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Quinolinic acid concentrations in striatal extracellular fluid reach potentially neurotoxic levels following systemic L-tryptophan loading

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Following a systemic tryptophan load, striatal extracellular fluid levels of quinolinic acid in the rat were quantified using intracerebral microdialysis. After an intraperitoneal dose of L-tryptophan (250 mg/kg), quinolinic acid levels in striatal perfusates increased by 230 fold. Peak concentrations of quinolinic acid exceeded 10^{-5} M, a concentration previously shown to be neurotoxic in vitro. These results indicate that quinolinic acid is markedly precursor responsive and that its concentration in striatal extracellular fluid may reach neurotoxic levels following an acute tryptophan load.

The relationship of the precursor amino acid L-tryptophan to its parent neurotransmitter, serotonin (5-hydroxytryptamine, 5-HT) is well established. Under a number of pharmacological and physiological manipulations, brain tryptophan availability has been linked to 5-HT synthesis and release[6,7,14]. Much less is known about an alternate tryptophan metabolic route — the kynurenine pathway[8,9]. A number of intermediates along this pathway are neuroactive. Of particular interest is quinolinic acid (2,3-pyridinedicarboxylic acid, QUIN), an endogenous ligand of the excitatory amino acid *N*-methyl-D-aspartate (NMDA) receptor[26], and a potent neurotoxin[23,25]. QUIN has been postulated to have an etiological role in a number of neurodegenerative diseases. When injected locally into the corpus striatum, a lesion is formed which may spare specific neuronal cell types, mimicking the pattern seen in Huntington's disease[2]. When infused intracerebroventricularly[13], or into rat hippocampus[22], QUIN acts as a potent epileptogenic compound. There is also evidence to implicate QUIN in the neuronal damage seen in glutaric aciduria[10] and hepatic encephalopathy[16] suggesting that it may have a broader role in neurological disorders.

Studies have shown that brain tissue levels of QUIN may rise significantly following tryptophan loading[10,15]. Because the most relevant compartment of the brain in terms of QUIN's excitoxicity is the extracellular fluid (ECF) space, it was of interest to determine whether QUIN concentrations were also elevated in this compartment following tryptophan administration. Recent development of in vivo perfusion techniques enables repeated measurements of the ECF. Of these techniques, perhaps the most physiological is microdialysis, using a probe which enables the passage of small ECF molecules across a membrane into the dialysate. The major limitation of this technique has been the development of sufficiently sensitive and specific assays to reliably measure low femtomolar amounts of the compounds of interest[28].

We have used intrastriatal microdialysis coupled to highly sensitive gas chromatograph–mass spectrometry (GC–MS) to monitor ECF QUIN in rat corpus striatum following tryptophan loading. QUIN was assayed by negative chemical ionization GC–

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MS. [^{18}O]QUIN was used as the internal standard[11].

Male Sprague–Dawley rats (280–300 g) were anaesthetized with chloralose–urethane (50:500 mg/kg) intraperitoneally (i.p.) and placed in a Kopf stereotaxic frame. The skull was exposed and holes drilled above the right and left corpus striata at coordinates AP: +0.5; L: 2.5[19]. Microdialysis probes of cannula design[5,21] and 4 mm exposed membrane surface were implanted at a depth of 7 mm (flat skull). Probes were perfused at 2.4 μl/min with an artificial ECF (Na^+ 125 mM, K^+ 2.8 mM, Ca^{2+} 1.2 mM, Mg^{2+} 1.2 mM, ascorbate 100 μM, pH 7.4) and left and right dialysates were pooled for subsequent QUIN assay. Continuous samples were taken at 30 min intervals and immediately frozen on dry ice and stored at −80 °C until analysis.

To obtain an adequate baseline, dialysates were collected for an initial 3 h. Rats were then administered L-tryptophan, 250 mg/kg, suspended in saline (4 ml/kg) or saline alone intraperitoneally (i.p.). Dialysis continued with repeated sampling for the next 180 min.

An additional experiment was designed to ensure that ECF QUIN as measured by dialysis reflected brain synthesis and release into the extracellular space and not simply a disruption of the blood–brain barrier. Two rats received an intravenous infusion of quinolinic acid (2.5 mmol/kg) through an indwelling femoral catheter over 5 min while simultaneously undergoing intrastriatral dialysis. Baseline plasma samples were taken at −15 min and at the commencement of the infusion, and at intervals of 15 min for the first hour and at 90, 120, 150 and 180 min following the infusion. Dialysate samples were taken at 30 min intervals.

To estimate ECF QUIN levels, probes were calibrated by placing them in a solution of quinolinic acid of known concentration (10 μM) and measuring the in vitro recovery of QUIN in the dialysate.

The intrafemoral infusion of QUIN resulted in a 100-fold increase in its plasma levels which slowly returned towards baseline over the following 3 h. In contrast, the striatal dialysate QUIN concentration increased slightly and only in the first 30 min sample, returning to baseline at 60 min (Fig. 1). These results suggest that: (1) the probe lies within the blood–brain barrier, as previous studies have also concluded[3,27] and (2) QUIN does not cross the blood–brain barrier to any appreciable extent.

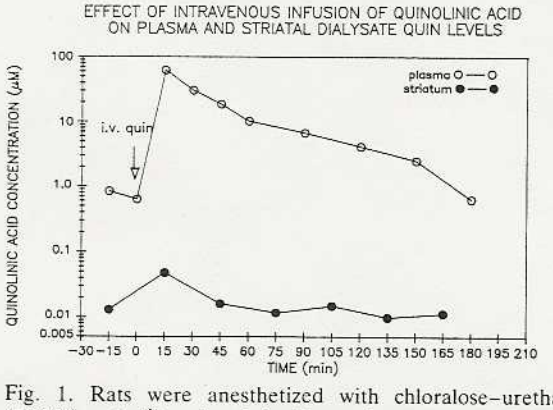

Fig. 1. Rats were anesthetized with chloralose–urethane (50:500 mg·kg^{-1}) and an indwelling catheter was placed in the left femoral vein through a small superficial incision. The rats were then placed in a Kopf stereotaxic frame and were implanted with microdialysis probes as described above. Two h following probe implantation, baseline plasma and dialysate samples were taken. Animals then received an intravenous infusion of QUIN (2.5 mmol/kg in 1 ml/kg 0.9% saline) through the indwelling femoral catheter over 5 min and plasma and dialysis sampling continued for the following 3 h. Results of the simultaneous plasma and dialysate measurements of QUIN for a representative animal are shown. Dialysate levels are uncorrected for recovery.

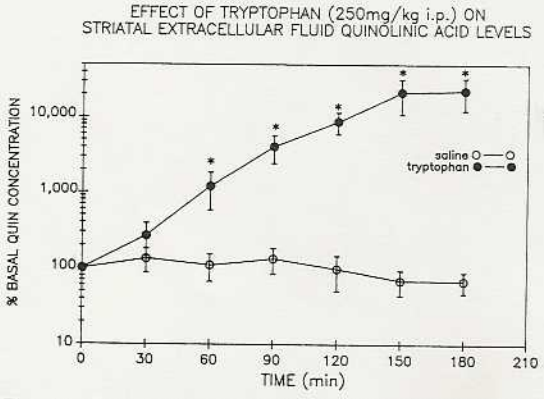

Fig. 2. Rats were anesthetized with chloralose-urethane (50:500 mg·kg^{-1}) i.p. and placed in a Kopf stereotaxic frame. Probes were implanted into left and right striata. At 120 min following probe implantation, 3 consecutive 30 min collections were made to obtain a baseline measurement. Rats then received either 0.9% saline ($n = 4$) or 250 mg/kg tryptophan ($n = 5$) i.o. Dialysates were collected for the following 3 h, with left and right samples pooled and assayed for QUIN using negative chemical ionization gas chromatography–mass spectroscopy. Basal QUIN levels in the saline treated rats were 5.5 ± 1.6 nM and in the tryptophan treated rats levels were 6.2 ± 1.8 nM (mean ± S.E.M.). Data represents QUIN concentration as a percentage of baseline values, vertical bars represent the S.E.M. *$P < 0.05$ using ANOVA with repeated measures.

In saline-treated rats, basal QUIN concentrations in striatal dialysates were 5.5 ± 1.6 nM and remained stable throughout the 3 h sampling period. In the tryptophan-treated animals, baseline QUIN levels were 6.2 ± 1.8 nM and increased progressively to concentrations of 1.4 ± 0.6 μM at 180 min, 230-fold greater than baseline (Fig. 2).

In vitro calibration of probes at the 2.4 μl/min perfusion rate gave a recovery efficiency of 10 ± 2%, enabling an estimation of actual extracellular fluid concentrations. If one were to assume the same diffusion kinetics in the brain as in vitro, the peak levels of QUIN would have exceeded 14 μM in rat striatum following the tryptophan load. However, diffusion kinetics in vivo are markedly different[18] and this calculation may underestimate actual ECF levels by as much as 5- to 10-fold[1].

Levels of QUIN of 10 μM have been shown to be toxic in vitro, in neuronal cell cultures[29], and in vivo, a QUIN injection of as low as 12 nmol results in striatal cell loss[24]. Thus, it is possible that an acute tryptophan load may cause neurotoxicity via QUIN formation. Indeed, chronic feeding studies have demonstrated central nervous system degeneration when portocaval-shunted rats were placed on a high tryptophan diet[4] and it is possible that this toxicity was mediated in part through QUIN. In normal rats, high tryptophan diets induce liver tryptophan pyrrolase which peripherally metabolizes tryptophan[12]; in animals with hepatic shunts, this (protective) route of metabolism is largely bypassed and the brain is exposed to much higher concentrations of tryptophan and QUIN[16].

In conclusion, corpus striatal ECF levels of QUIN reach potentially neurotoxic levels following a systemic tryptophan load in rats. Histological studies investigating this putative acute toxicity are currently underway; however chronic feeding studies have revealed CNS toxicity in portocaval-shunted rats[4]. In addition,the kynurenine pathway in brain appears to be markedly precursor responsive. Another kynurenine metabolite, kynurenic acid which acts as a NMDA receptor antagonist[20], has also been reported to be responsive to alterations in brain tryptophan availability[17]. However, the increase of 100% in brain kynurenic acid levels following a 300 mg/kg tryptophan load is much less dramatic than the QUIN response observed in the present study, although extracellular concentrations of kynurenic acid were not measured[17]. These data suggest that pharmacological effects of tryptophan may in part be mediated through intermediates of kynurenine and in particular QUIN.

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