Br. J. Pharmacol. (1988), **95**, 131–138

Stimulatory and inhibitory actions of excitatory amino acids on inositol phospholipid metabolism in rat cerebral cortex

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1 The effects of excitatory amino acids on [^{3}H]-inositol phosphate levels have been examined in rat cortical slices under basal conditions or following agonist stimulation.

2 Ibotenate and quisqualate provoked a substantial dose-dependent (EC_{50}, 30 μM and 20 μM respectively) increase in inositol phosphates; these responses were not additive suggesting a common site of action for the two amino acids. The responses to maximally effective concentrations of ibotenate and quisqualate were not blocked by verapamil, tetrodotoxin or Cd^{2+}, indicating that these effects are not indirect. Small, but significant, increases in inositol phosphates were also seen with glutamate and N-methyl-DL-aspartate (NMDLA); kainate and aspartate were ineffective.

3 Each excitatory amino acid tested reduced carbachol (1 mM) stimulated inositol phosphate formation. Kainate (IC_{50}, 20 μM) and NMDLA (IC_{50}, 20 μM) were the most effective inhibitors. Kainate also reduced the responses to noradrenaline, 5-hydroxytryptamine and 20 mM K^+.

4 The inhibitory action of NMDLA, but not kainate, could be reversed with the NMDA antagonists, DL-2-amino-5-phosphonovalerate (APV) and MK-801; DL-2-amino-4-phosphonobutyrate (APB) was without effect. Since MK-801 blocks the ion channels associated with the NMDA receptor, it appears that inhibition requires the entry of ions into the cell.

5 APV and MK-801 potentiated the stimulatory response to ibotenate but had no effect on the response to quisqualate. Potentiation was presumably the result of blocking the inhibition by ibotenate mediated through NMDA receptors.

6 In conclusion, excitatory amino acids appear to reduce agonist-mediated inositol phosphate formation in rat cerebral cortex by a non-specific action, possibly including the influx of Na^+ ions. In addition ibotenate and quisqualate substantially enhance inositol phosphate production: the pharmacology of the response suggests that it is mediated by a receptor distinct from previously defined excitatory amino acid receptor subtypes.

Introduction

It has now become established that breakdown of inositol phospholipids is a major signal transduction system in a wide variety of tissues including the central nervous system (Downes & Michell, 1985; Nahorski *et al.*, 1986; Fisher & Agranoff, 1987). In brain, stimulation of muscarinic cholinoceptors (Jacobson *et al.*, 1985), α_1-adrenoceptors (Kendall *et al.*, 1985; Minneman & Johnson, 1984), histamine-H_1-, (Donaldson & Hill, 1986) and 5-hydroxytryptamine- (Conn & Sanders-Bush, 1985; Godfrey *et al.*, 1988) receptors have all been shown to enhance phosphoinositide turnover. It has recently become apparent that excitatory amino acid (EAA) agonists can also enhance the production of inositol phosphates in rat hippocampus (Nicoletti *et al.*, 1986a,b), and primary cultures of striatal neurones (Sladeczek *et al.*, 1985) or cerebellar granule cells (Nicoletti *et al.*, 1987). In addition to these stimulatory effects, an inhibitory action of EAAs against agonist-stimulated inositol phospholipid breakdown has been observed in rat hippocampus (Baudry *et al.*, 1986; Nicoletti *et al.*, 1986a) and mouse striatal neurones (Schmidt *et al.*, 1987).

On the basis of electrophysiological findings, using the selective agonists N-methyl-D-aspartate (NMDA), kainate and quisqualate, excitatory amino

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acid receptors have been subdivided into three classes (Foster & Fagg, 1984; Watkins & Olverman, 1987). The kainate and quisqualate subtypes mediate fast excitatory ionic reponses carried by Na^+ ions whilst activation of NMDA receptors induces ionic changes that are longer in duration and involve movement of Ca^{2+} ions in addition to Na^+ (Cotman & Iversen, 1987). The activation of NMDA receptors can be selectively inhibited by the competitive antagonists DL-2-amino-5-phosphonovalerate (APV) and 3-(2-carboxypiperazine-4-yl)propyl- phosphonic acid (CPP) and the non-competitive antagonists MK-801 and phencyclidine (Foster & Fagg, 1984; Cotman & Iversen, 1987). Currently there are no selective antagonists of the kainate or quisqualate receptor subtypes. The EAA receptor that enhances inositol phospholipid turnover appears to have a pharmacological profile distinct from the subtypes defined above. Both quisqualate and ibotenate (a NMDA-selective agonist) are potent agonists while glutamate is less efficacious and kainate and NMDA are weak or ineffective (Sladeczek *et al.*, 1985; Nicoletti *et al.*, 1986b; Sugiyama *et al.*, 1987).

We show here that in slices of rat cerebral cortex, EAA agonists both enhance inositol phosphate production and inhibit the responses produced by other agonists. Some of this work has been presented to the British Pharmacological Society (Tyler *et al.*, 1988; Wilkins *et al.*, 1988).

Methods

Male Sprague-Dawley rats (weight 200–300 g) were used for all experiments. The rats were decapitated and the brain rapidly removed. The cortex was dissected out and cross-chopped slices (350 × 350 μm) were prepared with a McIlwain tissue chopper. The slices were then dispersed in 25 ml of Krebs Ringer HEPES buffer (composition mM: NaCl 150, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 1.2, Na_2HPO_4 1.2, HEPES 20 and glucose 2 mg ml^{-1}; pH 7.4) equilibrated with 100% O_2 at room temperature, or Krebs-bicarbonate buffer (composition mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, K_2HPO_4 1.2 and glucose 10) equilibrated with 95% O_2 : 5% CO_2; similar results were obtained in either medium.

The slices were washed with 100 ml of warmed buffer and then preincubated for 40 min at 37°C in a shaking waterbath with a change of buffer after 20 min. Following preincubation the slices were labelled with 5 μCi of [^{3}H]-inositol (specific activity 45–60 Ci $mmol^{-1}$) for 45 min in 5 ml buffer in a stoppered 25 ml flask, having been gassed with O_2 or 95% O_2 : 5% CO_2. The slices were then washed with 50 ml of buffer containing 10 mM LiCl (replacing NaCl) and allowed to settle under gravity. Fifty microlitre aliquots of the packed slices were then transferred into flat-bottomed tubes containing 250 μl of buffer, LiCl (final concentration 10 mM) and, where necessary, EAA antagonists. Agonists (10 μl) were added 10 min later, and the tubes were gassed, capped and transferred to a shaking water bath at 37°C for 30 min. In experiments investigating the inhibitory action of amino acids, the EAA was added 5 min after the tissue and the agonist after a further 10 min. For experiments with 5-hydroxytryptamine or noradrenaline, pargyline (10 μM) was also included in the buffer. When total [^{3}H]-inositol phosphates were determined the incubations were terminated by addition of 940 μl of chloroform/methanol/HCl (100 : 200 : 2); 310 μl of chloroform and 310 μl of water were then added to split the sample into two phases. Following vortexing and centrifugation (1000 *g* for 5 min) 750 μl of the upper phase was removed for analysis of inositol phosphates by Dowex anion exchange chromatography as described previously (Godfrey *et al.*, 1985). To estimate incorporation of radioactivity into phospholipids, 100 μl of the lower phase was removed, dried and counted after the addition of 5 ml of Instagel scintillation fluid (Packard Corp.).

A maximally effective concentration of carbachol (1 mM) was included in all experiments and results have been expressed relative to this. Statistical analysis of the data was performed using the unpaired Student's *t* test or the Wilcoxon rank order test.

Materials

[^{3}H]-myo-inositol was obtained from Amersham International (Amersham, U.K.), MK-801 (±)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate) and N-methyl-DL-aspartate (NMDLA) were kindly supplied by Merck Sharp and Dohme, all other drugs and fine chemicals were from Sigma (Poole, Dorset).

All the EAA were dissolved in buffer and the pH of the resultant solution were checked. Concentrations of glutamate above 1 mM significantly reduced the pH below 7.4 and this was corrected before use.

Results

Action of excitatory amino acids on resting and carbachol-stimulated inositol phosphates

All of the excitatory amino acids used in this study, including kainate, NMDLA and quisqualate, inhibited carbachol-induced formation of inositol phosphates (Table 1). The maximal extent of inhibition

Table 1 Action of excitatory amino acids on resting and carbachol-stimulated levels of inositol phosphates

EAA	[ ] (mM)	*Resting IPs* (% Carb max)	*Carbachol IPs* (% Carb max)
—	—	0 ± 1.8	100 ± 10
Aspartate	1	3.9 ± 2.4	—
Glutamate	1	12.0 ± 2.4*	46.3 ± 4.2***
Kainate	1	−2.3 ± 1.1	17.8 ± 4.0***
NMDLA	1	6.4 ± 1.0*	16.8 ± 3.4***
Ibotenate	0.5	34.4 ± 4.3**	33.6 ± 4.0***
Quisqualate	0.3	41.9 ± 1.7**	51.5 ± 5.0***

Rat cortical slices were labelled with [^{3}H]-inositol as described in the methods. Results are expressed as % increase in [^{3}H]-inositol phosphates relative to a maximally effective concentration of carbachol (1 mM; Carb max); the results are shown as means ± s.e.mean from 3–8 experiments performed in triplicate. The resting levels of inositol phosphates (IP) was 3453 ± 131 d.p.m. and carbachol (1 mM) increased their levels by 331 ± 31% during a 30 min incubation. The excitatory amino acids (EAA) were given either for a period of 30 min (column 1) or 10 min before the addition of carbachol (column 2). *$P < 0.05$; **$P < 0.01$, EAA compared with basal; ***$P < 0.01$, carbachol plus EAA compared with carbachol alone; statistical indications estimated by Wilcoxon rank test.

was amino acid- and concentration-dependent; maximally effective concentrations of kainate, glutamate and NMDLA inhibited the response to carbachol by more than 80%; quisqualate and ibotenate were less effective (Table 1; Figure 1a). Full dose-response curves for the inhibitory actions of glutamate, NMDLA and kainate are shown in Figure 1a; the IC_{50} for each agonist was approximately 1 mM, 20 μM and 20 μM respectively. Responses to NMDLA were not altered by the omission of Mg^{2+} from the Krebs buffer (not shown).

In the absence of carbachol a number of the excitatory amino acids caused a significant increase in inositol phosphate production (Table 1). The two most effective amino acids were ibotenate (0.5 mM) and quisqualate (0.3 mM) producing 33 and 41% respectively of the response to carbachol during a 30 min incubation (Table 1). The responses to maximally effective concentrations of ibotenate and quisqualate were not additive. Ibotenate (0.5 mM) increased the levels of inositol phosphates by 79 ± 5.2% above basal during a 30 min incubation and quisqualate (0.3 mM) increased them by 127 ± 9.6%; when given together, the response to ibotenate (0.5 mM) and quisqualate (0.3 mM) was 81 ± 6.7%. The results suggest that these agonists are acting at the same recognition site. The differences between the maximal responses to ibotenate and quisqualate may be due to ibotenate having a more powerful inhibitory action (Table 1).

Glutamate and NMDLA also caused a significant, although much smaller increase in inositol phosphate formation, while kainate and aspartate were inactive (Table 1). In fact, if anything, kainate decreased the resting level of inositol phosphates although this did not reach statistical significance. Full dose-response curves for the production of inositol phosphates by ibotenate, quisqualate and glutamate are shown in Figure 1b; the EC_{50} for each agonist was approximately 30 μM, 20 μM and 1 mM respectively.

Characterization of the inhibitory action of the amino acids

The specificity of the inhibitory action was investigated by studying the action of kainate on the formation of inositol phosphates induced by noradrenaline, 5-hydroxytryptamine and 20 mM K^+. Kainate was chosen for this study since it did not affect basal inositol phosphate levels. As shown in Table 2, kainate partially inhibited the formation of inositol phosphates by all three stimuli although the magnitude of this effect varied with the stimulus. For example kainate completely inhibited the formation of inositol phosphates induced by K^+ but only decreased the noradrenaline response by less than 50%. Similar results were observed with 100 μM NMDLA (not shown). This inhibitory action of kainate was not accompanied by any significant change in the amount of [^{3}H]-inositol radioactivity present in any of the individual inositol phospholipids, either in the absence or presence of carbachol (1 mM).

In order to examine the pharmacology of this inhibitory action the effect of several antagonists on the reponses to NMDLA and kainate were investigated. The NMDA receptor antagonists, APV and MK-801, partially reversed ($P < 0.01$) the inhibitory action of NMDLA but had no effect on the response to kainic acid (Table 3); 2-amino-4-phosphobutyrate (APB) did not antagonise either response (Table 3).

Characterization of the stimulatory actions of the amino acids

In order to rule out that the increase in inositol phosphates induced by ibotenate or quisqualate was not an indirect effect mediated, for example, through the release of neurotransmitters or the activation of phospholipase C by Ca^{2+}, the action of Cd^{2+}, verapamil and tetrodotoxin on the formation of inositol phosphates by these two agonists was investigated.

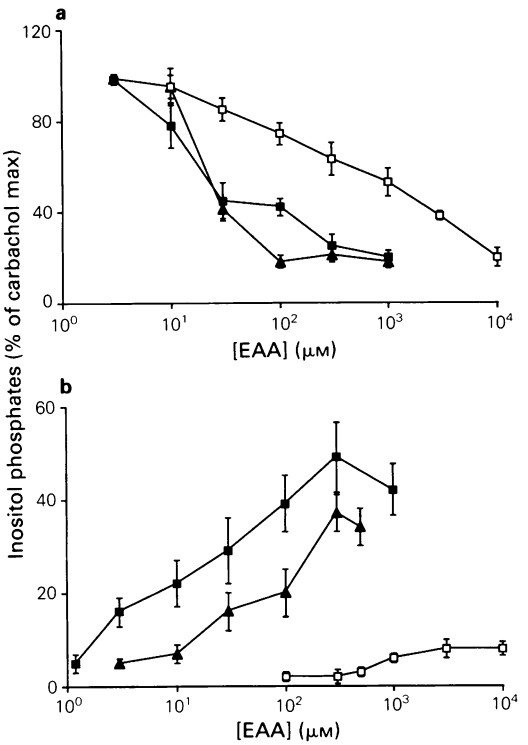

Figure 1 Dose-response curves showing excitatory and inhibitory actions of excitatory amino acids (EAAs) in rat cortical slices. Slices, labelled with [^{3}H]-inositol, were stimulated with the EAA in the presence (a) or absence (b) of 1 mM carbachol. Results are expressed as a % increase in inositol phosphates relative to that produced by 1 mM carbachol. The data are means from 3–5 experiments performed in triplicate with s.e.mean shown by vertical bars. Basal inositol phosphate levels were 3453 ± 131 d.p.m. and carbachol increased this by 331 ± 31% during a 30 min incubation. In (a): (□) glutamate; (■) kainate; (▲) NMDLA; in (b): (□) glutamate; (▲) ibotenate; (■) quisqualate.

Figure 2 shows that neither Cd^{2+}, verapamil or tetrodotoxin had any significant effect on the production of inositol phosphates induced by ibotenate. Similar results were observed for quisqualate (not shown). The small response to glutamate (1 mM) was, however, blocked by 300 nM tetrodotoxin (not shown).

The pharmacology of this excitatory action was further examined by studying the effect of several EAA receptor antagonists on the response to ibotenate and quisqualate. Neither APV or MK-801 had any significant affect on quisqualate-induced formation of inositol phosphates (Figure 3). In contrast, MK-801 and APV caused a significant ($P < 0.01$) potentiation of the formation of inositol phosphates induced by ibotenate (Figure 3). Neither 1 mM APB nor 100 μM kynurenic acid (a non-selective antagonist, Watkins & Olverman, 1987) had a significant effect on the ibotenate or quisqualate responses (data not shown).

Table 2 Kainate inhibits agonist-induced formation of inositol phosphates

Agonist	[ ] (mM)	*Inositol phosphates* (% basal) *No addition*	*Kainate*
Carbachol	1	512 ± 46	201 ± 26**
Noradrenaline	0.3	268 ± 22	195 ± 11*
5-HT	0.1	231 ± 13	121 ± 7.8**
K^+	20	164 ± 8.0	82 ± 8.9**

Rat cortical slices were prelabelled with [^{3}H]-inositol as described in the methods. Results are shown as % increase in inositol phosphates above basal during a 30 min incubation and represent the means ± s.e.mean from three experiments performed in triplicate. Kainic acid (100 μM) was given 10 min before the addition of agonist. *$P < 0.05$; **$P < 0.01$, kainic acid plus agonist compared with agonist alone; statistical indications estimated using Student's t test. 5-HT = 5-hydroxytryptamine.

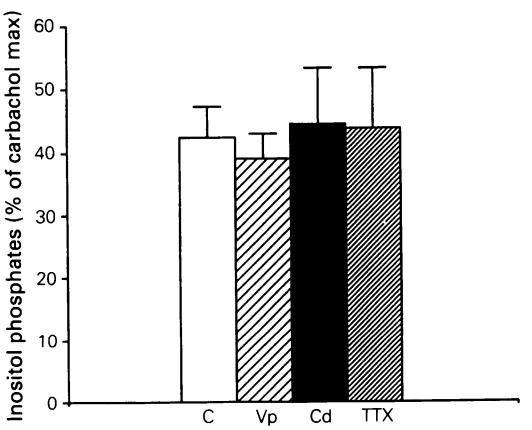

Figure 2 The actions of ion channel blockers on ibotenate-mediated production of inositol phosphates. Cortical slices prelabelled with [^{3}H]-inositol were stimulated with ibotenate (200 μM) for 30 min in the absence (C) or presence of verapamil (Vp 10 μM), cadmium ions (Cd 300 μM) or tetrodotoxin (TTX, 300 nM). The data are means from 3 experiments performed in triplicate (s.e. mean shown by vertical bars) and are expressed as a % of the response to 1 mM carbachol which increased inositol phosphate levels by 325 ± 37% over basal.

Table 3 Effects of amino acid antagonists on the inhibitory actions of kainic acid or N-methyl-D,L-aspartate (NMDLA)

Agonist	*Control*	*APB* (1 mM)	*APV* (1 mM)	*MK-801* (100 μM)
—	100 ± 5.8	102 ± 5.0	87.5 ± 6.5	132 ± 6.0
Carbachol	431 ± 33	352 ± 23	326 ± 20	360 ± 21
Carbachol + kainate	221 ± 17	158 ± 11	183 ± 11	209 ± 25
Carbachol + NMDLA	181 ± 15	165 ± 6.5	314 ± 27**	314 ± 33**

Rat cortical slices were prelabelled with [^{3}H]-inositol as described in the methods. Results are shown as % increase in inositol phosphates above basal during a 30 min incubation and represent the means ± s.e.mean from three experiments. D,L-2-Amino-5-phosphonovalerate (APV), D,L-2-amino-4-butyrate (APB) and MK-801 were given 5 min before kainate (100 μM) or N-methyl-D,L-aspartate (NMDLA; 100 μM); carbachol (1 mM) was added 10 min after the excitatory amino acid (EAA). **$P < 0.01$, carbachol and EAA compared with carbachol, EAA and EAA antagonist; statistical comparisons were made by Student's t test.

Discussion

Inhibitory action

The present study has demonstrated that EAAs are able to inhibit agonist-stimulated formation of inositol phosphates in rat cortex. The pharmacology of this inhibitory action suggests that it is unlikely to be linked to the activation of a single, established sub-type of EAA receptor i.e. the kainate, NMDA or quisqualate receptor sub-types. It would therefore seem that this response is mediated either through a novel sub-type of receptor or that it is a general response brought about by the activation of all excitatory amino acid receptors. The weaker inhibitory effects of ibotenate and quisqualate relative to the other amino acids e.g. kainate, glutamate, NMDA can be explained by their ability to induce the formation of inositol phosphates on their own (see below).

The blockade of carbachol-stimulated inositol phosphate formation in rat cerebral cortex by NMDLA, kainate and glutamate is in good agreement with previous observations in rat hippocampus (Baudry *et al.*, 1986). The IC_{50} values for NMDLA (20 μM), kainate (20 μM) and glutamate (1–2 mM) are very similar in both preparations. The inhibition curve to glutamate is relatively shallow compared to those obtained with kainate or NMDLA. This could be due either to glutamate acting on more than one receptor or to an active reuptake of glutamate into the slices. A similar shallow curve for glutamate was also observed by Baudry *et al.* (1986). The relative resistance of the noradrenaline response to inhibition by EAAs has also been seen in striatal neurones (Schmidt *et al.*, 1987) and hippocampus (Baudry *et al.*, 1986; but see Nicoletti *et al.*, 1986a). The reasons for the differential sensitivity of the noradrenaline response is unknown but could be brought about if the α_1-adrenoceptor and EAA receptors are situated on separate neurones.

There appear to be two possible explanations for this inhibitory action. Firstly, by analogy with the mechanism of agonist-induced inhibition of adenylate cyclase activity, it is possible that this effect is mediated through a novel inhibitory G-protein which blocks the activation of phospholipase C. As yet, such a protein has not been identified. Alternatively, the reduction of inositol phosphate formation may be related to the neurotoxic action of the EAAs (Schmidt *et al.*, 1987). It is well known that prolonged stimulation of EAA receptors results in the death of a large number of central neurones caused by the influx of Na^+ and Ca^{2+} ions leading to neuronal swelling and cell death (Rothman & Olney, 1987). The results obtained in the present study argue in favour of the latter hypothesis for the following reasons:

(i) This inhibitory action could also be observed against high K^+-induced inositol phosphate formation. The mechanism whereby the elevation of the K^+ concentration induces the hydrolysis of inositol phospholipids is through the entrance of extracellular Ca^{2+} via voltage-operated channels (Kendall & Nahorski, 1985). Ca^{2+} is then thought to activate phospholipase C directly without the need for a G-protein (Cockcroft, 1987).

(ii) MK-801 and APV partially reverse the inhibitory effect of NMDLA on carbachol-induced formation of inositol phosphates. Although it is likely that APV is acting as a competitive antagonist at the NMDA receptor (Cotman & Iversen, 1987), MK-801 is thought to act by blocking the NMDA-associated ion channel whilst in its open state, rather than affecting the binding of NMDA to its receptor (Kemp *et al.*,

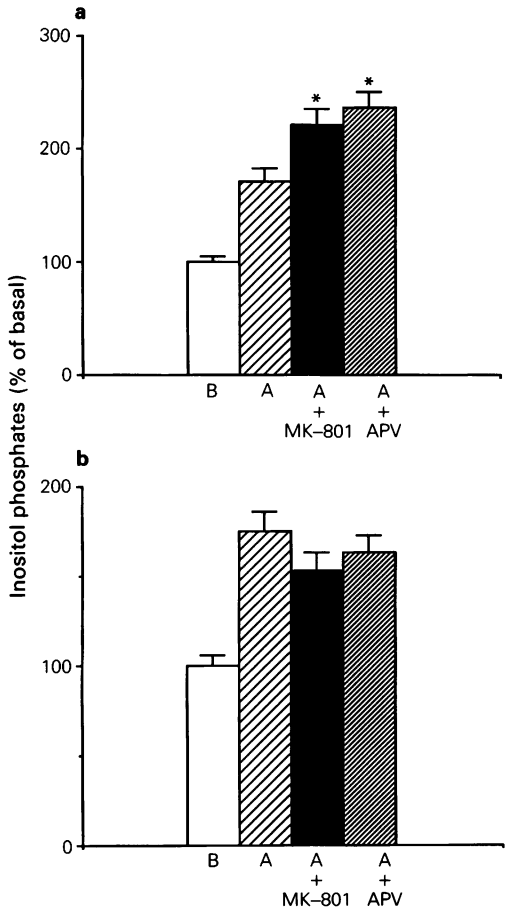

Figure 3 Effects of the excitatory amino acid antagonists 2-amino-5-phosphonovalerate (APV) and MK-801 on ibotenate-(a) and quisqualate (b)-stimulated production of inositol phosphates. Cortical slices prelabelled with [^{3}H]-inositol were stimulated with ibotenate (500 μM) or quisqualate (200 μM) for 30 min; APV or MK-801 were added 10 min before the agonist. Results are means of 3–4 experiments performed in triplicate with s.e.mean shown by vertical bars. B = basal; A = agonist alone; A + APV = agonist plus APV; A + MK-801 = agonist plus MK-801.

1987). Thus, the inhibitory action of NMDLA must, at least in part, be secondary to the opening of this channel rather than to a direct receptor-linked event. The partial reversal of the inhibitory action of NMDLA by MK-801 may be explained by the slow onset of action; binding of MK-801 requires prior opening of the channel (Foster & Wong, 1987; Kemp *et al.*, 1987).

The latter hypothesis is consistent with the suggestion of Baudry *et al.* (1986) that a build-up of intracellular Na^+ ions may be the cause of the inhibitory actions of the amino acids. They observed a good correspondence between the concentrations of amino acid needed for suppression of the PI responses and those required to promote Na^+ fluxes, and that removal of Na^+ ions prevented the inhibitory actions of the EAAs. Whether the inhibition of phospholipase C is a direct result of an elevation in intracellular sodium or is due to the onset of toxicity caused by Na^+ and Ca^{2+} influx requires further experiments.

Stimulatory actions

The ability of a number of the EAAs, particularly ibotenate and quisqualate, to stimulate the formation of inositol phosphates in rat cerebral cortex resembles previous observations made in rat hippocampus (Nicoletti *et al.*, 1986a,b), primary cultures from rat striatum (Sladeczek *et al.*, 1985; Schmidt *et al.*, 1987) and *Xenopus* oocytes injected with rat brain messenger RNA (Sugiyama *et al.*, 1987). It seems likely that the formation of inositol phosphates is mediated through a site which is distinct from previously defined sub-classes of EAA receptors. The evidence for this conclusion is as follows:

(i) The pharmacology of this response does not resemble that of previously described sub-types of EAA receptors. The two most efficacious agonists on inositol phosphate formation, quisqualate and ibotenate, are thought to act on distinct receptor subtypes (Foster & Fagg, 1984). Ibotenate has been reported to act as a potent NMDA agonist (Cotman & Iversen, 1987), though the lack of response to NMDA itself and inability of APV (see also Nicoletti *et al.*, 1986b) and MK-801 to antagonize the stimulatory action of ibotenate would strongly suggest that ibotenate is not generating the formation of inositol phosphates via this recognition site. In fact MK-801 and APV potentiated the response to ibotenate, presumably by preventing the neurotoxic action of this agonist, which is mediated through the NMDA receptor. Nicoletti *et al.* (1986b) reported that the stimulation of inositol phosphate production by both ibotenate and quisqualate could be antagonized by APB, and suggested that these agonists may be acting at the 'APB-sensitive' site previously characterized in electrophysiological studies (Foster & Fagg, 1984). However, our responses in cortex were resistant to APB, as was the response to quisqualate in *Xenopus* oocytes described recently by Sugiyama *et al.* (1987).

(ii) The stimulation of inositol phosphate formation by ibotenate or quisqualate does not appear to

be an indirect effect mediated through the release of other neurotransmitters since the addition of tetrodotoxin or Cd^{2+} had no significant effect on the responses to these agonists. Cd^{2+} blocks Ca^{2+} entry at the nerve terminal and thus prevents the secretion of neurotransmitters, while tetrodotoxin inhibits the conduction of the action potential down the nerve axon and therefore prevents the secretion of neurotransmitters induced by depolarization of the cell body. In contrast, the weak response to glutamate does appear to be indirect since it is sensitive to tetrodotoxin. This effect is probably due to release of transmitters which act on PI-coupled receptors.

(iii) This does not appear to be an indirect effect mediated through an increase in the levels of intracellular Ca^{2+} since verapamil and Cd^{2+}, at concentrations that prevent the entrance of extracellular Ca^{2+} through voltage-operated channels, and thereby inhibit K^+-induced formation of inositol phosphates (Kendall & Nahorski, 1985), have no significant effect on the response to ibotenate and quisqualate.

An increase in intracellular Ca^{2+} could also be brought about by the opening of a non-specific cation channel associated with the three recognised sub-types of excitatory amino acid receptors. However, this cannot explain the marked formation of inositol phosphates induced by ibotenate since blockade of the NMDA receptor by use of MK-801 or APV resulted in an increased formation of inositol phosphates. Further, the lack of response to kainate and aspartate would argue against a non-specific action of EAAs on inositide turnover.

Although our data are consistent with an action of quisqualate and ibotenate at a novel EAA receptor, this conclusion should only be considered as tentative at present. Since relatively high concentrations of ibotenate and quisqualate are required to produce these responses it is possible that their action may not be receptor-linked, though the lack of additivity would suggest that both are acting at the same site. The development of specific antagonists is required for the existence of this novel receptor to be fully substantiated.

Our observations that the response to glutamate is TTX sensitive and that aspartate failed to enhance inositol phosphate production would strongly suggest that these agents are not the endogenous mediators of the effects we see with ibotenate and quisqualate; the nature of this mediator remains to be elucidated.

Concluding remarks

This study has described two novel actions of excitory amino acids on the metabolism of inositol phospholipids in rat cerebral cortex. It appears that there is a new sub-type of EAA receptor in cortex which is coupled to inositol phospholipid metabolism; ibotenate and quisqualate are potent agonists at this receptor. Secondly, it appears that the activation of all EAA receptor sub-types markedly inhibits agonist or K^+-induced hydrolysis of inositol phospholipids; this may be due to influx of Na^+ ions with a consequent excitotoxic effect.

P.P.G. is supported by the Wellcome Trust. We would like to thank Zahra Taghavi and Tracey Powell for skilled technical assistance and Dr John Kemp for helpful discussions.

References

BAUDRY, M., EVANS, J. & LYNCH, G. (1986). Excitatory amino acids inhibit stimulation of phosphatidylinositol metabolism by aminergic agonists in hippocampus. *Nature*, **319**, 329–331.

COCKCROFT, S. (1987). Polyphosphoinositide phosphodiesterase: regulation by a novel guanine nucleotide binding protein, Gp. *Trends Biochem. Sci.*, **12**, 75–78.

CONN, P.J. & SANDERS-BUSH, E. (1985). Serotonin-stimulated phosphoinositide turnover: mediation by the S_2 binding site in rat cerebral cortex but not in subcortical regions. *J. Pharmacol. Exp. Therap.*, **239**, 195–203.

COTMAN, C.W. & IVERSEN, L.L. (1987). Excitatory amino acids in the brain-focus on NMDA receptors. *Trends Neurosci.*, **10**, 263–265.

DONALDSON, J. & HILL, S.J. (1986). Histamine-induced hydrolysis of polyphosphoinositides in guinea-pig ileum and brain. *Eur. J. Pharmacol.*, **124**, 255–265.

DOWNES, C.P. & MICHELL, R.H. (1985). Inositol phospholipid breakdown as receptor controlled generator of second messengers. In *Molecular Aspects of Transmembrane Signalling*, Vol. 4, pp. 3–56. ed. Cohen, P. & Houslay, M.D. Amsterdam: Elsevier.

FISHER, S.K. & AGRANOFF, B.W. (1987). Receptor activation and inositol lipid hydrolysis in neural tissues. *J. Neurochem.*, **48**, 999–1017.

FOSTER, A.C. & FAGG, G.E. (1984). Acidic amino acid binding sites in mammalian neuronal membranes: their characteristics and relationship to synaptic receptors. *Brain Res. Revs.*, **7**, 103–164.

FOSTER, A.C. & WONG, E.H.F. (1987). The novel anticonvulsant MK-801 binds to the activated state of the N-methyl-D-aspartate receptor in rat brain. *Br. J. Pharmacol.*, **91**, 403–409.

GODFREY, P.P., McCLUE, S.J., MINCHIN, M.C.W. & YOUNG, M.M. (1985). RU24969, a 5-HT_1 agonist, stimulates inositol phospholipid breakdown in rat brain slices. *Br. J. Pharmacol.*, **84**, 112P.

GODFREY, P.P., McCLUE, S.J., YOUNG, M.M. & HEAL, D.J. (1988). 5-Hydroxytryptamine-stimulated inositol phos-

pholipid hydrolysis in the mouse cortex has pharmacological characteristics compatible with mediation via 5-HT_2 receptors but this response does not reflect altered 5-HT_2 function after 5,7-dihydroxytryptamine lesioning or repeated antidepressant treatments. *J. Neurochem.*, **50**, 730–738.

JACOBSON, M.D., WUSTEMAN, M. & DOWNES, C.P. (1985). Muscarinic receptors and hydrolysis of inositol phospholipids in rat cerebral cortex and parotid gland. *J. Neurochem.*, **44**, 465–472.

KEMP, J.A., FOSTER, A.C. & WONG, E.F.H. (1987). Non-competitive antagonists of excitatory amino acid receptors. *Trends Neurosci.*, **10**, 294–298.

KENDALL, D.A. & NAHORSKI, S.R. (1985). Dihydropyridine calcium channel activators and antagonists influence depolarisation-evoked inositol phospholipid hydrolysis in brain. *Eur. J. Pharmacol.*, **115**, 31–36.

KENDALL, D.A., BROWN, E. & NAHORSKI, S.R. (1985). α-Adrenoceptor-mediated inositol phospholipid hydrolysis in rat cerebral cortex: relationship between receptor occupancy and response and effect of denervation. *Eur. J. Pharmacol.*, **114**, 41–52.

MINNEMAN, K.D. & JOHNSON, R.D. (1984). Characterisation of alpha-1 adrenoceptors linked to [^{3}H]-inositol metabolism in rat cerebral cortex. *J. Pharmacol. Exp. Therap.* **230**, 317–323.

NAHORSKI, S.R. KENDALL, D.A. & BATTY, I. (1986). Receptors and phosphoinositide metabolism in the central nervous system. *Biochem. Pharmacol.*, **35**, 2447–2453.

NICOLETTI, F., IADAROLA, M.J., WROBLEWSKI, J.T. & COSTA, E. (1986a). Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: Developmental changes and interaction with α_1-adrenoceptors. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 1931–1935.

NICOLETTI, F., MEEK, J.L., IADAROLA, M.J., CHUANG, D.M., ROTH, B.L. & COSTA, E. (1986b). Coupling of inositol phospholipid metabolism with excitatory amino acid recognition sites in rat hippocampus. *J. Neurochem.*, **46**, 40–46.

NICOLETTI, F., WROBLEWSKI, J.T. & COSTA, E. (1987). Magnesium ions inhibit the stimulation of inositol phospholipid hydrolysis in primary cultures of cerebellar granule cells. *J. Neurochem.*, **48**, 967–973.

ROTHMAN, S.M. & OLNEY, J.W. (1987). Excitotoxicity and the NMDA receptor. *Trends Neurosci.*, **10**, 299–302.

SCHMIDT, B.H., WEISS, S., SEBBEN, M., KEMP, D.E., BOCKAERT, J. & SLADECZEK, F. (1987). Dual action of excitatory amino acids on the metabolism of inositol phosphates in striatal neurones. *Mol. Pharmacol.*, **32**, 364–368.

SLADECZEK, F., PIN, J.P., RECASENS, M., BOCKAERT, J. & WEISS, S. (1985). Glutamate stimulates inositol phosphate formation in striatal neurones. *Nature*, **317**, 717–719.

SUGIYAMA, H., ITO, I. & HIRONO, C. (1987). A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature*, **325**, 531–533.

TYLER, W., WATSON, S.P. & GODFREY, P. (1988). Study of a novel phosphoinositide-linked excitatory amino-acid receptor sub-type in rat cerebral cortex. *Br. J. Pharmacol.*, **92**, (in press).

WATKINS, J.C. & OLVERMAN, H.J. (1987). Agonists and antagonists for excitatory amino acid receptors. *Trends Neurosci.*, **10**, 265–272.

WILKINS, C.J., GODFREY, P.P. & WATSON, S.P. (1988). MK-801 blocks NMDA but not kainate-mediated inhibition of stimulated inositide turnover in rat cerebral cortex. *Br. J. Pharmacol.* **92**, (in press).

(Received December 22, 1987
Revised March 30, 1988
Accepted April 11, 1988)