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Astrocyte-derived kynurenic acid modulates basal and evoked cortical acetylcholine release

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Abstract

We tested the hypothesis that fluctuations in the levels of kynurenic acid (KYNA), an endogenous antagonist of the α7 nicotinic acetylcholine (ACh) receptor, modulate extracellular ACh levels in the medial prefrontal cortex in rats. Decreases in cortical KYNA levels were achieved by local perfusion of *S*-ESBA, a selective inhibitor of the astrocytic enzyme kynurenine aminotransferase II (KAT II), which catalyses the formation of KYNA from its precursor L-kynurenine. At 5 mM, *S*-ESBA caused a 30% reduction in extracellular KYNA levels, which was accompanied by a two-threefold increase in basal cortical ACh levels. Co-perfusion of KYNA in the endogenous range (100 nM), which by itself tended to reduce basal ACh levels, blocked the ability of *S*-ESBA to raise extracellular ACh levels. KYNA perfusion (100 nM) also prevented the evoked ACh release caused by D-amphetamine (2.0 mg/kg). This effect was duplicated by the systemic administration of kynurenine (50 mg/kg), which resulted in a significant increase in cortical KYNA formation. Jointly, these data indicate that astrocytes, by producing and releasing KYNA, have the ability to modulate cortical cholinergic neurotransmission under both basal and stimulated conditions. As cortical KYNA levels are elevated in individuals with schizophrenia, and in light of the established role of cortical ACh in executive functions, our findings suggest that drugs capable of attenuating the production of KYNA may be of benefit in the treatment of cognitive deficits in schizophrenia.

Introduction

Cognitive deficits, affecting sensory gating, working memory, cognitive flexibility and attention, represent a core symptom cluster in schizophrenia, which is largely resistant to conventional pharmacotherapeutic interventions (Keefe, 2007; Kerns *et al.*, 2008). The pathophysiology of these broad and fundamental deficits is intricate and involves numerous brain regions, including the hippocampus (Boyer *et al.*, 2007), the frontal cortex (Lewis *et al.*, 2005; Sarter *et al.*, 2005; Tan *et al.*, 2007), and the striatal complex (Laruelle *et al.*, 2003). Likewise, impaired executive functions in schizophrenia have been linked to dysregulations in various neurotransmitter systems, including dopamine (Laruelle *et al.*, 2003; Toda & Abi-Dargham, 2007), glutamate (Krystal *et al.*, 2003; Moghaddam, 2003; Coyle, 2006), γ-aminobutyric acid (GABA) (Lewis & Moghaddam, 2006), and acetylcholine (ACh) (Hyde & Crook, 2001; Sarter *et al.*, 2005), throughout a diffusely distributed neural system.

Abnormal regulation of the nicotinic ACh receptor (nAChR), in particular the α7nAChR, is believed to play an especially significant role in the cognitive endophenotype of schizophrenia [for a review, see Martin *et al.* (2004)]. This is supported by studies in humans demonstrating an association between the α7nAChR gene and disease transmission (Leonard & Freedman, 2006; Martin *et al.*, 2007) and the fact that the α7nAChR protein is reduced in frontal, but not parietal, cortex in post-mortem tissue from individuals with the disease (Guan *et al.*, 1999; Severance & Yolken, 2008). These findings are complemented by a host of pre-clinical studies showing that nAChR function in the prefrontal cortex (PFC), an area of profound interest to schizophrenia research (Tan *et al.*, 2007; Kerns *et al.*, 2008), is critically involved in distinct cognitive phenomena such as working memory (Hasselmo & Stern, 2006) and attention (Sarter *et al.*, 2005). Together, this body of work has stimulated the development of drugs targeting the cholinergic system as possible adjunctive pharmacotherapeutics in schizophrenia. Indeed, partial and full nAChR agonists show some promise in the treatment of cognitive deficits in schizophrenia (Koike *et al.*, 2005; Olincy *et al.*, 2006; Olincy & Stevens, 2007; Buchanan *et al.*, 2008).

Manipulation of kynurenic acid (KYNA) offers an alternative approach to the modulation of α7nAChR function. A non-competitive α7nAChR antagonist (Hilmas *et al.*, 2001), KYNA acts as an allosteric inhibitory factor at a site that is very similar to that activated by the cognition enhancer galantamine (Lopes *et al.*,

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2007). KYNA is a metabolite of the kynurenine pathway (KP) of tryptophan degradation, and is formed by the irreversible transamination of the pivotal KP metabolite L-kynurenine (Turski *et al.*, 1989). In the mammalian brain, KYNA can be produced by several kynurenine aminotransferases (KATs), of which KAT II appears to be the most functionally relevant (Guidetti *et al.*, 2007a). This enzyme is almost exclusively localized in astrocytes (Guidetti *et al.*, 2007b), which promptly release newly formed KYNA into the extracellular milieu (Turski *et al.*, 1989). Interestingly, KYNA levels are significantly elevated in the PFC (Schwarcz *et al.*, 2001) and cerebrospinal fluid (CSF) (Erhardt *et al.*, 2001) of individuals with schizophrenia, and recent evidence suggests that an impairment in KP metabolism resulting in increased KYNA levels in the cerebral cortex may be causally involved in the cognitive endophenotype of the disease (Sathyasaikumar *et al.*, 2008).

Probably by virtue of its ability to antagonize presynaptic α7nAChRs, nanomolar concentrations of KYNA decrease extracellular glutamate and dopamine levels in various regions of the rat brain, including the medial PFC (mPFC) (Carpenedo *et al.*, 2001; Rassoulpour *et al.*, 2005; Grilli *et al.*, 2006; Wu *et al.*, 2006, 2008). Conversely, reductions in endogenous KYNA synthesis enhance the extracellular levels of these neurotransmitters (Wu *et al.*, 2006, 2008), suggesting an interesting bi-directional modulation of glutamatergic and dopaminergic transmission by endogenously formed KYNA.

Transmitter interactions within the PFC (Nelson *et al.*, 2005; Yang & Chen, 2005) and in the distributed neural system involving the PFC, the nucleus accumbens and the basal forebrain (Zmarowski *et al.*, 2005; Brooks *et al.*, 2007) suggest that fluctuations in KYNA levels, and subsequent changes in the activity of nAChR function, may also modulate the extracellular levels of ACh in the PFC. As such KYNA-induced changes in cortical cholinergic transmission would have interesting implications for cognitive processes and therapies. We tested this hypothesis in the present *in vivo* microdialysis study in rats using KYNA itself, the KYNA bioprecursor kynurenine and the specific KAT II inhibitor *S*-ethylsulfonylbenzoylalanine (*S*-ESBA) (Pellicciari *et al.*, 2006) as experimental tools.

Materials and methods

Chemicals

L-Kynurenine (sulfate form; 'kynurenine'), KYNA, D-amphetamine, lidocaine and chloramphenicol were obtained from Sigma Chemical Co. (St Louis, MO, USA). Conventional laboratory chemicals and reagents were purchased from commercial suppliers and were of the highest available purity. *S*-ESBA was synthesized as described previously (Pellicciari *et al.*, 2006).

Animals

Male Wistar rats (Charles River, Wilmington, MA, USA; 300–400 g) were used in all studies. Animals were maintained on a 12 : 12 h light/dark cycle (lights on: 0600 h) in a temperature- and humidity-controlled, AAALAC-approved animal care facility. Animals were individually housed in plastic cages lined with corn cob bedding (Harlan Teklad, Madison, WI, USA) and had access to food and water *ad libitum*. All procedures were approved by the Ohio State University or University of Maryland Institutional Animal Care and Use Committees in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Surgery

Animals were acclimated to the microdialysis testing environment for 3 days prior to surgery (minimum of 4 h/day) in clear plastic bowls (35 cm height × 38 cm diameter; CMA, Stockholm, Sweden) lined with corn cob bedding. The rats were anesthetized using isoflurane gas (2%, 0.6 L/min, O_2 delivery), and unilaterally implanted (hemispheres counterbalanced) with microdialysis guide cannulae (0.38 mm outside diameter; 3.0 mm membrane tip; Sci Pro Inc., Sanborn, NY, USA) into the mPFC ($A = 4.2$, $L = \pm 0.6$, $V = 0.6$ mm, 20º rostral angle relative to bregma and dura). All guide cannulae were fixed to the skull using stainless steel screws and dental acrylic. Stylets, ending flush with the guide cannula, were inserted to prevent cannula occlusion. The surgical site was swabbed with a topical antibiotic ointment (lidocaine, 5%), and animals received a prophylactic dose of the antibiotic chloramphenicol (100 mg/mL; 0.125 mL, subcutaneous). Following surgery, animals were returned to their home cages and allowed to recover for 1–4 days prior to microdialysis testing in an awake, freely moving state.

Microdialysis

In experiments designed to determine the effects of local *S*-ESBA or systemic kynurenine administration on extracellular KYNA levels, animals underwent a single microdialysis session. All studies measuring drug effects on extracellular ACh were conducted using repeated perfusions, with each animal receiving different treatments, in counterbalanced order, every other day. This testing paradigm has the advantage of decreasing variability among treatment conditions, because each subject serves as its own control (Bruno *et al.*, 1999; Zmarowski *et al.*, 2005; Brooks *et al.*, 2007).

Animals were introduced into the testing bowls, stylets were removed, probes (Sci Pro Inc., 0.2 mm outside diameter, 3 mm membrane tip for mPFC) were inserted into each guide, and inlet and outlet lines were attached to a two-channel liquid swivel (Instech, Plymouth Metting, PA, USA). Probes were continuously perfused with artificial CSF (aCSF) containing 166.5 mM NaCl, 27.5 mM $NaHCO_3$, 2.4 mM KCl, 1.2 mM $CaCl_2$, 0.5 mM Na_2SO_4, 0.5 mM KH_2PO_4 and 1.0 mM glucose (pH 7.1) at a flow rate of 1.25 μL/min, and microdialysate fractions were collected periodically as indicated. No acetylcholinesterase inhibitor was used in any experiment. A 3-h washout period was used after probe insertion to stabilize baseline values. Dialysis samples were stored at −80°C until the day of the assay. Data were not adjusted for recovery from the microdialysis probe.

High-performance liquid chromatography (HPLC) analysis

ACh was analysed using HPLC with electrochemical detection. Fifteen microliters of each microdialysis sample were injected using an autosampler (ESA Inc., Chelmsford, MA, USA), and ACh and choline were isocratically separated on a reverse phase UniJet microbore analytical column (1 × 530 mm, 10 μm; BAS Inc., West Lafayette, IN, USA), using a mobile phase containing 35 mM Na_2HPO_4, 0.44 mM EDTA and 1.0% of the microbicide Proclin, pH 8.5, at a flow rate of 0.15 mL/min. A post-column immobilized enzyme reactor (1 × 50 mm) containing acetylcholinesterase and choline oxidase was used to degrade ACh into H_2O_2 (Potter *et al.*, 1983), which was then quantified using a peroxidase-wired glassy carbon electrode (−200 mV). Peaks were quantified using EZCHROME ELITE software (ESA Inc.).

KYNA was determined by HPLC with fluorescence detection (excitation wavelength 344 nm; emission wavelength 398 nm; LC 240 fluorescence detector, Perkin Elmer, Beaconsfield, UK). To this end, 10 μL of each microdialysis sample was diluted with 5 μL of 0.1 M HCl, and 10 μL of the resulting solution was injected onto a 3-μm C_{18} reverse phase column (80 × 4.6 mm; ESA Inc.). KYNA was isocratically eluted at a flow rate of 1 mL/min, using a mobile phase containing 200 mM zinc acetate and 5% acetonitrile, pH 6.2 (Swartz *et al.*, 1990).

Histology

Following microdialysis, animals were deeply anesthetized with sodium pentobarbital [50 mg/kg, intraperitoneal (i.p.)] and transcardially perfused with 0.9% heparinized saline followed by 10% formalin. Brains were removed, stored in fixative for 24 h, and transferred to a 30% sucrose solution for 3 days. Cresyl violet-stained cryostat sections (50 μm) were examined under a light microscope. Only animals with injection cannula and microdialysis probe placements in the mPFC were used for analysis (Fig. 1).

Data analysis

Baseline values of extracellular ACh were compared using one-way ANOVA. When no significant differences were seen as a function of dialysis session or treatment group, drug effects were expressed as a percentage of the basal values. The results of experiments involving ACh efflux were analysed using a two-way, within-subjects ANOVA with drug group and time as within-subjects measures, and the Huynh–Feldt correction was utilized to reduce type I errors associated with repeated measures ANOVAs (Vasey & Thayer, 1987). Extracellular KYNA levels were analysed using a one-way, within-subjects (time) ANOVA. When necessary, *post hoc* comparisons were conducted using *t*-tests. All statistical tests were performed using SPSS for Windows (version 15.0), and significance was defined as $P < 0.05$ in all analyses.

Results

Effects of local KAT II inhibition on extracellular KYNA levels

The first experiment was designed to confirm the ability of *S*-ESBA to reduce extracellular cortical KYNA levels *in vivo*. This compound was previously shown to inhibit rat KAT II *in vitro* (IC_{50}, 6.1 μM) without affecting the activity of KAT I or other pivotal enzymes of the KP up to at least 1 mM (Pellicciari *et al.*, 2006). *S*-ESBA (5 mM) was introduced by reverse dialysis, assuming an effective concentration of ~1 mM in the tissue (Höcht *et al.*, 2007), and microdialysis samples were collected at 30-min intervals. After 2 h, the perfusion fluid was switched back to aCSF ($n = 4$).

As illustrated in Fig. 2, perfusion of the KAT II inhibitor decreased extracellular KYNA levels in the mPFC by ~30% within 1 h after the onset of drug perfusion ($F_{11,44} = 8.64$, $P < 0.01$). After the withdrawal of *S*-ESBA, KYNA levels gradually returned to baseline values, reaching pre-drug values 120 min later (fraction no. 12 in Fig. 2; $P > 0.05$).

Effects of KYNA fluctuations on basal ACh levels

Next, we examined the effects of *S*-ESBA on extracellular ACh levels ($n = 7$). As shown in Fig. 3, the KAT II inhibitor caused a dose-dependent increase in ACh levels (dose, $F_{3,12} = 9.21$, $P = 0.02$; dose × time, $F_{24,96} = 2.46$, $P = 0.04$). Pairwise comparisons between the doses revealed that each concentration of *S*-ESBA produced elevated ACh levels relative to aCSF controls. *Post hoc* analyses confirmed that perfusion of *S*-ESBA elevated ACh levels at each time point. In addition, the levels of ACh following the highest concentration of *S*-ESBA (5 mM) were significantly greater than those seen following the lowest concentration (500 μM; all *P*-values < 0.05).

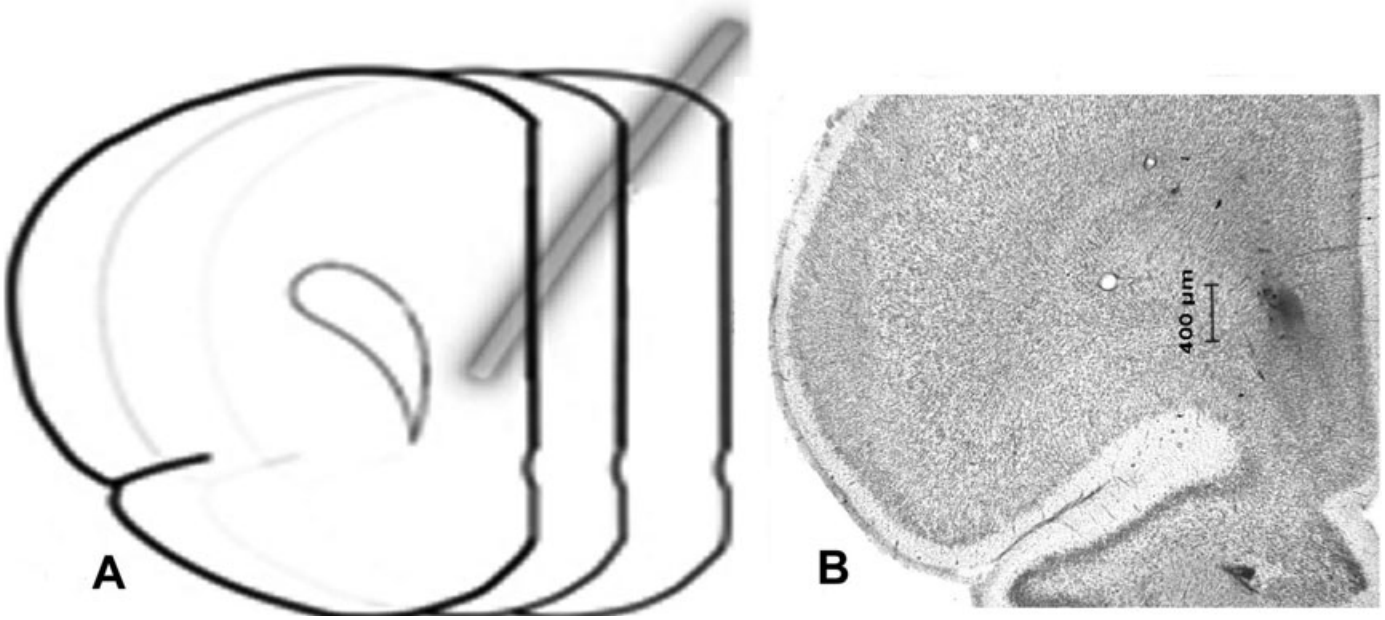

FIG. 1. Schematic diagram (A) and representative photomicrograph (B) of microdialysis probe placement in the medial prefrontal cortex (mPFC). Guides were implanted so that the membrane tip (2.0 mm) of the inserted probes terminated in the mPFC (area to right of scale bar in B).

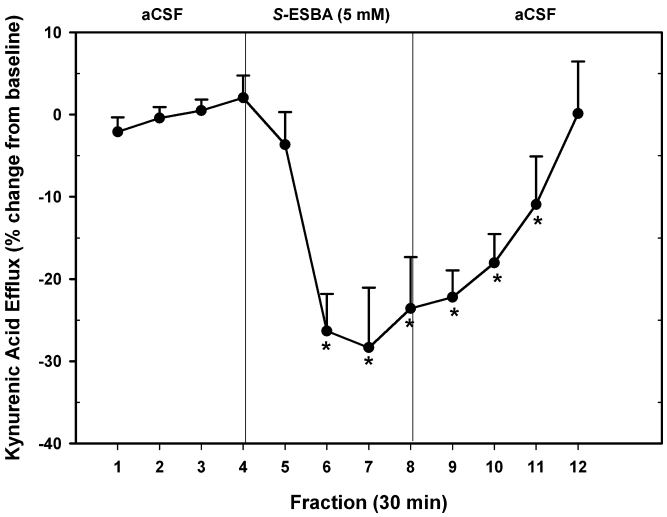

FIG. 2. Local perfusion of the kynurenine aminotransferase II inhibitor *S*-ethylsulfonylbenzoylalanine (*S*-ESBA) (5 mM) reversibly reduces kynurenic acid (KYNA) levels in the medial prefrontal cortex. KYNA levels gradually recover to basal levels following termination of the *S*-ESBA perfusion. Data are the mean + SEM of four animals. aCSF, artificial cerebrospinal fluid. $^*P < 0.01$ vs. the last baseline fraction.

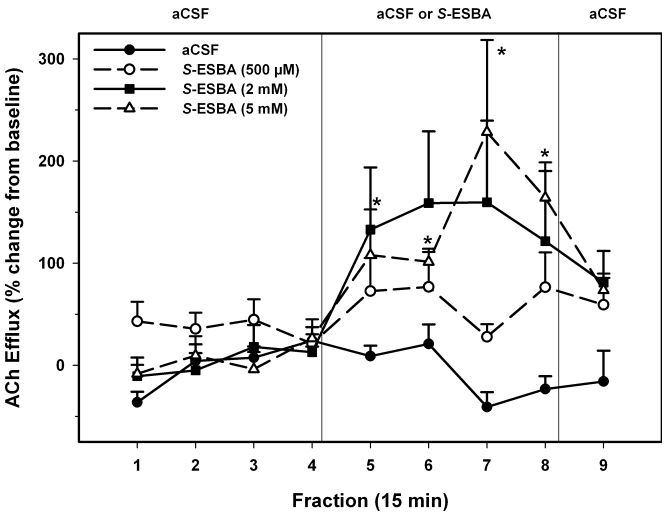

FIG. 3. Local perfusion of artificial cerebrospinal fluid (aCSF) (control) or the kynurenine aminotransferase II inhibitor *S*-ethylsulfonylbenzoylalanine (*S*-ESBA) (500 µM, 2 and 5 mM) increases extracellular acetylcholine (ACh) levels in the medial prefrontal cortex. ACh levels were elevated above aCSF control values during the infusion of each of the three concentrations of *S*-ESBA. Levels approached basal values soon after termination of the *S*-ESBA perfusion. Data are the mean + SEM of seven animals. $^*P < 0.05$, 5 mM vs. 500 µM *S*-ESBA.

To investigate the specificity of this effect, rats received, every other day and in counterbalanced order, intracortical perfusions of aCSF, KYNA (100 nM), *S*-ESBA (5 mM) or KYNA + *S*-ESBA ($n = 7$). After the collection of baseline samples, four microdialysates were collected at 15-min intervals, under various conditions, and this was followed by perfusion with aCSF for an additional three fractions.

As the basal values of recovered ACh (average of all samples in these rats: 4.1 ± 0.8 fmol per 15 µL) did not change significantly as a function of treatment ($F_{3,18} = 3.18$, $P = 0.08$) or dialysis session ($F_{3,18} = 0.81$, $P = 0.47$), the effects of experimental interventions were expressed as a percentage of baseline controls. As illustrated in Fig. 4, perfusion of aCSF did not alter ACh levels, whereas fluctuations in KYNA levels resulted in systematic changes in extracellular ACh (effect of compound, $F_{3,18} = 29.81$, $P < 0.001$; effect of compound × time, $F_{30,180} = 2.79$, $P = 0.002$). Perfusion of KYNA (100 nM) caused a trend towards reduced basal ACh levels, whereas a reduction of KYNA synthesis by perfusion with *S*-ESBA (see Fig. 2) produced a marked and sustained increase in cortical ACh levels ($F_{1,6} = 44.83$, $P = 0.001$), which was already evident in the first 15-min sample after the introduction of the enzyme inhibitor ($t_6 = -3.77$, $P = 0.01$). This ability of *S*-ESBA to stimulate cortical ACh levels appears to be directly linked to its capacity to decrease KYNA levels, as co-perfusion with 100 nM KYNA abolished the effect of *S*-ESBA ($F_{1,6} = 50.52$, $P < 0.001$). In other words, extracellular ACh levels did not differ from aCSF controls when the effect of the KAT II inhibitor on endogenous KYNA was neutralized ($P > 0.05$).

Effects of KYNA on amphetamine-induced ACh release

We next investigated the effect of an intracortical KYNA perfusion on the evoked release of ACh, stimulated by the systemic administration of D-amphetamine (Arnold *et al.*, 2000). Animals were tested four times, with the various treatments being administered every other day in a fully crossed, counterbalanced design ($n = 8$). Thus, following the four baseline collections, rats received an intracortical perfusion of aCSF + saline (0.9%, i.p.), KYNA (100 nM) + saline (0.9%), aCSF + D-amphetamine (2.0 mg/kg, i.p.), or KYNA + D-amphetamine, and four 15-min fractions were collected (Fig. 5). Three additional collections were taken, during which only aCSF was perfused into the mPFC.

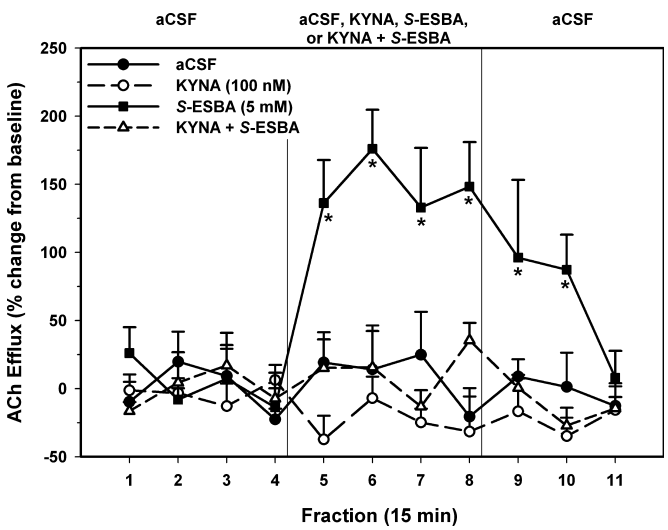

FIG. 4. Local fluctuations in kynurenic acid (KYNA) regulate extracellular acetylcholine (ACh) levels in the medial prefrontal cortex. Either KYNA was applied by reverse dialysis (100 nM) or its endogenous levels were decreased by local perfusion of *S*-ethylsulfonylbenzoylalanine (*S*-ESBA) (5 mM; see Fig. 2). Animals received each of the four drug treatments in counterbalanced order, as described in the text. Perfusion of KYNA alone caused a trend towards a decline in basal levels. The *S*-ESBA-induced increase in extracellular ACh was abolished by co-perfusion of KYNA (100 nM). Data are the mean + SEM of seven animals. $^*P < 0.01$ vs. artificial cerebrospinal fluid (aCSF) control or KYNA + *S*-ESBA.

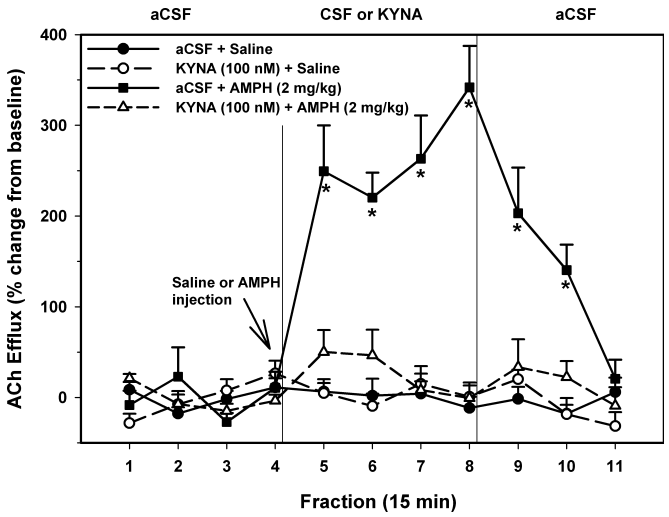

FIG. 5. Local perfusion of kynurenic acid (KYNA) (100 nM) blocks the ability of systemically administered D-amphetamine (AMPH; 2.0 mg/kg, intraperitoneal) to increase extracellular acetylcholine (ACh) levels in the medial prefrontal cortex. Rats were tested in each of four drug conditions, in counterbalanced order, as described in the text. Data are the mean + SEM of eight animals. $*P < 0.01$ vs. artificial cerebrospinal fluid (aCSF) or KYNA + D-amphetamine.

Mean basal ACh levels for all animals were 7.0 ± 1.9 fmol per 15 μL. No significant differences in baseline were observed between the four treatment conditions ($F_{3,21} = 0.31$, $P = 0.67$) or dialysis sessions ($F_{3,21} = 0.11$, $P = 0.92$). Thus, subsequent data were expressed and analysed as a percentage change from baseline values (Fig. 5). Overall, ACh levels differed significantly as a result of drug treatment (drug, $F_{3,21} = 79.12$, $P < 0.001$; drug × time, $F_{30,210} = 9.28$, $P < 0.001$). ACh levels following the local perfusion of KYNA/saline did not differ from those in the aCSF/saline control condition ($F_{1,7} = 0.02$, $P = 0.90$). In contrast, D-amphetamine caused a marked and long-lasting increase in extracellular ACh (drug, $F_{1,7} = 166.83$, $P < 0.001$; drug × time, $F_{10,70} = 12.30$, $P < 0.001$). ACh levels were already elevated in the first fraction after drug administration (collection no. 5, $t_7 = 4.59$, $P = 0.003$) and remained significantly increased until the final collection. Intracortical perfusion of KYNA antagonized the D-amphetamine-induced increase in ACh efflux (drug, $F_{1,7} = 69.14$, $P < 0.001$; drug × time, $F_{10,70} = 10.01$, $P < 0.001$). This blockade of evoked release was seen as early as the first collection following perfusion with KYNA ($t_7 = 3.20$, $P = 0.01$). Notably, no significant difference in ACh levels was observed between this and the aCSF/saline control condition ($P > 0.05$), indicating complete attenuation of the D-amphetamine effect by nanomolar KYNA (Fig. 5).

Effects of systemic kynurenine on extracellular KYNA in the absence and presence of d-amphetamine

In the second part of our study, we examined the possibility that systemically administered kynurenine (50 mg/kg, i.p., $n = 4$), after brain uptake and subsequent transamination to KYNA in astrocytes (see Introduction), would mimic the effects of local KYNA perfusions in the mPFC. First, we demonstrated (Fig. 6A) that kynurenine administration caused the expected marked increase in extracellular KYNA levels in the mPFC ($F_{15,45} = 124.82$, $P < 0.001$) (Swartz *et al.*, 1990). This elevation above baseline values (2.8 ± 0.1 nM) was already significant during the initial 30-min post-injection collection ($t_4 = -4.16$, $P = 0.02$) and reached a maximum by 2 h. KYNA levels reverted to basal values by fraction no. 15 ($P > 0.05$).

We next characterized, in a single microdialysis session, the effects of D-amphetamine on kynurenine-induced KYNA formation ($n = 5$). To this end, D-amphetamine (2.0 mg/kg, i.p.) was administered 75 min after kynurenine (50 mg/kg, i.p.), and extracellular KYNA levels in the mPFC were determined in 15-min microdialysate fractions. Overall, there was a clear, time-dependent increase in cortical KYNA levels ($F_{12,48} = 112.16$, $P < 0.001$) (Fig. 6B). In line with the results illustrated in Fig. 6A, kynurenine stimulated cortical KYNA as early as the first collection post-injection ($t_4 = -7.19$, $P < 0.002$), and KYNA levels reached a maximum of ~600% of basal values by the time that D-amphetamine was administered (arrow in Fig. 6B). KYNA levels then declined progressively, but remained significantly elevated above baseline for at least five additional collection intervals. These data are consistent with previous reports showing that the systemic administration of D-amphetamine lowers endogenous KYNA levels in the rat brain (Rassoulpour *et al.*, 1998).

Effects of systemic kynurenine on d-amphetamine-induced ACh release

Finally, we tested the hypothesis that newly produced KYNA will suppress D-amphetamine (2.0 mg/kg, i.p.)-stimulated ACh release, that is, that the results observed following local KYNA application (Fig. 4) can be replicated by the systemic administration of kynurenine (50 mg/kg, i.p.). To this end, rats were treated with saline/saline, kynurenine/saline, saline/D-amphetamine, and kynurenine/D-amphetamine, as indicated in Fig. 7 ($n = 6$). Mean basal levels of extracellular ACh were 5.3 ± 1.1 fmol per 15 μL. Again, no significant differences were found as a function of treatment condition ($F_{3,15} = 1.93$, $P = 0.22$) or dialysis session ($F_{3,15} = 3.03$, $P = 0.12$), so that all data were expressed and analysed as a percentage of baseline values. Overall analysis revealed significant differences in evoked ACh levels among the four conditions (effect of drug, $F_{3,12} = 9.54$, $P = 0.002$; time, $F_{12,48} = .35$, $P < 0.001$; effect of drug × time, $F_{36,144} = 5.86$, $P < 0.001$). The two saline injections did not affect basal ACh levels. As expected (see Fig. 5), administration of D-amphetamine produced a robust and enduring increase in cortical ACh levels ($F_{1,5} = 16.30$, $P = 0.01$), which was already evident during the first post-D-amphetamine collection period ($t_5 = -3.71$, $P = 0.01$) (Fig. 7). Whereas systemic kynurenine administration alone did not cause a significant change in basal ACh efflux ($F_{1,4} = 1.05$, $P = 0.36$), the KYNA precursor completely prevented the ability of D-amphetamine to increase ACh levels ($F_{1,4} = 94.89$, $P < 0.001$). As after a local perfusion of KYNA (Fig. 5), this blockade was already evident in the first fraction following D-amphetamine application ($t_5 = 3.92$, $P = 0.01$).

Discussion

Our study resulted in three new findings that are germane to the regulation of extracellular ACh levels in the rat mPFC. First, we showed that the basal levels of prefrontal ACh are modulated by fluctuations in endogenous KYNA. Thus, inhibition of KYNA biosynthesis by *S*-ESBA markedly elevated cortical ACh levels, and this effect was abolished by the co-administration of nanomolar concentrations of exogenous KYNA, which also offset the *S*-ESBA-induced reduction in endogenous KYNA. In support of the capacity of KYNA to attenuate extracellular ACh levels, we also noted a tendency of nanomolar KYNA to lower basal ACh levels, although the

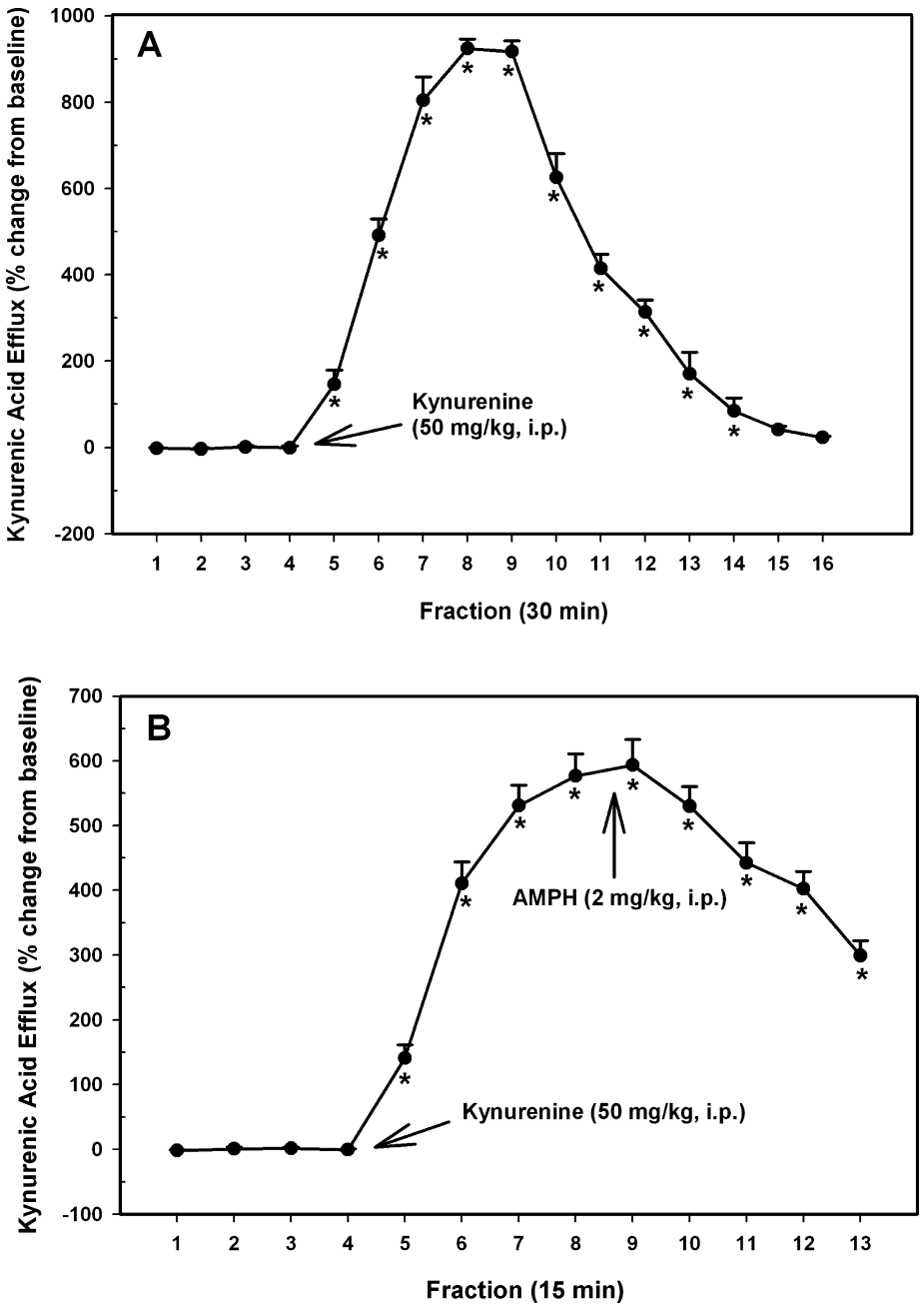

FIG. 6. (A) Systemic administration of kynurenine [50 mg/kg, intraperitoneal (i.p.)] causes a time-dependent increase in extracellular levels of kynurenic acid (KYNA) in the medial prefrontal cortex. Data are the mean + SEM of four animals. *$P < 0.05$ vs. the last baseline fraction. (B) Administration of D-amphetamine (AMPH; 2.0 mg/kg, i.p.) 75 min after kynurenine (50 mg/kg, i.p.) attenuates the kynurenine-induced increase in extracellular KYNA levels. Note the difference in collection intervals in A and B. Data are the mean + SEM of five animals. *$P < 0.05$ vs. the last baseline fraction.

experimental results did not attain statistical significance. Second, systemic administration of kynurenine, which substantially elevates extracellular KYNA levels in the brain, duplicated the effects of locally applied, exogenous KYNA. This indicates that astrocytes, which are responsible for most of the *de novo* synthesis of KYNA from kynurenine in the brain (Du *et al.*, 1992; Guidetti *et al.*, 2007a,b), deserve consideration as modulators of α7nAChR function and, by inference, cholinergic neurotransmission. Third, we demonstrated that the capacity of KYNA and kynurenine to modulate cortical cholinergic transmission is not limited to the tonic modulation of basal ACh levels. Thus, either local application of nanomolar concentrations of KYNA or systemic kynurenine administration strongly attenuated stimulated ACh release, here effected by an i.p. injection of the psychostimulant D-amphetamine (Arnold *et al.*, 2000).

Endogenous KYNA as a selective antagonist of α7nAChR

Following its irreversible transamination from kynurenine in astrocytes, newly formed KYNA is rapidly released into the extracellular milieu (Turski *et al.*, 1989; Swartz *et al.*, 1990) for possible interaction with neuronal membrane receptors. KYNA was

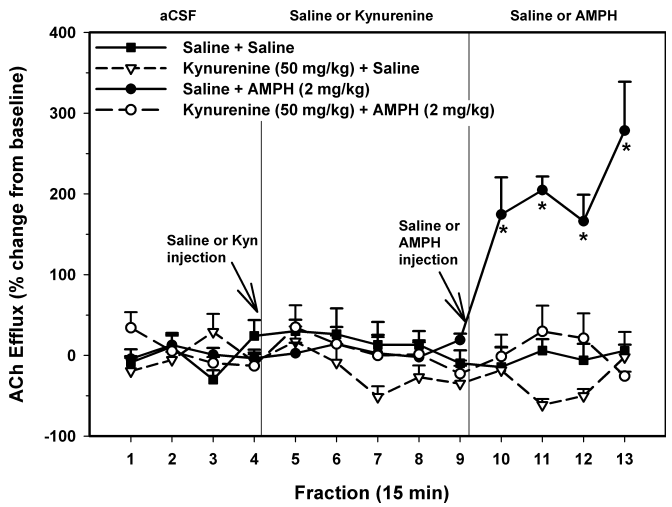

FIG. 7. Systemic administration of kynurenine [Kyn; 50 mg/kg, intraperitoneal (i.p.)] attenuates the ability of D-amphetamine (AMPH; 2.0 mg/kg, i.p.) to increase extracellular ACh levels in the medial prefrontal cortex. Rats were tested in each of four drug conditions, in counterbalanced order, as described in the text. Data are the mean + SEM of six animals. *$P < 0.01$ vs. saline + saline or kynurenine + D-amphetamine.

initially reported to function as a broad-spectrum antagonist of ionotropic glutamate receptors, with IC_{50} values around 1 mM (Perkins & Stone, 1982). It was later shown that KYNA exhibits a significantly higher affinity for the glycine co-agonist site of the *N*-methyl-D-aspartate (NMDA) receptor (IC_{50} ~10 μM and ~200 μM, in the absence of glycine and the presence of glycine, respectively) (Hilmas *et al.*, 2001). However, at physiological (i.e. nanomolar) concentrations, KYNA appears to preferentially, and non-competitively, inhibit α7nAChRs in the forebrain (Hilmas *et al.*, 2001; Rassoulpour *et al.*, 2005), although pharmacologically distinct NMDA autoreceptors may constitute an additional high-affinity target (Luccini *et al.*, 2007). KYNA's inhibition of α7nAChRs involves binding to an allosteric potentiating site similar to that activated by the cognition enhancer galantamine (Lopes *et al.*, 2007). Although some regional differences may exist (Linderholm *et al.*, 2007), the notion is supported by a number of *in vivo* and *in vitro* studies, demonstrating that the functional effects of nanomolar KYNA concentrations can be duplicated by specific α7nAChR antagonists such as methyllycaconitine or α-bungarotoxin (Rassoulpour *et al.*, 2005; Lopes *et al.*, 2007) but not by 7-Cl-KYNA, a selective antagonist of the $glycine_B$ site of the NMDA receptor (Hilmas *et al.*, 2001; Rassoulpour *et al.*, 2005). In line with this conclusion, hippocampal α7nAChR – but not NMDA receptor – function is altered in mice with a targeted deletion of KAT II (Alkondon *et al.*, 2004).

Mechanisms underlying KYNA regulation of prefrontal ACh levels

In the cerebral cortex, as in several other brain areas, nAChRs are mostly found as presynaptic heteroreceptors located on nerve terminals [for review, see Dani & Bertrand (2007)]. However, in the cortex and elsewhere, α7nAChRs also exist postsynaptically on somatodendritic structures of GABAergic interneurons (Alkondon *et al.*, 2000). The diverse location of these receptors enables ACh, choline and other endogenous nicotinic ligands to influence the release of a variety of neurotransmitters, including dopamine (Cao *et al.*, 2005; Shearman *et al.*, 2005) and glutamate (Gioanni *et al.*, 1999; Rousseau *et al.*, 2005).

As functional α7nACh autoreceptors in the cortex do not appear to exist at appreciable densities (Marchi *et al.*, 2002; Amtage *et al.*, 2004), it is likely that the intracortical modulation of ACh levels by KYNA described here is mediated indirectly via the most prominent of these α7nAChRs, which are associated with glutamatergic neurons. Indeed, a direct functional link between presynaptic α7nAChRs and glutamate release exists in the PFC (Gioanni *et al.*, 1999; Dickinson *et al.*, 2008), and we showed recently, using the same concentrations as in the present study, that perfusions of KYNA or *S*-ESBA bi-directionally modulate extracellular glutamate levels in the mPFC (Wu *et al.*, 2008). Fluctuations in extracellular glutamate, in turn, influence dopamine release from cortical afferents (Del Arco & Mora, 2005; Yang & Chen, 2005), and this sequence of neurochemical events may underlie the ability of KYNA and *S*-ESBA to modulate extracellular dopamine levels in the mPFC (Wu *et al.*, 2006). In other words, the effect of KYNA and *S*-ESBA on extracellular ACh described in the PFC may be caused by a local cascade of events involving glutamatergic and dopaminergic mechanisms. Such an indirect effect would also be in line with the fact that both glutamate (Giovannini *et al.*, 1997; Parikh *et al.*, 2008) and dopamine (Del Arco *et al.*, 2007) receptor activation in the PFC is associated with enhanced levels of cortical ACh.

Alternatively or in addition to intracortical events, the control of prefrontal ACh release by KYNA may involve a distributed system, in particular the PFC–nucleus accumbens–basal forebrain–PFC loop. Thus, glutamate release from cortical afferents to the nucleus accumbens stimulates cortical cholinergic transmission (Zmarowski *et al.*, 2005, 2007), as do other trans-synaptic manipulations of this circuit (Fadel *et al.*, 2001). Notably, additional extracortical mechanisms may include effects of KYNA itself, as exemplified by our recent observation that infusion of *S*-ESBA into the nucleus accumbens raises extracellular ACh levels in the mPFC (A. Zmarowski and J. P. Bruno, unpublished data).

Effects of KYNA on stimulated ACh release

Whereas locally applied KYNA or systemic kynurenine administration did not cause significant reductions in basal extracellular levels of ACh in the PFC, the same treatments abolished the ability of D-amphetamine to stimulate ACh levels. The psychostimulant was used here primarily as a tool to raise cortical ACh, allowing us to examine the effects of KYNA on the evoked release of the neurotransmitter. However, we chose D-amphetamine also for its pro-cognitive properties, which involve the PFC (Mattay *et al.*, 2000), as well as the fact that the drug's effect on cortical ACh may be mediated indirectly through the release of glutamate (Giovannini *et al.*, 1997; Del Arco & Mora, 2005; Parikh *et al.*, 2008) and dopamine (Del Arco *et al.*, 2007) In addition, D-amphetamine reduces the levels of KYNA in the frontal cortex and other brain areas (Rassoulpour *et al.*, 1998). As all of these qualities are similar to those of the KAT II inhibitor *S*-ESBA (see above), and as nanomolar concentrations of KYNA powerfully antagonize the effect of D-amphetamine (Figs 5 and 7), a reduction in KYNA may play a pivotal role in the drug's ability to stimulate cortical ACh release. This hypothesis, which is currently being tested further in our laboratories, implies that at least some of the cognition-enhancing effects of D-amphetamine might be duplicated by inhibitors of KYNA biosynthesis. More generally, the present results highlight the dynamic

range of KYNA's modulation of cortical ACh release and suggest that fluctuations in KYNA levels may affect ACh levels especially under conditions when the basal forebrain cortical cholinergic system is activated [i.e. during cognitive processing; see Kozak *et al.* (2007) for review].

Implications for cognitive deficits in schizophrenia

KYNA levels are significantly elevated in the PFC (Schwarcz *et al.*, 2001) and in the CSF (Erhardt *et al.*, 2001) of individuals with schizophrenia. Conceivably, these increases, which do not appear to be secondary to antipsychotic medication (Ceresoli-Borroni *et al.*, 2006), may dysregulate several cortical neurotransmitter systems that are implicated in the cognitive deficits in the disease, including ACh (Hyde & Crook, 2001; Sarter *et al.*, 2005) , glutamate (Krystal *et al.*, 2003; Moghaddam, 2003; Coyle, 2006), and dopamine (Laruelle *et al.*, 2003). In line with a role in cognitive phenomena, experimentally induced elevations in brain KYNA cause performance deficits in several behavioral tasks that require cognitive operations similar to those that are impaired in schizophrenia (Shepard *et al.*, 2003; Erhardt *et al.*, 2004; Chess & Bucci, 2006). Also of relevance in the context of the present study, mice with a genomic elimination of KAT II show significant improvements in cognitive tests *in vivo* (Potter *et al.*, 2006) and *ex vivo* (Bergeron *et al.*, 2007). Collectively, these data support the idea that KAT II inhibitors or other pharmacological manipulations leading to a reduction in cortical KYNA formation may provide clinical benefits, which may preferentially affect the cognitive deficits seen in schizophrenia. This concept, which is currently being explored further in animals with chronic elevations of brain KYNA levels, is in agreement with several recent reports of favorable effects of α7nAChR agonists and partial agonists in schizophrenia patients and relevant animal models (Pichat *et al.*, 2007; Feuerbach *et al.*, 2008) For example, galantamine, which activates the allosteric potentiating site of the α7nAChR that is inhibited by KYNA (Lopes *et al.*, 2007), results in significant improvements in attention in patients stabilized on the atypical antipsychotic risperidone (Schubert *et al.*, 2006), and the partial α7nAChR agonist DMXB-A improves scores on a composite neuropsychological scale and facilitates the P50 inhibitory component of the auditory gating test (Olincy *et al.*, 2006).

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Abbreviations

ACh, acetylcholine; aCSF, artificial cerebrospinal fluid; CSF, cerebrospinal fluid; GABA, γ-aminobutyric acid; HPLC, high-performance liquid chromatography; i.p., intraperitoneal; KAT, kynurenine aminotransferase; KP, kynurenine pathway; KYNA, kynurenic acid; mPFC, medial prefrontal cortex; nAChR, nicotinic acetylcholine receptor; NMDA, *N*-methyl-D-aspartate; PFC, prefrontal cortex; *S*-ESBA, *S*-ethylsulfonylbenzoylalanine.

References

Alkondon, M., Pereira, E.F., Eisenberg, H.M. & Albuquerque, E.X. (2000) Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neuronal networks. *J. Neurosci.*, **20**, 66–75.

Alkondon, M., Pereira, E.F., Yu, P., Arruda, E.Z., Almeida, L.E., Guidetti, P., Fawcett, W.P., Sapko, M.T., Randall, W.R., Schwarcz, R., Tagle, D.A. & Albuquerque, E.X. (2004) Targeted deletion of the kynurenine aminotransferase ii gene reveals a critical role of endogenous kynurenic acid in the regulation of synaptic transmission via alpha7 nicotinic receptors in the hippocampus. *J. Neurosci.*, **24**, 4635–4648.

Amtage, F., Neughebauer, B., McIntosh, J.M., Freiman, T., Zentner, J., Feuerstein, T.J. & Jackisch, R. (2004) Characterization of nicotinic receptors inducing noradrenaline release and absence of nicotinic autoreceptors in human neocortex. *Brain Res. Bull.*, **62**, 413–423.

Arnold, H.M., Nelson, C.L., Neigh, G.N., Sarter, M. & Bruno, J.P. (2000) Systemic and intra-accumbens administration of amphetamine differentially affects cortical acetylcholine release. *Neuroscience*, **96**, 675–685.

Bergeron, R., Wu, H.-Q., Potter, M.C., Guidetti, P. & Schwarcz, R. (2007) Mice with reduced brain kynurenic acid levels have high extracellular glutamate and show enhanced LTP in the hippocampus. *Soc. Neurosci. Abstr.*, **32**, 807.15.

Boyer, P., Phillips, J.L., Rousseau, F.L. & Ilivitsky, S. (2007) Hippocampal abnormalities and memory deficits: new evidence of a strong pathophysiological link in schizophrenia. *Brain Res. Rev.*, **54**, 92–112.

Brooks, J.M., Sarter, M. & Bruno, J.P. (2007) D2-like receptors in nucleus accumbens negatively modulate acetylcholine release in prefrontal cortex. *Neuropharmacology*, **53**, 455–463.

Bruno, J.P., Sarter, M., Arnold, H.M. & Himmelheber, A.M. (1999) *In vivo* neurochemical markers of cognitive processes: methodological and conceptual challenges. *Rev. Neurosci.*, **10**, 25–49.

Buchanan, R.W., Conley, R.R., Dickinson, D., Ball, M.P., Feldman, S., Gold, J.M. & McMahon, R.P. (2008) Galantamine for the treatment of cognitive impairments in people with schizophrenia. *Am. J. Psychiatry*, **165**, 82–89.

Cao, Y.J., Surowy, C.S. & Puttfarcken, P.S. (2005) Different nicotinic acetylcholine receptor subtypes mediating striatal and prefrontal cortical [3H]dopamine release. *Neuropharmacology*, **48**, 72–79.

Carpenedo, R., Pittaluga, A., Cozzi, A., Attucci, S., Galli, A., Raiteri, M. & Moroni, F. (2001) Presynaptic kynurenate-sensitive receptors inhibit glutamate release. *Eur. J. Neurosci.*, **13**, 2141–2147.

Ceresoli-Borroni, G., Rassoulpour, A., Wu, H.Q., Guidetti, P. & Schwarcz, R. (2006) Chronic neuroleptic treatment reduces endogenous kynurenic acid levels in rat brain. *J. Neural Transm.*, **113**, 1355–1365.

Chess, A.C. & Bucci, D.J. (2006) Increased concentration of cerebral kynurenic acid alters stimulus processing and conditioned responding. *Behav. Brain Res.*, **170**, 326–332.

Coyle, J.T. (2006) Glutamate and schizophrenia: beyond the dopamine hypothesis. *Cell. Mol. Neurobiol.*, **26**, 365–384.

Dani, J.A. & Bertrand, D. (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.*, **47**, 699–729.

Del Arco, A. & Mora, F. (2005) Glutamate–dopamine in vivo interaction in the prefrontal cortex modulates the release of dopamine and acetylcholine in the nucleus accumbens of the awake rat. *J. Neural Transm.*, **112**, 97–109.

Del Arco, A., Mora, F., Mohammed, A.H. & Fuxe, K. (2007) Stimulation of D2 receptors in the prefrontal cortex reduces PCP-induced hyperactivity, acetylcholine release and dopamine metabolism in the nucleus accumbens. *J. Neural Transm.*, **114**, 185–193.

Dickinson, J.A., Kew, J.N. & Wonnacott, S. (2008) Presynaptic alpha 7- and beta 2-containing nicotinic acetylcholine receptors modulate excitatory amino acid release from rat prefrontal cortex nerve terminals via distinct cellular mechanisms. *Mol. Pharmacol.*, **74**, 348–359.

Du, F., Schmidt, W., Okuno, E., Kido, R., Köhler, C. & Schwarcz, R. (1992) Localization of kynurenine aminotransferase immunoreactivity in the rat hippocampus. *J. Comp. Neurol.*, **321**, 477–487.

Erhardt, S., Blennow, K., Nordin, C., Skogh, E., Lindstrom, L.H. & Engberg, G. (2001) Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neurosci. Lett.*, **313**, 96–98.

Erhardt, S., Schwieler, L., Emanuelsson, C. & Geyer, M. (2004) Endogenous kynurenic acid disrupts prepulse inhibition. *Biol. Psychiatry*, **56**, 255–260.

Fadel, J., Sarter, M. & Bruno, J.P. (2001) Basal forebrain glutamatergic modulation of cortical acetylcholine release. *Synapse*, **39**, 201–212.

Feuerbach, D., Lingenhoehl, K., Olpe, H.R., Vassout, A., Gentsch, C., Chaperon, F., Nozulak, J., Enz, A., Bilbe, G., McAllister, K. & Hoyer, D. (2008) The selective nicotinic acetylcholine receptor alpha7 agonist JN403 is active in animal models of cognition, sensory gating, epilepsy and pain. *Neuropharmacology* 2008 Aug 28 (published ahead of print).

Gioanni, Y., Rougeot, C., Clarke, P.B., Lepousé, C., Thierry, A.M. & Vidal, C. (1999) Nicotinic receptors in the rat prefrontal cortex: increase in glutamate

release and facilitation of mediodorsal thalamo-cortical transmission. *Eur. J. Neurosci.*, **11**, 18–30.
Giovannini, M.G., Giovannelli, L., Bianchi, L., Kalfin, R. & Pepeu, G. (1997) Glutamatergic modulation of cortical acetylcholine release in the rat: a combined in vivo microdialysis, retrograde tracing and immunohistochemical study. *Eur. J. Neurosci.*, **9**, 1678–1689.
Grilli, M., Raiteri, L., Patti, L., Parodi, M., Robino, F., Raiteri, M. & Marchi, M. (2006) Modulation of the function of presynaptic alpha7 and non-alpha7 nicotinic receptors by the tryptophan metabolites, 5-hydroxyindole and kynurenate in mouse brain. *Br. J. Pharmacol.*, **149**, 724–732.
Guan, Z.Z., Zhang, X., Blennow, K. & Nordberg, A. (1999) Decreased protein level of nicotinic alpha7 subunit in the frontal cortex from schizophrenic brain. *Neuroreport*, **10**, 1779–1782.
Guidetti, P., Amori, L., Sapko, M.T., Okuno, E. & Schwarcz, R. (2007a) Mitochondrial aspartate aminotransferase: a third kynurenate-producing enzyme in the mammalian brain. *J. Neurochem.*, **102**, 103–111.
Guidetti, P., Hoffman, G.E., Melendez-Ferro, M., Albuquerque, E.X. & Schwarcz, R. (2007b) Astrocytic localization of kynurenine aminotransferase II in the rat brain visualized by immunocytochemistry. *Glia*, **55**, 78–92.
Hasselmo, M.E. & Stern, C.E. (2006) Mechanisms underlying working memory for novel information. *Trends Cogn. Sci.*, **10**, 487–493.
Hilmas, C., Pereira, E.F., Alkondon, M., Rassoulpour, A., Schwarcz, R. & Albuquerque, E.X. (2001) The brain metabolite kynurenic acid inhibits α7 nicotinic receptor activity and increases non-α7 nicotinic receptor expression: physiopathological implications. *J. Neurosci.*, **21**, 7463–7473.
Höcht, C., Opezzo, J.A. & Taira, C.A. (2007) Applicability of reverse microdialysis in pharmacological and toxicological studies. *J. Pharmacol. Toxicol. Methods.*, **55**, 3–15.
Hyde, T.M. & Crook, J.M. (2001) Cholinergic systems and schizophrenia: primary pathology or epiphenomena? *J. Chem. Neuroanat.*, **22**, 53–63.
Keefe, R.S. (2007) Cognitive deficits in patients with schizophrenia: effects and treatments. *J. Clin. Psychiatry*, **68**, (Suppl. 14), 8–13..
Kerns, J.G., Nuechterlein, K.H., Braver, T.S. & Barch, D.M. (2008) Executive functioning component mechanisms and schizophrenia. *Biol. Psychiatry*, **64**, 26–33.
Koike, K., Hashimoto, K., Takai, N., Shimizu, E., Komatsu, N., Watanabe, H., Nakazato, M., Okamura, N., Stevens, K.E., Freedman, R. & Iyo, M. (2005) Tropisetron improves deficits in auditory P50 suppression in schizophrenia. *Schizophr. Res.*, **76**, 67–72.
Kozak, R., Martinez, V., Young, D., Brown, H., Bruno, J.P. & Sarter, M. (2007) Toward a neuro-cognitive animal model of the cognitive symptoms of schizophrenia: disruption of cortical cholinergic neurotransmission following repeated amphetamine exposure in attentional task-performing, but not non-performing, rats. *Neuropsychopharmacology*, **32**, 2074–2086.
Krystal, J.H., D'Souza, D.C., Mathalon, D., Perry, E., Belger, A. & Hoffman, R. (2003) NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. *Psychopharmacology*, **169**, 215–233.
Laruelle, M., Kegeles, L.S. & Abi-Dargham, A. (2003) Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. *Ann. NY Acad. Sci.*, **1003**, 135–158.
Leonard, S. & Freedman, R. (2006) Genetics of chromosome 15q13–q14 in schizophrenia. *Biol. Psychiatry*, **60**, 115–122.
Lewis, D.A. & Moghaddam, B. (2006) Cognitive dysfunction in schizophrenia: convergence of gamma-aminobutyric acid and glutamate alterations. *Arch. Neurol.*, **63**, 1372–1376.
Lewis, D.A., Hashimoto, T. & Volk, D.W. (2005) Cortical inhibitory neurons and schizophrenia. *Nat. Rev. Neurosci.*, **6**, 312–324.
Linderholm, K.R., Andersson, A., Olsson, S., Olsson, E., Snodgrass, R., Engberg, G. & Erhardt, S. (2007) Activation of rat ventral tegmental area dopamine neurons by endogenous kynurenic acid: a pharmacological analysis. *Neuropharmacology*, **53**, 918–924.
Lopes, C., Pereira, E.F.R., Wu, H.-Q., Purushottamachar, P., Njar, V., Schwarcz, R. & Albuquerque, E.X. (2007) Competitive antagonism between the nicotinic allosteric potentiating ligand galantamine and kynurenic acid at α7* nicotinic receptors. *J. Pharmacol. Exp. Ther.*, **322**, 48–58.
Luccini, E., Musante, V., Neri, E., Raiteri, M. & Pittaluga, A. (2007) N-Methyl-D-aspartate autoreceptors respond to low and high agonist concentrations by facilitating, respectively, exocytosis and carrier-mediated release of glutamate in rat hippocampus. *J. Neurosci. Res.*, **85**, 3657–3665.
Marchi, M., Risso, F., Viola, C., Cavazzani, P. & Raiteri, M. (2002) Direct evidence that release-stimulating alpha7* nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals. *J. Neurochem.*, **80**, 1071–1078.
Martin, L.F., Kem, W.R. & Freedman, R. (2004) Alpha-7 nicotinic receptor agonists: potential new candidates for the treatment of schizophrenia. *Psychopharmacology*, **174**, 54–64.
Martin, K.F., Leonard, S., Hall, M.H., Tregelias, J.R., Freedman, R. & Olincy, A. (2007) Sensory gating and alpha7-nicotinic receptor gene allelic variants in schizoaffective disorder, bipolar type. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, **144B**, 611–614.
Mattay, V.S., Callicott, J.H., Bertolino, A., Heaton, I., Frank, J.A., Coppola, R., Berman, K.F., Goldberg, T.E. & Weinberger, D.R. (2000) Effects of dextroamphetamine on cognitive performance and cortical activation. *Neuroimage*, **12**, 268–275.
Moghaddam, B. (2003) Bringing order to the glutamate chaos in schizophrenia. *Neuron*, **40**, 881–884.
Nelson, C.L., Sarter, M. & Bruno, J.P. (2005) Prefrontal cortical modulation of acetylcholine release in posterior parietal cortex. *Neuroscience*, **132**, 347–359.
Olincy, A. & Stevens, K.E. (2007) Treating schizophrenia symptoms with an α7 nicotinic agonist, from mice to men. *Biochem. Pharmacol.*, **74**, 1192–1201.
Olincy, A., Harris, J.G., Johnson, L.L., Pender, V., Kongs, S., Allensworth, D., Ellis, J., Zerbe, G.O., Leonard, S., Stevens, K.E., Stevens, J.O., Martin, L., Adler, L.E., Soti, F., Kerm, W.R. & Freeman, R. (2006) Proof-of-concept trial of an alpha7 nicotinic agonist in schizophrenia. *Arch. Gen. Psychiatry*, **63**, 630–638.
Parikh, V., Man, K., Decker, M.W. & Sarter, M. (2008) Glutamatergic contributions to nicotinic acetylcholine receptor agonist-evoked cholinergic transients in the prefrontal cortex. *J. Neurosci.*, **28**, 3769–3780.
Pellicciari, R., Rizzo, R., Costantino, G., Marinozzi, M., Amori, L., Guidetti, P., Wu, H.-Q. & Schwarcz, R. (2006) Modulators of the kynurenine pathway of tryptophan metabolism. Synthesis and preliminary biological evaluation of (S)-4-(ethylsulfonyl)benzoylalanine, a potent and selective kynurenine aminotransferase II (KAT II) inhibitor. *ChemMedChem.*, **1**, 528–531.
Perkins, M.N. & Stone, T.W. (1982) An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Res.*, **247**, 184–187.
Pichat, P., Bergis, O.E., Terranova, J.P., Urani, A., Duarte, C., Santucci, V., Gueudet, C., Voltz, C., Steinberg, R., Stemmelin, J., Oury-Donat, F., Avenet, P., Griebel, G. & Scatton, B. (2007) SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (II) efficacy in experimental models predictive of activity against cognitive symptoms of schizophrenia. *Neuropsychopharmacology*, **32**, 17–34.
Potter, P.E., Meek, J.L. & Neff, N.H. (1983) Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. *J. Neurochem.*, **41**, 188–194.
Potter, M.C., Elmer, G.I., Guidetti, P., Calu, D. & Schwarcz, R. (2006) Improved behavioral performance of kynurenine aminotransferase II-deficient mice in hippocampus-based learning and memory paradigms. *Soc. Neurosci. Abstr.*, **32**, 265.9.
Rassoulpour, A., Wu, H.-Q., Poeggeler, B. & Schwarcz, R. (1998) Systemic *d*-amphetamine administration causes a reduction of kynurenic acid levels in rat brain. *Brain Res.*, **802**, 111–118.
Rassoulpour, A., Wu, H.-Q., Ferré, S. & Schwarcz, R. (2005) Nanomolar concentrations of kynurenic acid reduce extracellular dopamine levels in the striatum. *J. Neurochem.*, **93**, 762–765.
Rousseau, S.J., Jones, I.W., Pullar, I.A. & Wonnacott, S. (2005) Presynaptic alpha7 and non-alpha7 nicotinic acetylcholine receptors modulate [^{3}H]-D-aspartate release from rat frontal cortex in vitro. *Neuropharmacology*, **49**, 59–72.
Sarter, M., Nelson, C.L. & Bruno, J.P. (2005) Cortical cholinergic transmission and cortical information processing in schizophrenia. *Schizophr. Bull.*, **31**, 117–138.
Sathyasaikumar, K.V., Rassoulpour, A., Stachowski, E., Guidetti, P., Roberts, R.C. & Schwarcz, R. (2008) Impaired cortical kynurenine pathway metabolism in schizophrenia. *Soc. Neurosci. Abstr.*, **33**, 54.20.
Schubert, M.H., Young, K.A. & Hicks, P.B. (2006) Galantamine improves cognition in schizophrenic patients stabilized on risperidone. *Biol. Psychiatry*, **60**, 530–533.
Schwarcz, R., Rassoulpour, A., Wu, H.-Q., Medoff, D., Tamminga, C.A. & Roberts, R.C. (2001) Increased cortical kynurenate content in schizophrenia. *Biol. Psychiatry*, **50**, 521–530.
Severance, E.G. & Yolken, R.H. (2008) Novel alpha7 nicotinic receptor isoforms and deficient cholinergic transcription in schizophrenia. *Genes Brain Behav.*, **7**, 37–45.

Shearman, E., Rossi, S., Sershen, H., Hashim, A. & Lajtha, A. (2005) Locally administered low nicotine-induced neurotransmitter changes in areas of cognitive function. *Neurochem. Res.*, **30**, 1055–1066.

Shepard, P.D., Joy, B., Clerkin, L. & Schwarcz, R. (2003) Micromolar brain levels of kynurenic acid are associated with a disruption of auditory sensory gating in the rat. *Neuropsychopharmacology*, **28**, 1454–1462.

Swartz, K., During, M.J., Freese, A. & Beal, M.F. (1990) Cerebral synthesis and release of kynurenic acid: an endogenous antagonist of excitatory amino acid receptors. *J. Neurosci.*, **10**, 2965–2973.

Tan, H.Y., Callicott, J.H. & Weinberger, D.R. (2007) Dysfunctional and compensatory prefrontal cortical systems, genes and the pathogenesis of schizophrenia. *Cereb. Cortex.*, **17**(Suppl. 1), 171–181.

Toda, M. & Abi-Dargham, A. (2007) Dopamine hypothesis of schizophrenia: making sense of it all. *Curr. Psychiatry. Rep.*, **9**, 329–336.

Turski, W.A., Gramsbergen, J.B.P., Traitler, H. & Schwarcz, R. (1989) Rat brain slices produce and liberate kynurenic acid upon exposure to L-kynurenine. *J. Neurochem.*, **52**, 1629–1636.

Vasey, M.W. & Thayer, J.F. (1987) The continuing problem of false positives in repeated measures ANOVA in psychophysiology: a multivariate solution. *Psychophysiology*, **24**, 479–486.

Wu, H.-Q., Pellicciari, R. & Schwarcz, R. (2006) Bidirectional regulation of extracellular dopamine by endogenous kynurenic acid in the rat medial prefrontal cortex. *Soc. Neurosci. Abstr.*, **32**, 624.3.

Wu, H.-Q., Pellicciari, R., Bruno, J.P. & Schwarcz, R. (2008) Endogenous kynurenate modulates extracellular glutamate levels in the rat medial prefrontal cortex. *Soc. Neurosci. Abstr.*, **34**, 657.50.

Yang, C.R. & Chen, L. (2005) Targeting prefrontal cortical dopamine D1 and N-methyl-D-aspartate receptor interactions in schizophrenia treatment. *Neuroscientist*, **11**, 452–470.

Zmarowski, A., Sarter, M. & Bruno, J.P. (2005) NMDA and dopamine interactions in the nucleus accumbens modulate cortical acetylcholine release. *Eur. J. Neurosci.*, **22**, 1731–1740.

Zmarowski, A., Sarter, M. & Bruno, J.P. (2007) Glutamate receptors in nucleus accumbens mediate regionally selective increases in cortical acetylcholine release. *Synapse*, **61**, 115–123.