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Micromolar Brain Levels of Kynurenic Acid are Associated with a Disruption of Auditory Sensory Gating in the Rat

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Brain levels of kynurenic acid (KYNA), an endogenous antagonist of glycine$_B$/NMDA and α-7 nicotinic acetylcholine receptors, are elevated in individuals with schizophrenia. Both receptors are broadly implicated in the pathophysiology of this disease, particularly in the deficits many patients show in filtering the sensorium. In the present study, we sought to determine whether elevated brain levels of KYNA disrupt auditory gating in anesthetized rats. A mid-latency evoked potential was recorded from the hippocampus in response to a pair of auditory tones. Gating was assessed by determining the ratio of the amplitude of test and conditioning responses (T/C ratio) in rats that had received KYNA's precursor L-kynurenine (KYN; 150 mg/kg, i.p.) together with probenecid (PBCD; 200 mg/kg, i.p.) 2 h prior to the start of the recording session. KYNA levels in the hippocampus of KYN + PBCD-treated rats were increased 500-fold, and accompanied by a significant increase in T/C ratio consistent with a disruption in sensory gating. PBCD alone increased hippocampal KYNA 12-fold, but did not significantly elevate T/C ratio. L-701,324 (3–30 mg/kg, i.v.), a centrally acting glycine$_B$ site antagonist, also failed to disrupt gating; however, large quantities of the competitive NMDA receptor antagonist DL-2-amino-5-phosphopentanoate (200 nmol, i.c.v.) markedly increased T/C ratio. Thus, while total blockade of NMDA receptors disrupts auditory gating, partial blockade achieved by antagonism of its glycine coagonist binding site does not. These observations indicate that the disruption in auditory processing in rats with greatly elevated KYNA levels is not attributable to the compound's antagonist actions at the glycine$_B$ receptor.
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INTRODUCTION

Cognitive deficits have long been considered a core feature of schizophrenia. The failure of antipsychotic drugs to reverse these symptoms and their disproportionate impact on quality of life has led to a sustained effort to discover their neurobiological underpinnings (Buchanan *et al*, 1994, 1998). It has long been assumed that a reduction in the brain's capacity to filter irrelevant information from the sensorium contributes to these impairments (McGhie and Chapman, 1961; Venables, 1964). Indeed, individuals with schizophrenia exhibit pronounced deficits in psychophysiological measures of sensory gating. In one such paradigm, a pair of identical acoustic tones is presented during acquisition of electroencephalographic activity. Epochs of the recording bracketing the stimuli are averaged to generate a pair of event-related potentials (ERPs). When stimuli are applied in close succession, the amplitude of the ERP elicited by the first or conditioning stimulus exceeds the response evoked by the second or test stimulus, often by a factor of two or more. The diminished response to the second tone is thought to reflect the neural processes involved in gating sensory input and is typically quantified as the ratio of test to conditioning (T/C) response amplitude (Adler *et al*, 1999; Light and Braff, 1999). Numerous studies have demonstrated that persons with schizophrenia exhibit a blunted response to conditioning stimuli and a failure to show the characteristic reduction in ERP amplitude to the test stimulus (Adler *et al*, 1982; Boutros *et al*, 1991; Jin *et al*, 1997; Clementz *et al*, 1998a, b; Patterson *et al*, 2000). Notably, these deficits are strongly correlated with neuropsychological measures of attention (Erwin *et al*, 1998) and vigilance (Cullum *et al*, 1993). Abnormalities in sensory gating have been detected in unaffected family members of individuals with schizophrenia and in patients afflicted with schizotypal personality disorder, suggesting that anomalous sensory gating may represent an intermediate phenotypic marker of the illness (Siegel *et al*, 1984; Waldo *et al*, 1991; Clementz *et al*, 1998b; Cadenhead *et al*, 2000).

In addition to their role in clinical research, auditory ERP recording techniques are readily adapted for use in laboratory animals including the anesthetized rat. This preparation has been particularly valuable for studying the

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neurobiological basis of auditory gating. Results from a number of these studies have implicated both glutamatergic and cholinergic mechanisms in this phenomenon. Animals acutely administered the noncompetitive NMDA receptor antagonists, phencyclidine or dizolcipine (MK-801), exhibit deficits in auditory sensory gating that are qualitatively similar to those observed in schizophrenia (Adler *et al*, 1986; Miller *et al*, 1992a). Removal of hippocampal cholinergic afferents or administration of α-7 nicotinic acetylcholine receptor (α7nAChR) antagonists also disrupts auditory gating (Luntz-Leybman *et al*, 1992; Bickford and Wear, 1995). These data, together with clinical studies identifying a genetic linkage between auditory gating deficits in schizophrenic patients and the α7nAChR (Freedman *et al*, 1997), suggest that cholinergic neurons are an important and highly conserved component of the auditory gating pathway.

Although the neurobiological basis of sensory gating deficits in schizophrenia remains unknown, convergent lines of evidence suggest that glutamatergic and/or cholinergic neurotransmission is abnormally depressed. One possibility is that these impairments are related to an increase in brain levels of endogenous neuroactive compounds that modulate the activity of these neurotransmitters or their receptors. Brain and cerebrospinal fluid (CSF) levels of one such candidate, kynurenic acid (KYNA), a tryptophan metabolite and endogenous excitatory amino acid (EAA) receptor antagonist, are elevated in schizophrenic patients (Erhardt *et al*, 2001; Schwarcz *et al*, 2001). In addition to its well-characterized action as a competitive antagonist of the glycine coagonist binding site ($glycine_B$ site) on the NMDA receptor, KYNA also functions as a noncompetitive antagonist of the α7nAChR (Hilmas *et al*, 2001). Indeed, KYNA exhibits higher affinity for the α7nAChR than for the $glycine_B$ site, suggesting that α7nAChR blockade may account for some actions that had been previously ascribed exclusively to blockade of ionotropic EAA receptors.

In the present series of experiments, electrophysiological techniques were used to determine the effects of increased endogenous KYNA levels on auditory gating in the anesthetized rat. To accomplish this, L-kynurenine (KYN), the immediate precursor of KYNA, was administered together with probenecid (PBCD), an antagonist of organic acid transport (Miller and Ross, 1976; Suzuki *et al*, 1987). Experiments were also conducted comparing the effects of a centrally acting $glycine_B$ site antagonist (L-701, 324) with DL-2-amino-5-phosphopentanoate (DL-APV), a prototypical competitive antagonist of the glutamate binding site on the NMDA receptor. Our results indicate that while elevated brain levels of KYNA are capable of disrupting auditory processing, these effects are not due to competitive blockade of the $glycine_B$ site. A preliminary account of these results has been published in abstract form (Clerkin *et al*, 2001).

MATERIALS AND METHODS

Animals

All studies were conducted using adult, male Sprague–Dawley rats (300–500 g) obtained from Charles River Laboratories (Wilmington, MA). Animals were housed in a temperature-controlled vivarium under scheduled lighting conditions and were provided unrestricted access to food and water. All experiments were conducted in strict accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals (http://oacu.od.nih.gov/regs/guide/guidex.htm) and with the approval of the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine.

Drugs

L-Kynurenine sulfate and probenecid (Sigma, St Louis, MO) were dissolved in 2 N NaOH and diluted to a final concentration of 30 mg/ml with 0.1 M HEPES buffer. Both solutions were adjusted to a final pH of 8.4 with 2 N NaOH or 1 N HCl. L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)-phenyl-2(H)-quinolinone; Tocris, Ballwin, MO) was suspended in 20% polyethylene glycol and adjusted to pH 10 with 0.5 N HCl. Drugs were prepared fresh daily and stored at 4°C in amber bottles. DL-APV was dissolved in a few drops of 1 N NaOH and diluted to a final concentration of 40 mM in 0.9% saline (pH = 7). Vehicle solutions were prepared from the same solvents used to dissolve the active drug and adjusted to a comparable pH.

Evoked Potential Recording

Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic frame with hollow, atraumatic ear bars. Body temperature was maintained at 36.5°C using a feedback-controlled heating pad. A burr hole was drilled in the skull to permit placement of an epoxy-coated tungsten microelectrode (FHC, Bowdoinham, ME, 10 MΩ at 135 Hz) in the CA3 region of the hippocampus (3.8 mm posterior to bregma; 3.8 mm lateral to midline; 2.8–3.2 mm ventral to cortical surface; Paxinos and Watson, 1986). CA3 pyramidal neurons were identified by their characteristic pattern of discharge (Ranck, 1973; Bickford and Wear, 1995). Electrode potentials were amplified, filtered (0.01–1 kHz), digitized, and viewed in real time using *Axoscope* (Axon Instruments, Union City, CA). Auditory stimuli, consisting of two 3 kHz tones (10 ms duration), were digitally generated (Wavetek, Norfolk, UK) and amplified using a conventional stereo amplifier. The output of the audio amplifier was directed to a pair of insert earphones (Etymotic Research, Elk Grove Village, IL). Tone pairs were delivered binaurally via the ear bars every 10 s with an interstimulus interval of 500 ms. Recordings were digitized in 1 s epochs beginning 100 ms before presentation of the first stimulus and stored to disk as separate trials. Responses to 16 consecutive tone pairs (subsequently referred to as one 'episode') were averaged to generate a waveform for analysis. The N40 component of the auditory evoked potential was identified as the largest negativity occurring 30–60 ms after stimulus onset. Its amplitude was measured relative to the largest preceding positivity occurring at least 10 ms after stimulus onset. Gating was expressed as the ratio of the amplitude of the second (test) response to the first (conditioning) response, and T/C ratios were computed for each episode. In two animals per treatment group, the recording site was marked by passing a

small DC current (20 μA for 3 min) through the tip of the electrode. These animals were deeply anesthetized and perfused transcardially with saline followed by 10% neutral buffered formalin. Coronal sections (50 μm) were cut on a freezing microtome, counterstained with cresyl violet, and examined microscopically. All lesions were localized within the CA3 region of the hippocampus.

Biochemical Determinations

In the first group of experiments, tissue levels of KYN, KYNA and quinolinic acid (QUIN) in the hippocampus were measured in six animals per treatment group. Animals were killed by decapitation immediately after the recording session. Brains were rapidly removed, frozen on dry ice, and stored at −80°C until the day of the assay. The hippocampus was dissected out, and the tissue was sonicated (1 : 10, w/v, in ultrapure water) and acidified with 6% $HClO_4$.

After centrifugation (5 min), an aliquot of the supernatant was diluted in 0.2 M zinc acetate containing 5% acetonitrile (pH 6.2). KYN levels were determined by HPLC analysis with UV detection at 365 nm. For the measurement of KYNA, samples were subjected to HPLC analysis with fluorescence detection (excitation wavelength: 344 nm; emission wavelength: 398 nm) (Wu *et al*, 2000a).

QUIN levels were assayed by mass spectroscopy using an aliquot of the tissue homogenate used for the determination of KYN and KYNA. The homogenate was further diluted (1 : 2.5, v/v, in ultrapure water). Samples were prepared by adding 50 μl of an internal standard (pyridine-3,5-dicarboxylic acid, Sigma) to 50 μl of this homogenate, and 25 μl of 5 N HCl and 125 μl chloroform were added to eliminate fatty acids. After centrifugation in a microfuge (15 min), the supernatant was added to 125 μl of 50 mM tetrabutylammonium sulfate and lyophilized overnight. The lyophilized material was resuspended in acetonitrile containing diisopropylethylamine and pentafluorobenzylbromide (PFB). After incubation at 60–65°C, 25 μl of decane and 750 μl of water were added, and the mixture was centrifuged in a microfuge (15 min). In all, 20 μl of the decane phase was removed, and 1 μl was subjected to GC/MS analysis. Spectrographic analysis was performed using a trace gas chromatographer coupled to a quadrupole mass spectrometer (CG/ECNI-MS, ThermoFinnigan, San Jose, CA). Chromatographic separation was performed using a capillary column (0.25 mm i.d., film thickness 0.25 μm, 30 m DB-5ms, J&W Scientific, Folsom, CA) with helium as the carrier gas. The temperature was programmed as follows: 155°C for 1.25 min, 40°C/min to 270°C, 10°C/min to 320°C, 1 min at 320°C, injection port at 228°C. The ion source temperature was 220°C. Selected ion monitoring analyses were performed by recording signals of characteristic $(M\text{-}PFB)^-$ ions. The *m*/*z* value for $(QUIN\text{-}PFB)^-$ is 346.

Tissue concentrations of KYN, KYNA, and QUIN were compared between treatment groups using the Kruskal–Wallis ANOVA on ranks and Dunnett's *post hoc* method without adjustment for ties.

Experimental Design

Experiment 1: effect of elevated KYNA levels on N40 auditory gating. In these experiments, brain levels of KYNA were elevated by blocking organic acid transport with PBCD or by jointly administering PBCD and KYNA's bioprecursor KYN. Based on previous studies, we anticipated that KYNA levels would peak approximately 2 h after systemic administration of these compounds (Miller *et al*, 1992b; Vécsei *et al*, 1992). The prolonged post-treatment interval precluded the use of a repeated measures design. Accordingly, we adopted a parallel groups approach in which 32 animals were randomly assigned to one of four treatment groups including naïve, vehicle, PBCD (200 mg/kg), or PBCD (200 mg/kg) + KYN (150 mg/kg) ($n = 8$ per group). Vehicle or drugs were injected i.p. 60 min prior to administration of chloral hydrate, and recordings began 2 h after the drugs had been administered. Five consecutive episodes, each comprised of the average response to 16 stimulus trials, were acquired over a 20-min interval. The amplitudes of the conditioning and test waveforms (in μV) were used to determine the T/C ratio for each episode. Data from all five episodes were averaged to create a mean response for each animal. Grand means, representing the average response of subjects within each treatment group, were compared using a one-way analysis of variance (ANOVA). *Post hoc* pairwise comparisons were performed using a Bonferroni *t*-test. Individuals conducting these experiments remained unaware of the treatment condition of the animals until all of the data had been analyzed.

In addition to comparing the mean T/C ratio between treatment groups, we determined the frequency of occurrence of abnormal episodes in control and drug-treated rats. In accordance with previous studies (Adler *et al*, 1986; Miller *et al*, 1992a, 1995), T/C ratios > 0.5 were operationally defined as abnormal. Between-group comparisons were made using the Savage or log rank test (Lehman, 1974). *P*-values were calculated by a permutation procedure (SAS® PROC NPAR1WAY, SAS Institute, 1997) rather than relying on the asymptotic χ^2 distribution of the Savage statistic. In an effort to reduce Type I error rate, the closed testing procedure of Marcus *et al* (1976) was used. Accordingly, if the global null hypothesis was rejected at $P < 0.05$ (distribution of abnormal counts same in all groups), a pairwise null hypothesis of the form Group A = Group B would be rejected at $P < 0.05$ only if the three-way hypotheses (Group A = B = C and Group A = B = D) were also rejected at $P < 0.05$. All hypothesis tests were two-sided.

Experiment 2: effect of the glycine$_B$ site antagonist L-701,324 on N40 auditory gating. Dose–response studies were performed to assess the effects of L-701,324, a centrally acting glycine$_B$ antagonist, on auditory sensory gating. Rats ($n = 23$) were prepared for recording as described above with the addition of a cannula that was surgically implanted in the femoral vein. Two consecutive episodes were recorded to establish the baseline gating status of each animal. Only those animals that exhibited normal gating characteristics (T/C ratio < 0.5) were selected for further study. At 5 min after the last baseline recording, animals received one of three doses of L-701,324 (3, 10, or 30 mg/kg), or an equivalent volume of vehicle using an infusion pump programmed to inject the solutions at a rate of 0.2 ml/min. Evoked potential recordings were acquired in 5 min intervals beginning 10 min prior to drug infusion and continuing for 55 min. Data were smoothed by averaging

the conditioning and test response amplitudes obtained from two consecutive episodes. T/C ratios generated from these data were plotted in 10 min intervals. Differences between group means were evaluated using a two-way repeated measures analysis of variance (RMANOVA).

Experiment 3: effects of the competitive NMDA receptor antagonist DL*-APV on N40 auditory gating.* The effects of competitive blockade of the glutamate binding site on auditory gating was studied by comparing T/C ratios obtained before and after intracerebroventricular (i.c.v.) injection of DL-APV. Rats ($n = 12$) were prepared for recording as described earlier. An additional burr hole was drilled in the skull, and a stainless steel cannula was lowered into the lateral ventricle for drug administration (0.8 mm posterior to bregma, 1.2 mm lateral, 3.5 mm ventral; Paxinos and Watson, 1986). Injections were made ipsilateral to the recording electrode. Two consecutive episodes were recorded under baseline (preinjection) conditions. Animals that failed to gate normally (T/C ratio 0.5) were not tested further. At 5 min after acquisition of the second baseline episode, animals received 5 µl of DL-APV (40 mM) or vehicle (0.9% saline). Solutions were delivered at a rate of 1 µl/min. Recordings began immediately following the injection and continued in 5 min intervals for the next 45 min. As in the previous group of experiments, data were smoothed by averaging two consecutive episodes. Differences between treatment groups were evaluated using a two-way RMANOVA.

RESULTS

Experiment 1: Effect of Elevated KYNA Levels on N40 Auditory Gating

Peripheral KYN loading together with blockade of organic acid transport proved to be an effective strategy for increasing tissue levels of KYNA in the CNS (Table 1). KYNA levels in the hippocampus of untreated rats ranged from 20 to 60 nM and did not differ from the vehicle-treated group. Brain levels of KYN were also unaffected by vehicle treatment. PBCD (200 mg/kg) increased tissue levels of KYN and KYNA by 4- and 12-fold, respectively, whereas QUIN levels remained unchanged. Coadministration of KYN (150 mg/kg) and PBCD resulted in a 215-fold increase in the concentration of KYN in the hippocampus. The increased availability of the substrate together with blockade of organic acid transport also resulted in a significant increase in hippocampal KYNA levels, which reached 20 µM 2.5 h after drug treatment. QUIN levels in the hippocampus of KYN + PBCD-treated rats were not different from naïve animals.

Mean T/C ratios obtained from animals in each of the four treatment groups are illustrated in Figure 1. Naïve animals showed the smallest average T/C ratio (0.31 ± 0.04), indicative of a strong attenuation in the response evoked by the second of two auditory stimuli. Vehicle-injected controls and PBCD-treated rats showed comparable responses (0.39 ± 0.07 and 0.40 ± 0.08, respectively). Animals that received KYN together with PBCD exhibited a marked elevation in T/C ratio (0.64 ± 0.10). This fulfilled the criteria used to operationally define abnormal gating (i.e. $T/C > 0.5$) and differed significantly from naïve controls (ANOVA $F_{(3,28)} = 3.6$, $P = 0.025$; Bonferroni $t = 3.1$; $P < 0.02$).

The individual T/C ratios used to compile the grand means presented in Figure 1 represent the average of five consecutive episodes acquired over a 20-min interval immediately preceding sacrifice. This approach was adopted to reduce the possibility of an aberrant episode making a disproportionate contribution to the results. It also enabled us to compare the frequency of normal and abnormal trials among the subjects in each treatment group (Table 2). Naïve animals exhibited the fewest number of abnormal trials. Individual T/C ratios exceeded 0.5 in only six of the 40 episodes recorded (8 animals × 5 episodes/animal), and none of the naïve animals exhibited more than two abnormal episodes. Vehicle- and PBCD-treated rats showed a modest increase in the number of abnormal episodes, but these differences did not reach statistical significance. The highest incidence of abnormal trials was observed in the KYN + PBCD group in which the T/C ratio

Table 1 Effects of PBCD (200 mg/kg) with and without KYN (150 mg/kg) on the Tissue Concentration of KYN, KYNA, and QUIN in the Rat Hippocampus

Treatment	KYN (µM)	KYNA (nM)	QUIN (nM)
Naïve	0.9 ± 0.1	40 ± 6	92 ± 5
Vehicle	1.0 ± 0.1	36 ± 3	71 ± 4
PBCD	3.8 ± 0.3*	479 ± 48	70 ± 4
KYN+PBCD	193.0 ± 20.0*	20 ± 2 µM*	220 ± 26

Drugs were administered 150 min before sacrifice. Data are presented as the mean ± SEM of six rats per group. *$P < 0.05$ compared to uninjected controls, Dunnett's method without adjustment for ties.

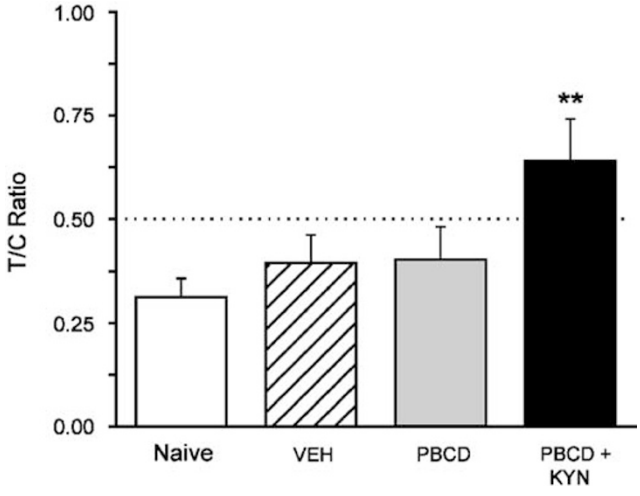

Figure 1 Effects of PBCD and KYN on auditory gating in the anesthetized rat. Data represent the mean ± SEM T/C ratio obtained from naïve rats and animals that received an i.p. injection of vehicle (VEH), PBCD (200 mg/kg), or PBCD + KYN (150 mg/kg) (eight animals per group). Values were acquired over a 20-min interval beginning 120 min after drug or vehicle treatment. T/C ratios above 0.5 (dashed line) denote disrupted sensory gating. **$P < 0.015$ compared to naïve animals, Bonferroni *t*-test.

Table 2 Distribution of Abnormal Episodes (T/C > 0.5) by Treatment Group

	KYN/PBCD		VEH/PBCD		VEH/VEH		Naïve	
No. of abnormal episodes	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
0	1	12.5	3	37.5	3	37.5	4	50.0
1	2	25.0	2	25.0	2	25.0	2	25.0
2	0	0.0	1	12.5	2	25.0	2	25.0
3	3	37.5	2	25.0	1	12.5	0	0.0
4	2	25.0	0	0.0	0	0.0	0	0.0
Abnormal episodes/rat	2.38 ± 0.53*		1.25 ± 0.45		1.13 ± 0.40		0.75 ± 0.31	

Global Savage test for differences: $\chi^2 = 8.68$, df = 3, permutation *P*-value = 0.022. *KYN/PBCD *vs* naïve, $\chi^2 = 4.88$, permutation *P*-value = 0.018.

exceeded 0.5 in nearly half of the episodes. Five of the eight animals tested exhibited three or more abnormal episodes. As a group, these animals exhibited a significantly greater proportion of abnormal episodes than uninjected controls ($\chi^2 = 4.9$, $P = 0.018$).

Experiment 2: Effect of the Glycine$_B$ Site Antagonist L-701,324 on N40 Auditory Gating

The disruption in auditory gating observed in animals with elevated brain levels of KYNA prompted us to assess the effects of L-701,324, a centrally acting glycine$_B$ site antagonist. Rats received a bolus injection of one of three doses of the drug (3, 10, or 30 mg/kg) administered intravenously to facilitate entry into the brain (Murray *et al*, 2000). The results of these experiments are illustrated in Figure 2. All rats exhibited normal gating (T/C ratio <0.5) prior to drug injection. RMANOVA revealed main effects for both dose ($F_{(3,95)} = 3.3$, $P < 0.05$) and time ($F_{(5,95)} = 4.5$, $P < 0.001$). However, there was no evidence of a dose × time interaction, indicating that none of the three doses of L-701,324 differed from one another at any of the intervals sampled.

Experiment 3: Effects of the Competitive NMDA Receptor Antagonist DL-APV on N40 Auditory Gating

The effects of 200 nmol DL-APV on N40 auditory gating are illustrated in Figure 3. Omnibus testing revealed main effects for treatment ($F_{(1,50)} = 7.9$, $P < 0.05$), time ($F_{(5,50)} = 4.6$, $P < 0.005$), and their interaction (treatment × time: $F_{(5,50)} = 4.8$, $P < 0.001$). The average T/C ratio obtained from vehicle-treated rats remained below 0.5 for the duration of the experiment and showed no evidence of a change over time. By contrast, animals treated with DL-APV exhibited a time-dependent increase in T/C ratio that exceeded 0.5 within 15 min after injection. Significant differences were observed between drug- and vehicle-treated animals beginning 20–25 min following administration and persisted for the duration of the experiment. Comparison of the relative contribution made by test and conditioning waveforms revealed that the increase in T/C ratio was due entirely to a decrease in the conditioning response amplitude (Table 3).

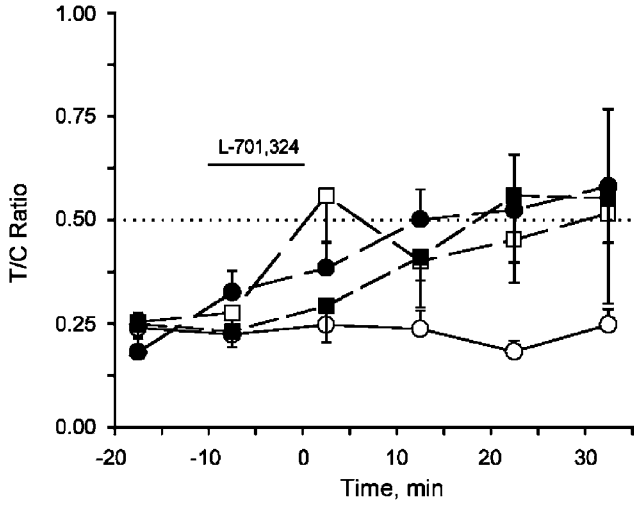

Figure 2 Effects of L-701,324, a competitive glycine$_B$ site antagonist, on auditory gating in the anesthetized rat. Data represent the T/C ratio (mean ± SEM) of animals that received an injection of vehicle ($n = 5$, open circles) or one of three doses of L-701,324 (3 mg/kg, $n = 7$, open rectangles; 10 mg/kg, $n = 6$, filled circles; 30 mg/kg, $n = 5$, filled rectangles). Solutions were injected intravenously during the interval denoted by the solid horizontal line.

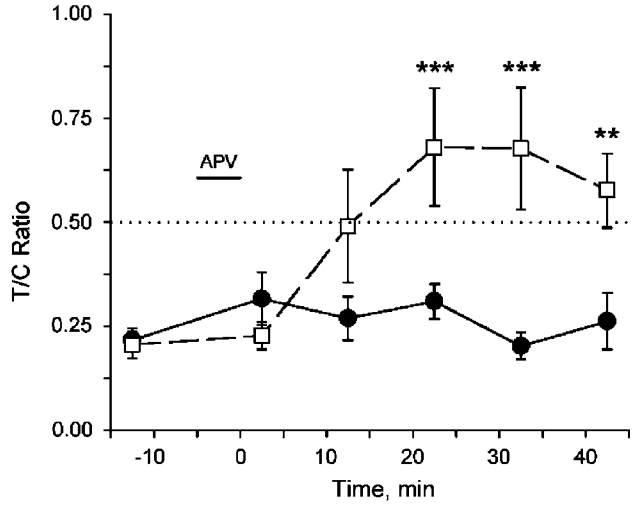

Figure 3 Effect of DL-APV, a competitive NMDA receptor antagonist on auditory gating in the anesthetized rat. Data represent the T/C ratio (mean ± SEM) of animals that received an i.c.v. injection of 5 µl vehicle ($n = 6$, filled circles) or 200 nmol DL-APV ($n = 6$, open squares). The solid horizontal line denotes duration of the injection. **$P < 0.01$, ***$P < 0.001$ compared to baseline (−12.5 min) using a two-tailed Bonferroni *t*-test.

DISCUSSION

Neuroactive metabolites of tryptophan along the kynurenine pathway have been implicated in a variety of neurological and psychiatric diseases including Huntington's and Parkinson's disease, stroke, epilepsy and schizophrenia (Stone, 2001; Schwarcz and Pellicciari, 2002). In the brain, both QUIN and KYNA are derived from KYN, which enters the CNS via the same carrier system as tryptophan (Fukui *et al*, 1991; Guidetti *et al*, 1995). However, unlike tryptophan, which serves as a substrate for serotonin biosynthesis in neurons, brain KYN is preferentially

Table 3 Conditioning and Test Response Amplitudes Before and After i.c.v. Infusion of DL-APV

	Conditioning response amplitude (μV)		Test response amplitude (μV)	
Injection interval (min)	Vehicle	APV	Vehicle	APV
−12.5	232 ± 28	248 ± 31	48 ± 5	49 ± 7
2.5	231 ± 25	153 ± 19**	69 ± 13	33 ± 4
12.5	250 ± 26	96 ± 18***	62 ± 9	37 ± 8
22.5	260 ± 20	70 ± 17***	78 ± 11	37 ± 6
32.5	247 ± 18	81 ± 19***	48 ± 5	51 ± 13
42.5	228 ± 26	104 ± 26***	54 ± 9	51 ± 6

Data represent mean ± SEM of six rats per group. $^{**}P<0.01$, $^{***}P<0.001$ compared to baseline (−12.5 min) values using a two-tailed Bonferroni *t*-test.

metabolized in astrocytes and microglial cells. Accordingly, these cells serve as the primary source of QUIN and KYNA in the brain (Guillemin *et al*, 2001; Schwarcz and Pellicciari, 2002). To date, most hypotheses involving the kynurenine pathway have emphasized the potential pathophysiological significance of elevations in brain QUIN, a potent excitotoxin and NMDA receptor agonist (Stone, 2001). Comparatively less attention has been paid to the possible pathogenic role of KYNA, a compound with significant anticonvulsant and neuroprotective properties (Schwarcz *et al*, 1992). KYNA is the end product in a side arm of the kynurenine pathway, and its formation is regulated by a number of distinct and, in some cases, brain-specific mechanisms (Gramsbergen *et al*, 1997; Hodgkins and Schwarcz, 1998). Changes in brain KYNA levels including those observed in schizophrenia, may be caused by impairment in one or more of these processes. Since KYNA cannot be cleared by cellular reuptake or enzymatic degradation, efflux through a nonspecific, PBCD-sensitive organic acid transporter appears to be the major mode of its elimination from the brain (Moroni *et al*, 1988; Turski and Schwarcz, 1988).

In the present study, we sought to test the premise that elevated brain levels of KYNA, an antagonist of both the glycine$_B$ site on the NMDA receptor and the α7nAChR, would produce a deficit in auditory sensory gating. Our hypothesis was based on evidence implicating glutamatergic and cholinergic receptors in sensory gating (Adler *et al*, 1986; Luntz-Leybman *et al*, 1992; Miller *et al*, 1992a; Bickford and Wear, 1995) and on data showing that cortical and CSF KYNA levels are elevated in schizophrenia (Erhardt *et al*, 2001; Schwarcz *et al*, 2001), a disorder characterized by marked auditory gating deficits (Adler *et al*, 1982; Boutros *et al*, 1991; Jin *et al*, 1997; Clementz *et al*, 1998a, b; Patterson *et al*, 2000). Since KYNA penetrates the blood–brain barrier very poorly (Fukui *et al*, 1991), an indirect approach was implemented, involving blockade of the PBCD-sensitive transporter alone and in conjunction with the administration of KYNA's immediate bioprecursor KYN. In accordance with previously published data, PBCD alone elevated hippocampal KYNA levels 12-fold (Miller *et al*, 1992b; Vécsei *et al*, 1992). As an inhibitor of the transport of all organic acids across epithelial barriers, PBCD also increases brain levels of several other compounds, including acidic products of indole and catecholamine degradation. In addition, PBCD causes nonspecific changes in brain chemistry, for example an increase in KYN levels (Table 1; cf also Vécsei *et al*, 1992). However, neither of these PBCD-induced effects were sufficient to modify auditory gating. By contrast, the 500-fold increase in hippocampal KYNA levels produced by PBCD + KYN, which occurred in the absence of changes in brain QUIN, significantly increased the T/C ratio in a manner that is typically interpreted as reflecting a disruption in sensory gating.

The most parsimonious interpretation of these data is that KYNA-mediated blockade of the glycine$_B$ site (IC_{50}: 10–15 μM; Birch *et al*, 1988a, b; Kessler *et al*, 1989; Danysz *et al*, 1989) is responsible for the observed deficit in auditory gating. This notion would be consistent with the fact that KYNA levels comparable to those attained in the present study are capable of blocking the convulsant and excitotoxic effects of NMDA receptor agonists (Vécsei *et al*, 1992; Santamaria *et al*, 1996). Similar antiexcitotoxic effects can be achieved by several specific glycine$_B$ site antagonists (Leeson and Iversen, 1994; Danysz and Parsons, 1998). In an effort to assess the role of glycine$_B$ receptor blockade on auditory gating, we tested the effect of L-701,324, a specific, centrally acting glycine$_B$ site antagonist (Bristow *et al*, 1996a, b; Grimwood *et al*, 1995). Although previous studies had shown that a single dose of 6 mg/kg (i.p.) results in behavioral changes indicative of NMDA receptor blockade (Bristow *et al*, 1996a, b), this dose had no effect on auditory gating (Clerkin *et al*, 2001). Subsequent studies were conducted using an intravenous route of administration to increase the drug's access to the brain. The doses of L-701,324 selected for testing exhibit widely divergent effects on [^{3}H]MK-801 binding *in vivo*, ranging from $<10\%$ inhibition at 3 mg/kg to $>80\%$ inhibition at 30 mg/kg (Murray *et al*, 2000). At all doses tested, a nonsignificant trend toward an increase in T/C ratio was observed but there was no evidence of a dose dependency of the response. Further evidence for a dissociation between glycine$_B$ receptor blockade and auditory gating mechanisms was provided by studies in which the selective glycine$_B$ receptor antagonist 7-chlorokynurenic acid (IC_{50}: 0.56 μM; Leeson and Iversen, 1994) was produced *in situ* following the i.p. administration of its precursor 4-chlorokynurenine (Wu *et al*, 2000b) and PBCD. These experiments, which were designed and performed in analogy to the studies described in this communication, showed that hippocampal 7-chlorokynurenic acid levels in excess of 4.5 μM had no effect on auditory gating (manuscript in preparation).

In contrast to glycine$_B$ receptor blockade, i.c.v. administration of 200 nmol DL-APV, a competitive antagonist of the NMDA binding site, significantly increased T/C ratio. In this study, we selected a drug concentration and route of administration that would reliably inhibit NMDA receptor function, as evidenced by the blockade of hippocampal LTP, a physiological process linked to activation of NMDA receptors (Leung and Shen, 1999). This effect of DL-APV was consistent with previous reports implicating NMDA receptors in sensory gating (Adler *et al*, 1986; Miller *et al*, 1992a). Notably, the α-APV-induced disruption in gating was not due to an injection artifact since the increase in T/C ratio did not occur during or immediately following α-APV but showed a delayed onset that closely

paralleled the latency to its effect on LTP (Leung and Shen, 1999).

Taken together, these data indicate that a near-complete inhibition of the glycine$_B$ site (using 30 mg/kg L-701,324) is without effect on T/C ratio, whereas a total blockade of NMDA receptor activity (using 200 nmol DL-APV) significantly attenuates auditory gating. Based on these conclusions, it is unlikely that the increase in T/C ratio observed in KYN + PBCD-treated rats is attributable to KYNA-induced blockade of the glycine$_B$ receptor. It is also unlikely that KYNA's disruptive effects on auditory gating are related to its ability to compete with glutamate at the NMDA recognition site or AMPA/kainate receptors since the brain levels of KYNA attained in KYN + PBCD-treated rats ($\sim$20 μM) remained 1–2 orders of magnitude below its IC_{50} values at these sites (Stone, 2001).

It is possible that the impairment of auditory gating observed in KYN + PBCD-treated rats is related to an effect of KYNA at nonglutamatergic receptors. The recently discovered anticholinergic properties of KYNA indicate one such possibility (Hilmas *et al*, 2001). Cholinergic projections from the septal nucleus to the hippocampus play an important role in auditory gating (Miller and Freedman, 1993; van Luijtelaar *et al*, 2001), and fimbria-fornix transections, which remove the cholinergic input to the hippocampus, increase the T/C ratio in anesthetized rats (Bickford and Wear, 1995). Notably, the lesion-induced gating deficit is reversed by nicotine and probably mediated by α7nAChRs. This pharmacological specificity is suggested by the fact that the α7nAChR antagonist α-bungarotoxin potently disrupts auditory sensory gating while other cholinergic receptor antagonists, including mecamylamine and scopolamine, are without effect (Luntz-Leybman *et al*, 1992). As a noncompetitive antagonist of the α7nAChR with a shallow dose–response curve (IC_{50}: 7 μM, Hilmas *et al*, 2001), KYNA could be in a position to significantly reduce auditory gating. The concentrations of KYNA reached in the hippocampus following KYN + PBCD treatment are certainly in a range that could be envisioned to affect α7nAChR function. Importantly, however, no effect on sensory gating was seen when endogenous brain KYNA levels were raised more than 10-fold by PBCD alone. This indicates that it is possible to dissociate KYNA's antiexcitotoxic/anticonvulsant properties, which can be realized at submicromolar concentrations (Pellicciari *et al*, 1994; Cozzi *et al*, 1999; Scharfman *et al*, 1999), from its potentially harmful effects on cognition at much higher levels.

Finally, it should be emphasized that the increase in T/C ratio observed in KYN + PBCD-treated animals and in rats that received DL-APV resulted from a reduction in the amplitude of the conditioning waveform, not an increase in test response amplitude. Therefore, it could be argued that these effects do not constitute an alteration in gating *per se* but rather a change in the animal's reactivity to the initial stimulus. While considerable effort continues to be directed toward understanding the physiological basis of auditory gating (for a review, see Leonard *et al*, 1996), a growing body of evidence suggests that a diminished response to conditioning stimuli may have functional and clinical importance. For example, significant reductions in conditioning response amplitude have been observed following blockade of α7nAChRs (Luntz-Leybman *et al*, 1992). In this study, as in ours, a significant amount of the variance in T/C ratio after drug treatment was attributed to a decrease in conditioning response amplitude. The loss of cholinergic afferents to the hippocampus following lesions of the fimbria-fornix also significantly reduces conditioning response amplitude, an effect that can be fully reversed by systemic administration of nicotine (Bickford and Wear, 1995; see above). Altered responsiveness to auditory stimuli has also been observed in humans. Thus, several laboratories have reported that the amplitude of the conditioning response is significantly reduced in schizophrenic patients (Jin *et al*, 1997; Patterson *et al*, 2000; Blumenfeld and Clementz, 2001). Moreover, in normal controls, the amplitude of the conditioning response is more tightly correlated with other indices of sensory gating (e.g. habituation and prepulse inhibition of the startle response) than T/C ratio (Schwarzkopf *et al*, 1993).

In summary, the results of the present study show that greatly elevated brain levels of KYNA in rats are associated with an altered responsiveness to auditory stimuli. This impairment does not appear to be attributable to the glutamate receptor antagonist properties of the compound but is likely to involve other mechanisms including an interaction with α7nAChRs. The recently documented increase in KYNA levels in the brain and CSF of schizophrenic patients (Erhardt *et al*, 2001; Schwarcz *et al*, 2001) could contribute to the well-characterized abnormality in sensory processing in the disease.

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