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In vivo release of [^{3}H]-purines by quinolinic acid and related compounds

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1 *In vivo* release of [^{3}H]-purines from the cortex of anaesthetized rats was measured and the actions of excitatory amino acids and analogues investigated.

2 High KCl, N-methyl-DL-aspartate (NMDLA) and quinolinic acid produced a large increase in basal release of labelled materials. Glutamate, quisqualate and kainate had less effect.

3 The N-methyl-D-aspartic acid (NMDA)-preferring receptor antagonist, 2-amino-7-phosphonoheptanoic acid, significantly reduced the release evoked by NMDLA and quinolinate but not that produced by the other agonists.

4 Kynurenic acid, a compound metabolically related to quinolinic acid, reduced the release due to NMDLA and quinolinate but not glutamate.

5 The results add further support to the suggestion that quinolinic acid acts on the NMDA-preferring receptor.

Introduction

Quinolinic acid is an excitant of cells in the cerebral cortex of the rat and in the caudate nucleus of the cat (Stone & Perkins, 1981; Birley, Collins, Perkins & Stone, 1982; Perkins & Stone, 1982; Herrling, Morris & Salt, 1983). This excitation appears to be due to preferential activation of the N-methyl-D-aspartic acid (NMDA)-preferring excitatory amino acid receptor (Stone & Perkins 1981: Perkins & Stone 1983a) a finding of considerable interest because quinolinic acid is an endogenous metabolite of L-tryptophan in the mammal (Gholson, Ueda, Ogasawara & Henderson, 1964; Mahler & Cordes 1971). Kynurenic acid is also a catabolite of L-tryptophan but, in contrast to quinolinic acid, antagonizes amino acid evoked excitation of central neurones (Perkins & Stone, 1982) and is also capable of antagonizing excitations produced by quinolinic acid (Perkins & Stone, 1982), In addition quinolinic and kynurenic acids are convulsant, when given intracerebroventricularly, in mice (Lapin 1981; 1982) but in rats only quinolinic acid is active when administered by this route (Lapin, Prakhie & Kiseleva, 1982). These data suggest that a complex relationship exists between tryptophan metabolites and amino acid and related excitants.

Jhamandas & Dumbrille (1980) have shown that L-glutamate and L-aspartate can evoke [^{3}H]-adenosine release from the rat cortical surface *in vivo* and have demonstrated that this technique provides a method by which the pharmacology of excitatory amino acids and their receptors may be studied *in vivo*. Therefore, in order to further our earlier observations on quinolinic and kynurenic acids we have investigated the effects of these compounds on [^{3}H]-purine release from rat cortex *in vivo*.

Methods

Male Wistar rats were anaesthetized (urethane 1.5 g kg^{-1} i.p.) placed in a stereotaxic head frame and the cortex exposed. Rectal temperature was monitored and maintained at 37 °C with a heating blanket. After removing the pia, a perspex cylinder (i.d. 5 mm) was carefully placed on the cortical surface and sealed in position with 4% agar in saline. The cup was then filled with 200 μl of saline.

A blunted 22 gauge needle was clamped with its tip just above the cortical surface and after 15 min the contents of the cup removed and replaced by 200 μl of [^{3}H]-adenosine (sp. act. 28 mCi mmol^{-1}) in buffer. After 60 min the radioactive solution was withdrawn and replaced by fresh buffer which was then changed every 10 min for the next 30 min, the solution being discarded each time.

After the washout period the radioactivity in samples taken every 5 min for 50 min was measured. This was taken as the basal release and then the effects of

the agonists were assessed by applying them, in the buffer, for two consecutive periods of 5 min. When antagonists were investigated (including verapamil) these were applied to the cortex in the preceding 5 min sample in addition to the 10 min period with the agonist.

The 200 μl samples were placed in 5 ml of scintillation fluid and the radioactivity converted from c.p.m. to d.p.m. using the ESR of quench correction in a Kontron SL 300 counter. When expressing the release of radioactivity the baseline was extrapolated by eye and the peak percentage increase in release calculated (see Figure 1). The following drugs were tested in these experiments; N-methyl-DL-aspartic acid (NMDLA), quinolinic acid, kynurenic acid, L-glutamic acid sodium salt, kainic acid (from Sigma), verapamil, quisqualic acid and 2-amino-7-phosphono-hepanoic acid (APH). The quisqualic acid and APH were kindly supplied by Drs H. Shinozaki and J. F. Collins respectively.

In order to establish the composition of the released labelled material, samples of perfusate from three experiments were analysed with thin layer chromatography. Samples of 20 μl were applied to silica gel plates containing a fluorescent additive (Merck), allowed to dry and then run in a mixture of ethylacetate: *n*-butanol: methanol: ammonia (4:7:3:4 v/v) (Shimizu, Creveling & Daly, 1970). Purine spots were subsequently located under u.v. light, scraped into scintillation vials and counted. The labelled material had the following proportionate compositions: adenosine 32%, hypoxanthine 16%, inosine 18% and nucleotides 18%.

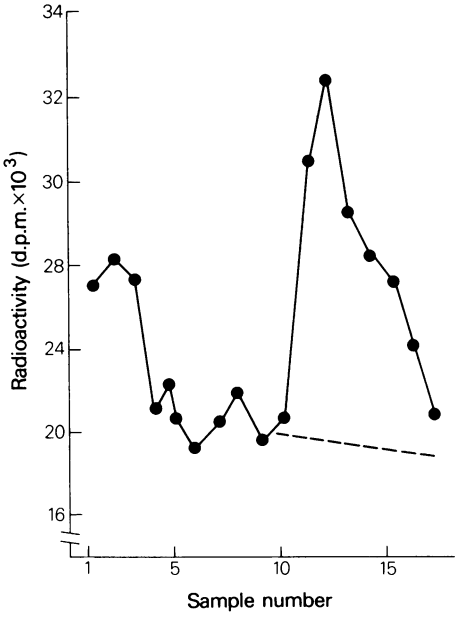

Figure 1 Typical curve showing the release of radiolabelled material from the rat cortical surface following application of 500 μM quinolinic acid for a 10 min period (samples 11 and 12).

Statistics

The statistical significance of the release evoked by agonists was assessed with the sign test and the probability of a significant difference existing between results was determined using Student's *t* test.

Results

The addition of a high concentration (56 mM) of KCl to the cup fluid caused an increase in release of radiolabelled material with a mean increase of 245 ± 48% (± s.e.mean $n = 21$) above basal release. Verapamil (100 μM) reduced this by 44% to 136 ± 36% ($n = 6$). However, the difference was not significant when examined with an unpaired *t* test. Verapamil (100 μM) on its own had no effect. L-Glutamate (5 mM) and kainate (1 mM) had less effect than KCl (Table 1). The low release obtained with kainate compared to KCl surprised us in view of its potency as a neuronal excitant and additional applications were made at lower doses to eliminate the possibility of neurotoxic effects of 1 mM. However, there was no consistent increase in release at the lower doses tested (see Table 1). Quisqualic acid was tested at 500 μM and gave a release comparable to kainate at 500 μM. (Table 1).

NMDLA and quinolinic acid were more effective than glutamate, kainate or quisqualate. NMDLA produced a dose-dependent increase in basal release with an increase in release of 152% and 277% at 1 mM and 5 mM respectively (Table 1). Quinolinate was tested at 5 concentrations giving a maximum release at 5 mM of 291% when there was evidence of plateauing of the dose-response curve (Figure 2).

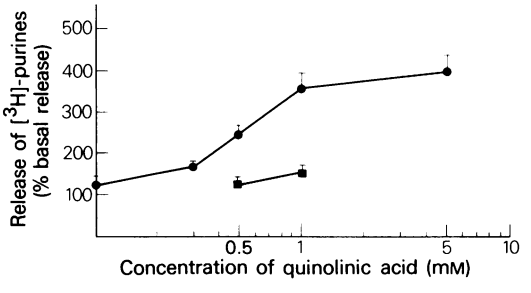

Figure 2 Dose-response curve showing the release of radiolabelled material (+ s.e.mean) from rat cortex following increasing concentrations of quinolinic acid alone (●), and in the presence of 1 mM 2-amino-7-phosphono-heptanoic acid (■). The reduction in release was significant at both points, $P < 0.05$.

Table 1 Effect of amino acids and KCl on [^{3}H]-purine release

compound	*Concentrations* (mM)	*Percentage increase in release above baseline*
KCl	56	245±48 (21)*
L-Glutamate	5.0	89±23 (8)*
Kainate	1.0	56± 7 (5)*
	0.5	40± 5 (5)*
	0.1	87± 9 (5)*
	0.05	40±12 (5)*
Quisqualate	0.5	51±10 (5)*
NMDLA	5.0	277±70 (5)*
	1.0	152±19 (8)*
Quinolinate	5.0	291±39 (11)*
	1.0	257±39 (11)*
	0.5	143±18 (4)
	0.3	64±11 (5)*

Results are expressed as the mean ± s.e. mean.
*Significant increase above basal release, examined using the sign test. $P<0.05$. The numbers in parentheses represent the number of observations.

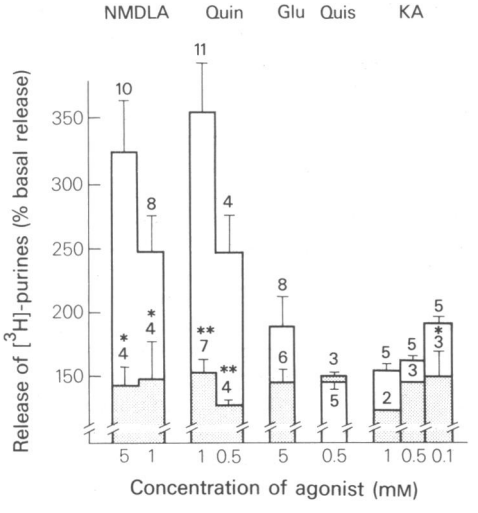

Figure 3 The release of radiolabelled material from rat cortices evoked by excitatory amino acids, N-methy-DL-aspartic acid, quinolinic acid, L-glutamic acid, quisqualic acid and kainate, applied for 10 min periods to the cortical surface. The shaded part of the column indicates the release following co-application of 1 mM 2-amino-7-phosphono heptanoic acid. (APH). Columns show values with s.e. mean bars and the number of experiments is above each column. Significant difference between mean release evoked by amino acid alone and in the presence of APH shown by * $P<0.05$; ** $P<0.01$.

Verapamil (100 μM) reduced the response to 5 mM NMDLA by 35% but, as with KCl, this was not a significant decrease.

Effects of APH

The selective NMDLA antagonist APH (Perkins, Stone, Collins & Curry, 1981; Perkins, Collins & Stone, 1982) significantly reduced the response to 1 mM NMDLA by 41% ($P<0.05$) and also reduced the response to 5 mM NMDLA by 56% (Figure 3). The responses to 1 mM and 500 μM quinolinate were significantly reduced by 1 mM APH (Figures 2 and 3).

In contrast, APH at 1 mM failed to reduce significantly the response to 5 mM glutamate (Figure 3) and the response to 500 μM quisqualate was virtually unaffected by a similar application of APH. APH at 1 mM also failed to modify the responses to 1 mM or 500 μM kainate, but did significantly reduce the response to 100 μM kainate by 23% ($P<0.01$).

Kynurenic acid at 5 mM significantly reduced the response to 5 mM NMDLA and 5 mM quinolinic acid (Figure 4). In addition, kynurenic acid at 1 mM antagonized the response to 1 mM quinolinic acid resulting in reduced increase in release of ^{3}H-purines by quinolinic acid; 124±38% ($n=7$) compared to 257±39% ($n=11$) ($P<0.05$). Kynurenic acid at 5 mM, however, failed to decrease significantly the release of labelled material evoked by 5 mM glutamate (see Figure 4).

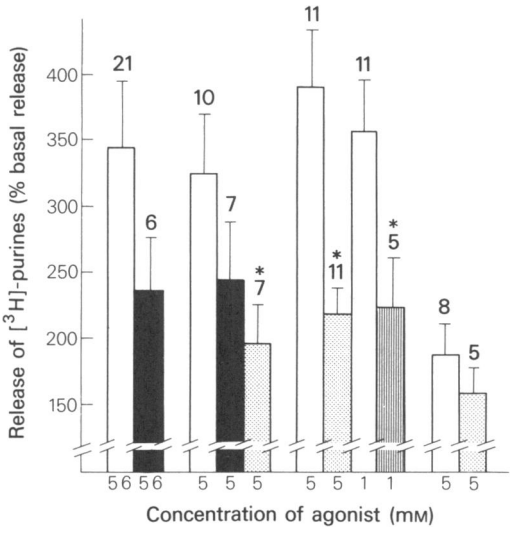

Figure 4 The release of radiolabelled material from rat cortices evoked by the agonists, KCl, N-methyl-DL-aspartic acid, quinolinic acid and L-glutamic acid, alone (open columns), in the presence of 100 μM verapamil (solid columns), 5 mM kynurenic acid (stippled columns) and 1 mM kynurenic acid (vertically hatched columns). Columns show mean values with s.e. mean bars and the number of experiments is above each column. A significant difference between means is shown by $*P<0.05$.

Discussion

There is only one previous study on amino acid-evoked [^{3}H]-purine release using cortical cups and the results are qualitatively similar to the data presented here. Interestingly, Jhamandas & Dumbrille (1980) found L-glutamate was more potent than KCl at promoting release whereas in our hands this was not the case. The results with verapamil are not conclusive in that although there was a consistent reduction in release due to KCl and NMDLA with this calcium antagonist the difference did not reach significant levels. This could represent either a failure to block completely all calcium movements with topically applied verapamil *in vivo* (this may be due to access difficulties) or that the release of labelled adenosine derivatives is not entirely calcium-dependent. As previous studies with cortical slices showed a substantial reduction in release of labelled adenosine or its derivatives (Lloyd & Stone, 1981) with verapamil, this suggests the former explanation is more likely. In addition, Jhamandas & Dumbrille (1980) showed calcium-dependency of glutamate-evoked release only when calcium-free medium *and* the addition of EGTA was employed, presumably reflecting the difficulty of depleting calcium in an *in vivo* system.

Our results add further support for APH being a selective antagonist at the NMDA receptor (Perkins *et al.*, 1981). The lack of effect on the responses to kainic acid and quisqualate is compatible with the view that kainate and quisqualate preferentially activate receptors other than those preferring NMDLA as an agonist. At 500 μM neither quisqualate not kainate showed any reduction at all with APH; indeed there was a slight increase with kainate. With glutamate there was a reduction in the presence of APH but this was not significant. This is probably a reflection of the mixed agonist action of glutamate (Watkins & Evans 1981), involving some activation of NMDLA- as well as quisqualate-preferring receptors. The significant reduction of responses to 100 μM kainate by APH at a concentration tenfold that of the agonist shows that at relatively high concentrations APH will block other excitatory compounds as has been observed in iontophoretic studies (Perkins *et al.*, 1981). The substantial reduction of responses to 1 mM and 5 mM NMDLA by 1 mM APH are in full agreement with previous work showing APH to be an effective NMDA antagonist.

Of particular interest was the 57% decrease in 500 μM quinolinate-evoked release of radiolabel by 1 mM APH. It adds further support to the suggestion that quinolinate is acting on NMDA-preferring receptors (Stone & Perkins 1981). In addition, the potency of quinolinate compared to NMDLA or glutamate in these experiments strengthens, in out view, the possibility that this naturally occurring metabolite of tryptophan may be of physiological importance in neuronal transmission.

These results also confirm the previous observation that kynurenic acid is an effective antagonist of quinolinic acid and NMDLA (Perkins & Stone, 1982). However, in this situation kynurenic acid did not significantly reduce the response to glutamate. As noted above, though, in this study glutamate was quite weak as an enhancer of the release of labelled adenosine whereas in a previous iontophoretic study it was slightly more effective than quinolinic acid in causing neuronal excitation (Perkins & Stone, 1983b). Caution must be taken, therefore, in comparing the results of such studies. Another explanation for the low potency of glutamate could be that it is taken up by the tissue such that there is much lower effective concentration than postulated. However, Jhamandhas & Dumbrille (1980) showed a clear response of cortical cups to glutamate using a similar volume which suggests that the reasons for this discrepancy lie elsewhere.

The lack of a similar agonist effect of kynurenic acid to that produced by quinolinate is consistent with the observation that convulsant activity is only evoked by quinolinic acid in the rat, kynurenic acid being inactive in this species (Lapin *et al* 1982).

Presumably, as in the iontophoretic studies (Perkins & Stone, 1982) only quinolinic acid is acting as a depolarizing agent and this direct excitatory action may be the reason for its convulsant properties. It should be noted here that the relationship between amino acid evoked depolarization and the release of adenosine is unclear. Indeed the physiological significance of the release of adenosine following amino-acid evoked depolarization of cells is obscure. Adenosine is an inhibitor of neuronal activity and, although speculative, it is possible that this compound is responsible for the post-excitatory depression of activity that has been reported to occur following amino-acid evoked depolarization of neurones (Peet, Malik & Curtis 1983). The ability of kynurenic acid and APH to antagonize both the depolarizing and adenosine releasing actions of quinolinic acid suggests a fairly intimate connection between these two events.

Thus the present results extend the evidence showing a similarity between the actions of quinolinate and NMDLA giving further support to the idea (Stone & Perkins, 1981, Perkins & Stone 1983a, 1983b) that quinolinic acid may have a role in the modulation of neuronal activity.

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