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Quinolinic acid lesion of the nigrostriatal pathway: effect on turning behaviour and protection by elevation of endogenous kynurenic acid in rattus norvegicus

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Abstract

Endogenous excitotoxins have been implicated in degeneration of nigral dopaminergic neurons in Parkinson's disease. It may be possible to reduce neurodegeneration by blocking the effects of these endogenous agents. The present study shows that contralateral turning seen following quinolinic acid-induced lesions of the nigrostriatal dopaminergic pathway was reversed by a treatment that increased brain levels of kynurenic acid, an endogenous excitatory amino acid antagonist. The treatment consisted of nicotinylalanine (5.6 nmol/5 μl i.c.v.), an inhibitor of kynureninase and kynurenine hydroxylase plus the precursor kynurenine (450 mg/kg i.p.) plus probenencid (200 mg/kg i.p.), an inhibitor of organic acid transport. Thus, neuroprotection by increasing brain kynurenic acid in vivo may be useful in retarding cell loss in Parkinson's and other neurodegenerative diseases involving excitotoxicity.

Keywords: Parkinson's disease; Quinolinic acid; Kynurenic acid; Substantia nigra; Excitotoxicity; Turning behaviour

Excitatory amino acids (EAA) and their receptors have been implicated in the neuronal loss observed in Parkinson's disease (PD) [16]. Thus, activation of *N*-methyl-D-aspartate (NMDA) receptors has been shown to be toxic to substantia nigra pars compacta (SNc) dopamine (DA) neurons in vitro [8] and in vivo in rats [4].

Certain endogenous excitotoxins may be involved in PD [11]. In the CNS, tryptophan metabolism via the kynurenine pathway yields quinolinic acid (QUIN) which activates NMDA receptors and has been shown to be neurotoxic [5]. Another tryptophan metabolite, kynurenic acid (KYNA), acts as an antagonist at EAA receptors [7]. Kynurenine amino transferase (KAT) and 3-hydroxyanthranilic acid oxygenase, the enzymes responsible for the production of KYNA and QUIN, respectively, have been found in astrocytes surrounding glutamatergic afferents and DA neurons in the SNc [12]. The concentrations of QUIN and KYNA in the SNc may be sufficiently high to influence EAA receptor function in vivo [12] and an increase in QUIN or a decrease in KYNA production may result in excessive activation of NMDA receptors. Thus, a balance may exist between the production of QUIN and KYNA in the SNc.

The level of KYNA in the brain can be influenced by nicotinylalanine (NICALA) [3], an inhibitor of kynureninase and kynurenine hydroxylase, enzymes critical to the biosynthesis of QUIN. Inhibition of this pathway elevates the concentration of kynurenine, a substrate for KAT. SNc infusion of QUIN is toxic to DA neurons, as reflected in the depletion of striatal tyrosine hydroxylase (TH) and the loss of SNc TH immunoreactivity [4]. Recently, we demonstrated that intracerebroventricular (i.c.v.) NICALA when coupled with systemic kynurenine and probenecid, elevates brain KYNA and prevents QUIN neurotoxicity [10]. At present, the possible functional significance of this neuroprotection is unknown.

In the present study, turning behaviour was used to assess

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the functional significance of NICALA-produced neuroprotection. Partial SNc lesions with QUIN led to contralateral turning. We assessed the effect of an elevation of endogenous KYNA produced by the administration of NICALA, kynurenine and probenecid on this turning.

Male Sprague–Dawley rats (Charles River, St. Constant, Quebec) weighing 100–125 g with free access to food (Purina rat chow) and water were housed individually and maintained on a 12:12 h light-dark cycle (lights on at 04:00 h) at a temperature of 20°C. Treatment was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and relevant University policy, and was approved by the University Animal Care Committee.

Rats were anaesthetized with a 2% halothane, 98% O_2 mixture and positioned in a stereotaxic apparatus. The treatment regimen and dose of NICALA employed here previously had been shown to produce the greatest increase in whole brain KYNA concentration [6]. NICALA (5.6 nmol/5 μl; Colour Your Enzyme, Kingston, Ontario) was dissolved in 0.9% saline (pH 7) and administered i.c.v. over 167 s at coordinates: 0.8 mm posterior to bregma, 1.45 mm lateral to the midline and 3.6 mm ventral to the surface of the skull with the incisor bar set at −3.3 mm. Probenecid (200 mg/kg) and kynurenine (450 mg/kg; Sigma, St. Louis, MO), dissolved in 1 N NaOH and titrated to pH 10 with 1 N HCl, were administered i.p. during the infusion of NICALA. Control animals received saline (5 μl i.c.v.) with kynurenine and probenecid. Three hours following injections of NICALA or saline, QUIN (60 nmol/0.5 μl), dissolved in 0.9% saline and titrated to pH 7.4 with 1 N NaOH, or saline (0.5 μl) was infused into the SNc over 75 s at respective coordinates: 5.3, 2.2 and 7.7 mm [4]. For attachment to the rotometer, an arborite chip was affixed to the skull with dental acrylic and screws.

The injection apparatus consisted of a stainless steel cannula (0.36 mm diameter) connected to an infusion pump-mounted Hamilton syringe via PE-20 polyethylene tubing. The cannula was left in place for an additional 2 min to allow for diffusion.

For behavioural testing a plastic cylinder (45 cm diameter, 30 cm high) was located inside a ventilated, illuminated, sound-attenuating box. The rotometer was a rotating disk with a single slot that moved past four infrared beams oriented at 90° intervals. A sliding stainless steel lead, clipped to the arborite chip in the rat's skull mount, allowed unrestricted movement. The number of full turns in each direction was recorded on a circuit board connected to a computer.

Testing was carried out at the beginning of the dark period. On days 4, 6, 8 and 10 after surgery, turning was measured for 20 min. Turning was quantified using the ratio of the number of turns toward (ipsilateral to) the lesioned side divided by the total number of turns. Thus, a ratio of 0.5 corresponds to no directional bias, whereas lower and higher ratios indicate contralateral and ipsilateral turning, respectively. Turning ratio has been shown to be a more reliable index of turning behaviour than the absolute number of turns, as the latter measure is highly influenced by overall motor activity which is unrelated to directional bias.

KYNA was measured according to the method of Russi et al. [13] with some modifications. Briefly, the rats were killed by decapitation and the brain rapidly removed. The SNc or striatum pooled from two rats was homogenized in 4 ml of a mixture (3:1) of ethanol and 1 N NaOH. After centrifugation (10 min, $5000 \times g$) the pellet was resuspended in 5 ml of 90% ethanol and centrifuged again (10 min, $5000 \times g$). The supernatants were pooled and placed at −80°C overnight to precipitate fatty materials which were discarded. Dowex AG1 Wx8 (acetate form 100–200 mesh, 200–300 mg) was added to the supernatant. The suspension was mixed for 5 min and centrifuged (10 min, $5000 \times g$). The supernatant was discarded and the resin washed in 2 ml of distilled water. The Dowex resin then was mixed with 1 ml of 10 N formic acid to recover the KYNA. Following centrifugation (10 min, $5000 \times g$), the supernatant was mixed with 100 mg of Dowex resin (AG50 Wx8 H^+ form). The resin was washed with 2 ml of water and KYNA extracted with 2 ml of 3 N ammonium hydroxide. To determine the percent recovery, a control sample containing a known amount of KYNA was taken through the same purification procedure. Routinely, the recovery of KYNA was 70%. The sample containing KYNA was lyophilized, resuspended in 200 μl of 50 mM Na^+ acetate (pH 6.2), and 50 μl applied to the HPLC column. A mobile phase consisting of 4.5% acetonitrile and 50 mM Na^+acetate (pH 6.2) was pumped through a CSC-Spherisorb-ODS2, 3 mm reverse-phase column at a flow rate of 1.0 ml/min. A solution of 0.5 M zinc acetate was delivered postcolumn at a flow rate of 1.0 ml/min to enhance the fluorescence signal. KYNA was detected using a fluorescence detector operating at excitation and emission wavelengths of 344 and 398 nm, respectively. The peak area under the curve was integrated and used for data analysis.

Striatal TH activity was determined two weeks following the infusion of QUIN or saline using a method described previously [4]. Protein was determined by the method of Bradford [2] and TH activity expressed as pmol L-dopa formed/mg protein per h. For each animal, the results were expressed as a percentage of the enzyme activity measured in the contralateral (uninjected) side. For untreated animals, these values were 500 ± 40 pmol/mg protein per h ($n = 3$), which agree with reported values [4].

The turning ratios for each group were subjected to a repeated measures analysis of variance (ANOVA) to assess rotational bias over the four experimental sessions. Between groups ANOVAs assessed the significance ($P < 0.05$) of treatment effects on KYNA.

Three hr following the administration of NICALA, kynurenine and probenecid, nigral KYNA was increased 2.2-fold when compared to untreated rats and 1.4-fold when compared to rats that received saline, kynurenine

and probenecid (Table 1). For striatal KYNA (measured in different groups of rats), the treatment produced a 3-fold increase compared to untreated rats. All of these effects were significant.

Turning behaviour assessed the function of DA neurons following the infusion of QUIN or saline into the SNc. The mean turning ratios of rats receiving nigral saline or QUIN, or rats pretreated with NICALA, kynurenine and probenecid 3 h prior to QUIN infusion into the SNc, are shown in Fig. 1. Turning ratios in rats that received nigral saline showed no significant change over the observation period. QUIN-treated rats showed a contralateral turning bias 4 days post-treatment that became progressively weaker across sessions (sessions effect, $P < 0.05$). Rats that received NICALA, kynurenine and probenecid 3 h prior to the infusion of QUIN showed no significant directional bias. Motor behaviour (total turns) did not change from day 4 to day 10 in any of the treatment groups (data not shown).

The 10-day period from surgery to the completion of behavioural tests precluded reliable identification of injection sites. TH activity in the striatum on the injected side, expressed as a percentage of that on the intact side, was used as a biochemical marker to confirm DA neurotoxicity in the SNc (Table 2). TH activity in the ipsilateral striatum 2 weeks following QUIN infusion was reduced significantly ($P < 0.01$), confirming the neurotoxic action of this NMDA receptor agonist. TH activity was not reduced significantly in the ipsilateral striatum of saline treated rats. Ipsilateral striatal TH activity in rats that received NICALA, kynurenine and probenecid 3 h prior to QUIN was not different from the contralateral side, indicating that elevated endogenous KYNA protected nigral DA neurons from QUIN-mediated toxicity.

The present study provides the first evidence that QUIN-induced partial lesions of the SNc produce turning behaviour that is prevented in rats pretreated with NICALA, kynurenine and probenecid. This treatment produced a significant elevation in brain KYNA and protected against

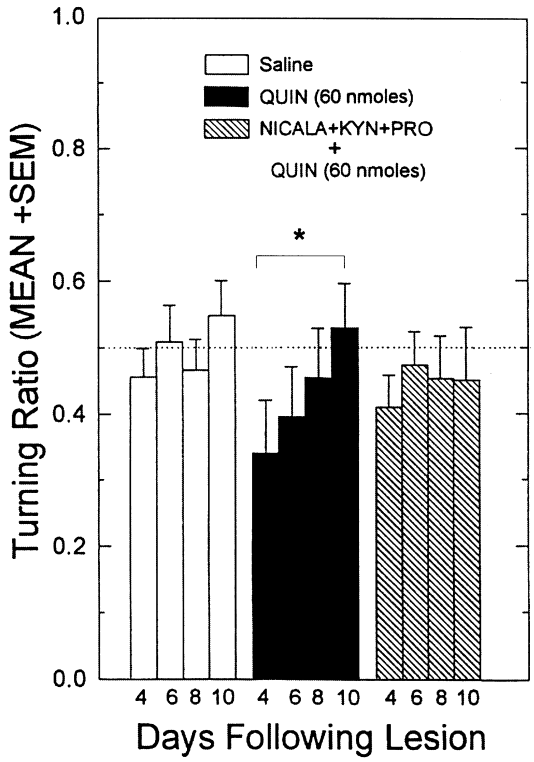

Fig. 1. Effect of nigral QUIN lesions on turning behaviour. The turning ratio was calculated by dividing the number of full turns made ipsilateral to the lesion side by the total number of full turns. Rats received an intranigral infusion of saline (0.5 μl, open bars, $n = 15$) or QUIN (60 nmol/0.5 μl, $n = 12$, filled bars). Hatched bars represent rats ($n = 13$) that were pretreated with NICALA (5.6 nmol in 5.0 μl), kynurenine (450 mg/kg i.p.) and probenecid (200 mg/kg i.p.) prior to the infusion of QUIN. Each bar represents the mean ± SEM. *Indicates a statistically significant day effect ($P < 0.05$) and post hoc tests (Newman-Keuls) showed day 4 differed from day 10.

Table 1

Concentration of kynurenic acid (KYNA) in the substantia nigra pars compacta and striatum in untreated rats (naive) or 3 h following the administration of nicotinylalanine (NICALA; 5.6 nmol i.c.v.) with kynurenine (450 mg/kg i.p.) and probenecid (200 mg/kg i.p.); or saline (5 μl i.c.v.) with kynurenine and probenecid. Data are expressed in pmol/g tissue and each point represents the mean ± SEM. Groups were compared using ANOVA followed by Newman-Keuls tests to determine differences between groups.

	Kynurenic Acid (pmol/g tissue)		
Brain Region	Naive Rats	Saline + Kynurenine + Probenecid	NICALA + Kynurenine + Probenecid
Striatum	964 ± 240 ($n = 3$)	1965 ± 560 ($n = 5$)	2893 ± 585* ($n = 3$)
Substantia nigra compacta	530 ± 33 ($n = 3$)	822 ± 55 ($n = 6$)	168 ± 61*+ ($n = 6$)

*Represents a significant difference from untreated rats ($P < 0.05$). +Represents a significant difference from rats that received saline instead of NICALA ($P < 0.05$).

Table 2

Effect of endogenous KYNA on striatal tyrosine hydroxylase (TH) 14 days following unilateral intranigral infusion of quinolinic acid (QUIN). TH activity in the injected side is expressed as a percentage of the activity in the uninjected side in the same animal. Animals received saline, QUIN (60 nmol) or QUIN 3 h following the administration of NICALA (5.6 nmol i.c.v.), kynurenine (450 mg/kg i.p.) and probenecid (200 mg/kg). Each value represents the mean ± SEM. Groups were compared using ANOVA followed by the Newman-Keuls test to determine specific differences between groups.

Treatment	Pretreatment	Tyrosine hydroxylase activity (percent of contralateral side)
Saline	–	94.8 ± 2.2 ($n = 15$)
QUIN (60 nmol)	–	65 ± 9.2* ($n = 12$)
QUIN (60 nmol)	NICALA + Kynurenine + Probenecid	90.5 ± 4.4+ ($n = 13$)

*Significantly different from the corresponding value in saline injected animals ($P < 0.05$); +significantly different from animals injected with QUIN alone ($P < 0.05$).

QUIN-induced neurochemical damage. Treatment with NICALA, kynurenine and probenecid also protected against QUIN-induced behavioural abnormalities.

In the present study, QUIN-induced partial lesions of SNc DA neurons (approximately 50% reduction in striatal TH activity) produced transient contralateral turning. A contralateral turning bias is associated with excitation of the nigrostriatal pathway on the lesion side [14,15]. Indeed, immediately following the infusion of QUIN a strong contralateral turning response was observed, presumably resulting from the excitation of DA neurons. However, neuronal death occurs within 24 h of exposure to excitotoxic concentrations of QUIN. Thus, the observation of contralateral turning 4 days following the infusion of QUIN may reflect transient hyper responsiveness of the remaining nigrostriatal DA neurons, as suggested previously [1]. Perhaps this effect results, in turn, from the loss of GABAergic inhibition of DA cells in SNc following QUIN.

The fact that a 2.2-fold increase of nigral KYNA protected nigrostriatal DA neurons from QUIN-induced toxicity is surprising. There are several possible explanations for this finding. One is that recent studies have shown that KAT, the enzyme responsible for the biosynthesis of KYNA, is present in astrocytes abutting excitatory synapses in the SNc [12]. Perhaps the small increase in nigral KYNA may result in a much higher local concentration of KYNA at critical synapses in the SNc.

Another explanation is related to the recent finding that kynurenine alone at a dose (500 mg/kg i.p.) similar to that used here (450 mg/kg i.p.), resulted in a significant increase in the concentration of QUIN in the brain [9]. However, in the present study, the inhibition of kynureninase and kynurenine hydroxylase provided by NICALA should have prevented QUIN synthesis. In animals receiving kynurenine alone, the pathway leading to the formation of QUIN would not be blocked. Therefore, it would be expected that an increase in KYNA would be paralleled by an increase in QUIN levels following kynurenine alone. Thus, the protective effects of elevated KYNA in control animals may have been offset by increases in QUIN. The protective effect of NICALA may have been due to a combination of an increase in KYNA and prevention of endogenous QUIN production.

A third alternative is that the protection against QUIN-mediated destruction of nigrostriatal DA neurons afforded by a 2.2-fold increase in nigral KYNA may be indicative of a steep dose-response relationship between the concentration of KYNA in the SNc and its ability to antagonize QUIN-mediated behavioural effects.

This study demonstrates that partial lesions of the nigrostriatal DA pathway induced by QUIN produce a reliable behavioural effect. Additionally, this behavioural effect can be prevented by the elevation of endogenous KYNA through the administration of NICALA, kynurenine and probenecid. This strategy may be able to protect neuronal populations in diseases that involve excitotoxic damage.

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