Identification of functional ionotropic glutamate receptor proteins in pancreatic β-cells and in islets of Langerhans

Elek Molnár[a,*], Anikó Váradi[b], R.A. Jeffrey McIlhinney[a], Stephen J.H. Ashcroft[b]

[a]*Medical Research Council, Anatomical Neuropharmacology Unit, Mansfield Road, Oxford OX1 3TH, UK*
[b]*Nuffield Department of Clinical Biochemistry, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK*

Received 22 July 1995

Abstract The presence of ionotropic glutamate receptor proteins in islets of Langerhans and pancreatic β-cell lines (MIN6, HIT T15, RINm5F) was investigated. For this purpose immunoblot analysis of β-cell membranes was performed with subunit-specific antibodies. We identified NMDAR1 subunits of the NMDA and KA-2 subunits of the kainate receptors, but did not detect GluR1 subunits of the AMPA receptor. The receptor subunits present were shown to be glycosylated. β-cell membranes contained specific binding sites for glutamate receptor ligands, and NMDA increased insulin secretion. These results demonstrate that ionotropic glutamate receptor proteins, similar to those in the central nervous system, are expressed in rat pancreatic β-cells.

Key words: Ionotropic glutamate receptor; Islets of Langerhans; RINm5F cells; MIN6 cells; HIT T15 cells; Insulin secretion

1. Introduction

Glutamate receptors are generally thought to be the principal neurotransmitter receptors responsible for excitatory neurotransmission in the central nervous system (CNS) [1,2]. The fast, excitatory postsynaptic responses of neurones to L-glutamate are evoked by ligand-gated ionotropic receptors, of which there are three subtypes. These subtypes, which are classified on the basis of pharmacological and electrophysiological responses to selective agonists and sequence homologies, are the AMPA (GluR1-4 or GluRA-D), the kainate (GluR5-7 and KA-1, KA-2) and the NMDA receptors (NMDAR1 and NMDAR2A-D). Ionotropic glutamate receptors are heterooligomeric protein complexes, composed of probably five subunits [3]. The receptor complexes are glutamategated non-selective ion channels, permeable to monovalent cations but, depending on their subunit composition, they exhibit differential permeabilities to divalent cations [3].

In addition to the important role that glutamate receptors play in the brain, recent evidence suggests a physiological role of glutamate in the endocrine pancreas. Neuronal ionotropic glutamate receptor mRNAs have been found in a pancreatic β-cell line, MIN6, [4] and in islets of Langerhans [5]. However, the presence of a receptor subunit mRNA in a cell may not necessarily indicate expression of the receptor protein [5,6]. It has for example, been reported that a pheochromocytoma cell line, PC12, expresses NMDA receptor mRNA but not receptor protein [8]. Therefore, it is important to investigate whether or not the ionotropic glutamate receptor mRNA found in β-cells is actually translated into protein. In the present study we used subunit-specific antibodies and ligand binding assays to determine that rat islets of Langerhans and β-cell lines express ionotropic glutamate receptor subunit proteins similar to those found in the CNS. The effect of glutamate and selective synthetic agonists on insulin secretion from islets of Langerhans and β-cell lines was also investigated.

2. Materials and methods

2.1. Production and purification of antibodies

Synthetic peptides corresponding to residues 436–450 of NMDAR1 (CTGPNDTSPGSPRHT [9]) and residues 950–965 of KA-2 (RPRPGPTGPRELTEHE [10]) were prepared using solid-phase synthesis. The NMDAR1 $_{(436-450)}$ peptide was coupled to mercaptosuccinylated ovalbumin carrier protein [11] via the N-terminal cysteine of the peptide which was conjugated to 5-thio-2-nitro benzoic acid (TNB) prior to coupling. The KA-2$_{(950-965)}$ peptide was conjugated to ovalbumin with glutaraldehyde [12]. The ovalbumin-coupled peptide antigens were co-adsorbed with an adjuvant peptide to colloidal gold particles (100 nm) [13]. Antibodies were raised by immunizing two New Zealand white female rabbits (4 to 6-month-old) with each antigen. Serum antibody titers were monitored ten days after the second and subsequent injections by ELISA using the appropriate peptide bound to the microtitration plates. The anti-NMDAR$_{(436-450)}$ antibodies were immunoaffinity purified using the synthetic peptide (5 mg) coupled to activated thiopropyl Sepharose 4B (Pharmacia). Antibodies directed against the KA-2$_{(950-965)}$ peptide were purified using BSA-coupled peptides immobilized on Affi-Gel 15 (Bio-Rad). Other steps of the purification procedure were performed as previously described [12].

2.2. Membrane preparation from β-cells, islets of Langerhans and brain

Culture of β-cell lines (MIN6, HIT T15, RINm5F), membrane preparation from cultured cells, and the isolation of rat islets of Langerhans were performed as described previously [14] Membranes from whole brains or dissected cortical, hippocampal, cerebellar and spinal cord areas of adult Wistar rats (250–300 g) were prepared as described earlier [12,15]. Before radioligand binding assays, all membrane fractions were washed a further three times by resuspension and centrifugation in 50 mM Tris-citrate (pH 7.4) at 4°C for 20 min at 54,000 × *g*.

2.3. Deglycosylation of membrane proteins

Membrane samples were resuspended in 50 mM sodium phosphate buffer (pH 7.0) to a final concentration of 10 mg protein/ml. The membranes were denatured by boiling in 1% SDS for 10 min, and then octyl-β-D-glycopyranoside (Sigma) was added to a final concentration of 2% octyl-β-D-glycopyranoside, 0.1% SDS and 1 mg protein/ml in 50 mM sodium phosphate buffer (pH 7.0). The preparation was then

*Corresponding author. Fax: (1865) 271 647.
Email: molnar@molbiol.ox.ac.uk

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate; BSA, bovine serum albumin; CNS, central nervous system; ELISA, enzyme-linked immunoadsorbant assay; HIT, HIT T15 β-cell line; IL, islets of Langerhans; MIN, MIN6 β-cell line; NMDA, *N*-methyl-D-aspartate; PAGE, polyacrylamide gel electrophoresis, PBS, phosphate buffered saline; PEG, polyethylene glycol; RT-PCR, reverse transcriptase polymerase chain reaction; RIN, RINm5F β-cell line.

SSDI 0014-5793(95)00890-X

incubated at 37°C with or without *N*-glycosidase F (6 units/ml, Boehringer Mannheim GMBH, Germany) for 4 h. Reactions were terminated by boiling in SDS-PAGE sample buffer.

2.4 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis, electrophoretic transfer of proteins and immunoblot analysis

SDS-PAGE was performed on 10% gels [16]. Proteins were transferred electrophoretically onto poly(vinylidene difluoride) microporous membrane (Immobilon; Millipore Co. Bedford, MA, USA) using a Multiphor II NovaBlot Electrophoretic Transfer Unit with a discontinous buffer system for 2 h at room temperature as recommanded by the manufacturer (LKB Produkter AB, Bromma, Sweden). Before immunostaining, the Immobilon sheets were blocked overnight at 4°C with 5% non-fat dry milk and 1.50 dilution of normal swine serum in PBS (blocking solution). The proteins on Immobilon sheets were reacted with different affinity-purified antibodies (2–5 μg/ml) in blocking solution for 12–16 h at 4°C. The bound antibodies were detected with an alkaline phosphatase-conjugated anti-rabbit IgG secondary antibody [12].

2.5 Radioligand binding

Radioligand binding studies were carried out by a centrifugation assay, using polyethylene glycol 6000 (PEG) precipitation. Membrane protein samples (0.2 mg) were incubated in 50 mM Tris-citrate (pH 7.4) for 30 min at 2°C, using either 20 nM [^{3}H]AMPA in the presence of 100 mM KSCN or 10 nM [^{3}H]KA. Non-specific binding was determined using 100 μM AMPA or KA, respectively. Following incubation, 2 mg/ml human γ-globulins and 15% (w/v) PEG were added and the samples centrifuged at 10,000 × *g* in an Eppendorf microfuge for 2 min. The supernatant was aspirated and the pellet rinsed twice superficially using 0.4 ml ice-cold 15% PEG. The pellets were suspended in 0.5 ml of 1% SDS and then transferred to scintillation vials and counted after the addition of 4 ml Liquiscint (National Diagnostics, Manville, NJ, USA).

2.6 Measurement of insulin release

Freshly isolated islets were preincubated for 1 h at 37°C in HEPES/Krebs buffer (119 mM NaCl, 4.75 mM KCl, 5 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 20 mM HEPES-Na, pH 7.4) containing 3.3 mM glucose and 2 mg/ml BSA [17]. Samples were then incubated for an additional 2 h in 0.6 ml HEPES/Krebs buffer containing 3.3 mM, 8.3 mM or 16.7 mM glucose in the presence or absence of various glutamate receptor agonists (0.5 and 2.5 mM glutamate, 0.5 mM kainate, 0.5 mM NMDA with 0.01 mM glycine) Insulin release was measured by radioimmunoassay as described by Ashcroft and Crossley [18], except that separation of free and bound insulin was achieved using anti-guinea-pig solid phase second antibody (Sac-Cel, IDS Ltd., Boldon, UK).

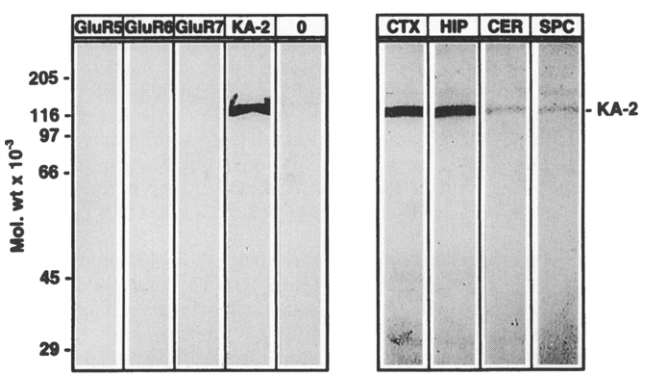

Fig. 1. Characterization of the specificity of anti-KA-2$_{(950–965)}$ polyclonal antibody using transfected cells, and distribution of KA-2 subunits in rat CNS. Aliquots (50 μg of protein/lane) of membrane proteins from COS-7 cells expressing the indicated glutamate receptor subunits following cDNA transfection (GluR5–7 and KA-2; 0, nontransfected control) and membrane fractions from various regions of the rat CNS (CTX, cerebral cortex; HIP, hippocampus; CER, cerebellum; SPC, spinal cord) were subjected to SDS-PAGE and immunoblotted with antibodies generated against C-terminal residues 950–965 of the KA-2 glutamate receptor subunit. The positions of the molecular weight markers are indicated on the left.

Table 1
Specific [^{3}H]AMPA and [^{3}H]KA binding activity of membranes prepared from rat brain, MIN6 and RINm5F β-cell lines

Membrane sample	[^{3}H]AMPA binding activity		[^{3}H]KA binding activity	
	(fmol/mg)	(% of brain)	(fmol/mg)	(% of brain)
Rat brain	381.8 ± 32.4	100	196.1 ± 12.7	100
MIN6	80.6 ± 5.9	21.1 ± 1.6	13.0 ± 0.6	6.6 ± 0.3
RINm5F	21.6 ± 1.1	5.7 ± 0.3	7.7 ± 0.7	4.0 ± 0.4

Specific [^{3}H]AMPA and [^{3}H]KA binding activities were determined by centrifugation assay using PEG precipitation. The binding assays were performed in triplicate, and the values are the mean ± S.D. from the three independent determinations.

3. Results

3.1. Characterization and specificity of the antibodies

To study the expression pattern of different ionotropic glutamate receptor subunit proteins, antipeptide antibodies were raised against the N-terminal domain of NMDAR1 (residues 436–450 [9]), the C-terminal region of KA-2 (residues 950–965 [10]) and N- and C-terminal epitopes of GluR1 (residues 253–267 and 877–889 [19]). The production, purification and characterization of anti-GluR1$_{(253–267)}$ and anti-GluR1$_{(877–889)}$ rabbit polyclonal antibodies have been described previously [12,20,21].

The specificity of the anti-KA-2$_{(950–965)}$ rabbit polyclonal antibody was tested on immunoblots of cDNA-transfected COS-7 cells expressing various kainate-preferring glutamate receptor subunits (Fig. 1). This antibody detected a 120 kDa protein only in those cells which were transfected with cDNA coding for the KA-2 protein. According to amino acid sequences reported by Herb et al. [10], the antigenic domain of the KA-2 subunit used here has no homology with the corresponding region in KA-1, which was confirmed previously with an antibody raised against residues 954–965 of KA-2 [22]; therefore the 120 kDa band represents only the KA-2 glutamate receptor subunit. A similar protein was labelled in membrane samples isolated from rat brain. The immunostaining with the anti-KA-2$_{(950–965)}$ antibody was stronger in samples prepared from cerebral cortex or hippocampus than with membranes from cerebellum or spinal cord (Fig. 1). The reactivity of the antibody with the native glutamate receptor protein was also tested by immunoprecipitation of the [^{3}H]kainate binding activity. Immunoprecipitation studies were carried out on rat brain (cerebral cortex) membranes solubilized in Triton X-100 under conditions which give soluble binding sites for both AMPA and kainate [12,23]. Solubilized preparations were incubated with the antibody coupled to protein A-Sepharose and antibody binding was determined by measuring the reduction in [^{3}H]kainate or [^{3}H]AMPA binding relative to controls [12]. Using different anti-KA-2$_{(950–965)}$ immune sera; 42 ± 11% (rabbit R15) and 39 ± 8% (rabbit R16) of the total [^{3}H]kainate binding activity was immunoprecipitated while no [^{3}H]AMPA binding activity was removed.

Antibody to NMDAR1 was raised against a unique region of the receptor (residues 436–450) which does not have corre-

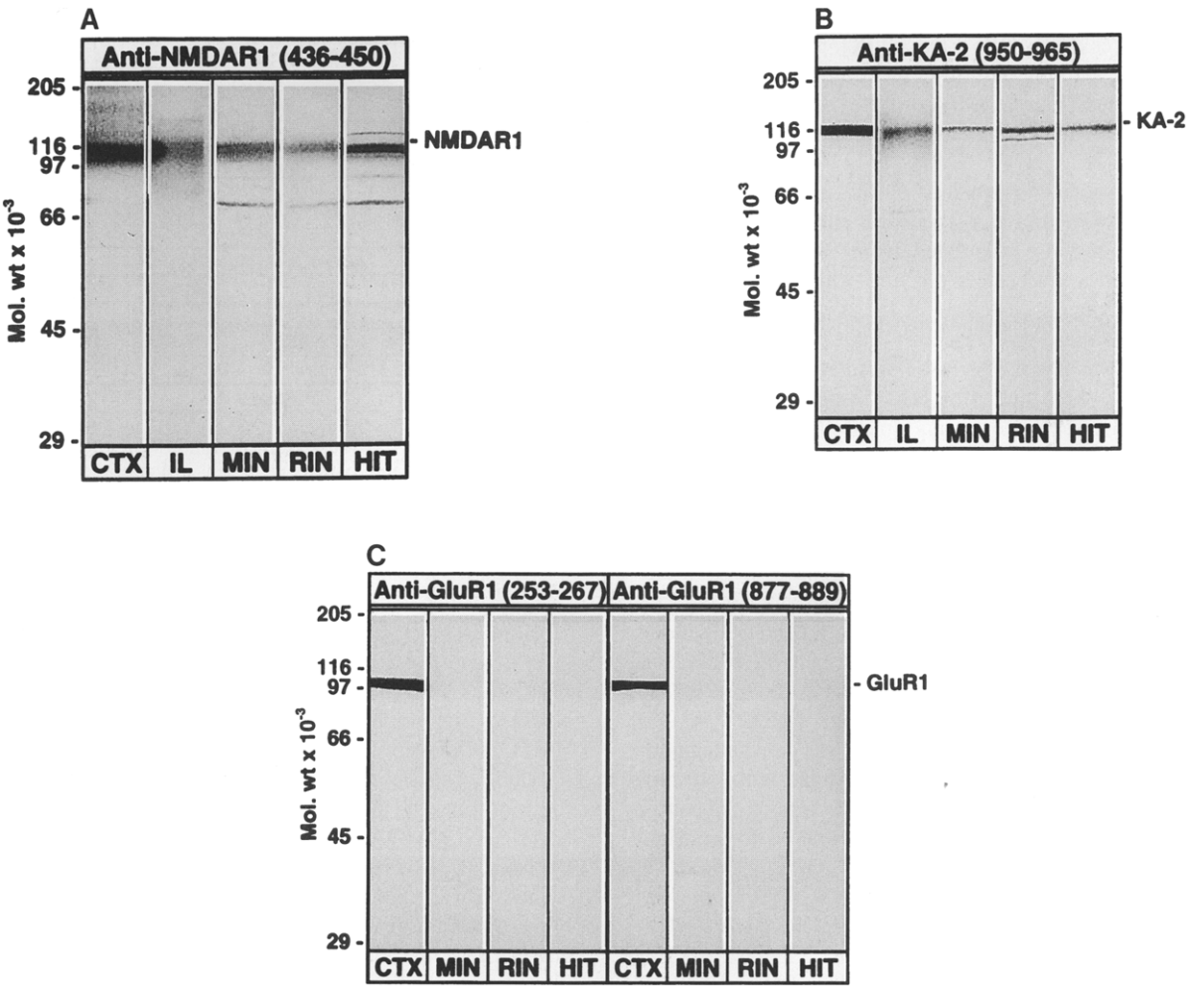

Fig. 2. Immunodetection of ionotropic glutamate receptor subunits in brain, pancreatic β-cells and islets of Langerhans Expression of NMDAR1, (A); KA-2, (B) and GluR1 (C) subunits was tested by subunit-specific antipeptide antibodies. Membrane fractions were prepared from rat cerebral cortex (CTX), rat islets of Langerhans (IL) and pancreatic β-cell lines. Membrane proteins (50 μg of protein or 100 islets/lane) were fractionated by SDS-PAGE, transferred to an Immobilon filter and probed with subunit-specific purified anti-NMDAR1$_{(436-450)}$ (A), anti-KA-2$_{(950-965)}$ (B), anti-GluR1$_{(253-267)}$ and anti-GluR1$_{(877-889)}$ (C) antibodies as described in section 2. The positions of the molecular weight markers are indicated on the left.

sponding regions in other non-NMDA ionotropic receptor proteins [24] and does not show sequence homology with the corresponding regions of other NMDA receptor subunits. To confirm the subunit specificity of the anti-NMDAR1$_{(436-450)}$ antibody, the corresponding regions of other NMDAR2 receptor subunits (residues 439–453 of NMDAR2A, residues 436–449 of NMDAR2B, residues 450–464 of NMDAR2C and residues 465–477 of NMDAR2D [25,26] were synthesized. Using a dot blot assay with these synthetic peptides, the anti-NMDAR1$_{(436-450)}$ antibody reacted only with the peptide derived from the NMDAR1 protein (not shown). On immunoblots, the anti-NMDAR1$_{(436-450)}$ polyclonal antipeptide antibody recognized a single band representing a protein with an apparent molecular weight of 115 kDa in membrane samples prepared from rat brain (Fig. 2A, CTX) or transfected COS-7 cells expressing the NMDAR1a receptor subunit (not shown).

Preimmune sera showed no immunoreacticity. For the immunoblot analysis, immunoaffinity purified antibodies were used routinely. The preincubation of these antipeptide antibodies with the respective synthetic peptide (20 μg peptide/ml) resulted in the disappearance of specific labelling.

3.2. *Immunochemical identification of different ionotropic glutamate receptor subunits in pancreatic β-cells and in islets of Langerhans*

Antibodies against the putative extracellular domain of the rat NMDAR1 glutamate receptor subunit detected a band of 115 kDa in membrane proteins prepared from rat brain, and showed a similar but weaker labelling of proteins in islets of Langerhans and β-cell lines (Fig. 2A). The molecular weight of these proteins closely approximates that expected for the NMDAR1 subunit on the basis of amino acid sequence (103,477 Da [9]) taking into account subunit glycosylation. By immunoblot analysis, the anti-KA-2 antibody detected a protein of 120 kDa which corresponds to the molecular weight of the glycosylated KA-2 subunit in rat brain membranes [22]. A lower amount of this subunit was also identified in islets of Langerhans and β-cell lines (Fig. 2B). Both antipeptide polyclo-

nal antibodies, which recognize different N- and C-terminal peptide sequences of the 105 kDa GluR1 protein [12,20,21], yielded the same staining pattern in membranes prepared from rat cerebral cortex (Fig. 2C). In contrast, neither anti-GluR1 antibody detected this receptor subunit in membranes prepared from β-cell lines (Fig. 2C).

Because the molecular weights of the immunochemically identified NMDAR1 and KA-2 receptor subunits were somewhat higher than those calculated from the amino acid sequences, we used a glycosidase enzyme, which specifically removes N-linked sugars, to determine the non-glycosylated molecular weight of these proteins. Enzymatic deglycosylation of NMDAR1 and KA-2 with PNGase F resulted in a lower molecular weight (103 kDa and 109 kDa, respectively) for these proteins both in cerebral cortex and in β-cell membranes (Fig. 3) consistent with the fact that both NMDAR1 and KA-2 have several consensus sites for N-linked glycosylation [9,10].

3.3. Determination of ligand binding activity of glutamate receptors in pancreatic β-cells

The presence of glutamate receptors β-cell was detected by [^{3}H]AMPA (in the presence of 100 mM KSCN) and [^{3}H]kainate binding in membrane samples prepared from rat brain and MIN6 or RINm5F β-cell lines (Table 1). Compared to the neuronal membrane fraction, much lower [^{3}H]AMPA and [^{3}H]kainate specific ligand binding activity was detected in pancreatic β-cell lines. These data are consistent with our immunoblotting studies in suggesting a lower amount of active receptor proteins in pancreatic β-cells compared to neuronal membranes.

3.4. The effect of ionotropic glutamate receptor agonists on insulin secretion

The rate of insulin secretion from rat islets of Langerhans at 3.3 mM glucose (3.0 ± 0.4 μU/h per islet) was not affected by 0.5 mM glutamate, NMDA or kainate. At 8.3 mM glucose, the rate of insulin secretion was increased by 0.5 mM NMDA (in the presence of the NMDA-receptor co-agonist glycine) from 65 ± 4 to 96 ± 8 μU/h per islet. Neither glutamate nor kainate (0.5 mM) had a significant effect. At 16.7 mM glucose 0.5 mM NMDA increased secretion from 130 ± 14 to 179 ± 20 μU/h per islet; 0.5 mM kainate elicited a smaller rise which did not achieve statistical significance; no effect of 0.5 mM glutamate was found. Even at 2.5 mM glutamate did not enhance insulin secretion at any of the glucose concentrations tested.

4. Discussion

4.1. Ionotropic glutamate receptor subunit proteins are expressed in pancreatic β-cells and in islets of Langerhans

Immunodetection with subunit-specific antibodies indicated the presence of NMDAR1 and KA-2 proteins in islets of Langerhans and the pancreatic β-cell lines MIN6, HIT T15 and RINm5F. The antibody reaction with the denatured receptor proteins in immunoblots and [^{3}H]AMPA and [^{3}H]kainate ligand binding assays of membranes suggest a significantly lower amount of receptor proteins in the islet and β-cell samples compared to membranes prepared from rat brain.

A limited population of the glutamate receptor subunit mRNAs was found in the mouse clonal β-cell line MIN6 using RT-PCR [4]. Detected subunits were GluR3, KA-2, NMDA1,

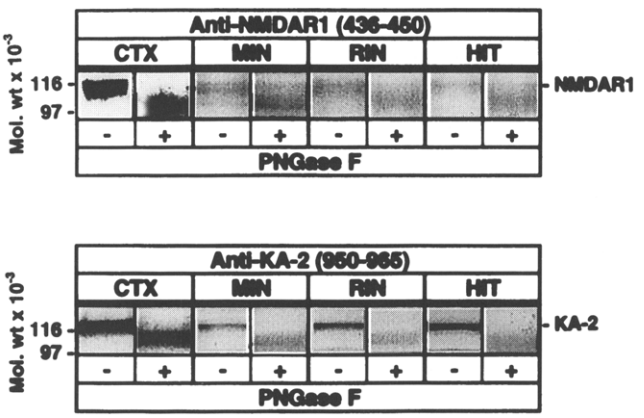

Fig. 3. Enzymatic deglycosylation of ionotropic glutamate receptor subunits in brain and pancreatic β-cells. Membranes (50 μg) from rat cerebral cortex (CTX), MIN6 (MIN), RINm5F (RIN) and HIT T15 (HIT) β-cell lines were incubated with (+PNGase F) or without (– PNGase F) glycosidases, subjected to SDS-PAGE and immunoblotted with anti-NMDAR1$_{(436-450)}$ or anti-KA-2$_{(950\ 965)}$ antibodies as indicated on the figure. The figure illustrates that NMDAR1 and KA-2 receptor subunits have approximately 10–12 kDa lower molecular weight after deglycosylation.

NMDAR2D; very low expression levels of GluR2 and NMDAR2C were also found. In the rat clonal β-cell line RINm5F, the same probes amplified a somewhat different combination of subunit mRNAs; GluR2, KA-1, KA-2, NMDAR1, NMDAR2D, and a very low signal for GluR5 [5]. In pancreatic islets the following receptor subunit mRNAs were found: GluR2, GluR3, GluR6, GluR7, KA-2, NMDAR1, NMDAR2D, and trace amounts of GluR1, NMDAR2A and NMDAR2C [5]. The two β-cell lines expressed different combinations of receptor subunit mRNAs probably because of the different species origin. However, RIN5mF, which originates from rat, also contained different mRNAs compared to rat islets of Langerhans. The different expression pattern of the receptor subtypes in this case can be explained by the presence of other cell types in pancreatic islets or by a change in the subunit pattern expressed on transformation of β-cells. Therefore from these PCR results it is difficult to conclude which subunits are present in the native pancreatic β-cells. Our data using subunit-specific immunoprobes indicate that GluR1 protein is not detectable in any of the β-cell lines studied whereas KA-2 and NMDAR1 subunit proteins are expressed in all studied β-cell lines and also in islets of Langerhans. This confirms the finding that mRNAs for KA-2 and NMDAR1 were present in MIN6, RINm5F and islet cells, and is consistent with the observation that GluR1 mRNA was not detected in β-cell lines [4,5].

Although some subunits can form functional homomeric receptors when expressed in oocytes or cultured cells, current evidence indicates that most functional receptor complexes found in brain are made up of more than one distinct subunit. Four related subunits (GluR1–4) make up the AMPA receptor hetero-oligomer complex. Electrophysiological experiments show that neither KA-1 nor KA-2 subunits form a functional receptor when expressed alone but do so when co-expressed with GluR5 or GluR6 [10,27], suggesting that functional ion channels in the central nervous system are made up of combinations of GluR5–7, KA-1 and KA-2. Expression of

NMDAR2A–D alone does not lead to formation of functional channels in *Xenopus* oocytes [25,28]. Formation of NMDA-operated channels, however, is greatly enhanced when any one of these NMDAR2 subunits is co-expressed with NMDAR1. Together these findings indicate that the NMDAR1 subunit may be essential for the formation of functional NMDA-operated channels in the CNS. Because one or more subunits in addition to NMDAR1 and KA-2 are probably necessary for expression of functional channels in mammalian cells, further studies with subunit protein specific probes are required to define the subunit composition of the functional β-cell glutamate receptor(s).

4.2. *Effect of ionotropic glutamate receptor agonists on insulin secretion*

We found that NMDA enhanced glucose-induced insulin secretion; however, under our experimental conditions the response was small and, moreover, glutamate, which is the endogenous ligand of the receptor, had no effect even at relatively high concentration (2.5 mM). In earlier studies, kainate, AMPA and NMDA in the presence of 3.3 mM glucose stimulated insulin secretion from MIN6 cells [4]. Both kainate and AMPA (but not NMDA) in the presence of 8.3 mM glucose moderately (1.2–1.8 fold) stimulated insulin secretion from rat islets of Langerhans [5]. In both of these two studies, the effect of glutamate was not reported, however, Bertrand et al. [29] reported a transient stimulation of insulin secretion by glutamate in perfused pancreas. This short transient insulin response to glutamate is probably blunted during the long-lasting static incubation applied for conventional insulin secretion measurements, which may reflect rapid desensitisation of these receptors [1]. The study of $[CA^{2+}]_i$ with the fluorescent Ca^{2+} indicator dye fura-2 and whole cell patch-clamp recording indicated the presence of functional NMDA, kainate and AMPA gated ion channels in β-cells, however, the frequency of glutamate receptor agonist-responsive β-cells was relatively low [4,5].

Our results clearly indicate that some of the ionotropic glutamate receptor subunit proteins are present in islets of Langerhans and in β-cell lines and may play a role in insulin secretion. However, the expression of varying receptor mRNAs in different β-cell lines, the rather modest effects of glutamate receptor ligands on insulin secretion, and the low incidence of glutamate-responsive β-cells suggest that caution should be exercised in ascribing major functional significance to these receptors in the regulation of insulin secretion. Further studies are needed to characterise and quantitate the various other ionotropic glutamate receptor subunits and their splice variants in β-cells in detail. The investigation of functional ionotropic glutamate receptors containing limited combinations of subunits in β-cell would be also a potentially useful experimental system.

Acknowledgements: We are grateful to Prof. Y. Miyazaki, University of Tokyo and Prof. C.B. Wollheim, University of Geneva, for providing MIN6 and RINm5F β-cells, respectively. The mammalian expression vectors containing the genes coding for the receptor GluR5–7 and KA-2 were the generous gift of Dr. Hannah Monyer at the Center for Molecular Biology, University of Heidelberg. The authors wish to thank Saul A. Richmond for the synthesis of the $KA\text{-}2_{(950\text{-}965)}$ peptide and are grateful to Mrs Gabrielle Johnson, Mrs Liz Norman and Mrs Linda Lawes for technical assistance. The authors thank Dr. Miguel Valverde and Dr. Simon Hardy for discussions. Dr. AnikóVáradi is an R.D. Lawrence Fellow of the British Diabetic Association.

References

[1] Mayer, M.L. and Westbrook, G.L. (1987) Prog. Neurobiol. 28, 197–276.
[2] Collingridge, G.L. and Lester, R.A. (1989) Pharmacol. Rev. 40, 143–210.
[3] Hollmann, M. and Heinemann, S. (1994) Annu. Rev. Neurosci. 17, 31–108.
[4] Gonoi, T., Mizuno, N., Inagaki, N., Kuromi, H., Seino, Y., Miyazaki, J. and Seino, S. (1994) J. Biol. Chem. 269, 16989–16992.
[5] Inagaki, N., Kuromi, H., Gonoi, T., Okamoto, Y., Ishida, H., Seino, Y., Kaneko, T., Iwanaga, T. and Seino, S. (1995) FASEB J. 9, 686–691.
[6] Tyndale, R.F., Hales, T.G., Olsen, R.W. and Tobin, A.J. (1994) J. Neurosci. 14, 5417–5428.
[7] Hales, T.S. and Tyndale, R.F. (1994) J. Neurosci. 14, 5429–5436.
[8] Sucher, N.J., Brose, N., Deitcher, D.L., Awobuluyi, M., Gasic, G.P., Bading, H., Cepko, C.L., Greenberg, M.E., Jahn, R., Heinemann, S.F. and Lipton, S.A. (1993) J. Biol. Chem. 268, 22299–22304.
[9] Moriyoshi, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N. and Nakanishi, S. (1991) Nature 354, 31–37.
[10] Herb, A., Burnashev, N., Werner, P., Sakmann, B., Wisden, W. and Seeburg, P.H. (1992) Neuron 8, 775–785.
[11] Klotz, I.M. and Heiney, R.E. (1962) Arch. Biochem. Biophys. 96, 605–612.
[12] Molnár, E., Baude, A., Richmond, S.A., Patel, P.B., Somogyi, P. and McIlhinney, R.A.J. (1993) Neuroscience 53, 307–326.
[13] Pow, D.V. and Crook, D.K. (1993) J. Neurosci. Methods 48, 51–63.
[14] Váradi, A., Molnár, E. and Ashcroft, S.J.H. (1995) Biochim. Biophys. Acta 1236, 119–127.
[15] Zukin, S.R., Young, A.B. and Snyder, S.H. (1974) Proc. Natl. Acad. Sci. USA 71, 4802–4807.
[16] Laemmli, U.K. (1970) Nature 227, 680–685.
[17] Christie, M.R. and Ashcroft, S.J.H. (1985) Biochem. J. 227, 727–736.
[18] Ashcroft, S.J.H. and Crossley, J.R. (1974) Diabetologia 11, 274–279.
[19] Hollmann, M., O'Shea-Greenfield, A., Rogers, S.W. and Heinemann, S. (1989) Nature 342, 643–648.
[20] Molnár, E., McIlhinney, R.A.J., Baude, A., Nusser, Z. and Somogyi, P. (1994) J. Neurochem. 63, 683–693.
[21] Baude, A., Molnár, E., Latawiec, D., McIlhinney, R.A.J. and Somogyi, P. (1994) J. Neurosci. 14, 2830–2843.
[22] Wenthold, R.J., Trumpy, V.A., Zhu, W.-S. and Petralia, R.S. (1994) J. Biol. Chem. 269, 1332–1339.
[23] Hunter, C., Wheaton, K.D. and Wenthold, R.J. (1990) J. Neurochem. 54, 118–125.
[24] Cockcroft, V.B., Ortells, M.O., Thomas, P. and Lunt, G.G. (1993) Neurochem. Int. 23, 583–594.
[25] Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B. and Seeburg, P.H. (1992) Science 256, 1217–1221.
[26] Ishii, T., Moriyoshi, K., Sugihara, H., Sakurada, K., Kadotani, H., Yokoi, M., Akazawa, C., Shigemoto, R., Mizuno, N., Masu, M. and Nakanishi, S. (1993) J. Biol. Chem. 268, 2836–2843.
[27] Sakimura, K., Morita, T., Kushiya, E. and Mishina, M. (1992) Neuron 8, 267–274.
[28] Meguro, H., Mori, H., Araki, K., Kushiya, E., Kutsuwada, T., Yamazaki, M., Kumanishi, T., Arakawa, M., Sakimura, K. and Mishina, M. (1992) Nature 357, 70–74.
[29] Bertrand, G., Gross, R., Puech, R., Loudatières-Mariani, M.-M. and Bockaert, J. (1992) Br. J. Pharmacol. 106, 354–359.