A Novel Variant of Ionotropic Glutamate Receptor Regulates Somatostatin Secretion From δ-Cells of Islets of Langerhans

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Many metabolic factors affect the secretion of insulin from β-cells and glucagon from α-cells of the islets of Langerhans to regulate blood glucose. Somatostatin from δ-cells, considered a local inhibitor of islet function, reduces insulin and glucagon secretion by activating somatostatin receptors in islet cells. Somatostatin secretion from δ-cells is increased by high glucose via glucose metabolism in a similar way to insulin secretion from β-cells. However, it is unknown how low glucose triggers somatostatin secretion. Because L-glutamate is cosecreted with glucagon from α-cells under low-glucose conditions and acts as a primary intercellular messenger, we hypothesized that glutamate signaling triggers the secretion of somatostatin. In this study, we showed that δ-cells express GluR4c-flip, a newly identified splicing variant of GluR4, an (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type ionotropic glutamate receptor of rat. After treatment with L-glutamate, AMPA, or kainate, secretion of somatostatin from isolated islets was significantly stimulated under low-glucose conditions. The glutamate-dependent somatostatin secretion was Ca²⁺ dependent and blocked by 6-cyano-7-nitroquinoxaline-2,3-dione. Somatostatin in turn inhibited the secretion of L-glutamate and glucagon from α-cells. These results indicate that L-glutamate triggers somatostatin secretion from δ-cells by way of the GluR4c-flip receptor under low-glucose conditions. The released somatostatin may complete the feedback inhibition of α-cells. Thus, α- and δ-cells may communicate with each other through L-glutamate and somatostatin signaling. Diabetes 53: 1743–1753, 2004

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AM, acetoxymethylester; AMPA, (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; GABA, γ-aminobutyric acid; GST, glutathione S-transferase; iGluR, ionotropic glutamate receptor; mGluR, metabotropic glutamate receptor; mGluR4, metabotropic glutamate receptor type 4; NMDA, N-methyl-d-aspartate; SSTR, somatostatin receptor.

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The pancreatic islet, a miniature endocrine organ for hormones regulating blood glucose, is composed of at least four kinds of cells: glucagon-secreting α-cells, insulin-secreting β-cells, somatostatin-secreting δ-cells, and pancreatic polypeptide—secreting F-cells. Insulin plays a central role in decreasing blood glucose, whereas glucagon has the opposite effect. The mechanisms underlying glucose regulation are very complex, but highly ordered, and deeply related to many metabolic disorders such as diabetes (1–4). Blood glucose is the most important regulator of the secretion of these hormones. In addition, neurons innervating islets release neurotransmitters such as acetylcholine, norepinephrine, and vasoactive intestinal peptides and thereby cause stimulation or inhibition of insulin and glucagon release (1). Recent studies have provided accumulating evidence that islet cells also secrete neurotransmitters and modulate hormone secretion (3,4). In β-cells, γ-aminobutyric acid (GABA) is stored in synaptic-like microvesicles, secretory vesicles distinct from insulin granules, and secreted through exocytosis (5,6). The released GABA seems to bind to ionotropic GABAA receptors on α-cells, resulting in an inhibition of glucagon secretion (7). GABA also binds to metabotropic GABAB receptors on β-cells to modulate insulin secretion (8,9). L-Glutamate, an excitatory neurotransmitter, is stored in glucagon-containing secretory granules and is cosecreted with glucagon under low-glucose conditions (10). Although the glutamatergic response in islets is not firmly understood, islet cells express ionotropic and metabotropic glutamate receptors (mGluRs) (9,11–13). We have previously shown that stimulation of the (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor on β-cells triggers GABA secretion without affecting insulin secretion (4,10). In contrast, α-cells express the mGluR type 4 (mGluR4), the stimulation of which inhibits glucagon secretion (13).

Somatostatin, a tetradecapeptide, is a potent paracrine modulator that inhibits the secretion of insulin and glucagon from islets (3,14,15). The inhibition is mediated through somatostatin receptor (SSTR)-1 and -5 in β-cells and SSTR-2 in α-cells (16–18). Given that the mechanism underlying glucose sensing by δ-cells is similar, if not identical, to that of β-cells, somatostatin secretion is upregulated by high blood glucose via cytosolic Ca²⁺ and cAMP (3,15,19). Somewhat paradoxically, the lowering of blood glucose levels also enhances somatostatin secretion...
A: Absence of immunoreactivity for the GluR4 COOH-terminal region in islet cells was revealed with a commercial antibody against GluR4 (GluR4-Chemicon). By comparison, the antibody stained cerebellar neurons. Bar = 20 μm.

B: Presence of immunoreactivity for the NH2-terminal half of GluR4 (GluR4N) in islet cells. Sections of islets of Langerhans were doubly immunostained with a mixture of antibodies against GluR4N (green), glucagon (red), insulin (red), somatostatin (red), or pancreatic polypeptide (PP; green). Merged pictures are also shown to clarify the colocalization of the peptides and GluR4N. Bar = 20 μm.

C: Immunoblotting with antibodies against GluR4N or GluR4-Chemicon revealed the presence of a GluR4 of a lower molecular mass in islets. Lanes 1 and 3, membrane fractions of rat brain (15 μg protein); lanes 2 and 4, islets (100 μg each); lane 5, membrane fractions of rat brain (20 μg); lane 6, islets (30 μg). In lanes 1 and 4, antibodies preabsorbed with antigenic peptides (2 mg) were used. The following antibodies were used: in lanes 1–4, GluR4N antibodies; in lanes 5 and 6, GluR4-Chemicon.

D: RT-PCR analysis of the expression of the GluR4 gene in brain (lanes 1 and 3) and isolated islets (lanes 2 and 4). No amplified products were obtained without the RT reaction (lanes 3 and 4). The amplified product, which has the expected molecular weight, is indicated by an arrow.
Although somatostatin secreted under low-glucose conditions might be responsible for somatostatin-evoked inhibition of glucagon secretion, it is unknown how low glucose enhances somatostatin secretion. We hypothesized that L-glutamate released from α-cells under low-glucose conditions triggers the secretion of somatostatin from δ-cells. This mechanism inevitably requires the presence of glutamate receptor(s) on δ-cells. However, it is unknown whether δ-cells express glutamate receptor(s).

Here we show several lines of evidence that islet cells, including δ-cells, express the functional GluR4c-flip receptor, an ionotropic GluR (iGluR) variant detected only in chicks at the transcript level (i.e., thus far not at the protein level) (21,22). Upon stimulation of the receptor, somatostatin secretion is triggered under low-glucose conditions, and somatostatin in turn inhibits secretion of glucagon and L-glutamate from α-cells. Based on these observations, an overall characterization of the L-glutamate–mediated paracrine signal network in islets is proposed.

RESEARCH DESIGN AND METHODS

Preparation of islets. Islets of Langerhans were isolated from male Wistar rats at 7–8 postnatal weeks by the collagenase digestion method combined with discontinuous Ficoll gradient centrifugation (23). Islets were then hand
picked and suspended in a bicarbonate-buffered Hank’s solution supplemented with 0.2% BSA. For experimental procedures, the islets were maintained for 1 day and washed with a culture medium comprised of RPMI-1640, 10% fetal bovine serum, 5 mmol/l glucose, 100 units/ml penicillin, and 100 μg/ml streptomycin. Animal care was performed under the authors’ institutional guidelines and was approved by the institutional review board.

**Antibodies.** Site-specific rabbit polyclonal antibodies against rat GluR2, GluR3, GluR4, GluR5, GluR6, GluR7, KA1, and KA2 were raised as follows. The synthetic peptides corresponding to the residues based on the published amino acid sequences (24-30) 854–864 [(C)NINPSSQNSQN] of GluR2, 859–869 [(C)QNFKPAPATNTQN] of GluR3, 395–405 [(C)KLVLIQDMPTL] of GluR4, 890–907 [(C)TKGKSSFTSILT] of GluR5, 406–429 [(C)WDPASGLNMTESQKGKPANITDSLSN] of GluR6, 859–871 [CSTVADEIRFSLT] of GluR7, 393–412 [(C)VAEGLSMDSRLYASNISDS] of KA1, and 392–512 [(C)YSNRTLAMNATTLDINLSQ] of KA2 were conjugated with keyhole-limpet hemocyanin with m-maleidobenzoyl-N-hydrosuccinimide ester and then injected into rabbits according to the standard immunization protocol. These site-specific antibodies were used after purification on an affinity column, on which the corresponding peptides were immobilized. (The GluR4-specific products, shown in Fig. 2B (700 bp) by white arrowheads, were digested with AvoI or DraI. The flip form digested with AvoI yielded DNA fragments of 438 and 262 bp, whereas the flop form digested with DraI yielded DNA fragments of 363 and 337 bp. D: Immunological detection of GluR4c. Immunological reactivity was examined with 100 μg protein of COS-7 cell membrane expressing the vector only (lane 1), GluR4c (lane 2), or islet membrane (lane 3). E: A section of an islet was doubly labeled with a mixture of antibodies against GluR4c (green), glucagon (red), insulin (red), or somatostatin (red). In an experiment, GluR4c and pancreatic polypeptides were shown as red and green, respectively. Merged pictures are also shown to clarify the colocalization of the peptides and GluR4c. Bar = 20 μm.
For the preparation of antibodies specific to GluR4c variants, DNA fragments encoding V849–I885 were amplified by PCR and then cloned into the EcoRI site of expression vector pGEX6X (Amersham Pharmacia Biotech) to form glutathione S-transferase (GST) fusion plasmids. After transformation, the GST fusion proteins encoding V849–I885 were purified on a glutathione-Sepharose 4B column (Amersham) and then injected into a rabbit with complete adjuvant twice with a 2-week interval between injections. The mouse monoclonal antibodies against glucagon were obtained from Progen, the rat monoclonal antibodies against somatostatin and rabbit polyclonal antibodies against GluR4 COOH-terminals from Chemicon, the antibodies against NR1 from Synaptic Systems, the guinea pig polyclonal antibodies against NR2 from Biogenesis, and the Alexa Fluor 568-labeled anti-mouse IgG and Alexa Fluor 488-labeled goat anti-rabbit IgG from Molecular Probes.

**Immunohistochemistry.** Indirect immunofluorescence microscopy was performed using an Olympus FV-300 confocal laser microscope, as previously described (10).

**RT-PCR.** RT-PCR was performed as previously described (10). Briefly, 30 temperature cycles were performed, with denaturation at 94°C for 30 s, annealing at 54°C for the common region of GluR4 variants (GluR4 common), 58°C for GluR4 COOH-terminal, and 4°C, and 57°C for GluR4d and GluR4s for 30 s, with an extension at 72°C for 1 min. The sequences of the oligonucleotides used as primers for each GluR4 variant were based on published sequences (21,22). The conditions for PCR were as follows: GluR4 common sense primer, 5′-CAGGCTCAGAAGGCTGGA-3′ (bases 586–605), and antisense primer, 5′-CATATCTTGAATCAAGACTA-3′ (bases 1187–1206) (accession no. M38063); GluR4 COOH-terminal sense primer, 5′-CCCATAGAAAGTGCAGAAGACC-3′ (bases 1960–1981), and antisense primer, 5′-CACTAGCACGGCAATCCGGAAC-3′ (bases 2672–2692) (accession no. M38063); GluR4 s sense primer, 5′-CCCTAGAAAGTGCAGAAGACC-3′ (bases 1960–1981), and antisense primer, 5′-GGTTCGTTGCATTTGTTGGGA-3′ (bases 2317–2338) (accession no. U65901) (22). For semiquantitative analysis of the expression of GluR4c-flip or -flop variants, the amplified products of GluR4c were treated with Avai or DraI to specifically digest flip and flop variants. The resultant mixture was analyzed by PAGE, and DNA fragments specific to the flip or flop variants were quantified with National Institutes of Health Image (version 1.63). The DNA sequences were determined by a standard method (33).

**Cloning and expression of cDNA encoding GluR4c-flip.** The amplified product of GluR4c-flip was used as a hybridization probe to isolate cDNA clones from rat islet cDNA libraries. The full-length cDNA was then constructed. cDNA encoding GluR4c-flip was subcloned into the XhoI and BamHI sites of expression vector pcDNA3.1(−) (Invitrogen, San Diego, CA). The resultant construct, GluR4c-flip, was transfected into COS-7 cells by the lipofection method.

**Western blotting.** Isolated islets (−500 pieces) were homogenized in a buffer containing 20 mmol/l 3-morpholinopropanesulfonic acid–Tris (pH 7.0), 0.3 mol/l sucrose, 5 mmol/l EDTA, 5 μg/ml leupeptin, and 5 μg/ml pepstatin A. The homogenate was centrifuged at 800g for 10 min, and the supernatant was again centrifuged at 266,000g for 30 min. The particulate fraction was suspended in SDS buffer and then subjected to SDS gel electrophoresis, followed by immunoblotting with enhanced chemiluminescence (10).

**Measurement of somatostatin, insulin, glucagon, and 1-glutamate secretion.** Isolated islets (40 pieces per assay) were washed three times with Dulbecco’s modified Eagle Medium’s, then incubated in Ringer solution containing 10 mmol/l HEPES (pH 7.4), 0.2% BSA, and glucose at the specified concentrations for 1 h at 37°C. The islets were then transferred to 350 μl of the Ringer solution containing glucose at the specified concentrations. In some experiments, 1-glutamate or its analogs at the indicated concentrations were added. At the times indicated, 70 μl of the Ringer solution was carefully removed, and the amounts of somatostatin, insulin, and glucagon were determined with enzyme-linked immunosorbent assay kits obtained from Phoenix Pharmaceuticals, Amersham Pharmacia Biotech, and Yanaihara, respectively (10). 1-GLutamate was determined by high-performance liquid chromatography on a RESOLVE C18 column (3.9 × 150 mm; Waters) and by fluorescence detection (10). The statistical significance of datasets was assessed using Student’s t test.

**RESULTS**

**Screening of iGluR expression in islets.** As the first step of this study, we examined the expression of various iGluRs in islet cells using site-specific antibodies. Overall our results confirmed the observations of Weaver et al. (12): 1) GluR2 immunoreactivity overlapped with glucagon or insulin, but not with somatostatin, confirming the presence of GluR2 subunits in α- and β-cells, but not in δ-cells; 2) coexpression of the kainate receptor subunits (i.e., KA2 and GluR5–7) was not observed, suggesting there was no functional kainate receptor in any islet cells; and 3) only background levels of immunoreactivity for

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GluR1, GluR3, KA1, and NR1 were observed, indicating that they are not expressed in islet cells or that their levels are under the detection limit of our assay. In addition, the expression of δ1 and δ2 receptors, a fourth class of iGluRs (34), was not detected in islets (data not shown).

However, one significant difference was found for GluR4. Weaver et al. (12) did not detect GluR4 expression using a commercial antibody that recognizes the COOH-terminal region, which was confirmed by our assay (Fig. 1A). On the other hand, our antibody exhibiting binding affinity to the common domain of the NH₂-terminal half of GluR4 (anti-GluR4N) intensely immunostained β-, δ-, and F-cells, and, to a lesser extent, α-cells (Fig. 1B). Similarly, Western blotting confirmed that the anti-GluR4N antibody recognized a single polypeptide of 93 kDa, which was slightly lower than brain GluR4. In contrast, the commercial antibody did not recognize any islet polypeptides (Fig. 1C). On RT-PCR analysis, a DNA fragment encoding the common region of the GluR4 variants from islets and brain was amplified, confirming the expression of GluR4 type receptor in islets (Fig. 1D). These results strongly suggest that a GluR4 variant(s), most probably GluR4 lacking the COOH-terminal region, is expressed in islet cells.

Expression of GluR4c-flip. GluR4 has seven possible splicing variants, some of which have not yet been detected at the protein level (Fig. 2A) (21,22). We performed...
RT-PCR to determine the DNA sequence of the COOH-terminal region of GluR4 variants. A DNA fragment (733 bp) encoding the GluR4 COOH-terminal region was not amplified from islet cDNA (Fig. 2B), confirming the absence of mRNA of authentic GluR4 in islets, as described above. In contrast, a DNA 700-bp fragment was amplified from islet cDNA, and the DNA sequence turned out to be that of a GluR4c-flip, the brain counterpart of which is GluR4c-flop (Fig. 2B). The nucleotide sequence of a cDNA cloned from an islet cDNA bank completely matched that of the GluR4c-flip. Neither GluR4d nor GluR4s mRNA was detected (Fig. 2B). Semiquantitative RT-PCR confirmed the dominant expression of GluR4c-flip in islets, whereas both GluR4c-flip and -flip variants were expressed to similar extents in the brain (Fig. 2C).

Next we prepared an antibody that specifically recognizes a recombinant GluR4c-flip and a single islet polypeptide band corresponding to the mass of the GluR4c-flip (Fig. 2D). As expected, immunohistochemical analysis indicated that islet cells, especially β- and δ-cells, expressed GluR4c subunits (Fig. 2E). The immunological localization was consistent with that of GluR4N immunoreactivity (Fig. 1B). These results demonstrated that δ-cells express GluR4c-flip at both the mRNA and protein levels.

**L-Glutamate-evoked somatostatin secretion.** The presence of iGluR on δ-cells strongly suggested that δ-cells can receive L-glutamate signals and that the resultant increase in cytoplasmic \([\text{Ca}^{2+}]\) may trigger somatostatin secretion. To test this hypothesis, we first measured the secretion of somatostatin derived from isolated islets under various glucose conditions. When islets were incubated with 3.3 mmol/l glucose throughout, the islets released a back-ground level of somatostatin (114.8 ± 26.9 pg · 40 islets \(^{-1} \cdot \text{h}^{-1} ; n = 4\) (Fig. 3A). When islets were first incubated with 3.3 mmol/l glucose and then transferred to 16.7 mmol/l glucose (i.e., high-glucose stimulation), an enhanced level of somatostatin release (200% of that with 3.3 mmol/l glucose throughout) was observed. Continuous incubation of islets with 16.7 mmol/l glucose evoked the release of some somatostatin (109.1 ± 40.7 pg · 40 islets \(^{-1} \cdot \text{h}^{-1} ; n = 4\). When the islets were first incubated with 16.7 mmol/l glucose and then transferred to 3.3 mmol/l glucose (i.e., low-glucose stimulation), somatostatin secretion was significantly enhanced, corresponding to 147% of that with 16.7 mmol/l glucose throughout. Thus, high- and low-glucose stimulation enhanced somatostatin secretion, as has been observed in previous studies (3,15). Under similar assay conditions, the secretion of insulin and glucagon was enhanced by high- and low-glucose stimulation, respectively, indicating a well-known islet cell—specific response to glucose (Fig. 3A).

Next we examined the effect of exogenous L-glutamate on somatostatin secretion and found that 1 mmol/l L-glutamate enhanced somatostatin secretion (321.8 ± 31.1 pg · 40 islets \(^{-1} \cdot \text{h}^{-1} ; n = 4\), which was inhibited up to 60% by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Fig. 3B). AMPA and kainate also stimulated somatostatin release ∼1.5- to 1.9-fold in a CNQX-sensitive manner. Neither N-methyl-d-aspartate (NMDA) nor MK-801, antagonists of the NMDA receptor, affected somatostatin secretion (Fig. 3B). CNQX alone inhibited somatostatin secretion by 55%, suggesting that endogenous L-glutamate cosecreted with glucagon from α-cells participates in the inhibition (Fig. 3B). L-Glutamate inhibited glucagon secretion without affecting insulin secretion, as previously described (10,13) (Fig. 3C). Somatostatin secretion evoked by endo- and exogenous L-glutamate was strongly inhibited by EGTA-acetoxymethylster (EGTA-AM) (Fig. 3D). These results strongly suggested that stimulation of the AMPA-type receptor triggers somatostatin secretion via an increase in \([\text{Ca}^{2+}]\). It is noteworthy that the stimulatory effect of AMPA, kainate, and L-glutamate and the inhibitory effect of CNQX were not observed when islets were first incubated at 3.3 mmol/l glucose and then transferred to 16.7 mmol/l glucose (Fig. 3E). Essentially the same results were observed when islets were incubated at 16.7 mmol/l glucose throughout. Subsequently, the islets were then transferred to 3.3 mmol/l glucose, causing essentially the same stimulation by L-glutamate (data not shown). These results indicated that glutamatergic signaling was not involved in somatostatin secretion after high-glucose stimulation.

**Somatostatin-evoked inhibition of glucagon and L-glutamate secretion.** Finally, we checked whether exogenous somatostatin in turn could inhibit the secretion of glucagon and L-glutamate from islets. The secretion of both L-glutamate and glucagon was enhanced by low-glucose stimulation, a finding that was consistent with a previous observation (10). Somatostatin at 100 nmol/l decreased the secretion of both glucagon and L-glutamate to control levels (Fig. 4). Secretion of L-glutamate and glucagon was also enhanced by the addition of isoproterenol at 1 μmol/l; this secretion was inhibited by somatostatin as well (data not shown).

**DISCUSSION**

Besides insulin, glucagon, and pancreatic polypeptides, islets secrete L-glutamate from α- and possibly β-cells, GABA and Zn\(^{2+}\) from β-cells, and somatostatin from δ-cells as paracrine-like modulators (3,4,10,15,35). Although the modes of action of these paracrine modulators are less characterized, we recently showed that α- and β-cells communicate with each other through L-glutamate and GABA, which act as intercellular transmitters, to precisely regulate their endocrine functions (4). In the present study, we obtained evidence that α- and δ-cells also communicate through L-glutamate— and somatostatin-mediated signaling pathways.

We first examined whether δ-cells express iGluRs and possess the ability to receive glutamate signals. iGluRs are ligand-gated cation channels that are classified into AMPA, kainate, NMDA, and δ-type receptors based on their primary structure and pharmacology (36,37). The AMPA receptor is a homomeric or heteromeric complex consisting of GluR1 to -4 subunits. We showed that a splicing variant, GluR4c-flip, was actually expressed and present in islet cells; δ-cells express only GluR4c-flip, and β-cells express both GluR4c-flip and GluR2. As to the expression of mGluR, among known mGluRs, only mGluR4 has been shown to be present in α-cells (13). Collectively, it is quite likely that GluR4c-flip is the only GluR expressed in δ-cells.

The GluR4c-flip has also been detected in brain. In brain, the flop type seems to be dominant, whereas the flip type...
FIG. 3. Glutamatergic signals stimulate somatostatin secretion from isolated islets under low-glucose conditions. A: Secretion of somatostatin, insulin, and glucagon under various glucose conditions. Islets were incubated in a Ringer solution containing 16.7 or 3.3 mmol/l glucose for 1 h, as indicated. The glucose concentration was then changed to 3.3 or 16.7 mmol/l, as indicated, and the release of somatostatin, insulin, or glucagon was measured. *P < 0.01; **P < 0.001. B: Effect of L-glutamate, glutamate receptor agonists, and glutamate receptor antagonists on somatostatin secretion. Islets were incubated in a Ringer solution containing 16.7 mmol/l glucose for 1 h, as indicated, and then transferred to the medium containing 3.3 mmol/l glucose in the presence or absence of the listed compounds. *P < 0.01; **P < 0.001. C: Effect of L-glutamate on secretion of insulin and glucagon. **P < 0.001. D: Effect of intracellular Ca²⁺ buffers on L-glutamate-evoked somatostatin secretion. Islets were incubated with a medium containing 16.7 mmol/l glucose in the presence or absence of EGTA-AM at 50 μmol/l for 30 min. Islets were then transferred to the medium containing 3.3 mmol/l glucose with or without L-glutamate at 1 mmol/l. After islets were incubated for 1 h, the released somatostatin was quantified. **P < 0.001. E: Absence of the stimulatory effect of glutamatergic stimulation under high-glucose conditions. Islets were incubated in a Ringer solution containing 3.3 mmol/l glucose for 1 h, as indicated, and then transferred to a medium containing 16.7 mmol/l glucose in the presence or absence of the listed compounds for 1 h. All data are means ± SE (n = 4). CNQX was used at 50 μmol/l, L-glutamate at 1 mmol/l, kainate at 1 mmol/l, AMPA at 1 mmol/l, NMDA at 1 mmol/l, and MK-801 at 50 μmol/l.
is more abundant in islets, with only a trace level of the flop type. The significance of such selective expression of the flip type in islets is unknown at present, but it may be related to a specific demand of islet glutamatergic systems.

We further explored the relationship between L-glutamate and somatostatin signaling. Somatostatin is confined to secretory granules and secreted upon increase in intracellular [Ca^{2+}] and cAMP (14,15). We have shown that L-glutamate triggers the exocytosis of somatostatin, which is dependent on an increase in intracellular [Ca^{2+}]. The glutamate effect was mimicked by AMPA and kainate and blocked by CNQX, suggesting the activation of AMPA-type GluRs in δ-cells. Even in the absence of exogenous L-glutamate, CNQX partially inhibited somatostatin secretion, probably by blocking endogenous glutamatergic signals from islet α-cells (Fig. 3B). Because L-glutamate becomes active as an intercellular transmitter only under low-glucose conditions (10), the present observations explain the mechanism by which somatostatin is secreted from δ-cells under such conditions.

It is noteworthy that under high-glucose conditions, δ-cells were not responsive to L-glutamate (Fig. 3). Similar glucose-dependent changes in glutamate signal reception have been observed for L-glutamate—evoked GABA secretion from β-cells (10). Although we do not know the molecular mechanisms underlying the glucose-dependent change in glutamate signal receptiveness, we assume desensitization of GluR4c-flip occurs because of a change in the metabolic state. It is known that flip/flop variants differ from each other in their agonist sensitivity and desensitization rate (38). Another plausible explanation is that, like GluR2 in neurons (39), GluR4c-flip at the plasma membrane is recycled depending on the glucose conditions, causing a loss or gain of sensitivity to glutamate.

Altogether, secretion of somatostatin from δ-cells may be regulated through two distinct signaling pathways: glucose-dependent gating of the ATP-sensitive potassium channels under high-glucose conditions, which is similar to insulin secretion from β-cells (3), and glutamate receptor—mediated signaling under low-glucose conditions, which is similar to GABA secretion from β-cells. Because L-glutamate is cosecreted with glucagon in islets under low-glucose conditions (4), the present results suggest the occurrence of L-glutamate— and somatostatin-mediated bidirectional signaling between α- and δ-cells. Thus, taking the previous observations into consideration, we propose that α-, β-, and δ-cells, as well as F-cells, may communicate with each other using L-glutamate, GABA, and somatostatin as intercellular transmitters (Fig. 5). L-Glutamate may act as a primary signaling molecule and thereby trigger secondary peptidergic and GABAergic responses in islets. Very recently, it has been shown that L-glutamate may act as an autocrine signaling molecule by way of mGluR4 on α-cells (13). These three distinct signaling pathways must regulate glucagon secretion from α-cells. In addition, recent evidence indicates that the vesicular inhibitory amino acid transporter, which is responsible for vesicular storage of GABA or glycine, is expressed in α-cells (40) and associated with glucagon-containing secretory granules (41). Thus either GABA or glycine may be costored and cosecreted with L-glutamate and glucagon and then may participate in the paracrine signaling shown above, although secretion of GABA or glycine from α-cells remains to be detected. Further studies, especially ones to reveal the components of the paracrine signal network at the molecular level and realtime imaging of the signal transmission, will be helpful for understanding the overall features of the complex, but well-ordered, regulation of secretion of blood glucose-regulating hormones.

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FIG. 5. L-Glutamate-mediated cross-talk among islet cells. L-glutamate functions as a primary intercellular transmitter under low-glucose conditions and triggers secondary GABAergic and peptidergic signaling so as to inhibit glucagon secretion in islets of Langerhans. See DISCUSSION for details. Pi, inorganic phosphate; SG, secretory granule; SLMV, synaptic-like microvesicle.


