Biochemical Pharmacology, Vol. 35, No. 20, pp. 3631–3635, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

THE EFFECT OF KAINIC, QUINOLINIC AND β-KAINIC ACIDS ON THE RELEASE OF ENDOGENOUS AMINO ACIDS FROM RAT BRAIN SLICES

J. H. CONNICK and T. W. STONE

Dept. of Physiology, St. George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE, U.K.

(*Received* 11 *March* 1986; *accepted* 6 *May* 1986)

Abstract—It has been suggested that the neurotoxic properties of quinolinic acid and kainic acid may, at least in part, involve an indirect action on nerve terminals containing glutamate. In the present study it is confirmed that kainate causes the release of endogenous glutamate from rat hippocampal slices, but that quinolinic acid does not share this activity. In addition β-kainic acid was found to depress the potassium evoked release of endogenous glutamate at relatively high concentrations and this effect may underlie the anticonvulsant properties of this substance.

The receptors responsible for eliciting neuronal excitation due to glutamate and aspartate have been subdivided into three main types [1, 2]; those which respond preferentially to *N*-methyl-D,L-aspartate (NMDLA), quisqualic acid and kainic acid respectively. Quinolinic acid is an endogenous compound with relatively selective effects on the NMDLA receptor, or at least a proportion of them [3, 4].

Both quinolinate and kainate have been exploited in models of various human neuropathological conditions, such as Huntingtons disease and epilepsy, both amino acids being potent convulsants [5, 6], and both being able to produce neurotoxicity after intracerebral injections [7, 8].

The underlying mechanisms of these toxic effects are obscure. Whilst NMDLA and the related neurotoxin, ibotenate, are able to produce toxicity in rats of any age [9], quinolinic acid and kainate are ineffective in immature rats [7, 10], and in rats in which putatively glutamatergic afferents have been lesioned [11]. Thus it would appear that either glutamate, aspartate or some unknown factor associated with such afferent pathways is required for the degeneration produced by quinolinic and kainic acids. To date, however, a direct stimulant effect on amino acid release has only been demonstrated for kainic acid [12]. Kainate has also been shown to inhibit the reuptake mechanisms for glutamate and aspartate [13], whereas quinolinic acid has no effect [14].

The release of endogenous amino acids has also been proposed as an important factor in the spread and generalization of neuronal excitation seen during epileptic seizures [15]. The analogue of kainic acid with the C2 carbon in the D-configuration, β-kainic acid, has been shown to act as an anticonvulsant [16, 17]. This action does not appear to be due to a blockade of the postsynaptic receptor for NMDLA, quisqualate or kainic acid on cortical or hippocampal neurones [18]. This raises the possibility that β-kainate might produce its anticonvulsant action by a presynaptic mechanism, such as a depression of excitatory amino acid release. The present study was therefore designed to examine the effects of quinolinate, kainate and β-kainic acid on the release of endogenous amino acids from rat brain slices. A sensitive HPLC technique has been used to examine the release of a range of endogenous amino acids and to avoid the interpretative problems associated with the use of radiolabelled compounds [19].

MATERIALS AND METHODS

Male Wistar rats (200–250 g) were decapitated and their brains rapidly removed into ice-cold oxygenated buffer (KH_2PO_4 2.2 mM; $MgSO_4$ 1.2 mM; KCl 2.0 mM; Glucose 10.0 mM; $NaHCO_3$ 25.0 mM; NaCl 115.0 mM; $CaCl_2$ 2.5 mM). Hippocampal slices (400 μm thick) were prepared using a McIlwain tissue chopper and preincubated for 30 min in 100 ml buffer gassed with 95% O_2–5% CO_2 at 37° in order to allow slices to recover and achieve a stable level of amino acid efflux. Release of amino acids was determined by placing single slices into vials containing 1 ml oxygenated buffer for 15 min at 37°. Depolarization was produced by incubation with the compound of interest or in the presence of 44 mM potassium, an equivalent reduction of sodium concentration being made in the latter case, to maintain isotonicity. The calcium dependency of aspartate and glutamate efflux was investigated by incubating slices as above, calcium ions having been replaced by 1 mM ethyleneglycol-bis (β-amino ethyl ether) *N*,*N*′-tetra-acetic acid (EGTA). The effect of magnesium ions on the stimulation of glutamate release produced by quinolinic acid and NMDLA was investigated by omitting this ion from the incubation medium. Aliquots of medium were then removed and frozen at −20°. Tissue slices were homogenized and the protein concentration measured by the method of Lowry *et al.* [20] with bovine serum albumin as standard.

Aliquots (100 μl) of each sample were rapidly

mixed with 10 μl of an *o*-phthalaldehyde/2-mercaptoethanol reagent (Pierce, U.K.) and allowed to stand for 30 sec before injection onto the chromatographic column. Analysis was performed using a gradient HPLC system (Gilson UK) and derivatized amino acids were detected with a Gilson Spectra/Glo fluorimeter; excitation wavelength 390 nm, emission cut off filter at 475 nm. Injections were made using a Rheodyne valve fitted with a 20 μl loop. Separation was performed on a reverse phase "μ-Bondapac" C_{18} analytical column (Waters, U.K.) fitted with a C_{18} guard column. The solvents used were a modification of those used by Turnell and Cooper [21]. Solvent A contained 12.5 mM Na_2HPO_4/8% Acetonitrile in aqueous solution; Solvent B contained water, methanol and acetonitrile (4:3:3). The gradient program expressed as time in minutes from injection (% solvent B) was: 0(0); 10(0); 30(100). The flow rate was 1.5 ml/min at room temperature. Derivatized amino acid concentrations were quantified by peak height relative to an external standard mixture.

RESULTS

A number of amino acids were separated sufficiently well by the analytical system used here to allow good quantification of their efflux. These included aspartate, glutamate, γ-aminobutyric acid (GABA), serine, glutamine, glycine, alanine and taurine. Depolarization induced by high potassium tended to increase the efflux of all amino acids to some degree, although this was only significant in the cases of aspartate, glutamate, glycine and GABA (Table 1 and Fig. 1).

The calcium dependency of both glutamate and aspartate efflux was examined by chelation of calcium with EGTA. The ability of both 44 mM potassium and 5 mM kainic acid to stimulate the release of both these compounds was markedly attenuated in the absence of calcium (Table 1). The calcium dependency of efflux of other amino acids was not determined.

Potassium failed to increase significantly aspartate efflux in the absence of calcium, whilst the potassium stimulated release of glutamate was reduced by 53.3% ($P < 0.05$) in the absence of calcium. Removal of calcium did not effect the basal release of either amino acid.

Kainic acid (5 mM) was similarly ineffective in eliciting a significant release of aspartate in the absence of calcium. Glutamate efflux produced by 5 mM kainate was reduced to 47.4% of that produced in the presence of calcium, although the amount released was still greater than control in the absence of calcium.

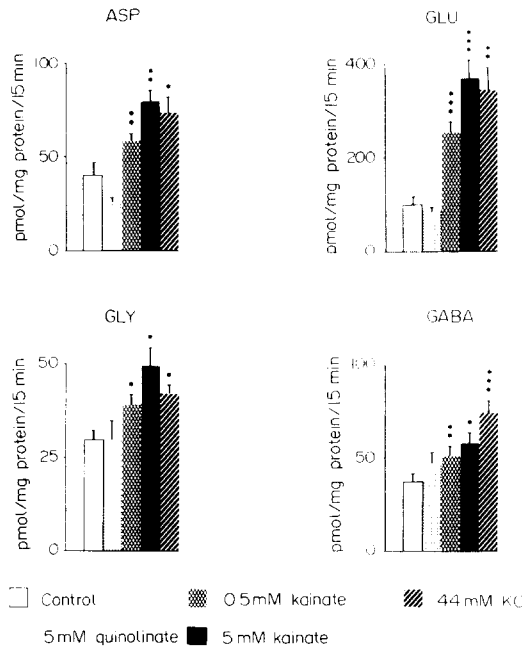

Fig. 1. Effect of 0.5 mM and 5 mM kainate, 5 mM quinolinic acid and 44 mM potassium on the release of transmitter amino acids from rat hippocampal slices. Results are expressed as amount of amino acid released (pmol/mg protein/15 min) ± SEM from 11 animals. Statistical significance of the release was assessed using a Students *t*-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

At a concentration of 0.5 mM, kainic acid caused a significant increase in efflux of aspartate, glutamate, GABA and glycine summarized in Fig. 1. In the case of 5 mM kainate the increase was comparable with that elicited by 44 mM potassium in the case of glutamate, aspartate and glycine. The release of GABA produced by 5 mM kainic acid was significantly less than that produced by potassium ($P < 0.05$).

Quinolinic acid had no detectable effect on the release of any of the amino acids when studied at concentrations of up to 5 mM (Fig. 1). Omission of

Table 1. The effect of calcium on aspartate and glutamate efflux

		Control	44 mM KCl	5 mM Kainate
ASP	$+Ca^{2+}$	35.1 ± 4.6	45.0 ± 4.9	36.2 ± 8.3
	$-Ca^{2+}$	39.0 ± 7.4	72.4 ± 7.8†	78.0 ± 6.4†
GLU	$+Ca^{2+}$	152.3 ± 5.7	215.0 ± 9.9*	248.0 ± 15.0*
	$-Ca^{2+}$	138.0 ± 15.8	460.7 ± 36.6†	472.3 ± 41.0†

Results are expressed as mean (pmol/mg protein/15 min) ± SEM from six animals.

* Represents the statistical difference ($P < 0.05$) relative to control release in the absence of calcium.

† Represents the statistical difference ($P < 0.05$) relative to control release in the presence of calcium, and control and stimulated release in the absence of calcium.

Statistical significance was assessed using a Student's *t*-test.

Table 2. The effect of magnesium on glutamate efflux

Condition	$-Mg^{2+}$	$+Mg^{2+}$
Control	123.5 ± 6.7	130.6 ± 10.8
44 mM KCl	501.7 ± 15.3*	531.5 ± 40.3*
5 mM Quinolinate	130.7 ± 5.1	141.2 ± 7.8
5 mM NMDLA	142.0 ± 9.3	132.4 ± 12.5

Results are expressed as mean (pmol/mg protein/15 min) ± SEM from four animals.

* Represents the statistical difference ($P < 0.05$) between control and stimulated release in the presence and absence of magnesium. Statistical significance was assessed using a Student's *t*-test.

magnesium from the incubation medium, a procedure found to be essential for the demonstration of acetylcholine release by quinolinate [22], had no apparent effect on efflux in the presence or absence of quinolinate (Table 2). NMDLA (1 mM) was similarly ineffective in inducing amino acid release either in the presence or absence of magnesium (Table 2).

At a concentration of 250 μM β-kainate significantly potentiated the potassium stimulated efflux of glutamate (Fig. 3), although it was without effect on control glutamate release or on the release of other amino acids (Fig. 2). At a concentration of 1 mM, however, β-kainate significantly diminished the potassium stimulated release of glutamate by 49.0% with no effect on the efflux of glycine or GABA (Figs 2 and 3). Potassium induced aspartate efflux also appeared to be reduced by β-kainate at 1 mM but this was not significant (Fig. 3).

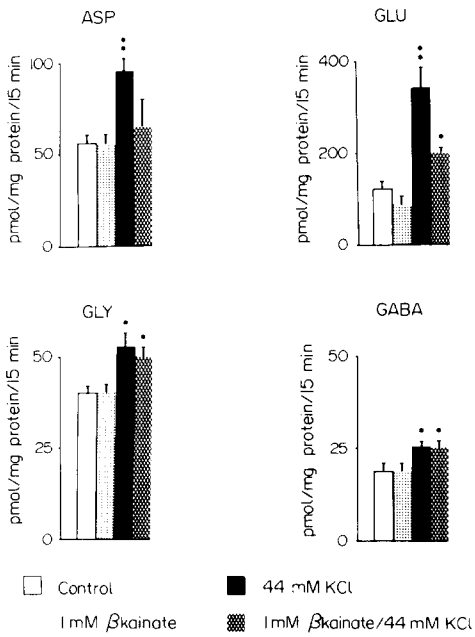

Fig. 2. Effect of 1 mM β-kainic acid on the resting and potassium stimulated release of transmitter amino acids from rat hippocampal slices. Results are expressed as amount of amino acid released (pmol/mg protein/15 min) ± SEM from 10 animals. Statistical significance of the release assessed using a Student's *t*-test: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

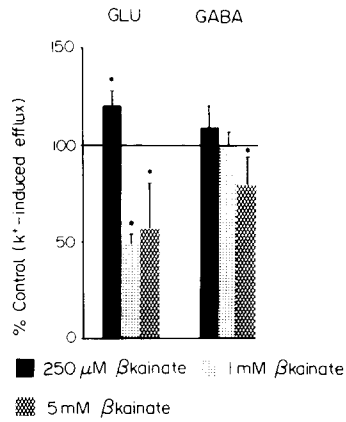

Fig. 3. Effect of concentration of β-kainate on the potassium evoked release of glutamate and GABA from rat hippocampal slices. Results are expressed as mean % potassium induced release ± SEM from between 3 and 8 animals. Statistical significance of the release was assessed using a Student's *t*-test: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

When tested at 5 mM, β-kainate produced a significant reduction of the potassium induced release of GABA in addition to its action on glutamate efflux.

DISCUSSION

Both kainate and quinolinic acid share a number of similarities as neurotoxins [24]. Notably, they are both incapable of exerting their toxic effects in neonatal animals [7, 10] or adult rats in which certain afferent projections are absent [11]. In contrast the related compounds NMDLA and ibotenate are potent neurotoxins in animals of all ages [9]. The implications of this observation for the mechanism by which degeneration is mediated have been extensively discussed in the literature [25], one proposed explanation being that kainate and quinolinate cause the release of some compound or compounds from the afferent projections which act either alone or in an additive manner with the neurotoxins to produce neuronal degeneration [9, 11]. Evidence suggests that glutamate is the most likely contributor to kainate toxicity [26]. This model is supported by the finding that kainic acid stimulates the release of endogenous glutamate from both brain slices [12, 19] and synaptosome preparations [27]. Whilst these various authors found that only endogenous glutamate and not radiolabelled, preloaded D-aspartate were released, Pastuszko *et al.* [28] found that D-aspartate was released in parallel with endogenous aspartate and glutamate. The source of the released glutamate is therefore unclear, Pastuszko *et al.* have suggested that the reason for the apparent difference in release patterns between the endogenous and exogenous amino acids might be due to glial activity in the brain slice preparation, but this is unlikely to explain the results obtained by Poli *et al.* using synaptosomes.

The calcium dependency of amino acid release is suggestive of a neuronal origin, though glial con-

tamination, which is present in all preparations means that this cannot be used as an absolute indicator of neuronal origin. Calcium dependency may, however, be of some use in differentiating between the true stimulation of release of transmitter or the blockade of reuptake of that transmitter.

Omission of calcium has no effect on the uptake of ^{3}H-D-aspartate (Connick and Stone, unpublished observations). This is of particular importance in the case of kainate, which has been shown to be a moderately good inhibitor of glutamate uptake [13]. Since kainate stimulated glutamate release is reduced by 47.4% in the absence of calcium (Table 1), this implies that at least 47.4% of the glutamate efflux produced by 5 mM kainate is a true release and is not due to an inhibition of reuptake. The remaining 52.6% consists of a mixture of calcium independent release and the inhibition of reuptake by 5 mM kainate. The glutamate release caused by 5 mM kainic acid (Fig. 1) may therefore be considered as having a minimum value represented by the calcium dependent component.

Whilst 5 mM kainic acid may be considered to be a comparatively high dose, it is typical of doses injected in order to produce neuronal degeneration [29]. Nicklas has suggested that this might be diluted 10–100-fold when injected focally in brain [30], and as kainate is not actively accumulated by brain [13], it is reasonable to assume that concentrations on the range of 0.1–1 mM may be found under pathological conditions.

Schwarcz *et al.* [32] have noted in comparing kainic and quinolinic acids that quinolinic acid is toxic in the range of 5–50 μg compared with 0.5–2 μg for kainic acid. It might therefore be supposed that a dose ratio of around 10 should pertain for those mechanisms involved in neurotoxicity. Since quinolinate, like kainate is not taken up by brain [31], it is reasonable to compare 0.5 mM kainate with 5 mM quinolinate.

However, whereas 0.5 mM kainate showed a clear stimulatory effect on glutamate and aspartate release, neither quinolinic acid nor NMDLA was able to modify the release of endogenous glutamate or aspartate at 5 mM, even in the absence of magnesium ions, which are known to block the activation of NMDLA receptor mechanisms [22, 33]. It seems unlikely, therefore, that these amino acids are important in mediating quinolinate neurotoxicity and therefore that quinolinate differs from kainate in its neurotoxic mechanism. Since acetylcholine [34] and serotonin [35] have also been proposed as possible candidates in this role, it is possible that these or related compounds present in the presynaptic terminals may contribute to the degenerative process.

The reduction of the potassium induced glutamate (and aspartate) efflux by β-kainate at 1 mM may suggest a mechanism for its anticonvulsant properties [16]. It is established that some anticonvulsants may act by preventing the spread and generalization of excitation found during an epileptic discharge [15]. It is, therefore, probable that any drug tending to reduce the release of an excitatory transmitter, such as glutamate, would display anticonvulsant properties.

In order to produce reliable anticonvulsant effects in mice, Collins *et al.* [16] needed to inject at least 10 μl of 0.33 μmol β-kainate (i.e. 33 mM) into the cerebral ventricles, 1.0 μmol (i.e. 100 mM) being necessary to abolish both the tonic and clonic phases of the seizure. This concentration would undoubtedly be reduced by dilution in the CSF and diffusion from the injection site to an even greater extent than that discussed in the case of focal injections. The concentration of 1 mM β-kainate found in the present study to be an effective depressant of glutamate release cannot therefore be considered high relative to those found effective in behavioral experiments.

Consistent with the present demonstration of reduced glutamate release, β-kainate has been found to be significantly more active in depressing orthodromically evoked potentials as compared with antidromic potentials in the CA1 region of the hippocampus, (Stone, unpublished observations). The favoured candidate as a transmitter in the Schaffer collateral–commissural input to this hippocampal region is L-glutamate; NMDLA receptors are thought not to be of importance in the mediation of synaptic potentials in this pathway [36].

Although an inhibition of glutamate and aspartate release may adequately explain the ability of β-kainate to prevent seizure activity, it is not immediately clear why the pharmacological profile of its anticonvulsant activity is that of an NMDLA antagonist [17]. It has been noted elsewhere that β-kainate is not an antagonist at post-junctional NMDLA receptors [18]. One implication of the present work may therefore be that endogenous glutamate or aspartate, released during seizure activity, can contribute significantly to the seizure process by acting on NMDLA receptors. It is alternatively possible that β-kainate is depressing the efflux of other excitatory amino acids which cannot be detected by the HPLC system used here, but which act at the NMDLA receptor, such as quinolinic acid [37] or homocysteic acid [38].

Acknowledgements—We are grateful to the Wellcome Trust and National Fund for Research into Crippling Diseases for grant support.

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