented than telencephalic afferents.

Recently, Fadel et al. [10], using the same darkness/cereal stimulus described in the present Experimental procedures section, demonstrated that increases in cortical ACh release produced by this behavioral stimulus were also attenuated by intra-basalis infusions of the same dose of kynurenate (1.0 mM). In addition, intra-basalis infusions of kynurenate have been demonstrated to block increases in cortical ACh release produced by electrical stimulation of the PPT [39].

The observation that intra-basalis administration of kynurenate (1.0 mM) did not suppress basal levels of cortical ACh is not unique to the current experiments. In the experiments reported by Rasmusson et al. [39], even higher doses of kynurenate administered into the basal forebrain (5.0 and 10.0 mM) were shown to have no impact on basal levels of cortical ACh. Similarly, in their study on glutamatergic regulation of behaviorally-stimulated ACh release, Fadel et al. [10] reported that intra-basalis administration of kynurenate (1.0 mM), while attenuating behaviorally-stimulated ACh release, resulted in only a modest decline in basal efflux. Moreover, these authors reported no decline in basal cortical ACh efflux following intra-basalis administration of 1.0–5.0 mM of the more selective AMPA/KA antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX), yet cortical ACh efflux stimulated by kainic acid was suppressed by the antagonist. Intra-basalis perfusion of 100 μM of the NMDA selective antagonist 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) has been reported to decrease basal efflux of cortical ACh by approximately 50% [13], although Fadel et al. [10] failed to see any effect of NMDA on basal cortical ACh efflux. These studies suggest that while there may be some tonic glutamatergic regulation of basal cortical ACh efflux mediated through NMDA receptors, kynurenate fails to reveal this more selective modulation of cholinergic corticopetal neurons. Alternatively, the level of behavioral activation may have differed in those studies showing an effect of NMDA antagonism [13], such that the decrease observed in ACh levels actually reflected a decrease in stimulated cortical ACh efflux. This would then be consistent with the studies cited above demonstrating that perfusion of glutamatergic antagonists into the basal forebrain are effective in modulating stimulated efflux produced by a variety of mechanisms.

4.3. Chlordiazepoxide and amphetamine-stimulated cortical acetylcholine release

Positive modulation of the GABAergic transmission through bilateral infusion of the BZR agonist CDP into the nBM/SI attenuated the amphetamine-stimulated increase in cortical ACh release, suggesting that, like the effect of kynurenate in Experiment 2, the excitability of nBM/SI cholinergic neurons following systemic amphetamine is a component of the increased release of ACh in the cortex. The present data correspond with the findings of Moore et al. [32] that demonstrated that intra-basalis infusion of CDP attenuates the increase in cortical ACh release produced by a behavioral stimulus (darkness/cereal).

As a BZR agonist, CDP positively modulates the effects of endogenously released GABA and for this reason it was preferred over the use of direct $GABA_A$ receptor agonists which have been shown to decrease a number of markers of ACh transmission (for review see [43]). Negative modulators of the $GABA_A$ receptor complex have also been shown to have effects on cortical ACh release. For example, it has been shown recently that the neurosteroid pregnenolone sulfate (a negative modulator of $GABA_A$, and positive modulator of NMDA) infused into the basal forebrain results in increased ACh release in frontal cortex and amygdala [36]. In addition, the systemic administra-

tion of the β-carboline ZK 93426, a BZR selective inverse agonist, potentiates stimulated cortical ACh release [29].

5. Conclusions

The current findings, in consideration with other investigations, suggest that there is no single site that accounts for the increase in cortical ACh release produced by systemic amphetamine administration. The increase in cortical ACh release produced by systemic amphetamine probably reflects the synchronous effects of amphetamine on DA release, as well as other non-dopaminergic transmitter systems, throughout a distributed, multisynaptic neuronal network that likely includes the amygdala, basal forebrain, nucleus accumbens, hippocampus, prefrontal cortex, and ventral tegmentum. Dopamine receptor activity in the NAC [1] and the basal forebrain, when studied independently, have not proven to be necessary for the effects of systemic amphetamine on cortical ACh release. The current studies demonstrating that local modulation of corticopetal cholinergic neurons can attenuate amphetamine-stimulated cortical ACh release, suggest that amphetamine involves complex modulation of the cell bodies of the basal forebrain cholinergic neurons. Future studies designed to unravel this complex modulation will add insight into how these neurons are involved in modulating attentional functions and contribute to the cognitive symptoms of neuropsychiatric disorders.

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