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The Role of Gap Junction Membrane Channels in Secretion and Hormonal Action

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Connexins, gap junctions, and coupling are obligatory features of both endocrine and exocrine glandular epithelia. Evidence from these two types of tissues, and particularly from pancreatic islets and acini, indicates that cell-to-cell communication via gap junction channels is required for proper biosynthesis, storage, and release of specific secretory products. However, endocrine and exocrine glands express a different set of connexins and show opposite connexin and coupling changes in relation with the activation and inhibition of their secretory function. Also, several hormones modulate connexin and coupling expression, and junctional coupling affects hormonal stimulation. These observations indicate that gap junction channels play an important role in the control of secretion and hormonal action.

KEY WORDS: Connexins; junctional coupling; secretion; endocrine glands; exocrine glands; pancreas; insulin; β-cell; amylase, hormones.

GAP JUNCTIONS IN GLAND EPITHELIA

Most endocrine and exocrine secretions are multicellular events which depend on the coordinated activity of numerous cells. To achieve this coordination, secretory cells cross-talk in a variety of ways, including by interacting with hormones, neuromediators, and other signals which diffuse in the extracellular spaces. In these cases, coordination is achieved by the simultaneous activation of specific receptors, metabolic pathways, or effector systems in several cells. However, most secretions remain regulated events under *in vitro* conditions which perturb the native blood supply, innervation, and flux of extracellular fluid, implying that other communication mechanisms can ensure proper cross-talk of secretory cells. The observation that the release of several secretory products is markedly altered after dispersion of secretory cells, and rapidly improves after cell reaggregation (Salomon and Meda, 1986; Bosco *et al.*, 1989, 1994), further suggests that these mechanisms depend on cell-to-cell contacts. The further observation that cell adhesion cannot alone account for this secretory improvement, has led to the hypothesis that cell-to-cell coupling mediated by gap junction channels is an important event to achieve a proper biosynthesis, storage, and release of specific secretory products.

Much of the evidence supporting this view has been gathered in the endocrine and exocrine cells of the rodent pancreas, even though gap junctions and cell-to-cell coupling are obligatory features of secretory cells in all the endocrine and exocrine multicellular glands investigated so far (Friend and Gilula, 1972; Meda *et al.*, 1984b).

EFFECTS OF JUNCTIONAL COUPLING ON INSULIN BIOSYNTHESIS, STORAGE, AND RELEASE

Within the islets of Langerhans, which collectively form the endocrine pancreas, the insulin-producing β-cells are connected to each other by numerous and minute gap junctions, made of Cx43 (Meda *et al.*, 1991). In a resting β-cell, there are 800–2000 gap junction channels which, altogether, occupy less than

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1 μm^2 of the plasma membrane (Meda *et al.*, 1979). These channels allow for the intercellular spread of electrotonic potential changes (Meissner, 1976; Eddlestone *et al.*, 1984), the detection of junctional conductances at individual β-to-β-cell interfaces (Meda *et al.*, 1991), and the synchronization of free Ca^{2+} oscillations (Valdeolmillos *et al.*, 1993) and membrane potentials across the islets (Meda *et al.*, 1984a). Gap junction channels also mediate the exchange between β-cells of molecules which do not permeate the cell membrane, such as exogenous fluorescent probes, nucleotides, and glycolytic intermediates (Kohen *et al.*, 1979; Meda *et al.*, 1981, 1983, 1991). Metabolic coupling and gap junctions are not observed between all β-cells, suggesting that the 10–3,000 β-cells which form a pancreatic islet are functionally grouped in multiple communication territories rather than in a single syncitial unit (Meda, 1995). This view, which is also supported by dual patch clamp measurements of junctional conductance at individual β-to-β-cell interfaces (Meda *et al.*, 1991), has recently been challenged by microelectrode studies on intact isolated mouse islets (Mears *et al.*, 1995).

At any rate, several lines of evidence indicate a contribution of β-cell coupling to the control of insulin secretion. First, single β-cells show a perturbed functioning, as indicated by increased basal release of insulin, poor responsiveness to secretagogues, decreased protein biosynthesis, decreased basal expression of the insulin gene, and loss of its normal cAMP-dependent control, whereas restoration of β-to-β-cell contacts is paralleled by a rapid improvement of these defects (Salomon and Meda, 1986; Bosco *et al.*, 1989; Pipeleers, 1984; Bosco and Meda, 1992; Philippe *et al.*, 1992) (Fig. 1). The finding that alkanols which block gap junction channels prevent the increase in insulin secretion that normally occurs under the latter conditions (Meda *et al.*, 1990), indicates that coupling may play a major role in these changes (Fig. 1). Preliminary *in vitro* studies indicate that similar changes are seen after exposure to antisense oligonucleotides designed to specifically hybridize to the endogenous Cx43 mRNA (unpublished data).

Second, *in vivo* as well as *in vitro*, sustained stimulation of insulin release is associated with increased β-cell coupling (Kohen *et al.*, 1979; Meda *et al.*, 1979, 1983, 1991) due, at least in some cases, to enhanced expression of gap junctions and Cx43 (Meda *et al.*, 1991). Other experiments indicate that β-cell coupling may also increase during an acute glucose stimulation, as judged by an enhanced coupling coefficient and

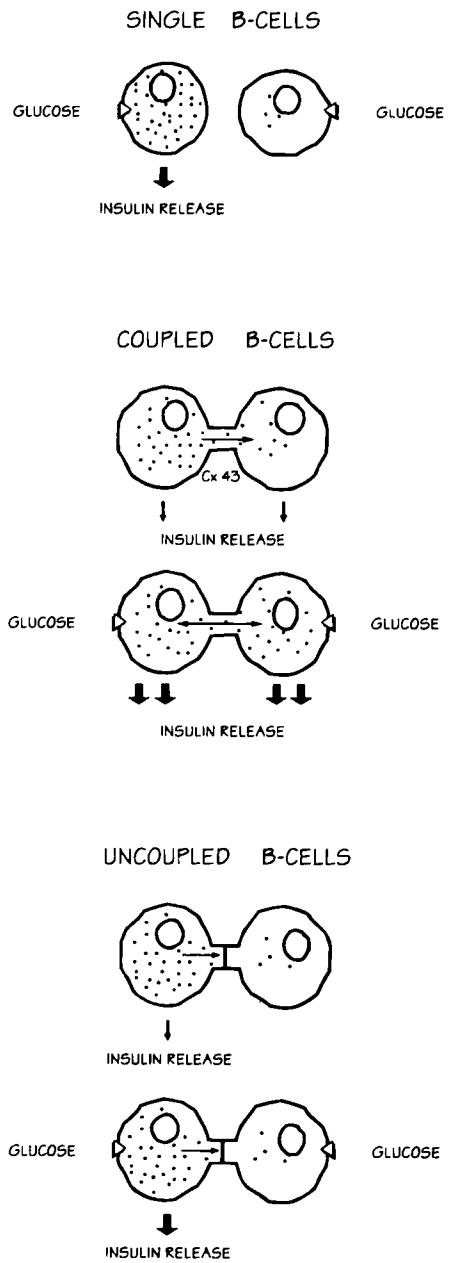

Fig. 1. Gap junctional communication and insulin secretion. Upper panel: Natural secretagogues, including glucose, directly stimulate only those β-cells in which the concentrations of critical ions and molecules (represented by black dots) reach appropriate threshold levels. Middle panel: Establishment of gap junctional communication via Cx43-made channels permits the diffusion-driven passage of these ions and molecules from secreting into nonsecreting cells. As a result, the latter cells become activated and synchronized with those already functioning, even in the absence of external stimuli. Addition of secretagogues further increases the response of gap junction-sharing cells, presumably because the rapid equilibration of ionic and molecular concentrations across junctional channels optimizes the threshold level for activation. Lower panel: The acute and temporary blockade of gap junction channels prevents this equilibration. As a result, uncoupled β-cells secrete little insulin, and with a heterogeneous pattern, like single cells.

synchronization of both electrical activity and Ca^{2+} oscillations of islet cells (Valdeolmillos *et al.*, 1993; Eddlestone *et al.*, 1984). However, it is still unclear whether these changes are solely due to improved junctional communication.

Third, conditions that inhibit insulin release decrease or abolish β-to-β-cell coupling *in vitro* (Meda *et al.*, 1983; Kohen *et al.*, 1983). *In vivo*, however, such conditions result in hyperglycemia and increased β-cell coupling (Meda *et al.*, 1983), suggesting that the level of circulating glucose and the ability of β-cells to properly recognize the sugar may independently influence junctional channels.

Fourth, the acute pharmacological blockade of gap junction channels markedly alters β-cell function, as indicated by increased basal insulin release and loss of stimulated insulin secretion from both isolated islets of Langerhans and intact pancreas (Meda *et al.*, 1990). These alterations, which cannot be accounted for by changes in the main second messengers that control insulin secretion, are rapidly and fully reversible after washout of the uncoupling drugs and are not observed in single β-cells (Meda *et al.*, 1990), indicating that they may well be a specific result of cell uncoupling. This view is further supported by preliminary observations showing that antisense oligonucleotides which block the transcription of Cx43 mRNA, also decrease in a similar way the secretory response of clustered, but not of single, β-cells (unpublished data).

Fifth, at least six independent tumor-derived and transformed β-cell lines which produce abnormally low amounts of insulin, and feature defective glucose sensitivity, do not express connexins and gap junctions and are essentially uncoupled (Vozzi *et al.*, 1995). These defects cannot be simply explained by the *in vitro* downregulation of gap junctional channels since primary β-cells in monolayer culture consistently express Cx43, gap junctions, and coupling for prolonged time periods (Kohen *et al.*, 1979; Meda *et al.*, 1981; Kohen *et al.*, 1983; Vozzi *et al.*, 1995). Nor can these defects be ascribed solely to the proliferation capacity of the cell lines, since the glucose-sensitive cells of a rat insulinoma, from which two of the communication-deficient lines were derived, still express Cx43, gap junctions, and coupling *in vivo* (Meda *et al.*, 1991). Hence, coupling defects appear to be a shared attribute of cell lines showing abnormal insulin production and release, irrespective of their origin and metabolic characteristics. This view is further supported by the observation that the actively proliferating cells of another tumor-derived β-cell line which has retained at least some of the normal sensitivity to glucose, show small gap junctions made of Cx43 (unpublished data). Also, the stable transfection of the gene coding for Cx43 in at least some of the connexin and communication incompetent cells, makes them able to express gap junctions and coupling like primary β-cells, to display a qualitatively normal glucose responsiveness, a markedly increased insulin content, and an enhanced expression of the insulin gene (Vozzi *et al.*, 1995). *In vivo*, these coupled tumor-derived cells grow at a lower rate and secrete more insulin than wild type, noncommunicating partners (unpublished data).

EFFECTS OF JUNCTIONAL COUPLING ON PANCREATIC ENZYME BIOSYNTHESIS, STORAGE, AND RELEASE

The enzyme-producing cells which form the exocrine acini of pancreas are also coupled by numerous gap junctions, made of both Cx32 and Cx26 (Meda *et al.*, 1991, 1993). In a resting acinar cell, there are 60,000–138,000 gap junction channels occupying about 5 μm^2 of the cell membrane (Meda *et al.*, 1983). The ionic and molecular exchanges which occur through this large number of junctional channels are extensive, as judged by the high junctional conductance (Chanson *et al.*, 1989), and the large spread of both electrotonic potentials (Iwatsuki and Petersen, 1977) and low molecular weight tracers that is observed at individual cell-to-cell interfaces (Iwatsuki and Petersen, 1979; Meda *et al.*, 1986). As a result, the 10–50 cells that form a pancreatic acinus are electrically and metabolically coupled to each other, thus forming a truly syncitial unit.

Several lines of evidence indicate that coupling is involved in the secretion of pancreatic amylase. First, dispersed acinar cells secrete poorly under basal conditions and after stimulation by natural cholinergic stimuli, and markedly increase their response to secretagogues after re-establishment of junctional contacts (Bosco *et al.*, 1994) (Fig. 2). The pattern of junctional coupling which is observed under such conditions is analogous to that observed in the intact pancreas (Bosco *et al.*, 1994).

Second, the most efficient secretagogues of the exocrine pancreas rapidly uncouple acinar cells *in vitro* (Chanson *et al.*, 1989; Iwatsuki and Petersen, 1977, 1979; Meda *et al.*, 1986, 1987). At least for

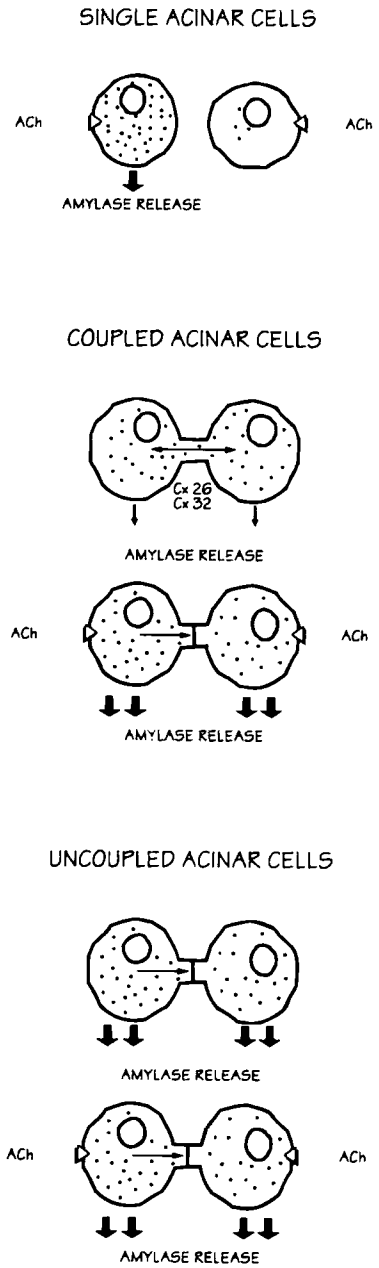

Fig. 2. Gap junctional communication and amylase secretion. Upper panel: Natural secretagogues, including acetylcholine (ACh), directly stimulate only those acinar cells in which the concentrations of critical ions and molecules (represented by black dots) reach appropriate threshold levels. Middle panel: Establishment of gap junctional communication via Cx32- and Cx26-made channels permits the diffusion-driven passage of these ions and molecules from secreting into nonsecreting cells. As a result, the latter cells become activated and synchronized with those already functioning, in the absence of external stimuli. Addition of maximally stimulatory concentrations of secretagogues further increases the response of gap junction-sharing cells while causing their acute uncoupling, possibly to prevent that the factors which control secretion become excessively diluted with diffusion into increasingly larger cytoplasmic volumes. Lower panel: Conditions resulting in the acute and temporary blockade of gap junction channels also markedly stimulate the amylase secretion of acinar cells, to levels similar to those observed during maximal stimulation by natural secretagogues.

cholinergic agonists, we know that this effect is due to the activation of muscarinic receptors, and is mediated by a Ca^{2+}-, pH-, and PKC-independent gating of gap junction channels (Chanson *et al.*, 1989; Iwatsuki and Petersen, 1977, 1979; Meda *et al.*, 1986, 1987). An identical uncoupling is elicited *in vivo* by the endogenous cholinergic neuromediators that are released within the pancreas during vagal nerve stimulation (Chanson *et al.*, 1991), supporting the view that this coupling modulation is of physiological relevance.

Third, the acute pharmacological blockade of gap junction channels increases amylase release within seconds in the absence of other stimuli, *in vitro* as well as *in vivo* (Chanson *et al.*, 1989; Meda *et al.*, 1986, 1987) (Fig. 2). This stimulation is not associated with detectable alterations of the nonjunctional conductances and of the major second messengers which control acinar cell secretion. Furthermore, it is prevented under low temperature conditions that inhibit exocytosis, and is rapidly reversed after washout of the uncoupling drugs and restoration of normal junctional communication (Chanson *et al.*, 1989; Meda *et al.*, 1986, 1987). Together, these data suggest that the uncoupling drugs act specifically on gap junctions. This interpretation is further supported by the lack of effect of these drugs on acinar cells that cannot form gap junctions after physical dispersion or are already uncoupled due to maximal stimulation by cholinergic secretagogues (Chanson *et al.*, 1989; Meda *et al.*, 1986; Bosco *et al.*, 1994). In contrast, during stimulation by secretagogues which do not perturb acinar cell coupling, drugs blocking gap junctions potentiate amylase secretion up to the maximal levels observed during cholinergic stimulation (Meda *et al.*, 1987). Since this potentiation is seen irrespective of the intracellular mechanism whereby noncholinergic secretagogues activate acinar cells, the pharmacological uncoupling probably results in the activation of a step which is rather distal in the chain of events which leads to exocytosis of pancreatic enzymes.

Fourth, prolonged exposure to dexamethasone of cells of the AR4-2J line, which was derived from a pancreatic acinar cell carcinoma, increases amylase production and secretion as well as junctional coupling, and differentially modulates the expression of Cx32 and Cx26 (Meda *et al.*, 1995). Thus, hormonal conditions favoring differentiation of specific acinar cell markers, also selectively modulate junctional communication and the expression of distinct connexins.

JUNCTIONAL COUPLING IN THE FUNCTIONING OF OTHER ENDOCRINE AND EXOCRINE GLANDS

Few studies have thoroughly investigated the participation of gap junctional coupling in the biosynthesis, storage, and release of other hormonal or enzymatic secretory products. Where endocrine functions are concerned, some evidence for such a participation has been obtained for cells of both thyroid (Munari-Silem *et al.*, 1990, 1991) and adrenal glands (Munari-Silem *et al.*, 1995), in which coupling increases with hormone release, similarly to what is observed in pancreatic β-cells. Also, a couple of studies on liver have shown that preservation of the native pattern of hepatocyte connexins and coupling is essential for the proper biosynthesis and release of multiple blood-transported products, including albumin, α-fetoprotein, and glucose (Seseke *et al.*, 1992; Stutenkemper *et al.*, 1992; Iwai *et al.*, 1995).

Regarding exocrine functions, there is some evidence supporting a role of coupling in the secretion of the major salivary glands (Sasaki *et al.*, 1988; Sugita *et al.*, 1995; Kanno *et al.*, 1987), in which coupling decreases with stimulation of saliva output, in a way analogous to that observed in pancreatic acini.

MECHANISM OF COUPLING EFFECTS WITHIN GLANDS

The available data provide compelling evidence that changes in junctional coupling closely parallel changes in secretion of both endocrine and exocrine glands. Coupling appears implicated in the regulation of both the moment-to-moment release of secretory products and their longer-term cell specific expression. However, how coupling affects these secretory parameters remains to be established.

Compared to other forms of cell-to-cell communication, gap junctional coupling is unique in that it provides a mechanism for direct equilibration of ionic and molecular gradients between nearby cells. In such a system, an increase of a cytoplasmic ion or molecule smaller than 900 Da in one cell, is followed by its diffusion-driven passage into nearby cells. At steady state, this passage leads to the equilibration of electrochemical concentrations on the two sides of the gap junction channels. If the resulting concentration reaches the threshold level for activation of an effector mechanism, functioning will be simultaneously modified in all coupled cells. In this way, junctional coupling could ensure the functional recruitment of many cells that, otherwise, would not be directly activated. Such a recruitment role has been experimentally verified in both the β-cell and acinar cell populations of pancreas (Salomon and Meda, 1986; Bosco *et al.*, 1989, 1994; Bosco and Meda, 1992). Insulin and amylase secretion of coupled cells is larger than that of uncoupled cells (Bosco *et al.*, 1989, 1994), indicating that the coupling-induced equilibration is also likely to optimize the concentration of the factors which control secretion. This view is consistent with the observation that the threshold level for activation of insulin secretion and biosynthesis is lowered under conditions promoting β-cell coupling (Sorenson and Parsons, 1985). Eventually, coupling would also be expected to synchronize cell functions which are modulated by factors exchanged through gap junctions. This expectation has been verified, at least for Ca^{2+}, in both endocrine and exocrine pancreatic cells (Valdeolmillos *et al.*, 1993; Petersen and Petersen, 1991).

This model implies that junctional coupling may be particularly advantageous in systems comprising functionally different cells. Increasing evidence shows that this is the case in the pancreas, since individual β-cells and acinar cells are substantially different in their ability to release secretory products, *in vitro* and *in vivo* (Salomon and Meda, 1986; Bosco *et al.*, 1989, 1994; Stefan *et al.*, 1987) (Figs. 1 and 2). Furthermore, β-cells are also heterogeneous in their ability to biosynthesize proteins, including insulin, and in some of the metabolic features which are critical for the early processing of glucose (Bosco and Meda, 1992; Heimberg *et al.*, 1993; Schuit *et al.*, 1988). Conceivably, these disparities explain the asynchronous functioning of individual cells. By equilibrating ionic and molecular gradients across communication territories, junctional coupling could balance and coordinate these disparities, thus permitting the coordinated functioning of intrinsically different cells (Figs. 1 and 2).

The identification of an intrinsic heterogeneity of both β- and acinar cells permits one to conceptualize why and how junctional coupling is beneficial to promote the endocrine and exocrine secretion of pancreas. What is not determined is the molecular mechanism underlying this modulation. A first possibility is that coupling enhances the secretagogue-induced changes in free intracellular Ca^{2+}. Indeed, the levels and oscillations of this cation, which plays a critical role in the control of both insulin and amylase secretion, are differentially affected by the establishment of contacts

between both β-cells and acinar cells (Valdeolmillos *et al.*, 1993; Petersen and Petersen, 1991).

Second, coupling may modulate the electrophysiological characteristics of secretory cells. Resting membrane potentials and individual conductances become larger and more stable after establishment of cell-to-cell contacts, presumably because individual β-cells and acinar cells are equipped with such limited numbers of K^+ and Ca^{2+} channels that they cannot ensure stable ionic fluxes, when these channels fluctuate between the open and the closed state (Atwater *et al.*, 1983; Petersen and Findlay, 1987). By mediating the intercellular equilibration of current-carrying ions, junctional coupling may result in the functional sharing of these channels, resulting in a continuous provision of sufficient numbers of channels to all coupled cells. Such a channel sharing may markedly promote the responsiveness of β-cells and acinar cells, since the activation of the distal steps of their secretory machinery depends on proper control of membrane potentials and ionic fluxes.

Third, intercellular exchanges of both Ca^{2+} and K^+ could account for the electrical synchronization of β-cells and acinar cells, which has been observed in intact islets of Langerhans and acinar cell assemblies (Meissner, 1976; Valdeolmillos *et al.*, 1993; Petersen and Petersen, 1991).

Fourth, coupling could ensure the spreading of signals controlling secretion across large cell populations. Secretagogue-induced Ca^{2+} waves, indicating a temporally and spatially coordinated change in the levels of this ion, have been observed throughout intact islets and acini (Valdeolmillos *et al.*, 1993; Petersen and Petersen, 1991). These waves may result from the intercellular exchange of Ca^{2+} via gap junctions, and may mediate the rapid recruitment of secreting cells distant from the site of signalling. An analogous mechanism has been shown to ensure the hormonal stimulation of cells devoid of cognate receptors, provided these cells were coupled to neighbors able to recognize the signal (Lawrence *et al.*, 1978; Murray and Fletcher, 1984). This effect, which is probably due to a gap junction-mediated transfer of second messengers, most likely cAMP, may operate in the pancreas to functionally recruit cells deficient in factors which rate-limit secretion. Some acinar cells lack receptors for gut hormones and neuromediators (Bosco *et al.*, 1994). By sharing gap junctions with cells equipped with these receptors, defective cells may acquire the ability to exhibit an adequate secretory response.

DIFFERENTIAL EXPRESSION OF CONNEXINS IN ENDOCRINE AND EXOCRINE GLANDS

Screening of several glands using antibodies and cDNA probes to connexins, has shown that whereas exocrine glands express high levels of Cx32, variable levels of Cx26, but not Cx43, endocrine glands express high levels of the latter connexin, variable levels of Cx26, but not Cx32 (Meda *et al.*, 1993) (Fig. 3). The differential distribution of Cx43 and Cx32 was observed in glands producing peptide, glycoproteic, and lipidic products, indicating that the alternative expression of these two gap junction proteins is not related to the type of metabolic differentiation of secretory cells. Rather, it could be required to ensure specific characteristics of gap junctions and cell-to-cell communications, as well as a specific regulation of junctional coupling during secretion changes, as certainly it is the case for the endocrine and exocrine cells of pancreas.

Further observations have revealed that the main secretory cells of thyroid, an endocrine gland which releases its products into an extracellular lumen, like exocrine glands, express both Cx43 and Cx32. Conversely, they have shown that two evolutionarily

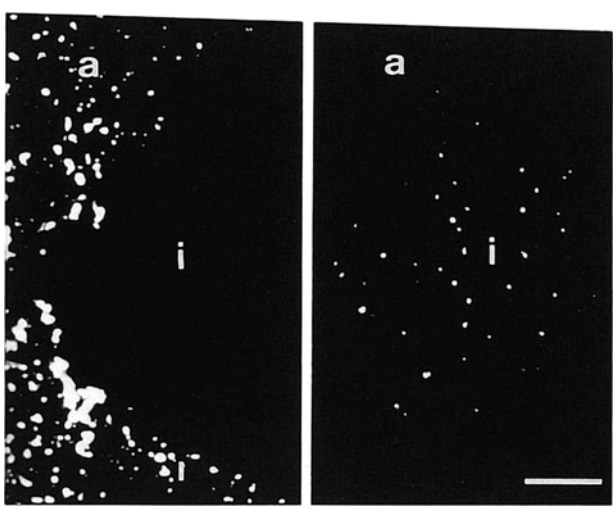

Fig. 3. Differential distribution of Cx32 and Cx43 in exocrine and endocrine cells. Incubation of two consecutive sections of a normal rat pancreas with antibodies to Cx32 (left panel) and Cx43 (right panel) resulted in a distinctly different labeling of the exocrine and endocrine portions of the gland. Cx32 was found to be extensively expressed in pancreatic acini (a), but absent in a nearby islet of Langerhans (i). In contrast, Cx43 was found to be sparsely present in the islet tissue, but not in acini. The bar represents 15 μm in both photographs.

related glands (rat prepucial and human sebaceous) which show a histological organization typical of an exocrine gland, in spite of a likely (phero)hormonal function, express Cx43 and no Cx32 (Meda *et al.*, 1993). Thus, the differential expression of Cx32 and Cx43 may contribute to the still undetermined events, whereby a secretory cell is eventually determined to discharge its secretory products in the vascular network rather than into an excretory system of ducts, hence defining its endocrine or exocrine function.

HORMONAL ACTION AND JUNCTIONAL COUPLING

Numerous hormones have been reported to affect the expression of connexins, gap junctions, and coupling in a large variety of cell systems (Meda *et al.*, 1984b; Stagg and Fletcher, 1990). Different hormones elicit different effects on coupling, and the effect of a given hormone is influenced by a combination of parameters, including the type of target cell, the isoform of connexin, and the physiological or experimental condition prevailing at the time of hormone application both inside and outside the cells (Meda *et al.*, 1984b; Stagg and Fletcher, 1990). Thus, the only general conclusion which can be so far derived from these studies, is that regulation of gap junctional coupling is a frequent and ubiquitous target of most hormones.

In most cases, the mechanism whereby hormones affect gap junction channels and cell-to-cell communication has not been investigated and/or remains elusive. The long time course required for some effects on the expression and functioning of connexin-made channels does not allow for a unambiguous distinction between direct and indirect hormonal effects. Hence, a number of the gap junction changes which have been putatively attributed to hormonal regulation may actually be a secondary consequence of other cell alterations primarily induced by the hormone. However, a direct effect of some hormones is certainly conceivable on at least some connexin isoforms. Thus, the promoter region of Cx43 contains an estrogen-responsive element (Yu *et al.*, 1994) that could account for the transcriptional regulation of the expression of this connexin after estrogen administration (Ramondt *et al.*, 1994). Other direct hormonal effects on connexins may occur at a posttranslational level, via a hormone-induced phosphorylation/dephosphorylation of the Ser and Thr residues, which are present on the C-terminus of some, but not all, connexins (Stagg and Fletcher, 1990). Connexin phosphorylation has been implicated in the membrane insertion of newly formed connexons, in their clustering within gap junction plaques, and in the regulation of the conductance and permeability of gap junction channels (Stagg and Fletcher, 1990; Musil and Goodenough, 1990).

In only a few cases, however, has the time course and dose-dependence curve of the hormonal effects on junctional coupling been found to be consistent with physiologically relevant conditions. A well-documented case is that of smooth muscle cells of uterus, which in the nonpregnant female and up to partum show a low contractile activity, which is spatially restricted and asynchronous in different regions of the myometrium. Under these conditions, the expression of connexins, gap junctions, and coupling is essentially undetectable. In contrast, as soon as labor starts, contractions and action potentials are markedly intensified and become transmitted over much larger distances and synchronized across large areas of the uterus. These changes are obligatorily associated with a parallel increase in the expression of Cx43, gap junctions, and coupling, and both the mechanical and junctional events are induced by concentrations of estrogens, progesterone, and prostaglandins that mimic those observed at the end of pregnancy (Miller *et al.*, 1989; Ramondt *et al.*, 1994).

Eventually, coupling has also been implicated in the transmission of hormonal signaling across multicellular systems (Lawrence and Gilula, 1978). Likely, this transmission is accounted for by the connexin-mediated cell-to-cell exchange of hormone-induced second messengers, such as Ca^{2+}, IP_3, or the catalytic subunit of hormone-responsive and cAMP-dependent protein kinases (Lawrence and Gilula, 1978; Murray and Fletcher, 1984; Saez *et al.*, 1989). Conceivably, such exchanges could ensure the amplification of a hormonal signal in at least two ways. First, coupling promotes the diffusion of minute amounts of second messengers, which typically have a rather short half-life, at distance from the initial site of hormone–target interaction. Such a diffusion would be greatly restricted in the absence of gap junction channels, due to the rapid dilution, binding, and/or degradation of the second messengers in the extracellular spaces. Second, coupling allows for the response of cells that lack appropriate hormone receptors and/or are deficient in the metabolic steps that are activated by the binding of hormones to cognate receptors. These noncompetent cells may gain responsiveness to hormonal stimulation after receiving, via

connexin-made channels, appropriate second messengers, generated within nearby hormone-competent cells (Bosco and Meda, 1992; Bosco *et al.*, 1989, 1994). Evolutionarily, the coupling-induced amplification of hormonal signaling has probably been advantageously selected, since it permits peripheral target tissues to be affected by minute concentrations of hormones, hence reducing the biosynthetic, storage, and release work of endocrine glands, while promoting tissue specificity of the hormonal effects.

CONCLUSIONS AND PERSPECTIVES

Secretion of both endocrine and exocrine pancreas is the result of the activity of numerous and functionally heterogeneous cells, whose integration obligatorily depends on proper communication. Full control of β-cell and acinar cell function cannot be ascribed to a sole regulatory mechanism, but rather to the interaction between multiple mechanisms that involve cell-to-cell signaling by nutrients, intrinsic and extrinsic neural inputs, local and circulating hormones, as well as direct interactions between adjacent cells. The multiplicity of mechanisms for intercellular coordination, permits the pancreas to properly adapt its hormonal and enzymatic secretion to the needs of the organism, which continuously change throughout the day, depending on the physiological condition. It also provides pancreatic cells with a regulatory system that, even though redundant and costly for the cells, allows for preservation of a normal secretory function under most conditions.

The precise contribution of different communication mechanisms to the net response of the pancreas remains to be fully understood, as does the hierarchic organization of pancreatic controls. In this respect, the finding of a gap junction-mediated modulation of pancreatic functions, under conditions abolishing indirect cell-to-cell communication, indicates a fundamental, hitherto disregarded role of cell-to-cell coupling. In view of the now well-established functional heterogeneity of both β-cells and acinar cells, it is probable that this mechanism has become an obligatory feature in evolution since it provides the most direct way for compensating intrinsic metabolic and effector differences of secretory cells. Hence, this equilibrating mechanism may be essential for building an appropriate output of hormones and enzymes, starting from the highly heterogeneous β-cell and acinar cell populations.

At this time, however, the molecular mechanism underlying the relationship between the control of coupling and pancreatic secretion remains to be unraveled, and our understanding of whether such a relationship is causal is limited by several unknowns. It remains also to be assessed whether and how coupling defects participate in the early and still obscure pathogenesis of pancreatic dysfunctions of major relevance to human medicine. As yet, such a possibility has not been investigated.

Previous studies have revealed that gap junction channels and their constitutive connexin proteins may be regulated by a variety of hormones. Again, the mechanism of such a regulation, and the way changes in gap junctional coupling may in turn affect the peripheral action of endocrine products, remain to be assessed under physiologically relevant conditions similar to those prevailing *in vivo*. The recent availability of novel cell and molecular biology tools, and of strategies with which to interfere with specific steps of junctional coupling and secretion, offers now the exciting perspective of directly addressing these questions, *in vitro* as well as *in vivo*.

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REFERENCES

Atwater, I., Rosario, L., and Rojas, E. (1983). *Cell Calcium* **4**, 451–461.

Bosco, D., and Meda, P. (1992). *Endocrinology* **129**, 3157–3166.

Bosco, D., Orci, L., and Meda, P. (1989). *Exp. Cell Res.* **184**, 72–80.

Bosco, D., Soriano, J. V., Chanson, M., and Meda, P. (1994). *J. Cell Physiol.* **60**, 378–388.

Chanson, M., Bruzzone, R., Bosco, D., and Meda, P. (1989). *J. Cell Physiol.* **139**, 147–156.

Chanson, M., Orci, L., and Meda, P. (1991). *Am. J. Physiol.* **261**, G28–G36.

Eddlestone, G. T., Gonçalves, A., Bangham, J. A., and Rojas, E. (1984). *J. Membr. Biol.* **77**, 1–14.

Friend, D. S., and Gilula, N. B. (1972). *J. Cell Biol.* **53**, 758–776.

Heimberg, H., De Vos, A., Vandercammen, A., Van Schaftingen, E., Pipeleers, D., and Schuit, F. (1993). *EMBO J.* **12**, 2873–2879.

Iwai, M., Miyashita, T., and Shimazu, T. (1995). In *Progress in Cell Research*, Vol. 4 (Kanno, Y. Kataoka, K., Shiba, Y., Shibata, Y., and Shimazu, T., eds.), Elsevier Science B.V., Amsterdam, pp. 297–300.

Iwatsuki, N., and Petersen, O. H. (1977). *Nature* **268**, 147–149.

Iwatsuki, N., and Petersen, O. H. (1979). *Pflügers Arch. Eur. J. Physiol.* **380**, 277–281.
Kanno, Y., Sasaki, Y., and Shiba, Y. (1987). *Arch. Oral Biol.* **32**, 43–46.
Kohen, E., Kohen, C., Thorell, B., Mintz, D. H., and Rabinovitch, A. (1979). *Science* **204**, 862–865.
Kohen, E., Kohen, C., and Rabinovitch, A. (1983). *Diabetes* **32**, 95–98.
Lawrence, T. S., Beers, W. H., and Gilula, N. B. (1978). *Nature* **272**, 501–506.
Mears, D., Sheppard, N. F., Jr., Atwater, I., and Rojas, E. (1995). *J. Membr. Biol.* **146**, 163–176.
Meda, P. (1995). In *Pacemaker Activity and Intercellular Communication* (Huizinga, J. D., ed.), CRC Press, Boca Raton, pp. 275–290.
Meda, P., Perrelet, A., and Orci, L. (1979). *J. Cell Biol.* **82**, 441–448.
Meda, P., Amherdt, M., Perrelet, A., and Orci, L. (1981). *Exp. Cell Res.* **133**, 421–430.
Meda, P., Michaels, R. L., Halban, P. A., Orci, L., and Sheridan, J. D. (1983). *Diabetes* **32**, 858–868.
Meda, P., Atwater, I., Gonçalves, A., Bangham, A., Orci, L., and Rojas, E. (1984a). *Q. J. Exp. Physiol.* **69**, 719–735.
Meda, P., Perrelet, A., and Orci, L. (1984b). In *Modern Cell Biology*, Vol. 3 (Satir B. H., ed.), Alan Liss, New York, pp. 131–196.
Meda, P., Bruzzone, R., Knodel, S., and Orci, L. (1986). *J. Cell Biol.* **103**, 475–483.
Meda, P., Bruzzone, R., Chanson, M., Bosco, D., and Orci, L. (1987). *Proc. Natl. Acad. Sci. USA* **84**, 4901–4904.
Meda, P., Bosco, D., Chanson, M., Giordano, E., Vallar, L., Wollheim, C., and Orci, L. (1990). *J. Clin. Invest.* **86**, 759–768.
Meda, P., Chanson, M., Pepper, M., Giordano, E., Bosco, D., Traub, O., Willecke, K., El Aoumari, A., Gros, D., Beyer, E., Orci, L., and Spray, D. C. (1991). *Exp. Cell Res.* **192**, 469–480.
Meda, P., Pepper, M., Traub, O., Willecke, K., Gros, D., Beyer, E., Nicholson, B., Paul, D., and Orci, L. (1993). *Endocrinology* **133**, 2371–2378.
Meda, P., Vozzi, C., Ullrich, S., Dupont, E., Charollais, A., Sutter, E., and Bosco, D. (1995). In *Progress in Cell Research*, Vol. 4 (Kanno, Y. Kataoka, K., Shiba, Y., Shibata, Y., and Shimazu, T., eds.), Elsevier Science B.V., Amsterdam, pp. 281–287.
Meissner, H. P. (1976). *Nature* **262**, 502–504.
Miller, S. M., Garfield, R. E., and Daniel, E. E. (1989). *Am. J. Physiol.* **256**, C130–C141.
Munari-Silem, Y., Mesnil, M., Selmi, S., Bernier-Valentin, F., Rabilloud, R., Rousset, B. (1990). *J. Cell Physiol.* **145**, 414–427.
Munari-Silem, Y., Audebet, C., and Rousset, B. (1991). *Endocrinology* **128**, 3299–3309.
Munari-Silem, Y., Lebrethon, M. C., Morand, I., Rousset, B., and Saez, J. M. (1995). *J. Clin. Invest.* **95**, 1429–1439.
Murray, S. A., and Fletcher, W. H. (1984) *J. Cell Biol.* **98**, 1710–1719.
Musil, L. S., and Goodenough, D. A. (1990). *Curr. Opin. Cell Biol.* **2**, 875–880.
Petersen, C. C., and Petersen, O. H. (1991). *FEBS Lett.* **284**, 113–116.
Petersen, O. H., and Findlay, I. (1987). *Physiol. Rev.* **67**, 1054–1116.
Philippe, J., Giordano, E., Gjinovci, A., and Meda, P. (1992). *J. Clin. Invest.* **90**, 2228–2233.
Pipeleers, D. (1984). *Experientia* **40**, 1114–1126.
Ramondt, J., Verhoeff, A., Garfield, R. E., and Wallenburg, H. C. (1994). *Eur. J. Obstet. Gynecol. Reprod. Biol.* **54**, 63–69.
Sáez, J. C., Connor, J. A., Spray, D. C., Bennett, M. V. L. (1989). *Proc. Natl. Acad. Sci. USA* **86**, 2708–2712.
Salomon, D., and Meda, P. (1986). *Exp. Cell Res.* **162**, 507–520.
Sasaki, Y., Shiba, Y., and Kanno, Y. (1988). *Jpn. J. Physiol.* **38**, 531–543.
Schuit, F. C., In't Veld, P. A., and Pipeleers, D. G. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 3865–3869.
Seseke, F. G., Gardemann, A., and Jungermann, K. (1992). *FEBS Lett.* **301**, 265–270.
Sorenson, R. L., and Parsons, J. A. (1985). *Diabetes* **34**, 338–341.
Stagg, R. B., and Fletcher, W. H. (1990). *Endocr. Rev.* **11**, 302–325.
Stefan, Y., Meda, P., Neufeld, M., and Orci, L. (1987). *J. Clin. Invest.* **80**, 175–183.
Stutenkemper, R., Geisse, S., Schwarz, H. J., Look, J., Traub, O., Nicholson, B. J., and Willecke, K. (1992). *Exp. Cell Res.* **201**, 43–54.
Sugita, M., Shiba, Y., and Kanno, Y. (1995). In *Progress in Cell Research*, Vol. 4 (Kanno, Y., Kataoka, K., Shiba, Y., Shibata, Y., and Shimazu, T., eds.), Elsevier Science B. V., Amsterdam, pp. 313–316.
Valdeolmillos, M., Nadal, A., Soria, B., and Garcia-Sancho, J. (1993). *Diabetes* **42**, 1210–1214.
Vozzi, C., Ullrich, S., Charollais, A., Philippe, J., Orci, L., and Meda, P. (1995). *J. Cell Biol.* **131**, 1561–1572.
Yu, W., Dahl, G., and Werner, R. (1994). *Proc. R. Soc. London B. Biol. Sci.* **255**, 125–132.