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Roles of connexins and pannexins in endocrine/neuroendocrine physiology

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Running title: connexin and pannexin signaling in (neuro)endocrine function.

Abbreviations: adrenocorticotropic hormone (ACTH), arcuate (ARC), adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP), corticotrophin-releasing hormone (CRH), connexin (Cx), folliculostellate (FS), follicle-stimulating hormone (FSH), growth hormone (GH), growth hormone-releasing hormone (GHRH), gonadotrophin-releasing hormone (GnRH), luteinizing hormone (LH), pannexin (Panx), paraventricular nucleus (PVN), parvocellular (PV), pituitary adenylate cyclase-activating peptide (PACAP), prolactin (PRL), supraoptic nucleus (SON), triiodothyronin (T3), thyroid-stimulating hormone (TSH), thyrotrophin-releasing hormone (TRH), thyroxine (T4), zona fasciculata (ZF), zona glomerulosa (ZG), zona reticularis (ZR).

Abstract

To ensure appropriate secretion in response to organismal demand, (neuro)endocrine tissues liberate massive quantities of hormone, which act to coordinate and synchronize biological signals in distant secretory and non-secretory cell populations. Intercellular communication plays a central role in this control. With regard to molecular identity, junctional cell-cell communication is supported by connexin (Cx)-based gap junctions. In addition, connexin hemichannels, the structural precursors of gap junctions, as well as pannexin (Panx) channels have recently emerged as possible modulators of the secretory process. This review focuses on the expression of connexins and pannexins in various (neuro)endocrine tissues, including the adrenal cortex and medulla, the anterior pituitary, the endocrine hypothalamus and the pineal, thyroid and parathyroid glands. In response to a physiological or pathological situation, junctional intercellular coupling can be acutely modulated or persistently remodelled, thus offering multiple regulatory possibilities. The functional role(s) of gap junction-mediated intercellular communication in endocrine physiology, as well as the involvement of connexin/pannexin-related hemichannels are also discussed.

Keywords: connexin, pannexin, hemichannel, endocrine, adrenal gland, pituitary gland, endocrine hypothalamus, pineal gland, thyroid and parathyroid glands.

Introduction

The neuro(endocrine) system regulates body-wide homeostasis in mammals by dynamically integrating environmental cues and modifying the functional set point of downstream effectors accordingly [1]. To achieve this, secretory cell/neuron populations must act in unison to release either peptide hormone or neurotransmitter messengers [2]. Target organs then decode the information contained within the signal to mount an appropriate response (*e.g.* stress, growth, metabolism and reproduction). As a consequence, mechanisms have evolved to ensure coordinated responses to stimuli by streamlining cell-cell communication. Chief among these are the connexins and pannexins, which provide a relatively cell-specific pathway for the rapid exchange of information [3]. Indeed, these channels are able to modulate tissue output through the passage of ions and molecules between cells/neurons, as well as from cells/neurons into the extracellular space. Providing strong evidence for a critical role of connexins and pannexins in neuro(endocrine) regulation, studies in models with impaired channel function consistently present with altered intercellular communication and hormone/neurotransmitter release [4]. Thus, connexins and pannexins appear to be an intrinsic component of many neurohormonal axes and, as such, their structural and functional description is important to properly understand organismal homeostasis. The aim of the present paper is to review the tissue expression and localization of connexins and pannexins, as well as their contribution to neuro(endocrine) physiology.

Adrenal gland

Adrenal cortex: dual contribution of gap junctional communication in steroidogenesis and cell proliferation

The adrenal cortex is a secretory tissue, which constitutes the outer part of the adrenal gland. It is involved in the stress response through the secretion of mineralocorticoids (*i.e.* aldosterone) by the zona glomerulosa (ZG) and glucocorticoids (*i.e.* cortisol/corticosterone) by the zona fasciculata (ZF). The third zone, the zona reticularis (ZR) cortex is dedicated to androgen synthesis and release. Interestingly, the adrenocortical cells can display neuroendocrine properties [5].

Connexin expression and distribution

Adrenocortical gap junctions were structurally identified in the early seventies by freeze-fracture electron microscopy performed in the rat [6]. As shown in Table 1, Cx43 emerges as the major, if not exclusive, gap junction protein expressed in the adrenal cortex. With the exception of the human adrenal cortex, which expresses Cx26, Cx32 and Cx50 in addition to Cx43 [7], no signal was detected for Cx26, Cx31, Cx32, Cx36,

Cx37, Cx40 and Cx46 [8-12] in mammals. Of note, we recently identified Cx37, Cx40 and Cx45 transcripts in the mouse cortex (unpublished results). Abundant Cx43-built gap junction plaques are present in the ZF and ZR, while cells within the ZG exhibit few, if any, gap junctions [8, 9, 13, 14] (Table 2). Single cell RT-PCR experiments have also revealed the presence of Cx43 mRNA in the ZF and ZR [15]. Cx43 is not only expressed in the normal adrenocortical tissue, but also in benign and malignant neoplastic tissues, in which Cx43 expression is dramatically reduced [11].

In mammals, the presence of gap junctions is not restricted to adults, but is also detected in neonates and fetuses of various species, including rat, mouse, rabbit, sheep and human [16-20]. In neonatal rats, gap junctions are already well differentiated in the ZF and ZR. In the ZG, they become detectable 2 weeks postnatal [16].

Connexin intercellular channels

The first electrophysiological study of gap junction-mediated electrical coupling between cortical cells was reported over 40 years ago in rabbit adrenal slices [18]. As hypothesized [21], gap junctional communication in the adrenal cortex plays a pivotal role in a number of interactive cell processes, including differentiation, steroidogenesis and hormone responsiveness, migration and proliferation (reviewed in [22-24]). It is noteworthy that Cx43 exhibits a differential distribution pattern within the three zones of the adrenal cortex that correlates with divergent proliferation rates and responsiveness to ACTH. Through cAMP diffusion between cortical cells, ACTH enhances Cx43 protein expression and gap junction plaque formation in the ZF and ZR, resulting in an increased gap junction number and size [10, 13, 25], enhanced steroidogenesis, at least in cultured cells [10, 26], and decreased cell proliferation rate [10, 25, 26]. Altogether, this clearly indicates that expression of adrenocortical gap junctions is under hormonal influence. Strengthening further the evidence that adrenocortical gap junctions are hormonally regulated is the finding that hypophysectomy leads to a robust decrease in Cx43 expression in the ZF and ZR, and that Cx43-mediated cell-cell communication is restored by subsequent ACTH treatment [27]. The physiological contribution of gap junction-mediated cell-cell communication to adrenocortical hormone release is demonstrated by the tissue response to a low ACTH concentration [22, 28]. At submaximal ACTH concentrations, only a fraction of cortical cells responds to the stimulus by producing cAMP. By mediating intercellular communication, gap junctions allow the transfer of cAMP from responsive to non-responsive cells, thus resulting in increased cortisol secretion. This finding uncovers gap junctions as a mechanism whereby cortical cells modify their responsiveness to low physiological ACTH concentrations [28].

More recently, adrenocortical gap junctions were reported as modulators of cell migration [29]. To date, there are no studies examining connexin hemichannel structure and/or function in the adrenal cortex.

Pannexin channels

The presence of Panx proteins in adrenocortical cells has not yet been reported. Nevertheless, we recently detected Panx1, but not Panx2 and Panx3, in the rat cortex (unpublished data).

Adrenal medulla: gap junctional communication as an adaptive pathway to regulate stimulus-secretion coupling

The neuroendocrine chromaffin cells are responsible for catecholamine secretion and are notably stimulated upon stressful situations, with a marked involvement in the 'fight or flight' response. The traditional scheme of stimulus-secretion coupling in the adrenal medulla, stating that catecholamine release is chiefly, if not exclusively, controlled by synaptically-released acetylcholine at the splanchnic nerve terminal-chromaffin cell synapses, has prevailed for many decades. It was revisited in the early 2000s, with the first description, in rat, of the functional role of connexin-mediated gap junctional coupling between chromaffin cells in the secretory process [15] (Fig. 1).

Connexin expression and distribution

In the adrenal medulla, connexin-composed gap junctional plaques were originally described in the 1980s from observations of freeze-fractured specimens [30]. As summarized in Table 3, diverse connexins are expressed in the normal adrenal medullary tissue, coupling both endocrine (*i.e.* chromaffin cells) and non-endocrine cells (*i.e.* satellite cells and sustentacular cells). Unlike the cortex in which Cx43 is the main connexin isoform expressed, the rodent medulla expresses Cx29, Cx43 and neuronal Cx36, consistent with a neural crest-derived tissue [31]. In humans, the medullary tissue does not show presence of Cx36, but rather of Cx50, a neuronal connexin robustly expressed in the horizontal cells of the retina [32]. While Cx36 and Cx43 are present in the neurosecretory chromaffin cells, Cx29 couples S100-positive cells, likely targeting the non-secretory glial-like sustentacular cell population, as well as surrounding the preganglionic sympathetic nerve fibers that innervate the medulla [33]. Inmunoreactivity for Cx26, Cx31, Cx32, Cx37, Cx40 and Cx46 remains absent in normal adrenal medullary tissue [7-9]. Of note, we recently identified in rat and mouse, Cx37 and Cx40 transcripts, two connexins exhibiting a vascular tropism, and Cx45 (unpublished data). Connexin expression depends on the

physiological/pathological status of the medullary tissue, as illustrated by the *de novo* expression of Cx26, Cx32 and Cx43 in pheochromocytomas [7].

Connexin intercellular channels

The presence of connexins between chromaffin cells, mainly Cx36 and Cx43 in rodents [12, 15, 34, 35], strongly suggests the involvement of gap junction membrane channels in hormone secretion [36]. In a paper published in the early 2000s, Martin and colleagues [15] described, for the first time, the presence of functional gap junctional communication between rat chromaffin cells in acute adrenal slices and its role as an additional component of stimulus-secretion coupling. Due to electrical coupling, a single stimulated cell can propagate its stimulus (*e.g.* electrical or nicotine/acetylcholine-evoked depolarization) to its neighbors, resulting in synchronous multicellular cytosolic rises in intracellular calcium concentrations and catecholamine release (Fig. 1).

The prevalence of gap junctional coupling in the adrenal medulla is highly plastic and depends on various factors (Table 4 and reviewed in [37]), including age [38, 39], species [30, 40], gender [15, 34, 35], physiological (*i.e.* stressed/unstressed) state [12, 34, 41] and splanchnic innervation competence [38, 39].

Supporting the role of gap junctions in adrenal medulla endocrine function are data reporting an upregulated gap junctional communication between chromaffin cells in response to a pharmacological or surgical impairment of splanchnic innervation [38], or in the neonatal adrenal medulla in which the innervation is not yet fully competent [39]. Similarly, when hormone demand is high, such as in stressful situations, the adrenal medullary tissue triggers an adaptive remodelling, enabling the organism to cope with stress (Fig. 1). Among the determinants remodelled in response to stress [41], gap junctional coupling is dramatically enhanced (*i.e.* 80% of coupled chromaffin cells in cold-exposed rats *versus* 20% in unstressed animals [34]). This is associated with an increased expression of both Cx36 and Cx43 [34]. The plasticity of gap junction communication observed in response to stress is not restricted to rat, but is also found in mouse following cold-exposure [12] or application of the pituitary adenylate cyclase-activating peptide (PACAP) [35], a non-cholinergic splanchnic-derived neurotransmitter selectively released upon high frequency nerve firing [42, 43]. Importantly, the recent description of the contribution of gap junctions to catecholamine secretion *in vivo* [12] significantly advances the knowledge of endocrine/neuroendocrine tissue physiology. Hence, within the medullary tissue, gap junctional signaling between chromaffin cells is central to proper adrenal neuroendocrine function by acting as a lever to dynamically adjust hormone release to organism needs (Fig. 1). This implies that mechanisms exist for the fine-tuning of gap junction activity. Accordingly, the modulation of adrenal gap junctional coupling by synaptically-

released neurotransmitters or neuromodulators is a striking example. The ability of acetylcholine to tonically inhibit [38, 44] or PACAP to enhance [35] gap junctional communication between chromaffin cells likely represents key a regulatory check point for catecholamine secretion. At rest, when moderate catecholamine release is required, the cholinergic inhibitory control of gap junctions limits adrenal medullary tissue stimulation. Conversely, in response to increased sympathetic activity, as observed during stressful episodes, catecholamine need intensifies and is critical for the 'fight or flight' response. As observed in stressed rats [34], electrical coupling is upregulated, probably in response to stress-evoked splanchnic PACAP release [35].

Apart from a role in hormone secretion, no other obvious physiological function has been attributed to medullary gap junctions, but many processes, such as embryonic development, stem cell function, cell growth/differentiation and aging, remain to be investigated. In particular, the role of Cx29-based gap junctional coupling in S100-positive cells (*i.e.* satellite or Schwann cells and sustentacular cells) is still unknown, but the current hypothesis is that these non-endocrine cells may form a large-scale network, which regulates chromaffin cell function, similar to that described for glial cells and neurons. Indeed, the sustentacular cell network may coordinate the exchange and/or propagation of instructive signals, as recently reported for calcium ions [45]. By taking an active part in adrenal medulla calcium homeostasis, the sustentacular cell network regulates the synthesis and release of catecholamines from chromaffin cells. Also, the expression of gap junction channels at early embryonic stages indicates that they may contribute to function during development. In the adrenal medullary tissue, Cx36 deficiency results in a dramatic decrease of nerve stimulation-evoked catecholamine release [12], revealing an unanticipated role for Cx36 at the splanchnic nerve-chromaffin cell synapse.

Connexin hemichannels

Whereas connexin-mediated adrenal cell-to-cell communication is well-documented and unequivocally fulfills a function in hormone secretion, the role of connexin hemichannels in adrenal physiology is unknown. A single study performed in chromaffin cells reports connexin hemichannel-mediated enhanced neurite outgrowth in transfected PC-12 cells, likely through ATP release and signaling [46]. Since chromaffin cells express Cx36 and Cx43, and as these connexins can form functional hemichannels [47, 48], it is conceivable that connexons may have a functional role in the medullary tissue. By forming a transmembrane conduit allowing the exchange of ions and molecules between the cytosol and the extracellular milieu, connexin hemichannel opening can mediate the spread of cellular signals within a tissue through autocrine/paracrine mechanisms [49], and may therefore modulate physiological and/or pathological functions.

Pannexin channels

Studies addressing the expression and role of Panx channels in the adrenal medulla are scarce. Unlike connexins, the ability of Panx proteins to form junctional membrane channels is still controversial. As recently shown [50], the formation of Panx-mediated intercellular coupling is cell-specific and depends on Panx glycosylation. In tumoral chromaffin PC-12 cells, stable expression of Panx1 and Panx3 does not result in functional gap junctions [50], consistent with the current view that Panx channels function as single membrane channels rather than intercellular junctional channels. Regarding endogenous Panx expression in the medullary tissue, we recently detected the presence of Panx1 and Panx2, but not Panx3 RNA transcripts in macrodissected medulla (unpublished data). A very recent study reports Panx1 protein expression in bovine chromaffin cells [51]. Because of their calcium permeability [52], Panx1 channels may contribute to the regulation of intracellular calcium homeostasis and calcium-dependent cellular mechanisms. In this respect, activity of Panx1-based pannexons participate in catecholamine secretion by chromaffin cells [51] and thus should be considered as new players in endocrine function (Fig. 1).

Panx expression in other adrenal medulla cells still remains to be explored. In particular, whether non-endocrine glial-like sustentacular cells express Panx proteins is unknown, but the presence of Panx1 in astrocytes [53] strongly suggests that these channels may also be resident between sustentacular cells.

Anterior pituitary gland: gap junctions as a long-range signaling mechanism

In mammals, the anterior pituitary gland (*i.e.* adenohypophysis) originates early in embryogenesis from the ectoderm of the Rathke's pouch, an epithelial depression in the roof of the mouth. Endocrine cells then differentiate from precursors following a pathway tightly regulated by tissue-specific and cell-specific transcription factors, and are typified by the hormone that they produce [54]. Thus, corticotrophs, somatotrophs, lactotrophs, thyrotrophs and gonadotrophs secrete ACTH, GH, PRL, TSH and gonadotrophins (*i.e.* FSH and LH), respectively. The anterior pituitary also houses non-endocrine cell types, including stem/progenitor cells (*i.e.* SOX2-positive cells) and FS cells. The latter are thought to play a supporting role similar to that glial cells in the brain, with which they share surface expression markers in common, such as S100b protein [55, 56]. In response to hypothalamic input, the anterior pituitary liberates hormones, which underlie growth and metabolism, lactation, reproduction and stress. This is aided by the organization of most endocrine and non-endocrine cell populations into three-dimensionally intermingled networks, with thyrotrophs being a notable

exception [57-59,54,60]. Through the integration and amplification of signaling processes, these homotypic pituitary networks drive the complex electrical and transcriptional dynamics required to generate a 'gain of function' in hormone release [54, 61-66]. While the mechanisms underlying intercellular/intra-network communication remain poorly characterized, they have invoked a role for cell-cell coupling *via* gap junctions [61, 65, 67] (Fig. 2,). This particularly holds true for transmission of the secretagogue-triggered intracellular calcium signals which underlie calcium-dependent exocytosis [68- 70].

Connexin expression and distribution

Gap junctions were first identified in the mammalian pituitary gland last century based upon their ultrastructural features as observed by electron microscopy [71-73]. Indicative of gap junction functionality is dye coupling between cells in organotypic pituitary cultures [74]. Immunohistochemical studies have shown that Cx43 is the major connexin subtype within the pituitary, being preferentially expressed in FS cells and gonadotrophs [75] (Table 5). In addition to Cx43, northern blot and immunostaining of rat pituitaries have demonstrated the presence of Cx26, although the cell type localization is not well defined [8]. Likewise, Cx36 is expressed in a subset of anterior pituitary cells, demonstrating that this connexin isoform is not restricted to neuroectodermal tissues [76]. By contrast, Cx32 is absent in the anterior pituitary [8]. While the identity of the connexin remains elusive, dye coupling is present in somatotrophs and lactotrophs [77, 78], suggesting that these endocrine cells communicate *via* gap junctions. We recently detected Cx43 in the pituitary glands of sheep (unpublished data), which is in line with findings in rats [75, 79] and mink [80]. Of note, the pars intermedia, which borders the anterior and posterior pituitaries and that contains melanocyte-stimulating hormone-secreting melanotrophs, is immunopositive for Cx43, but not for Cx26 or Cx32 [8].

Connexin intercellular channels

While direct evidence for a role of connexin signaling in pituitary hormone release is lacking, numerous studies have suggested that gap junctions are an integral component of glandular cell-cell communication (Fig. 2,). Focusing on the individual cell populations, the known functions of connexin intercellular channels within the pituitary are discussed below.

FS cells

These S100b-expressing cells form a large-scale electrically-coupled network capable of transmitting calcium waves from one end of the pituitary gland to the other [56-58, 61, 81]. FS cells abundantly express Cx43 [75, 79], and pre-treatment with the gap junction uncoupler carbenoxolone impairs the extent of signal propagation [61]. Expression of Cx43 is highest between FS cells during the annual peak in PRL secretion in mink, suggesting that junctional exchanges between these cells may contribute to the intra-pituitary control of the lactotroph axis [80]. Moreover, evidence for bidirectional interplay between endocrine and non-endocrine populations is provided by studies showing that adenosine released by somatotrophs and lactotrophs is able to modulate Cx43 expression and dye coupling in FS cells [82].

Lactotrophs

During lactation in mice, lactotrophs double in size to form a highly connected structural and functional network tasked with coordinating calcium signals [54, 65, 83]. This allows the high levels of PRL required to drive mammary gland development and output in mammals. Rather than returning to the *status quo* following weaning, the network stores a functional template, allowing repeated episodes of lactation to be met with evolved behavior and further improved tissue output [54, 65]. Gap junctions are implicitly involved in such experience-dependent plasticity, since homotypic and heterotypic gap junctional contacts increase in number during lactation, as identified using electron microscopy and immunogold labeling for hormone, and dye coupling is enhanced during lactation, remaining high even after weaning. Furthermore, gap junction inhibition using 18α-glycyrrhetinic acid reduces dye coupling and prevents the network from displaying lactating-like wiring patterns during demand, most likely due to blockade of long-range signal entrainment [65]. Similarly, recent electron microscopy studies in the ovine pituitary have shown that lactotroph-lactotroph junctional contacts increase in line with the circannual peak of PRL during the non-breeding season [84]. Therefore, episodes of structural and functional plasticity within the lactotroph population/network are associated with alterations in gap junctional signaling in both mice and sheep.

Gonadotrophs

Small gonadotroph clusters respond to gonadotrophin-releasing hormone (GnRH) stimulation with synchronous calcium rises, and this may be important for information transfer within the gonadotroph network [57, 67]. However, such activity profiles do not appear to be gap junction-dependent, since they are not be blocked by 18α-glycyrrhetinic acid [67]. Nonetheless, mice in which the coding region of Cx43 is replaced with Cx26 present infertility, suggesting that the former gap junction isoform plays a key role in gonadotroph axis output in rodents [85].

Somatotrophs

The pattern of GH secretion differs between males and females of most species, which may explain the phenotypic divergence in body mass detected between the sexes [86]. While generally attributed to sexual imprinting of hypothalamic growth hormone-releasing hormone (GHRH) neuron number, structure and function [87, 88], the somatotroph network itself also gives rise to sex differences in GH output. In response to GHRH, female somatotrophs display highly coordinated calcium-spiking activity, which subsides following stimulus wash-out [57, 63]. By contrast, male somatotrophs respond to an identical challenge with synchronous oscillations that persist beyond stimulation. At the level of GH secretion, this presents as marked differences in pulse width and amplitude [63]. There are a number of clues that gap junctions may mediate the display of coordinated behavior between somatotrophs. First, somatotrophs isolated *in vitro* on coverslips, or *in situ* in pituitary slices, display asynchronous intracellular calcium rises in response to GHRH, commensurate with a decrease in cell-cell contacts [63, 89]. Secondly, co-activated cells are dye coupled, with a predominance of transfers between somatotrophs [78]. Thirdly, tracer spread between co-activated cells can be reduced using halothane, a gap junction blocker [78]. To date, there are no studies examining connexin hemichannel structure and/or function in the pituitary gland.

Pannexin channels

Pannexins are abundantly expressed in the pituitary tissue where they act as plasma membrane channels for the delivery of ATP, an essential signaling mediator in the purinergic pathway [90, 91] (Fig. 2). Panx1 and Panx2, but not Panx3, mRNA and protein expression are observed throughout the anterior pituitary, with the former being mainly localized to corticotrophs and some somatotrophs, and the latter being detected in FS cells [90, 92]. Suggesting that Panx proteins constitute ATP-permeant channels in pituitary cells is the observation that silencing of Panx1 in AtT-20 corticotrophs lowers basal release of ATP [90]. Moreover, full-length Panx1, as well as its truncated splice variants Panx1c and Panx1d physically associate with P2X2, P2X3, P2X4 and P2X7 ATP-gated purinergic channel subtypes [92]. While the role of pannexins in pituitary cell function are not well defined, they may modulate gonadotrophin, GH and PRL release, given that activation of P2X receptors in gonadotrophs, somatotrophs and lactotrophs induces depolarization and calcium fluxes [93-96] (Fig. 2). In the pars intermedia, melanotrophs express Panx2 [90], yet its role is unknown.

Neuroendocrine hypothalamus and posterior pituitary

Neurons with cell bodies in the arcuate (ARC) nucleus and parvocellular (PV) neurons in the paraventricular nucleus (PVN) of the hypothalamus project axons to the median eminence and secrete releasing factors into the portal vasculature for the blood-borne regulation of pituitary hormone release [97]. Thus, GnRH, thyrotrophin-releasing hormone (TRH), corticotrophin-releasing hormone (CRH), GHRH and dopamine control gonadotrophin, TSH, corticotrophin, GH and PRL release, respectively. By contrast, magnocellular neurosecretory neurons in the supraoptic and PV hypothalamic nuclei terminate in the posterior pituitary gland (*i.e.* neurohypophysis) and release oxytocin and vasopressin, primarily tasked with milk let down and solute balance [98, 99]. The posterior pituitary can be regarded as an extension of the hypothalamus from where it outpouches during development and, in addition to neurosecretory nerve boutons, also houses pituicytes. These glial-derived cells ensheath the descending hypothalamic nerve terminals and may provide a barrier function, modifying hormone access to the circulation as a function of demand [100].

Connexin expression and distribution

As summarized in Table 6, Cx30, Cx36 and Cx43 are expressed in the hypothalamus and posterior pituitary. Gap junction signaling in the neonatal hypothalamus is widespread, but decreases dramatically during postnatal development in line with a reduction in Cx36 expression [76, 101]. Nonetheless, homotypic dye coupling has been shown to be present between oxytocinergic and vasopressinergic neurons in adults [98, 102-104]. This is, however, unlikely to be attributable to intercellular communication *via* Cx36-based gap junctions, since this connexin isoform is only detected in PVN neurons expressing somatostatin and CRH [105]. In female rat, hypothalamic GnRH neurons display Cx32 immunoreactivity, which is distributed in the soma, and, very occasionally, in axon terminals of the median eminence [106]. While Cx26 and Cx43 are undetectable in GnRH neurons of female rat [106], some GnRH neurons have been shown to exhibit Cx43-immunopositive puncta in male rats [107]. Likewise, few Cx26 and Cx32 immunolabelings were described in the median eminence of male rat [107]. In the mouse, Cx36 and Cx43 are present in the hypothalamus and median eminence, but both proteins are absent from the GnRH population. However, high levels of Cx36 were detected in kisspeptin neurons in the hypothalamic anteroventral periventricular nucleus [108]. Irrespective of the species investigated (*i.e.* rat or mouse), gap junctional coupling has not been observed between adjacent GnRH neurons, but connects GnRH neurons and their closely apposed neuronal inputs [106, 108]. In addition to neurons, the hypothalamus contains glial or supporting cells, including astrocytes. Cx43, an isoform known to be enriched in astrocytes, tends to be expressed in the vicinity of capillaries in the ARC and ventromedial hypothalamus, and is modulated by both

blood glucose concentration and GH levels [91, 109]. Conversely, Cx30, which usually forms channels with Cx43 in astrocytes, is also expressed in the mediobasal hypothalamus, but with no clear relationship to the vasculature [109]. Lastly, pituicytes in the posterior pituitary express Cx43, with greater density at the periphery [75, 107]. Although less frequently encountered, heterotypic gap junctions can also be observed in the hypothalamus, as reported for a Cx32/Cx43-mediated coupling between neurons and astrocytes in the rat SON [110].

Connexin intercellular channels

In the developing brain, connexin channels comprise electrical synapses responsible for generating synchrony between neuronal ensembles [111] (Fig. 2). In adult rats, astrocyte-astrocyte and astrocyte-neuron gap junctional communication underlies the transmission of calcium waves [112]. By contrast, relatively little is known about the contribution of connexin-based signaling to neuroendocrine hypothalamic function and is discussed below.

Glucose homeostasis

Inhibition of astroglial Cx43 expression in the mediobasal hypothalamus has been shown to impair the central regulation of glucose homeostasis, as evidenced by decreased insulin secretion following brain glucose challenge [109].

Hydration

Dye coupling between neurons in the PVN is upregulated by *in vivo* hydration status, as well as by extracellular osmolality [113], although the connexin species involved remains unknown. The hydration status not only influences dye coupling between vasopressin neurons, but also modifies gap junctional communication between neurons and astrocytes, as illustrated by the increased number of Cx32/Cx43 gap junction plaques in the rat SON following hyperosmotic stimuli [110].

Lactation

In lactating rats, burst firing in oxytocin neurons of the SON is critical for milk ejection in response to suckling. Implicating a role for gap junctions in organizing this activity at the magnocellular population level is the observation that Cx32 mRNA expression increases during lactation [114], alongside enhanced dye coupling between oxytocin neurons induced by maternal behavior [99, 115, 116,].

Reproduction and gonadal steroid effects

Mice conditionally deleted for Cx36 exhibit altered oestrous cyclicity in the face of normal puberty and fecundity [108]. This probably is not related to gap junctional communication within the GnRH neuron network,

since electrical coupling was absent in paired patch-clamp recordings, and no dye transfer could be detected between identified GnRH neurons [108]. Cx36-expressing kisspeptin neurons may thus offer an alternative and attractive explanation for disrupted cyclicity in the mouse. Nevertheless, the presence of connexin immunoreactive puncta distributed between GnRH fibres indicates the possibility that gap junctions play a role in GnRH release at the median eminence, at least in the rat [107]. Among magnocellular neurosecretory cells, the frequency of dye coupling is reduced in male rats following castration [117], but is enhanced in female rats following ovariectomy [118]. This clearly indicates that gonadal steroids influence gap junctional communication between SON peptidergic neurons.

Connexin hemichannels

Hexameric hemichannels comprised of Cx43 are present in hypothalamic tanycytes, specialized ependymal-glial cells involved in glucosensing [119, 120] and fasting-refeeding responses [121]. Following exposure to glucose, tanycytes display elevations in intracellular calcium concentrations [119, 109], with macroscopic conductance being abolished following Cx43 hemichannel blockade, probably due to decreased purinergic signaling [119, 120]. The functional consequences of perturbing tanycyte Cx43 hemichannel expression *in vivo* remain elusive. In the endocrine hypothalamus, astrocytic Cx43 hemichannels have been reported to participate in the increased glutamate release after hypertonic stimulus [122]. This is consistent with previous studies showing that glutamate can diffuse through Cx43 hemichannels [123] and that Cx43 hemichannels can be induced by hyperosmolarity *in vivo* [124].

Pannexin channels

Within the posterior pituitary, Panx2 is abundantly detected in vasopressin-containing axons and nerve endings [90], with some Panx1 localized to S100-positive pituicytes [90]. In the endocrine hypothalamus, Panx1 mRNA is expressed in the magnocellular neurons of the PVN and SON [125], including vasopressin-containing neurons [126]. In these cells, the pharmacological blockade of pannexin channels results in a decreased ATP-induced current [126], demonstrating that pannexin channels may be involved in the regulation of hypothalamic neuronal activity.

Pineal gland

The pineal gland is an endocrine gland located within the brain, which contains neuron-like cells (*i.e.* pinealocytes) of the same embryonic origin as eye photoreceptors, and is directly sensitive to light in birds and reptiles. This light sensitivity is lost with evolution and, in mammals, the gland secretes melatonin only during darkness under direct influence of the hypothalamic suprachiasmatic nucleus. This pathway thus controls the circadian rhythmicity, which typifies hormone secretion and many downstream body functions [127]. The pineal gland is composed of two main cell categories, namely A pinealocytes that display characteristics close to those of astrocytes, and B pinealocytes that secrete melatonin. The pineal gland releases melatonin with a circadian rhythmic pattern and unsurprisingly gap junctions are present between pineal cells in many species (see [128] for a review), as putative synchronizers. However, direct evidence of the function of pineal gap junctions is still lacking.

Connexin expression and distribution

Gap junctions have been morphologically identified in the pineal gland of various species, including the chicken [128], rat [76, 129, 130], mouse [131], guinea pig [132], monkey [133] and human [134]. Interestingly, gap junctions are present at both homocellular and heterocellular junctions between pinealocytes and astrocytes [128, 135]. In chicken, gap junctions are mainly composed of Cx43 in astrocytes (*i.e.* A pinealocytes) and Cx45 in B pinealocytes [128], suggesting the presence of heterotypic Cx43/Cx45 gap junctional channels. In rat, Cx43 has been identified in astrocytes and its increased expression during development follows the differentiation of this cellular category [136]. Connexin expression is maintained in cultured pineal cells, pinealocytes and astrocytes expressing Cx26 and Cx43, respectively [137]. More recently, a sizable expression of neuronal Cx36 has also been detected in the pineal gland of adult rat [76].

Connexin intercellular channels

The function of pineal cell-cell gap junctional coupling still remains to be elucidated. A commonly assigned function of gap junctional coupling within an excitable cell network is the synchronization of the electrical firing discharges between cells. Although pineal cell clusters exhibit a rhythmic bursting activity associated with synchronized firing, it is apparently unrelated to gap junctions, at least in rat [138]. The description of heterotypic communication between pinealocytes and neighboring astrocytes [128] suggests that pineal gap junctions are important players in the regulation of pineal tissue homeostasis. In particular, it can be hypothesized that gap junctional communication may coordinate metabolic functions within pinealocytes and/or

astrocytes, as well as between astrocytes and pinealocytes, as reported in some regions of the central nervous system [139, 140]. Astrocytic gap junctions would be expected to distribute glucose, metabolites and nutrients within the pineal gland, and contribute to the clearance of substances whose concentrations increase in the extracellular environment during pinealocyte activity [141].

Another putative contribution of gap junctions to pineal gland function relates to the regulation and/or amplification of melatonin secretion. In this light, chicken is an interesting model, since its pinealocytes are photoreceptive. Thus, a light cue received by one pinealocyte might conceivably be transferred through the gap junctional network to other pinealocytes, allowing melatonin release to be coordinated between all secreting cells. This situation is reminiscent of the rodent adrenal gland in which gap junctions contribute to signal synchronization and hormone release [12, 15]. In cultured rat pinealocytes, the incidence of dye coupling and the expression of Cx26 are increased by norepinephrine application, a mechanism that may plausibly contribute to neurotransmitter-regulated melatonin secretion [137].

Although definitive studies are lacking, it is likely that gap junction-mediated intercellular communication is an important determinant for synchronizing the input (*i.e.* light entrainment) and output (*i.e.* melatonin secretion) pathways of the pineal gland, and thereby of circadian rhythmicity. To date, there are no studies examining connexin hemichannel and pannexin channel structure and/or function in the pineal gland.

Thyroid and parathyroid glands

The thyroid gland secretes triiodothyronin (T3), thyroxine (T4) and calcitonin to control many body functions, such as body growth, metabolism, thermoregulation and calcium homeostasis, under the regulation of hypothalamic TSH. The thyroid tissue is composed of follicular cells secreting T3 and T4, and parafollicular cells secreting calcitonin. Although TSH and thyroid hormones have been reported to regulate connexins in a variety of target tissues [142-144], the role of connexins in mediating thyroid function itself is not well documented. Indeed, connexin expression in the thyroid gland mainly controls cell differentiation and gland development, and most studies have been focused on the role of connexins in pathological situations [145-147].

The parathyroid glands consist of four to eight small endocrine glands located close to the thyroid, which secrete parathyroid hormone to maintain blood calcium levels within a tightly controlled range. This is achieved by facilitating osteolysis and renal calcium reabsorption.

Connexin expression and distribution

The rat thyroid gland displays immunoreactivity for Cx26, Cx32 and Cx43 with labeling varying from sparse to abundant depending on the cell type studied [8]. In the follicles, the three connexins are present, with a more robust expression detected for Cx32, whereas parafollicular cells (*i.e.* C-cells) express only Cx26 [8]. Unlike rat follicular cells, pig polarized thyroid cells do not express Cx26 [148]. Freshly isolated rat thyrocytes express high levels of Cx32 [149]. By contrast, in pig thyroid gland, both Cx32 and Cx43 are co-expressed in the same epithelial cells, but with a polarized distribution. In particular, Cx32 is found throughout the basolateral membrane domain of the follicular cell, while Cx43 is co-localized with zonula occludens-1 in tight junctions in the upper juxtapical pole of the lateral cell membrane [8, 148]. This subcellular connexin compartmentalization points to distinct regulatory mechanisms and functions. In addition, by co-expressing Cx32 and Cx43, the thyroid gland shares features of both endocrine (*i.e.* Cx43 expression) and exocrine (*i.e.* Cx32 expression) tissues. This is consistent with the fact that thyroid cells display both an exocrine function by exporting thyroglobulin into the follicular lumen and an endocrine function by releasing thyroid hormones into the vascular compartment [22, 150].

In the parathyroid glands, gap junctions were morphologically identified using freeze-fractured replicas in the early 1980s [151]. A few years later, an electrophysiological study eluded to the presence of electrically-coupled parathyroid cells [152]. The endocrine cells of the parathyroid glands exhibit robust immunostaining for Cx26 and Cx43 but, unlike the thyroid secretory cells, no staining for Cx32 was evident [8].

Connexin intercellular channels

The first indication in literature for a contribution of connexins to the differentiation and organization of thyrocytes in follicles came from experiments showing the persistence of Cx32 in these cells when cultured with TSH, which favors the reconstitution of follicles [149]. Cx32 contributes to thyroid development, in particular to epithelial morphogenesis. Indeed, pig thyrocyte-derived cells form three dimensional follicle-like structures *in vitro* only if they are forced to express Cx32, but not Cx43 [153]. Cx32-mediated intercellular communication also participates in the control of thyroid cell growth and proliferation. In thyroid-derived cell lines, overexpression of Cx32 [154], but not Cx43 [155], reduced cell proliferation, in line with the observed thyroid hypoplasia in mice in which Cx32 was upregulated selectively in thyroid cells [156]. Collectively, these findings argue for a critical role of Cx32 in the development of the thyroid gland.

In the parathyroid glands, although gap junctions have been identified for a long time [151], there are no studies reporting their functional role within the tissue.

Concluding remarks and future perspectives

Although morphologically reported several decades ago in many tissues, the functional role of connexins in endocrine/neuroendocrine glands, and especially in hormone secretion, is still a matter of debate. This particularly holds true for connexin hemichannels for which many fundamental issues remain to be addressed, as well as the parathyroids in which connexin function is yet to be studied. When investigated at the functional level, the anatomical network formed by gap junction-coupled secretory cells consistently appears to be a relevant determinant in the coordination and/or synchronization of hormone release from endocrine and neuroendocrine tissues. This is especially well documented in the pancreatic beta-cells [3, 4, 157], and in the adrenal medullary tissue, with *in vivo* studies clearly demonstrating that gap junctional communication contributes to insulin [158] and catecholamine secretion [12]. Although less well studied, gap junction-coupled glial-like cell networks must also be taken into consideration. Indeed, unlike secretory cell networks, which tend to be spatially restricted or compact, they support large-scale communication at low wiring cost, enabling integration of signals throughout the gland and concerted hormone release [61, 65].

Though not reviewed in this paper, it is worth noting that gap junctional communication is commonly dysregulated or even 'loosened' in tumor tissues [159], including endocrine gland neoplasms [24, 147], complying with its involvement in the control of cell metabolism, proliferation, growth and death. This strengthens the critical role of intercellular communication in the maintenance of vital physiological functions and body homeostasis [3].

Other fields lacking anatomical and functional data in endocrine/neuroendocrine tissues deal with connexin hemichannels and pannexin channels, first described many years ago [160, 161]. Because these transmembrane channels support ion and molecule exchanges between the cytosolic compartment and the extracellular environment, it is likely that they participate in the regulation of cell function. In the context of endocrine tissues, connexin hemichannels and pannexin channels may contribute to signal transduction associated with secretory function. Unveiling their expression and roles in secretory tissues would therefore significantly improve the knowledge of the physiological mechanisms that drive hormone release.

In summary, gap junctions, connexin hemichannels and pannexin channels are all intrinsic components of (neuro)endocrine axis structure and function. It is anticipated that their further experimental dissection will yield important insights into hormone release or tissue turnover, which can then be targeted to ameliorate pathologies associated with (neuro)endocrine dysfunction.

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Figure legends

Figure 1: Contribution of connexins and pannexins to adrenal catecholamine secretion. Under basal conditions (*i.e.* low hormonal need), connexin channels engaged in cell-cell coupling support information transfer (*i.e.* electrical and associated calcium signals) from a stimulated cell to adjacent coupled cells, leading the latter to exocytose. Coupled chromaffin cells (*i.e.* grey cells *versus* light grey cells for non-coupled cells) exhibit either a weak coupling, which supports the propagation of small potential fluctuations, or a robust coupling, which allows action potentials to be fully reflected into the connected cells (*i.e.* red potential traces). In addition, pannexin channels, through their contribution to nicotine-evoked rise in intracellular calcium concentration, also contribute to catecholamine release. In response to an increased catecholamine demand (*e.g.* in stressful situations), the adrenal medulla gap junctional communication remodels such that both the number of

gap junction-coupled chromaffin cells and the coupling strength are enhanced (*i.e.* disappearance of a weak coupling in favor of a robust coupling). Because the robust coupling supports the propagation of action potentials (and ensuing rises in intracellular calcium concentration) between cells, it appears as a key determinant in the increased catecholamine secretion observed in response to stress. Data collected from experiments performed in rat [15,34], mouse [12] and bovine [51] adrenal medullary tissue.

Figure 2: Connexin and pannexin function in the pituitary gland and hypothalamus. Connexin channels facilitate specific intercellular communication between pituitary cells and may be important for regulating hormone release during periods of demand or plasticity. By contrast, pannexin channels, through the liberation of ATP, may constitute an important mode of short-range paracrine signaling. In the hypothalamus, connexin channels couple neurosecretory neurons and have been shown to allow electrical synchronisation. This likely contributes to neuropeptide release, an important determinant of pituitary hormone release, as well as other downstream processes (*e.g.* lactation and hydration). Figure was produced using Servier Medical Art.

Table 1: Connexin expression profiles in the normal adrenal cortex

	Cx26		Cx32		Cx43		Cx50	
species	expression level	references	expression level	references	expression level	references	expression level	references
rat					+	[8, 9, 13, 15]		
mouse					+	[9, 12, 14, 27]		
guinea pig					+	[9]		
cow					+	[9, 10, 28]		
rhesus monkey					+	[14]		
human	+	[7]	+	[7]	+	[7, 11, 28]	+	[7]

Table 2: Divergent Cx43 protein expression in the different cortical zones

species	capsula	zona glomerulosa	zona intermedia	zona fasciculata	zona reticularis	references
rat		+/- +/- +/-	 +	+ ++ +	++ ++ ++	[8] [13] [9]
mouse	 + +	+/- +/- - -	no ZI +	+ + ++ +	++ + + ++	[9] [27] [14] [12]
guinea-pig		+/-	no ZI	+	+	[9]
cow		 +		+ +	 +	[28] [9]
rhesus monkey	+	-	+	+	+	[14]
human	 +	 +/-		+ +	+ +	[28] [11]

+/-: sparse staining, +: moderate staining, ++: robust staining

Table 3: Connexin expression in the normal adrenal medulla

	Cx29			Cx36			Cx43			Cx50		
species	expression level	cell type	references	expression level	cell type	references	expression level	cell type	references	expression level	cell type	references
rat				+	CC	[15, 34]	+	CC	[15, 34]			
							+/-	CC?	[8]			
							+/-	islets of cortical cells?	[9]			
mouse	+	S100-positive cells (Schwann cells, ST cells?)	[33]	+	ND	[12, 162, 163]	+/-	ND	[9, 12]			
	+	ND	[12, 162, 163]	+	CC	[35]	+	CC	[35]			
guinea pig							+/-	ND	[9]			
human										+	CC	[7]

+/-: sparse, +: abundant, ND: not determined, CC: chromaffin cells, ST cells: sustentacular cells

Table 4: Gender dimorphism in electrical coupling between rat and mouse chromaffin cells and connexin expression with changes in response to different physiological conditions

species	gender	electrical coupling	gap junction channels	connexin hemichannels	pannexin channels
adult unstressed rat	♂	+/- [34]	+/- [34]	ND	ND
	♀	+ [15]	+ [15]	ND	ND
adult unstressed mouse	♂	+ [35]	+ [12, 35]	ND	ND
	♀	+/- [164, 165] ++ [35]	++ [35]	ND	ND
adult stressed rat	♂	++ [34]	++ [34]	ND	ND
	♀	ND	ND	ND	ND
adult stressed mouse	♂	ND	++ [12]	ND	ND
	♀	ND	ND	ND	ND
neonate rat	ND	++ [39]	++ [38, 39]	ND	ND

+/-: weak, +: moderate, ++: robust, ND: not determined

Table 5: Connexin expression in the anterior pituitary gland

	Cx26			**Cx36**			**Cx43**		
species	expression level	cell type	references	expression level	cell type	references	expression level	cell type	references
rat	+/-	ND (secretory cells)	[8]	+	ND	[76]	+	ND (secretory cells)	[8]
							+	FS cells	[75]
							+/-	gonadotrophs	[75]
mouse							+	TtT/GF immortalized FS cells	[82]
mink							+	FS cells	[80]
sheep							+	ND	(DJ Hodson, personal observation)

+/-: sparse, +: abundant, ND: not determined, FS cells: folliculostellate cells

Table 6: Connexin expression in the hypothalamus and posterior pituitary

	Cx30			**Cx36**			**Cx43**		
species	expression level	cell type	references	expression level	cell type	references	expression level	cell type	references
rat	+/-	ND throughout the mediobasal hypothalamus	[109]	+	ND (disappears postnatally)	[76]	+/-	gonadotropin-releasing hormone-containing cells	[107]
							+	ND close to capillaries	[109]
							+	pituitary pituicytes	[75, 107]
mouse				+	corticotrophin-releasing hormone-containing cells	[105]			
				+	somatostatin-containing cells	[105]			
				+	kisspeptin-containing cells	[108]			

+/-: sparse, +: abundant, ND: not determined