The Local Paracrine Actions of the Pancreatic α-Cell

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Secretion of glucagon from the pancreatic α-cells is conventionally seen as the first and most important defense against hypoglycemia. Recent findings, however, show that α-cell signals stimulate insulin secretion from the neighboring β-cell. This article focuses on these seemingly counterintuitive local actions of α-cells and describes how they impact islet biology and glucose metabolism. It is mostly based on studies published in the last decade on the physiology of α-cells in human islets and incorporates results from rodents where appropriate. As this and the accompanying articles show, the emerging picture of α-cell function is one of increased complexity that needs to be considered when developing new therapies aimed at promoting islet function in the context of diabetes.

PARACRINOLOGICAL CONSIDERATIONS

α- and β-Cells: From This Day Forward, for Better, for Worse, for Richer, for Poorer, in Sickness and in Health, to Love and to Cherish

Hormone secretion from the pancreatic islet is central to the regulation of glucose metabolism. Responsible for most of the islet's hormonal output are the insulin-secreting β-cells and the glucagon-secreting α cells. Because these hormones have opposite effects on plasma glucose levels, α- and β-cells have long been considered functional antagonists. The islet also contains other endocrine cells that secrete hormones, but their actions may be more local. Recent genome-wide association studies point out that variants of genes expressed in the islet are associated with diabetes. This, combined with the increased availability of human islets for research (1), has revitalized the field of diabetes research and led to a renaissance in islet biology studies. Even the basic anatomy of the islet is being revisited, with studies showing that there are vast species differences in islet cytoarchitecture (2–6). A remarkable feature in all vertebrate species is that, despite their variety, islets always contain α- and β-cells. In our research group, we always wonder why it makes evolutionary sense to have these two cells packed together. The most common explanation is that it is important for β-cells to inhibit α-cell activity so that release of hormones with antagonistic effects does not overlap (7). Using findings on human α-cells published over the last decade, this article will try to show that this is probably not the whole story.

Most, if Not All, Islet Cells Release Paracrine Signals

A common feature of islet endocrine cells is that they release molecules that serve as paracrine signals. In many cases, even the hormones they secrete have local effects. This paracrine function may not be exclusive to endocrine cells. Indeed, secretions from islet pericytes and local macrophages have been shown to exert trophic effects on β-cells (8–10). The effects of paracrine signals are being investigated intensely because they will help us understand how hormone secretion is orchestrated in the islet. The paracrine signaling pathways could in principle also be targeted for therapeutic intervention. Before we delve deeper into the subject, however, we need some terminological and conceptual definitions.

In the pancreatic islet, paracrine signals can influence other cells within the same islet by diffusing through the interstitial space or circulating in local blood vessels. In autocrine signaling, by contrast, a cell secretes a signaling molecule that binds to receptors on the same cell or other cells of the same cell type (e.g., β-cell to β-cell communication). The same signaling molecule can be used in different contexts such as endocrine, paracrine, autocrine, or synaptic signaling. What then are the criteria to consider

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that a molecule is a paracrine signal? By adopting standards used for decades in the neurosciences (11), we propose that to be a bona fide paracrine signal 1) the molecule must be present or produced in the islet cell, 2) the molecule must be released in response to cell stimulation, and 3) specific receptors for the molecule must be present on target cells in the islet. These criteria seem obvious, but for many signaling candidates they have not been met.

A first step is to detect the signaling molecules in islet cells, but often it is easier to show that the cells express components of the synthetic machinery producing the molecule. Because many of the signaling molecules also participate in general cellular metabolism (e.g., GABA or glutamate), demonstrating the presence of a candidate molecule or its biosynthetic pathway may not be sufficient. Perhaps the most rigorous approach is to show that secretory granules or vesicles contain the molecule, which requires challenging methodologies such as postembedding immunoelectron microscopy. A cell's secretory phenotype can also be defined by its expression of vesicular transporters for transmitters. A cell expressing the vesicular acetylcholine transporter (vAChT) can thus be considered cholinergic (12,13). This approach, however, may overlook cells that use different mechanisms to secrete paracrine molecules (e.g., ATP release through pannexins) (14).

A second, more challenging criterion is to demonstrate that the paracrine signal is released from the islet cell. This is difficult because the molecule is very likely secreted at low concentrations, degraded by enzymes, or taken up by membrane transporters. Another challenge is to selectively stimulate the islet cell in question and to locate the source for the paracrine signal in the intact islet. This may require measuring molecules secreted from single, dispersed islet cells, at a great loss of detectability. Here, islet cells of a given type could be enriched using cell sorting, with such protocols being recently established for human islets. For several candidate molecules, these technical limitations have prevented demonstrating that they are bona fide paracrine signals in the pancreatic islet.

A third criterion is that the target cell must express receptors for the signaling molecule. The problem here is that many receptor genes are expressed at very low levels and may not be detected, and there are few reliable antibodies for immunohistochemical visualization. A common approach is to stimulate islets pharmacologically and examine the effects on hormone release, Ca^{2+} responses, or changes in membrane potential. Most of the candidate paracrine signals have been proposed based on pharmacological stimulation of receptors on islet cells. However, meeting this criterion alone is not enough to identify a bona fide paracrine signal because the receptors may be receptors for humoral or neural communication.

Islet Endocrine Cells Are Great Targets for Paracrine Signals

Islet endocrine cells are notable glucose sensors. In a process termed stimulus-secretion coupling, these cells use a series of metabolic reactions to convert glucose stimulation into changes in membrane potential: glucose transport into the cells via low-affinity glucose transporters, glucose phosphorylation by the enzyme glucokinase, and the subsequent metabolism of glucose that increases the intracellular ATP-to-ADP ratio. This elevated ratio closes K_{ATP} channels, depolarizes the membrane, and opens voltage-gated Ca^{2+} channels, triggering hormone secretion (15). The complex stimulus-secretion coupling in islet cells not only ensures that glucose metabolism is coupled to membrane electrical activity but also provides multiple points where external signals can modulate hormone secretion. Because islet endocrine cells display complex electrical activity with bursts of action potentials, many signals impinging on islet endocrine cells can change the secretory behavior of the cells by altering membrane electrical activity. Function of endocrine cells can also be regulated at the level of the secretory pathway. Many processes related to granule exocytosis are dependent on second messenger cascades (e.g., cAMP, Ca^{2+}) that can be activated by ligands binding to membrane receptors. Paracrine or autocrine signals can therefore target receptors to manipulate different intracellular signaling cascades and thus regulate islet hormone secretion.

The Particular Case of the Human Islet

α- and δ-cells are not segregated to the periphery in human islets as they are in rodent islets (4–6). Most β-cells in the human islet are close to α-cells, δ-cells, or both. The association of β-cells with α-cells in human islets is so close that, even after dispersion of islets into single cells, most β-cells remain attached to an α-cell (16). This has profound implications for islet function. The close apposition allows endocrine cells to interact using membrane-bound molecules, an interaction that promotes function and survival (17). Because of these close contacts, electrical coupling between α- and β-cells is also conceivable (18). Although this remains to be established in the human islet, this direct communication could contribute to control the biosynthesis and release of secretory products, as well as to cell survival (19). Because many signaling molecules are rapidly degraded in the interstitial space and in the bloodstream, the proximity of β-cells with α-cells also promotes paracrine interactions.

There are additional players that could play a role in α-cell to β-cell communication. Molecules released by α-cells can be degraded by enzymes or bound by molecules residing in the extracellular matrix (e.g., cholinesterases [see below]), which likely shapes the duration and magnitude of the effects on the target cell. Of course, we cannot rule out the contribution of a third party such as the somatostatin-secreting δ-cell. In the human islet, δ-cells are strategically positioned to play this role (20). Thus, in addition to a direct effect on β-cells, signaling molecules released from α-cells can recruit δ-cells to modulate the net effect on β-cells (e.g., acetylcholine [21]).

At this point, it is important to note that until recently it was thought that the impact of α-cells on β-cell function is negligible (22–25). This can be explained by the smaller proportion and spatial segregation of α-cells in mouse and rat islets, the most common research models. It is also possible that the fluid dynamics in the rodent islet hinder paracrine signals from reaching β-cells. Indeed, a study using the perfused rat pancreas model showed that glucose-induced insulin secretion is not affected by signals from neighboring α-cells (23). In the human islet, by contrast, the percentage of α-cells is higher, α-cells and β-cells are aligned randomly along blood vessels, and most β-cells face α-cells (>70%) (4), making it likely for β-cells to be directly exposed to α-cell secretion. As discussed below, α-cell–derived signals can indeed alter β-cell function in the human islet and, according to new studies, also in the rodent islet.

THE ROLE OF GLUCAGON AS A PARACRINE SIGNAL

The In Vivo Effects of Intraislet Glucagon

Glucagon is a major hyperglycemic hormone in the organism that counters decreases in plasma glucose levels. Glucagon secretion is thought to provide the first line of defense in glucose counterregulation (26). Glucagon, however, has also been known for decades as a strong amplifier of insulin secretion (27). We recently confirmed that glucagon input increases insulin secretion from human β-cells (28). In in vitro perifusion studies of hormone secretion, we found that the glucagon receptor antagonists L-168,49 and des-His1-[Glu9]-glucagon (1-29) amide decreased insulin secretion stimulated by increases in glucose concentration (see also 29). It is well established that human β-cells express glucagon and glucagon-like peptide 1 (GLP-1) receptors (30,31), but the in vivo role of paracrine glucagon signaling had remained elusive until recently.

Determining local glucagon concentration in the islet in vivo is beyond what current methods can detect, but there is nevertheless a strong case to be made that local glucagon amplifies β-cell activity in the living organism. We found that local glucagon affects insulin secretion from human islets using an in vivo model that reproduces blood flow, capillarity, and ultrastructural features of the islet vasculature in the pancreas (28). In mice, glycemic levels are lower when the percentages of α-cells are higher, suggesting that if glucagon input is increased it may lead to similar effects (28). The strong insulinotropic effects of glucagon (27–29), the increased insulin secretion from β-cells overexpressing glucagon receptors (32,33), and the association of glucagon receptor mutations with reduced insulin secretion and type 2 diabetes (34,35) all support the notion that intraislet glucagon influences insulin secretion.

A series of recent studies in rodents also point out that intraislet glucagon is needed for adequate insulin secretion in vivo (36–38). These studies found that paracrine glucagon stimulates insulin secretion by activating glucagon and GLP-1 receptors on β-cells. These findings contrast with previous reports that ruled out an influence of α-cells on β-cells based on the theory of core-to-mantle blood flow (39,40) but are consistent with studies that revised these older notions about perfusion of the mouse islet (41–43). It is now clear from the various sophisticated mouse models used in the different studies that local glucagon signaling is required for appropriate insulin secretion, to preserve glucose tolerance during the metabolic stress induced by high-fat feeding, and to maintain proper glucose homeostasis in vivo (36–38). That previous studies using ablation of α-cells failed to show this important intraislet cross talk is probably due to the deployment of compensatory mechanisms or to an incomplete reduction in α-cells (44).

Homeostatic Considerations

How can the stimulatory effects of glucagon on the hypoglycemic hormone insulin be reconciled with the hyperglycemic function of glucagon? One answer to this conundrum is to consider glucagon as a hormone that participates in different regulatory circuits. In one circuit, activated during normoglycemia, glucagon secretion reaches concentrations large enough to amplify insulin secretion from neighboring β-cells. As we recently showed, this local secretion is needed to maintain the human glycemic set point (28). Glucagon secretion under these circumstances is probably not strong enough to reach plasma levels that produce systemic responses. By contrast, when glycemia drops, a second circuit is activated in which glucagon secretion becomes strong enough to produce systemic, hyperglycemic effects. Under these hypoglycemic conditions, glucagon cannot stimulate β-cells because glucose levels are no longer permissive for insulin secretion.

The function of paracrine glucagon under normoglycemic conditions can be characterized in engineering terms. To maintain glucose homeostasis, the regulatory system must include sensors, disturbance detectors, an integrator, and effectors. Both α- and β-cells are specialized glucose detectors endowed with mechanisms to sense glucose. Any change in the regulated variable, glucose concentration, produces changes in α- and β-cell physiology that can be considered disturbance signals (e.g., cell membrane depolarization or hyperpolarization, changes in intracellular Ca^{2+} concentration). These error signals eventually converge on insulin granule exocytosis, which is the integrator (controller) that uses the disturbance signals to send out the control signal insulin to the effector organs (liver, muscles, and fat). By increasing cAMP concentration in β-cells (37), glucagon secretion produces a disturbance signal that is one of the input signals for insulin exocytosis. When activated during glucose counterregulation, by contrast, α-cells become integrators (controllers) themselves, and glucagon acts as a control signal that directly instructs effector organs to produce and release glucose.

It is likely that the glycemic set point results from the dynamic interactions between α- and β-cells. Theoretical models of glucose homeostasis estimate that interactions between α- and β-cells provide a better control of glycemia (45,46). These models posit that the interactions between α- and β-cells need to be asymmetric to provide a negative feedback loop. In fact, all known secretory products of β-cells inhibit glucagon secretion, whereas glucagon and acetylcholine, both secreted from α-cells in human islets, stimulate insulin secretion (47). This arrangement attenuates exacerbated responses and works best with the prevailing small fluctuations in plasma glucose levels. Interrupting this feedback loop by inhibiting glucagon receptors on β-cells acutely destabilizes the glycemic set point (28), confirming the predictions made by the mathematical models.

That the human glucostat depends not solely on the β-cell but on the functional cooperation between α- and β-cells has implications for therapies aimed at reconstituting the β-cell population to treat diabetes. It is likely that the glycemia levels set by β-cells without glucagon input would be characteristic of prediabetes. Because inhibiting glucagon receptors systemically may also eliminate this crucial local input to the β-cell, new approaches to inhibit the contribution of glucagon to hyperglycemia need to be reexamined.

THE α-CELL AS A LOCAL SOURCE OF ACETYLCHOLINE

Acetylcholine: A Shifting Story

Acetylcholine is a major amplifier of β-cell secretion: it stimulates insulin release by increasing the cytoplasmic free Ca^{2+} concentration, $[Ca^{2+}]_i$, via inositol phosphate production and enhancing the effects of Ca^{2+} on exocytosis via protein kinase C in β-cells (48). Metabotropic receptors for acetylcholine, called muscarinic receptors, expressed in pancreatic β-cells are essential for maintaining proper insulin secretion and glucose homeostasis in mice (49,50). Cholinergic agonists have been reported to restore defective glucose-stimulated insulin secretion (51,52). In human islets, several endocrine cells express muscarinic receptors (31). Variations in the gene that encodes the muscarinic receptor M3 are associated with increased risk for early-onset type 2 diabetes (53). It is generally believed that acetylcholine is released during the cephalic phase of food ingestion from parasympathetic nerve endings in the pancreatic islet to prepare the β-cell for the upcoming rise in nutrient levels (48,49,54). The consensus is that the endocrine pancreas is richly innervated by the autonomic nervous system (54,55), with studies based on the cholinesterase technique revealing dense parasympathetic innervation in cat, rat, rabbit, and human islets (56–59).

A decade ago we started examining human pancreatic islets for the presence of prototypical cholinergic markers and found to our surprise that vAChT and choline acetyltransferase (ChAT) were expressed in α-cells (60). We further determined that α-cells release acetylcholine

using a biosensor cell approach (60,61). These results have been replicated using analytical methods and in monkeys (Fig. 1). While acetylcholine also has paracrine effects on other cells within the human islet (e.g., δ-cells) (21), we propose that acetylcholine serves as a feed-forward signal to keep the β-cell responsive to future challenges, thus limiting plasma glucose fluctuations. In this sense, its role may be similar to that of glucagon but using different second messenger systems (cAMP versus Ca^{2+} release from intracellular stores). Moreover, the intracellular signaling pathways activated by acetylcholine may promote long-term survival of β-cells (62).

Because acetylcholine is efficiently and rapidly degraded by cholinesterases, the paracrine interactions of acetylcholine are extremely local and may occur preferentially via the interstitial space between endocrine cells and not
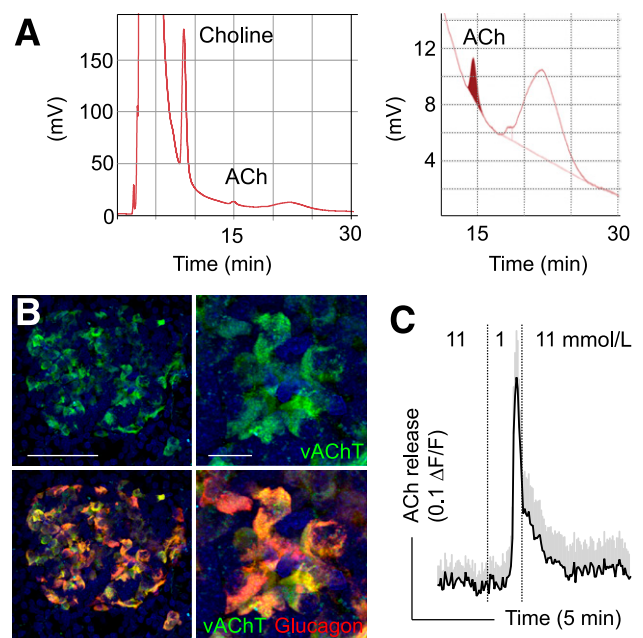
Figure 1—Detection of acetylcholine (ACh) secretion from human islets using high-performance liquid chromatography and biosensor cells. *A*: Chromatogram showing acetylcholine detected from a 20-μL human islet sample. The acetylcholine peak area corresponds to ~1–3 nmol/L and is accompanied by a larger choline peak. A blow up is shown on the right. Human islets were homogenized, protein was denatured, spun down, and placed in high-performance liquid chromatography column. Data contributed by Dr. Ed Phelps, University of Florida. *B*: Confocal images of vAChT (green) and glucagon (red) immunostaining in pancreas section from a monkey (*Macaca fascicularis*). As in the human, α-cells are labeled for this cholinergic marker. Panels on the right are higher magnifications of those on the left; bottom panels are merged images of vAChT and glucagon immunostaining. Scale bars = 100 μm (left), 20 μm (right). *C*: Trace of Ca^{2+} responses in biosensor cells expressing the muscarinic receptor M3 to monitor acetylcholine secretion from monkey islets (*M. fascicularis*). Reducing the glucose concentration from 11 mmol/L to 1 mmol/L elicited strong acetylcholine secretion. The dotted line denotes time of changes in glucose concentration. For these experiments, monkey islets were placed on a carpet of biosensor cells (trace shows mean ± SEM of 15 biosensor cells).

through the vascular route. The effects of acetylcholine on insulin secretion from isolated human islets are greatly amplified in the presence of cholinesterase blockers (60,61,63), indicating *1*) that acetylcholine is released endogenously and *2*) that cells in the islet express cholinesterases. The interplay among acetylcholine, its receptors, and cholinesterase is the subject of active research in our laboratory because it may represent a target for intervention to promote insulin secretion. Indeed, there are many clinically approved cholinesterase blockers that could be repurposed to enhance insulin secretion in type 2 diabetes.

Is There a Neural Source of Acetylcholine in the Human Islet?

Several studies, including our own, show cholinergic innervation of the human islet, but this innervation is sparse compared with that of the mouse islet (64–66). This is consistent with studies showing that the contribution of neural input to insulin secretion during the cephalic phase is relatively small in humans (67,68). Along similar lines, vagotomized patients have normal postprandial serum insulin levels (69), and patients with type 1 diabetes who have undergone pancreas transplantation (and thus have denervated islets) remain euglycemic without therapy (70–72). The human β-cell may thus rely on local paracrine acetylcholine to get cholinergic input. Because it is not exclusively dependent on nervous input, cholinergic signaling in human islets is probably activated under circumstances other than those elicited by activation of the parasympathetic system.

Of course, it is not possible to rule out that parasympathetic nerves contribute cholinergic input to the human islet. It has been reported extensively that activation of parasympathetic nerves increases plasma levels of pancreatic polypeptide, which is released from pancreatic polypeptide–secreting islet γ-cells (73,74). Indeed, this physiological effect is widely used to demonstrate the impact of parasympathetic input on islet function during the cephalic phase. Interestingly, the highest levels of muscarinic receptor expression can be found in the γ-cell (31). We are revisiting the cholinergic innervation of the human islet with a particular emphasis on the γ-cell and found in preliminary experiments that cholinergic innervation of this cell is particularly strong (Fig. 2). If confirmed, these findings would explain why parasympathetic input predominantly affects the secretion of pancreatic polypeptide, a hormone with strong effects on pancreatic acinar cells and motility of the gastrointestinal tract (75). There are already indications that in humans the cephalic phase response may be strongest for pancreatic polypeptide and minor for insulin, glucagon, GLP-1, GIP, and ghrelin (76,77). Whether parasympathetic nerves can regulate other islet cells indirectly via γ-cells remains to be determined.

Revealing a Cholinergic Phenotype Is Not Easy

It is important to mention here that confirming a cholinergic phenotype is challenging. The levels of transcripts for
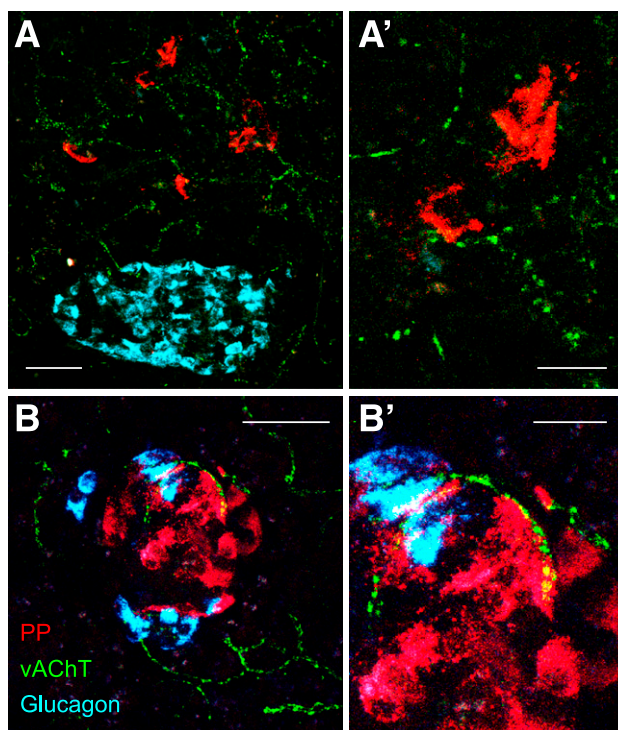

Figure 2—Cholinergic innervation of pancreatic polypeptide (PP)-secreting islet γ-cells. *A*: Maximum projection of a z-stack of confocal images showing cholinergic nerves labeled for vAChT (green) in a human pancreas section. Note that the density of nerves is low in the islet region demarcated by α-cells (glucagon [cyan]). By contrast, many cholinergic axons can be seen in pancreatic polypeptide–rich regions (red [at low and high magnification in *A* and *A*']). *B*: As in *A*, cholinergic axons can be seen closely apposed to pancreatic polypeptide–labeled cells. Shown is a rare islet mainly composed of pancreatic polypeptide cells from the posterior part of the human pancreatic head. Scale bars: 50 μm (*A* and *B*), 10 μm (*A*' and *B*').

acetylcholine-synthesizing enzymes can be extremely low. Cells may contain only four molecules of ChAT transcripts and still exhibit ChAT enzymatic activity that produces acetylcholine (78). Visualizing the presence of ChAT with immunostaining is also not straightforward because the enzyme is found at low amounts (79,80). In our hands, visualizing ChAT required signal amplification with either the avidin-biotin complex method or tyramide signal amplification (Fig. 3). We successfully used antibodies to vAChT to reveal the cholinergic phenotype of α-cells, but the immunostaining results depended on the processing and quality of the human pancreas preparation (60,64). It is likely that these technical limitations have precluded critical demonstrations of the cholinergic phenotype in the islet and other tissues.

α-CELLS RELEASE GLUTAMATE AS A POSITIVE AUTOCRINE SIGNAL

What Is the Major Brain Neurotransmitter Doing in the Islet?

Glutamate is the major excitatory neurotransmitter in the central nervous system. Even though it has been proposed
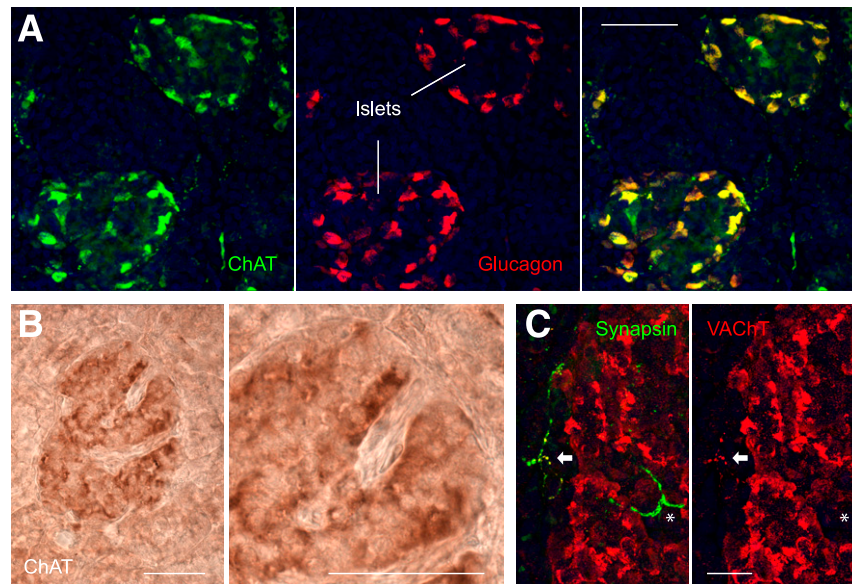
Figure 3—Cholinergic markers in the human pancreatic islet. *A*: Confocal images showing immunostaining for the acetylcholine-synthesizing enzyme ChAT (green [shown alone in left panel]) in human pancreas sections using tyramide signal amplification. Glucagon staining is shown in middle panel (red). Most of the ChAT staining colocalizes with glucagon (merged image in right panel). Note that some ChAT-labeled axons can be seen in the exocrine regions. *B*: Brightfield images showing immunostaining for ChAT using the avidin-biotin complex method. Note that a subset of islet cells is stained in the islet. Right panel is a magnification of left panel. *C*: Confocal images showing staining for the axonal marker synapsin (green) and the cholinergic marker vAChT (red). An axon close to the islet is labeled for both markers (arrow [staining appears yellow in the merged image on the left]). By contrast, an axon penetrating the islet is only labeled for synapsin (*). Scale bars: 100 μm (*A* and *B*), 10 μm (*C*).

as an islet paracrine signal for >20 years (81), the functional role of glutamate in the islet is still unresolved. One problem is that glutamate may originate from different sources such as the plasma, nerve terminals, or endocrine cells. Glutamate has been shown to present in glucagon secretory granules (81), and vesicular glutamate transporters (vGluT1-3) are expressed in α-cells of rodent, monkey, and human islets (82,83). Initial studies of rodent islets indicated that glutamate is secreted together with glucagon (81). Findings in human islets later confirmed that glutamate is released under conditions that also stimulate glucagon secretion (83). However, glutamate derived from metabolic pools, and not from granules, may be also released via membrane glutamate transporters by uptake reversal (84), suggesting multiple mechanisms for glutamate secretion.

Extracellular glutamate can act on ionotropic (AMPA/kainate and N-nitrosodimethylamine [NMDA] receptors) and metabotropic receptors. Results of the expression of glutamate receptors in islet cells are conflicting (85). Databases show that all endocrine cells, but not exocrine cells, of the human pancreas express one or more glutamate receptors (31). Glutamate has been reported to activate α-cells via AMPA/kainate receptors (83,86), inhibit α-cells via metabotropic receptors (87), activate AMPA/kainate and metabotropic receptors in β-cells to increase insulin secretion (88,89), or stimulate δ-cells via AMPA/kainate receptors (90). We compared glutamate signaling in human, monkey, and mouse islets and found that in all three species glutamate activated AMPA/kainate receptors

on α-cells and stimulated glucagon secretion. β-cells and insulin secretion, by contrast, could not be stimulated with glutamate agonists, either at basal or stimulatory glucose concentrations. These results have been confirmed for mouse islets (86). Activation of AMPA/kainate receptors leads to membrane depolarization, opening of voltage-gated Ca^{2+} channels, and an increase in glucagon secretion due to a rise in intracellular Ca^{2+} (83,86). We further found that blocking AMPA/kainate receptors in vivo reduces glucagon release and exacerbates insulin-induced hypoglycemia in mice (83). These results suggest that α-cells use glutamate to potentiate its secretory activity.

As always, the effects of extracellular glutamate in the islet may be more complex. The concentrations of glutamate in the islet may be strongly affected by reverse transport from a cell's metabolic pool (84). Glutamate derived from such a mechanism could act as an extracellular signal as well as an intracellular metabolite that affects hormone secretion. Also, involvement of metabotropic receptors cannot be dismissed (87), and other endocrine cells within the islet (e.g., δ-cells) may also respond to glutamate (90). Clearly, more research is needed to further define the function of glutamate in the islet. Last but not least, glutamate may activate NMDA receptors to regulate insulin secretion and thus blood glucose control (91).

An Autocrine Loop That Boosts Glucagon Secretion
The findings described above indicate that glutamate participates in an autocrine positive feedback loop. This

autocrine positive feedback ensures that even small decreases in plasma glucose concentration elicit glucagon secretion sufficient for avoidance of further hypoglycemia. Many of the paracrine and autocrine signals in the islet are involved in regulatory circuits that use feedback. Interestingly, most of the autocrine signals described for the islet provide positive feedback. That is, the autocrine signals reinforce the effects produced by the initial perturbation (i.e., a change in glucose concentration). As a result, a small perturbation at the input causes a much larger effect at the output. Small deviations in plasma glucose concentration (∼10%) are thus counteracted by sharp increases in insulin and glucagon secretion (threefold) (92). Positive feedback is often used in the rising phase of a physiological response to a perturbation. Not surprisingly, autocrine feedback loops in the islet are activated near the threshold for hormone responses to changes in glucose concentration. This helps make hormonal responses fast and robust.

In type 1 diabetes, α-cells lose their ability to sense decreases in glucose concentration. As a consequence of what is called glucose blindness, glucagon secretion is not stimulated when insulin treatment induces hypoglycemia. α-Cells, however, still respond to other stimuli and retain their potential to secrete glucagon. AMPA/kainate receptors expressed in the human α-cell thus represent a putative target for pharmacological intervention to prevent hypoglycemia. Safe drugs that positively modulate AMPA receptors (e.g., ampakines) are currently being developed for potential therapeutic applications to improve memory and cognition as well as to treat schizophrenia. Using these drugs to activate AMPA/kainate receptors in α-cells could be an adjuvant therapy in the management of drug-treated diabetes.

CONCLUDING REMARKS

Recent studies are revealing a new paracrine role for the α-cell in the islet (Fig. 4). For many years this role was overlooked, mostly because of technical limitations such as the inability to measure or manipulate glucagon signaling selectively in the islet. This led to the consensus that the contribution of α-cells to β-cell activity is negligible. Studies on human islets then showed that glucagon input from the α-cell to the β-cell affects insulin secretion and glucose homeostasis. These findings have now been confirmed and extended using novel genetic tools in mice. There is no doubt now that the α-cell impacts β-cell output, which is also reflected in mathematical models of glucose homeostasis. While we focused here on the direct actions on β-cells, the secretory products from α-cells also activate δ-cells. Indeed, glucagon, glutamate, and acetylcholine stimulate somatostatin secretion. Because δ-cells are activated by β-cell secretory products such as GABA, urocortin, and ATP, δ-cells may provide bidirectional communication between α- and β-cells. This can include electrical communication. Indeed, β-cells have been shown to regulate glucagon secretion through gap junction communication with δ-cells (93). Future models
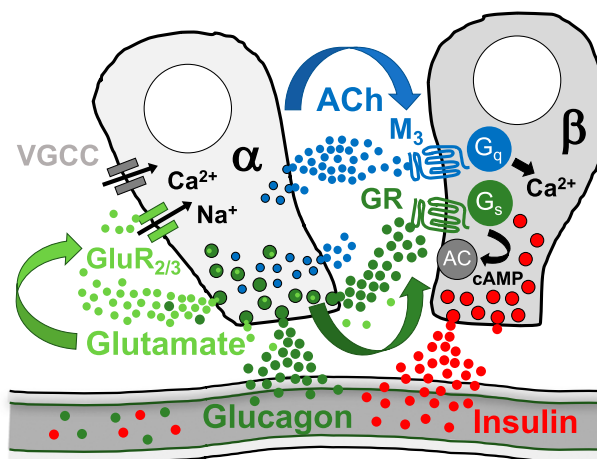
Figure 4—Schematic for paracrine signaling originating from human α-cells. α-Cells secrete glucagon, acetylcholine, and glutamate, which all have been shown to have local paracrine or autocrine excitatory effects. Not depicted are additional receptors or non–β-cell targets for these signaling molecules within the islet (for more information, see ACETYLCHOLINE: A SHIFTING STORY). AC, adenylate cyclase; ACh, acetylcholine; GluR₂/₃, glutamate AMPA receptor 2/3; G$_q$, G$_q$ alpha unit; GR, glucagon receptor; G$_s$, G$_s$ alpha unit, G stimulatory; M₃, acetylcholine muscarinic receptor 3; VGCC, voltage-gated Ca^{2+} channel.

of how hormone secretion is orchestrated in the islet will have to include this ever-increasing complexity. It seems that the more we learn, the less we know.

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