Br. J. Pharmacol. (1988), **93**, 868–876

Quinolinic acid effects on amino acid release from the rat cerebral cortex *in vitro* and *in vivo*

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1 The effect of quinolinic acid, N-methyl-D,L-aspartate (NMDLA) and kainate on the release of endogenous and exogenous amino acids from the rat cerebral cortex *in vitro* and *in vivo* was studied.

2 Neither quinolinic acid nor NMDLA had any effect on the basal or potassium-evoked release of $[^3H]$-D-aspartate from slices of rat cerebral cortex either in the presence or absence of magnesium. Kainic acid failed to modify the basal efflux of $[^3H]$-D-aspartate but significantly inhibited (by 34.4% ± 0.04%, $P < 0.05$) the potassium-evoked release.

3 Neither quinolinate nor NMDLA had any effect on the basal efflux of endogenous amino acids from rat cortical slices either in the presence or absence of magnesium ions at concentrations between 10 μM and 5 mM.

4 Both NMDLA (1 mM) and quinolinate (5 mM) produced an efflux of endogenous aspartate (371.4% ± 11.6%; 389.3% ± 12.1%) and glutamate (405.4% ± 13.6%; 430.1 ± 8.7%) respectively from the rat cerebral cortex *in vivo* ($P < 0.01$). The quinolinic acid-evoked efflux was abolished by the NMDLA antagonist, 2-amino-5-phosphonovaleric acid (200 μM).

5 Kainic acid also caused an efflux of endogenous amino acids from the rat cerebral cortex *in vivo*. However, the profile of this release was different from that produced by quinolinate and NMDLA.

6 The results add further support to the suggestion that quinolinic acid acts at the NMDLA-preferring receptor and may also explain the requirement for intact afferent projections for the neurotoxic effects of quinolinate to be manifested.

Introduction

There is now considerable electrophysiological and biochemical evidence to suggest that the endogenous tryptophan metabolite, quinolinic acid, acts selectively on the N-methyl-D,L-aspartate (NMDLA) type of amino acid receptor to produce excitation of central neurones (Stone & Connick, 1985, Stone *et al.*, 1987). In addition to its potent excitatory activity, quinolinic acid is capable of producing convulsions when given intracerebroventricularly to mice (Lapin, 1981) and is a kainate-like neurotoxin producing axon-sparing lesions following injection into several regions of the CNS (Schwarcz *et al.*, 1983).

Kainic acid, however, has been proposed to act, at least in part, by inhibition of the reuptake of released acidic amino acids (McGeer & McGeer, 1982) thereby increasing the concentrations of these amino acids in the synaptic cleft. In addition, Ferkany & Coyle (1983) have shown that kainate can promote a calcium-dependent release of glutamate and aspartate from both rat and mouse hippocampus and cerebellum. These findings have been tendered in partial explanation of the requirement for putative glutamatergic afferent pathways to be present in order for kainate to produce toxicity (Biziere & Coyle, 1978).

Quinolinic acid has also been shown to require such afferent projections in order to manifest its neurotoxic effects (Schwarcz *et al.*, 1984). The possibility existed therefore that quinolinic acid might also act in part indirectly, either inhibiting the reuptake of acidic amino acids, or causing their release, in addition to any direct postsynaptic effects. We have therefore investigated the possible modulation of amino acid release and uptake by quinolinic acid and other related compounds.

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Methods

Preparation of rat cortical brain slices

Male Wistar rats were killed by decapitation after stunning and the occipital, interparietal, parietal and frontal bones of the skull removed. The dura was removed with a pair of fine forceps and the neocortex of each cerebral hemisphere separated by a cut parallel to the surface of the cortex. The slice (approximately 1.5 mm thick) was then placed in ice cold Krebs-bicarbonate solution (composition, mM: KH_2PO_4 2.2, $MgSO_4$ 1.2, KCl 2.0, glucose 10.0, $NaHCO_3$ 25.0, NaCl 115.0, $CaCl_2$ 2.5). The neocortical slice was then trimmed to give a square slice approximately 5 mm by 5 mm and tissue sections (400 μm) prepared on a McIlwain tissue chopper. Individual slices were next separated with glass seekers and then transferred to appropriate incubation conditions.

Release of [^{3}H]-D-aspartate by rat brain cortical slices

Cortical slices were preincubated for 15 min in 1 ml of Krebs-bicarbonate solution, before addition of [^{3}H]-D-aspartate to a final concentration of 10^{-8} M. The incubation was continued for 20 min before separation of the slices from the incubation medium by gentle filtration onto GF/C filters and washed with 10 ml of Krebs bicarbonate medium at 34°C. Pairs of slices were rapidly transferred to nylon gauze holders in perfusion chambers. Oxygenated Krebs-bicarbonate solution was warmed and perfused the chambers at 34°C and a rate of 0.5 ml min^{-1}. Fractions were collected every 3 min. Four perfusion chambers were used simultaneously in parallel. Slices were washed with Krebs-bicarbonate medium for 30 min and then twenty 3-min fractions were collected, the compound of interest being included in fractions 10 and 11, or in fractions 6 to 11 in experiments in which the compound of interest and potassium were applied.

In vitro *release of endogenous amino acids*

Cortical slices were placed in a 100 ml conical flask, containing 75 ml Krebs-bicarbonate solution and incubated with gassing at 34°C for 30 min. Individual slices were then transferred to a series of 1.5 ml tubes each containing 1 ml of Krebs-bicarbonate solution with or without the compound of interest and incubated with gassing in a 34°C water bath for 15 min. Aliquots (200 μl) of supernatant were then removed from each tube, and immediately frozen at −20°C for subsequent analysis by high performance liquid chromatography (h.p.l.c.).

Slices were then sedimented by brief centrifugation and the supernatant discarded. Pellets were then stored at −20°C for protein determination (Lowry *et al.*, 1951).

Surgical procedure for the preparation of a cortical cup

Male Wistar rats were anaesthetized with urethane (1.3–1.7 mg kg^{-1}). The rat was mounted in a stereotaxic head frame and the body temperature (rectal) maintained at approximately 37°C by an overhead lamp.

The neck muscles were dissected and the cerebrospinal fluid (CSF) drained from the cisterna magna through a puncture made in the posterior atlanto-occipital membrane. This usually prevented the development of cerebral oedema throughout the experiment. When there was a cerebral oedema in spite of CSF drainage, the results were rejected.

A craniotomy was performed and the dura reflected to expose the cortical surface between the coronal and lambdoid sutures. A truncated Gilson pipette tip of diameter about 4 mm was carefully placed on the cortical surface and sealed in position with 4% agar in saline. If any damage to the cortex was observed during this procedure the results were discarded. A push pull pair of nylon cannulae was inserted into the cup and the cortex superfused with buffer (either Krebs-bicarbonate medium, Krebs-bicarbonate medium without added magnesium or 50 mM Tris-HCl pH 7.4 in 0.9% saline) by a peristaltic pump at a rate of 100 μl per 10 min for at least 2 h before the experiment started. The cup was then drained and 100 μl of oxygenated medium placed in the cup. After 10 min, this solution was removed and frozen at −20°C. A second 100 μl of medium containing the compound of interest was similarly introduced into the cup and incubated for 10 min before collection and storage. The cortex was then superfused with medium for at least 1 h between release experiments. A maximum of two release experiments were conducted on one animal. The concentration of amino acids within each sample was determined by h.p.l.c. analysis.

Procedure for the determination of endogenous amino acids

Endogenous amino acids were measured by h.p.l.c. using a method based upon that of Turnell & Cooper (1982): *o*-phthalaldehyde (OPT)/2-mercaptoethanol derivatives were produced by taking 100 μl of OPT reagent solution (Sigma) and mixing with 100 μl of amino acid mixture (Standards or sample). The mixture was immediately vortexed to produce the 1-alkyl-thio-2-alkyl-substituted iso-

Table 1 Effect of kainic acid on the efflux of [^{3}H]-D-aspartate from the rat cerebral cortex *in vivo*

Evoked release ratio (S_t/S_b)			
K$^+$ 44 mM	*Kainate*	*Kainate concentration*	n
2.15	0.98	100 mM	8
2.48	1.16	1 mM	8
2.41	1.50*	1 mM (+K$^+$ 44 mM)	8

S_t/S_b is the ratio of mean d.p.m. from the 6 min following stimulation (S_t) to the mean d.p.m. from the 15 min before stimulation (S_b). In all cases s.e. mean was less than 15% of the mean.

indole derivative (Roth, 1971). After 30 s, 20 µl of the mixture was injected onto the chromatographic column for analysis.

A Gilson gradient system was used, and detection performed by a fluorimeter with an excitation wavelength of 390 nm and emission cut off filter at 475 nm, at maximum sensitivity. Separation of the derivatised amino acids was performed on a reverse phase 'µ-Bondapak' C_{18} analytical column (Waters) fitted with a C_{18} 'guard pak' precolumn (Waters). Solvent A was made each day by diluting 125 ml of 50 mM disodium hydrogen phosphate, pH 7.2, to 460 ml with water, and making up to 500 ml with acetonitrile. This mixture was then filtered through a 0.45 µm Durapore filter (Millipore) under vacuum. Solvent B consisted of water (400 ml), acetonitrile (300 ml) and methanol (300 ml) premixed and filtered under vacuum.

Chromatographic conditions: the gradient programme consisting of a series of linear steps; expressed as time in min from injection (% solvent B) was; 0(0), 10(0), 30(100). The flow rate was 1.5 ml min^{-1} at room temperature.

Quantification: amino acid derivatives were identified by their retention times relative to a reference injection of standard amino acids injected every 10 samples. The amino acid concentrations were quantified by comparing the peak heights to those obtained in the reference injection. This method was found to provide an accurate and reliable means of quantification (Connick, 1987). In some cases the resolution of the peaks for γ-aminobutyric acid (GABA) and alanine was not sufficient for accurate quantification (i.e. when peaks were not separated to the baseline) and in these cases the offending amino acid has been omitted from the results.

Control for the assessment of cortical damage

These experiments were conducted upon completion of an *in vivo* release experiment.

An aliquot of [^{51}Cr]-EDTA (10 µCi) in approximately 100 µl was injected i.p. After 20 min fresh neutral saline (100 µl) was placed in the cortical cup and allowed to equilibrate for 10 min before collection. The cortical surface was then disrupted with 3 pricks from a hypodermic needle. Another 100 µl aliquot of neutral saline was then placed in the cup and removed after 10 min.

The rat was then killed by cervical dislocation, and samples of plasma, liver, brain cortex, and hindlimb muscle collected. Each sample was weighed and the accumulated activity in each sample was determined by Cherenkhof counting in a LKB model 80000 γ-counter.

Statistics

Throughout this study, statistical significance has been assessed relative to control conditions by use of either a paired or unpaired Student's *t* test as appropriate. Levels of significance are indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Results

[^{3}*H*]-D-*aspartate release* in vitro

Neither quinolinic acid nor its more potent analogue NMDLA was able to influence either the resting or the potassium-stimulated efflux of [^{3}H]-D-aspartate (data not shown). Omission of magnesium from the medium, the presence of which has been found to attenuate NMDLA receptor-mediated responses (Evans *et al*., 1977; Lehmann *et al*., 1983a), did not change this lack of activity. Kainic acid might have been expected to show some stimulatory action on [^{3}H]-D-aspartate release, if only by virtue of its inhibition of [^{3}H]-D-aspartate uptake (Connick & Stone, 1985). Even at 1 mM, however, kainate had no effect on the basal efflux of [^{3}H]-D-aspartate (Table 1), and actually inhibited the potassium-evoked release of [^{3}H]-D-aspartate (by 34.4% ± 0.04%, $n = 8$, $P < 0.05$).

Release of endogenous amino acids in vitro

The concentrations of endogenous amino acids released into the medium under control conditions are

Table 2 Effect of quinolinic acid or N-methyl-D,L-aspartate (NMDLA) on the efflux of endogenous aspartate and glutamate from rat cerebral cortex *in vitro*

	Control		*Experimental*		
Compound	*Aspartate*	*Glutamate*	*Aspartate*	*Glutamate*	n
Quinolinic acid:					
5 mM	100.0 ± 12.5	100.0 ± 15.0	85.0 ± 11.0	93.1 ± 14.5	6
5 mM ($-Mg^{2+}$)	100.0 ± 6.7	100.0 ± 9.1	112.0 ± 12.1	95.9 ± 11.3	6
NMDLA:					
1 mM	100.0 ± 5.6	100.0 ± 5.9	98.6 ± 11.4	116.4 ± 14.5	6
1 mM ($-Mg^{2+}$)	100.0 ± 8.7	100.0 ± 11.3	108.9 ± 11.2	87.8 ± 8.9	6

Aspartate and glutamate efflux was determiend as described in the methods section. Results are expressed as mean % efflux ± s.e. mean from 6 experiments conducted in triplicate.

presented in Table 2. The release of endogenous amino acids stimulated by potassium showed a dependence on the presence of calcium ions in the surrounding medium. Whilst the basal efflux of glutamate and aspartate was unaffected by removal of calcium, the potassium-stimulated release was reduced by 42.8% ± 5.2% ($n = 4$, $P < 0.05$) and 48.1% ± 7.4% ($n = 4$, $P < 0.05$) respectively. Neither quinolinic acid (5 mM) nor NMDLA (1 mM) was able to modify the basal efflux of endogenous glutamate or aspartate, even in the absence of magnesium ions (Table 2). Lower concentrations of both NMDLA and quinolinate (10 μM and 100 μM) also failed to produce any observable effect (data not shown). This lack of activity was also found in rat hippocampal slices (Connick & Stone, 1986). In order to ensure that the lack of activity of quinolinic acid in inducing amino acid release *in vitro* was not due to any problems associated with the preparation of the tissue, similar experiments were repeated *in vivo*.

Release of endogenous amino acids in vivo

In this study we have used the cortical cup technique to follow the overflow of endogenous amino acids from the cortex. Preliminary experiments emphasised the need for extreme care during the surgery involved. Even the smallest damage to the pial surface produced variable results, and generated a vastly greater acid release. In addition great care was necessary in attaching the plastic cylinder to the cortex, so as not to damage the superficial blood supply.

In order to ensure that the pial surface was intact, and to show that any changes in amino acid levels were not due to plasma contamination, the impermeant extracellular space marker $[^{51}Cr]$-EDTA was used in a number of experiments. Only 0.6% ± 0.1% ($n = 4$) of the label present in the plasma was found in the cup with the intact cortex. This rose to 9.2% ± 1.2% ($n = 4$) following mild damage to the cortical surface with a hypodermic needle. This amount was equivalent to the activity found in both the cortex itself and hind limb muscle.

Effect of quinolinic acid in vivo

The concentrations of endogenous amino acids released into the medium under control conditions are presented in Table 3. Whilst 1 mM quinolinic acid slightly increased the mean percentage efflux of both aspartate and glutamate, the results were not significant (data not shown). At 2 mM, however, quinolinate caused a large and highly specific increase in the release of aspartate (191.5% ± 15.6% of mean) and glutamate (180.2% ± 13.4% of mean). The efflux of other amino acids (serine, glycine, glutamine, alanine, and GABA) was not significantly changed.

At 5 mM quinolinate produced a greater increase in the release of aspartate (389.3% ± 12.1% of mean) and glutamate (430.1% ± 8.7% of mean) (Figure 1), and in addition at this concentration, the efflux of serine, glycine and taurine was also increased. In contrast to the results obtained in neutral saline,

Table 3 Typical basal levels of endogenous amino acid efflux from the rat cerebral cortex *in vivo*

Compound	*Concentration* (μmol 10 min^{-1} cm^{-2})	n
Aspartate	1.32 ± 0.17	10
Glutamate	2.95 ± 0.33	10
Glutamine	5.34 ± 0.06	10
Glycine	0.72 ± 0.01	10
Alanine	0.82 ± 0.08	10
GABA	0.34 ± 0.05	10
Taurine	0.80 ± 0.16	10

Values are given ± s.e. mean.
Amino acid efflux was determined as described in the text. Results are expressed as mean basal amino acid efflux (μmol 10 min^{-1} cm^{-2}) ± s.e. mean from 10 animals.

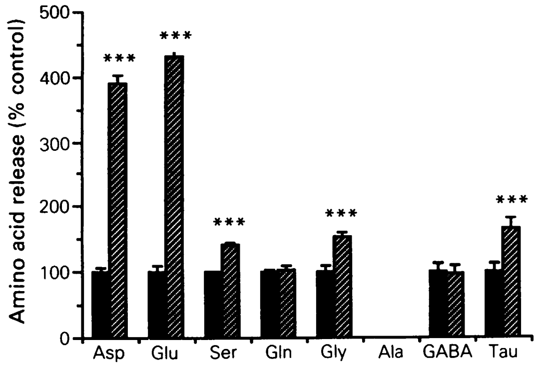

Figure 1 The effect of 5 mM quinolinic acid on the basal efflux of endogenous amino acids from the rat cerebral cortex *in vivo*. Amino acid efflux was determined as described in the text with the inclusion of 5 mM quinolinic acid in the neutral saline solution for the second 10 min 'stimulation' period. Results are mean % control efflux from 11 experiments with s.e. mean shown by vertical lines. Solid columns, control; hatched columns, 5 mM quinolinic acid. Asp = aspartate; Glu = glutamate; Ser = serine; Gly = glycine; Gln = glutamine; Ala = alanine; Tau = taurine.

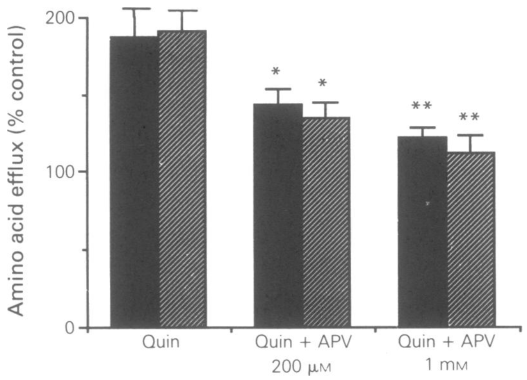

Figure 3 The effect of 200 μM and 1 mM (±)-D,L-2-amino-5-phosphonovaleric acid (APV) on the 2 mM quinolinic acid (Quin) stimulated efflux of endogenous aspartate (solid columns) and glutamate (hatched columns) from the rat cerebral cortex *in vivo*. Amino acid efflux was determined as described in the text with the inclusion of 2 mM quinolinic acid, or 2 mM quinolinic acid with 200 μM or 1 mM APV in the neutral saline solution for the second 10 min 'stimulation' period. Results are mean % control efflux from 8 experiments with s.e. mean shown by vertical lines.

5 mM quinolinic acid in Krebs-bicarbonate medium had no effect (Figure 2) (or even a slight depressive effect), on the efflux of any of the amino acids with the exception of taurine, which was significantly increased to 141.6% ± 16.3% of mean. Repeating this experiment with the omission of magnesium from the Krebs medium again revealed a large increase in the efflux of aspartate (293.7% ± 13.4% of mean) and glutamate (324.2% ± 9.1% of mean) (Figure 2). Thus even nominally magnesium-free Krebs-bicarbonate medium attenuated the relative increase in aspartate and glutamate release with respect to saline, though it should be noted that no attempt was made to remove the trace amounts of magnesium present in the other constituents of the Krebs-bicarbonate solution.

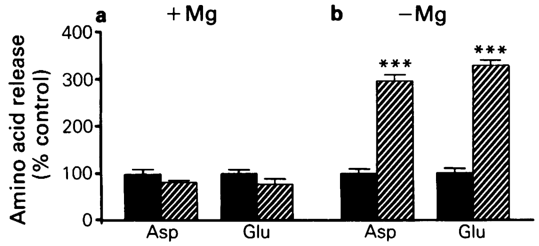

Figure 2 The effect of magnesium on the 5 mM quinolinic acid-evoked efflux of endogenous aspartate (Asp) and glutamate (Glu) from the rat cerebral cortex *in vivo*. Amino acid efflux was determined as described in the text with the inclusion of (a) 5 mM quinolinic acid in Krebs-bicarbonate solution, or (b) 5 mM quinolinic acid in nominally magnesium-free Krebs-bicarbonate solution for the second 10 min 'stimulation' period. In (b) magnesium was omitted from all solutions used during the experiment. Results are mean % control efflux from 15 and 6 experiments respectively; s.e. mean shown by vertical lines. Solid columns, control; hatched columns, plus 5 mM quinolinic acid.

The release of aspartate and glutamate obtained with 5 mM quinolinic acid in neutral saline was abolished in the presence of APV (Figure 3).

Effect of NMDLA

NMDLA (1 mM) evoked a similar specific release of aspartate (371.4% ± 11.4% of mean) and glutamate (405.4% ± 13.6% of mean) to that evoked by 5 mM quinolinate. Whilst the response to NMDLA was severely reduced in Krebs-bicarbonate medium (by over 60%), in common with quinolinic acid (Figure 2), it remained significantly different from control ($P < 0.05$). Removal of magnesium ions from the medium, however, increased the response to NMDLA to over 75% of that obtained in saline.

Effect of kainic acid

Whereas the NMDLA-receptor agonists tested showed a response *in vivo* but not *in vitro*, kainic acid at 2 mM, which produced a significant increase

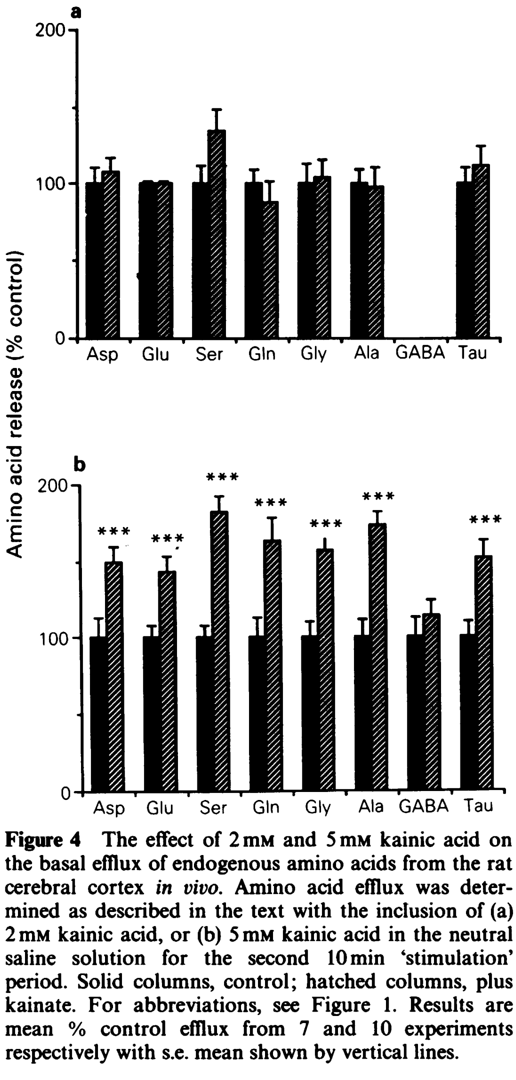

Figure 4 The effect of 2 mM and 5 mM kainic acid on the basal efflux of endogenous amino acids from the rat cerebral cortex *in vivo*. Amino acid efflux was determined as described in the text with the inclusion of (a) 2 mM kainic acid, or (b) 5 mM kainic acid in the neutral saline solution for the second 10 min 'stimulation' period. Solid columns, control; hatched columns, plus kainate. For abbreviations, see Figure 1. Results are mean % control efflux from 7 and 10 experiments respectively with s.e. mean shown by vertical lines.

in amino acid efflux *in vitro* in rat hippocampal slices (Connick & Stone, 1986) was without demonstrable effect *in vivo* (Figure 4). Increasing the dose of kainate to 5 mM, however, caused a large efflux of almost all the amino acids detected; aspartate was increased to 150.1% ± 9.8% of control, glutamate to 144.4% ± 9.9%, serine to 183.0% ± 10.3%, glutamine to 163.3% ± 15.1%, glycine to 157.3% ± 9.3%, alanine to 174.1% ± 9.1% and taurine to 151.8% ± 12.5%. Unlike NMDLA receptor agonists, the release of aspartate and glutamate in response to 5 mM kainate was not changed in Krebs-bicarbonate medium (data not shown).

Discussion

Lehmann *et al.* (1983a) have demonstrated the release of acetylcholine from striatal slices by both NMDLA and quinolinate *in vitro*. These authors reported the absolute dependence of this release on the absence of magnesium ions from the medium. In the presence of 1 mM magnesium, no release could be demonstrated. However, no effect of either agonist against amino acid release could be demonstrated in our system, either in the presence or absence of magnesium. Our results thus confirm the absence of an NMDLA response reported by Ferkany & Coyle (1983). The different results obtained on acetylcholine release and amino acid release may reflect activation of different cell populations, or a difference in sensitivity of striatal and neocortical tissue.

It is most surprising that in such comparatively high doses neither NMDLA nor quinolinate produce any tissue damage, which might be expected to cause the leakage of amino acids into the medium at the very least. It is possible that in high concentrations these compounds cause desensitization of their receptors (Addae & Stone, 1986), or over-depolarization of the tissue. Since much lower concentrations of quinolinate and NMDLA also had no effect on release, this would appear unlikely.

Excitatory amino acids and release in vivo

In contrast to the lack of effect of quinolinic acid and NMDLA in evoking release of either radiolabeled D-aspartate or endogenous glutamate and aspartate *in vitro*, both agonists proved to have powerful effects *in vivo*.

Preliminary experiments using the agonists dissolved in pregassed neutral saline showed the largest stimulation of release by both compounds. In a study of the effects of topically applied exogenous amino acids on evoked potentials in the rat cerebral cortex Addae & Stone (1986) found no difference between the use of 0.9% sodium chloride and Krebs solution, saline being more convenient due to problems of precipitation encountered during static incubations with Krebs-bicarbonate solution. Whilst the use of saline might be criticised on the grounds that ion levels in the cortex might become depleted, such depletion of ion levels in the cortex has been shown to be exceedingly difficult (Fagg & Lane, 1979).

The resting release of all the amino acids studied here (Table 3) were similar to those obtained in an earlier paper by Clark & Collins (1976). Thus aspartate accounted for 10.7% of the total resting amino acid efflux and glutamate 24%, both very similar to the results of Clark & Collins (1976). GABA release was 2.8% of the total, however, approximately twice that found by Clark & Collins (1976) while taurine was 6.5% of the total, compared with 10% found in the earlier study (Clark & Collins, 1976).

In these experiments, rats have been anaesthetized with urethane. This anaesthetic has been reported to have weak amino acid antagonist activity (Evans & Smith, 1982). Thus it is possible that the values of evoked release are an underestimate of those which may occur in awake animals.

The maximum response to both NMDLA and quinolinate was obtained in neutral saline, whilst Krebs-bicarbonate solution severely attenuated the response. An increased response could be restored by omitting magnesium. The magnesium sensitivity of quinolinic acid-evoked efflux, together with the action of APV (Figure 3), confirm that quinolinate is indeed acting at the NMDLA receptor subclass, or at least at a proportion of them.

Whereas the NMDLA receptor agonists tested showed a response *in vivo* but not *in vitro*, kainic acid at 2 mM, which produced a significant increase in amino acid efflux *in vitro* (Ferkany and Coyle, 1983; Connick & Stone, 1986) was without demonstrable effect *in vivo*. Increasing the dose of kainate to 5 mM, however, caused a large efflux of almost all the amino acids detected.

Autoradiographic evidence concerning the distribution of kainate receptors suggests that whereas NMDLA displaces bound glutamate in the upper layers of the cortex, kainate receptors are only found in the deeper cortical layers (Greenamyre *et al.*, 1985). Thus whilst 2 mM kainate had no demonstrable effect on amino acid release monitored at the cortical surface, it is possible that a similar release to that which occurs *in vitro* took place in the deeper layers, but was masked by the reuptake of the released amino acids in more superficial layers. The increase of extracellular taurine may reflect its reuptake by brain tissue, which is far less avid than that for glutamate and aspartate (Richelson & Thomson, 1973). Kainate at a dose of 5 mM however, may have caused sufficient tissue damage *in vivo* to allow the cell membrane to become permeable to all amino acids, and the high concentration of kainate may also have prevented the reuptake of aspartate and glutamate.

The most popular recent method of investigating the releasing properties of the excitatory amino acids *in vivo* has been by the tissue dialysis probe described by Hamberger *et al.* (1982). Whilst this technique does provide major advantages, in that it allows the investigation of various deep neuronal structures in freely moving or anaesthetized animals, the inevitable tissue damage associated with passing a steel probe vertically through the brain of an animal produces a number of problems, notably considerable glial invasion of the damaged tissue (Figure 2 in Jacobson & Hamberger, 1984). A barrier of highly active tissue is therefore produced between the intact brain and the dialysis probe, which may not therefore provide a true reflection of the area of brain under investigation.

Vezzani *et al.* (1985) using this dialysis technique, found that focal injections of high concentrations of quinolinate into the rat hippocampus caused an increase in the efflux of taurine (224% of control), with no change in the efflux of the two other amino acids monitored (glutamate and glycine).

Lehmann *et al.* (1985), reported a marked increase in taurine efflux from rabbit hippocampus in response to both NMDLA and quinolinate. In addition the increased efflux of phosphoethanolamine (PEA) was demonstrated. These increases were large; taurine was increased to 1200%, and PEA to 2400% of their resting levels in response to 5 mM NMDLA. Although the efflux of other amino acids was not reported in detail, the authors observed that 'most other amino acids rose by 20–100%'. In comparison with 5 mM NMDLA, 5 mM quinolinate increased extracellular taurine to 800% of control and PEA by 1500%. It should be noted that the ratio of taurine to PEA for NMDLA was 0.5, whilst in the case of quinolinate it was 0.53. Addition of 5 mM APV reduced the 5 mM NMDLA-induced efflux of both taurine and PEA by 90%.

It is interesting that the same authors were unable to detect any effect of NMDLA (up to 5 mM) on taurine (and probably other amino acid) efflux from a synaptosome preparation (Lehmann *et al.*, 1985).

Lehmann *et al.* (1983b), had previously investigated the effect of kainic acid on the efflux of amino acids from the rabbit hippocampus using dialysis techniques. Again, the efflux of aspartate was not monitored (or at least not reported), but an increased efflux of glutamate, taurine and PEA was found in response to 1 mM kainate. Long perfusion periods with higher concentrations of kainate increased the levels of 'virtually all amino acids', which may be interpreted as reflecting the inability of brain tissue to retain low molecular weight substances in the presence of kainate. This may represent an early sign of cell damage.

The only other *in vivo* study on the effect of NMDLA and quinolinic acid on release was an examination of tritiated purine release. Perkins & Stone (1983) found that both NMDLA and quinolinate produced the release of tritiated purines from the rat cerebral cortex *in vivo*. Kynurenic acid did not evoke any release by itself, but did act to block the effect of both quinolinate and NMDLA. Attempts to repeat this work *in vitro* with brain slices, were without success (H.G.E. Lloyd and T.W. Stone, unpublished observations).

Jacobson & Hamberger (1985) found that kainate caused a rapid increase in the release of aspartate, glutamate, GABA, PEA and taurine in the rabbit olfactory bulb *in vivo*. Whilst slices of olfactory cortex also showed an increased efflux of aspartate and glutamate in response to kainate, other amino acids were unaffected. The authors therefore concluded that certain tissue properties were not reflected *in vitro*.

In summary, with the exception of the quinolinic acid- and NMDLA-evoked release of acetylcholine from striatal slices (Lehmann *et al*., 1983a), all other attempts to produce an effect on amino acid release using agonists at the NMDLA receptor *in vitro* have failed. The reasons for these obvious differences between the results obtained *in vitro* and those *in vivo* are unknown. It is possible that some factor which facilitates amino acid release is lost during the preparation of the brain slices, or that some inhibitory factor is released. Alternatively the differences may simply reflect the higher level of excitability *in vivo* resulting from the constant afferent stimulation.

When considering quinolinic acid as an endogenous agonist at the NMDLA receptor, these negative effects *in vitro* may be used as supporting evidence, since none of the results differentiate quinolinate from NMDLA; i.e. they are pharmacologically identical. In addition both compounds can be blocked by APV *in vivo* and the results thus clearly discriminate between quinolinate and kainate, which evokes a pronounced amino acid release even *in vitro*. Thus whilst some of the neurodegenerative properties of quinolinate resemble those of kainate more than NMDLA (Stone *et al*., 1987), this cannot be explained by a releasing action upon the same amino acid pool.

The excitotoxic hypothesis states that the neurotoxic effects of acidic amino acids result from a direct interaction with specific receptors, located on susceptible neurones, causing excessive depolarization and neuronal death (Olney, 1980). The neurodegeneration produced by quinolinic acid, like that of kainate, cannot be explained simply in these terms since it is dependent upon intact (glutamatergic) synaptic input and it shows greater than expected neurotoxic potency as compared with the apparent potency of other NMDLA agonists when tested electrophysiologically (Stone & Connick, 1985).

Clearly these requirements suggest an interaction between quinolinate and a non synaptic region, located either on the dendrites or on the cell body. Quinolinate may activate presynaptic terminals, directly or indirectly to cause increased release of the neurotransmitter, which in turn interacts with postsynaptic NMDLA receptors to produce cell death. The first direct support for this hypothesis is the present demonstration of the release of aspartate and glutamate by quinolinate *in vivo*. This mechanism explains why quinolinate induced neurotoxicity requires the involvement of an excitatory amino acid releasing input.

This work was supported by a grant from the National Fund for Research into Crippling Diseases.

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(Received October 7, 1987
Revised November 11, 1987
Accepted November 19, 1987)