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ARTICLE

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Elevated levels of kynurenic acid change the dopaminergic response to amphetamine: implications for schizophrenia

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Abstract

Kynurenic acid (KYNA) is an endogenous compound implicated in the pathophysiology of schizophrenia. This tryptophan metabolite antagonizes both the *N*-methyl-D-aspartate (NMDA) receptors and the nicotinic α_7* receptors at micromolar concentrations. In the present study the effects of amphetamine on dopamine (DA) release in the nucleus accumbens and on firing of DA neurons in the ventral tegmental area (VTA) were investigated in rats treated with kynurenine, the precursor of KYNA, in order to elevate brain KYNA levels. In rats subchronically treated with kynurenine (90 mg/kg.d for 6 d via osmotic minipumps, resulting in a 2-fold increase in whole-brain KYNA), the amphetamine-induced (2 mg/kg i.p.) increase in accumbal DA release was clearly enhanced compared to controls. Furthermore, subchronic treatment with kynurenine reduced the inhibitory action of amphetamine (0.2–25.6 mg/kg i.v.) on firing rate and burst firing activity of VTA DA neurons. A single dose of kynurenine (5 mg/kg s.c., 60 min, resulting in a 3-fold increase in whole-brain KYNA) did not alter the amphetamine-induced effects on DA neurotransmission compared to control rats. Present data are in agreement with the increased striatal DA release by amphetamine as observed by brain-imaging studies in patients with schizophrenia. Thus, subchronic elevation of rat brain KYNA, may rationally serve as an animal model similar to a pathophysiological condition of schizophrenia. It is proposed that the reduced responsivity of VTA DA neurons to the inhibitory action of amphetamine observed in rats with subchronically elevated KYNA levels may partly account for the increase in terminal DA release.

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Introduction

Schizophrenia is a severe and debilitating psychiatric disorder and one of the world's most important public health problems. This devastating mental illness typically strikes maturing people just when they are entering adulthood. The lifetime prevalence is approximately 1%, independently of geographic, cultural and socioeconomic status (Carpenter and Buchanan, 1994). Our knowledge on the pathophysiological mechanisms leading to schizophrenia is still fragmentary. However, genetic, biological and environmental factors are all believed to be risk factors for the disease. The dopamine (DA) hypothesis of schizophrenia proposes that an increase in dopaminergic transmission in brain limbic areas is responsible for positive symptoms of the disorder, e.g. delusions and hallucinations. For many years, this idea was traditionally supported by indirect, pharmacological evidence, i.e. that DA receptor antagonists reverse some of the psychotic symptoms of schizophrenia and that frequent abuse of high doses of amphetamine may cause a toxic psychosis. Although clinical support of the DA hypothesis has been sparse, achievements during the last decade in positron emission tomography (PET) techniques have revealed an abnormal, excessive DA release

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following administration of amphetamine in untreated patients with schizophrenia (Abi-Dargham et al., 1998; Laruelle, 1998; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996, 1999).

Despite decades of effort, antipsychotic medications of today are essentially without effect on cognitive and negative symptoms of schizophrenia. Therefore, it has been proposed that DA only plays an intermediary role in the pathophysiology of the disease and that, in particular, deficits in brain glutamatergic systems are of major importance for its manifestation and progress (Carlsson et al., 2001; Javitt, 2004; Javitt and Zukin, 1991; Jentsch and Roth, 1999). Clinical support for this hypothesis consists of studies showing elevated cerebrospinal fluid (CSF) (Erhardt et al., 2001a; Nilsson et al., 2005) and post-mortem brain (Schwarcz et al., 2001) concentrations of kynurenic acid (KYNA), an endogenous tryptophan metabolite antagonizing *N*-methyl-D-aspartate (NMDA) receptors and nicotinic α_7* receptors (Birch et al., 1988; Ganong and Cotman, 1986; Hilmas et al., 2001; Kessler et al., 1989; Parsons et al., 1997). Low levels of glutamate have been found in CSF from patients with schizophrenia (Kim et al., 1980), although this initial finding has not been consistently replicated (Hashimoto et al., 2005). In addition, phencyclidine (PCP), ketamine and other compounds blocking the NMDA receptor, induce schizophrenia-like symptoms in healthy humans, including positive and negative symptoms as well as cognitive deficits, and furthermore, exacerbate the symptoms observed in patients with schizophrenia (Adler et al., 1999; Allen and Young, 1978; Jentsch and Roth, 1999; Malhotra et al., 1997). Abnormalities in NMDA receptor expression (Akbarian et al., 1996; Dracheva et al., 2001) and phosphorylation (Emamian et al., 2004) have also been found post mortem in schizophrenia patients, and a number of polymorphisms of NMDA receptor subunit genes increase susceptibility to the disease (Itokawa et al., 2003; Ohtsuki et al., 2001; Rice et al., 2001). Experimental studies show that disruption of glutamatergic neuronal systems, e.g. by administration of PCP, MK-801 or elevated endogenous levels of KYNA, distorts prepulse inhibition (PPI) (Erhardt et al., 2004; Geyer et al., 2001; Mansbach and Geyer, 1989, 1991), a behavioural model of schizophrenia. Moreover, systemically administered NMDA receptor antagonists all increase midbrain dopaminergic cell firing (Erhardt and Engberg, 2002; Erhardt et al., 2001b; French, 1994; French et al., 1993; Linderholm et al., 2007; Nilsson et al., 2006; Schwieler et al., 2006). Interestingly, a hypoglutamatergic condition, induced in healthy volunteers by administration of ketamine, potentiates the DA response to an amphetamine challenge (Kegeles et al., 2000). These findings suggest that an underlying dysfunction in glutamatergic circuits controlling brain dopaminergic activity account for the increased amphetamine-induced DA release observed in patients with schizophrenia (Abi-Dargham et al., 1998; Laruelle, 1998; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996, 1999).

The aim of the present study is to investigate the amphetamine-induced effects on terminal DA release as well as on neuronal dopaminergic firing under conditions of acutely and subchronically elevated levels of brain KYNA. For this purpose, kynurenine, the immediate precursor of KYNA, was administered to rats in a single-dose or subchronically via osmotic minipumps.

Materials and methods

Animals and surgery

Male Sprague–Dawley rats (Scanbur BK, Sollentuna, Sweden) weighing minimally 180 g (at time of minipump implantation) and maximally 250 g (at the time of the experiment) were housed in groups of 3–4. Free access to food and water was provided. Environmental conditions were checked daily and maintained under constant temperature (25 °C) and humidity (40–60%) in a room with a regulated 12-h light/dark cycle (lights on 06:00 hours). Experiments were approved by and performed in accordance with the guidelines of the Ethical Committee of Northern Stockholm, Sweden. All efforts were made to minimize the number of animals used and their suffering.

To subchronically elevate endogenous KYNA concentration rats were administered kynurenine for 6 d via two osmotic minipumps (volume 2 ml) with continuous flow of 10 μl/h. The osmotic pumps (2ML1 Alzet®, Cupertino, CA, USA) were filled under aseptic conditions with either vehicle (0.9% NaCl) or kynurenine (dissolved in deionized water; pH adjusted to 5.7 with $NaHCO_3$) in a concentration equivalent to a dose of 90 mg/kg.d (at day of implantation). All solutions were passed through a sterile filter (Acrodisc Syringe Filter 13 mm with 0.2 μm Supor® membrane) before the filling of pumps.

Rats were anaesthetized in a Plexiglas chamber filled continuously with 4.8% isoflurane in air using a vaporizer (Univentor 400 Anaesthesia Unit; Univentor Ltd, Zejtun, Malta) and positioned on a heating pad maintaining body temperature at 37 °C throughout the surgery (Temperature Control Unit, HB 101/2, AgnTho's AB, Lidingö, Sweden). Anaesthesia was

maintained using a nose cone delivering 2.4% isoflurane. Before surgery, 0.5 ml bupivacain (5 mg/ml) was administered subcutaneously (s.c.) to provide post-operative analgesia. The osmotic minipumps were inserted through an incision in the neck and placed s.c. on the back of the rats. To verify an effect of the kynurenine administration on KYNA concentration, blood was collected from the lateral tail vein at the end of surgery. These samples were then compared with blood collected at day 6. After surgery rats were placed in single cages for 24 h before being reunited in groups of three. After 6 d electrophysiological or microdialysis experiments were performed.

Microdialysis

Rats were anaesthetized as described above. The skull was exposed, and two shallow holes were drilled for insertion of anchor screws. A hole was drilled above the nucleus accumbens and following careful removal of the dura a guide cannula (MAB 9.IC, AgnTho's AB) was directed to the region of nucleus accumbens and fixed with stainless-steel screws and dental cement (Dentalon®, AgnTho's AB). Before each guide cannula implantation the incisor bar was adjusted so that the skull was set in a horizontal flat plane. Stereotaxic coordinates for the implantation of the guide cannula with reference to bregma and brain surface, respectively, were: AP +1.6, ML ±1.4, DV +6.2, meaning that the tip of the guide cannula was placed 2 mm above the vertical target for the final position of the microdialysis probe. Following surgery, 0.5 ml bupivacain (5 mg/ml) was administered s.c. to provide post-surgical analgesia. Rats were then allowed to recover, singly housed, for 24 h with food and water ad libitum. On the day of experiment, microdialysis was performed in unanaesthetized freely moving rats. The guide was removed and a microdialysis probe (MAB 9.14.2, polyether sulphone, 2 mm dialysing length, 0.56 mm diameter and a 15 kDa cut-off membrane; AgnTho's AB) was inserted into the cannula. Rats were connected to microdialysis swivels. The probes were perfused with perfusion fluid CNS (CMA Microdialysis AB, Solna, Sweden) delivered via polyethylene tubing from an infusion pump (Univentor 864, Univentor Ltd) at a flow rate of 2 μl/min. Thirty-minute fractions were collected using a microfraction collector (Univentor 820, Univentor Ltd) and manually injected (Rheodyne, Cotati, CA, USA) into a high-performance liquid chromatography (HPLC) system. To minimize the inter-individual variation due to differences in probe recovery, the dialysate concentrations were transformed to percent of baseline before statistical analysis. A stable baseline, consisting of three consecutive samples with a maximal variation of 10%, was usually obtained after 2–3 h and defined as 100%. Results for subsequent samples were calculated as percentages of this average basal release. Rats were then administered d-amphetamine intraperitoneally (i.p.) and DA concentration was measured for 180 min. One group of rats was pretreated with a single dose of kynurenine (5 mg/kg s.c.) 60 min prior to amphetamine administration. Separation of DA was achieved by reversed-phase liquid chromatography using a 55 mM sodium acetate buffer (pH 4.1, 10% methanol) with 0.8 mM octanesulfonic acid and 0.01 mM Na_2EDTA. The mobile phase was delivered by an HPLC pump (Bischoff Chromatography, Leonberg, Germany) through a ReproSil-Pur C18 column (4 × 150 mm, Dr Maisch GmbH, Ammerbuch, Germany) at a rate of 0.8 ml/min. Following separation, the analysate was first passed through a guard cell with an oxidizing potential of 50 mV. Samples were then quantified by sequential oxidation and reduction in a high-sensitivity analytical cell (ESA 5011; ESA Inc., Chelmsford, MA, USA) controlled by a potentiostat (Coulochem III; ESA Inc.) with an applied potential of −200 mV for detection of DA. The signals from the detector were transferred to a computer for analysis (Datalys Azur, Grenoble, France). The retention time of DA was approximately 8 min.

Histology

At the end of the microdialysis experiment, rats were decapitated and brains rapidly removed and stored in 4% paraformaldehyde in phosphate buffer for at least 5 d. Serial coronal sections (50 μm) were made using a cryostat (Slee Medical GmbH, Mainz, Germany), and histological verification of probe placement was confirmed with reference to the stereotaxic atlas of Paxinos and Watson (1998). No distinction could be made between the core and the shell of the nucleus accumbens. Data are reported only from animals where probe membranes were correctly positioned in the nucleus accumbens.

Electrophysiology

Electrophysiological experiments were performed as previously described (Linderholm et al., 2007; Nilsson et al., 2006). Briefly, rats were anaesthetized (chloral hydrate; 400 mg/kg i.p.) and mounted onto the ear bars of a conventional stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). For i.v. administration of drugs, a cannula was inserted into a lateral tail vein. The skull surface was exposed and a 3 mm

Table 1. Whole-brain concentrations of kynurenic acid

	Controls ($n=15$)	Acute kynurenine (5 mg/kg s.c., 60 min) ($n=9$)	Subchronic kynurenine (90 mg/kg.d s.c., for 6 d) ($n=13$)
Kynurenic acid (nM)	20.7 ± 3.5	66.1 ± 9.0***	40.6 ± 8.1*

Values represent mean ± S.E.M.
* $p<0.05$, *** $p<0.001$ vs. controls (Kruskal–Wallis analysis of variance followed by Mann–Whitney U test).

burr hole was drilled with its centre located 3.0 mm anterior to lambda and 0.7 mm lateral to the midline. A glass microelectrode was lowered into the region of the ventral tegmental area (VTA), according to the stereotaxic coordinates from the atlas of Paxinos and Watson (1998). All DA neurons were found 7.5–8.5 mm from the brain surface and fulfilled the neurophysiological characteristics (i.e. triphasic action potentials with a duration >2.0 ms, basal firing rates between 1 and 10 Hz and frequent occurrence of burst firing, including progressively decreasing spike amplitude) previously described for DA neurons in the VTA (Wang, 1981). Cells were considered silenced when no firing occurred during 30 consecutive seconds after d-amphetamine administration. To further confirm that recordings had been made on only DA neurons, the inhibitory action of a single dose of the DA agonist apomorphine (100 μg/kg i.v.) was, when experiments allowed, verified at the end of the experiments.

Data analysis

The distribution of spikes was analysed online utilizing a Spike II software program. In order to avoid artifacts in the sampling procedure, the spike analyser was set to ignore time- intervals <20 ms. The onset of a burst was determined as an inter-spike interval (ISI) <80 ms and its termination by the next interval >160 ms (Grace and Bunney, 1984a,b). Cells were considered to be bursting if at least one inter-spike time-interval of 100 recorded spikes was <80 ms. DA cell firing and burst firing activity was generally calculated over consecutive periods of 100–300 ISIs. At very low firing frequencies (<2 Hz) of cells following amphetamine administration, burst firing activity was occasionally calculated over consecutive periods of <100 ISIs.

Analysis of KYNA

Immediately after the electrophysiological experiments rats were decapitated. Blood was collected and brains were removed and immediately stored at −18 °C for subsequent analysis of KYNA with an isocratic reversed-phase HPLC system. For details regarding preparation and analysis of brain and blood see Linderholm et al. (2007) and Nilsson et al. (2006).

Drugs and chemicals

The following drugs were used: chloral hydrate (Merck, Darmstadt, Germany), L-kynurenine (Sigma, St Louis, MO, USA), d-amphetamine sulphate (Apoteksbolaget, Göteborg, Sweden). Chemicals used were as follows: zinc acetate, KYNA, methanol, octanesulfonic acid, Na_2EDTA (Sigma), sodium acetate (Riedel-de Haen, Seelze, Germany), perchloric acid (Kebo Laboratory, Stockholm, Sweden), and acetonitrile (Labasco, Partille, Sweden). Perfusion fluid composition (CMA Microdialysis AB, Solna, Sweden) (mM): 147.0 NaC1, 2.7 KCl, 1.2 $CaCl_2$, and 0.85 $MgCl_2$.

Statistical analysis

The statistical software package GraphPad Prism® 4.03 (GraphPad Software Inc., San Diego, CA, USA) was used. All data are expressed as mean ± S.E.M. for at least four rats per treatment group. No differences between the naive control groups and the groups treated with saline for 6 d were observed in either of the tested parameters. For ease of presentation, all control data presented were pooled into one control group. A p value of <0.05 was considered statistically significant.

Results

Concentrations of KYNA following administration of kynurenine

Whole-brain KYNA levels in controls and in kynurenine-treated rats are summarized in Table 1. A single dose of kynurenine (5 mg/kg s.c., 60 min, $n=9$) elevated brain KYNA levels 3-fold whereas subchronic treatment with kynurenine (90 mg/kg.d at day of surgery for 6 d, $n=13$) was associated with a 2-fold increase in brain KYNA levels. In a subset of animals KYNA was measured in microdialysates from nucleus accumbens. Basal levels of KYNA were 1.28 ± 0.21 nM in vehicle-treated rats ($n=6$) vs. 2.68 ± 0.50 nM ($p=0.038$) in rats subchronically treated with kynurenine (90 mg/kg.d at day of surgery for 6 d, $n=4$). Analysis of blood levels of KYNA revealed that

Table 2. Blood concentrations of kynurenic acid

	Day 0	Day 6	Day 6	
	Minipump surgery ($n=20$)	Subchronic vehicle (0.9% NaCl s.c.) ($n=7$)	Subchronic kynurenine (90 mg/kg.d s.c.) ($n=13$)	Acute kynurenine (5 mg/kg s.c., 60 min) ($n=9$)
Kynurenic acid (nM)	61.8 ± 15.6	52.6 ± 6.9	200.7 ± 43.5***	496.5 ± 113.3***

Values represent mean ± S.E.M.
*** $p<0.001$ vs. day 0 values (Kruskal–Wallis analysis of variance followed by Mann–Whitney U test).

kynurenine-treated rats (90 mg/kg.d at day of surgery for 6 d, $n=13$) displayed a 3-fold increase in KYNA levels at day 6 compared to blood levels on the day of implantation, whereas blood concentration of KYNA in vehicle-treated rats ($n=7$) did not change during the treatment period (see Table 2).

Microdialysis experiments

The mean baseline concentration of DA in a perfusate collected over 30 min from nucleus accumbens in awake, freely moving rats was 1.61 ± 0.23 nM ($n=25$). Data were not corrected for in-vitro dialysis probe recovery. Basal DA concentration in rats subchronically administered kynurenine (90 mg/kg.d at day of surgery for 6 d) did not differ from basal concentrations detected in control rats (data not shown). Administration of a single dose of kynurenine (5 mg/kg s.c., $n=4$) was associated with a slight non-significant decrease in DA output after 60 min (−25%, $p=0.13$).

The amphetamine-induced release of nucleus accumbens DA is summarized in Figure 1. Systemic administration of amphetamine (2 mg/kg i.p.) was associated with a marked increase in DA release in the nucleus accumbens in awake, freely moving control rats as well as in rats pretreated with a single dose of kynurenine (5 mg/kg s.c., 60 min prior to administration of amphetamine). Maximal increase in DA release was obtained 60 min after the amphetamine injection (+374% in control rats, $n=13$ and +461% in rats pretreated with a single dose of kynurenine, $n=4$). Administration of amphetamine (2 mg/kg i.p.) to rats subchronically treated with kynurenine (90 mg/kg.d at day of surgery for 6 d) was associated with a potentiated extracellular DA efflux. This effect reached its maximum 60 min after the amphetamine injection (+814%, $n=8$) and was significantly enhanced compared to amphetamine-induced DA release observed in control rats.

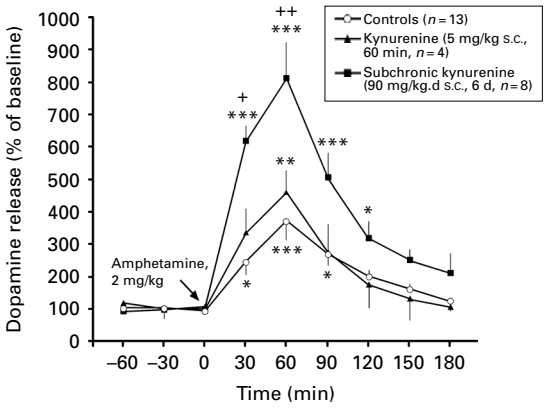

Figure 1. Effects of amphetamine (2 mg/kg i.p.) administration on dopamine output in the nucleus accumbens in awake, freely moving rats. Each point represents the mean ± S.E.M. percent of baseline ($n=4$–13). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to pre-drug value (one-way ANOVA followed by Bonferroni multiple comparison test); $^{+}$ $p<0.05$, $^{++}$ $p<0.01$ between-group comparisons [two-way ANOVA for repeated measurements (time × treatment) followed by Bonferroni post-hoc tests].

Electrophysiological experiments

Effects of kynurenine on neuronal firing of VTA DA neurons

Basal average firing rate and percent burst firing activity measured in 33 VTA DA neurons from 24 control rats was 4.3 ± 0.3 Hz and 19.7 ± 3.9%, respectively. A single dose of kynurenine as well as subchronic administration of the drug was associated with increased firing of VTA DA neurons. Thus, in rats treated with a single dose of kynurenine (5 mg/kg s.c., 60 min; 32 VTA DA neurons from 13 rats), basal average firing rate and percent burst firing activity were 4.8 ± 0.4% Hz and 37.2 ± 5.9%, respectively. In rats subchronically treated with kynurenine (90 mg/kg.d

Table 3. Effects of kynurenine on the firing rate and spike distribution of dopamine neurons in the ventral tegmental area

	Controls (33 neurons)	Acute kynurenine (5 mg/kg s.c., 60 min) (32 neurons)	Subchronic kynurenine (90 mg/kg.d s.c., for 6 d) (19 neurons)
Firing rate (Hz)	4.3±0.3	4.8±0.4	6.1±0.4**
Mean % burst firing	19.7±3.9	37.2±5.9*	37.9±7.0*

Values represent mean±S.E.M. from 24 control rats, 11 rats treated with acute kynurenine and 13 rats subchronically treated with kynurenine.
* $p<0.05$, ** $p<0.01$ vs. corresponding control value (Kruskal–Wallis analysis of variance followed by Mann–Whitney U test).

at day of surgery for 6 d; 19 VTA DA neurons from 13 rats) the basal average firing rate and percent burst firing activity was 6.1±0.4% Hz and 37.9±7.0%, respectively (see Table 3).

Amphetamine-induced inhibition of VTA DA neurons

A previous study demonstrated a relationship between basal firing of a single VTA DA neuron and the responsiveness of this neuron to amphetamine. Thus, for faster firing of the VTA DA neuron, a higher dose of amphetamine is required to silence the neuron (White and Wang, 1984). In the present study, efforts were therefore made to record from DA neurons with similar frequency and burst firing activity within the three groups. No significant difference in the pre-drug firing rate and the average percent burst firing activity was noted between controls and rats treated with kynurenine prior to amphetamine administration (data not shown).

Systemic administration of amphetamine (0.2–25.6 mg/kg i.v.) was found to dose-dependently reduce firing rate and percent burst firing activity of VTA DA neurons. In control rats, the firing rate of nine VTA DA neurons out of 13 was totally suppressed following administration of 1.6 mg/kg amphetamine (Figures 2, 3a). The DA neurons that were still firing following administration of 1.6 mg/kg amphetamine were quiescent after administration of 3.2 mg/kg (two neurons) or 6.4 mg/kg (two neurons). Rats pretreated with a single dose of kynurenine (5 mg/kg s.c., 60 min) did not change the inhibitory action of amphetamine (Figure 2). In rats with subchronically administered kynurenine (90 mg/kg.d at day of surgery for 6 d), larger doses of amphetamine were required to induce inhibition of firing rate and percent burst firing activity (Figures 2, 3b). Thus, 1.6 mg/kg amphetamine was not able to totally suppress firing in any DA neuron

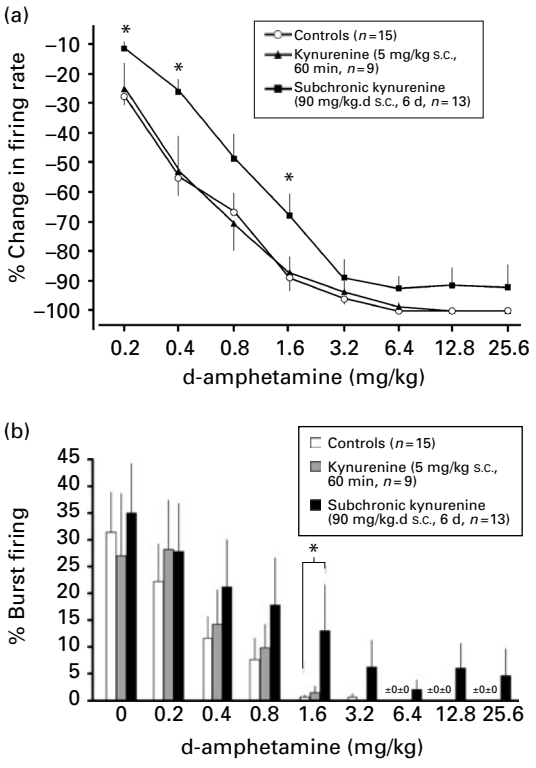

Figure 2. Cumulative dose–response curves illustrating the action of intravenously administered amphetamine on (a) the firing rate, and (b) the burst firing activity of VTA DA neurons in control rats and kynurenine-treated rats, either a single dose or subchronically. Each point respresents the mean±S.E.M. obtained from 8–15 neurons. * $p<.05$, between-group comparisons (Kruskal–Wallis analysis of variance followed by Mann–Whitney U test).

recorded from in these rats. Following 12.8 mg/kg amphetamine two neurons were still firing and bursting, one of them even at the dose 25.6 mg/kg and this neuron was not quiescent until apomorphine (100 μg/kg i.v.) was administered.

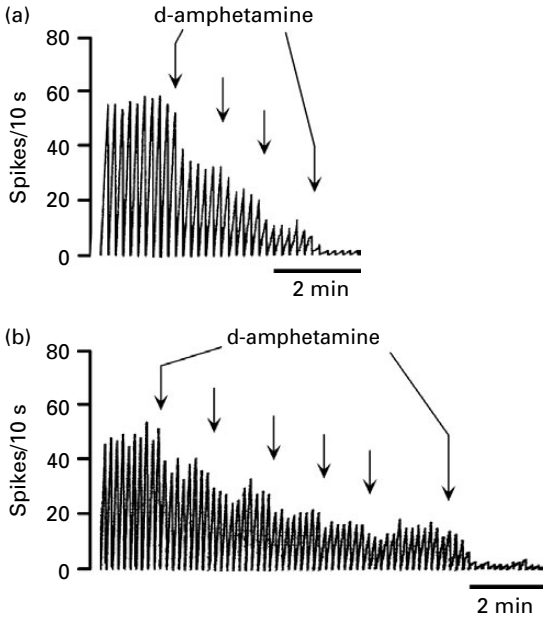

Figure 3. Extracellular single-unit recordings from DA neurons in the VTA following intravenous administration of d-amphetamine in cumulative doses (0.2 + 0.2 + 0.4 + 0.8 + 1.6 + 3.2 mg/kg at arrows) in (a) a control rat, and (b) a rat after subchronic treatment with kynurenine (90 mg/kg.d s.c., for 6 d).

Discussion

The major finding of the present study is that subchronic treatment with kynurenine, in contrast to a single dose of the compound, enhances the amphetamine-induced DA release in the rat nucleus acccumbens. Given the augmented effect of amphetamine on DA neurotransmission seen in patients with schizophrenia (Abi-Dargham et al., 1998; Laruelle, 1998; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996, 1999), the present results suggest that hypoglutamatergia, here induced by elevated levels of KYNA, may resemble a state similar to what is proposed in the disease. A rational explanation for the potentiated DA release may be the attenuated effect of amphetamine to inhibit firing of VTA DA neurons in a situation of subchronically elevated brain KYNA.

In the present study, kynurenine was given in order to elevate brain KYNA. Kynurenine is the precursor of several neuroactive metabolites, among them KYNA, 3-hydroxykynurenine (3-HK) and quinolinic acid. Although only KYNA concentrations were measured in the present study, 3-HK and quinolinic acid levels may be affected as a result of the kynurenine treatment and could, thus, theoretically contribute to the presently observed interaction with amphetamine. However, 3-HK is a free-radical generator (Eastman and Guilarte, 1989) and appears not to directly interact with glutamatergic or dopaminergic processes, making it less likely to participate in the effects induced by kynurenine. Quinolinic acid is an NMDA receptor agonist and an increased brain concentration of this compound might lead to excitotoxic lesions and neuronal cell death (Stone, 1993). Administration of kynurenine has previously been found to attenuate quinolinic acid-induced neurotoxicity in the rat, a finding in all probability related to increased levels of KYNA and its anticonvulsant and neuroprotective properties (Nozaki and Beal, 1992; Santamaria et al., 1996; Vecsei et al., 1992). In the present study we demonstrated that administration of kynurenine increases endogenous concentrations of KYNA in whole brain, accumbal microdialysate and blood. In addition, both acute and subchronic administration of kynurenine was associated with increased firing of VTA DA neurons, a finding in line with previous studies (Linderholm et al., 2007; Nilsson et al., 2006). Furthermore, increased VTA DA firing has also been observed following administration of PNU 156561A (Erhardt and Engberg, 2002; Erhardt et al., 2001b, 2002), a kynurenine 3-hydroxylase inhibitor that prevents the conversion of kynurenine to 3-HK and quinolinic acid and thereby more selectively increases brain levels of KYNA. Hence, the present results obtained following kynurenine treatment are likely to be exclusively mediated via increased levels of endogenous brain KYNA.

A question of importance is whether the effects of KYNA are mediated via glutamatergic or cholinergic neurotransmission since KYNA is able to antagonize both the glycine site of the NMDA receptor and the nicotinic α_7* receptor at low concentrations. Recently we demonstrated that the increase in firing of VTA DA neurons following acutely elevated levels of KYNA is specifically related to blockade of the NMDA receptor (Erhardt and Engberg, 2002; Linderholm et al., 2007; Schwieler et al., 2006). In consonance, systemic administration of other NMDA receptor antagonists, e.g. PCP, ketamine, MK-801, SDZ 220-581 as well as specific antagonists of the NMDA/glycine receptor (e.g. L-701,324) produce excitation of midbrain DA neurons (Erhardt and Engberg, 2002; French, 1994; French et al., 1993; Linderholm et al., 2007; Schwieler et al., 2006), whereas a blockade of the nicotinic α_7* receptor is associated with a slight decrease in firing (Linderholm et al., 2007). One may also speculate whether elevated KYNA concentrations would activate VTA DA neurons via other glutamate receptors, e.g. through direct interaction with AMPA receptors

(Prescott et al., 2006) or indirectly via increased glutamate release (Mathe et al., 1998; Svensson et al., 1998). However, such mechanisms seem implausible since local administration of KYNA reduces glutamate in the rat caudate nucleus and the striatum (Carpenedo et al., 2001; Rassoulpour et al., 2005). Moreover, a putative potentiation of AMPA receptors by KYNA appears to be restricted to *Xenopus* oocytes (Prescott et al., 2006). Rather, a disrupted gamma aminobutyric acid (GABA)-ergic inhibitory input should account for the paradoxical increase in firing of these neurons following acute systemic administration of NMDA receptor antagonists (French, 1994; French et al., 1993) or acutely increased levels of endogenous KYNA (Erhardt and Engberg, 2002). Thus, NMDA receptor antagonists may primarily reduce the activity of GABAergic projections to the VTA, thereby disinhibiting the VTA DA neurons. In support of this view GABAergic interneurons, compared to glutamatergic pyramidal neurons, display a particular vulnerability to NMDA receptor antagonists (Grunze et al., 1996; Li et al., 2002).

Despite the excitation of VTA DA neurons observed following acute and chronic elevation of KYNA, basal extracellular levels of DA in the nucleus accumbens were not affected. This discrepancy may be related to differences in sensitivity between the methodological techniques used or by an antagonistic action of KYNA at nicotinic α_7* receptors located on glutamatergic afferents modulating terminal DA release (Rassoulpour et al., 2005). Notably, also in patients with schizophrenia basal dopaminergic output appears to be unaltered (Abi-Dargham et al., 1998; Laruelle, 1998; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996, 1999). In line with previous studies (Fuentealba et al., 2007; Guix et al., 1992; Moghaddam and Bunney, 1989), administration of amphetamine to control rats increased accumbal DA efflux by nearly 400%. The present results also show an enhanced amphetamine-induced accumbal DA release under conditions of subchronic elevation of brain KYNA. In consonance, dopaminergic response to an amphetamine challenge is enhanced in the prefrontal cortex following subchronic administration of PCP (Balla et al., 2003). A potentiated DA release following subchronic pretreatment with PCP or elevated levels of KYNA, resembles the response to amphetamine exposure observed in patients with schizophrenia (Abi-Dargham et al., 1998; Laruelle, 1998; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996, 1999) as well as the amphetamine-induced response seen in healthy volunteers pre-exposed to an NMDA receptor antagonist (Breier et al., 1998; Kegeles et al., 2000).

Increased terminal DA concentrations induced by cytoplasmatic/vesicular release and efflux through the DA transporter, constitute the most prominent effects of amphetamine (Kahlig et al., 2005; Kuczenski and Segal, 1989). However, a synaptic elevation of DA simultaneously activates feedback mechanisms that suppress DA firing and synthesis/release via both presynaptic inhibitory DA D_2 receptors and postsynaptic DA D_1 receptors located on GABAergic afferents controlling VTA DA neurons (White, 1996). The mechanism underlying the potentiated DA release following amphetamine administration is thus puzzling in view of the divergent and even opposite effects on DA transmission by the drug. Furthermore, acute systemic administration of amphetamine increases the release of glutamate (Giorgetti et al., 2001, 2002; Wolf et al., 2000) and acetylcholine (Arnold et al., 2001) while it decreases the concentration of KYNA (Rassoulpour et al., 1998). However, substantial differences in amphetamine response were observed between acute and subchronic KYNA elevation, suggesting that adaptive mechanisms are involved. In order to analyse whether attenuated feedback mechanisms could underlie the potentiated DA release, electrophysiological recordings from VTA DA neurons were performed. Indeed, rats with subchronically elevated KYNA levels required higher doses of amphetamine to inhibit firing of these neurons. As previously proposed, the hyper-reactivity of dopaminergic systems seen in schizophrenia (Abi-Dargham et al., 1998; Laruelle, 1998; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996, 1999) may reflect a dysfunction in NMDA-mediated regulation of GABAergic afferents impinging upon DA cell soma and terminals (Carlsson et al., 1999; Coyle, 2006; Javitt, 2007). An attenuated GABAergic tone may thus underlie the increased basal firing of VTA DA neurons (see above) as well as the reduced efficacy of amphetamine in inhibiting these neurons in rats with elevated levels of KYNA. Thus, the attenuated inhibitory response in firing of VTA DA neurons to amphetamine may functionally contribute to the potentiated accumbal DA release observed in rats with subchronically elevated levels of KYNA. Other mechanisms may also be involved in the substantial DA release seen following subchronic KYNA elevation. Thus, subchronic elevation of brain KYNA, concurrent with the observed prolonged activation of dopaminergic neuronal activity, may induce long-term alterations in synaptic plasticity, including e.g. effects on axonal transport and vesicular trafficking, or increase the cytoplasmic/vesicular pools of DA. Alternatively, subchronically elevated levels of

KYNA might also induce adaptive changes in receptor expression. In this regard, the nicotinic α_7* receptor is known to rapidly adapt following chronic nicotine exposure (Anand et al., 1993; Marks et al., 1985). Since KYNA blocks presynaptic nicotinic α_7* receptors located on glutamatergic terminals (Carpenedo et al., 2001; Rassoulpour et al., 2005), receptor adaptation following prolonged KYNA elevation might influence glutamate release and consequently DA output.

The present results may have potential behavioural implications and growing evidence suggest that KYNA per se impairs cognitive functions (Chess and Bucci, 2006; Chess et al., 2007), auditory sensory gating (Shepard et al., 2003) and PPI (Erhardt et al., 2004) in the rat. Hence pharmacologically elevated levels of KYNA mimic symptoms of schizophrenia where deficits in working memory and PPI are core neuropsychological dysfunctions (Geyer et al., 2001; Silver et al., 2003).

In conclusion, subchronic elevation of endogenous rat brain KYNA induces a markedly enhanced amphetamine-provoked DA release. This finding, tentatively induced by changes related to a long-lasting blockade of NMDA receptors and/or nicotinic α_7* receptors, resembles the enhanced response to amphetamine seen in patients with schizophrenia. Although underlying mechanisms need to be more thoroughly investigated, reduced responsiveness of VTA DA neurons to the inhibitory actions of amphetamine might partly explain the excessive DA efflux. Given the similarities in amphetamine response between patients with schizophrenia and rats with subchronically elevated levels of KYNA, one may propose that the latter condition may serve as a possible animal model of schizophrenia.

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Statement of Interest

None.

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