Differential Expression of Glutamate Receptor Subtypes in Rat Pancreatic Islets*

(Received for publication, November 3, 1995, and in revised form, March 8, 1996)

C. David Weaver†, Tom L. Yao‡, Alvin C. Powers§, and Todd A. Verdoorn¶

From the †Department of Pharmacology and the ‡Division of Endocrinology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-6600

Pancreatic islets of Langerhans are self-contained miniature organs responsible for maintaining metabolic homeostasis through the release of the hormones insulin, glucagon, somatostatin, and pancreatic polypeptide (PP). Control of hormone release involves complex interactions between circulating fuels and hormones, autocrine and paracrine regulation, and neuronal input. However, the final common pathway to glucagon secretion from the same preparation (7). Preliminary descriptions of functional glutamate receptors on a β cell line (8) and on isolated islet cells have also appeared (9). These observations raise important questions about the role of glutamate receptors in regulating hormone secretion from islet cells. For example, it is unclear which islet cell types actually contain glutamate receptors, and the functional characterization of these receptors was insufficient to clearly classify the glutamate receptors that appear on these cells. If glutamate serves as a mediator of communication between islet cells or between the central nervous system and the endocrine pancreas, it is essential to precisely define the cellular localization and functional properties of these receptors.

Ionotropic glutamate receptors subserve a large proportion of excitatory neurotransmission in the central nervous system. Three classes of glutamate receptors exist, named according to the agonists: N-methyl-D-aspartate (NMDA), AMPA, and kainate. Receptors of each class are comprised of heteromeric combinations of homologous subunits (10, 11). NMDA receptors are made of NMDAR1 plus one of seven NMDAR2 variants. AMPA receptors consist of combinations of GluRA, GluRB, GluRC, and GluRD. The GluR5, GluR6, GluR7, KA1, and KA2 subunits form neuronal kainate receptors. The exact subunit configuration of receptors in neurons is uncertain, but we do know that different combinations of subunits expressed in host cells produce receptors having unique functional properties. For example, AMPA receptors lacking a GluRB subunit are permeable to calcium and have current-voltage (I-V) relations with strong inward rectification (12, 13). When GluRB is present the I-V relation is more linear, and the channels have lower permeability to calcium. Functional kainate receptors are formed increasing intracellular calcium and activating calcium-dependent secretion mechanisms (1, 2). Islet cell ion channels can be regulated by intracellular second messengers, G-proteins, and energy levels, but very few of them respond directly to extracellular stimuli. One mechanism for rapidly translating extracellular chemical signals into electrical signals is through ligand-gated ion channels. γ-amino butyric acid (GABA) A receptors have been observed in many pancreatic islet cell types. Activation of these receptors inhibits glucagon secretion (3) and depolarizes some types of β cell lines (4). Since the GABA synthesizing enzyme, glutamic acid decarboxylase, is found in β cells (5), it has been postulated that GABA is co-released with insulin from the β cells and that it may mediate communication among islet cells themselves (3).

Mechanisms whereby glutamate receptor activation may control hormone secretion from pancreas or preparations of pancreatic cells have recently been described. Glutamate was found to potentiate glucose-stimulated secretion of insulin from perfused pancreas via actions at α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-type glutamate receptors (6). Interestingly, AMPA receptor agonists were also shown to increase glucagon secretion from the same preparation (7).

Immunocytochemistry was carried out on sections of rat pancreas to localize the expression of glutamate receptor subunits and the major pancreatic peptide hormones. Glutamate receptor expression was concentrated in pancreatic islets, and each islet cell type expressed different neuronal glutamate receptors of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate classes. AMPA receptor subunits were expressed in α, β, and pancreatic polypeptide cells, whereas kainate receptors were found predominantly in α and δ cells. Patch clamp electrophysiology was used to measure the functional properties of islet cell glutamate receptors. L-glutamate and other glutamate receptor agonists evoked currents in islet cells that were blocked by the selective AMPA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione and potentiated by cyclothiazide in a manner indistinguishable from that of neuronal AMPA receptors. Activation of islet cell AMPA receptors produced steady-state current cations that depolarized the cells an average of 20.7 ± 5.4 mV (n = 6). Currents mediated by functional kainate receptors were also observed in a line of transformed pancreatic α cells. Thus, L-glutamate probably regulates islet physiology via actions at both AMPA and kainate receptor classes. The pattern of receptor expression suggests that glutamate receptor activation may have multiple, complex consequences for islet physiology.

1. This work was supported by the Vanderbilt Diabetes Research and Training Center, RO1 NS 30945 (to T. A. V.) and Individual National Research Service Award NS 09788 (to C. D. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
2. To whom correspondence should be addressed: Dept. of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232-6600. Tel.: 615-322-2207; Fax: 615-343-6532; E-mail: verdoota@ctrvax.vanderbilt.edu.
3. The abbreviations used are: PP, pancreatic polypeptide; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; FITC, fluorescein isothiocyanate; GABA, γ-amino butyric acid; Cy3, indocarbocyanine; NMDA, N-methyl-D-aspartate; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; MOPS, 3-(N-morpholino)propanesulfonic acid.
when individual GluR5 or GluR6 subunits are expressed alone (14, 15), but they also can combine with KA2 to form heteromeric receptors (16). Glutamate is probably the natural agonist for AMPA and kainate receptors in the brain. Although AMPA appears selective for the AMPA class, kainate will activate both kainate and AMPA receptor classes. AMPA and kainate receptors can be distinguished based on the desensitization behavior of kainate-induced currents and their sensitivity to allosteric potentiators, such as cyclothiazide (17).

We have examined glutamate receptors in pancreatic islets using histochemical and physiological approaches. Immunocytochemistry was used to identify particular islet cell types that express glutamate receptor subunits, and patch clamp electrophysiology was used to characterize the functional glutamate receptors found on islet cells. We show for the first time that AMPA receptors consisting of GluRB, GluRC, and perhaps GluRA subunits are functionally expressed on α and β cells in pancreatic islets. In addition, α cells express functional kainate receptors, whereas δ cells appear to express a kainate receptor subunit protein KA2, whose functional significance is uncertain. These results indicate that glutamate receptors subserve specialized roles in islet physiology.

**MATERIALS AND METHODS**

GluR5/67 antibody was purchased from Pharmingen. GluRA, GluRB/C, GluRD, and NMDAR1, antibodies were purchased from Chemicon. GluR6/7 and KA2 antibodies were a gift from Dr. Robert Wenthold. Guinea pig anti-insulin antiserum was purchased from ICN Biochemicals. Guinea pig anti-glucagon and guinea pig anti-pancreatic polypeptide antisera was purchased from Linco Research. Sheep anti-somatostatin antisera was purchased from Cortex Biochem. Indocarbocyanine (Cy3)-conjugated anti-rabbit IgG, ML grade; Cy3-conjugated donkey anti-rabbit IgG, IgM specific; fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG, ML grade; FITC-conjugated donkey anti-sheep IgG, ML grade; normal rabbit IgG, and normal mouse IgG were purchased from Jackson ImmunoResearch. Alkaline phosphatase-labeled goat anti-rabbit IgG was purchased from DAKO. Triton X-100 Surface-Amps and bichininonic acid protein assay reagents were purchased from Pierce. EM grade 16% paraformaldehyde solution and tissue-freezing medium were purchased from Electron Microscopy Sciences. Poly-Aqua/Mount was purchased from Electron Microscopy Sciences. Protein assay reagents were purchased from Pierce. EM grade 16% sucrose solution was purchased from Sigma. All other chemicals were of reagent grade or higher.

Islet Isolation and Cell Culture—Pancreatic islets of Langerhans were isolated from 200–250 g male Sprague-Dawley rats using the collagenase digestion technique essentially as described (18). Islets were cultured at 37 °C in RPMI 1640 containing 5 mM glucose, 10% fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin for up to 1 week on Matrigel-coated glass coverslips. When dissociated islet cells were used, they were prepared as described (19) and cultured for up to 2 days in 35-mm plastic dishes (Falcon). αTC-6 and αTC-9 cells were cultured as described previously (20).

Immunocytochemistry—Sprague-Dawley rats (200–250 g) were killed by decapitation. Pancreata were removed and immersed in ice for 4–6 h in phosphate-buffered saline (PBS) containing (in mM) 137 NaCl, 2.7 KCl, 1.5 KH2PO4, 100 Na2HPO4 and 4% paraformaldehyde. Pancreata were then equilibrated in 30% sucrose dissolved in PBS containing (in mM) 137 NaCl, 2.7 KCl, 1.5 KH2PO4, 100 Na2HPO4 overnight at 4 °C, embedded in tissue-freezing medium (OCT compound), cryosectioned (5 μm), and mounted on glass slides. For immunostaining, slides were rehydrated in PBS. Sections stained with the GluR5/67 antibody were treated according to the supplied protocol. For all other antibodies, sections were permeabilized in 0.2% Triton X-100 in PBS and blocked for 1 h in 5% normal donkey serum in PBS. The blocking solution was decanted, and the sections were incubated overnight at 4 °C with affinity-purified anti-glutamate receptor antibodies diluted to the following concentrations in PBS containing 0.1% Triton X-100 and 1% bovine serum albumin: GluRA, 1 μg/ml; GluRB/C, 0.5 μg/ml; GluRD, 1 μg/ml; GluR6/7, 0.5 μg/ml; NMDAR1, 0.5 μg/ml. As a control anti-glutamate receptor antibodies were omitted or replaced with normal IgG. Following the incubation, the sections were washed three times for 10 min per wash with PBS containing 0.1% Triton X-100. For detection of tissue-bound anti-glutamate receptor antibody the sections were incubated for 1 h with a 1:500 dilution of Cy3-conjugated donkey anti-rabbit IgG or donkey anti-ML IgM (IgG-specific) (Cy3-conjugated donkey anti-rabbit IgG, IgM specific; fluorescein isothiocyanate (FITC)-conjugated donkey anti-sheep IgG, ML grade; normal rabbit IgG, and normal mouse IgG were purchased from Jackson ImmunoResearch. Alkaline phosphatase-labeled goat anti-rabbit IgG was purchased from DAKO. Triton X-100 Surface-Amps and bichininonic acid protein assay reagents were purchased from Pierce. EM grade 16% sucrose solution was purchased from Sigma. All other chemicals were of reagent grade or higher.

Western blots, islets, αTC-6/9 cells, or rat brain cortex tissue were extracted on ice in an extraction buffer that contained (in mM) 20 TRIS, pH 7.5, 500 NaCl, 1 phenylmethylsulfonyl fluoride, 0.001 leupeptin, 0.001 pepstatin A, 0.001 pepstatin A sonicated on ice twice for 15 s at 1-min intervals and centrifuged at 100,000 × g for 1 h at 4 °C. The supernatant fraction was discarded, and the pellet was rinsed twice with extraction buffer. The pellet was resuspended in a buffer containing (in mM) 20 MOPS, pH 7.5, 0.1 M glycerol, 1 phenylmethylsulfonyl fluoride, 0.001 leupeptin, 0.001 pepstatin A, 0.001 pepstatin A. Western blots were obtained using the bichininonic acid assay method (21). Proteins were separated on 7.5% (w/v) polyacrylamide gels using the method described by (22) and transferred to nitrocellulose membranes. The membranes were blocked with 10% (w/v) nonfat dry milk in PBS for 1 h, washed three times for 10 min in PBS containing 0.05% (v/v) Tween 20, and probed for 1 h with anti-glutamate receptor antibodies diluted in PBS containing 0.5% (w/v) goat serum. The dilution factors for the anti-glutamate receptor antibodies were the same as those used for the immunocytochemistry studies. Following incubation with primary antibody the blots were washed three times for 10 min in Tris-buffered saline (TBS) solution, which contained (in mM) 25 Tris, pH 8.0, 137 NaCl, 2.7 KCl. The blots were then incubated with a 1:10,000 dilution of alkaline phosphatase-labeled goat anti-rabbit IgG in TBS containing 0.05% (v/v) Tween 20 and 0.5% (v/v) goat serum, washed three times for 10 min in TBS, and washed once for 10 min in alkaline phosphatase buffer, which contained (in mM) 100 Tris, pH 8.0, 100 NaCl, 5 MgCl2. The blots were developed using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates dissolved in alkaline phosphatase buffer.

Electrophysiology—Patch clamp electrophysiology was performed on intact and dissociated islets at room temperature using either an Axopatch 200 (Axon Instruments) or a Dagan Connector (Dagan Corporation) amplifier. Signals were filtered using a Frequency Devices 8-pole Bessel filter, recorded on a Macintosh Quadra 800 or 110x computer, and analyzed using Igor and Microsoft Excel computer programs. Voltage clamp recordings were made using either conventional whole cell mode or the perforated patch technique. All current clamp recordings were made with perforated patches. Only cells showing a combined seal/input resistance of greater than 1 GΩ were included for current clamp studies, and measurements were made only after the series resistance had dropped to a stable value usually less than 20 MΩ. For control experiments, pipettes were filled with (in mM) KCl, 135 CsCl, 1 MgCl2, 11 EGTA, 10 HEPES, pH 7.3. For perforated patch, pipettes were filled with (in mM) 10 KCl, 10 NaCl, 70 KSO4, 2 MgCl2, 10 HEPES, pH 7.3, and 240 μM amphotericin B. The extracellular solution contained (in mM) 150 NaCl, 2.5 KCl, 2.5 CaCl2, 1 MgCl2, 5 glucose, 10 HEPES, pH 7.3. Ligands were dissolved in the extracellular solution except for those used to test for NMDA receptors. These solutions did not contain Mg2+. In most experiments ligands were applied by controlled superfusion using a multibarreled pipette. Rapid agonist application was performed as described (23).
AMP messenger RNA (mRNA) and kainate receptor decay constants were calculated from curves fit to single exponential equations. Current-voltage relations were obtained by ramping the voltage from -80 mV to +80 mV at a rate of 40 mV/s. Currents measured in the absence of agonist were digitally subtracted from those measured in the presence of agonist to yield the agonist-evoked I-V curve. Reversal potentials were determined from a least-squares fit of the I-V relation to third order polynomial equations. Rectification ratios were calculated by dividing the conductance measured at +50 mV by that measured at -50 mV.

RESULTS

Immunocytochemistry was used to examine the expression pattern of glutamate receptors in cryosections of whole pancreas. To identify the types of islet cell that expressed glutamate receptors, each section was also stained with an antibody specific for one of the major pancreatic islet hormones. An antibody against insulin stained the core of the islets and was used as marker for \( \beta \) cells, an antibody that recognizes gluta-gon specifically stained \( \alpha \) cells, \( \delta \) cells were labeled with a somatostatin antibody, and cells positive for pancreatic polypeptide were referred to as PP cells. Laser scanning confocal microscopy was used to identify cells in which glutamate receptor immunoreactivity co-localized with that of the islet hormones. Sections were made from three different adult rats, and except where noted the results did not differ between animals. An antibody specific for the AMPA receptor subunits GluRB and GluRC (GluRB/C) strongly stained large portions of every islet examined but did not stain the surrounding pancreatic acinar tissue. Islets were labeled in the center and the mantle, but the cells in the core of the islet showed more intense staining than did the cells near the edges. Based on double labeling with antibodies against GluRB/C and hormone markers, GluRB/C subunits appeared in \( \alpha, \beta \), and PP cells but not in \( \delta \) cells (Fig. 1). Although the levels of GluRB/C varied between
islet cells in the core, we were unable to detect core cells that were positive for only insulin or GluRB/C. Similar co-localization of GluRB/C immunoreactivity with that of glucagon (Fig. 1B) and PP (not shown) was also apparent in the mantle of islets. GluRB/C antibody staining did not co-localize with immunoreactivity for somatostatin (Fig. 1C). An antibody specific for the AMPA receptor subunit GluRA also stained sections from two rats (not shown). However, the staining with the GluRA antibody was generally weaker than that of the GluRB/C antibody, and in one of the two rats staining could only be visualized using immunoperoxidase detection with a metal-enhanced dianinobenzidine substrate. In both rats the GluRA staining was restricted to the islet core. In the rat where immunofluorescent double labeling was possible, the GluRA staining co-localized only with insulin.

Kainate receptor subunits were also detected in these pancreatic sections and were concentrated in islets but not pancreatic acinar cells. Strong immunoreactivity for the kainate receptor subunits, GluR6/7, was observed in $\alpha$ cells but not in $\beta$ cells (Fig. 2, A and B). GluR6/7 immunoreactivity was not observed in $\delta$ cells (Fig. 2B, inset), nor was GluR6/7 protein detected in PP cells (not shown). To assure the specificity of the GluR6/7 labeling we also stained sections with a monoclonal antibody directed against the GluR5/6/7 subunits. This antibody was made against a different epitope of these kainate receptor subunits but stained the same population of cells recognized by the GluR6/7 polyclonal antibody. This supports the assertion that GluR6/7 staining represents kainate receptor subunits. Islet cells were also labeled with an antibody specific for the KA2 subunit of kainate receptors. This immunoreactivity appeared only in $\delta$ cells (Fig. 2C) and not in $\beta$ (Fig. 2C, inset), $\alpha$, or PP cells (not shown).

Other glutamate receptor subunits were notably absent from the pancreatic islets. An antibody specific for the AMPA receptor subunit, GluRD (Fig. 1D) did not stain pancreas sections above background, indicating that the AMPA receptors in islets

**FIG. 2.** Kainate receptor subunits but not NMDAR1 are expressed in pancreatic islet cells. Shown are superimposed images of anti-glutamate receptor immunoreactivity (red) and neuroendocrine cell marker immunoreactivity (green) detected by the indirect fluorescence technique. Yellow represents co-localization of the two antigens. Illustrated are the following: A, GluR6/7 and insulin (scale bar = 50 $\mu$m); B, GluR6/7 and glucagon (scale bar = 50 $\mu$m); inset, GluR6/7, and somatostatin (scale bar = 25 $\mu$m); C, KA2 and somatostatin (scale bar = 50 $\mu$m); inset, KA2 and insulin (scale bar = 50 $\mu$m); D, NMDAR1 and glucagon (scale bar = 50 $\mu$m); inset, normal rabbit IgG and glucagon (scale bar = 50 $\mu$m). GluR6/7 appears to be exclusively coexpressed with glucagon in islet cells, while KA2 coexpression is only visible with somatostatin. NMDAR1 staining was not detectable in islet cells.
anti-glutamate receptor antibodies recognize the same proteins in brain, islets, and islet cell lines. Shown is a Western blot of crude membrane proteins from brain (25 μg of total protein/lane), islets (25 μg of total protein/lane), and αTC-6 cells (50 μg of total protein/lane). Proteins were resolved on 7.5% (w/v) polyacrylamide gels, blotted to nitrocellulose, and probed with anti-glutamate receptor antibodies. Immunoreactivity was detected using alkaline phosphatase-labeled goat anti-rabbit IgG with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates. Anti-GluRB/C antibodies recognized proteins of the same apparent molecular weight in both brain and αTC-6 cells. GluR6/7 and KA2 immunoreactivity was identical to that of αTC-6 cells. Molecular weights (left side) were determined from standards.

AMPAR and kainate subunit receptors were also detected in Western blots of crude membrane fractions from brain, islets, and islet cell lines (Fig. 3). GluR6/7 and KA2 immunoreactivity was observed in brain and αTC-6 membranes. The apparent molecular weight of these proteins was appropriate for glutamate receptor subunits. We were unable to detect GluR6/7 or KA2 immunoreactivity in isolated islets, perhaps due to limiting amounts of islet tissue, the low proportion of α and β cells, or proteolytic breakdown of these antigens caused by the collagenase digestion technique.

To examine the electrophysiological consequences of glutamate receptor activation in pancreatic islet cells we used the patch clamp technique and examined the effects of glutamate receptor agonists on membrane potential and ionic currents in these cells. Current clamp measurements of membrane potential were made using the perforated patch technique and revealed a variety of cell types in our preparation. The average resting membrane potential of islet cells in 5 mM glucose (a concentration considered to be nonstimulating for β cells) was −59 ± 2.5 mV (n = 22). Of seven cells challenged with 16.7 mM glucose, three depolarized and fired action potentials, three gave no response to glucose, and one was firing spontaneous action potentials that were inhibited by high glucose. Two other cells also fired spontaneous action potentials, but they were not tested with high glucose. The observations suggest that our isolated islet preparations contained α and β cells in addition to some cells that were more difficult to classify.

A subpopulation of islet cells (6 of 22) also responded to the application of 300 μM L-glutamate with marked depolarization (Fig. 4A). The average glutamate-induced depolarization was 20.7 ± 5.4 mV (n = 6). In one case the depolarization evoked by L-glutamate was sufficient to induce firing of action potentials. One of the six glutamate receptor positive cells also depolarized in response to high glucose. We were unable to test the other five with high glucose. When we measured the current generated by 300 μM L-glutamate in these cells under voltage clamp at −70 mV we found it was often little more than an increase in membrane noise. We gave a measurable inward current at −70 mV (amplitude = 3.1 pA). In 12 cells measured under voltage clamp the average amplitude of inward current evoked by 300 μM L-glutamate at −70 mV was 2.0 ± 0.7 pA. We reasoned that rapid desensitization attenuated the currents induced by our relatively slow application of agonist, and therefore we measured L-glutamate currents in the presence of cyclothiazide, which blocks desensitization of neuronal AMPAR receptors (24). Cyclothiazide strongly potentiated the steady-state current evoked by L-glutamate (Fig. 4B, average potenti-
Agonist response characteristics of native islet cells
Islet cells were held at −70 mV under voltage clamp and challenged with 300 μM L-glutamate, 100 μM AMPA, or 300 μM kainate each in the presence of 50 μM cyclothiazide. NMDA (30 μM) plus 10 μM glycine in the absence of extracellular Mg2+ was used to screen for NMDA receptors, and GABA-A receptors were examined with 30 μM GABA. In eight cells tested with 300 μM kainate in the absence of cyclothiazide the average current had a mean amplitude of 4.1 ± 1.5 pA. The mean amplitude represents the average response (± S.E.M.) in cells producing measurable currents.

| Agonist          | Number of cells tested | Percentage positive | Mean amplitude    |
|------------------|------------------------|---------------------|-------------------|
| L-Glutamate      | 146                    | 26                  | 51.0 ± 12.2 (n = 36) |
| Kainate          | 147                    | 19                  | 24.7 ± 8.0 (n = 26) |
| AMPA             | 130                    | 20.8                | 29.1 ± 9.9 (n = 25) |
| NMDA + glycine   | 128                    | 0.8                 | 87.2 (n = 1)       |
| GABA             | 208                    | 5.3                 | 20.7 ± 5.5 (n = 7) |

**Fig. 5. Functional AMPA and kainate receptors are present on αTC-9 cells.** A, rapid application of L-glutamate (holding voltage = −60 mV) to these cells produced a desensitizing peak current followed by a much smaller steady-state component. B, cyclothiazide (50 μM) prevented observable desensitization and potentiated both the peak and steady-state current in this cell. C, shown is a rapidly desensitizing current evoked by kainate (holding voltage = −60 mV) in a different αTC-9 cell. D, the L-glutamate current in this cell shows rapid desensitization as well, but the ratio of the peak and steady-state amplitude differed from that seen in cells expressing only AMPA receptors (see A and B above). Cyclothiazide had no effect on the currents in this cell (not shown).

**Discussion**

Using complementary approaches we have shown that pancreatic islet cells express glutamate receptors of the AMPA and kainate classes. Immunocytochemistry using double labeling techniques showed that AMPA receptor subunits were expressed in α, β, and PP cells but were generally absent from δ cells. Kainate receptor subunits were expressed in α and δ cells and were not found in β or PP cells. Patch clamp of islet cells revealed that functional AMPA-type receptors appeared on the plasma membrane in about 25% of the cells tested. A trans-

![Image](http://www.jbc.org/)
formed line of α cells also expressed functional kainate-type receptors that could be detected with rapid agonist application techniques. The expression of different glutamate receptor types on islet cells specialized to secrete different hormones suggests that communication within a islet is mediated by machinery similar to that used by the central nervous system.

Unambiguous identification of receptor expression in pancreatic islets depends on the specificity of the antibodies used here. The NMDAR1, GluR, GluR, and GluRB/C antibodies have been extensively characterized using transfected cells and brain tissue (29–31). The NMDAR1 antibody recognizes splice variants 1a, 1b, 2a, and 2b. While these are the major splice variants expressed in brain, it remains possible that others are expressed in islets. In our hands, the GluRB/C antibodies only stained proteins of the appropriate molecular weight in membranes isolated from brain, islets, and islet cell lines when present in sufficient quantities. The GluR6/7 and KA2 antibodies also show appropriate staining patterns in brain tissue (32) and recognize bands of the appropriate size in Western blots with membrane proteins from brain and αTC-6 cells. The low density of these subunits in islet cell membranes prevented detection of GluR6/7 and KA2 antigens in Western blot experiments. This may have been due to loss or damage of α and δ cells as a result of the collagenase islet isolation technique. However, a monoclonal antibody selective for GluR5/6/7 subunits (33) strongly stained islet mantle cells supporting the localization of kainate receptor subunits in α and δ cells.

Similar to the situation in the central nervous system, the role of the KA2 subunit in δ cells of islets is uncertain. We were unable to study putative KA2-containing receptors with electrophysiology, since δ cells are in low abundance in islets and no δ cell line was available. The apparent expression of KA2 in the αTC-6 cell line may result from dedifferentiation of these cells, since no KA2 was detected in native α cells.

The current densities in isolated islet cells were low enough to make complete characterization of the receptors difficult. It is possible that islet isolation procedures, which involve proteolytic digestion of the pancreatic acinar tissue, could have damaged or destroyed some of the receptors before our patch clamp experiments. Even so, the steady-state currents were large enough to depolarize islets when agonist was applied slowly, long after desensitization was complete. If glutamate is released rapidly onto the receptors, similar to what happens at synapses in the central nervous system, the much larger desensitizing component of the response should have larger, albeit brief, depolarizing effects.

The functional glutamate receptors in islet cells have properties similar to those of receptors found in neurons or expressed in host cells from cloned subunits. Islet cell AMPA receptors respond to L-glutamate, AMPA, and kainate, are blocked by the competitive antagonist, CNQX, and are potentiated by cyclothiazide. These properties are shared by neuronal AMPA receptors. The current-voltage relation of islet cell AMPA receptors indicate that they are typical nonsel ective cation channels. Some cells showed noticeable inward rectification, which is a hallmark of AMPA receptors lacking a GluRB subunit. Such receptors would be expected to have higher permeability to divalent cations such as calcium. Because the currents were so small, it was difficult to determine the calcium permeability of these receptors with standard ion substitution studies. In one cell showing inward current we did detect a very small inward current at −70 mV after replacing all extracellular monovalent cations with Ca2+. The possibility that some AMPA receptors in islets flux Ca2+ suggests that AMPA receptor activation could bypass the voltage-dependent Ca2+ channels and directly influence hormone secretion by changing intracellular [Ca2+]. Imaging of intracellular calcium in islet cells is currently in progress to address this possibility.

The staining pattern of GluRB/C subunits indicates that the vast majority of islet cells express AMPA receptor subunits. By contrast, our functional studies indicate that only a subpopulation of them actually have significant numbers of functional receptors on the plasma membrane. The proportion of cells showing functional receptors remained consistent between preparations and is not markedly different from that seen in the αTC6 and αTC9 cell lines or the insulinoma cell line, GKP3.2. The perinuclear pattern of receptor subunit staining suggests that much of the protein is intracellular as opposed to the plasma membrane (34). Similar observations have been made using these antibodies in brain sections (35). The intracellular location of glutamate receptor subunit proteins and the fact that only some islet cells express functional receptors suggest that the presence of functional receptors may be regulated by environmental or metabolic factors. Alternatively, the presence or absence of functional glutamate receptors could be a manifestation of diversity among cells of a given islet cell type. For example, heterogeneity in glucose sensitivity among β cells has been described (36). This heterogeneity could extend to the presence or absence of functional L-glutamate receptors.

Although formally possible, it seems unlikely that glutamate receptors are functional only in one type of islet cell (α, β, δ, or PP). Some cells that were depolarized by L-glutamate showed spontaneous action potentials similar to those seen in α cells, whereas other cells depolarized and fired action potentials in response to glucose, which is typical of β cells. The fact that L-glutamate depolarizes islet cells via actions at AMPA receptors supports the view that AMPA receptor activation can modulate hormone secretion from pancreatic islets. In a perfused pancreas preparation Bertrand et al. have shown that activation of AMPA, but not NMDA receptors, can influence secretion of insulin (6) and glucagon (7). In these studies perfusion of agonists stimulated secretion of both hormones, which are generally considered to have opposing physiological actions. Since AMPA receptors are found on both glucagon- and insulin-secreting cells this result is not surprising. However, it seems unlikely that the natural mode of receptor activation involves changes in plasma glutamate levels because that would stimulate the release of two physiological antagonistic hormones. Recent reports also support the presence of functional glutamate receptors on islet cells and cell lines derived from transformed β cells (8, 9). Besides showing that glutamate receptor activation depolarizes some islet cells these workers also showed that receptor activation can increase intracellular calcium. The postulated presence of functional NMDA receptors on islet cells awaits confirmation. The pharmacology of the NMDA responses, in particular the effects of antagonists, were incompletely described, and even though AMPA and perhaps kainate receptor activation stimulated insulin secretion in islet cells, NMDA could not (9). NMDA receptor ligands were likewise unable to modulate hormone secretion in perfused pancreas preparations (6, 7).

Islet cells have much in common with neurons. The most notable similarity involves the expression of proteins specialized for synaptic transmission. Islet cells contain presynaptic proteins associated with vesicular secretion such as synaptotagmin (37). They also express GABA-A (3, 4) and glutamate receptors, which are major postsynaptic receptors in the mammalian central nervous system. The presence of such specialized synaptic machinery in islets suggests that synaptic-like

2 C. D. Weaver, J. G. Partridge, T. L. Yao, J. M. Moates, M. A. Magnuson, and T. A. Verdoorn, manuscript in preparation.
communication is important for normal islet physiology. This machinery may subserve communication between the central nervous system and islets. For example, GABA-containing nerve fibers have been detected in or near islets (38). Differences in electrical activity between isolated islets and those measured in vivo may be due to neuronal inputs that are present in vivo but lacking in vitro (39). Islet cell AMPA and kainate receptors would be well suited for translating neuronal inputs into electrical signals that modify secretory properties. It will be of interest to determine if the coeliac ganglia, which contain glutamatergic nerve fibers have been detected in or near islets (38). Differentiation of this manuscript, Dr. Tom J. et al. advise and support with the immunohistochemistry, and Anita Perry-Lane, Keivan Shahroki, and Raymond Green for excellent technical assistance.

**REFERENCES**

1. Ashcroft, F. M., Proks, P., Smith, P. A., Aamala, C., Bokvist, K., and Rorsman, P. (1994) J. Cell. Biochem. 55, 54–65
2. Rorsman, P., Ashcroft, F. M., and Berggren, P.-O. (1991) Biochem. Pharmacol. 41, 1783–1790
3. Rorsman, P., Berggren, P.-O., Bokvist, K., Ericson, H., Mohler, H., Ostenson, C.-G., and Smith, P. A. (1989) Nature 341, 233–236
4. Von Blankenfeld, G., Turner, J., Ahnert-Hilger, G., John, M., Enkvist, M. O. K., Stephenson, F., Kettenmann, H., and Wiedemann, B. (1995) Pflugers Arch. Eur. J. Physiol. 430, 381–388
5. Vincent, S. R., Høkfelt, T., Wu, J.-Y., Elde, R. P., Morgan, L. M., and Kimmel, J. R. (1983) Neuroendocrinology 36, 197–204
6. Bertrand, G., Gross, R., Puech, R., Lobalber-Mariani, M. M., and Boeckart, J. (1992) Br. J. Pharmacol. 106, 354–359
7. Bertrand, G., Gross, R., Puech, R., Lobalber-Mariani, M. M., and Boeckart, J. (1993) Eur. J. Pharmacol. 237, 45–50
8. Goncal, T. Muzino, N., Inagaki, N., Kurony, H., Seino, Y., Miyazaki, J., and Seino, S. (1994) J. Biochim. 269, 1689–16992
9. Inagaki, N., Kuroni, H., Goncal, T., Okamoto, Y., Ishida, Y., Kaneko, Y., Iwano, T., and Seino, S. (1995) J. Biochim. 268, 686–691
10. Nakarisi, S. (1992) Science 258, 597–603
11. Schoepfer, R., Monyer, H., Sommer, B., Widen, W., Sprengel, R., Kuner, T., Lomeli, H., Herb, A., Köhler, M., Burnashev, N., Gütter, W., Ruppersberg, P., and Seeburg, P. (1994) Proc. Natl. Acad. Sci. U.S.A. 42, 353–357
12. Verdoorn, T. A., Burnashev, N., Monyer, H., Seeburg, P. H., and Sakmann, B. (1992) Science 253, 1715–1718
13. Hume, R. L., Dingledine, R., and Heinemann, S. F. (1991) Science 253, 1028–1031
14. Sommer, B., Burnashev, N., Verdoorn, T. A., Kainz, K., Sakmann, B., and Seeburg, P. H. (1992) EMBO J. 11, 1651–1656
15. Egebjerg, J., Better, B., Hermans-Borgmeyer, I., and Heinemann, S. F. (1991) Nature 351, 745–748
16. Herb, A., Burnashev, N., Werner, P., Sakmann, B., Widen, W., and Seeburg, P. H. (1992) Neuron 8, 75–85
17. Partin, K. M., Patneau, D. K., Winters, C. A., Mayer, M. L., and Buonanno, A. (1993) Neuron 11, 1069–1082
18. Lapie, E. P., and Kostianovsky, M. (1967) Diabetes 16, 35–39
19. Lernmark, A. (1974) Acta Diabetologica 12, 431–438
20. Powers, A. C., Efra, S., Mijusk, S., Spector, D., Habener, J. F., and Hanahan, D. (1990) Diabetes 40, 406–414
21. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Rabin, D. C. (1985) Anal. Chem. 50, 75–80
22. Laemmli, U. K. (1970) Nature 227, 680–685
23. Verdoorn, T. A. (1994) Mol. Pharmacol. 45, 475–480
24. Wong, L. A., and Mayer, M. L. (1993) Mol. Pharmacol. 44, 504–510
25. Jonas, P., Racca, C., Sakmann, B., Seeburg, P. H., and Monyer, H. (1994) Neuron 12, 1281–1289
26. Hamaguchi, K., and Letter, E. H. E. (1990) Diabetes 39, 415–425
27. Huettner, J. E. (1990) Neuron 5, 253–266
28. Boxall, A. R., and Garthwaite, J. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 2965–2981
29. Petralia, R. S., Winters, C. A., Niedzielski, A. S., and Wenthold, R. J. (1993) Neuroscience 57, 943–964
30. Petralia, R. S., Yokotani, N., and Nishino, T. J. (1994) J. Neurosci. 14, 667–696
31. Petralia, R. S., Wang, Y.-X., and Woldhers, R. J. (1994) J. Comp. Neurol. 349, 85–110
32. Huntley, G. W., Rogers, S. W., Moran, T. J., Janssen, W., Archim, N., Nix, C., Cauley, K., Heinemann, S. F., and Morrison, J. H. (1993) J. Neurosci. 13, 2956–2981
33. Jetton, T. L., and Magnuson, M. A. (1991) Proc. Natl. Acad. Sci. U.S.A. 89, 2619–2623
34. Petralia, R. S., and Woldhers, R. J. (1992) J. Comp. Neurol. 318, 329–354
35. Heinberg, E., De Vo, D., Vansandem, A., Pipeliers, E., and Schult, F. (1993) EMBO J. 12, 2873–2879
36. Jacobson, G., Bean, A. J., Scheller, R. H., and Juntti-Berggren, L., Deeney, J. T., Berggren, P.-O., and Melster, B. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 1247–1294
37. Sorensen, R. L., Garry, D. G., and Breite, T. C. (1991) Diabetes 40, 1365–1374
38. Sánchez-Andrés, J. V., Gorris, A., and Valdiviemos, M. (1995) J. Physiol. (Lond.) 486, 223–228
39. Santos, R. M., Rosario, L. M., Nadal, A., Garcia-Sancho, J., Soria, B., and Valdiviemos, M. (1991) Pflugers Arch. Eur. J. Physiol. 418, 417–422
40. Valdiviemos, M., Santos, R. M., Contreras, D., Soria, B., and Rosario, L. M. (1989) FEBS Lett. 250, 19–23
41. Gion, P., and Henquin, J. C. (1992) J. Biol. Chem. 267, 2073–20720
42. Luo, E. A., Tornheim, K., Deeney, J. T., Varnum, B. A., Tillotson, D., Proctor, L., and Corkey, B. E. (1991) J. Biol. Chem. 266, 9134–9139
43. Rogers, S. W. (1992) Diabetes 41, 755–762
44. Gilon, P., and Henquin, J. C. (1992) J. Biol. Chem. 267, 2073–20720
45. Longo, E. A., Tornheim, K., Deeney, J. T., Varnum, B. A., Tillotson, D., Proctor, L., and Corkey, B. E. (1991) J. Biol. Chem. 266, 9134–9139
46. Rogers, S. W. (1992) Diabetes 41, 755–762
Differential Expression of Glutamate Receptor Subtypes in Rat Pancreatic Islets
C. David Weaver, Tom L. Yao, Alvin C. Powers and Todd A. Verdoorn

J. Biol. Chem. 1996, 271:12977-12984.
doi: 10.1074/jbc.271.22.12977

Access the most updated version of this article at http://www.jbc.org/content/271/22/12977

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 45 references, 18 of which can be accessed free at
http://www.jbc.org/content/271/22/12977.full.html#ref-list-1