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Review

Involvement of gap junctional communication in secretion

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Abstract

Glands were the first type of tissues in which the permissive role of gap junctions in the cell-to-cell transfer of membrane-impermeant molecules was shown. During the 40 years that have followed this seminal finding, gap junctions have been documented in all types of multicellular secretory systems, whether of the exocrine, endocrine or pheromonal nature. Also, compelling evidence now indicates that gap junction-mediated coupling, and/or the connexin proteins per se, play significant regulatory roles in various aspects of gland functions, ranging from the biosynthesis, storage and release of a variety of secretory products, to the control of the growth and differentiation of secretory cells, and to the regulation of gland morphogenesis. This review summarizes this evidence in the light of recent reports.

Keywords: Exocrine gland; Endocrine gland; Enzyme; Hormone; Ca^{2+}; Synchronization

Contents

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1. Introduction

Secretion is a widespread and diversified cell function that, in most cases, is accomplished by multi-cellular glands. Within these organs, secretory cells coordinate their functioning by exchanging information with each other, and possibly also with nearby vascular and mesenchymal cells, via both indirect (i.e., neurotransmitter-, hormone-, calcium-mediated, . . .) and direct communication mechanisms (i.e., cell adhesion molecule-, integrin-, junction-mediated, . . .) [1–3]. In vertebrates, a consistent, if not obligatory feature of this communication network, is the cell-to-cell channels made of connexin proteins, that cluster to form gap junctions [1–10]. Thus, it has long been known that these specialized membrane micro-domains connect a variety of secretory cells, irrespective of their endocrine or exocrine nature, the type of secretory product made, the mechanism of its release and the type of regulation by stimuli and inhibitors [1–10]. Since, a large body of circumstantial evidence has accumulated, indicating that gap junctions are required for the fine regulation of the biosynthesis, storage and release of various secretory products [1–10]. With the increasing knowledge on the connexin family, some of this work has been recently revisited with more specific tools, and in more physiologically relevant models, confirming that distinct connexin isoforms are implicated in the fine in vivo tuning of the biosynthesis and release of secretory products [2,3,9,10]. Recent work has also provided some insight about the reason why different connexins have been preferred by some types of secretory cells, while being excluded by others [11–13].

Here, we have reviewed the distribution and function of these connexins that are expressed by endocrine and exocrine

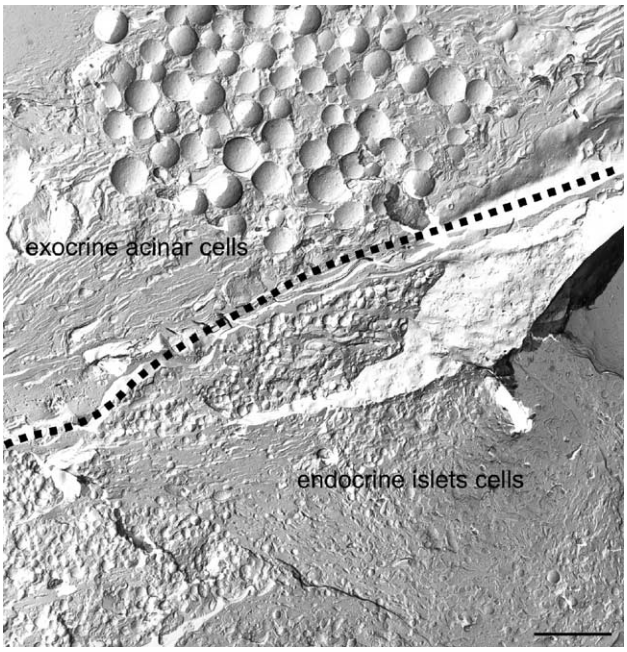

Fig. 1. The exocrine and endocrine cells of pancreas are organized within independent, closely apposed units. Under the electron microscope, a freeze-fracture replica of a rat pancreas reveals the close apposition of exocrine acinar cells, identified by the large, spherical secretory granules containing a mixture of digestive enzymes (above the dotted line), and the endocrine cells of a pancreatic islet identified by the much smaller secretory granules containing either insulin, glucagon, somatostatin or pancreatic polypeptide (below the dotted line). Scale bar, 1.5 μm.

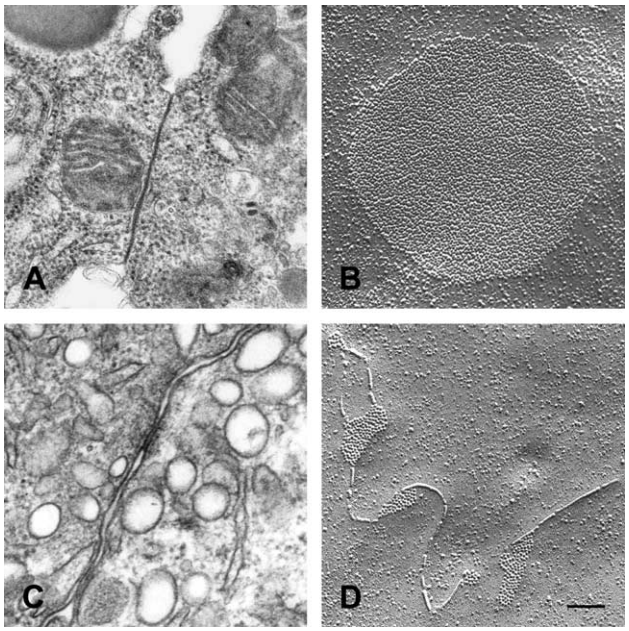

Fig. 2. Gap junctions differ between exocrine and endocrine cells of pancreas. (A) Electron microscopy shows that the membranes of adjacent pancreatic acinar cells, identified by the typical ultrastructure of enzyme-containing secretory granules (white arrow) come into contact at large gap junctions (between the black arrows). (B) Freeze-fracture reveals that these junctions usually comprise several hundreds-thousands connexons, which are know by immunolabeling to be made of Cx26 and Cx32. (C) Gap junctions are much more minute between the insulin-producing β-cells of pancreatic islets. (D) Freeze-fracture reveals that each β-cell plaque comprises only a few dozen connexons, which are know by immunolabeling to be made of Cx36. Typically, β-cell gap junctions are clustered in restricted domains of the cell membrane, in close contact with tight junction fibrils. Bar, 300 nm in panels A and C, and 100 nm in panels B and D.

glands, in light of these recent studies. The reader is referred to previous reviews [1–10] for a comprehensive coverage of the early studies on this topic.

2. Exocrine and endocrine cells express different connexin isoforms

It has long been documented that whereas most secretory cells of exocrine glands express Cx26 and Cx32 but usually not Cx43, the latter protein is the predominant connexin isoform of endocrine cells [11], including when the two types of cells closely interact within a very same organ (Fig. 1). Hence, and with the exception of glands which have both endocrine–exocrine features, for phylogenetic, embryological or physiological reasons [3,11], there appears to be an almost alternative choice of different connexin isoforms depending on whether secretory cells discharge their products into the external environment or into the blood. Often, the various connexins chosen to form the connexons also pack into morphologically different gap junction plaques in exocrine and endocrine cells (Figs. 2 and 3). No data yet dispute this differential distribution, which is observed in various animal species, even though Cx26 has now been documented between the endocrine cells of the pineal gland [14] and the anterior pituitary [15]. However, other connexin isoforms have now been added to the list of proteins making gap junctions of secretory cells. Thus, Cx36, Cx40 and Cx45 have been documented in selected endocrine cells [3], whereas Cx30 has been shown to be co-expressed with Cx26 and Cx32 in an exocrine gland [16]. Thus, if there is not a specific “endocrine” or “exocrine” connexin, there is certainly a highly conserved pattern of

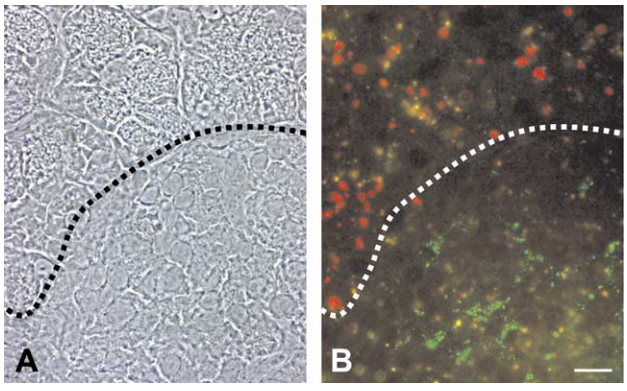

Fig. 3. Exocrine and endocrine pancreatic cells express different connexins. (A) Phase-contrast microscopy reveals the close apposition of the exocrine acinar cells and of the endocrine islet cells, in a cryo-section of a control mouse pancreas. (B) Immunolabeling of the same field with antibodies to Cx32 (red) and Cx36 (green) reveal that the former protein forms large gap junction plaques in the exocrine acini (above the dotted line), whereas the latter forms minute plaques in the central, β-cell-rich region of an endocrine islet (below the dotted line). Note the paucity of Cx36 at the islet periphery, which is formed mostly by glucagon-, somatostatin- and pancreatic polypeptide-producing cells. Scale bar, 12 μm.

expression, that varies in the two main types of secretory systems. In view of recent studies relating different connexin isoforms to different conductance, permeability and regulatory characteristics of gap junctional channels [17–20], it is most likely that this differential selection was imposed, or became selected during evolution, in order to match the requirements for specific gap junction-permeant molecules to the various needs of different types of secretory cells. It will be difficult to test this hypothesis experimentally, inasmuch as most of the ions and metabolites that permeate gap junction channels are also the very same molecules that are central to the proper control of the main steps of several secretory processes. However, the two studies in which Cx32 was expressed ectopically, either by knock-in of the *Gjb1* gene at the *Gja1* locus [21; the nomenclature of the connexin genes is given at http://www.informatics.jax.org/] or by transgenic over expression of the cognate cDNA in a cell type that only expresses Cx36 [22], have documented an altered function of various target cells, including the exocrine mammary gland, the exocrine and endocrine cells of testis and the endocrine insulin-producing cells of pancreatic islets [21,22]. These data indicate that specific connexin patterns are required for proper in vivo secretion of various products. Recent studies have provided some insight about the mechanism whereby exocrine and endocrine cells may select different connexins. Thus, the characterization of the 5′ regulatory region of the human *Gja9* gene has led to the identification of a promoter region sufficient to restrict the expression of the cognate connexin to the insulin-secreting β-cells and neurons [12]. Within this regulatory region, a conserved neuron-restrictive silencer element binds the NRSF/REST factor, which functions as a potent repressor of neuronal genes. NRSF/REST is widely expressed in most cell types, except β-cells and most neurons, and its ectopic expression reduces that of Cx36 in an insulin-producing cell line [12]. Thus, endocrine cells and secretory neurons lacking REST can produce a Cx36 mRNA, whereas most other cells, including numerous types of gland cells, cannot. In a parallel study, mice lacking the basic helix–loop–helix transcription factor Mist1, which is normally expressed in most exocrine cell types, were shown to feature altered architecture and function of pancreatic acini, as well as a severe transcriptional down-regulation of the *Gjb1* gene [13]. Strickingly, the acinar cells of Mist1 KO mice were fully uncoupled, due to loss of Cx32 and to a prominently cytosolic localization of Cx26 [13]. These findings are at variance with those made in the Cx32 KO mice, whose pancreatic structure was normal, and acinar cell coupling as well as membrane localization of Cx26 were at least partly preserved [24]. These findings point to transcription as a key step in the differential choice that secretory cells make between various connexin isoforms.

An additional regulatory event is suggested by the alterations in the levels of several connexins, which are frequently

Table 1
Distribution of connexins in secretory systems

Connexin	Organ	Cell type	Secretory product	Mode of secretion
Cx26 and Cx32	Pancreas	Acinar cells	20 enzymes (amylase, ...), ions	Exocrine
	Lacrymal glands	Acinar cells	Ions, (glyco)proteins	
	Liver	Hepatocytes	Bile salts, proteins, cholesterol, ...	
	Stomach	Parietal cells	Hydrochloric acid, intrinsic factor	
	Salivary glands	Acinar cells	Enzymes (amylase), ions, bicarbonate	
	Mammary glands	Alveolar cells	Proteins, immunoglobulins, ions	
	Testis	Sertoli cells	Peptides	
Cx30	Mammary glands	Alveolar cells	Proteins, immunoglobulins, ions	
Cx43	Testis	Sertoli cells	Peptides	
	Ovary	Granulosa cells	Proteins, ions	
Cx43	Adrenal	Spongiocytes	Mineralocorticoids, glucocorticoids, androgens	Endocrine
	Testis	Leydig cells	Testosterone	
	Ovary	Thecal cells	Androgens	
		Luteal cells	Progesterone	
	Pituitary gland	Prolactin cells	Prolactin	
		Basophil cells	ACTH	
	Placenta	Throphoblast cells	B-hCG, hCS	
	Thyroid	C cells	Calcitonin	
	Parathyroid	Principal cells	Parathormone	
Cx36	Pancreas	β-cells	Insulin	
	Hypothalamus	Neurons	GnRH, TRH, somatostatin, GH, GHRH, PIH, PRF, ADH	
	Adrenal	Chromaffin cells	Epinephrine, norepinephrine	
Cx32 and Cx43 and Cx26	Thyroid	Follicular cells	Thyroxine, triiodothyronin	
Cx43 and Cx26	Pituitary gland	Acidophil cells	Growth hormone, prolactin	
Cx26	Pineal gland	Pinealocytes	Melatonin	
	Hypothalamus	Neurons	GnRH, TRH, somatostatin, GH, GHRH, PIH, PRF, ADH	
	Pituitary gland	Acidophil cells	GH, Prl	
Cx40	Kidney	Myoepithelial cells	Renin	
Cx43	Sebaceous glands	Sebocytes	Dead cells, pheromones, complex lipids, ...	Pheromone

observed after the selective deletion of only one of their coding genes. Thus, mice lacking Mist1 not only showed decreased transcription of the *Gjb1* gene but also decreased levels of the Cx26 protein [13], indicating a differential transcriptional, translational and/or post-translational control of 2 connexins, that are co-expressed by a very same cell type. Similarly, deletion of the *Gjb1* gene resulted in altered membrane levels of Cx26 in liver hepatocytes [23], acinar cells of pancreas [24] and lacrimal glands [25], which all normally co-express Cx26 and Cx32 [11]. Thus, some stringent, still to be defined mechanism links the expression of these beta type connexin isoforms in various tissues.

3. Gap junctions and exocrine secretion

Forty years after the first identification of dye coupling between the cells of an exocrine gland [26], an event that undoubtedly gave a novel, since uninterrupted impetus to the field of gap junctions and coupling, no multicellular exocrine gland has been found in which secretory cells are not joined by connexin channels [2,5,7,9,11]. The following sections, arranged as a function of the connexin isoform expressed in various glands (Table 1), summarize the present status of our knowledge on the effect of the signaling dependent on these connexins on exocrine secretion. Cx26, Cx32, Cx40, Cx43 and Cx45 have been reported in exocrine glands after analysis of either total RNA or proteins [2,11]. However, immunolabeling of sections has consistently shown that the latter three proteins are restricted to vessels, connective tissue, contractile cells and excretory ducts. Thus, only Cx26 and Cx32 are expressed by parenchymal secretory cells, most of which co-express both gap junction proteins.

3.1. Cx26 and Cx32

Analysis of transgenic mice deleted for either one of these proteins [27,28] indicated that neither Cx26 nor Cx32 are obligatory for a normal prenatal development of exocrine glands, and for the close to normal differentiation of their main secretory cells [27,28]. However, recent data indicate that this conclusion may not hold true, at least in the case of the mammary glands, when *Gjb2* is deleted in the post-natal period [29]. Furthermore, transgenic animals lacking Cx26 and/or Cx32 show impaired function of many glands [24,25,27,28].

3.1.1. Pancreas, acinar cells

The acinar cells of pancreas, which secrete a mixture of about 20 (pro)enzymes, including the predominant amylase, are coupled by Cx32- and Cx26-made channels [11]. Loss of Cx32 in transgenic mice had no effect on acinar architecture, but increased basal amylase secretion, presumably as a result of a decrease in the basal cell-to-cell coupling [24]. Thus, whereas virtually all cells are coupled within resting acini of control mice [7,30], this coupling decreased by more than 60% in acini of knock-out Cx32 animals [24]. Strikingly, the maximal stimulation of this system by physiologic cholinergic stimuli, normally results in at least a partial uncoupling of the acinar cells [7,30] (Fig. 4). Accordingly, the amylase release measured under such conditions was similar in control mice expressing native levels of Cx32 and in knock-out animals that lacked this protein [24,30]. In the latter animals, some reduction in the levels of Cx26, which presumably accounted for the residual coupling of acinar cells, was also detected after loss of Cx32 [24], suggesting some link in the expression of the two gap junction proteins. A recent study has provided some insight about the regulation of this link. Thus, mice lacking the transcription factor Mist1 showed defective dye and electrical coupling of acinar cells due to complete loss of transcription of the *Gjb1* gene [13]. Strikingly, and in spite of a normal transcription of the Cx26 mRNA, the cognate protein was no more detected in the acini of the knock-out mice [13]. These studies point to a dual role of Cx26 and Cx32 in ensuring the coupling of acinar cells (both protein contribute), to an essential role of Cx32 in the control of basal enzyme secretion (Cx26 cannot substitute for this function), and a differential regulation of the expression of the two gap junction proteins, which are usually co-expressed by the very same acinar cells.

3.1.2. Lacrimal glands, acinar cells

The acinar cells of the exorbital lacrimal glands, which secrete the various components of tears, are also coupled by Cx32 and Cx26 channels [31]. Again, in this system, loss of Cx32 in transgenic mice altered the distribution of Cx26, which appeared retained in the cytosolic compartment of

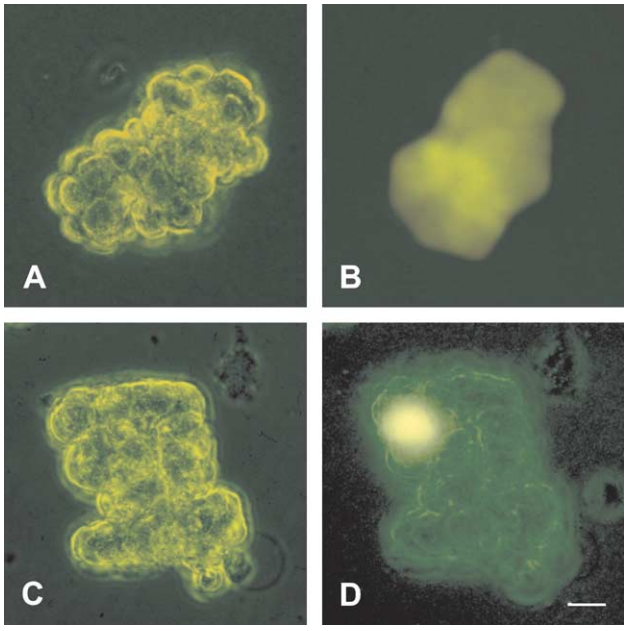

Fig. 4. Pancreatic acinar cells are extensively coupled in the resting state and uncouple during stimulation by secretagogues. (A and B) Microinjection of Lucifer Yellow revealed that acinar cells are all dye coupled to each other within a resting pancreatic acinus. (C and D) Upon stimulation by a secretagogue, the tracer was retained by the injected cell, indicating uncoupling of the acinar cells. Scale bar, 15 μm.

acinar cell of female, but not male animals [25]. This finding correlated with the persistence of acinar cell coupling in male but not female animals, and with an abnormally low fluid output of the glands of female mice, after stimulation by topical doses of carbachol, in spite of a normal protein content of the lacrymal secretion [25]. The data suggest a role of gap junctions in fluid secretion, consistent with previous findings in another exocrine system [32]. They also imply that knock-out mice, and specifically female, should be much more prone to develop corneal erosions and ulcers after loss of Cx32. So far, this implication has not been documented.

3.1.3. Liver, hepatocytes

Hepatocytes, which secrete the various components of bile, are coupled by gap junctions containing Cx26 and Cx32 [11], even though the relative abundance of the 2 proteins varies in centrolobular and periportal cells [33]. Mice knock-out for the *Gjb1* gene also showed decreased expression of Cx26 at the hepatocyte membrane [23]. The absence of Cx32 and/or the mis-localization of Cx26 altered the hepatic function of Cx32-deficient mice. Thus, after stimulation of the hepatic nerves, the liver of these animals released significantly less glucose from glycogen than that of wild type littermates [28]. Moreover, electrical stimulation of hepatic nerves also resulted in a reduced bile flow in Cx32-deficient mice [23], a function in which Cx43, the connexin isoform that couples the cells of intra- and extra-hepatic biliary ducts may also play a significant role [34]. In vitro, transfection of a Cx32 cDNA improved the dye coupling of the hepatoma-derived HepG2 cells, and enhanced liver-specific functions, such as albumin secretion and ammonium removal [35]. All together, these results show an important role of Cx32 in the secretory function of hepatocytes. The role of Cx26 in this context appears less relevant, inasmuch as this connexin isoform cannot substitute for lack of Cx32 [23,28]. However, Cx26 is deemed to have other essential roles in the functions of hepatocytes, e.g., in the control of their proliferation [36].

3.1.4. Stomach, parietal cells

Cx26 and Cx32 are co-expressed in the HCl and intrinsic factor producing parietal cells of gastric glands [37]. Cx32 is mainly detected at the cell membrane, whereas Cx26 is mostly localized in the cytoplasm [37]. Inhibition of cell-to-cell coupling by 18alpha-glycyrrhetinic acid inhibited the increase in cytosolic-free Ca^{2+} which is normally caused by gastrin stimulation, suggesting a physiological role of gap junction channels in the rapid onset of acid secretion [37].

3.1.5. Salivary glands, acinar cells

The acinar cells of submandibular and sublingual glands, which secrete the various components of saliva, are also coupled via Cx26 and Cx32 channels [2,11], which mix within the same gap junction plaques [38,39]. So far, however, only circumstantial evidence suggests a role of gap junctional communication in the secretion of saliva. Thus, cholinergic stimuli induce in vitro a rapid increase in cytosolic-free Ca^{2+} and salivary secretion from rat mandibular glands, two events which are inhibited by octanol in both the intact isolated gland and acini dispersed thereof [40]. The data suggest that gap junction channels may control salivary secretion via the inhibition of capacitative Ca^{2+} entry.

3.1.6. Mammary glands, alveolar cells

Contrasting with the exocrine glands mentioned above, the alveolar cells of mammary glands express Cx26 and Cx32 [41,42], as well as Cx30 [16] (Table 1). The expression of the 2 former proteins changes as a function of the development and function of mammary glands [41,42]. Thus, Cx26 is barely detectable in the glands of virgin animals, increases during the early steps of pregnancy and is seen at steady, maximal levels throughout lactation. In contrast, Cx32 is not detectable in glands of virgin and pregnant rodents, but is dramatically induced after delivery [16,41,42]. In a variety of transgenic mice, normal mammary development was observed after loss of Cx32 or full replacement of this protein by Cx43 [30], consistent with the observation that these animals can at least sustain the minimal requirements of milk production, content and release which are needed to raise a litter. In contrast, mice in which the *Gjb2* gene was specifically ablated in the mammary epithelium before puberty, featured abrogated alveolar development and increased cell death during pregnancy, resulting in impaired lactation [43]. This alteration was developmentally controlled, inasmuch as it was not observed when Cx26 was ablated during the later part of pregnancy [30]. Interestingly, Cx32 was reported to initially organize with Cx26 into heteromeric hemichannels, and to form both heteromeric (Cx26–Cx32) and homomeric channels (Cx32–Cx32) only at later developmental stages [43], a change which was paralleled by differences in the cell-to-cell transfer of second messengers implicated in milk production [43]. Strikingly, channels made exclusively or predominantly of Cx26 were inhibited by taurine, an osmolyte accumulating in mammary cells during lactation and which is required for milk synthesis [43]. The data indicate that the stoichiometry of connexins in gap junction channels changes as a function of mammary gland development, presumably to maximize the specific molecular and electrical exchanges which are needed to support proper milk secretion.

3.1.7. Testis, Sertoli cells

A few gap junctions containing Cx26 and Cx32 have been described in spermatids and Sertoli cells of the seminiferous epithelium [44].

3.2. Cx30, Cx37, Cx43 and Cx45

3.2.1. Mammary glands, alveolar cells

Cx30 has recently been reported to be expressed by the alveolar cells of mammary glands, at levels which increased during pregnancy and reached a maximum at the onset of lactation [16]. In agreement with these findings, the expression of Cx30 was induced in vitro by lactogenic hormones [16]. Even though transgenic mice knock-out for the *Gjb6* gene and

lacking the cognate protein have been recently reported [45], no lactation defect was investigated or reported.

Levels of Cx43 varying with developmental and functional stages have also been reported in the mammary gland. Thus, Cx43 was barely expressed in virgin mice, increased from pregnancy to lactation and, then, decreased with the gland involution [16,46,47]. These changes further correlated with post-translational modification, inasmuch as the protein is mostly non-phosphorylated in the glands of virgin and pregnant females but is essentially phosphorylated in lactating animals [16,46,47]. Still, the presence of Cx43 in the mammary glands does not contradict its usual absence between the secretory cells of exocrine glands, inasmuch as the protein is exclusively found in the myoepithelial cells, the contractile cells which surround the alveolae and the excretory ducts [46]. Such a localization is in agreement with the presence of Cx43 in many types of contractile cells [48], as well as its detection in other ductal, excretory systems of exocrine glands [34].

3.2.2. Testis, Sertoli cells

Glands such as testis and ovary, which comprise cells fulfilling both an exocrine (secretion of fluid within the lumen of seminiferous tubules and the autrum of ovarian follicles, respectively) and endocrine function (production of testosterone, and of androgens and progesterone, respectively) express mostly Cx43. Cx43 couples Sertoli cells to spermatogonia and spermatocytes [49,50] as well as to basal germ cells [51]. This connexin appears required for the synchronization of local functions of Sertoli cells, presumably controlling spermatogenesis [52]. Accordingly, mice lacking Cx43, feature at birth unusually small gonads, due at least in part to a deficiency in the prenatal expansion of the germ cell line [21,50,53]. Grafting of fetal testis from mice lacking Cx43 under the kidney capsules of wild type SCID mice, revealed that this defects persisted postnatally [53].

3.2.3. Ovary, granulosa cells

The granulosa cells of the ovarian follicle have been reported to be mostly coupled by Cx43 gap junctions [54–57], even though expression of Cx32, Cx37 and Cx45 have also been documented [58–60]. The relevance of the Cx43 role is demonstrated by the abnormal postnatal folliculogenesis in mice lacking this isoform [50], and by the delayed growth of oocytes in follicles showing reduced [61] or nil levels of Cx43 [62]. Cx37 channels are restricted to the interface between the oocytes and the granulosa cells forming the cumulus [60]. This connexin also plays a major role in the functional development of ovaries, inasmuch as mice lacking Cx37 feature a premature interruption of follicle development and oocytes which cannot complete their normal meiotic maturation [60].

4. Gap junctions and endocrine secretion

In vertebrates, the hormone-producing cells of all multicellular endocrine glands are also connected by functional gap junctions. The reason for this apparently obligatory requirement is presumably related to the functional heterogeneity of individual cells, which differ within a very same gland in several respects, including the ability to biosynthesize, store and release hormones [63–66]. Recent work further indicates that connexins are also essential to ensure the accurate synchronization of cell events, which is required to release the hormones in a regularly, rhythmic burst pattern, that should follow specific frequency and circadian rhythms to avoid over effects and run-down of the peripheral receptors [67–71]. To achieve the proper cell-to-cell communication that underlies these functions, most endocrine cells have utilized Cx43 (Table 1), the connexin isoform which, as summarized above, has usually been excluded by the parenchymal cells of exocrine glands. In the endocrine glands, Cx43 either expressed alone, providing for only homomeric and homotypic channels, or is co-expressed with other connexin isoforms, usually Cx36 and Cx40, leading to the possibility of at least some type of heteromeric and/or heterotypic channels (Cx43–Cx40). In a few endocrine glands, that embryologically originate as exocrine adnexae to epithelia, an unusual situation is found in which Cx32 and Cx26 are co-expressed with Cx43 by the very same secretory cell [2,3,11], even though not in the same membrane compartment. Under these circumstances, the gland may be endowed with a variety of rather different homomeric channel types.

4.1. Cx43

4.1.1. Adrenal gland, cortex spongiocytes

Cx43 is expressed by the spongiocytes of the adrenal cortex, which produce corticoids and sexual hormones [11,72–77]. The abundance of gap junctions varies in the different regions of the cortex. Thus, whereas there is little or no Cx43 in the outer, mineralocorticoid-producing region (zona glomerulosa), the protein is abundant in the inner glucocorticoid- (zona fasciculata) and androgen-producing regions (zona reticularis) [72,73]. This distribution correlates with regional differences in dye coupling, which is barely detectable in the outer region and conspicuous in the inner regions [76]. The number and size of gap junction plaques increased in bovine adrenal cortical cell lines [74] and primary rat adrenal cortical cells [75] after exposure to ACTH, the predominant hormone in the physiologic control of steroidogenesis. Conversely, drugs blocking Cx43 channels and antisense constructs decreasing their numbers, impaired the ACTH-induced secretion of cortisol in vitro [74,76,77]. Eventually, Cx43 was found decreased in the inner cortical regions of adrenal glands of hypophysectomized mice, and was restored at about normal levels when the animals were treated with ACTH, indicating a rapid and direct hormonal regulation of the in vivo assembly of Cx43-made gap junctions [78]. However, potentially conflicting results have been recently published with regard to the Cx43 induction by exogenous ACTH, since the infusion of this hormone in sheep adrenal glands decreased Cx43 expression [79]. Furthermore, the effect of gap junction blockade on

steroidogenesis is also questioned by experiments testing 18 alpha-glycyrrhetinic acid, which stimulated this anabolic pathway by a mechanism distinct from the protein kinase A-dependent one which physiologically controls steroidogenesis of adrenal cells [80]. Future studies should carefully address whether these apparently contradictory observations reflect a difference in the animal, cell model or experimental protocol tested in various studies. At any rate, the mechanism whereby gap junctions and/or cell coupling may be implicated in steroidogenesis remains to be elucidated. The finding that the production of cAMP increases in cortical cell lines after ACTH exposure, and that an experimental elevation in the cytosolic levels of cAMP reproduces in these cultures the steroidogenic induction as well as the increase in gap junction plaques observed with ACTH implicates the cyclic nucleotide in the regulatory pathway triggered by Cx43 [74].

4.1.2. Testis, Leydig cells

Even though six connexin transcripts have been reported to be expressed in the testis [81–83], the testosterone-producing cells of Leydig [84–86] appear mostly coupled by Cx43-made gap junctions [45,82–84], which decrease in confluent cell cultures, presumably as a result of activation of pathways dependent on protein kinase A and C [85]. A comparable decrease was observed during LH-induced stimulation of testosterone production, suggesting a tonic inhibitory influence of the connexin signaling [85]. In agreement with these observations, stimulation of testosterone production by hCG was also associated with a decrease in Cx43 mRNA levels both in vitro and in vivo [86]. This effect was mimicked by cAMP [86], suggesting that this second messenger somehow links the connexin-dependent signaling to the production of testosterone.

4.1.3. Ovary, thecal and luteal cells

The thecal cells of the ovarian follicles, which produce androgens, are connected by Cx43 [54,55,60]. The expression of this connexin varies with the stage of follicle development, consistent with an involvement of connexins in the endocrine function of thecal cells [56,57,87]. Conversely, the expression of follicular Cx43 appears to be under a tight hormonal control. Thus, treatment of hypophysectomized rats with estrogens up-regulated Cx43 expression, whereas treatment with gonadotropins, which stimulated ovulation and the formation of corporea lutea, down-regulated it [88]. Moreover, Cx43 levels were shown to decrease after the ovulatory LH surge [88].

The cells of corporea lutea, which produce progesterone, are also coupled by Cx43 [89,90]. Pharmacological treatments enhancing this coupling increase progesterone production, whereas treatments uncoupling luteal cells decrease steroid production [54]. Consistent with a relationship between connexin signaling and steroid production, the experimental inhibition of Cx43 expression decreased the LH-induced steroid production of luteal cells [91]. Strikingly, mice lacking Cx37, the connexin that forms gap junctions between oocytes and granulosa cells, also developed numerous and abnormal corpora lutea, by a mechanism which has not yet been elucidated [60].

4.1.4. Anterior pituitary, prolactin-producing cells

In rodents, Cx43 couples several endocrine cell types within the anterior pituitary [11,92–95]. This coupling increases the intercellular synchronization of Ca^{2+} transients [96]. Ca^{2+} waves are also propagated throughout the entire gland via the network of folliculo-stellate cells that are extensively coupled by Cx43 channels [94,97]. Annual screening of minks showed that the levels of Cx43 and the number of gap junction increased in the anterior pituitary during period of high prolactin content, under which conditions, the cellular distribution of the connexin was modified [98].

4.1.5. Placenta, cytotrophoblast cells

Cx43 connects the cells of placental cytotrophoblast, and these cells to those of the syncytio-trophoblast [99–101]. Recent studies have reported that the pharmacological blockade of gap junctions, as well as the antisense interference with the Cx43 transcript, uncouple cytotrophoblast cells, impairing their fusion and, thus, the formation of the syncytio-trophoblast [102,103]. These alterations were associated with a decrease in both the expression of trophoblast-specific genes, including those coding for β-hCG and human chorionic somatotropin [102,103], and the secretion of the former placental hormone [103].

4.1.6. Sebaceous glands, sebocytes

Pheromone glands, which are the phylogenetic precursors of multicellular endocrine systems, combine an exocrine mode of secretion with chemical signaling typical of hormones [2,3]. So far, Cx43 is the only connexin, which has been described in a pheromone-producing gland of rodents [11], as well as in the sebaceous glands of skin that represent their human homologs [104]. Whether Cx43 plays any role in the secretion of volatile pheromones has not yet been investigated.

4.2. Cx36

4.2.1. Pancreas, β-cells

Cx36 channels couple the insulin-producing β-cells of pancreatic islets [105–107] (Fig. 5) at sites of minute gap junctions [108]. Contrasting with most other types of cells, β-cells have not been convincingly shown to express other connexin isoforms, at least in normal adult rodents [109]. While it is not questionable that a Cx43 transcript is consistently found in pancreatic islets [105,109], the present evidence points to an endothelial and fibroblastic, rather than endocrine cell localization of the cognate protein [109]. However, a couple of reports have suggested that some induction of Cx43 may occur under certain conditions [110,111]. However, until such an induction can be documented in vivo, the available in vitro data should be evaluated in

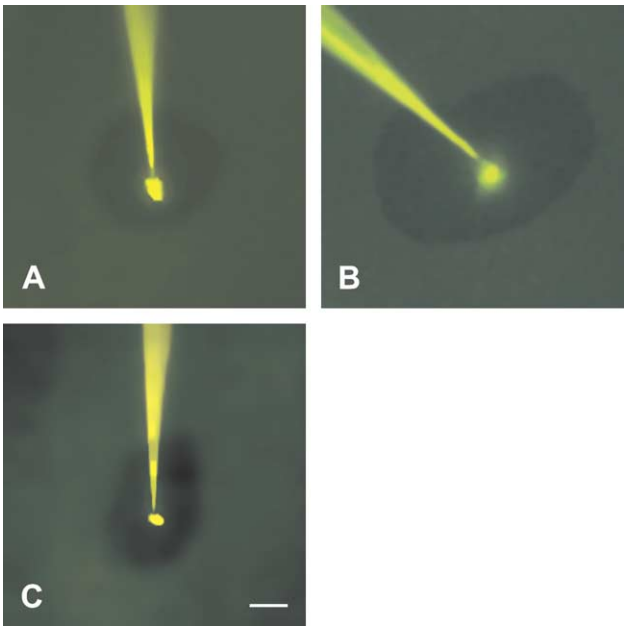

Fig. 5. Pancreatic β-cells are coupled in the resting state and do not uncouple during stimulation by secretagogues. (A) Microinjection of Lucifer Yellow revealed that small groups of β-cells are dye coupled within a resting pancreatic islet of Langerhans. (B) Upon stimulation by a secretagogue, the tracer was still transferred between several coupled cells which, as quantitated in serial sections of islets, were usually more numerous than under basal condition. (C) Such a transfer was not observed in an islet isolated from a homozygous KO-Cx36 mouse, demonstrating that β-cell coupling is mediated by Cx36 channels. Scale bar, 45 μm.

view of the well established "default shift" which cells towards the ectopic expression of Cx43, which most cells face when exposed to standard culture conditions. The functional importance of Cx36 is demonstrated by the impairment of glucose-induced insulin secretion and of its causal calcium waves after transfection of both primary β-cells and insulin-producing cell lines with Cx36 antisense constructs [112–116] or cDNA coding for other connexin species [22,115]. Recently, these observations have been extended to the in vivo situation. Thus, β-cells of knock-out transgenic mice lacking Cx36 were uncoupled (Fig. 5) and failed to respond to glucose stimulation with regular and synchronized calcium waves [67]. As a result, islets lacking Cx36 did not release insulin in a normal pulsatile fashion, and showed an increased basal release of insulin [67]. These pancreatic alterations are similar to those which are observed in type II diabetics [1–3,67], and indicate that loss of β-cell coupling alters insulin secretion. Strikingly in vitro experiments indicate that adequate levels of Cx36 are required for proper insulin secretion. Thus, the excess of Cx36 appears as deleterious as the lack of the connexin for proper insulin secretion [114,115].

4.2.2. Hypothalamus, neuroendocrine cells

Many types of secretory neurons are also coupled by Cx36 [117–120], which is one of the many proteins that are specifically shared by neurons and pancreatic β-cells [12]. Electrophysiological studies testing gap junction blockers have indicated that this coupling is implicated in the control of the pulsatile release of several neuropeptides, including GHRH [68], GnRH [69] and LHRH [70].

4.2.3. Adrenal gland medulla, cathecolamine-producing cells

Cx36 is expressed by the chromaffin cells of the adrenal gland medulla [71,106,107], which release catecholamines. Studies in rats demonstrate that the signaling dependent on Cx36 controls the basal release of both epinephrine and norepinephrine [71] and amplifies it after nicotinic stimulation by a cholinergic-dependent mechanism [121].

4.3. Cx32

Cx43, Cx32 and Cx26 are coexpressed in rodent thyroid glands [11,122–124]. In vitro, exposure of primary thyrocytes to TSH increased dye coupling in a time- and dose-dependent manner [124], whereas a mutation of Cx32 which leads to uncoupling, decreased thyroxine release [125]. Moreover, forced expression of Cx32 in a communication-incompetent thyroid cell line induced the up-regulation of the thyroglobulin gene [126]. Strikingly, however, the production of thyroxin was found unaffected in fetuses of Cx43/Cx32 double knock-out mice [127], indicating that the in vitro effects of connexins on thyroid function may not necessarily reflect the in vivo relevance of the connexin-dependent mechanisms. Presumably, this indicates that compensatory mechanisms enter into the play, in order to sustain a vital secretory function in the absence of the connexin regulation.

4.4. Cx26

Beside the case of the thyroid gland mentioned in the previous paragraph, a few other endocrine glands have been shown to express Cx26.

4.4.1. Pineal gland, melatonin-producing cells

Pinealocytes have been reported to be coupled by Cx26 channels, which are up-regulated in vitro by norepinephrine, possibly to improve melatonin secretion [14].

4.4.2. Anterior pituitary, GH- and PRL-producing cells

The acidophile cells of the anterior pituitary, which produce growth hormone and prolactin, are coupled [92–95] via channels made predominantly of Cx43 and Cx26 [11,92]. While there is some evidence for a secretion modulatory role of Cx43 (see above Section 4.1.4), the specific function of Cx26 in the function of the pituitary remains to be established.

4.4.3. Hypothalamus, neurosecretory neurons

GnRH-secreting cells express Cx36 [128], the only connexin which has yet been unambiguously mapped to the gap junction connecting neurons [129]. However, these cells have been reported to also express Cx32, Cx43 and Cx26 in situ [130], and Cx26 is the predominant connexin isoform coupling the GnRH-secreting cells of the GT1 line [15]. These cells feature a synchronized release of the hormone only when

Table 2
Connexins of exocrine and endocrine glands

Type of secretion	Organ	Cell type	Secretory product	Connexin
Enzymes	Pancreas	Acinar cell	Amylase, lipase and other digestive enzymes	Cx26 Cx32
	Salivary glands	Acinar cell	Amylase, lipase	Cx26 Cx32
	Prostate	Alveolar cell	fibrinolysin, other enzymes, peptides	Cx26 Cx32
Milk	Mammary glands	Alveolar cell	Casein, immunoglobulins, other proteins, lipids, ions	Cx26 Cx30 Cx32
Bile	Liver	Hepatocyte	Biliary salts, proteins, bilirubin, ions	Cx26 Cx32
Fluid	Salivary glands	Acinar cell	Water, ions	Cx26 Cx32
	Lacrymal glands	Acinar cell	Water, ions, peptides, (glyco) proteins	Cx26 Cx32
	Stomach	Parietal cell	Water, intrinsic factor, HCl, HCO_3^-	Cx26 Cx32
	Pancreas	Acinar cell (in rodents)	Water, ions	Cx26 Cx32
		Duct cells	Water, ions	Cx45 ?
	Ovary	Granulosa cell	Water, ions, glycoproteins	Cx37 Cx43
	Testis	Sertoli cells	Water, ions, inhibin, activins, ...	Cx43
Mucus	Salivary glands	Acinar cell	mucins	Cx26 Cx32
Sugars	Liver	Hepatocyte	Glucose	Cx32 Cx26
	Seminal vesicle	Alveolar cell	Fructose, peptides, prostaglandins	Cx26 Cx32
Peptide hormones	Heart	Auricular myoendocrine cell	Atrial natriuretic hormone	Cx43 Cx45
	Pancreas	β-cell	Insulin	Cx36
		α-cell	Glucagon	Cx36 ? Cx43 ?
	Thyroid	C cell	Calcitonin	Cx43
	Parathyroid	Chief cell	Parathormone	Cx43
	Anterior pituitary	Acidophil cell	Growth hormonel Prolactin	Cx43 Cx26
	Posterior pituitary		Oxytocin, Vasopressin	Cx36
	Hypothalamus	Neuroendocrine cell	Gonadotropin releasing hormone, Corticotropin releasing hormone, Thyrotropin releasing hormone, Growth hormone releasing hormone, Somatostatin	Cx36
			Prolactin release inhibiting hormone, Prolactin releasing factor, ...	Cx26 ?
Indolamine hormones	Pineal	Pinealocyte	Melatonin Serotonin	Cx26
Glycoprotein hormones	Kidney	Myoepithelial cell of afferent arteriole	Renin	Cx40
	Thyroid	Follicular cell	Triiodothyronine, Thyroxine	Cx43 Cx32 Cx26
	Anterior pituitary	Basophil cell	Follicle stimulating hormone; Luteinizing hormone; Thyroid stimulating hormone; Adrenocorticotropic hormone	Cx43
	Placenta	Throphoblast cell	Human chorionic gonadotropin, Human chorionic somatomammotropin	Cx43
Steroid hormones	Adrenal cortex	Spongiocyte	Corticoids, Mineralocorticoids, Androgens	Cx43

(continued on next page)

Table 2 (*continued*)

Type of secretion	Organ	Cell type	Secretory product	Connexin
	Testis	Leydig cell	Testosterone	Cx43
Steroid hormones	Ovary	Thecal cell	Androgens	Cx43
		Luteal cell	Progesterone	Cx43
Cathecolamines	Adrenal medulla	Chromaffin cell	Epinephrine, Norepinephrine	Cx36
Pheromones	Skin	Sebaceous cells	Sebum components	Cx43
	Preputial glands	Alveolar cells	Farnesenes, other lipids	Cx43

functional gap junctional communication is established [69], providing some clue that gap junctions are involved in the pulsatile release of the hormone. A single patient affected by congenital deafness because of a Cx26 mutation has so far been documented with a form of hypogonadotrophic hypogonadism of hypothalamic origin [130]. Thus, until additional cases are documented, the endocrine disorder reported in this study cannot be considered more than an associated phenotype.

4.5. Cx40

In kidneys, the renin-producing cells are joined to each other by Cx40 gap junctions, which also connect these endocrine cells to nearby endothelial cells [131]. Rats made hypertensive after clipping of one renal artery, showed a selective increase in kidney renin and Cx40, implying that the connexin is involved in the control of renin secretion and/or in the vasomotor control of kidney vessels [131]. However, in a strain of spontaneously hypertensive rats, Cx40 was found decreased between the endothelial cells of digestive arteries, and the pharmacological blockade of the renin-angiotensin system increased the levels of the protein while decreasing the hypertension [132,133]. These studies concur to indicate a link between the Cx40-dependent signaling and renin secretion, but also point to a different impact of the connexin pathway in renin-dependent and -independent forms of hypertension.

5. A gland-based view of connexin distribution

Table 2 revisits the distribution of connexins as a function of the type of exocrine and endocrine secretion. When compared to Table 1, which focuses on connexin isoforms, Table 2 shows that the selection of these isoforms does not directly relate to the biochemical nature of the main product of each gland, the rate of its secretion and the type of control of its release and biosynthesis. This selection is also not immediately linked to the embryological origin of the glands, the chronology of their development and/or their adult architecture. The organ-based classification further outlines that the main secretory cells of most exocrine and endocrine glands express multiple connexins, with the notable exception of gland cells connected by Cx36 that usually do not use any other additional connexin species (Table 2). Eventually, the organ-based classification stresses the notion that most exocrine cells use in combination multiple connexins of the β group, whereas most endocrine glands use a single connexin of either the α or the γ group [11].

Since the main function of glands is the regulated biosynthesis, storage and release of secretory products, particular attention has been taken to relate the expression of connexins and/or the extent of the cell-to-cell communications these proteins permit to these secretory parameters. The data gained on this respect in various glands are summarized in the following sections.

5.1. Enzyme-producing glands

5.1.1. Pancreatic acinar cells

The deletion of the *Gjb1* gene, which encodes Cx32, increases the basal secretion of amylase from pancreatic acinar cells, in spite of the retained expression of Cx26 and of a reduced coupling [24]. These changes were associated to an increase in the circulating levels of the digestive enzyme, providing direct evidence that Cx32 significantly affects the in vivo functioning of the gland [24]. These findings are consistent with the previous observations that pancreatic acinar cells are extensively coupled under resting conditions but rapidly uncouple during maximal stimulation [7,30], and that pharmacological blockade of coupling increases amylase release in the absence of other secretagogues [140]. Thus, acute uncoupling of acinar cells is somehow required to initiate, maintain or enhance the increased secretion of pancreatic enzymes which is elicited by endogenous hormones and neurotransmitters, as well as by agonist drugs.

Since the secretagogues that uncouple acinar cells increase cytosolic-free Ca^{2+} levels and induce waves of the cation whose intercellular propagation and frequency are at least partly dependent on gap junction channels [141–143], the secretagogue-induced uncoupling presumably takes place after the initiation of the agonist stimulation. The persistence of coupling at the beginning of this stimulation allows for the transmission of a threshold signal produced in few acinar cells to all other cells of each pancreatic acinus, thus ensuring the functional recruitment of many additional secreting cells [64]. The subsequent uncoupling may be needed to terminate the agonist action, as indicated by the decrease in the junctional conductance of acinar cell pairs after the agonist-induced Ca^{2+} peak [144]. However, it is equally possible that some of the endogenous molecules that permeate connexin channels may negatively control the secretion of enzymes produced by acinar cells (Fig. 6). By hindering the intercellular exchange of these signals, uncoupling could then allow for a number of acinar cells to escape inhibition and enter activated secretion. Eventually, uncoupling may be needed to functionally isolate those acinar cells that are highly sensitive to secretagogues, in order to prevent the dilution of the agonist-induced second messengers into less sensitive cells of the acinus [64,144]. It has indeed been shown that, when exposed simultaneously to a

secretagogue, individual acinar cells show a rather variable secretory function and that this functional heterogeneity decreases as acinar cells aggregate and express connexins [64,145].

5.1.2. Salivary acinar cells

The connexin involvement in salivary secretion appears similar to that of pancreas. Thus, individual cells of the major salivary glands show a heterogeneous function [146], become uncoupled within the acini during stimulation by cholinergic agonists [7,147] and show inhibited secretion after drug-induced uncoupling [40]. However, the specificity of the latter effect remains to be determined, inasmuch as the alkanol used to cause uncoupling also inhibited the capacitive entry of Ca^{2+} into acinar cells [40]. The use of knock-out transgenic mice lacking one or another of the connexins connecting the acinar cells of salivary glands [11,38,39] should allow to address this question.

5.2. Milk-producing glands

The alveolar cells of mammary glands change the expression of Cx26, Cx30 and Cx32 as a function of their

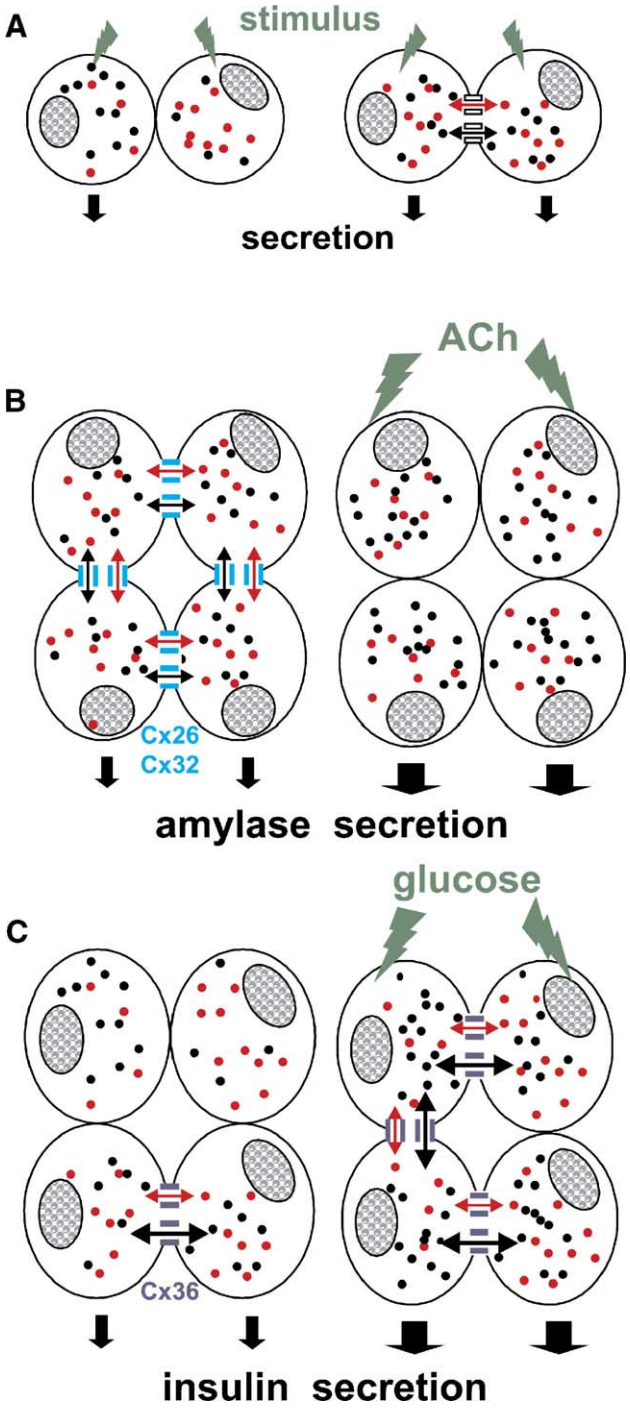

Fig. 6. Working model of the contribution of connexins to secretion. Based on the data gathered in the exocrine and endocrine pancreas, a working model can be proposed to account for the connexin–secretion relationships that have been experimentally observed. (A) In glands made of functionally heterogeneous cells (left panel) that either lack gap junctions or in which connexin channels are blocked, stimuli activate only those cells that feature adequate levels of relevant receptors and second messengers acting as either positive (black dots) or negative regulators of secretion (red dots). Establishment of gap junctional communication (right panel) permits the diffusion-driven passage of these critical ions and molecules between the coupled cells and, at steady state, their equilibration. The resulting increase in positive regulators, and/or decrease in negative regulators, could account for the recruitment of increasing numbers of secretory cells that is observed with cell aggregation and coupling of both the acinar and islet cells of pancreas. Under these conditions, these cells also function in synchrony with the companion cells forming a communication territory within the exocrine acini and the endocrine islets, respectively. (B) Under resting conditions (left panel), coupling is large between the cells of pancreatic acini, providing for the equilibration of the signal molecules that positively and negatively regulate secretion. This equilibration ensures the secretory recruitment and synchronous function of the coupled cells, leading to an amylase secretion larger than that observed from uncoupled and single acinar cells, some of which may not be appropriately equipped to secrete individually. Acute stimulation by secretagogues (right panel), including the main physiological neurotransmitter acetylcholine (ACh), provides for the activation of the secretory machinery of individual cells, which increases the levels of signals upregulating secretion (black dots). ACh also uncouples acinar cells, preventing the dilution of these positive regulators, which would reduce their concentration below the threshold level for stimulation of secretion, and which would result from their diffusion across the highly permeant Cx26 and Cx32 channels, and into the large cytosolic volume of coupled acinar cells. As a result of these two concomitant changes, amylase release is markedly promoted. (C) Under resting conditions (left panel), groups of pancreatic β-cells are coupled within each pancreatic islet, providing for only a partial equilibration of the signal molecules that regulate secretion. As a result, basal insulin release is higher than that of single (or uncoupled) β-cells. Acute stimulation by secretagogues (right panel), including the main physiological nutrient glucose, activates the secretory machinery of most β-cells and, by increasing their coupling, enhances their Cx36-dependent synchronization. As a result of these two concomitant changes, insulin release is markedly promoted. Thus, several factors contribute to the effect of connexins on secretion, including cell volume, type of connexin isoform, permeability of the junctional channels to positive and negative regulators of the secretory machinery, levels of expression of the connexins, extent of the resulting communication compartment, and effect of secretagogues on the latter. Conceivably, differences in one or more of these factors account for the different behaviour of the exocrine and endocrine cells of pancreas, under resting and stimulated conditions. However, it should be stressed that, until the endogenous gap junction-permeant molecules (the dots in the figure) are identified, this model remains at best an academic hypothesis. Also, this model does not consider additional contributions of connexins, by way of their interactisms with other proteins, including transcription factors and cytoskeleton proteins, that are essential to the control of secretion. As evidence will become available for such interactions in pancreatic systems, sizable notification of the present working model may be required.

developmental and functional state [41–43,46,148], suggesting that precise ratios of these isoforms are required for proper production and/or storage of milk. So far, however, only the deletion of the Gjb2 gene, which encodes for Cx26, has been reported to result in impaired lactation, pointing to a central role of this connexin at least before pregnancy [29]. The mechanism underlying this role remains to be investigated. Cyclic changes in the levels of Cx43, which links myoepithelial cells, have also been documented in mammary glands [16,46,47], suggesting that a connexin-dependent mechanism is also implicated in the control of cell contraction [48], which is relevant to the release of milk, during lactation.

5.3. Bile-producing gland

Pharmacological uncoupling of hepatocytes alters the bile flow induced by glucagon and vasopressin, respectively, without affecting basal biliary secretion [149]. Since the uncoupling treatment did not alter bile flow when the levels of the second messengers used by the two hormones were increased across the entire hepatic lobule, the data suggest that junctional communication is important for the diffusion towards peri-portal hepatocytes of signals generated in centro-lobular cells [149]. Accordingly, electrical stimulation of sympathetic nerves in mice lacking Cx32 lead to an abnormally low decrease of bile flow, but not of total bile output, suggesting that the contraction of the bile canaliculi formed by the hepatocyte membranes is somehow impaired in the absence of this connexin [23].

5.4. Fluid-producing glands

5.4.1. Lacrymal acinar cells

The deletion of the *Gjb1* gene, encoding for Cx32, induced the uncoupling of acinar cells of exorbital lacrymal glands and decreased their fluid secretion in response to low, but not to high doses of carbachol [25]. Intriguingly, this secretory defect was restricted to female mice and did not affect the resistance of the cornea of the knock-out animals [25].

5.4.2. Gastric parietal cells

In vitro, the stimulation of the parietal cells of gastric glands by gastrin increases the permeability of their junctional channels to both Lucifer Yellow and Ca^{2+}, under conditions that also increase the acid (in the gastric lumen) and HCO_3^- (in the gastric mucosa) release from the same cells [37].

5.4.3. Pancreatic acini and ducts

The in situ vascular perifusion of pancreas with drugs blocking connexin channels resulted in a stimulation of the amylase and fluid secretion from exocrine acinar cells and ducts, and in a parallel decrease of the insulin secretion from pancreatic islets, showing the different regulation of coupling by secretagogues in this mixed exocrine and endocrine gland [32].

5.5. Sugar-producing gland

Livers of transgenic mice lacking Cx32 showed reduced coupling of hepatocytes [150] and decreased glucose output in response to noradrenaline and glucagon, as a result of decreased hydrolysis of the liver glycogen stores [151]. The latter alteration was not observed when the two stimulating hormones were infused at saturating concentrations, suggesting that the residual Cx26 channels were able to propagate both a neural (sympathetic) and a hormonal signal (possibly mediated by cAMP ?) from peri-portal to centro-lobular hepatocytes [151].

5.6. Peptide hormone-producing glands

5.6.1. Pancreatic insulin-producing β-cells

Transgenic mice lacking Cx36 comprise uncoupled β-cells without gap junctions, that fail to show the intercellular synchronization of $[Ca^{2+}]_i$ transients and the pulsatile release of insulin which are induced during glucose stimulation of control β-cells [67]. Furthermore, islets lacking Cx36 show an increased basal release of insulin, accounting for the absence of a further secretion increase during stimulation by postprandial sugar concentrations [67]. These observations show that Cx36-dependent signaling is essential for proper regulation of insulin release in vivo [67], thus extending the observations previously made after the transgenic expression of the islet-ectopic Cx32 [22] or in several in vitro models [113–116]. The latter experiments were instrumental to document the specificity of the secretion control achieved by the Cx36 signaling, and to show that this control requires adequate amounts of connexin channels [113,115]. These observations are also in agreement with the findings that several cell lines featuring defective insulin secretion do not express connexins [112,113], and that single, uncoupled β-cells show alterations in the transcription of the insulin gene and in the secretion of the cognate hormone, which are rapidly corrected after restoration of β-cell contacts [63,65,66,152], and which are mimicked by the pharmacological blockade of connexin channels [153].

Recent in vitro and in vivo data show that the mechanism underlying these secretory defects is the remarkable dependence of β-cells on Cx36 to synchronize throughout the islets the glucose-induced oscillations in $[Ca^{2+}]_i$ which, in turn, drive the oscillations in insulin output [67,113]. Glucose stimulation induces secretory and metabolic responses from either intact pancreatic islets or clusters of islet cells, which are markedly more uniform than those of single β-cells [154–158]. Thus, coupling between pancreatic β-cells appears essential to synchronize the activity of individual cells which, taken individually, are metabolically and secretory heterogeneous [1,5,6]. Nevertheless, the reason why asynchrony is deleterious for β-cell function remains to be validated by a direct experimental testing. Irregular Ca^{2+} oscillations could conceivably alter the expression of specific β-cell genes [158], the resistance of β-cells to apoptosis [136], or other events [3].

5.6.2. Hypothalamic neuroendocrine cells

Using pharmacological gap junction blockers, the coupling of several hormone-producing neurons has been related to the control of the pulsatile release of GHRH [68], GnRH [69] and LHRH [70].

5.6.3. Pituitary prolactin-producing cells

Seasonal changes in the expression of pituitary Cx43 have been associated to changes in prolactin secretion [98].

5.7. Glycoprotein hormone-producing glands

5.7.1. Thyroid T3- and T4-producing follicular cells

The involvement of connexins in thyroid secretion is supported by the observation that TSH stimulation increases the coupling of thyrocytes in a time- and concentration-dependent manner [124], whereas loss of coupling, due to a Cx32 mutation, reduces the release of thyroxin [125]. Furthermore, transfection of Cx32 in thyroid-derived cell lines also resulted in increased expression of the thyroglobulin gene [126].

5.7.2. Placental-producing cells

The pharmacological blockade of gap junctions as well as the antisense interference with Cx43 transcripts, which uncouple the cytotrophoblast cells of the placenta, are associated with a decrease in the expression of the genes coding for β-hCG and human chorionic somatomammotropin [102,103], and with a reduced secretion of the former placental hormone [103].

5.8. Steroid hormone-producing glands

5.8.1. Adrenal corticoid-producing cells

In vitro, a decrease in the coupling of adrenal cells, as a result of either the exposure to drugs blocking connexin channels or the transfection of a Cx43 antisense construct, was paralleled by impaired ACTH-stimulated release of cortisol [74–76]. However, another gap junction blocker was recently reported to stimulate steroid production, via the activation of two pathways (an extracellular signal-related kinase and a calcium/calmodulin-dependent kinase) which are distinct from that (protein kinase A-dependent) which controls steroidogenesis of primary cells [80]. Stimulatory concentrations of the natural stimulatory hormone ACTH increase Cx43 expression of adrenal cells, in vivo and in vitro [10].

5.8.2. Testicular testosterone-producing cells

The cells of a testosterone-producing cell line become partially uncoupled during LH-induced stimulation of testosterone secretion, suggesting a tonic inhibitory influence of connexin signaling on the release of the steroid hormone [85].

5.8.3. Ovarian luteal cells

Consistent with a relationship between connexin signaling and steroid production, the experimental inhibition of Cx43 expression [91], as well as drug causing uncoupling, decreased the LH-induced steroid secretion of luteal cells [54]. Conversely, pharmacological treatments enhancing the coupling of corporea lutea cells were associated with increased progesterone release [54].

5.9. Cathecolamine-producing glands

The coupling of the chromaffin cells of the adrenal medulla is enhanced during stimulation of catecholamine release by nicotine, indicating that a Cx36-dependent signaling amplifies the secretion of epinephrine and norepinephrine [71]. The pharmacological blockade of synaptic transmission, as well as the surgical denervation of the adrenal glands, resulted in increased coupling of chromaffin cells, indicating that the Cx36-dependent signaling is tonically inhibited by cholinergic synaptic inputs [121].

6. A working model of connexin functions

The studies summarized above provide compelling evidence for a physiologically relevant role of cell-to-cell communication mediated by connexins in the secretion of a variety of exocrine and endocrine glands. However, it should be stressed that only in a few glands has the molecular mechanism underlying such a role began to be elucidated. Many of the endogenous molecules that permeate connexin channels are also important signals for many types of secretion [1,5,7,9,18,19,138], complicating the identification of the signal(s) that couple the changes in connexin-dependent communication to the changes in secretion. The most direct evidence on this respect, is so far available for only pancreas and the adrenal medulla, and points to a central role of stimuli-induced Ca^{2+} transients that require connexin channels to become synchronized in different cells. However, recent studies suggest that connexins may control cell functioning, and particularly gene expression and cell growth even in the absence of significant cell-to-cell communication [139]. The underlying mechanism which may involve the passage of second messengers through hemi-connexin channels and/or the interaction of connexins with other membrane and cytosolic proteins is not yet elucidated. At any rate, the reason why connexins contribute so significantly to the complex signaling network that controls most secretory processes remains to be understood.

In view of the above, any model regarding the function of connexins in glands is admittedly highly speculative and should not be regarded more than a framework on which to build working hypotheses to be tested by direct experimentation. As compared to other forms of cell-to-cell communications, which involve the interaction of cells with either signal molecules (hormones, neurotransmitters, ATP, NO, etc.) diffusing in the intercellular spaces, or adjacent cells (via cell adhesion molecules, integrins, etc.), the intercellular communication mediated by gap junctions is unique in that it is driven by diffusion and, thus, may achieve a rapid equilibration of ionic and molecular electrochemical gradients between coupled cells (Fig. 6). If the resulting concentration of signal molecules reaches a threshold level for activation (or inhibition) of an effector mechanism, functioning will be

modified not only in the cell in which the ionic and molecular change first occurred, e.g., as a result of secretagogue stimulation, but also in the cells coupled to it. In this case, junctional coupling could result in the functional recruitment of cells that, otherwise, may not be directly activated (or inhibited) [63–66]. In these cases, the secretion of the coupled cells was actually larger than that which the same number of cells show when they are isolated from each other, indicating that coupling also optimizes the levels of the signal molecules which stimulate the effector mechanisms essential for xsecretion (Fig. 6). Eventually, the coupling-induced equilibration of cytoplasmic constituents would be expected to also synchronize those functions which are modulated by gap junction-permanent molecules (Fig. 6), an expectation which as been verified experimentally [67,71,113]. Conversely, uncoupling, as well as the use of connexins imparting to cell-to-cell channels a selective permeability to certain cytosolic molecules [18–20,159] could result in the establishment of specific efslectrochemical gradients (Fig. 6), which may be beneficial to avoid the dilution of essential factors regulating secretion in glands where large numbers of cells are extensively coupled (Fig. 6). Such a dilution could cause the levels of these molecules to fall below the threshold level of activation, hence resulting in decreased secretory outputs (Fig. 6). This pattern has been observed in several exocrine acinar cells [7,24,30].

These considerations imply that junctional coupling may be of particular value in tissues comprising of cells with substantial structural, metabolic or functional heterogeneity. Increasing evidence shows that such a heterogeneity exists in may secretory systems [63–66,160–165]. Disparities in intrinsic structural and functional properties will conceivably result in the asynchronous function of individual cells (Fig. 6). Junctional coupling could decrease or correct these localized disbalances, thus permitting distinct cell subpopulations to function simultaneously and/or at the same rate [67,71,113].

7. Non-secretory functions of gland connexins

While this review has mostly focused on the involvement of gap junction proteins and coupling in the secretory function of glands, other roles of connexins should not be undervalued, which are relevant to the development, morphogenesis, growth, differentiation, renewal and repair of glands. The ablation of 1–2 connexin genes expressed in secretory systems has usually shown an apparently normal development of the targeted glands, implicating that either the connexin-dependent signaling has no essential function in gland development or that, some in vivo mechanism can fully compensate the lack of this signaling [28,67,109,127,133], at least in most cases. This presumably reflects the obligatory requirement of secretory systems which are essential for life sustainment during both the pre- and post-natal periods. However, the conditional and cell-specific ablation of the *Gjb2* gene in young mice altered the development and function of mammary glands [29], indicating a significant role of the connexin in the post-natal morphogenesis of an exocrine gland system. Analogous observations have been made in vitro with thyroid-derived cells. Thus, Cx32 and coupling are lost with passages in culture, together with the ability of the cells to form follicular structures [125,134], and these alterations are reverted by exposing cell monolayers to TSH [134] or by transfecting communication-incompetent thyrocytes with Cx32, but not Cx43 [135]. While these data implicate Cx32 in the morphogenesis of thyroid follicles, no obvious thyroid defect has been reported in mice knock-out for the *Gjb1* gene [28,127].

Several studies also implicate gap junctions in the control of secretory cell proliferation. Thus, altered growth of the endocrine pancreas was detected in transgenic mice whose insulin-producing β-cells were selectively forced to over express either the ectopic Cx32 [22] or the native Cx36 [136]. Transfection of Cx26 reduced the growth of hepatoma cells [36], as did that of Cx32 in communication-incompetent thyroid-derived cells [135], an effect which was not reproduced with Cx43 [137]. Pharmacological gap junction inhibition increased the growth of adrenocortical cells [138], whereas the exposure of adrenal cells to an antisense cDNA, which reduced the levels of Cx43, increased their growth rate [77]. Mice lacking Cx43 have also been shown to have hypodeveloped gonads [21,50], featuring delayed or reduced growth of the germ cells [61,62]. Thus, in different types of glands, connexins may contribute to control the size of the secretory cell population. As yet, however, the relevance of the latter control for primary cells in vivo has only been shown in a few cases [21,22,43,50].

8. Conclusions

The work summarized above provides compelling evidence for a central role of gap junction channels in the function of both endocrine and exocrine glands. The junctions appear relevant for both the function of the main parenchymal secretory cells, as well as for that of the associated vessels and ducts (in exocrine systems), which cannot be functionally dissociated from secretory cells when the gland is considered as a whole functional unit. This review mostly focused on the main physiological function of glands, which is the synthesis, storage and release of specific products, and has barely touched on other functions in which gap junctions may also play a significant role, such as the developmental growth and acquisition of terminal differentiation characteristics of secretory cells, their regeneration in the postnatal life, which takes place in fully differentiated glands such as liver or exocrine pancreas, their interaction with some of their close by target cells.

Several questions remain to be addressed by a direct experimental testing, in order to elucidate the reasons why the connexin-dependent signaling is obligatory for secretory systems, a stringent requirement that glands share with many, if not all other multicellular systems. First of all, the molecular mechanism linking the expression of connexins and/or the gap junction-dependent coupling of gland cells to their secretion is not established. Many ions (Ca^{2+}, K^{+}, ...) and metabolites (IP_3, glycolytic intermediates, nucleotides,

...) could certainly be considered candidate second messengers. However, if there is ample evidence that cAMP and Ca^{2+} are involved in the control of both gap junction and secretion [1,5–7,9,19,138], it remains to be demonstrated whether either one of these two second messengers actually links in a direct and causal manner the gap junction and the secretion changes. Future studies should also investigate whether connexins may control secretion by a mechanism independent of cell coupling [139] and, if so, whether this implies some hitherto neglected role of hemichannels, the interaction of connexins with other proteins and specific transcriptions factors, or some other mechanism.

Second, the importance of the gap junction-dependent signaling relative to that ensured by the many other mechanisms that interplay to control secretion remains to be established [1,6]. The finding that chronic alterations of β-cell connexins are sufficient to reproduce in vivo the defects of insulin secretion which are observed in type II diabetes [22,67] indicates a prominent role of the gap junction signaling, at least in the endocrine pancreas, and raises the intriguing possibility that connexin defects may contribute to secretory diseases [1–3,9].

Therefore, a third challenge is to determine whether gap junction alterations are implicated in the pathogenesis of secretory disorders. If various alterations of connexin expression and/or cell-to-cell coupling have been reported in glands under pathological conditions [1–3,9], no secretory defect has yet been proven to be associated to either a mutation or a pathogenic polymorphism of a connexin gene, or to quantitative alterations in the levels of specific connexins, in their trafficking through the cytoplasm or their clustering and functioning after insertion into the cell membrane. Eventually, recent work has outlined the striking similarities, as well as the significant differences that exist between the connexin, innexin and pannexin proteins that form gap junctions in vertebrates, invertebrates and at least some mammals, including humans, respectively [166–169]. In spite of the fact that some of the initial work on gap junction function was carried out in invertebrate glands [26], almost nothing is known about the functional roles innexins and pannexins may have on secretory processes, and/or on the development, differentiation, growth and morphogenesis of exocrine and endocrine glands. A direct approach of these questions is now timely to define the hierarchical relationship, if any, that exists between distinct families of gap junction-forming proteins, which are co-expressed in a variety of secretory systems. All these questions open new exciting avenues for investigations to come.

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