

Prolonged Nicotine Administration Results in Biphasic, Brain-Specific Changes in Kynurenate Levels in the Rat

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The content of the endogenous NMDA and $\alpha 7$ nicotinic acetylcholine receptor antagonist kynurenate (KYNA) is increased in the cerebral cortex and cerebrospinal fluid of patients with schizophrenia. In view of the very high incidence of smoking in schizophrenic individuals, a study was designed to examine the effect of acute and prolonged nicotine administration on brain KYNA levels in experimental animals. Adult male rats received subcutaneous nicotine injections twice daily for up to 10 days, and animals were routinely killed 1 h after the last injection. Neither acute treatment nor a 2-day regimen with 1 mg/kg nicotine (=0.35 mg/kg pure base) caused changes in cerebral KYNA levels. Four- or 6 day-treatment with this dose resulted in 20–40% decreases in cerebral KYNA content. Animals treated with 1 or 10 mg/kg nicotine for 10 days showed dose-dependent, significant increases in KYNA in hippocampus, striatum, and cortex, but not in the serum. Discontinuation of nicotine treatment for 7 days restored brain KYNA to control levels. Separate animals, implanted with osmotic minipumps delivering 2 mg/kg of nicotine/day for 10 days also showed significant elevations in brain KYNA. Hippocampal microdialysis, performed in animals receiving nicotine (1 mg/kg) for 10 days, revealed a significant increase in basal extracellular KYNA levels compared to controls, whereas acute treatment with this dose produced no such change. Measurements of KYNA's bioprecursor kynurenine in brain or blood did not reveal any nicotine-induced changes. These results indicate that nicotine has a brain-specific, biphasic effect on the transamination of kynurenine to KYNA. Such nicotine-induced fluctuations in brain KYNA may cause functional changes in processes that regulate glutamatergic and cholinergic neurotransmission in the normal and diseased brain. *Neuropsychopharmacology* (2005) **30**, 697–704, advance online publication, 20 October 2004; doi:10.1038/sj.npp.1300583

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INTRODUCTION

Several lines of evidence suggest a role of nicotinic acetylcholine receptors (nAChRs) in the pathophysiology of schizophrenia (Adler *et al*, 1998; Guan *et al*, 1999; Breese *et al*, 2000; Leonard *et al*, 2000). This connection was initially proposed because of the high incidence of heavy smoking in schizophrenic individuals (Hughes *et al*, 1986; Lohr and Flynn, 1992). It was found later that nicotine can normalize auditory gating and visual attention deficits, which are prominent pathological features of schizophrenia (Adler *et al*, 1993; Sherr *et al*, 2002). This could conceivably be mediated by the $\alpha 7$ subtype of the nAChR, which is known to be critically involved in cognitive functions (Levin and Simon, 1998). An intuitively attractive inference of

these studies is, therefore, that patients smoke heavily in an attempt to self-medicate, that is to correct various sensory abnormalities that are associated with the disease (Sandyk and Kay, 1991; Adler *et al*, 1993; Olincy *et al*, 1998). In line with this reasoning, auditory gating in animals can be specifically disrupted by selective antagonists of the $\alpha 7$ nAChR (Luntz-Leybman *et al*, 1992; Stevens *et al*, 1996, 1998). Conversely, $\alpha 7$ nAChR stimulation normalizes the auditory gating deficit that is observed in rats that have been reared in social isolation (O'Neill *et al*, 2003). These considerations have stimulated the development of $\alpha 7$ nAChR agonists such as ARR-17779 (Mullen *et al*, 2000), which interact directly with the binding site for acetylcholine, or of drugs such as galantamine (Reminyl[®]), which increase nAChR activity by interacting with a site close to, but distinct from, the acetylcholine-binding site (Pereira *et al*, 1994, 2002; Samochocki *et al*, 2003). It is also noteworthy that one measure of sensory gating abnormalities, diminished inhibition of the P50 evoked response to repeated auditory stimuli, has been linked to the chromosome 15q14 locus of the $\alpha 7$ nAChR gene (Freedman *et al*, 1997; Riley *et al*, 2000).

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Compared to control subjects with similar smoking habits, $\alpha 7$ nAChR density is reduced in the cerebral cortex and hippocampus of individuals with schizophrenia (Freedman *et al*, 1995; Adler *et al*, 1998; Leonard *et al*, 2000; Guan *et al*, 1999). Therefore, since cerebral $\alpha 7$ nAChRs are normally upregulated in heavy smokers (Benwell *et al*, 1988; Breese *et al*, 2000) and after chronic nicotine administration in animals (Olale *et al*, 1997; Sparks and Pauly, 1999), $\alpha 7$ nAChRs in patients appear to have an abnormally blunted reaction to excessive smoking. This could be due to a dysfunction in any of several distinct endogenous mechanisms, which normally control $\alpha 7$ nAChR expression and activity in the brain (Albuquerque *et al*, 1997; Pereira *et al*, 2002).

In a first attempt to investigate the possible role of one of these mechanisms, we examined the effect of nicotine on kynurenic acid (KYNA), a tryptophan metabolite that is present in the mammalian brain in nanomolar concentrations (Moroni *et al*, 1988; Turski *et al*, 1988). KYNA, long known as an antagonist of the glycine coagonist site of the NMDA receptor (Kessler *et al*, 1989), blocks $\alpha 7$ nAChR activity at even lower, endogenous brain concentrations (Hilmas *et al*, 2001), and reductions in brain KYNA levels were recently found to increase $\alpha 7$ nAChR function (Alkondon *et al*, 2004). It is therefore conceivable that nicotine-induced fluctuations in brain KYNA levels influence the activity of $\alpha 7$ nAChRs (Hilmas *et al*, 2001). This concept, and the recent demonstration that KYNA levels are elevated in cortical brain regions and cerebrospinal fluid of schizophrenic patients (Erhardt *et al*, 2001a; Schwarcz *et al*, 2001) and that elevations in brain KYNA disrupt auditory sensory gating (Shepard *et al*, 2003), prompted us to examine the consequences of acute and prolonged nicotine administration on the disposition of KYNA and its bioprecursor kynurenine in rats. Our data, some of which have been communicated in a preliminary fashion (Hilmas *et al*, 2001), revealed that nicotine causes biphasic, brain-specific changes in KYNA levels without affecting the brain concentrations of kynurenine.

MATERIALS AND METHODS

Materials

KYNA, L-kynurenine, and nicotine (bitartrate salt) were purchased from Sigma Chemical Co. (St Louis, MO). All other chemicals were of the highest commercially available purity.

Animals

Adult male Sprague-Dawley rats (200–220 g) were purchased from Charles River Laboratories (Kingston, NY). The animals were housed in an AAALAC-approved animal facility under standard laboratory conditions, that is, a 12/12 h light/dark cycle with free access to food and water. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Maryland, Baltimore.

Drug Administration

Nicotine was dissolved in phosphate-buffered saline (PBS; pH 7.4) and administered either subcutaneously (s.c.) twice

daily (every 12 h) or via osmotic minipumps (Alzet, Alza Corp., Palo Alto, CA; delivering 2 mg/kg of nicotine/day). Control animals received appropriate vehicle treatments. Animals treated s.c. were killed 1 h after the final injection, and animals treated with osmotic minipumps were killed on the morning of the final day of treatment.

Microdialysis

Microdialysis was performed as reported previously (Wu *et al*, 1992). Briefly, the animals were anesthetized with chloral hydrate (360 mg/kg, i.p.) and mounted in a David Kopf stereotaxic frame. A guide cannula (outer diameter: 0.65 mm) was positioned on top of the hippocampus (AP: 3.4 mm posterior to bregma, L: 2.3 mm from the midline, V: 1.5 mm below the dura) and secured to the skull with anchor screws and acrylic dental cement. At least 20 h after surgery, a microdialysis probe (CMA/10, membrane length: 2 mm, Carnegie Medicin, Stockholm, Sweden) was inserted through the guide cannula, extending throughout the hippocampus. The probe was connected to a microperfusion pump (CMA/100, Carnegie Medicin) set to a speed of 1 μ l/min and perfused with Ringer solution containing (in mM): NaCl, 144; KCl, 4.8; MgSO₄, 1.2; CaCl₂, 1.7; pH 6.7. In acute experiments, dialysate was collected every 30 min for a total of 7 h. In experiments where nicotine was administered for 10 days, animals received an additional injection of nicotine (1 mg/kg) 2 h after the collection of baseline samples, and microdialysis continued for an additional 7 h.

KYNA and Kynurenine Determination

Animals were killed by decapitation, and trunk blood was collected when indicated. The brain was removed, and hippocampus, striatum, and frontal cortex were rapidly dissected out, placed on dry ice and stored at -80°C . On the day of the assay, the tissue was thawed and homogenized (1:10, w/v) in ultrapure water. A 300 μ l aliquot of the homogenate was acidified with 75 μ l of 6% perchloric acid. After centrifugation (10 min, 12 000g), an aliquot of the supernatant was diluted (1:1, v/v) with HPLC mobile phase (200 mM zinc acetate containing 3.5% acetonitrile, pH 6.2). A 200 μ l aliquot of the sample was applied to a C₁₈ reverse-phase HPLC column (150 \times 4.6 mm; Alltech Associates, Deerfield, IL, USA; flow rate: 1.0 ml/min), and KYNA was eluted isocratically with a retention time of approximately 5 min. KYNA was detected fluorimetrically using a Perkin-Elmer LC 240 Fluorescence Detector (Beaconsfield, UK; excitation wavelength: 344 nm; emission wavelength: 398 nm).

To determine extracellular hippocampal KYNA levels, 30 μ l microdialysate fractions were directly applied to the HPLC column, and KYNA was measured as described above.

For the measurement of kynurenine, 200 μ l of the diluted acidic supernatant used for KYNA determination were applied to the same HPLC system described above. Kynurenine was eluted with a retention time of approximately 4 min and detected by UV spectroscopy at 365 nm (Beckman 160 Absorbance detector, Fullerton, CA, USA).

To determine serum levels of kynurenine and KYNA, trunk blood was immediately centrifuged (10 min, 12 000g). The supernatant plasma was diluted (1:5, v/v) with ultrapure water and acidified with 75 μ l of 6% perchloric acid. After centrifugation (5 min, 12 000g), an aliquot of the serum was further diluted (1:1, v/v) with HPLC mobile phase, and 200 μ l were subjected to HPLC analysis as described above.

All chromatographic data were recorded using a Hewlett-Packard 3390 A integrator.

Protein Determination

Protein was determined in aliquots of the original tissue homogenate according to the method of Lowry *et al* (1951).

Data Analysis

Microdialysis data were not corrected for recovery from the probe (~20%; Wu *et al*, 1992). For all experiments, a repeated-measures analysis of variance (ANOVA) with appropriate *post hoc* analysis was used. A *p*-value of <0.05 was considered significant in all analyses.

RESULTS

Acute Nicotine Administration

To determine the acute effects of nicotine on cerebral KYNA levels, animals received a single injection of nicotine (1 mg/kg (= 0.35 mg/kg free base)). Examined after 1 or 2 h, this treatment did not cause significant changes in forebrain tissue KYNA (in fmol/mg protein: control, 163.5 \pm 17.3; 1 h, 164.1 \pm 28.6; 2 h, 166.2 \pm 33.0; *n* = 5 per group). Measurement of extracellular KYNA concentrations in hippocampal dialysates, too, revealed no acute effect of nicotine (1 mg/kg) for up to 5 h (*n* = 5).

Repeated Nicotine Administration: Dose Dependency

A 10-day treatment with nicotine caused dose-dependent increases in tissue KYNA levels in hippocampus, striatum, and frontal cortex. Whereas 0.1 mg/kg of nicotine was ineffective, 1 mg/kg of nicotine resulted in a 34–45% increase, and 10 mg/kg of nicotine caused an 82–107% increase in KYNA content in the three brain areas (Figure 1). These differences were found to be statistically significant in all brain regions sampled.

The tissue content of KYNA's bioprecursor kynurenine, too, was determined in the brains of animals receiving PBS or 1 mg/kg nicotine for 10 days (*n* = 5 per group). The levels of kynurenine (in pmol/mg protein) in nicotine-treated rats (hippocampus, 24 \pm 8; striatum, 23 \pm 3; frontal cortex, 20 \pm 6) were not significantly different from those in PBS-treated rats (hippocampus, 20 \pm 3; striatum, 22 \pm 5; frontal cortex, 24 \pm 6).

Repeated Nicotine Administration: Time Dependency

The time course of nicotine-induced changes in tissue KYNA content was examined in separate animals (Figure 2). After repeated administration of nicotine (1 mg/kg) for 2, 4,

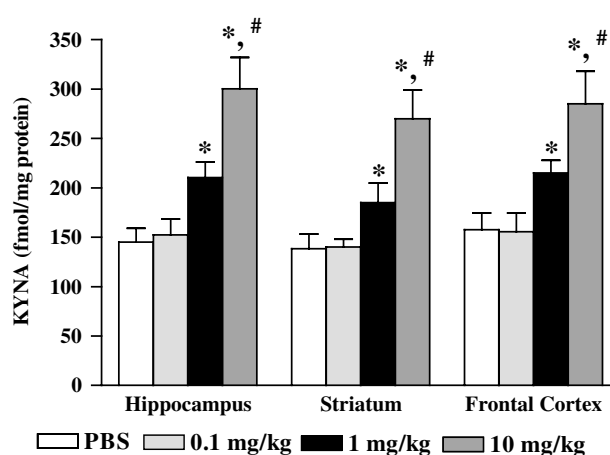


Figure 1 Dose-dependent effects of repeated nicotine administration (s.c., b.i.d. for 10 days) on KYNA levels in various brain regions. Experiments were conducted as described in the text. Data are expressed as the mean \pm SEM (*n* = 5 per group). **p* < 0.05 vs PBS-injected animals, #*p* < 0.05 vs 1 mg/kg nicotine (repeated measures ANOVA followed by Bonferroni's *post hoc* test for multiple comparisons).

6, 8, or 10 days, KYNA levels in hippocampus, striatum, and frontal cortex underwent biphasic changes over time. Repeated injections for 4 or 6 days resulted in 14–35% reductions in KYNA levels, which reached statistical significance in hippocampus and striatum. In contrast, a 10-day treatment with nicotine caused an approximately 40% increases in KYNA levels in all three brain areas (cf Figure 1). Treatment for 2 or 8 days had no significant effect on endogenous KYNA.

Repeated Nicotine Administration: Effect on Extracellular KYNA Levels

To examine if changes in tissue levels are paralleled by changes in extracellular KYNA, hippocampal microdialysis was performed in rats treated with nicotine (1 mg/kg) for 10 days (Figure 3). The basal levels of KYNA in the nicotine-treated animals (3.6 \pm 0.4 nM) were significantly higher than in PBS-treated controls (2.4 \pm 0.5 nM). An additional acute injection of 1 mg/kg nicotine after the fourth sample collection, that is after 2 h of baseline determination, did not cause a significant change in extracellular KYNA levels in either nicotine- or PBS-treated rats.

Prolonged Nicotine Infusion Via Minipumps

Administration of nicotine (2 mg/kg (= 0.7 mg/kg free base) per day) via osmotic minipumps for 10 days resulted in a significant, approximately 70%, increase in KYNA content in hippocampus, striatum, and frontal cortex, compared to PBS-treated controls (Figure 4).

Repeated Nicotine Administration: Effect of Drug Discontinuation

To determine the reversibility of the nicotine effect, repeated treatment with nicotine (1 mg/kg) was stopped after 10 days, and KYNA levels were determined in

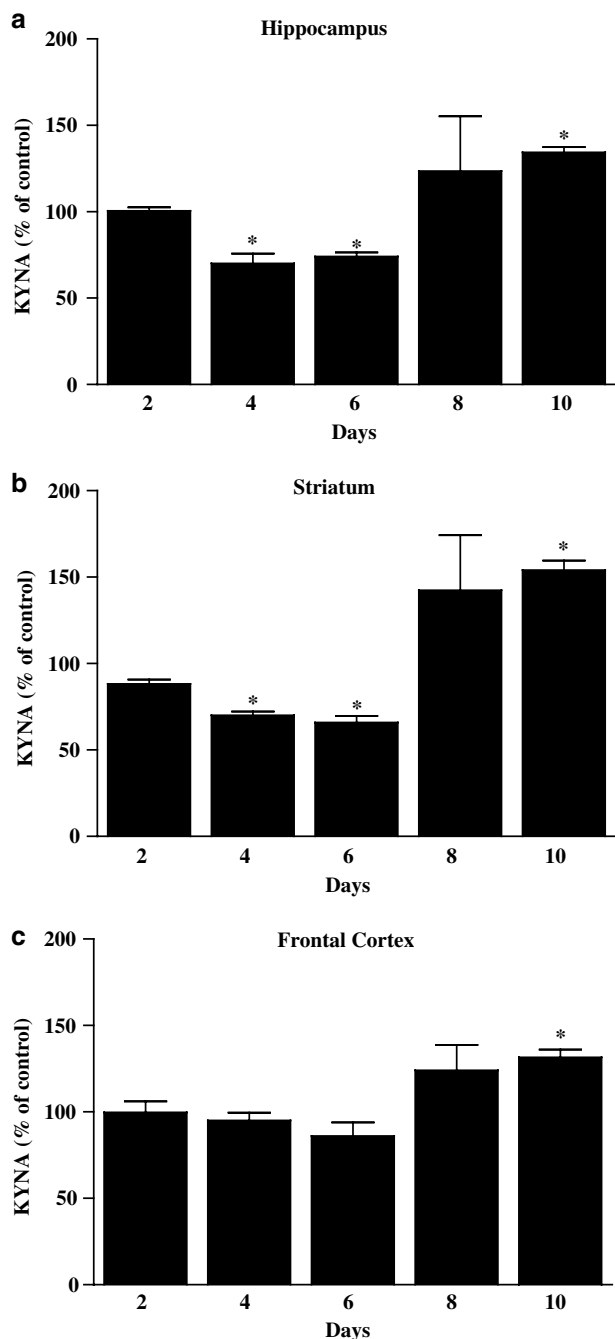


Figure 2 Time-dependent effects of nicotine (1 mg/kg, s.c., b.i.d. for 2, 4, 6, 8, or 10 days) on KYN levels in the hippocampus (a), striatum (b), and frontal cortex (c). PBS-injected control animals contained 146.4 ± 18.4 (hippocampus), 131.1 ± 17.4 (striatum), and 135.2 ± 16.2 (frontal cortex) fmol KYN/mg protein. Experiments were conducted as described in the text. Data are expressed as the mean \pm SEM ($n=5$ per time point). * $p < 0.05$ vs PBS-injected animals (repeated measures ANOVA followed by Bonferroni's *post hoc* test for multiple comparisons).

hippocampus, striatum, and frontal cortex 7 days later. Compared to animals tested immediately after 10 days of repeated nicotine treatment, brain KYN in these rats was significantly reduced and had in fact returned to control levels (Figure 5). In separate animals, an additional single challenge with nicotine (1 mg/kg) 7 days after nicotine discontinuation failed to affect brain KYN levels (Figure 5).

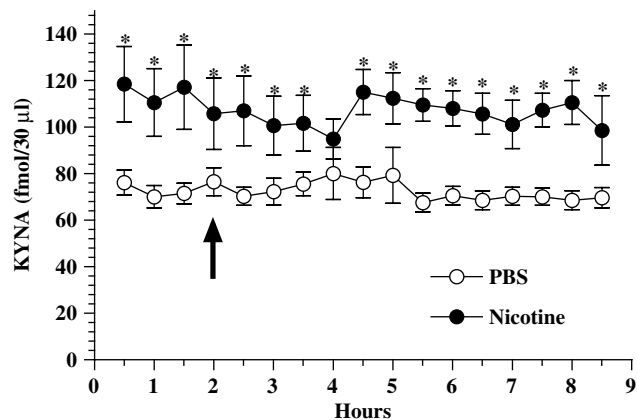


Figure 3 Effect of repeated nicotine (1 mg/kg, s.c., b.i.d. for 10 days) or PBS administration on extracellular KYN levels in the hippocampus. Experiments were conducted as described in the text. Data are the mean \pm SEM of five animals per group. Nicotine treatment significantly elevated baseline extracellular KYN concentrations ($p < 0.05$ vs PBS-injected animals; repeated measures ANOVA followed by Bonferroni's *post hoc* test for multiple comparisons). No changes in extracellular KYN were seen after an additional acute injection of nicotine (1 mg/kg, s.c.) in these animals (arrow; $p > 0.05$ vs the respective baseline; repeated measures ANOVA followed by Bonferroni's *post hoc* test for multiple comparisons).

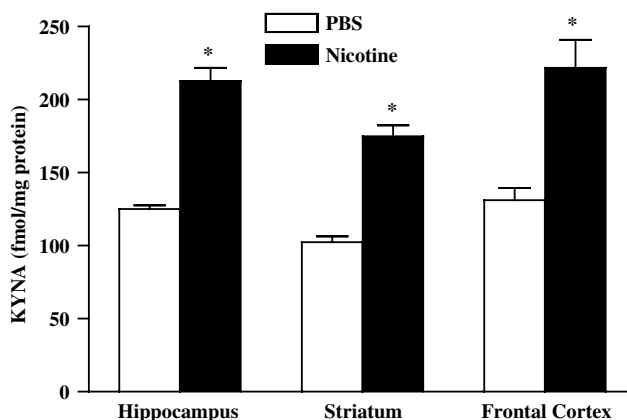


Figure 4 Effects of prolonged nicotine infusion (2 mg/kg/day for 10 days) on KYN levels in various brain regions. Experiments with osmotic minipumps were conducted as described in the text. Data are expressed as the mean \pm SEM ($n=5$ per group). * $p < 0.05$ vs PBS-treated animals (repeated measures ANOVA followed by Bonferroni's *post hoc* test for multiple comparisons).

Repeated Nicotine Administration: Kynurenine and Kyna Content in Serum

Serum levels of kynurenine and KYN were measured in animals that received repeated injections of nicotine (1 mg/kg) for 2, 4, 6, 8, or 10 days. None of these treatment regimens caused changes in the levels of either metabolite (Table 1).

DISCUSSION

The present study demonstrated that prolonged, but not acute, nicotine administration causes significant changes in KYN levels in the rat brain. In agreement with an earlier, preliminary experiment (Hilmas *et al*, 2001), these nicotine-induced changes were biphasic in nature, that is an initial

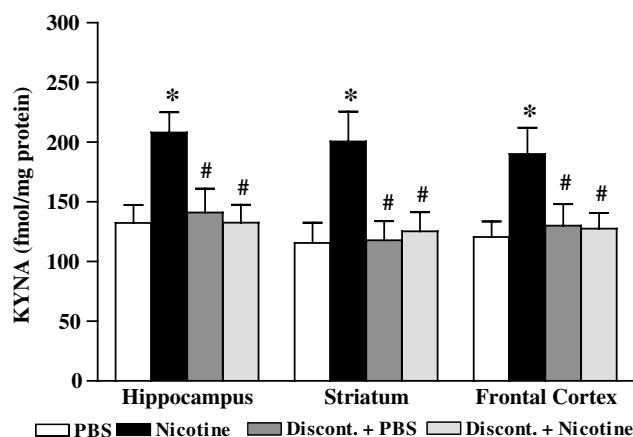


Figure 5 Effect of discontinuation of prolonged nicotine treatment. Two groups of animals received either PBS or nicotine (1 mg/kg, s.c., b.i.d.) for 10 days ($n=5$ per group). Two other groups received nicotine (1 mg/kg, s.c., b.i.d.) for 10 days. Nicotine administration was then discontinued. After 7 days, the animals received a single s.c. injection of either PBS ('Discont. + PBS'; $n=5$) or nicotine (1 mg/kg; 'Discont. + nicotine'; $n=5$) and were killed 1 h later. * $p < 0.05$ vs PBS; # $p < 0.05$ vs nicotine (repeated measures ANOVA followed by Bonferroni's *post hoc* test for multiple comparisons). No changes were seen between 'Discont. + PBS' and 'Discont. + nicotine' animals ($p > 0.05$; repeated measures ANOVA followed by Bonferroni's *post hoc* test for multiple comparisons).

Table 1 Blood Kynurenic acid and KYNA Levels in Nicotine-Treated Rats

Days	PBS (pmol/mg protein)		Nicotine (pmol/mg protein)	
	Kynurenic acid	Kynurenic acid	Kynurenic acid	Kynurenic acid
2	31 ± 4	0.313 ± 0.030	27 ± 7	0.328 ± 0.028
4	28 ± 5	0.300 ± 0.018	32 ± 5	0.323 ± 0.030
6	34 ± 6	0.317 ± 0.028	28 ± 6	0.330 ± 0.020
8	22 ± 4	0.328 ± 0.030	30 ± 8	0.328 ± 0.010
10	29 ± 3	0.313 ± 0.020	31 ± 5	0.330 ± 0.038

Animals were killed 1 h after the final injection of nicotine (1 mg/kg, s.c., b.i.d.), and the blood was processed as described in the text. Data are the mean ± SEM ($n=5$ per group). No significant differences between PBS- and nicotine-treated rats were found (repeated measures ANOVA followed by Bonferroni's *post hoc* test for multiple comparisons).

significant reduction in KYNA was observed after 4 or 6 days of repeated injections, whereas a significant increase in KYNA was seen when nicotine was given for 10 days. These nicotine-induced fluctuations were not brain region-specific since similar effects were observed in the hippocampus, striatum, and frontal cortex. However, they were not accompanied by changes in the serum levels of KYNA. The increases in brain tissue KYNA levels observed after 10 days of nicotine treatment were reversible, dose-dependent and, as documented by hippocampal microdialysis, associated with quantitatively similar elevations in the extracellular compartment. These results also resembled the increases in brain KYNA levels that were seen when the drug was injected s.c. twice daily (mimicking smoking) or administered by chronic infusion (mimicking a nicotine patch) for 10 days. Finally, nicotine treatment was found to

have no effect on the concentration of KYNA's immediate bioprecursor, kynurenine, in brain or serum.

KYNA is a product of the kynurenine pathway of tryptophan degradation. In the mammalian brain, KYNA is formed through irreversible transamination of kynurenine by kynurenine aminotransferases (Guidetti *et al*, 1997). These enzymatic processes take place predominantly in astrocytes (Ceresoli-Borroni *et al*, 1999a; Guillemain *et al*, 2001; Kiss *et al*, 2003), which then readily liberate newly synthesized KYNA into the extracellular space for possible receptor interactions (Turski *et al*, 1989). Notably, KYNA production is controlled by several distinct factors and mechanisms. Some, such as kynurenine, competing amino-acid substrates, and 2-oxoacids, operate both in the periphery and in the brain. Others, for example the decrease in KYNA formation effected by reduced cellular energy metabolism or by depolarizing agents such as potassium or veratridine, are brain-specific (Gramsbergen *et al*, 1997). It remains to be seen if and to what extent astrocytic $\alpha 7$ nACh (Sharma and Vijayaraghavan, 2001) or NMDA (Krebs *et al*, 2003) receptors can directly influence cerebral KYNA formation.

KYNA formation can also be reduced by dopaminergic compounds such as amphetamine (Rassoulpour *et al*, 1998), L-DOPA (Wu *et al*, 2002), or selective dopamine receptor agonists (Poeggeler *et al*, 1998). These effects, too, are brain-specific and therefore qualitatively similar to the effect of prolonged nicotine administration described here. In fact, it is possible that dopaminergic mechanisms participate in the biphasic effects of nicotine on brain KYNA reported in the present study. Thus, nAChRs show both desensitization (Marks *et al*, 1983; Castro and Albuquerque, 1995) and subsequent supersensitivity (Schwartz and Kellar, 1983, 1985; Wonnacott *et al*, 1990) in response to repeated or chronic nicotine treatment, and these adaptive changes may influence the nAChRs-mediated regulation of extracellular dopamine (Harsing *et al*, 1992; Marshall *et al*, 1997). In other words, nicotine could cause biphasic changes in brain KYNA formation indirectly by controlling dopamine release linked to nAChR activation.

Alternatively or in addition, the effects of prolonged nicotine administration on brain KYNA levels may involve glutamatergic mechanisms. Thus, activation of presynaptic $\alpha 7$ nACh receptors is associated with enhanced glutamatergic transmission (Alkondon *et al*, 1996; Gray *et al*, 1996). Chronic nicotine treatment results in changes in astrocytic glutamate transporters (Lim and Kim, 2001) and causes the functional upregulation of ionotropic glutamate receptors (Risso *et al*, 2004). These phenomena result in abnormal glutamatergic activity in response to prolonged nicotine treatment and could, in turn, compromise cerebral KYNA formation (Wu *et al*, 1992).

In neurobiological research, high concentrations of KYNA (≥ 1 mM) are frequently used as a tool to block all ionotropic glutamate receptors (Perkins and Stone, 1982). Consequently, intracerebral application of large amounts of KYNA has long been known to exert neuroprotective and anticonvulsant effects in experimental animals (Foster *et al*, 1984). At much lower concentrations, KYNA inhibits the strychnine-insensitive glycine coagonist site of the NMDA receptor (IC_{50} : 8 μ M; Kessler *et al*, 1989), and recent evidence favors a physiological action of KYNA as an

allosteric, noncompetitive antagonist of the $\alpha 7$ nAChR (Hilmas *et al*, 2001; Alkondon *et al*, 2004). Endogenous KYNA may therefore affect neuronal excitability and vulnerability by directly or indirectly interfering with both cholinergic and glutamatergic neurotransmission (Harris *et al*, 1998; Poeggeler *et al*, 1998; Cozzi *et al*, 1999; Wu *et al*, 2000; Pereira *et al*, 2002; Schwarcz and Pellicciari, 2002; Sapko *et al*, 2003). Further, the changes in cerebral KYNA levels observed after prolonged nicotine administration may account for the ability of nicotine to influence neuronal viability *in vivo* (Akaike *et al*, 1994; Marin *et al*, 1994; O'Neill *et al*, 1998).

The present findings are also relevant for the pathophysiology of schizophrenia since both glutamate receptor and $\alpha 7$ nAChR dysfunction have been implicated in the disease process (Carlsson and Carlsson, 1990; Freedman *et al*, 1995; Tamminga, 1998; Leonard *et al*, 2000; Schilström *et al*, 2000; Coyle and Tsai, 2004). Thus, the elevated levels of KYNA measured in the brain and cerebrospinal fluid (Erhardt *et al*, 2001a; Schwarcz *et al*, 2001) may contribute to the presumed hypoglutamatergic and hypocholinergic tone in schizophrenic individuals. Since brain KYNA levels are decreased 4 and 6 days after repeated nicotine administration, excessive smoking in the schizophrenic population could constitute an attempt to self-medicate (cf Introduction). Indeed, a reduction in brain KYNA enhances nicotinic and glutamatergic transmission (Alkondon *et al*, 2004) and could thus normalize gating (Adler *et al*, 1993) and eye-tracking (Olincy *et al*, 1998; Avila *et al*, 2003), deficit, and improve cognitive function (Mori and Mishina, 2003). However, the present data suggest that more prolonged exposure to nicotine may have the opposite, detrimental effects on sensory and cognitive modalities since brain KYNA levels are elevated (cf Shepard *et al*, 2003). In fact, even relatively modest increases in brain KYNA are known to significantly influence the electrophysiological properties of monoaminergic neurons (Erhardt *et al*, 2000, 2001b, 2002; Schwieler and Erhardt, 2003).

The results described here raise several interesting issues for future research. For example, further studies will be required to elucidate the molecular and cellular mechanisms that underlie the biphasic effects of extended nicotine administration on cerebral KYNA formation and the reversal of brain KYNA to normal levels upon treatment cessation. In addition, the functional consequences of fluctuations in cerebral KYNA levels in response to prolonged exposure to nicotine, especially the effects on cholinergic and glutamatergic neurotransmission, need to be explored in detail. It will be especially important to evaluate whether concurrent treatment with antipsychotic drugs influences nicotine-induced changes in brain KYNA. Thus, neuroleptics are known to have reciprocal interactions with nicotine (Jann *et al*, 1986; Miller *et al*, 1990; Lee *et al*, 2001), affect smoking behavior (George *et al*, 1995) and, when administered chronically, reduce brain KYNA levels in rats (Ceresoli-Borroni *et al*, 1999b; cf also Schwieler and Erhardt, 2003). Evidence for interactions between prolonged nicotine and neuroleptic treatments in determining brain KYNA formation would further support the suggestion (Court *et al*, 1998) that smoking history be carefully assessed and considered in future biochemical studies in schizophrenic individuals.

In summary, the present results demonstrate that prolonged nicotine administration in rats has a brain-specific, biphasic and reversible effect on KYNA levels. Nicotine-induced fluctuations in brain KYNA may cause changes in glutamatergic and cholinergic neurotransmission and may play a role in the beneficial effects of nicotine in patients suffering from schizophrenia or other brain diseases. By inference, our study suggests possible clinical benefits from direct pharmacological manipulations of cerebral KYNA levels, which can be achieved by targeting enzymes of the kynurenine pathway (Schwarcz and Pellicciari, 2002).

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