ROLE OF ACCUMBENS AND CORTICAL DOPAMINE RECEPTORS IN THE REGULATION OF CORTICAL ACETYLCHOLINE RELEASE

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Abstract—Cortical acetylcholine, under resting and stimulated conditions, was measured in frontoparietal and prefrontal cortex using in vivo microdialysis in freely-moving rats. Cortical acetylcholine efflux was stimulated by systemic administration of the benzodiazepine receptor partial inverse agonist FG 7142. Administration of FG 7142 (8.0 mg/kg, i.p.) significantly elevated acetylcholine efflux in both cortical regions (150-250% relative to baseline) for 30 min after drug administration. The ability of endogenous dopamine to regulate cortical acetylcholine efflux under resting or stimulated conditions and the relative contributions of D₁- and D₂-like dopamine receptor activation was also assessed. In a first series of experiments, systemic administration of the antipsychotic drug haloperidol (0.15, 0.9 mg/kg, i.p.) blocked FG 7142-stimulated acetylcholine efflux in frontoparietal, cortex while the D₂-like antagonist, SCH 23390 (0.1, 0.3 mg/kg), was less effective in attenuating stimulated acetylcholine efflux. In a second series of experiments, the effects of infusions of these antagonists and of the D₂-like antagonist sulpiride (10, 100 μM) into the nucleus accumbens were assessed. Infusions of haloperidol and sulpiride significantly blocked FG 7142-stimulated acetylcholine efflux while SCH 23390 did not. By contrast, a third series of experiments demonstrated that perfusion of these antagonists (100 μM) locally into the cortex (through the probe) did not affect FG 7142-stimulated acetylcholine efflux. Moreover, none of these dopamine receptor antagonists, whether administered systemically or perfused into the nucleus accumbens or cortex, affected basal cortical acetylcholine efflux.

These results reveal similarities in stimulated cortical acetylcholine release across frontoparietal cortex and suggest a prominent role for D₂-mediated accumbens dopamine transmission in the regulation of cortical acetylcholine release. The findings provide evidence in support of a neural substrate that links dysregulation of mesolimbic dopaminergic transmission to changes in cortical cholinergic transmission. Dysregulation within this circuit is hypothesized to contribute to the etiology of disorders such as schizophrenia, dementia and drug abuse. © 1998 IBRO. Published by Elsevier Science Ltd.

Key words: acetylcholine, cortex, dopamine, nucleus accumbens, microdialysis, FG 7142.

All cortical areas and layers are innervated by cholinergic projections originating in the basal forebrain. As aberrations in the integrity of this projection have been implied in the development of cognitive symptoms of major neuropsychiatric disorders, including dementia and schizophrenia, the afferent regulation of basal forebrain cholinergic neurons has been increasingly investigated. The regulation of cortical acetylcholine (ACh) release by telencephalic and brainstem projections to basal forebrain cholinergic neurons has provided a fruitful research avenue toward the understanding of the normal functions of cortical cholinergic inputs and the development of novel pharmacological strategies aimed at the attenuation of disease-associated dysregulation of this widespread cortical input system.

Particularly relevant to the role of corticopetal cholinergic neurons in psychiatric disorders is the regulation of this system by its GABAergic inputs. A major source of the GABAergic inputs to basal forebrain neurons is the nucleus accumbens. The GABA<sub>A</sub> receptor complex, of which there is a high density in the basal forebrain, is allosterically modulated by ligands for the benzodiazepine site in that GABA-gated chloride flux is enhanced by benzodiazepine receptor (BZR) agonists and reduced by BZR inverse agonists, resulting, respectively, in an enhancement and reduction in GABA-mediated inhibition of cell excitability. As would be predicted by the anatomical relationship between GABAergic terminals and cholinergic neurons in the basal forebrain, systemic administration or local basal forebrain infusions of BZR agonists and inverse agonists results in reduction and augmentation of...
cortical ACh efflux, respectively.\textsuperscript{54-49} Importantly, these studies revealed that the effects of BZR ligands on cortical ACh release interact with activity in the cholinergic system, reflecting the ability of GABA to regulate the excitability of these neurons.\textsuperscript{61,63}

The role of the GABAergic projections from the nucleus accumbens (NAC) in the regulation of cortical ACh release is of particular interest in the context of theories that predict that over-reactivity of cortical cholinergic inputs mediate the development of positive symptoms in schizophrenia.\textsuperscript{60,63,65} This prediction is based in part on the traditional hypothesis that mesolimbic dopaminergic hyperactivity represents a critical link in the chain of neuropathological mechanisms in schizophrenia.\textsuperscript{7,26,69} and on the presumed consequences of dopaminergic hyperactivity in the NAC for the GABAergic regulation of basal forebrain cholinergic neuronal excitability. Bourdelais and Kalivas\textsuperscript{5,4} demonstrated that systemic administration of amphetamine lowers extracellular GABA levels in the basal forebrain, thus supporting previous data by Mogenson and co-workers which, collectively, suggested that dopamine (DA) receptor stimulation in the NAC decreases the activity of the efferent GABAergic projection to the basal forebrain.\textsuperscript{39,42,73}

DA receptor stimulation in the NAC is hypothesized to decrease GABAergic outflow into the basal forebrain, resulting in disinhibition of corticopetal cholinergic neurons and increased cortical ACh release. Systemic or intraventricular administration of DA or DA receptor agonists have been repeatedly demonstrated to increase cortical ACh outflow.\textsuperscript{2,15,16,54} Furthermore, Duy et al.\textsuperscript{17} suggested that mesolimbic DA contributes significantly to the increases in cortical ACh release following systemic administration of amphetamine. While these data suggest a major role of mesolimbic DA in the regulation of cortical ACh, the contribution of DA receptor subtypes in the NAC and locally in the cortex to the effects of these systemically administered drugs is unsettled.

In the present study, the effects of DA receptor antagonists on both resting (basal) and stimulated cortical ACh efflux were determined. Cortical ACh efflux was stimulated via the systemic administration of the BZR partial inverse agonist FG 7142, a manipulation which reliably enhances cortical ACh efflux.\textsuperscript{50,49} While there may be multiple neuronal mechanisms underlying the ability of FG 7142 to stimulate cortical ACh efflux, a major mechanism is likely to be the allosteric reduction of GABA\textsubscript{\textalpha} mediated inhibition of cholinergic neurons in the basal forebrain, similar to that shown for other BZR inverse agonists.\textsuperscript{21,28} Another contributing mechanism involves the ability of FG 7142 to increase DA turnover or efflux in the NAC.\textsuperscript{50} FG 7142 was thus used as a pharmacological tool to increase cortical ACh release and to assess the potential of DA receptor antagonists to attenuate activated ACh efflux via a decrease in GABAergic transmission in basal forebrain. It was predicted that DA antagonists would attenuate the FG 7142-induced increase in cortical ACh efflux, and, furthermore, that the blockade of DA receptors in the NAC would be sufficient for this effect. However, because FG 7142 also increases DA efflux in the medial prefrontal cortex (mPFC)\textsuperscript{75} and because there are cells in the mPFC that project to the NAC and/or basal forebrain cholinergic neurons\textsuperscript{23,28,66,62} and are modulated by cortical DA,\textsuperscript{12} the effects of DA antagonists perfused into the mPFC on cortical ACh efflux were also assessed. Finally, we compared basal and FG 7142-stimulated ACh efflux in two regions, frontoparietal cortex (FPC) and mPFC. The anatomical organization of cortical cholinergic afferents and recent microdialysis studies have suggested a rather widespread release of ACh in all cortical areas and layers (see Ref. 63 for a review).

**EXPERIMENTAL PROCEDURES**

**Animals and surgery**

Four- to eight-month-old male Fisher 344/Brown Norway rats (National Institute of Aging Colony, Charles River, Wilmington, MA) were maintained in a temperature- and humidity-controlled environment on a 12:12 h light:dark cycle (lights on at 06:00) with food and water freely available. All animals were extensively handled and habituated to the microdialysis testing environment prior to surgery for microdialysis guide cannula implantation. 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.

Animals were anesthetized with sodium pentobarbital (60.0 mg/kg, i.p.) and were stereotaxically implanted with a microdialysis guide cannula (0.65 mm o.d., CMA 10, Carnegie Medicine, Acton, MA) into the FPC or dual implantation into the mPFC and shell of the ipsilateral NAC. For placement of probes in the FPC, the microdialysis guide cannula was rotated in the stereotaxic apparatus to an angle of 50° away from vertical, towards the midline. The cannula was then inserted 1.0 mm beyond the dural surface through a burr hole drilled at 1.0-2.0 mm anterior to bregma and 1.0 mm lateral to the midline. For placement into the mPFC, the cannula was rotated approximately 10° away from the midline and 10° anterior and inserted 0.7 mm from a point on the dural surface. 3.0 mm anterior to bregma and 0.6 mm lateral to the midline. For animals implanted in the mPFC, an additional guide cannula was implanted into the NAC by rotating the stereotaxia than 5% of their pre-surgery body weight as a result of the surgery and fully recovered to their pre-surgery weight within three days.

**Microdialysis sessions**

Following surgery, habituation of the animals to the microdialysis testing chambers (clear plastic parabolic bowls; 35.0 cm height; 38.0 cm diameter, Carnegie Medicine, Stockholm, Sweden) continued for three days.
The first microdialysis session was performed on the fourth post-surgical day. On each microdialysis test day, animals were allowed to habituate to the testing chambers for 30 min prior to insertion of the removable concentric dialysis probe(s) (0.5 mm o.d., 0.3 mm i.d., 1 mm membrane tip length; CMA Microdialysis, Acton, MA). Probes were perfused at a flow rate of 1.3 μl/min with an artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 126.5, NaHCO₃ 27.5, KCl 2.4, Na₂SO₄ 0.5, KH₂PO₄ 0.5, CaCl₂ 1.1, MgCl₂ 0.8, p-glucose 4.9, and neostigmine bromide (0.5 μM; Sigma Chemical, St Louis, MO). In animals with mPFC and NAC probes, neostigmine perfusion was halted for the ANC probe. In all experiments, collection of dialysates began 3 h after probe insertion, a time-point at which basal cortical ACh efflux is stable and dependent on axonal depolarization.⁵⁵ Four consecutive 15-min baseline dialysates were collected prior to any drug manipulations.

**Acetylcholine analysis**

ACh in each dialysate was quantified by high-performance liquid chromatography with electrochemical detection.⁵⁶ ACh and choline were separated by a C-18 carbon polymer column (530 × 1 mm; Bioanalytical Systems (BAS), W. Lafayette, IN) using a mobile phase (pH 8.5) containing 30.0 mM NaH₂PO₄ and 12.0 mM NaCl. Post-column derivatization of ACh and choline was achieved by an immobilized enzyme reactor column (BAS) containing covalently-bound acetylcholinesterase and choline oxidase. The hydrogen peroxide generated by the enzymatic degradation of ACh and choline was further broken down and detected by a peroxidase-coated glassy carbon working electrode coupled to a LC-4C electrochemical detector (BAS). Detector output was recorded and analysed using “Chromograph” software (BAS). The peak corresponding to ACh was quantified by integration of the peak area and comparison with a four-point external standard curve bounding the expected range of dialysate ACh levels. The detection limit for ACh by this method was approximately 20.0 fmol/10.0 μl injected on column.

**Verification of probe placement**

Three days following the last microdialysis session, animals were given a sublethal dose of sodium pentobarbital and transcardially perfused with 0.2% heparin in 0.9% saline followed by 10% formalin. The brains were removed, blocked rostral to the cerebellum, and stored in 10% formalin at 4°C with transfer to 30% sucrose phosphate buffer at least three days prior to sectioning. Several 40.0 μm sections surrounding the dialysis probe tract(s) were processed for Nissl staining for placement verification.

**Drugs**

The BZR partial inverse agonist FG 7142 (FG; Research Biochemical International, RBI; Natwick, MA) was solubilized in 10% Creomphor EL (CEL; BASF, Ludwigshafen, Germany) for systemic injections. The dose used (8.0 mg/kg) produces reliable increases in cortical ACh efflux,⁵⁷,⁵⁸ and is comparable to doses shown to increase DA efflux in the NAC.⁵⁷ The systemic doses used for the D₂-like antagonist SCH 23390 (0.1 and 0.3 mg/kg, RBI) and for the D₂-like antagonist haloperidol (0.15 and 0.9 mg/kg, McNeil Pharmaceuticals, Springhouse, PA) are similar to those demonstrated to antagonize the increase in cortical ACh efflux produced by systemic amphetamine.⁶⁰ The systemic vehicle solution for both of these DA antagonists was 0.9% saline. The intracranial doses administered via the dialysis probes were 10.0 and 100.0 μM (for intracranial sessions 100 μM only). While there are no data on the effects of these ligands perfused intracranially on cortical ACh release, the doses used here are comparable to doses of DA antagonists shown to locally modulate release of ACh in the dorsal striatum.⁶² The more selective D₂-like antagonist l-sulpiride (RBI) was perfused via the probe at the same doses as SCH 23390 and haloperidol. The vehicle solution for all drugs administered into the accumbens via the dialysis probe was aCSF.

**Experimental designs**

**General design and comparison of basal and FG 7142-stimulated acetylcholine efflux in frontoparietal and medial prefrontal cortex**. Following cannula implantation into either the FPC or dural implantation into the FPC and NAC (see above), animals were allowed to recover for three days during which time habituation to the testing chamber was continued. Following this recovery period, each animal was tested on four microdialysis sessions (between 10.00–16.00), separated by a 24-h "washout" period. On each session, four baseline samples were collected (see above) and then a DA receptor antagonist or its vehicle (see below) was administered. After two or three more 15-min collections, FG (8.0 mg/kg, i.p.) or its vehicle was administered and three more collections were taken. For all experiments, individual baseline cortical ACh efflux (pmol/min) for each session was defined as the median value of the four baseline collections with efflux at subsequent time-points expressed as percent change from this median baseline. Comparisons between basal ACh efflux in FPC vs PFC were conducted using an independent t-test. Analysis of the relative effect of FG in different cortical regions was performed using a mixed ANOVA on the effects of CORTICAL AREA (FPC and PFC) and COLLECTION INTERVAL (last baseline collection, 15 min and 30 min post-FG) as the between- and within-subject factors, respectively.

**Effects of systemic administration of dopamine antagonists on cortical acetylcholine efflux**. This experiment was designed to test the hypothesis that enhanced dopaminergic transmission is a necessary condition for the ability of FG to increase ACh efflux in the frontal cortex. The relative contributions of D₁- and D₂-like receptor activation were also investigated. The effects of the DA antagonists were also assessed in the “absence” of FG in order to assess whether DA receptor activity tonically mediates basal cortical ACh release. Each animal was implanted with a guide cannula into the FPC as described above and randomly assigned to one of two groups according to the DA antagonist administered (SCH 23390 or haloperidol). Immediately following the fourth baseline collection, a systemic injection (i.p.) of SCH 23390 (0.5 [saline], 0.1 or 0.3 mg/kg) or haloperidol (0 [saline], 0.15 or 0.9 mg/kg) was given. Following one (for SCH 23390) or two (haloperidol) subsequent 15-min collection periods, FG (8.0 mg/kg, i.p.) or its vehicle was administered. Each animal, thus, received the following four drug combinations, in random order, across four microdialysis sessions: vehicle (saline)+FG, DA antagonist (low dose)+FG, DA antagonist (high dose)+FG, and DA antagonist (high dose)+vehicle (CEL).

The effects of SCH 23390 and haloperidol were tested in separate groups of animals and analysed separately. The ability of FG to stimulate cortical ACh release was analysed by one-way ANOVAs of the effects of FG on ACh efflux 15 and 30 min following the administration of the drug compared to baseline ACh efflux. The effects of the DA antagonists alone were also analysed by similar ANOVAs of the effects of the higher dose of either antagonist administered together with the vehicle for FG (see Figs 2 and 3). The ability of the antagonists to attenuate the effects of FG was analysed by two-way ANOVAs on the effects of the two doses of the antagonists on the FG-induced increase in ACh efflux at the 15- and 30-min collection intervals. Multiple comparisons were conducted using modified Bonferroni
tests as described in Keppel in order to reduce the probability of Type I errors.

Effects of dopamine antagonists administered intracranially on cortical forebrain efflux. The BZR partial inverse agonist FG has been demonstrated to increase indices of DA transmission in both NAC and PFC. Therefore, we investigated the necessity of DA transmission in NAC and PFC by determining the effects of localized injections of D2 and D3 antagonists following systemic administration of FG. In this experiment, we also examined an additional D3-like agonist, sulpiride, because of concerns about the affinity of haloperidol for non-D2 receptors.

Intra-accumbens dopamine antagonists. Animals received microdialysis guide cannula in both the left PFC and ipsilateral NAC according to the procedures described above in the Experimental Procedures. Animals were then assigned to one of three DA antagonist groups (SCH 23390, haloperidol, or sulpiride). Immediately following collection of the final baseline dialysate in each session, the inlet line of the probe was switched to a syringe containing the DA antagonist dissolved in ACSF. Following a 15-min discard period, to account for the "dead volume" of the perfusion line, two additional 15-min cortical dialysates were collected. FG (8.0 mg/kg, i.p.) was then administered, followed by collection of two additional cortical dialysates. Once begun, perfusion of DA antagonist through the NAC dialysis probe was not terminated until the end of the test session. Separate groups of animals were tested for each of the DA antagonists using the following three conditions in randomized order; intra-accumbens DA antagonist vehicle (aCSF)+FG, intra-accumbens DA antagonist (100 μM)+FG, and intra-accumbens DA antagonist (100 μM)+FG.

As described above, the effects of FG on cortical ACh efflux were analysed by one-way ANOVAs on ACh efflux during the two post-FG collection intervals. The effects of the antagonists alone were determined by ANOVAs on the effects of the two doses of either antagonist and vehicle on ACh efflux before FG was administered. As before, the ability of the antagonist to attenuate FG-induced increases in ACh efflux was determined by two-way ANOVAs on the effects of the two doses of either antagonist during the two post-FG collection intervals. Multiple comparisons were Bonferroni-corrected as described above.

Intra-cortical dopamine antagonists. In order to assess the potential contribution of cortical DA receptors to the effects of systematically-administered FG on cortical ACh efflux, a subset of animals from the experiment utilizing intra-accumbens perfusions, received an "additional" microdialysis session in which the higher dose of the DA antagonist (100 μM) was perfused through the PFC probe prior to FG administration. Half of the subjects were tested prior to the series of intra-accumbens dialysis sessions and half were tested after the intra-accumbens sessions. Once again, the effects of FG were determined by one-way ANOVAs, and the ability of the individual antagonist to modify the FG-induced increase in ACh release was determined by two-way ANOVAs, followed by Bonferroni-corrected multiple comparisons in the event of significant interactions.

RESULTS

Probe placements

The active zone of the probes in all animals used to measure the effects of systemic DA antagonists on FPC ACh efflux were located between 1.4–2.2 mm anterior to bregma, within layers 2–6 of cortical areas Fr1, Fr3 or Par1 in Paxinos and Watson. All rats in which the effects of intra-accumbens or intra-cortical DA antagonists on frontal cortical ACh efflux were measured were confirmed to have one microdialysis probe situated within the prelimbic, anterior cingulate or dorsal portion of the infralimbic cortex and one probe in the ipsilateral NAC. The NAC probes lay primarily within the shell region, in some cases extending to the border of the core of the NAC, olfactory tubercle, or rostral ventral pallidum. No systematic differences in the efficacy of the antagonists to attenuate FG-stimulated ACh efflux were observed as a function of probe placement.

Basal and FG 7142-stimulated acetylcholine efflux in different cortical regions

Median baseline ACh efflux did not differ between FPC (0.08 ± 0.02 pmol/min) and mPFC (0.15 ± 0.03 pmol/min; t_{12} = 1.94, P = 0.062). Systemic administration of FG (8.0 mg/kg) resulted in an elevation of ACh efflux beyond baseline levels in both cortical regions (F_{2,64} = 25.75, P = 0.0001). Multiple comparisons revealed that ACh efflux in both the 15 min (t_{13} = 5.12, P = 0.001 < 0.033) and 30 min (t_{13} = 7.86, P = 0.001 < 0.033) collections after FG were significantly greater than the last baseline interval. The magnitude of this stimulation (% change from baseline) was comparable between the two regions at both the 15 min (FPC: 164 ± 34%; mPFC: 192 ± 51%) and 30 min (FPC: 177 ± 27%; mPFC: 235 ± 40%) timepoints. These regional similarities are supported by the absence of a significant effect of CORTICAL AREA (F_{1,32} = 0.31, P = 0.583) and of an interaction between CORTICAL AREA and COLLECTION INTERVAL (F_{2,64} = 0.76, P = 0.474).

Effects of systemic SCH 23390

Figure 1 illustrates that in the "absence" of the D3-like antagonist SCH 23390, systemic administration of FG significantly elevated cortical ACh efflux during the 15- and 30-min collection intervals by approximately 150–200% above baseline (F_{2,30} = 7.03, P = 0.012). Multiple comparisons indicated that ACh efflux was significantly higher than baseline efflux during both post-FG collection intervals (15 min: t_{5} = 3.27, P = 0.022 < 0.033; 30 min: t_{5} = 6.054, P = 0.002 < 0.033). In the absence of FG, administration of the higher dose of SCH 23390 (0.3 mg/kg), did not significantly affect ACh efflux (F_{2,30} = 3.64, P = 0.065). Although the data in Fig. 1 suggest that systemic administration of SCH 23390 attenuated the ability of FG to stimulate ACh efflux, the analysis of the main effect of dose of SCH 23390 on the FG-induced increase in cortical ACh efflux did not reveal a significant effect (F_{2,10} = 2.856, P = 0.104). Likewise, the interaction between dose of SCH 23390 and collection interval was not significant (F_{2,10} = 0.142, P = 0.869).
**Effects of systemic haloperidol**

As in the previous experiment, systemic administration of FG significantly elevated cortical ACh efflux ($F_{2,10}=10.537$, $P=0.003$) during the 15- and 30-min collection intervals by approximately 150–200% above baseline (15 min: $t_{5}=3.974$, $P=0.011<0.033$; 30 min: $t_{5}=3.969$, $P=0.011<0.033$). Administration of the higher dose of haloperidol, together with the vehicle for FG did not affect ACh efflux ($F_{2,10}=0.323$, $P=0.731$). However, co-administration of FG and haloperidol revealed a significant attenuation of the FG-induced increase in ACh efflux by haloperidol ($F_{2,10}=4.603$, $P=0.038$). This effect did not differ between the two collection intervals ($F_{2,10}=0.597$, $P=0.569$). Multiple comparisons of the effects of dose of haloperidol revealed a significant effect of the lower dose of haloperidol ($t_{5}=3.096$, $P=0.027<0.033$) while the attenuating effects of the higher dose were not significant ($t_{5}=2.374$, $P=0.064>0.033$). Figure 2 suggests that administration of haloperidol almost completely attenuated the FG-induced increase in cortical ACh efflux. This observation was substantiated by a post hoc analysis indicating that cortical ACh efflux was not significantly elevated, relative to the last baseline collection, during the two post-FG collections following either the low or high dose of haloperidol (all $P>0.10$).

**Effects of locally-administered dopamine antagonists on cortical acetylcholine efflux**

**Intra-accumbens perfusions of SCH23390, haloperidol, or sulpiride.** Similar to the experiments described above, administration of FG alone reliably increased cortical ACh efflux in all three separate experiments on the effects of intra-accumbens infusions of DA antagonists (SCH: $F_{2,14}=6.134$, $P=0.012$; haloperidol: $F_{2,14}=9.675$, $P=0.002$; sulpiride: $F_{2,10}=13.061$, $P=0.002$; see Figs 3–5). Furthermore, neither antagonist affected cortical ACh efflux during the collection interval before FG was administered (order as above: $F_{2,14}=2.538$, $P=0.115$; $F_{2,14}=2.041$, $P=0.167$; $F_{2,10}=1.509$, $P=0.267$; see Figs 3–5).
Fig. 4. Effects of intra-accumbens perfusions of the D2-like antagonist haloperidol on basal and FG 7142-stimulated ACh efflux in medial prefrontal cortex (for details on the figure labels see the legend of Fig. 3). Intra-accumbens perfusion of haloperidol potently attenuated the FG-induced increases in cortical ACh efflux.

Fig. 5. Effects of intra-accumbens perfusion of the D1-like antagonist sulpiride on basal and FG 7142-stimulated ACh efflux in medial prefrontal cortex (for details on the figure labels see the legend of Fig. 3). Both concentrations of sulpiride significantly reduced the FG-stimulated cortical ACh efflux.

Fig. 6. Effects of local cortical perfusion of the D2-like antagonist SCH 23390 on basal and FG 7142-stimulated ACh efflux in medial prefrontal cortex. Perfusions of SCH 23390 (100 μM) or ACSF began immediately after the last baseline collection interval (BSL) and continued throughout the duration of the session (Figs 6–8 are constructed similar to the description in the legend of Fig. 3). The significant increase in cortical ACh efflux produced by FG was not altered by the perfusion of SCH through the cortical probe.

Similar to the effects of systemically administered SCH 23390, infusions of this D1 antagonist into the NAC did not significantly attenuate the FG-induced increases in ACh efflux ($F_{2,14}=3.388$, $P=0.063$). While Fig. 3 appears to suggest a rather potent attenuation of the effects of FG by SCH, the apparent variability of the FG-induced increases in ACh release prevented the effects of SCH to reach statistical significance (note that the figures depict S.E.Ms). The effects of SCH did not differ between collection intervals as indicated by the absence of a significant interaction between the effects of dose and collection interval ($F_{2,14}=0.0331$, $P=0.724$).

Infusions of haloperidol into the NAC, similar to the effects of systemic administration, potently attenuated the effects of FG ($F_{2,14}=11.574$, $P=0.001$). Multiple comparisons confirmed that both concentrations of haloperidol significantly reduced the FG-induced levels of ACh efflux (10 μM: $t_5=2.971$, $P=0.021<0.033$; 100 μM: $t_5=3.943$, $P=0.006<0.033$). The effects of haloperidol were identical across the two post-FG collection intervals ($F_{2,14}=1.954$, $P=0.179$; see Fig. 4).

Similar to the effects of haloperidol, infusions of sulpiride into the NAC blocked the FG-induced increases in cortical ACh efflux ($F_{2,10}=7.472$, $P=0.011$; 10 μM: $t_3=3.206$, $P=0.024<0.033$; 100 μM: $t_3=4.096$, $P=0.009<0.033$). As illustrated in Fig. 5, the potency of sulpiride did not differ between the two post-FG collection intervals ($F_{2,10}=0.667$, $P=0.534$).

**Effects of intracortical infusions of SCH23390, haloperidol, or sulpiride.** Once again, administration of FG alone reliably increased cortical ACh efflux (see open bars in Figs 6–8; SCH: $F_{2,6}=8.426$, $P=0.018$; haloperidol: $F_{2,6}=4.674$, $P=0.046$; sulpiride: $F_{2,6}=15.723$, $P=0.002$). Furthermore, similar to the effects of the antagonists following infusions into the NAC before FG was co-administered, infusions of these compounds into the cortex did not affect ACh efflux during the collection interval before FG was administered (sequence as above: $F_{1,5}=2.974$, $P=0.185$; $F_{1,4}=1.083$, $P=0.357$; $F_{1,4}=0.352$, $P=0.585$). Furthermore, neither agonist affected the FG-induced increases on cortical ACh efflux when given through the probe (sequence as above: effects of the antagonist versus ACSF over the two post-FG collection intervals: $F_{1,5}=0.681$, $P=0.470$;
**D_1-D_2** regulation of cortical ACh release

![Graph showing effects of local cortical perfusion of D_1-like antagonist haloperidol on basal and FG 7142-stimulated ACh efflux in medial prefrontal cortex.](image)

**Fig. 7.** Effects of local cortical perfusion of the D_1-like antagonist haloperidol on basal and FG 7142-stimulated ACh efflux in medial prefrontal cortex. Perfusion of haloperidol through the cortical probe did not affect the FG-induced increase in ACh efflux.

![Graph showing effects of local cortical perfusion of D_2-like antagonist sulpiride on basal and FG 7142-stimulated ACh efflux in medial prefrontal cortex.](image)

**Fig. 8.** Effects of local cortical perfusion of the D_2-like antagonist sulpiride on basal and FG 7142-stimulated ACh efflux in medial prefrontal cortex. Similar to the other two antagonists, perfusion of sulpiride through the cortical probe did not affect FG-stimulated ACh efflux.

\[ F_{1,4} = 5.014, P = 0.089; F_{1,5} = 0.414, P = 0.555 \]. Collection interval did not affect ACh release in these experiments, and interactions between the effects of collection interval and the intracortical infusion of the DA antagonists were not found (all \( P > 0.12 \); see Figs 6–8).

**DISCUSSION**

These experiments were designed to assess the D_1- and D_2-receptor regulation of basal and stimulated frontal cortical ACh release. Several conclusions can be drawn from the results of these experiments. First, regardless of the route of administration, D_1 or D_2 antagonists did not affect “basal” ACh efflux in frontal cortex. Second, and consistent with our previous studies, administration of the BZR partial inverse agonist FG 7142 markedly enhanced ACh efflux in frontal cortex. Third, the magnitude and time-course of FG 7142-stimulated ACh efflux was comparable between FPC and mPFC. Fourth, systemic administration of D_2, but not D_1, antagonists significantly attenuated FG 7142-stimulated cortical ACh efflux. Fifth, the intra-accumbens perfusion of D_2 antagonists blocked FG 7142-stimulated ACh efflux. Finally, neither class of DA antagonist affected FG 7142-stimulated ACh efflux when perfused locally into the mPFC although intracortical haloperidol exhibited a trend towards attenuation of stimulated ACh efflux. The discussion below focuses on methodological issues related to the validity of these findings, the mechanisms underlying FG 7142 stimulated ACh release, neurotransmitter interactions within NAC that might mediate the effects of DA antagonists on cortical ACh release, and the relevance of these transmitter interactions to contemporary theories of psychopathology.

**Methodological issues**

The first issue relates to the use of the within-subject, repeated perfusion microdialysis design. The power of this experimental design lies in the ability to use each animal as its own experimental control. Thus, each animal received the vehicle as well as the two doses of antagonist (for its respective drug group). Moreover, the relative potencies of intra-accumbens vs intracortical antagonist administrations were compared within the same subjects. We have repeatedly assessed the validity of this design in microdialysis studies of cortical ACh efflux as well as the dopaminergic modulation of striatal ACh or GABA efflux. In each of these studies, the ability of behavioral or pharmacological manipulations to increase or decrease transmitter efflux did not interact with dialysis session. Moreover, in each case, the ability of local tetrodotoxin perfusions to suppress transmitter efflux was nearly identical in the first and fourth dialysis sessions. Thus, the repeated perfusion design employed in the present experiments appears to be a valid and sensitive method for assessment of the dopaminergic regulation of cortical ACh efflux.

A related methodological issue is the use of a cholinesterase inhibitor in the dialysis studies and its impact on the responsivity of cortical cholinergic inputs. The use of concentric dialysis probes and relatively short collection intervals for studying cortical ACh efflux necessitated the inclusion of a cholinesterase inhibitor in the aCSF. The use of a cholinesterase inhibitor such as neostigmine is not without controversy as the non-physiological increases in extracellular ACh may dampen the responsivity of cholinergic neurons via either auto-receptor stimulation or potential “long-loop”
feedback mechanisms. This consideration was underscored in a recent study on the dopaminergic modulation of striatal ACh efflux on which “qualitatively” different results were obtained as a function of the concentration of neostigmine employed.\textsuperscript{18} While this issue remains unsettled, we have recently determined the ability of tactile stimulation to increase frontoparietal and mPFC ACh efflux under two concentrations of neostigmine (0.05 and 0.5 μM).\textsuperscript{29} While “basal” ACh efflux was predictably different under the two neostigmine conditions, “stimulated” efflux (as a percent change from baseline) did not differ (in either region) under the two concentrations. Similar results have recently been reported with respect to handling-induced increases in hippocampal ACh efflux.\textsuperscript{42} In addition, the ability of BZR ligands to bidirectionally modulate stimulated cortical ACh efflux has also been replicated at different levels of acetylcholinesterase inhibition.\textsuperscript{46,47} and the ability of FG 7142 to stimulate cortical ACh efflux has been observed with aCSF solution containing up to 5 mM neostigmine (unpublished observations). Collectively, these data suggest that the use of neostigmine in the present experiment did not limit the validity of these experiments in documenting DA-mediated changes in cortical ACh efflux.

**FG 7142-stimulated acetylcholine efflux**

The ability of the systemically administered BZR partial inverse agonist FG 7142 to stimulate ACh efflux in mPFC was found to be similar in magnitude to the drug’s ability to increase efflux in frontoparietal cortex.\textsuperscript{20,40} The FG 7142-induced increases in cortical ACh release may be based on multiple neuronal mechanisms. FG 7142 might stimulate cortical ACh efflux directly by reducing GABAergic transmission in the basal forebrain. This hypothesis was corroborated by the finding that intrabasalis injections of another, more soluble BZR inverse agonist, β-CCM increased cortical ACh efflux.\textsuperscript{48}

In addition to these more direct effects on basal forebrain cholinergic neurons, systemic injections of FG 7142 have been shown to increase indirect measures of dopaminergic transmission\textsuperscript{14,24} or dopamine efflux\textsuperscript{6} in PFC. The available data on the effects of FG 7142 on NAC DA transmission are less consistent. Several microdialysis studies have reported that systemic administration of FG 7142 or β-CCE, a BZR full inverse agonist, increased DA efflux in NAC.\textsuperscript{29,37,51} although Brose and colleagues,\textsuperscript{8} using the voltammetric signal for the DA metabolite homovanillic acid as an index of DA release, found that FG 7142 did not enhance dopaminergic transmission in NAC. Interestingly, local administration of FG 7142 directly into the NAC also did not increase DA efflux, although only a single dose (10 μM) was tested.\textsuperscript{81} One potential source for these inconsistent findings may be the subregion of the NAC being studied. Horger and colleagues\textsuperscript{29} reported that while systemic administration of FG 7142 did not increase DA utilization in tissue punches from whole accumbens or the core region, there was an increase in the dihydroxyphenylacetic acid/DA ratio in the shell region. This is important because the shell region of the accumbens is more closely linked to mesolimbic afferents\textsuperscript{29} and is the principal site of GABAergic efferents to basal forebrain.\textsuperscript{78,80} Finally, FG 7142 may enhance DA release directly in basal forebrain\textsuperscript{53} and thereby enhance cortical ACh efflux as a result of a D\textsubscript{1}-mediated disinhibition of corticopetal neurons.\textsuperscript{43} Thus, while the mechanisms underlying the ability of FG 7142 to stimulate cortical ACh release are complex, it is important to recognize that the goal of this study was to use FG 7142 as a means for stimulating cortical ACh efflux so that the potential role for cortical and NAC D\textsubscript{1} or D\textsubscript{2} modulation could be assessed.

**Basal and stimulated acetylcholine efflux in different cortical regions**

One of the issues addressed by these experiments was the extent to which ACh efflux, under basal and stimulated conditions, was comparable in different cortical regions. The selection of PFC was directed by our previous studies on the ability of BZR ligands to bidirectionally modulate cortical ACh efflux whether administered systemically\textsuperscript{35,47} or directly into basal forebrain.\textsuperscript{48} The rationale for the selection of mPFC was two-fold. First, the PFC provides inputs to the basal forebrain,\textsuperscript{27,66,67} to the shell region of the NAC and to the ventral tegmental area which provides dopaminergic inputs to mPFC and NAC. As such, the mPFC may be yet another site in which FG 7142 or DA receptor ligands may transsynaptically modulate cortical ACh release. Second, our initial studies on the attentional and instrumental performance-associated increases in cortical ACh release have been conducted in the mPFC and data from both human\textsuperscript{51} and other primate\textsuperscript{14} studies strongly implicate the medial frontal cortical areas in cognitive processing.\textsuperscript{31,38,50,55,56}

The anatomical organization suggests that activation of cortical cholinergic afferents results in a rather widespread release of ACh in all cortical areas and layers.\textsuperscript{63} Recent microdialysis studies have reported that electrical stimulation of basal forebrain,\textsuperscript{33} the transition from light to dark phases,\textsuperscript{32} or tactile stimulation\textsuperscript{44} result in comparable ACh efflux in primary visual, somatosensory, and motor cortical areas. The present results support this general assertion by demonstrating that both the magnitude and time-course of FG 7142-stimulated ACh were nearly identical in frontoparietal and prefrontal cortex. While it remains to be “simultaneously tested” in the same animal, accumbens DA-mediated regulation of FG 7142-stimulated release (see below) is likely
to yield a similar modulation of cortical ACh throughout the cortical mantle.

**Dopamine modulation of cortical acetylcholine efflux**

Ample evidence documenting the dopaminergic regulation of cortical ACh release is available. Systemic injections of amphetamine, apomorphine, or selective D₁ agonists (CY 208-243, A-77636) stimulate ACh efflux in frontal cortex. The potential contributions of D₂ receptor activity are not as clear. Systemic administration of the D₂ agonists quinpirole or (+)-4-propyl-9-hydroxynaphthoxazine failed to stimulate ACh efflux in frontal cortex. The contributions of “endogenous” DA to modulate cortical ACh release has been largely studied by determining the effects of systemic and intracortical administration of selective D₁ and D₂-like antagonists on amphetamine-stimulated ACh efflux. Systemic administration of either the D₁ antagonist SCH 23390 or the D₂ antagonist haloperidol attenuated amphetamine-induced cortical ACh efflux suggesting a role for both DA receptor subtypes in the effects of amphetamine. Unexpectedly, while SCH 23390 attenuated apomorphine-induced cortical ACh efflux, the D₂ antagonist raclopride was not effective. It is not clear how much should be made of this distinction between D₁ and D₂ antagonism as only a single dose of each drug was tested and there was a marked trend toward a blockade following raclopride.

Our data on the effects of systemically administered D₁ and D₂ antagonists on FG 7142-stimulated cortical ACh release are somewhat consistent with the results discussed above. Administration of the D₂ antagonist haloperidol markedly attenuated stimulated ACh efflux whereas the D₁ antagonist SCH 23390 did not. Neither D₁ or D₂ antagonists affected “basal” cortical ACh efflux, a finding not consistent with previous studies demonstrating that the D₁ antagonist SCH 23390 or haloperidol slightly decreased basal cortical ACh efflux. While the doses of SCH 23390 and haloperidol in these earlier studies were similar to the doses in the present study, differences in the microdialysis procedures and the degree of habituation of animals prior to drug administration, as well as the various drugs used to stimulate ACh efflux, may account for these differences.

**Role of accumbens dopamine receptors in the regulation of cortical acetylcholine release**

The results of these experiments strongly support the position that dopaminergic transmission within the NAC plays an important modulatory role in FG 7142-stimulated cortical ACh efflux. The data indicate that D₂ receptors participate in this regulation as local perfusion of NAC with the D₂ antagonist sulpiride markedly attenuated stimulated ACh efflux and haloperidol completely blocked the effects of FG 7142. These data are consistent with the more indirect demonstration that unilateral depletions of mesotelencephalic DA with the neurotoxin 6-hydroxydopamine (6-OHDA) attenuate the ability ofamphetamine to stimulate ACh efflux in frontal cortex. Infusions of the D₁ antagonist SCH 23390 also appeared to attenuate stimulated ACh efflux however, the variability in the magnitude of the FG effect precluded observing significant differences.

The neuronal circuitry that may underlie the ability of NAC DA receptors to modulate basal forebrain corticopetal cholinergic innervation has been fairly well-characterized. The principal output pathway of the NAC is the GABAergic projection to the basal forebrain. Direct infusions of DA agonists into the NAC increase the firing rates of basal forebrain neurons, presumably via a reduction in GABA release. Consistent with this model, amphetamine lowers GABA efflux in ventral pallidum (VP). Moreover, the ability of apomorphine to reduce GABA in VP was enhanced following 6-OHDA-induced DA depletions in NAC. Recently, it has been demonstrated that the acute systemic administration of haloperidol or clozapine increases ACh efflux in VP. While dopaminergic transmission within NAC appears to ultimately inhibit GABA release in basal forebrain, the precise anatomical substrates responsible for the release of similar effects of D₁ and D₂ receptor activity on these NAC efferents, as well as the relative roles of the excitatory inputs driving these GABA projections neurons remain to be determined.

**Role of cortical dopamine receptors in the regulation of cortical acetylcholine release**

Local perfusion of the prefrontal cortex with D₁ or D₂ antagonists did not attenuate FG 7142-induced cortical ACh efflux. There was a trend toward an inhibitory effect of haloperidol, however this did not reach significance and was not replicated by local perfusion with the more selective D₂ antagonist sulpiride. While care should be taken not to overinterpret the results of a single dose of antagonist, these doses, when delivered into the NAC, did attenuate stimulated cortical ACh efflux. Moreover, the ineffectiveness of intracortical perfusions of D₁ or D₂ antagonists in this study are consistent with previous reports on the inability of locally-administered amphetamine to stimulate cortical ACh efflux.

**Modulation of cortical acetylcholine release in psychopathology**

The present results indicate that the neuropharmacological effects of FG 7142, which have been widely considered to model DAergic responses to psycho-
logical stress\textsuperscript{30} include profound changes in the cholinergic as well as the dopaminergic inputs to the cerebral cortex. Moreover, the activation of mesolimbic and mesocortical DA systems postulated to occur with stress,\textsuperscript{29,30,32} psychostimulant use\textsuperscript{15,16} and psychosis\textsuperscript{31} is likely to interact with the activation of cortical ACh\textsuperscript{58,60,64} in the mediation of the cognitive deficits that accompany or mediate these syndromes. Contemporary theories on the neurobiology of schizophrenia postulate that dysfunctions in the developing telencephalon alter the inputs to NAC, resulting in an abnormal upregulation in DA receptor activity.\textsuperscript{70} Consequent changes in basal forebrain GABAergic transmission in schizophrenia are consistent with the observation that disruptions of prepulse inhibition following intra-accumbens administration of DA agonists are reversed by infusions of the GABA agonist muscimol into the basal forebrain.\textsuperscript{28} The role of GABAergic projections from NAC in the regulation of cortical ACh release is of particular interest given hypotheses about the role of cortical cholinergic inputs in the development of positive symptoms in schizophrenia.\textsuperscript{58,60,64}

CONCLUSION

In summary, the results of these experiments demonstrate that the BZR partial inverse agonist, FG 7142, stimulates ACh efflux in FPC and mPFC. This stimulated efflux can be attenuated by systemic or intra-accumbens, but not intracortical, administration of D\textsubscript{2} antagonists. These data provide the first "direct" functional linkage between dopaminergic receptor activity in NAC and the release of ACh in prefrontal cortex and, as such, provide a basis for an extension of current theories of schizophrenia beyond mesolimbic dopaminergic mechanisms.

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