Br. J. Pharmacol. (1992), **106**, 354–359

Evidence for a glutamate receptor of the AMPA subtype which mediates insulin release from rat perfused pancreas

[1†]G. Bertrand, [*]R. Gross, [*]R. Puech, [*]M.M. Loubatières-Mariani & [†]J. Bockaert

†Centre CNRS-INSERM de Pharmacologie-Endocrinologie UMR 6, Rue de la Cardonille, 34094 Montpellier Cedex, France and *Faculté de Médecine, Laboratoire de Pharmacologie, URA 599 du CNRS, Institut de Biologie, Boulevard Henri IV, 34060 Montpellier Cedex, France

1 The effect of L-glutamate has been studied on insulin secretion by the isolated perfused pancreas of the rat. The glutamate receptor subtype involved has been characterized.

2 In the presence of a slightly stimulating glucose concentration (8.3 mM), L-glutamate (5×10^{-5}–4×10^{-3} M) induced an immediate, transient and concentration-dependent insulin response. On the other hand, in the presence of a non stimulating glucose concentration (2.8 mM), L-glutamate (10^{-3} M) did not modify the basal insulin secretion.

3 The three non-NMDA receptor agonists, kainate (10^{-4}–10^{-3} M), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, 5×10^{-5}–10^{-4} M) and quisqualate (5×10^{-6}–5×10^{-5} M) all provoked a transient and concentration-dependent insulin response from pancreas perfused with 8.3 mM glucose. Compared with glutamate, kainate exhibited a similar efficacy, whereas AMPA and quisqualate elicited only a 3 fold lower maximal insulin response. In contrast, NMDA (10^{-4}–10^{-3} M) was ineffective.

4 An antagonist of non-NMDA receptors, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 5×10^{-5} M) totally prevented the stimulatory effect of L-glutamate (4×10^{-4} M) and kainate (2×10^{-4} M). In contrast, the NMDA receptor antagonist, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine ((+) MK801) was without effect.

5 The insulin secretory effect of glutamate (4×10^{-4} M) was not affected by atropine (3×10^{-7} M) or tetrodotoxin (3×10^{-6} M).

6 Quisqualate at a high maximally effective concentration (4×10^{-4} M) inhibited glutamate (10^{-3} M) or kainate (4×10^{-4} M)-induced insulin release.

7 This study shows that L-glutamate stimulates insulin secretion in rat pancreas, by acting on an excitatory amino acid receptor of the AMPA subtype.

Keywords: Glutamate; AMPA receptor; insulin release; rat pancreas

Introduction

L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. It plays an important role in many neuronal functions such as fast synaptic transmission (Tang *et al.*, 1989), neuronal plasticity (Collingridge & Singer, 1990) and also in neurodegenerative diseases (Meldrum & Garthwaite, 1990).

Many effects of glutamate are mediated by three major receptor subtypes termed according to their most selective agonists: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (previously termed quisqualate receptors) and kainate receptors (Watkins *et al.*, 1990). However, it is now clear that most responses to kainate are mediated by the AMPA receptor subtype where kainate acts as a full agonist, whereas AMPA (or quisqualate) gives only partial responses. These receptors are gated ionic channels permeable to Na^+, K^+ and for the NMDA subtype, to Ca^{2+} as well.

Despite extensive research on glutamate receptors in the central nervous system, little is known about the existence of peripheral glutamate receptors. A NMDA receptor subtype has been characterized in the myenteric plexus of the guinea-pig ileum (Moroni *et al.*, 1986; Shannon & Sawyer, 1989).

Much evidence shows that pancreatic islets cells, particularly insulin secreting β cells, share with neurones common characteristics; they contain tyrosine hydroxylase (Teitelman & Lee, 1987), neurone specific enolase (Polak *et al.*, 1984), Go protein (Terashima *et al.*, 1987), glutamic acid decarboxylase, high levels of γ-aminobutyric acid (GABA) (Okada, 1986) as well as synaptic-like microvesicles (Reetz *et al.*, 1991).

The present study was designed to investigate an eventual effect of L-glutamate on insulin secretion and to characterize the receptor subtype involved. This work, performed on the isolated perfused pancreas of the rat, shows that L-glutamate stimulates insulin release by activating a glutamate receptor of the AMPA subtype with similar pharmacological characteristics to that described in the central nervous system.

Methods

Our experiments were performed on male Wistar rats fed *ad libitum* and weighing 330 to 350 g. The surgical procedure for the rat isolated perfused pancreas has been previously described (Loubatières *et al.*, 1969; Bertrand *et al.*, 1986). After anaesthesia with sodium pentobarbitone (60 mg kg^{-1}, i.p.), the pancreas was totally isolated from all neighbouring tissues; it was perfused through its own arterial system with a Krebs Ringer bicarbonate buffer containing 2 g l^{-1} pure bovine serum albumin (fraction V) and glucose 8.3 mM. The Krebs buffer had the following ionic composition (mM): NaCl 108, KH_2PO_4 1.19, KCl 4.74, $CaCl_2$ 2.54, $MgSO_4$ 7, H_2O 1.19 and $NaHCO_3$ 18. A mixture of O_2 (95%) and CO_2 (5%) was continuously bubbled through this medium; the pH was about 7.4. The preparation was maintained at 37.5°C. Each organ was perfused at a constant pressure: the pressure

[1] Author for correspondence at: Centre CNRS-INSERM de Pharmacologie-Endocrinologie UMR6, Rue de la Cardonille, 34094 Montpellier Cedex, France.

was selected so as to produce a flow rate of about 2.5 ml min^{-1} at the start of the experiments.

In all the experiments, a 30 min adaptation period was allowed before taking the first sample. Glutamate or other agonists, were infused for 20 min from time 45 min in the absence or from 55 min in the presence of blockers. The blockers were added from 40 min, 15 min before the agonist infusion. Samples were collected during 1 min and were then immediately frozen for insulin radioimmunoassay. Insulin in the pancreatic effluent was assayed by the method of Herbert *et al.* (1965) using the antibody supplied by Miles Laboratories (Paris). [^{125}I]-insulin was obtained from International CIS (Gif-Sur-Yvette, France). Pure rat insulin (NOVO, Copenhagen, Denmark) was used as the reference standard, the biological activity of which was 22.3 $\mu u\ ng^{-1}$. The intra- and inter-assay variations were respectively 9 and 13%.

For the kinetics of insulin output rate the results for each point are expressed as changes in relation to the value recorded before agonist administration at time 45 or 55 min (taken as 100%). Data are expressed as mean ± standard error of the mean (s.e.mean).

In order to establish the concentration-response curves for agonists we used the increase of mean insulin output rate as a percentage. This value was obtained as follows: AUC/5 (AUC = area under the curve during the first 5 min of infusion).

The data were submitted to analysis of variance followed by the multiple comparison test (Zar, 1974).

Drugs

L-Glutamic acid (monosodium salt), kainic acid, N-methyl-D-aspartic acid, glycine, atropine and tetrodotoxin were obtained from Sigma. (**RS**)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were from Tocris Neuramin. Quisqualic acid was purchased from Cambridge Research Biochemicals. (+) -5- Methyl-10,11- dihydro-5H-dibenzo[a,d]cyclohepten-5, 10-imine hydrogen maleate ((+)-MK801) was from Research Biochemicals Inc.

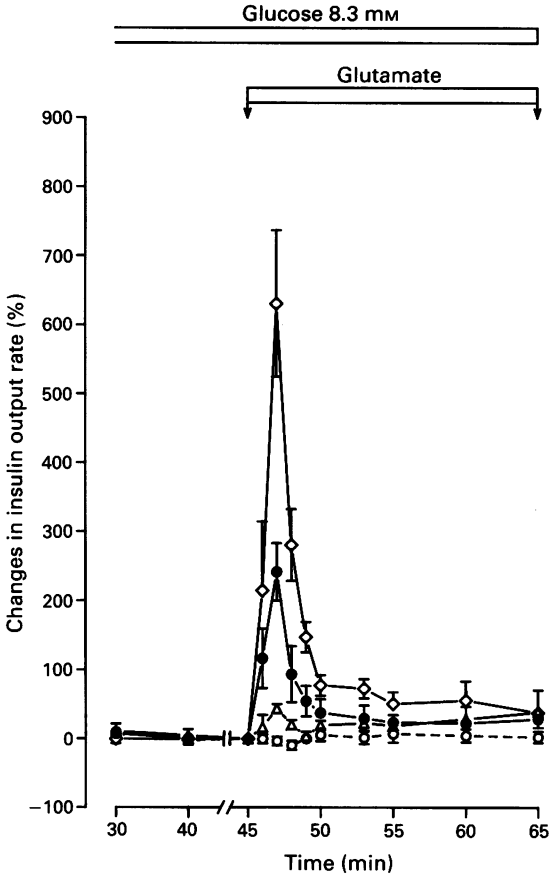

Figure 1 Effects of glutamate on insulin secretion from the isolated perfused pancreas of the rat: (△) glutamate 5×10^{-5} M ($n = 4$), (●) 2×10^{-4} M ($n = 4$), (◇) 4×10^{-3} M ($n = 5$), controls (○) ($n = 6$). The insulin output rate (ng min^{-1}) at 45 min for each set of experiments was: 14.2 ± 3.2; 14.5 ± 4.9; 12.3 ± 2.2; 15.1 ± 2.8 respectively. Each point represents the mean with s.e.mean shown by vertical lines.

Results

Effect of L-glutamate

In pancreata perfused in the presence of a slightly stimulating glucose concentration (8.3 mM), the control rate of insulin release was stable, reaching 15.1 ± 2.8 ng ml^{-1} at 45 min (the reference value).

L-Glutamate was studied at concentrations ranging between 10^{-5} and 4×10^{-3} M. At 10^{-5} M, glutamate did not significantly affect insulin release (results not shown). In the range 5×10^{-5} to 4×10^{-3} M, this amino acid provoked an immediate and transient insulin response which was concentration-dependent. The insulin response occurred in a peak form which culminated at the 2 min and lasted about 5 min although the infusion was maintained for 20 min (Figure 1). The increase of mean insulin output rate averaged a maximum of +270 ± 44% with glutamate 4×10^{-3} M (Figure 4).

On the other hand, in the presence of a non-stimulating glucose concentration (2.8 mM), L-glutamate even at 10^{-3} M, did not affect basal insulin release. The insulin output during the first 5 min of glutamate infusion was 1.2 ± 0.8 ng versus 1.1 ± 0.5 ng in controls (result not shown).

Effect of glutamate receptor agonists

Non-NMDA receptor agonists Three non-NMDA receptor agonists, kainate, AMPA and quisqualate were studied.

In the presence of 8.3 mM glucose, all three agonists elicited a concentration-dependent peak-shaped insulin response as did glutamate (Figures 2 and 3). Kainate exhibited a comparable efficacy to that of glutamate. As shown in Figure 4 the maximal increase in the mean insulin output rate obtained with kainate at 10^{-3} M (+358 ± 48%), was not significantly different from that induced by glutamate (+270 ± 44%). In contrast, quisqualate and AMPA elicited weak maximal insulin responses (+74 ± 15% and +87 ± 15% respectively), about 3 fold lower than that obtained with glutamate. However quisqualate was more potent than glutamate; it was concentration-dependently effective in the range 5×10^{-6} to 5×10^{-5} M (Figure 4).

NMDA receptor agonist NMDA, in the range 10^{-4}–10^{-3} M did not affect insulin release: the mean changes in insulin output rates during the first 5 min of NMDA infusion were +9 ± 6%; +12 ± 5% and +4 ± 2% at NMDA 10^{-4}, 4×10^{-4} and 10^{-3} M respectively versus +2 ± 5% in controls (Figure 4). These results were obtained in presence of glycine (10^{-6} M) known to potentiate NMDA effects in brain (Johnson & Asher, 1987). Mg^{2+} is known to inhibit NMDA receptor channels (Nowak *et al.*, 1984). However, in the absence of Mg^{2+}, NMDA (4×10^{-4} M) remained ineffective on insulin secretion: the mean change in insulin output rates was +2 ± 3%.

Effects of blockers of glutamate receptors on the insulin responses to glutamate and kainate (Figure 5)

In these experiments, the two most effective agonists glutamate and kainate were used at concentrations inducing about half maximal insulin response i.e. 4×10^{-4} M and 2×10^{-4} M respectively.

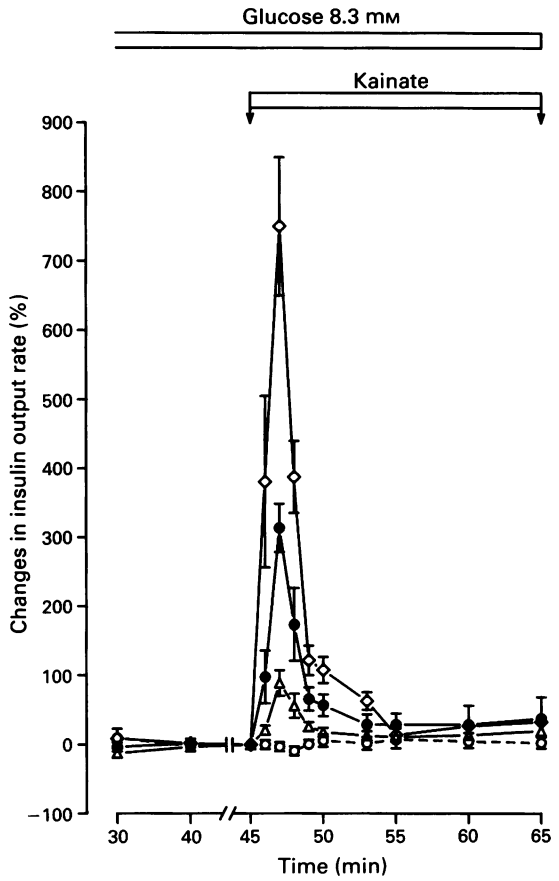

Figure 2 Effects of kainate on insulin secretion from the isolated perfused pancreas of the rat: (△) kainate 10^{-4} M ($n = 4$), (●) 2×10^{-4} M ($n = 6$), (◇) 10^{-3} M ($n = 6$). The insulin output rate (ng min^{-1}) at 45 min for each set of experiments was: 13.3 ± 6.7; 17.2 ± 1.5; 13.2 ± 1.7 respectively. Each point represents the mean with s.e.mean shown by vertical lines.

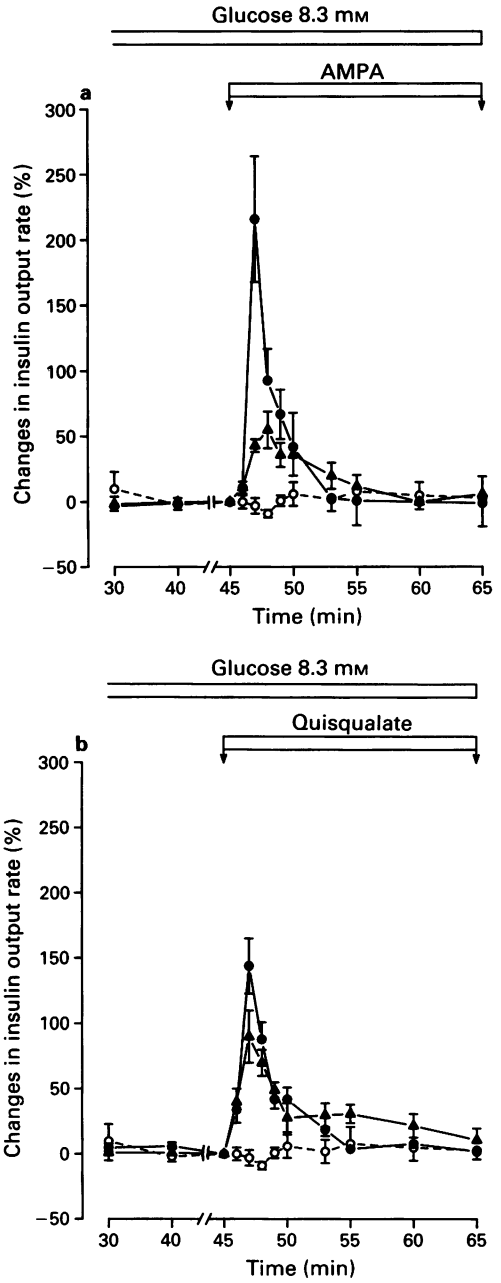

Figure 3 Effects of non-NMDA receptor agonists on insulin secretion from the isolated perfused pancreas of the rat: (a) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA): (▲) 5×10^{-5} M ($n = 4$) and (●) 2×10^{-4} M ($n = 5$); (b) quisqualate: (▲) 10^{-5} M ($n = 4$) and (●) 5×10^{-5} M ($n = 5$). Each point represents the mean with s.e.mean shown by vertical lines.

The antagonists were introduced 15 min before glutamate or kainate and were present during the 20 min of agonist infusion.

CNQX

6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) is a competitive antagonist of non-NMDA receptors (Honoré *et al.*, 1988). CNQX at 5×10^{-5} M ineffective *per se* on insulin release during the 15 min pretreatment, totally prevented the stimulating effects of both glutamate and kainate (Figure 5a, b).

MK801

(+) -5- Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5, 10-imine ((+)-MK801) is a highly potent and non competitive blocker of NMDA receptors (Wong *et al.*, 1986). (+)-MK801 at 10^{-6} M did not affect the insulin response induced by glutamate or kainate (Figure 5a, b).

Inhibition by quisqualate of the insulin responses to glutamate and kainate (Figure 6)

In order to characterize further the non-NMDA receptor subtype involved in glutamate and kainate effects, we investigated the interactions between these agonists and quisqualate to trigger insulin release. Quisqualate was used at 4×10^{-4} M, a maximally effective concentration.

The responses to glutamate (10^{-3} M) and kainate (4×10^{-4} M) were both inhibited by the simultaneous infusion of quisqualate (Figure 6). Thus, in the presence of quisqualate, the insulin secretory responses to glutamate ($+69 \pm 10\%$) and kainate ($+100 \pm 21\%$) were not significantly different from the maximal response to quisqualate ($+72 \pm 5\%$).

Effect of atropine and tetrodotoxin (TTX) on the insulin response to glutamate and kainate (Figure 7)

We investigated a possible involvement of a nervous component in glutamate effect.

Atropine at 3×10^{-7} M, a concentration used to block acetylcholine-induced insulin release in our preparation (Loubatières *et al.*, 1973), did not significantly modify the responses to glutamate. In addition TTX at 3×10^{-6} M, a concentration used to prevent nerve action potentials (Stag-

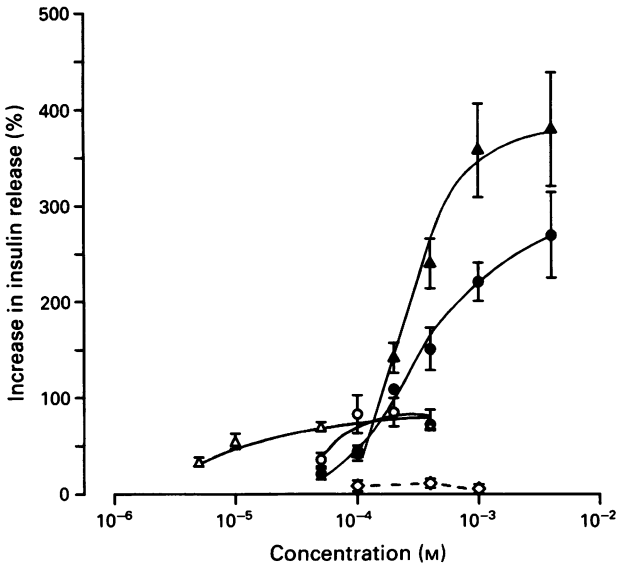

Figure 4 Concentration-response curves for the effects of glutamate (●), kainate (▲), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (○), quisqualate (△) and N-methyl-D-aspartate (NMDA) (◇) on insulin release. Each point represents the mean of 4–6 experiments and vertical lines indicate the s.e.mean.

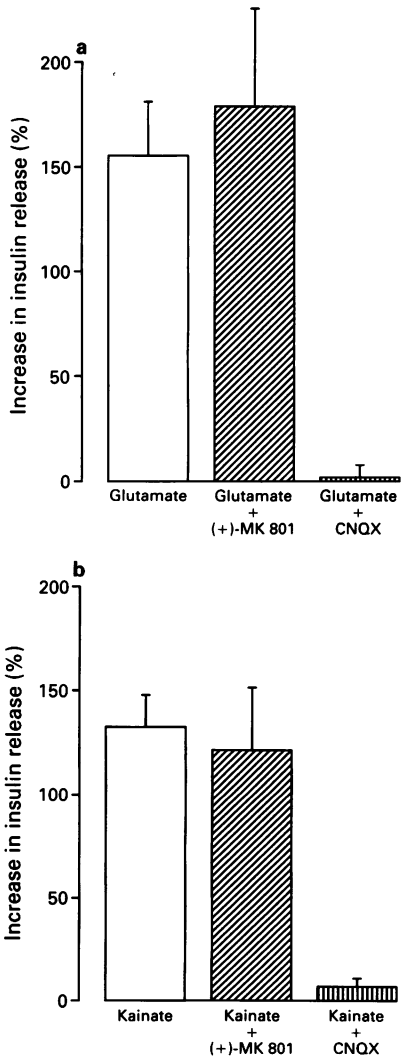

Figure 5 Effects of blockers of glutamate receptors on the insulin response induced by (a) glutamate (4×10^{-4} M) or (b) kainate (2×10^{-4} M). Each column represents the mean of 4–6 experiments and vertical lines indicate s.e.mean.

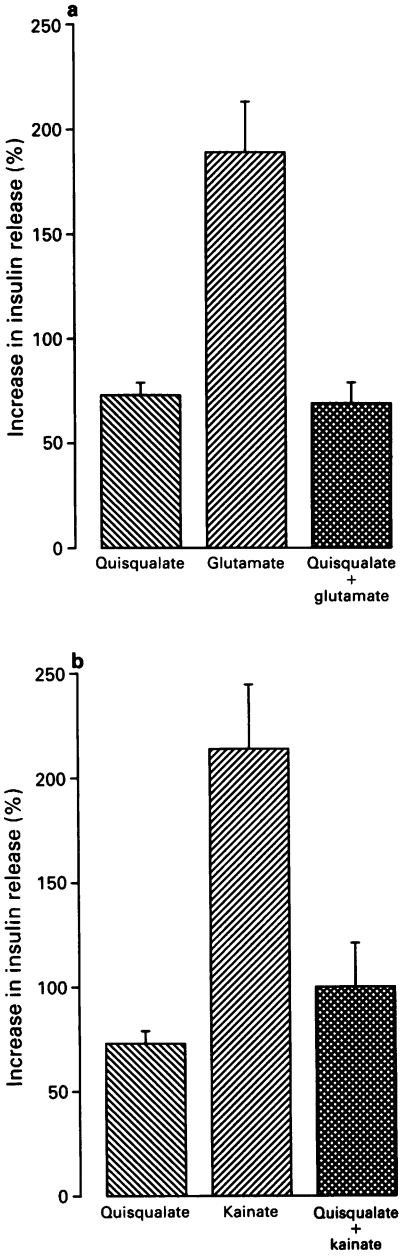

Figure 6 Inhibition by quisqualate of the insulin response induced by (a) glutamate (10^{-3} M) and (b) kainate (4×10^{-4} M). Each column represents the mean of 4–6 experiments and vertical lines indicate s.e.mean.

ner & Samols, 1985a), was ineffective *per se* on insulin release and did not alter glutamate-induced insulin release.

Discussion

The present study shows that L-glutamate transiently stimulates insulin secretion from the rat isolated pancreas. This effect appears to be mediated by a glutamate receptor of the AMPA subtype. This is the first time that the presence of such a glutamate receptor subtype has been reported in the periphery.

The stimulatory effect of glutamate was dependent on the glucose concentration: glutamate stimulated insulin secretion in the presence of a slightly stimulating concentration of glucose (8.3 mM) but not in the presence of a low concentration (2.8 mM). Thus, this amino acid is not an initiator but a potentiator of glucose-induced insulin release.

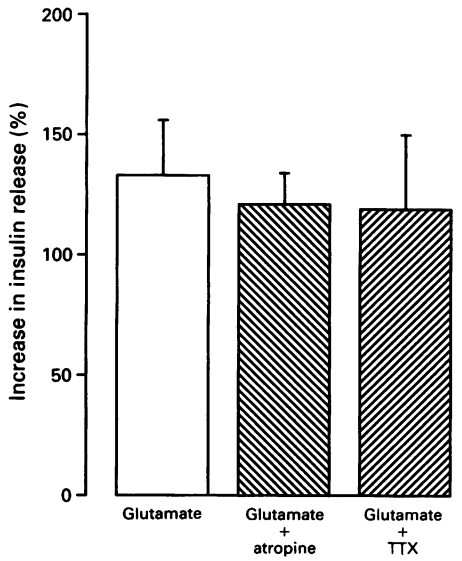

Figure 7 Effect of atropine (3×10^{-7} M) or tetrodotoxin (TTX, 3×10^{-6} M) on the insulin responses induced by glutamate (4×10^{-4} M). Each column represents the mean of 4–6 experiments and vertical lines indicate s.e.mean.

Glutamate has previously been reported not to stimulate insulin secretion, even in the presence of a high glucose concentration, in mouse pancreatic islets (Sehlin, 1972). However these studies were performed during static incubation for 60 min and therefore the transient insulin response observed in our kinetic study might have been blunted in such long-lasting static experiments.

Glutamate induced an immediate, transient insulin response in a concentration-dependent manner in the range 5×10^{-5}–4×10^{-3} M. As this effect was immediate and transient, and occurred at micromolar concentrations, a metabolic action of the amino acid is unlikely. Furthermore glutamate is very poorly taken up by islet cells (Sehlin, 1972).

Glutamate is known to exert excitatory effects in brain by acting on specific receptors classified as NMDA and non-NMDA types, including kainate and AMPA receptors (Watkins *et al.*, 1990). Our data are consistent with a non-NMDA receptor mediating glutamate-induced insulin release. Glycine is known to potentiate the action of NMDA by binding to a regulatory site of the NMDA receptor complex (Johnson & Ascher, 1987). In the presence of glycine (10^{-6} M), NMDA was ineffective whereas the response to glutamate remained unchanged (results not shown). Furthermore, even in the absence of Mg^{2+}, known to block NMDA receptor channels (Nowak *et al.*, 1984), NMDA was unable to stimulate insulin release. The involvement of a non-NMDA receptor was confirmed by the inability of (+)-MK801, a potent antagonist of NMDA receptors (Wong *et al.*, 1986), to block insulin response to glutamate. On the other hand, the three non-NMDA receptor agonists, kainate, quisqualate and AMPA, like glutamate exhibited a stimulating effect on insulin release. Furthermore the competitive and selective antagonist of non-NMDA receptors, CNQX, totally blocked the insulin response to glutamate or kainate.

The pharmacological characteristics of the insulin response induced by glutamate analogues suggest the involvement of an AMPA receptor subtype. The apparent agonist potency order on insulin secretion (quisqualate > AMPA > kainate) is in agreement with the agonist potencies reported for AMPA receptors mediating excitatory responses in brain neurones and for [^{3}H]-AMPA binding sites (see Monaghan *et al.*, 1989). In addition, the concentration-response curves show that quisqualate and AMPA induced lower maximal insulin responses than did kainate or glutamate. The response to quisqualate was not additive to those obtained with glutamate or kainate; in contrast, quisqualate inhibited insulin responses to both glutamate and kainate. Similar inhibition of the response to kainate by quisqualate, but also by AMPA, has been reported in various studies e.g. in cultures of chick motor neurones (O'Brien & Fischbach, 1986), mouse striatal neurones (Pin *et al.*, 1989; Charpentier *et al.*, 1990) and in *Xenopus* oocytes injected with rat brain mRNA (Rassendren *et al.*, 1989). These authors concluded that quisqualate and AMPA behave as apparent partial agonists at a receptor fully activated by kainate; the behaviour as full or partial agonist of kainate and AMPA (or quisqualate) would be due to the fact that they produce non desensitizing and desensitizing responses respectively.

AMPA receptors are widely distributed in mammalian brain, especially in hippocampus, cortex, striatum, septum and the molecular layer of cerebellum (Monaghan *et al.*, 1989; Young & Fagg, 1990). In contrast, AMPA receptors have never been described outside the central nervous system. A glutamate receptor displaying pharmacological characteristics of the NMDA subtype has been reported in isolated ileal longitudinal muscle-myenteric plexus of the guinea-pig (Moroni *et al.*, 1986). The activation of such receptors by glutamate or NMDA led to contraction of the ileum; this response was blocked by tetrodotoxin and a blocker of muscarinic receptors, indicating an action on the myenteric plexus. It is well known that the pancreatic islets are richly innervated by autonomic nerve fibres: parasympathetic nerve stimulation results in an increase in insulin secretion, whereas sympathetic stimulation decreases this secretion (via α_2-adrenoceptors) (see Miller, 1981). The intrapancreatic nervous system appears to be composed of a complex system of connected nerves and ganglia dispersed in the parenchyma. It has been reported that pancreatic ganglia share some functional characteristics with the myenteric plexus (Stagner & Samols, 1985b; 1986). However, unlike results obtained with the ileum, in our study glutamate does not appear to stimulate insulin release by promoting the release of acetylcholine from pancreatic cholinergic nerves, since atropine did not affect the response to the amino acid. In addition, tetrodotoxin, known to block nerve action potentials, did not prevent the insulin response to glutamate. These findings do not provide any evidence that glutamate could stimulate insulin release through an action on the intrinsic pancreatic neurones, and thus a direct action on islet cells appears more likely.

The presence in the pancreas of glutamate receptors mediating insulin release might be of physiological relevance. Indeed, it is noteworthy that glutamate is effective in circulating plasma concentrations (ranging between 30–100 μM) reported in rats (Christophe *et al.*, 1971) and man (Schmid *et al.*, 1989; Stegink *et al.*, 1991). Furthermore, monosodium glutamate is largely used to enhance food flavour. The rapidity in onset and transient nature of insulin response to glutamate in the presence of a physiological stimulating glucose concentration, suggests that this amino acid could act as an amplifier in the initiation of insulin release during food intake.

In conclusion, glutamate stimulates insulin release in rat pancreas, by acting on an excitatory amino acid receptor of the AMPA subtype similar to that described in the central nervous system.

We wish to thank M. Roye for expert technical assistance and V. Montesinos for secretarial assistance.

References

BERTRAND, G., CHAPAL, J. & LOUBATIERES-MARIANI, M.M. (1986). Potentiating synergism between adenosine diphosphate or triphosphate and acetylcholine on insulin secretion. *Am. J. Physiol.*, **251**, E416–E421.

CHARPENTIER, N., DUMUIS, A., SEBBEN, M., BOCKAERT, J. & PIN, J.P. (1990). On concanavalin A-treated striatal neurones quisqualate clearly behaves as a partial agonist of a receptor fully activated by kainate. *Eur. J. Pharmacol.*, **198**, 241–251.

CHRISTOPHE, J., WINAND, J., KUTZNER, R. & HEBBELINCK, M. (1971). Amino acid levels in plasma, liver, muscle, and kidney during and after exercise in fasted and fed rats. *Am. J. Physiol.*, **221**, 453–457.

COLLINGRIDGE, G.L. & SINGER, W. (1990). Excitatory amino acid receptors and synaptic plasticity. *Trends Pharmacol. Sci.*, **11**, 290–296.

HERBERT, V., LAW, K.S., GOTTLIEB, C.W. & BLEICHER, S.J. (1965). Coated charcoal immunoassay of insulin. *J. Clin. Endocr.*, **25**, 1375–1384.

HONORE, T., DAVIES, S.N., DREJER, J., FLETCHER, E.J., JACOBSEN, P., LODGE, D. & NIELSEN, F.E. (1988). Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. *Science*, **241**, 701–703.

JOHNSON, J.W. & ASCHER, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature*, **325**, 529–531.

LOUBATIERES, A.L., MARIANI, M.M., DE MALBOSC, H., RIBES, G. & CHAPAL, J. (1969). Etude expérimentale d'un nouveau sulfamide hypoglycémiant particulièrement actif, le HB 419 ou glibenclamide. I. Action bêtacytotrope et insulino-sécrétrice. *Diabetologia*, **5**, 1–10.

LOUBATIERES-MARIANI, M.M., CHAPAL, J., ALRIC, R. & LOUBAITIERES, A.L. (1973). Studies of the cholinergic receptors involved in the secretion of insulin using isolated perfused rat pancreas. *Diabetologia*, **9**, 439–446.

MELDRUM, B. & GARTHWAITE, J. (1990). Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmacol. Sci.*, **11**, 379–387.

MILLER, R.E. (1981). Pancreatic neuroendocrinology: peripheral neural mechanisms in the regulation of the islets of Langerhans. *Endocrine Rev.*, **2**, 471–494.

MONAGHAN, D.T., BRIDGES, R.J. & COTMAN, C.W. (1989). The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.*, **29**, 365–402.

MORONI, F., LUZZI, S., FRANCHI-MICHELI, S. & ZILLETTI, L. (1986). The presence of N-methyl-D-aspartate-type receptors for glutamic acid in the guinea pig myenteric plexus. *Neurosci. Lett.*, **68**, 57–62.

NOWAK, L., BREGESTOVSKI, P., ASCHER, P., HERBERT, A. & PROCHIANTZ, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature*, **307**, 462–465.

O'BRIEN, R.J. & FISCHBACH, G.D. (1986). Characterization of excitatory amino acid receptors expressed by embryonic chick motoneurons in vitro. *J. Neurosci.*, **6**, 3275–3283.

OKADA, Y. (1986). Localization and function of GABA in the pancreatic islets. In *GABAergic Mechanisms in the Mammalian Periphery*. ed. Erdo, S.L. & Bowery, N.G. pp. 223–240. New York: Raven Press.

PIN, J.P., VAN VLIET, B.J. & BOCKAERT, J. (1989). Complex interaction between quisqualate and kainate receptors as revealed by measurement of GABA release from striatal neurons in primary culture. *Eur. J. Pharmacol.*, **172**, 81–91.

POLACK, J.M., BLOOM, S.R. & MARANGOS, P.J. (1984). Neuron-specific enolase, a marker for neuroendocrine cells. In *Evolution and Tumor Pathology of the Neuroendocrine System*. ed. Falkmer, S., Hakanson, R. & Sundler, F. pp. 433–542. Amsterdam: Elsevier.

RASSENDREN, F.A., LORY, P., PIN, J.P., BOCKAERT, J. & NARGEOT, J. (1989). A specific quisqualate agonist inhibits kainate responses induced in Xenopus oocytes injected with rat brain RNA. *Neurosci. Lett.*, **99**, 333–339.

REETZ, A., SOLIMENA, M., MATTEOLI, M., FOLLI, F., TAKEI, K. & DE CAMILLI, P. (1991). GABA and pancreatic β-cells: colocalization of glutamic acid decarboxylase (GAD) and GABA with synaptic-like microvesicles suggests their role in GABA storage and secretion. *EMBO J.*, **10**, 1275–1284.

SEHLIN, J. (1972). Uptake and oxidation of glutamic acid in mammalian pancreatic islets. *Hormones*, **3**, 156–166.

SHANNON, H.E. & SAWYER, B.D. (1989). Glutamate receptors of the N-methyl-D-aspartate subtype in the myenteric plexus of the guinea pig ileum. *J. Pharmacol. Exp. Ther.*, **251**, 518–523.

SCHMID, R., SCHUSDZIARRA, V., SCHULTE-FROHLINDE, E., MAIER, V. & CLASSEN, M. (1989). Role of amino acids in stimulation of postprandial insulin, glucagon, and pancreatic polypeptide in humans. *Pancreas*, **4**, 305–314.

STAGNER, J.I. & SAMOLS, E. (1985a). Perturbation of insulin oscillations by nerve blockade in the in vitro canine pancreas. *Am. J. Physiol.*, **248**, E516–E521.

STAGNER, J.I. & SAMOLS, E. (1985b). Role of intrapancreatic ganglia in regulation of periodic insular secretions. *Am. J. Physiol.*, **248**, E522–E530.

STAGNER, J.I. & SAMOLS, E. (1986). Modulation of insulin secretion by pancreatic ganglionic nicotinic receptors. *Diabetes*, **35**, 849–854.

STEGINK, L.D., FILER, L.J., BRUMMEL, M.C., BAKER, G.L., KRAUSE, W.L., BELL, E.F. & ZIEGLER, E.E. (1991). Plasma amino acid concentrations and amino acid ratios in normal adults and adults heterozygous for phenylketonuria ingesting a hamburger and milk shake meal[1-3]. *Am. J. Clin. Nutr.*, **53**, 670–675.

TANG, C.M., DICHTER, M. & MORARD, M. (1989). Quisqualate activates a rapidly inactivating high conductance ionic channel in hippocampal neurons. *Science*, **243**, 1474–1477.

TEITELMAN, G. & LEE, J.K. (1987). Cell lineage analysis of pancreatic islet cell development: glucagon and insulin cells arise from catecholaminergic precursors present in the pancreatic duct. *Dev. Biol.*, **121**, 454–466.

TERASHIMA, T., KATADA, T., OINUMA, M., INOUE, Y. & UI, M. (1987). Endocrine cells in pancreatic islets of Langerhans are immunoreactive to antibody against guanine nucleotide-binding protein (G_o) purified from rat brain. *Brain Res.*, **417**, 190–194.

WATKINS, J.C, KROGSGAARD-LARSEN, P. & HONORE, T. (1990). Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends Pharmacol. Sci.*, **11**, 25–33.

WONG, E.H.F., KEMP, J.A., PRIESTLEY, T., KNIGHT, A.R., WOODRUFF, G. & IVERSEN, L.L. (1986). The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonists. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 7104–7108.

YOUNG, A.B. & FAGG, G.E. (1990). Excitatory amino acid receptors in the brain: membrane binding and receptor autoradiographic approaches. *Trends Pharmacol. Sci.* **11**, 126–133.

ZAR, J.H. (1974). *Biostatistical Analysis*. Englewood Cliffs, N.J.: Prentice-Hall.

(Received October 22, 1991
Revised February 5, 1992
Accepted February 10, 1992)