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PICOLINIC ACID BLOCKS THE NEUROTOXIC BUT NOT THE NEUROEXCITANT PROPERTIES OF QUINOLINIC ACID IN THE RAT BRAIN: EVIDENCE FROM TURNING BEHAVIOUR AND TYROSINE HYDROXYLASE IMMUNOHISTOCHEMISTRY

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Abstract—Previous results suggest that the tryptophan metabolite, picolinic acid may have the unusual properties of antagonizing the neurotoxic but not the neuroexcitant effects of another tryptophan metabolite, quinolinic acid in the central nervous system. The present experiments tested this possibility utilizing behavioural and tyrosine hydroxylase immunohistochemical techniques. In the first series of experiments, rats received injections of relatively high concentrations of 6-hydroxydopamine (12 μg in 1 or 2 μl), quinolinic acid (120 nmol in 0.5 μl), picolinic acid (480 nmol in 0.5 μl) or co-treatments (0.5 μl) with quinolinic (120 nmol) plus picolinic acid (480 nmol) into the region of the substantia nigra. Results revealed that 6-hydroxydopamine and quinolinic acid alone produced a large loss of tyrosine hydroxylase-positive cells in the pars compacta of the substantia nigra. Behavioural results for all 6-hydroxydopamine ($n = 10$) and for some quinolinate-treated rats ($n = 5$) revealed ipsi- and contraversive circling following amphetamine (1 mg/kg, i.p.) and apomorphine (0.5 mg/kg, s.c.), respectively, consistent with unilateral loss of dopamine cells in the substantia nigra. The remaining quinolinate-treated rats ($n = 9$) circled ipsiversively following either stimulant suggesting damage to the pars reticulata. Groups treated with picolinic acid alone ($n = 6$) or co-injected ($n = 6$) showed no loss of tyrosine hydroxylase-positive cells in the substantia nigra and no circling response to the stimulants. In the second series of experiments, low concentrations of quinolinic acid (2.5, 5.0, 7.5 nmol), picolinic acid (10, 20, 30 nmol), or the two together (7.5 plus 30 nmol, respectively) were microinjected (0.5 μl) into the dorsal striatum and circling behaviour evaluated. These results revealed dose-dependent contralateral circling with either quinolinate or picolinate; co-injection of the two tryptophan metabolites also produced contralateral circling. It was concluded that picolinic acid blocks the neurotoxic but not the neuroexcitant effects of quinolinic acid.

Recently, a number of endogenous metabolites of tryptophan generated via the kynurenine pathway have been found to influence excitatory amino acid neurotransmission in the CNS. One such metabolite, quinolinic acid, has been reported to excite neurons by activation of glutamate receptors of the *N*-methyl-D-aspartate (NMDA) subtype.[6,12] Another tryptophan metabolite, kynurenic acid, while producing no postsynaptic effects on its own, was found to antagonize the neuroexcitant effects of quinolinic acid.[12]

Higher concentrations of quinolinic acid, like ibotenic or kainic acid, were found to be neurotoxic in the striatum[15,16] and subsequently in the basal forebrain.[4] Like its neuroexcitant effects, the neurotoxic effects of quinolinic acid were antagonized by kynurenic acid.[1,5] Recently, Jhamandas *et al.*[8] showed that several other tryptophan metabolites, viz. quinaldic, hydroxyanthranilic, anthranilic and picolinic acid, also were effective in antagonizing quinolinate neurotoxicity. This anti-quinolinic acid effect of picolinic acid, a pyridine monocarboxylic acid, is of particular interest for several reasons. A recent study from our laboratory showed that the neuroprotective effects of picolinic acid were seen only against excitotoxins that require intact glutamatergic afferent inputs for their neurotoxic effect. Additionally, the anti-quinolinic acid effect of picolinic acid showed structural specificity.[2] These findings suggested that picolinic acid may act at a presynaptic site to modulate neurotoxicity. However, in electrophysiological studies, picolinic acid, unlike kynurenic acid, failed to block synaptic responses produced by stimulation of glutamate receptors.[14] Thus, picolinic acid appears to prevent excitotoxicity without influencing excitation.

The anti-neurotoxic effects of picolinic acid that have been observed thus far have focused entirely on brain cholinergic neurons; the effects of this agent on other neuronal systems remain unknown. Also, the evidence for the ability of picolinate to prevent excitotoxicity without blocking excitation remains sparce. The goal of the present study was to examine

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Abbreviations: NMDA, *N*-methyl-D-aspartate; 6-OHDA, 6-hydroxydopamine; SN, substantia nigra; TH, tyrosine hydroxylase.

the action of picolinic acid in a non-cholinergic neuronal population, and to explore the possibility of its differential action on neurotoxicity versus neuroexcitation using quinolinic acid to produce both of these actions.

The actions of picolinic acid on quinolinate-induced neurotoxicity were examined by focal injections into the zona compacta of the substantia nigra (SN), using tyrosine hydroxylase (TH) immunohistochemistry to assess the damage to dopaminergic neurons that are located in this region and that project to the striatum. To assess the function of the nigral dopaminergic neurons after quinolinate injections alone or in combination with picolinic acid, the rotational model was used.[13] Ungerstedt[19,20] showed that amphetamine or apomorphine, injected systemically after unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal dopaminergic cells of the SN pars compacta, produced differential turning responses. Amphetamine, stimulating dopamine release from intact neurons on the non-lesion side, produced ipsilateral or ipsiversive turning, i.e. turning towards the side of the lesion. Apomorphine, on the other hand, stimulating supersensitive dopamine receptors on the lesion side, produced contralateral or contraversive turning, i.e. turning away from the lesion side. In the present investigation we replicated this effect in animals receiving 6-OHDA lesions and evaluated the effects of amphetamine and apomorphine in animals receiving intra-nigral neurotoxic doses of quinolinic acid alone, picolinic acid, or a combination of the two tryptophan metabolites. We hypothesized that picolinic acid would antagonize the neurotoxic actions of quinolinic acid on nigral dopaminergic neurons.

To evaluate the action of picolinic acid on quinolinate-induced excitation, the experiments performed here were based on recent behavioural observations that acute activation of striatal NMDA receptors produced dose-dependent contralateral turning.[18] In the present study, dose-dependent turning behaviour was produced following intrastriatal injections of quinolinic acid and the ability of picolinic acid to influence this motor response was tested in co-injection experiments. From the previous observations with picolinic acid,[14] it was hypothesized that picolinic acid would fail to antagonize the quinolinate-induced turning response.

EXPERIMENTAL PROCEDURES

Treatment of the rats in the present study 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 Queen's University Animal Care Committee.

Subjects

Sixty-two male Wistar rats were purchased from Charles River Canada. Rats weighted between 225 and 250 g at the time of arrival and were individually housed in hanging wire cages in a temperature controlled (21 ± 1°C) colony room maintained on a 12 h light/dark cycle (lights on at 0700 h). Food and water were available *ad libitum* in the home cage.

Surgery

Rats were anaesthetized with sodium pentobarbital (Somnotol, 65 mg/kg, i.p.) and secured in a stereotaxic frame. In Experiment 1, 36 rats received injections unilaterally into the region of the SN zona compacta. Co-ordinates were: 5.3 mm posterior (P) to bregma, 2.2 mm lateral (L) to the midline, and 7.7 mm ventral (V) to the surface of the skull with the incisor bar set at 3.3 mm below the horizontal plane passing through the interaural line.[11] In Experiment 1A, 10 rats were pretreated with desmethylimipramine (15.0 mg/kg, s.c.) 15 min prior to central infusions. Six rats received 12.0 μg of 6-OHDA in a volume of 2.0 μl and four rats received the same dose in a 1.0 μl volume. Eighteen rats received injections into the right side of the brain and 18 into the left side. In Experiments 1B, C and D, 14, six and six rats, respectively, received intranigral infusions (0.5 μl) of quinolinic acid (120 nmol), picolinic acid (480 nmol) or a mixture of quinolinic acid (120 nmol) plus picolinic acid (480 nmol). All injections were delivered with the use of an infusion pump (Sage Instruments model no. 355) at a rate of 1.0 μl per min; cannulae (0.31 mm diameter) were left in place for 5 min following injection to allow time for drug diffusion. The cannula was then removed and the wound closed with sutures.

In Experiment 2, 26 rats were implanted unilaterally with chronic indwelling guide cannulae (0.64 mm diameter) aimed at the dorsal striatum. Co-ordinates were: 0.3 mm P, 3.0 mm L and 3.5 mm V. Thirteen animals received cannulae on the right side of the brain, 13 on the left. The cannulae were anchored to the skull with stainless steel screws and dental acrylic cement. Between injections, the guide cannulae were occluded with stainless steel wire pins.

Apparatus

Testing was carried out in four polyurethane-sealed circular wooden bases (30 cm diameter) with wire mesh sides 30 cm in height, fitted with Plexiglas covers.

Drugs

6-OHDA hydrobromide [Research Biochemicals Inc. (RBI), Natick, MA, U.S.A.] was dissolved in 0.9% saline with 0.2 mg/ml of ascorbic acid in concentrations of 6.0 or 12.0 μg/μl. Desmethylimipramine hydrochloride (RBI) was dissolved in 0.9% saline at a concentration of 15.0 mg/ml. Quinolinic acid (RBI) and picolinic acid [Sigma Chemical Co. (Sigma), St Louis, MO, U.S.A.] were suspended in 0.9% saline and solubilized by adjusting pH to 7.4 by addition of 0.1 M NaOH. Concentrations of quinolinic acid in Experiments 1 and 2 were 20.1 μg (120 nmol)/0.5 μl and 0.42 μg (2.5 nmol), 0.84 μg (5.0 nmol) or 1.25 μg (7.5 nmol)/0.5 μl, respectively. Concentrations of picolinic acid in Experiments 1 and 2 were 59.1 μg (480 nmol)/0.5 μl and 1.23 μg (10 nmol), 2.46 μg (20 nmol) or 3.69 μg (30 nmol)/0.5 μl, respectively.

(+)-Amphetamine sulphate (Smith, Kline and French, Canada, Ltd) was dissolved in 0.9% saline at a concentration of 1.0 mg/ml. Apomorphine hydrochloride (Sigma) was dissolved in 0.9% saline with 1.0 mg/ml of ascorbic acid at a concentration of 0.5 mg/ml. All drugs were freshly prepared each day.

Striatum injections

In Experiment 2, microinjections were delivered in a volume of 0.5 μl with a 10 μl Hamilton microsyringe. Injection cannulae were constructed with stainless steel tubing (0.31 mm diameter), cut to extend 1.0 mm beyond the tip of the guide cannulae and were attached to the microsyringe by a length of polyethylene tubing. The injections were delivered sequentially with the use of an infusion pump,

each injection taking 30 s, and the cannula was left in place for an additional 60 s to allow for diffusion.

Procedure

Substantia nigra injections (*Experiment* 1). Behavioural testing began approximately one week following surgery and involved six sessions per animal as follows: (i) no injection; (ii) a randomly selected half of the rats received an i.p. injection of amphetamine vehicle (0.9% saline) and the other half received a s.c. injection of apomorphine vehicle (1.0 mg/kg ascorbate in 0.9% saline); (iii) a randomly selected half of the rats received an i.p. injection of amphetamine (1.0 mg/kg) and the other half received a s.c. injection of apomorphine (0.5 mg/kg); (iv) the rats that received amphetamine in session three received apomorphine and vice versa; (v) the rats that received amphetamine vehicle in session two received apomorphine vehicle and vice versa; (vi) no injection. Test sessions were separated by 72 h.

All complete turns (360°) were recorded by an observer during four 5-min observation periods (0–5, 15–20, 30–35, 45–50 min) comprising a 50-min session. The use of this time-sampling procedure made it possible to test three animals at a time, each starting at staggered intervals of 5 min with the clock stopped during the time required to make an injection. Subsequently, the direction of each turn with respect to the side of the lesion (ipsiversive versus contraversive) was determined. Circling behaviour was expressed as a ratio of ipsilateral turns over the total number of turns (ipsilateral + contralateral) during the 20 min observation period. Values greater than 0.5 indicated ipsilateral circling. Thus, the dependent measures were circling ratio and total number of turns.

All rats had received unilateral injections into the region of the SN. Experiment 1A included 10 rats that had received 6-OHDA lesions. Animals in experiments 1B ($n = 14$), 1C ($n = 6$) and 1D ($n = 6$) had received quinolinic acid, picolinic acid or the two together, respectively.

Striatum injections (*Experiment* 2). Behavioural testing began approximately one week after surgery. Experiment 2A involved seven sessions per animal ($n = 10$) as follows: (i) no injection; (ii) central injection of 0.9% saline; (iii–v) each of three doses of quinolinic acid (2.5, 5,0, 7.5 nmol) administered in a counterbalanced order across rats over three sessions; (vi) replication of saline; (7) replication of no injection. Experiment 2B ($n = 8$) similarly involved seven sessions with the exception that for sessions iii–v, picolinic acid (10.0, 20.0, 30.0 nmol) was given. Experiment 2C ($n = 8$) included seven sessions with the first and seventh being no injection and the second and sixth being saline; for sessions iii–v, rats received either quinolinic acid (7.5 nmol), picolinic acid (30.0 nmol) or quinolinic acid (7.5 nmol) plus picolinic acid (30.0 nmol), the order being counterbalanced across rats. Test sessions were separated by 48–96 h.

Rats were tested four at a time in separate arenas that were videotaped for 20 min following injections. Subsequently, the number of turns (360°) and the direction of each turn was recorded by an observer who was blind both to the treatment conditions of the animal and to the side of the cannula. The direction of each turn with respect to the side of the cannula (ipsilateral or contralateral) then was determined. Circling behaviour was expressed as a ratio of ipsilateral turns over the total number of turns (ipsilateral + contralateral) during the 20 min observation period. Values less than 0.5 indicated contralateral circling. Thus, the dependent measures were circling ratio and total number of turns.

Histology

All rats from both experiments 1 and 2 were injected with a lethal dose of sodium pentobarbital and exsanguinated with saline (0.9%) followed by 4% formalin. Brains were extracted and stored in sucrose (30%) for 48 h. For the brains from the animals in Experiment 1, frozen coronal sections (50 μm) were prepared for immunohistochemical identification of TH-containing cells using the avidin–biotin complex technique.[7] For the animals from Experiment 2, frozen coronal sections (60 μm) were mounted, stained with Thionin and cannulae placements determined.

Statistical analyses

For the turning ratio and total turns data, *t*-tests for correlated measures were conducted on the first and second no-injection scores and the first and second sessions of saline or vehicle. Values are expressed as mean ± S.E.M. Where no differences were found, scores were averaged across those conditions. ANOVAs then were conducted to analyse treatment effects. Individual comparisons employed Dunnett's tests using saline or vehicle sessions as the control condition.

RESULTS

With two exceptions, for both turning ratios and total turns, for all experiments, the comparison of the first and second no-injection or vehicle treatment data failed to yield a significant difference and the data for each rat from the two sessions were averaged for further analyses. The exceptions were the mean turning ratios for the first and second no-injection treatments for quinolinic acid lesions (see below) and the mean total turns for the first and second no-injection treatments for 6-OHDA lesions. In these cases, the data for the two treatments were not combined for further analyses.

6-*Hydroxydopamine lesions of the substantia nigra*

6-OHDA produced a large loss of TH staining in the SN zona compacta on the injected side. Intact and injected coronal sections looked like those shown in Fig. 1A and B, respectively, for quinolinic acid. There appeared to be no systematic differences in the extent of cell loss in animals receiving 6-OHDA in 1.0 or 2.0 μl volumes and the data for the two subgroups were combined for analyses of turning data.

The turning ratios for the combined no-injection, combined vehicle, amphetamine and apomorphine treatments are shown in Table 1. Amphetamine produced a small increase in ipsiversive turning over the ipsiversive bias seen in these animals with unilateral 6-OHDA lesions; apomorphine, on the other hand, produced a strong contraversive turning bias. ANOVA revealed a significant treatment effect, $F_{(3,27)} = 16.97$, $P < 0.0001$. *Post hoc* tests showed that the difference between the vehicle and apomorphine treatments was significant.

The total turns for the four 5-min observation periods combined during the first and second no-injection, combined vehicle, amphetamine and apomorphine treatments are shown in Table 2. Results appeared to show an increase in turning following treatments with amphetamine or apomorphine. ANOVA confirmed that differences in total turns occurred, yielding a significant treatments effect, $F_{(5,36)} = 3.2$, $P < 0.05$. However, Dunnett's tests failed to reveal any significant differences when each treatment was compared to the vehicle condition.

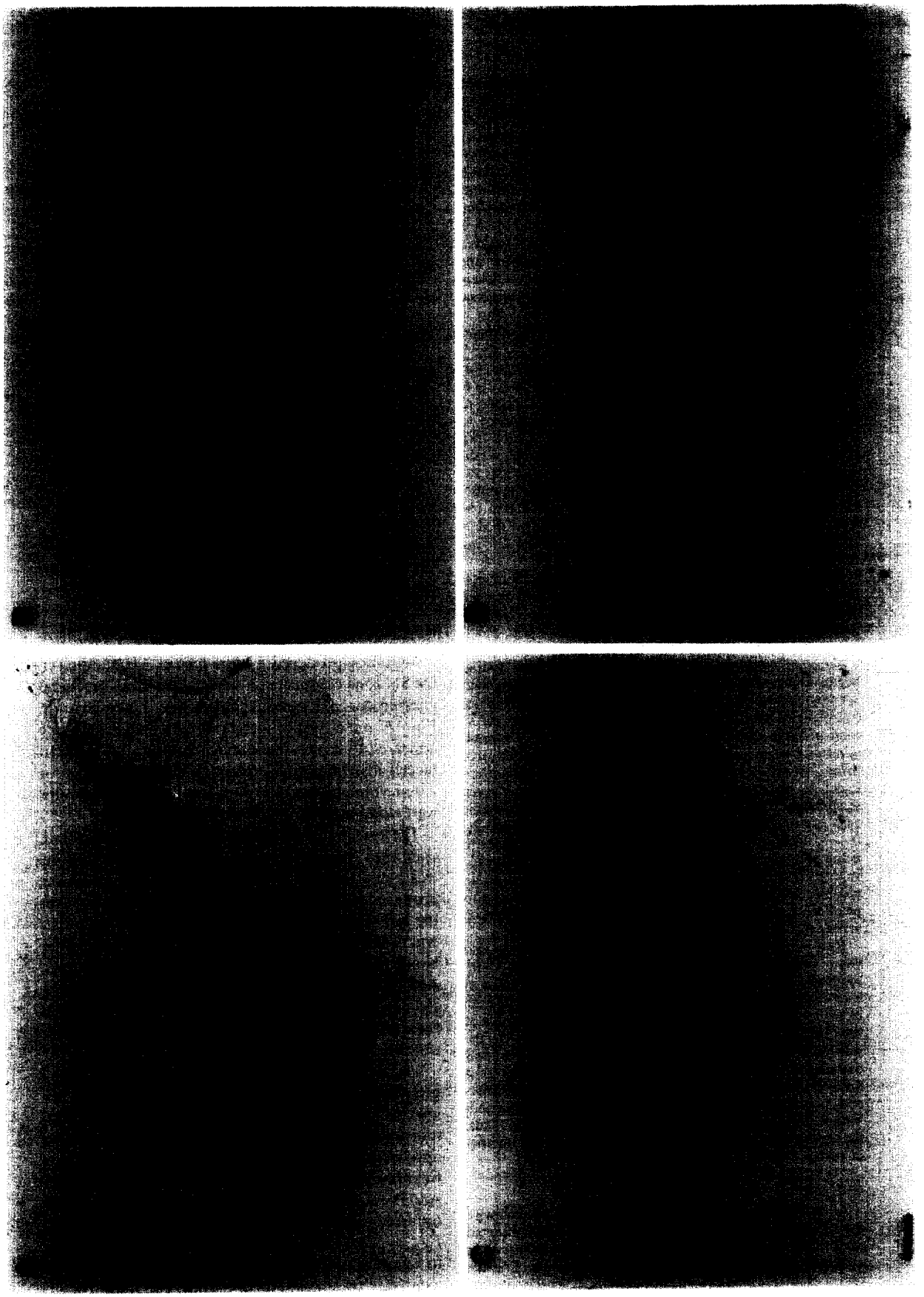

Table 1. Mean (±S.E.M.) turning ratios (ipsilateral/total turns) for animals with substantia nigra injections

Experiment	(*n*)	No injection	Vehicle	Amphetamine	Apomorphine
1A: 6-OHDA	(10)**	0.78 ± 0.04	0.77 ± 0.06	0.85 ± 0.06	0.19 ± 0.10*
1B-1: Quinolinic acid	(5)**	0.31 ± 0.12 0.67 ± 0.14†	0.56 ± 0.12	0.88 ± 0.10*	0.10 ± 0.07*
1B-2: Quinolinic acid	(9)**	0.35 ± 0.07	0.51 ± 0.08	0.90 ± 0.06*	0.95 ± 0.03*
1C: Picolinic acid	(6)	0.44 ± 0.04	0.43 ± 0.05	0.44 ± 0.04	0.61 ± 0.09
1D: Co-injection	(6)	0.38 ± 0.06	0.47 ± 0.07	0.36 ± 0.10	0.59 ± 0.16

1B-1, Quinolinic acid lesions of the SN, rats showing contralateral turning to apomorphine; 1B-2, Quinolinic acid lesions of the SN, rats showing ipsilateral turning to apomorphine.
*Dunnett's tests showed a significant ($P < 0.05$) difference from vehicle.
†Significantly ($P < 0.05$) different from no injection no. 1 by a *t*-test.
**ANOVA revealed a significant ($P < 0.05$) treatment effect.

Table 2. Mean (±S.E.M.) total turns per 20 min for animals with substantia nigra injections

Experiment	(*n*)	No injection	Vehicle	Amphetamine	Apomorphine
1A: 6-OHDA	(10)**	15.1 ± 1.9 8.7 ± 1.3†	12.6 ± 1.7	37.0 ± 14.9	36.6 ± 11.2
1B-1: Quinolinic acid	(5)	13.9 ± 3.3	10.7 ± 1.8	70.4 ± 45.7	50.0 ± 17.0
1B-2: Quinolinic acid	(9)**	8.4 ± 0.9	7.7 ± 1.2	150.8 ± 39.0*	89.6 ± 31.2*
1C: Picolinic acid	(6)	7.4 ± 1.6	7.2 ± 0.9	15.7 ± 3.1	12.5 ± 7.4
1D: Co-injection	(6)	7.8 ± 0.7	7.9 ± 0.4	15.8 ± 2.8	7.7 ± 4.1

Abbreviations as in Table 1.
*Dunnett's tests showed a significant ($P < 0.05$) difference from vehicle.
†Significantly ($P < 0.05$) different from no injection no. 1 by a *t*-test.
**ANOVA revealed a significant ($P < 0.05$) treatment effect.

Thus, 6-OHDA produced a large decrease in TH-positive cells in the SN on the side of injection. Animals receiving 6-OHDA injected unilaterally into the SN showed a strong ipsilateral turning bias that was increased slightly by amphetamine and reversed by apomorphine.

Quinolinic acid lesions of the substantia nigra

Quinolinate produced a large loss of TH staining in the SN zona compacta on the injected side. Coronal sections from the intact and injected side of a representative animal are shown in Fig. 1A and B, respectively.

Examination of the behavioural results following injections of apomorphine revealed two distinct effects: five rats showed strong contralateral turning and nine rats showed strong ipsilateral turning. As previous studies have shown that the direction of turning produced by apomorphine in animals with lesions of the SN zona compacta depends critically upon the extent of damage to the zona reticulata,[3] these animals were treated as two separate groups for the purposes of analyses.

For the five rats showing contralateral turning to apomorphine, mean (±S.E.M.) turning ratios for the first and second no-injection, combined vehicle, amphetamine and apomorphine treatments are shown in Table 1. Amphetamine produced a large increase in ipsiversive turning over values seen in no-injection and vehicle sessions; apomorphine, on the other hand, produced a strong contralateral turning bias. ANOVA revealed a significant treatment effect, $F_{(4,16)} = 12.53$, $P < 0.0001$. Dunnett's tests showed that both the amphetamine and apomorphine treatments differed significantly from vehicle.

For the five rats showing contralateral turning to apomorphine, mean (±S.E.M.) total turns are shown in Table 2 (1B-1). Results showed an apparent increase in turning following treatments with amphetamine or apomorphine although the standard errors associated with the values for these treatments were very large. As a result of the large variability in total turns and the small number of animals in this group, no significant treatment effect was found in the ANOVA, $F_{(3,12)} = 1.77$, $P > 0.05$.

For the nine rats showing ipsilateral turning to apomorphine, mean (±S.E.M.) turning ratios for the combined no-injection, combined vehicle, amphetamine and apomorphine treatments are shown in Table 1. Both amphetamine and apomorphine produced a large increase in ipsiversive turning over values seen in no-injection and vehicle sessions. ANOVA revealed a significant treatment effect, $F_{(3,24)} = 27.67$, $P < 0.0001$. Dunnett's tests showed

Fig. 1. Coronal sections from representative animals showing TH-immunoreactive cells in the substantia nigra from an uninjected animal (A) and from animals receiving 120 nmol quinolinic acid (B), 480 nmol picolinic acid (C), or co-injection of the two tryptophan metabolites (D). Quinolinic acid produced a large loss of TH staining on the injected side. Picolinic acid alone had little effect and appeared to antagonize the neurotoxic effect of quinolinic acid when co-injected with it. Scale bar = 200 μm.

that both the amphetamine and apomorphine treatments differed significantly from vehicle.

For the nine rats showing ipsilateral turning to apomorphine, mean (±S.E.M.) total turns (Table 2, IB-2) showed a large increase following treatments with amphetamine or apomorphine and the ANOVA revealed a significant treatments effect, $F_{(3,24)} = 10.10$, $P < 0.001$. Dunnett's tests showed that the two drug treatments differed significantly from the combined vehicle condition.

In summary, quinolinic acid produced a large decrease in TH-positive cells in the SN on the side of injection. A subset of animals treated with quinolinic acid showed ipsilateral circling following amphetamine and contralateral circling following apomorphine. The remaining quinolinate-treated animals circled ipsilaterally following either stimulant.

Picolinic acid injections into the substantia nigra

Picolinate failed to produce a loss of TH staining in the SN zona compacta on the injected side. A representative coronal section is shown in Fig. 1C.

Mean (±S.E.M.) turning ratios for the combined no-injection, combined vehicle, amphetamine and apomorphine treatments are shown in Table 1. Apomorphine appeared to produce a small increase in ipsiversive turning over the values seen in the other treatment conditions. However, ANOVA revealed no significant treatment effect, $F_{(3,15)} = 2.19$, $P > 0.05$. Similarly, ANOVA of total turns (Table 2, part 1C) showed no significant treatments effect, $F_{(3,15)} = 1.39$, $P > 0.05$. Thus unlike quinolinic acid, picolinic acid did not produce significant changes in turning behaviour following stimulant administration.

Co-injection of quinolinic and picolinic acid into the substantia nigra

Quinolinate, when co-injected with picolinate failed to produce a loss of TH staining in the SN zona compacta on the injected side. A coronal section from a representative animal, given a combination of quinolinic (120 nmol) plus picolinic acid (480 nmol), is shown in Fig. 1D.

The turning ratios for each treatment are shown in Table 1. Apomorphine appeared to produce a small increase in ipsiversive turning over the values seen in the other treatment conditions. However, ANOVA revealed no significant treatment effect, $F_{(3,15)} = 1.54$, $P > 0.05$. Nor did the ANOVA of total turns (Table 2, part 1D) show a significant treatments effect, $F_{(3,15)} = 2.71$, $P > 0.05$. Thus, co-injection of picolinic acid with quinolinic acid led to an antagonism of the neurotoxic effects of quinolinic acid.

Cannulae placement for multiple injections

The location of cannulae tip for each rat from each experiment is shown in Fig. 2. All cannulae were located in the dorsal–anterior region of the caudate–putamen, ranging over a rostro-caudal distance of about 1.5 mm.

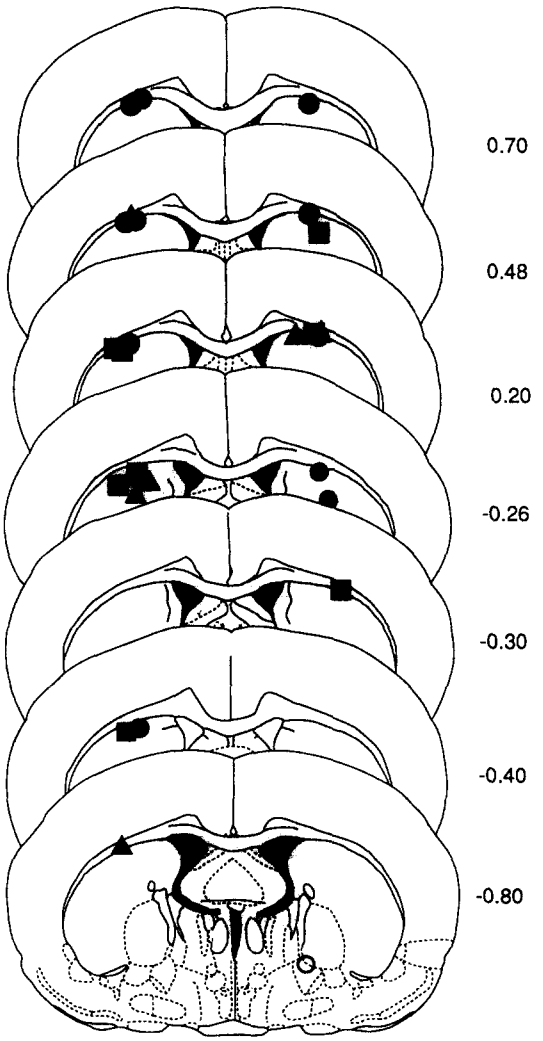

Fig. 2. Location of intracerebral injection sites for animals from Experiment 2. The position of cannulae tips aimed at the dorsal striatum for Experiments 2A ($n = 10$; circles), 2B ($n = 8$; triangles), and 2C ($n = 8$; squares) are shown on coronal sections from Paxinos and Watson.[11] The anterior–posterior co-ordinates, relative to bregma, are located to the right of the sections.

Quinolinic acid-induced circling behaviour

The turning ratios for the combined no-injection, combined vehicle and 2.5, 5.0 and 7.5 nmol doses of quinolinic acid unilaterally injected into the dorsal striatum are shown in Table 3. There appeared to be no directional bias in the no-injection or saline conditions; on the other hand, quinolinic acid appeared to produce a dose-dependent increase in contralateral turning. This description of the data was supported by the results of the ANOVA which revealed a significant treatment effect, $F_{(4,36)} = 3.95$, $P < 0.01$. Dunnett's tests showed that each of the two higher doses was significantly different from saline.

The total turns per 20 min during each treatment are shown in Table 4. Total turns appeared to increase with increasing doses of quinolinic acid and the ANOVA revealed a significant treatment effect,

Table 3. Mean (±S.E.M.) turning ratios (ipsilateral/total turns) for animals with striatum injections

Experiment	(*n*)	No injection	Vehicle	Drug dose		
				Low	Medium	High
2A: Quinolinic acid	(10)**	0.49 ± 0.06	0.53 ± 0.05	0.41 ± 0.04	0.33 ± 0.07*	0.31 ± 0.05*
2B: Picolinic acid	(8)**	0.55 ± 0.05	0.54 ± 0.06	0.41 ± 0.05	0.46 ± 0.03	0.33 ± 0.03*
				Quin	Pic	Quin + Pic
2C: Co-injection	(8)**	0.45 ± 0.04	0.46 ± 0.06	0.29 ± 0.07*	0.42 ± 0.03	0.26 ± 0.01*

Pic, picolinic acid; Quin, quinolinic acid. Low, medium and high drug doses were 2.5, 5.0 and 7.5 nmol of quinolinic acid in Experiment 2A, and 10, 20 and 30 nmol of picolinic acid in Experiment 2B. In Experiment 2C, the doses of quinolinic and picolinic acid were 7.5 and 30 nmol, respectively.
*Dunnett's tests showed a significant ($P < 0.05$) difference from vehicle.
**ANOVA revealed a significant ($P < 0.05$) treatment effect.

Table 4. Mean (±S.E.M.) total turns per 20 min for animals with striatum injections

Experiment	(*n*)	No injection	Vehicle	Drug dose		
				Low	Medium	High
2A: Quinolinic acid	(10)**	7.7 ± 1.0	11.0 ± 2.1	18.1 ± 1.5	33.9 ± 4.5*	34.4 ± 5.1*
2B: Picolinic acid	(8)	8.9 ± 1.9	10.9 ± 2.0	13.5 ± 3.2	17.1 ± 4.1	16.6 ± 4.4
				Quin	Pic	Quin + Pic
2C: Co-injection	(8)**	9.4 ± 1.6	13.1 ± 1.3	17.3 ± 3.0	13.5 ± 1.9	24.9 ± 3.0*

Abbreviations as in Table 3.
*Dunnett's tests showed a significant ($P < 0.05$) difference from vehicle.
**ANOVA revealed a significant ($P < 0.05$) treatment effect.

$F_{(4,36)} = 15.73$, $P < 0.0001$. Dunnett's tests showed that the two higher doses differed significantly from saline.

Picolinic acid-induced circling behaviour

The turning ratios for each treatment (Table 3) indicate little turning bias in the no-injection or saline conditions, generally with greater contralateral circling with increasing doses of picolinate. ANOVA confirmed a significant treatment effect, $F_{(4,28)} = 4.02$, $P < 0.01$, and Dunnett's tests showed the 30 nmol dose to be significantly different from saline. Total turns (Table 4, part 2B) were not significantly affected by treatments, $F_{(4,28)} = 1.27$, $P > 0.05$.

Co-injection of quinolinic and picolinic acid

The turning ratios for each treatment are shown in Table 3. The no-injection and saline conditions revealed a small contralateral turning bias. Quinolinic acid produced contralateral turning as did picolinic acid although the latter effect was small. Co-injection of picolinic acid with quinolinic acid yielded contralateral turning of the same magnitude as that seen with quinolinic acid alone. ANOVA revealed a significant treatment effect, $F_{(4,28)} = 3.45$, $P < 0.05$ and Dunnett's tests showed that quinolinic acid alone or in combination with picolinic acid produced significantly more contralateral turning than seen in the saline condition.

Mean (±S.E.M.) total turns, shown in Table 4, part 2C were higher in the quinolinic acid alone and in the co-injection condition. ANOVA revealed a significant treatment effect, $F_{(4,28)} = 7.33$, $P < 0.001$. Only the co-injection condition differed significantly from the saline in *post hoc* tests.

DISCUSSION

The results of the present study showed that quinolinic acid injected in a relatively high dose into the SN pars compacta, like 6-OHDA, produced a large decrease in cells containing TH immunosensitivity. Whereas dense TH staining was seen in the region of the SN zona compacta on the intact side, only sparce TH staining was observed in this region following injections of either 6-OHDA or quinolinic acid. Like animals treated with 6-OHDA, quinolinate-injected rats showed turning responses following amphetamine or apomorphine. The immunohistochemical and behavioural effects of the quinolinic acid-induced lesion were blocked in animals co-injected with picolinic acid. Low doses of quinolinic acid microinjected locally into the dorsal striatum produced contralateral turning in a dose-dependent manner. However, co-injection with picolinic acid produced no significant change in the turning response seen with quinolinate alone. Thus, picolinic acid blocked the neurotoxic effects of quinolinic acid in the SN but failed to affect the neuroexcitant effects of quinolinic acid in the dorsal striatum.

In both turning studies—those carried out following injections of the SN and those carried out following injections of the striatum—evaluations of drug effects were preceded and followed by saline or vehicle and no-injection treatments. With the exception of one comparison of the first and second no-injection turning ratios (Experiment 1B-1), none of these values was seen to differ significantly from the pre- to the post-drug treatments. This showed that neither chronic cannulation (Experiment 2) nor the series of systemic (Experiment 1) or cental

(Experiment 2) injections had significant enduring effects on the directional bias; this observation is in agreement with numerous previous studies from this laboratory.[9,18] In the case where the two no-injection treatments were seen to differ, it is noteworthy that neither was found to differ significantly from the combined vehicle treatment in the *post hoc* comparisons whereas both the amphetamine and apomorphine treatments did (Table 1). Thus, it would appear that this one exception can be attributed to sampling error.

With only one exception, mean total turns during the first and second no-injection sessions and the first and second vehicle sessions did not differ significantly. This is in agreement with the findings of our previous studies[9,18] and provides further evidence that drug treatments, although significantly affecting behaviour during the test sessions, had little effect on the days following injection. In general, amphetamine and apomorphine produced an increase in total turns in Experiment 1 in those cases where they also produced changed in turning ratios. In Experiment 2, injections of low concentrations of quinolinic acid produced increases in total turns. In most previous turning studies from this laboratory, a strong relationship between changes in total turns and changes in turning ratios has not been seen.[9,18]

In most of the present turning experiments, mean turning ratios for the no-injection and vehicle treatments were about 0.5, indicating no directional bias. The one notable exception was the strong ipsiversive bias seen in the 6-OHDA lesion animals from Experiment 1A. Normally, animals undergoing unilateral depletions of dopamine initially circle ipsilaterally but then learn to compensate and circling is no longer seen; however, these animals can be induced to circle if exposed to a novel environment.[19] This may provide an explanation for the circling behaviour seen in our 6-OHDA-treated animals when they were placed into the (novel) turning areas. One problem with this interpretation is that the quinolinate-lesion animals in Experiment 1B, that were observed to endure a level of dopamine cell loss comparable to that seen in the 6-OHDA-treated animals, did not show a directional bias in no-injection and vehicle sessions. Perhaps this difference between the two SN lesion groups is related to the different neurotoxins since quinolinate, unlike 6-OHDA, would affect non-dopaminergic cells. Further studies will be needed to assess this possibility.

In spite of the strong ipsiversive turning bias observed in the 6-OHDA-treated animals, differential effects of amphetamine and apomorphine were seen. Amphetamine produced a nonsignificant increase in ipsiversive turning whereas apomorphine produced strong contralateral turning. These results are in agreement with the original observations of Ungerstedt.[19,20] Ipsiversive turning was attributed to the dopamine-releasing action of amphetamine on the intact side whereas contraversive turning was attributed to the action of apomorphine on supersensitive dopamine receptors on the lesion side.

Like 6-OHDA-treated animals, some rats receiving quinolinate injections into the region of the SN pars compacta showed differential turning responses to amphetamine and apomorphine (Experiment 1B-1). These rats showed a large loss of TH-positive cells and their results appear to be consistent with our findings with 6-OHDA injections and the observations of Ungerstedt.[19,20] The observation that some quinolinate-lesion animals showed ipsiversive turning following either stimulant can be understood with reference to the effects of electrolytic lesions of the SN. Thus, it has been found that rats receiving lesions restricted to the zona compacta show differential turning to amphetamine and apomorphine whereas rats receiving lesions that invade the zona reticulata circle ipsilaterally to either stimulant.[3] In either case, large losses of dopamine cells were seen. Although we do not have histological confirmation, previous results[3] would suggest that in a subgroup of our quinolinate-lesion animals, the zona reticulata may have been damaged in addition to the zona compacta. It is noteworthy that animals co-treated with quinolinic plus picolinic acid failed to show significant turning in either direction following amphetamine or apomorphine.

The present study found that local infusion of quinolinic acid produced a large loss of TH-positive cells in the SN. One previous study[15] reported that injections of quinolinic acid (120 nmol) into the SN produced some degeneration detected in thionine-stained material. Perhaps the more extensive cell loss seen in the present study was related to the use of TH immunohistochemistry.

Of direct importance to the hypothesis under investigation in Experiment 1 was the observation that picolinic acid, while having no apparent effect on its own, antagonized the neurotoxic actions of quinolinate in the SN when the two were co-injected in a molar ratio of 4:1. These results are in agreement with our earlier finding that picolinic acid protected cholinergic cells of the basal forebrain from the toxic actions of quinolinic acid.[2,8] In one of those studies,[2] we showed that another pyridine monocarboxylic acid, nicotinic acid, structurally related to picolinic acid, did not significantly alter the neurotoxic action of quinolinic acid when co-injected in a molar ratio of 4:1 with it. This result rules out the possibility that the neuroprotective effects of picolinic acid may be related to its osmotic effects. It should be noted that the concentrations of picolinic acid in the brain remain to be established but that they are unlikely to be in the concentrations employed here. The present results show that the neuroprotective actions of picolinic acid seen previously in cholinergic neurons extend to non-cholinergic (dopaminergic) neurons.

Results obtained from unilateral intrastriatal injection studies in Experiment 2 revealed that quinolinic acid produced a dose-related contralateral circling

response, an observation consistent with our earlier finding that intrastriatal injections of NMDA similarly produced contralateral circling.[18] The quinolinic acid response occurs as a result of activation of NMDA receptors[10,12,16,17] which, in turn, activate dopaminergic neurons in the striatum.[18] Interestingly, picolinic acid injections also produced circling behaviour. However, co-injection of picolinic acid with quinolinic acid failed to block the turning response, indicating that picolinic acid does not attenuate the neuroexcitant action of quinolinic acid. This result agrees with the findings of Robinson *et al.*[14] that picolinic acid failed to block synaptic responses produced by stimulation of glutamate receptors in electrophysiological studies. Thus, the antagonism of the action of quinolinate, apparent in neurotoxicity experiments, was not seen in behavioural experiments using low doses. It should be noted that the doses of quinolinic and picolinic acid used in the former experiments were much greater than those used in the latter experiments. However, the molar ratios of quinolinate to picolinate (1:4) in both types of experiments were similar.

The excitotoxicity and excitation experiments revealed a significant difference in regard to the actions of picolinic acid when injected alone. The neuroprotective dose of picolinic acid, which was fourfold greater than the quinolinic acid dose, failed to produce depletion of TH immunoreactivity in the SN. A similar lack of action of picolinic acid on choline acetyltransferase activity in the cortex following injection into the nucleus basalis magnocellularis has been observed in earlier studies.[8] In contrast, in Experiment 2, picolinic acid injected into the dorsal striatum elicited turning responses similar to those induced by injections of quinolinic acid. The mechanism underlying this stimulatory action of picolinic acid is unknown. In a recent experiment,[21] involving the study of the effects of picolinic acid on the release of glutamate from striatal slices, picolinic acid (100 μM) produced a weak stimulatory action on this release. This action may account for the stimulation of contralateral turning seen following injection of picolinic acid into the striatum. This profile of results might suggest that picolinic acid acts as a partial agonist at the quinolinate site; thus, low doses produce a quinolinate-like neuroexcitant effect while high doses fail to produce a neurotoxic effect because they fail to fully activate the site. In conclusion, picolinic acid, while producing excitatory responses, appears not to produce excitotoxicity.

CONCLUSIONS

The results of the present study thus appear to provide support for the hypothesis that picolinic acid can influence quinolinic acid-induced neurotoxicity without significantly influencing quinolinic acid-induced excitation. In this regard, the action of picolinic acid differs from that of kynurenic acid, another tryptophan metabolite, which effectively blocks excitotoxicity as well as excitation.

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