A High Affinity Glutamate/Aspartate Transport System in Pancreatic Islets of Langerhans Modulates Glucose-stimulated Insulin Secretion

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To examine the role of glutamatergic signaling in the function of pancreatic islets, we have characterized a high affinity glutamate/aspartate uptake system in this tissue. The islet [3H]glutamate uptake activity was Na+-dependent, and it was blocked by L-trans-pyrrolidine-2,4-dicarboxylic acid, a blocker of neuronal and glial glutamate transporters. Islet glutamate transport activity exhibited a V_{max} of 8.48 ± 1.47 fmol/min/islet (n = 4), which corresponds to 102.2 ± 17.7 pmol/min/mg islet protein. The apparent K_{m} of islet glutamate transport activity depended on the glucose concentration used in the assay. In the presence of glucose concentrations that do not stimulate insulin secretion (2.8 mM), the apparent K_{m} was 34.7 ± 7.8 μM (n = 3). However, in high glucose (16.7 mM) the apparent K_{m} increased to 112.7 ± 16.5 μM (n = 3) with little or no change in V_{max}. Like most known plasma membrane glutamate transporters, islet glutamate transporters also transported d-aspartate. Anti-d-aspartate immunoreactivity showed that the islet glutamate/aspartate transport activity was localized to the non-β cell islet mantle. In perifusion experiments with isolated islets in the absence of exogenous amino acids, L-trans-pyrrolidine-2,4-dicarboxylic acid in the presence of 8.3 mM glucose potentiated insulin secretion 23.3 ± 2.3% (n = 3) compared with 8.3 mM glucose alone. This effect was abolished in the presence of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor antagonist, 6-cyano-7-nitroquininaline-2,3-dione. Furthermore, 6-cyano-7-nitroquininaline-2,3-dione alone inhibited glucose-stimulated insulin secretion in isolated islets by 15.9 ± 5.9% (n = 3). Taken together these data suggest that a high affinity glutamate transport system exists in pancreatic islets and that this system contributes to a glutamatergic signaling pathway that can modulate glucose-inducible insulin secretion.

Although the role of glutamate as a signaling molecule is well established in the central nervous system, a similar role in the periphery has only recently been suggested. We (1) and others (2) have detected functional glutamate receptors in the pancreatic islets of Langerhans. These miniature organs, found dispersed throughout the exocrine pancreas, are composed of four major cell types as follows: the insulin-secreting β cell, the glucagon-secreting α cell, the pancreatic polypeptide-secreting PP cell, and the somatostatin-secreting δ cell. The electrically excitable β cells are stimulated to secrete insulin in response to changes in serum glucose concentrations. Secretion of insulin, and the three other major peptide hormones found in islets, is also believed to be affected by other metabolic and neuronal signals (reviewed in Refs. 3 and 4). Bertrand et al. (5, 6) have shown that α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)1 receptor agonists can potentiate both insulin and glucagon secretion from a perfused pancreas preparation and that oral or intravenous glutamate can increase insulin secretion and glucose tolerance in vivo (7).

We have localized AMPA-type glutamate receptors to β, α, and PP cells and kainate receptors in α cells using immunohistochemistry and electrophysiology (1). To elucidate the role of glutamatergic signaling in islet physiology, we examined islets for the presence of a high affinity uptake system similar to those described in the central nervous system. Glutamate transporters in the central nervous system allow glutamate to act as a specific signaling molecule despite its relatively high concentration in the cerebrospinal fluid because they reduce the concentration of glutamate in the vicinity of receptors.

Earlier studies have suggested that islets do not possess a high capacity for glutamate uptake and utilization; however, these studies were focused on the possible role of glutamate as a carbon source or as a fuel secretagogue (8). The millimolar concentrations of glutamate used in these studies did not adequately address the possibility of a high affinity glutamate uptake system in islets, the presence of which might serve to support a role for glutamate receptors in islet signal transduction.

By using a [3H]glutamate uptake assay, we detected glutamate transport activity in isolated rat pancreatic islets. The uptake observed in islets had properties similar to those of central nervous system transporters. It had high affinity for glutamate, was Na+-dependent, and was blocked by L-trans-pyrrolidine-2,4-dicarboxylic acid (L-trans-PDC), a compound that blocks neuronal and glial transporters. Furthermore, the apparent K_{m} of islet glutamate uptake was markedly increased by increasing glucose concentrations. Visualization of d-aspartate immunoreactive signals (reviewed in Refs. 3 and 4).

1 The abbreviations used are: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNQX, 6-cyano-7-nitroquininaline-2,3-dione; L-trans-PDC, L-trans-pyrrolidine-2,4-dicarboxylic acid; PBS, phosphate-buffered saline; TBS, Tris-buffered saline.

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whereas blockade of AMPA receptors with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) resulted in inhibition of insulin secretion. These observations indicate that glutamate transporters are important components of a glutamatergic signaling system within the pancreatic islets of Langerhans.

EXPERIMENTAL PROCEDURES

Materials—Percoll was purchased from Pharmacia Biotech Inc. Culture dishes were purchased from Corning. Ham's F-12 tissue culture media, fetal bovine serum, horse serum, penicillin, and streptomycin were purchased from Life Technologies, Inc. L-[3,4-3H]Glutamate and D-[2,3-3H]Asparagine were purchased from New ENSE Science Products. t-trans-PDC, bicineulline methiodide, and CNQX were purchased from Research Biochemicals. t-Cystine was purchased from Sigma. Alkaline phosphatase-conjugated goat anti-rabbit antibody was purchased from DAKO. Alkaline phosphatase-conjugated donkey anti-guinea pig antibody was purchased from Jackson ImmunoResearch. Triton X-100 Surf- fact-Amphs was purchased form Pierce. Electron microscopy grade glutaraldehyde, paraformaldehyde, and tissue freezing medium were purchased from Electron Microscopy Sciences. Poly(Aqua/Mount was purchased from Polysciences. All other chemicals were of reagent grade or higher. Pregnant Sprague-Dawley rats were obtained from Harlan.

Islet Isolation and Culture—Islets were isolated and cultured by a modification of the method described by DeLarco et al. (10) with the exception that the Percoll solutions were made in 1.060 g/ml Percoll. Five ml of culture medium was changed again. Two days following the last medium change, the islets were harvested from the culture dish. The islets were pelleted as described above and resuspended in 1 ml of ice-cold uptake buffer. The islets were pelleted by centrifugation as described above. The supernatant solution was aspirated, and the islets were resuspended in 1 ml of ice-cold uptake buffer. This wash procedure was repeated a total of three times. After the final wash, the islets were lysed in 100 µl of 0.5 N NaOH for 5 min with occasional vortexing. The samples were centrifuged as above and the pellet containing 3 ml of aqueous scintillation mixture, mixed by inversion, and counted using a Packard model 1600 TR liquid scintillation counter. Values obtained from the scintillation counter were converted into uptake activity units using L-[3H]glutamate or t-[3H]aspartate standards. For calculation of $K_\text{m}$ and $V_\text{max}$ values obtained at 0 °C were treated as a blank and subtracted from the values obtained at 37 °C. Uptake data were analyzed using Igor (Wave- metrics), Excel (Microsoft), and Instat (GraphPad Software). Statistical comparisons were paired two-tailed t tests. t-trans-PDC inhibition curves were fit, and t-trans-PDC IC_50 was calculated using the logistic equation. Figures were constructed using Igor.

Immunocytochemistry—Two to five hundred islets were placed in a 50-µl perfusion chamber. The islets were washed for 30 min at a flow rate of 100 µl/min for 5 min. Uptake buffer containing control glucose or glucose concentration was then added to the perfusion chamber and incubated for 3 days at 37 °C. Following the wash the islets were perfused for 15 min at the same flow rate and temperature with uptake buffer containing 2.8 mM glucose (control) or uptake buffer containing 2.8 mM glucose and 100 µM α-aspartate. The islets were washed for 15 min at 200 µl/min with ice-cold uptake buffer. The islets were then fixed for 1 h in the fixative at room temperature. At the end of the incubation the chambers were disassembled, and the frits with fixed islets attached were removed. The islet-bearing frits were transferred to 1.5-ml centrifuge tubes containing uptake buffer with 0.25% (v/v) glutaraldehyde and 0.1% (v/v) paraformaldehyde and incubated overnight at 4 °C. The frits were then washed 3 times for 20 min in phosphate-buffered saline (PBS) containing 0.1% (v/v) paraformaldehyde and 200 µl/min for 5 min. The perfusion was halted, and the islets were incubated for 1 h in the fixative at room temperature. The islets were then washed 3 times for 10 min each in fresh culture medium. The islets were then washed 3 times for 1 h in fresh culture medium. The islets were then washed 3 times for 10 min each in fresh culture medium. The islets were then washed 3 times for 20 min in phosphate-buffered saline (PBS) containing 1% (v/v) paraformaldehyde and 1% (v/v) glutaraldehyde and 200 µl/min for 5 min. The perfusion was halted, and the islets were incubated for 1 h in the fixative at room temperature. The islets were then washed 3 times for 10 min each in fresh culture medium. The islets were then washed 3 times for 20 min in phosphate-buffered saline (PBS) containing 1% (v/v) paraformaldehyde and 1% (v/v) glutaraldehyde and 200 µl/min for 5 min. The perfusion was halted, and the islets were incubated for 1 h in the fixative at room temperature. The islets were then washed 3 times for 10 min each in fresh culture medium. The islets were then washed 3 times for 20 min in phosphate-buffered saline (PBS) containing 1% (v/v) paraformaldehyde and 1% (v/v) glutaraldehyde and 200 µl/min for 5 min.
min at a flow rate of 100 μl/min at 37 °C with a buffer containing (in mM) 128 NaCl, 1.19 MgSO4, 18 NaHCO3, 2.54 CaCl2, 1.19 KH2PO4, 4.74 KCl, and 0.1% (w/v) bovine serum albumin (perfusion buffer) plus 2.8 mM glucose ( bubbled for 1 h prior to perfusion with 95% O2/5% CO2). Following this wash, the perfusion was stopped for 5 min. At the end of the 5 min, a fraction was collected by perfusing the islets at 1 ml/min for 30 s. Perfusion was allowed to continue at 100 μl/min for 2.5 min after which the flow was stopped for an additional 5 min. Another fraction was collected as above. The islets were then perfused for 2.5 min at 100 μl/min with perfusion buffer containing 2.8 mM glucose or perfusion buffer containing 2.8 mM glucose plus either 500 μM L-trans-PDC, 10 μM CNXQ, or both L-trans-PDC, and CNXQ. Flow was stopped and fractions were collected as above. Following this treatment, the islets were perfused for 2.5 min at 100 μl/min with perfusion buffer containing 8.3 mM glucose or perfusion buffer containing 8.3 mM glucose plus either L-trans-PDC, CNXQ, or both L-trans-PDC and CNXQ. Flow was stopped and fractions were collected as above. Insulin concentrations in the perifusate were determined by a core facility using the Linco insulin radioimmunoassay with appropriate standards. Data were analyzed using Instat and Excel. Statistical comparisons are from paired two-tailed t tests.

RESULTS

We have identified a high affinity glutamate uptake system in isolated, intact pancreatic islets using a [3H]glutamate uptake assay. Islet glutamate uptake exhibited saturable kinetics (Fig. 1) and an affinity for [3H]glutamate that was nearly identical to glutamate transporter activities observed in the central nervous system. Lineweaver-Burk replots of glutamate uptake activity revealed an apparent $K_m$ of 34.7 ± 7.8 μM ($n = 3$) in the presence of 2.8 mM glucose. This [3H]glutamate uptake activity displayed a $V_{max}$ of 8.48 ± 1.47 fmol/min/islet (corresponding to 102.2 ± 17.7 pmol/min/mg islet protein) ($n = 4$). Islet glutamate uptake activity showed other properties similar to central nervous system glutamate transport activities. In particular, islet [3H]glutamate uptake activity was dependent on temperature. Uptake at 0 °C averaged only 13.1 ± 4.1% ($n = 3$) of that measured at 37 °C. Islet [3H]glutamate uptake was also strongly dependent on the presence of Na+ in the uptake buffer. In experiments where Na+ was replaced with choline, the glutamate uptake activity was 28.7 ± 3.0% ($n = 3$) that measured in the presence of normal external Na+. A Na+-independent, Cl−-dependent glutamate transport activity has been reported in the periphery (13, 14) and in neurons (15). This activity, designated system $X_-$, is inhibited by excess cystine. Islet glutamate uptake decreased only modestly (6.1%, $n = 2$) when Cl− was replaced by gluconate in the uptake assay buffer. However, the addition of 500 μM cystine in Na+-free conditions further decreased [3H]glutamate uptake activity to 11.3% of control. Since system $X_-$ is believed to be primarily involved in the uptake of cystine, we focused our study on the more prominent Na+--dependent [3H]glutamate uptake activity in islets.

To determine which islet cell types were responsible for the observed glutamate uptake activity, we examined transport of D-aspartate. As in the case of most other known plasma membrane glutamate transporters, islet D-[3H]aspartate transport was quite similar to L-[3H]glutamate transport for both $K_m$ and $V_{max}$ (Fig. 2). D-[3H]Aspartate uptake was also sensitive to temperature and to the removal of external Na+ (Fig. 2, inset). The similarities between L-[3H]glutamate and D-[3H]aspartate uptake suggested that both were transported by the same transporter proteins and thereby D-aspartate uptake offered a way to determine which islet cells harbored glutamate transporters. In these experiments islets were incubated in the presence and absence of 100 μM D-aspartate and fixed with glutaraldehyde and paraformaldehyde to covalently bind amino acids in a form accessible for immunostaining. This technique was first demonstrated as indicated in Ref. 11, and the degree of retention of labeled L-glutamate and D-aspartate by different fixatives was determined (16). Samples were...
Islet Glutamate Transport System

FIG. 2. Islet glutamate transporters also transport d-aspartate. The plot represents an uptake assay with points being the average of uptake measured in triplicate samples for either l-[3H]glutamate (filled circles) or d-[3H]aspartate (open triangles). The apparent $K_m$ values in this experiment are 29.8 and 29.2 $\mu M$ for l-glutamate and d-aspartate, respectively. The $V_{\text{max}}$ values are 7.3 and 7.5 fmol/min/islet for l-glutamate and d-aspartate, respectively. The inset shows a comparison of d-aspartate uptake in standard uptake buffer at 37 °C (control), in Na+-free uptake buffer at 37 °C (Na+-free), or in standard uptake buffer at 0 °C (0 °C). Data are the average of triplicate samples (±S.E.).

FIG. 3. Islet d-aspartate (l-glutamate) uptake is localized to the cells of the islet mantle. Shown are light micrographs of 2.5% paraformaldehyde-fixed, isolated islet sections. A, islet incubated with 100 $\mu M$ d-aspartate prior to fixation and probed with anti-d-aspartate antibody. B, islet probed with anti-glucagon antibody. Note the similar staining pattern of the islet mantle cells with both the anti-d-aspartate and anti-glucagon antibodies. C, islet incubated in the absence of d-aspartate prior to fixation, probed with anti-d-aspartate antibody. Scale bar, 25 $\mu M$.

Insulin secretion.

It has been suggested that down-regulation of glucagon secretion in $\alpha$ cells exposed to high glucose may be mediated by GABA$_A$ receptors expressed in these cells (18, 19). The proposed source of GABA is $\beta$ cells, which may coordinately release it with insulin (20). Since glutamate/aspartate uptake activity was predominantly in $\alpha$ cells, we tested whether glucose inhibition of glutamate transport might be mediated through this mechanism. To do this we repeated the uptake experiments with 2.8 and 16.7 mM d-glucose in the presence of the GABA receptor antagonist, bicuculline. However, the degree of inhibition (31.7 ± 1.7%, $n = 3$) was the same as that measured in the absence of bicuculline (Fig. 4A). The addition of 50 $\mu M$ bicuculline alone had no effect on [3H]glutamate uptake (102.7 ± 11.2% of uptake in the absence of bicuculline, $n = 3$).

Previously, Bertrand et al. (5, 7) have demonstrated that AMPA receptor agonists potentiate glucose-induced insulin secretion in a perfused pancreas preparation (5) and in vivo (7). To identify potential roles of glutamate transporters in islets, we examined the effects of glutamate transport inhibition on glucose-stimulated insulin secretion. l-trans-PDC is an inhibitor of glutamate transport in the central nervous system (21) and showed an $IC_{50}$ of 129.2 $\mu M$ against islet [3H]glutamate transport. In the presence of 35 $\mu M$ glutamate the maximum amount of inhibition we observed in islets was 66.0% with 2 mM l-trans-PDC. The average inhibition of glutamate transport (35 $\mu M$) was 55.0 ± 3.9% ($n = 4$) in the presence of 500 $\mu M$ l-trans-PDC (Fig. 5A). This concentration of l-trans-PDC has been shown to potentiate glutamate receptor activity in hippocampal CA1 neurons (22).

FIG. 5B shows the results from a representative experiment where isolated islets, in the absence of exogenous amino acids, were exposed to 2.8 and 8.3 mM d-glucose in the absence and presence of either 500 $\mu M$ l-trans-PDC, the AMPA receptor antagonist CNQX (10 $\mu M$), or both l-trans-PDC and CNQX. We have previously demonstrated that 10 $\mu M$ CNQX is sufficient to inhibit insulin secretion (6).
FIG. 4. Islet glutamate uptake is sensitive to the metabolizable glucose concentration. A, shown is glutamate uptake (35 μM) in the presence of 2.8 mM d-glucose, 16.7 mM d-glucose, 16.7 mM L-glucose, or 16.7 mM d-glucose plus 50 μM bicuculline. Data are the average of three to five determinations from separate islet preparations (±S.E.). B, the plot represents a typical transport assay with points being the average of triplicate samples in the presence of either 2.8 mM d-glucose (closed circles) or 16.7 mM d-glucose (open triangles). The apparent Km values in this experiment are 50 and 140 μM for 2.8 and 16.7 mM glucose, respectively. The measured Vmax values for both conditions are 12.6 fmol/min/islet. The axes are 1/V (fmol/min/islet) and 1/S (L-glutamate, μM).

completely block the AMPA receptor-activated currents in islet cells (1), and 500 μM l-trans-PDC has no effect on the activation of islet glutamate receptors. In the presence of 8.3 mM glucose and 500 μM l-trans-PDC, insulin secretion increased significantly, 23.3 ± 2.3% (n = 3 separate islet preparations) (p = 0.0099), over the level induced by the 8.3 mM glucose control which averaged 4.8 ± 1.1 ng/ml (n = 3). CNQX (10 μM) completely blocked the potentiating effect of l-trans-PDC (75.6 ± 10.8% of control, n = 3), and furthermore, 10 μM CNQX alone decreased the level of insulin secretion in the presence of 8.3 mM glucose by 15.9 ± 5.9% (n = 3). Neither l-trans-PDC nor CNQX had any effect on insulin secretion in the presence of 2.8 mM glucose.

DISCUSSION

We (1) and others (2) have shown that ionotropic glutamate receptors are functionally expressed in pancreatic islets and that activation of these receptors may have implications for peptide hormone secretion (5, 6) and glucose tolerance (7). Glutamate receptors in the central nervous system rely on a high affinity uptake system to maintain low extracellular glutamate concentrations so that glutamate can act as a neurotransmitter. To elucidate the physiological relevance of glutamate receptors in pancreatic islets, we have investigated the presence of a glutamate uptake system in isolated, intact islets. The majority of islet [3H]glutamate transport is Na⁺-dependent, and the kinetic properties of this transport are similar to those described in the central nervous system. Because islet glutamate transporters also used α-aspartate as a substrate, it was possible to use anti-α-aspartate antibodies to localize islet glutamate/aspartate uptake to the non-β cells of the islet mantel. Antagonists of glutamate receptors and transporters had inhibitory and potentiating effects on insulin secretion, respectively, even in the absence of exogenous amino acids. Therefore, islets appear to contain an entire glutamatergic signaling system including activity-dependent release of glutamate, specific glutamate receptors, and a high affinity uptake system.

Manfras et al. (23) have detected glutamate transporter mRNA in human islets. However, attempts to measure glutamate uptake in islets have been focused on the possible role of glutamate as a metabolite or fuel secretagogue and not as a signal for cell to cell communication (8). The millimolar concentrations of glutamate used in this study were inappropriate to address the existence of glutamate uptake systems like those described in the central nervous system. Traditionally these transporters have been characterized based on their sensitivity to temperature, external Na⁺ concentration, and on their affinity for glutamate. The almost complete dependence of islet glutamate uptake on temperature suggests that the activity that we report was not the result of l-trans-PDC binding to glutamate receptors. Islet glutamate uptake activity also displayed a dependence for external Na⁺; however, this dependence was incomplete (71.3 ± 3.0%). The presence of a Na⁺-independent, Cl⁻-dependent, system Xc⁻, has been reported in the periphery (13, 14) and in neurons (15). In islets we observed a 6.1% decrease in [3H]glutamate uptake in the absence of external chloride, and we found that cystine, an inhibitor of system Xc⁻ glutamate transport, substantially blocked the Na⁺-independent uptake activity. We chose not to characterize further the system Xc⁻ activity since it is believed to be primarily involved in cystine uptake and since the majority of the [3H]glutamate transport activity in islets is Na⁺-dependent.

The apparent Km for glutamate in islets (34.7 ± 7.8 μM) is within the range measured in preparations from the central nervous system as follows: 42 μM in cerebellar granule cells, 58 and 66 μM in cortical astrocytes, and 31 μM in C6 glioma cells (24). The Vmax value that we report for islet glutamate transport 102.2 ± 17.7 pmol/min/mg protein is also similar to those measured for synaptosomal glutamate transporters from the cerebellum (530 ± 280 pmol/min/mg protein) and brain stem (570 ± 290 pmol/min/mg protein) (25). Based on our immunohistochemical data, islet glutamate transporters are only found...
in 25–30% of islet cells. Therefore, our measurement of $V_{\text{max}}$ is an underestimate. Our data suggest that islet glutamate transporters, like their central nervous system counterparts, are capable of lowering extracellular glutamate concentrations to produce an environment favorable for glutamatergic signal transduction.

Most known plasma membrane glutamate transporters are also capable of transporting D-aspartate (26). This observation has provided a means to visualize uptake in fixed cells after incubation in the presence of D-aspartate using stereoselective anti-D-aspartate antibodies (12, 17). Since D-aspartate is not normally found in, metabolized by, or released from most known cell types, it makes a convenient exogenous marker for glutamate transport activity. Islet $l$-[3H]glutamate and D-[3H]aspartate transport activities exhibited similar properties suggesting that the same transporter was responsible for both uptake activities. Incubation of islets in the presence of D-aspartate followed by fixation and probing with anti-D-aspartate antibodies revealed that islet D-aspartate ($l$-glutamate) transport was restricted to the islet mantle that consists primarily of $\alpha$ cells in our preparation. Islets that were not incubated in the presence of D-aspartate prior to immunostaining failed to show reactivity with the D-aspartate antibodies. These data demonstrate that islet cells, like most other cells examined thus far, contain little or no detectable endogenous D-aspartate. Furthermore, since the anti-D-aspartate antibodies used in this study do not generally detect concentrations of fixed D-aspartate below 500 $\mu$M (17), the D-aspartate signal detected in the mantle cells after exposure of the islets to 100 $\mu$M D-aspartate is due to the concentration of D-aspartate by these cells.

One of the chief roles of islets is to act as physiological glucose sensors. The mechanism by which $\beta$ cells sense and respond to serum glucose levels is linked to glucose metabolism. The coordinate regulation of peptide hormone secretion from other islet cell types is poorly understood. We have observed that $\beta$-glucose inhibits [3H]glutamate uptake in the non-$\beta$ cells of intact islets. Our results clearly show that the effects of glucose on glutamate uptake were not due to increased osmolarity in the presence of 16.7 mM glucose since the non-metabolizable $l$-glucose had no effect on islet glutamate uptake. These data suggest that changes in glutamate uptake activity in non-$\beta$ cells are linked to the metabolism of glucose. Although the response of $\alpha$ cells, the major mantle cell type in our preparation, to changes in glucose concentrations is poorly described, it has been suggested that GABA released by $\beta$ cells conveys glucose sensitivity to $\alpha$ cells (18, 19). To test the hypothesis that GABA mediates the glucose effect on glutamate uptake, we used the GABAA receptor antagonist bicuculline to block $\alpha$ cell GABA receptors. Blockade of these receptors had no effect on the glucose modulation of glutamate uptake. Therefore, non-$\beta$ cells are either capable of linking glucose metabolism directly to changes in their physiology or effects of glucose in $\beta$ cells are being transduced to the cells of the islet mantle by an undescribed mechanism. In either case these observations represent a novel way that glucose metabolism may be related to changes in islet physiology.

The replots of glutamate uptake in the presence of 2.8 and 16.7 mM glucose revealed that increased glucose concentration resulted in a change in the apparent $K_{\text{m}}$ of glutamate transport. These data are consistent with a competitive mode of

**Fig. 5.** Block of glutamate transporter and receptor systems modulates glucose-stimulated insulin secretion. A, shown is the inhibition of glutamate transport by $l$-trans-PDC. Control, glutamate uptake (35 $\mu$m) in uptake buffer containing 2.8 mM glucose. $l$-trans-PDC, glutamate uptake (35 $\mu$m) in uptake buffer containing 2.8 mM glucose and 500 $\mu$m $l$-trans-PDC. Data are the average of four determinations from separate islet preparations (±S.E.). B, shown are the results of a representative experiment where ~100 islet/experimental condition were exposed first to 2.8 mM glucose followed by 2.8 mM glucose alone or 2.8 mM glucose plus either 500 $\mu$m $l$-trans-PDC, 10 $\mu$m CNQX, or both 500 $\mu$m $l$-trans-PDC and 10 $\mu$m CNQX. The islets were then exposed to 8.3 mM glucose alone or 8.3 mM glucose plus $l$-trans-PDC, CNQX, or both $l$-trans-PDC and CNQX. Bars represent the average of two 0.5-ml fractions collected for each of the experimental conditions.
inhibition. ° cells are known to possess phosphate-activated glutaminase (2), and this enzyme is a characteristic constituent of glutamate-releasing nerve endings in the central nervous system (27). Therefore, ° cells may be a possible source of releasable intra-islet glutamate. Glucose-inducible release of this glutamate may explain the apparent competitive inhibition of glutamate uptake and may provide a mechanism by which ° cells can communicate with ° cells through their AMPA-type glutamate receptors. Preliminary data obtained using high pressure liquid chromatography to detect released glutamate suggest that glucose does indeed increase the release of glutamate from isolated islets.

Other possible explanations for the observed glucose effect on glutamate uptake include glucose-mediated depolarization of non-° cells resulting in a decreased driving force for glutamate or modulation of transporter activity by posttranslational modification. In other systems, however, neither a decreased driving force for glutamate nor posttranslational modification have been shown to result in kinetics that resemble competitive inhibition (24, 28–30). Finally, these observations could result from glucose-induced stimulation of non-° cell metabolism leading to the metabolism of glutamate and the release of the °H label outside of the cell.

The data reported by Bertrand and colleagues (5, 7) in perfused pancreas preparations (5) and in vivo (7) have pointed to a possible role for glutamate in modulating peptide hormone secretion through its action at AMPA receptors. Their data do not address the source of glutamate acting at these receptors. Our data show that the blockade of glutamate uptake increases insulin secretion and confirm that AMPA receptors mediate the actions of glutamate. Since these experiments were performed in isolated islets in the absence of any exogenous amino acids, the effects on insulin secretion caused by °-trans-PDC and CNQX must be due to modulation of a glutamatergic signal that originates in the islets themselves. These observations are intriguing in light of the fact that while islets isolated from both the dorsal and ventral portions of the pancreas contain the same amount of insulin, dorsal islets have been reported to secrete 40–50% more insulin in response to 16.7 mM glucose than ventral islets (31, 32). Dorsal islets contain up to 25% ° cells, but ventral islets may only contain 5% ° cells. As mentioned above, ° cells, but not the other cell type cells, express phosphate-activated glutaminase. The presence of this enzyme in ° cells, taken together with our data that suggest islets release glutamate from an intra-islet source, provides a possible explanation for the discrepancy in the amount of glucose-stimulated insulin secretion from dorsal and ventral islets.

Multiple components of a glutamatergic signaling pathway appear to exist in islets: 1) specific glutamate receptors, 2) a high affinity uptake system to control the levels of glutamate, and 3) suggestions based on uptake behavior that glutamate is released by relevant physiological stimuli. The precise role of a glutamatergic signaling system in islet physiology or pathology is not completely understood, but a number of possibilities present themselves. Glutamate may play a similar role to the one postulated by Rorsman et al. (18, 19) for GABA in mediating communication between ° and ° cells. Glutamate may also subserve communication between islets and the central nervous system. Our demonstration of glucose-sensitive glutamate transport in the non-° cells of pancreatic islets adds to the growing evidence that glutamate is an important signaling molecule in this endocrine tissue.

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