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Revisiting the stimulus-secretion coupling in the adrenal medulla: role of gap junction-mediated intercellular communication

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Abstract

The current view of stimulation-secretion coupling in adrenal neuroendocrine chromaffin cells holds that catecholamines are released upon trans-synaptic sympathetic stimulation mediated by acetylcholine released from the splanchnic nerve terminals. However, this traditional vertical scheme would merit to be revisited in the light of recent data. Although electrical discharges invading the splanchnic nerve endings are the major physiological stimulus to trigger catecholamine release *in vivo*, growing evidence indicate that intercellular chromaffin cell communication mediated by gap junctions represents an additional route by which biological signals (electrical activity, changes in intracellular Ca²⁺ concentration,...) propagate between adjacent cells and trigger subsequent catecholamine exocytosis. Accordingly, it has been proposed that gap junctional communication efficiently helps synapses to lead chromaffin cell function, and in particular hormone secretion. The experimental clues supporting this hypothesis are presented and discussed with regards to both interaction with the excitatory cholinergic synaptic transmission and physiopathology of the adrenal medulla.

Index Entries: Gap junctions, adrenal medulla, innervation, chromaffin cells, cholinergic synaptic transmission, catecholamine release, stimulus-secretion coupling, development, stress, physiopathology.

Overview of the neurogenic control of catecholamine release in the adrenal medulla

The adrenal medulla is the latest link of the sympathoadrenal axis and is involved in the ability of the organism to generate appropriate reaction to stressful situations. This adaptive response to stressor agents involves the secretion of adrenal catecholamines (adrenaline and noradrenaline) by the neuroendocrine chromaffin cells. Once released into the blood circulation, catecholamines contribute to the regulation of multiple functions including cardiovascular function as well as energy metabolism, thus helping the organism to cope with a changing environment. With regards to catecholamine secretion by chromaffin cells, the common view is that it is chiefly controlled by synaptically released acetylcholine at the splanchnic nerve terminal-chromaffin cell synapses (1,2; Fig. 1). The first demonstration of a neuronal control of the release of catecholamines from the adrenal gland comes from the pioneer work of Dreyer (3), showing that the stimulation of the splanchnic nerve caused an outflow of the “pressor activity” from the adrenal. This “pressor activity” has been further identified as being mediated by the release of adrenaline (4). In the early twentieth century, numerous studies have confirmed that catecholamine release from the adrenal medulla is controlled by the splanchnic nerve activity (5,6). Within the medulla, chromaffin cells are organized into discrete cell complexes (7) and each chromaffin cell receives its own synaptic inputs, up to five synapses per cell (8-10). Because it is currently proposed that each chromaffin cell cluster functions as an independent unit to release catecholamines after activation of the splanchnic nerve (11), it was of physiological interest to characterize

stimulus-secretion coupling at the cell cluster level. In particular, recent studies have focused on the functional role of direct intercellular communication between chromaffin cells.

It has long been thought that, since the triggering of hormone release is chiefly achieved by synaptic neurotransmission at the splanchnic nerve-chromaffin cell contacts, there was no need for an additional stimulatory signal exchanged between chromaffin cells. The present article reviews the evidence supporting the presence of cell-to-cell coupling mediated by gap junctions between adrenal chromaffin cells and the consequences of this coupling on hormone secretion, an emerging area of potential physiological/physiopathological interest that has received too little attention so far.

Gap junctions in the adrenal medullary tissue

Gap junctions are specialized regions of the plasma membrane in which protein oligomers establish a contact between adjacent cells, thereby forming conduits for intercellular communication that allows coupled cells to exchange nutrients, metabolites, ions, second messengers and small molecules under ~1000 Da. With regards to molecular identity, gap junctions in chordate are chiefly assemblies of connexins (12-14). Indeed, regarding the more recently discovered pannexin family (15-18), their contribution to functional gap junctions in vertebrates is still unclear. Their functional roles are only beginning to emerge. To date, expression of pannexins in the adrenal gland has not been reported, neither in the cortex nor in the medulla. By contrast, connexins are widely present in the adrenal gland, with a preferential expression in the zona fasciculata and zona reticularis of the cortex (19). In the medulla, connexin-built gap junctional plaques have been originally described in the eighties from observations of freeze-fractured specimens (20). By using this experimental procedure, gap junction clusters have been found in several species including mouse, guinea-pig, hamster and rabbit but, surprisingly not in rat. Later on, the use of immunohistofluorescence and

immunoblot techniques revealed the presence of connexins in the rat adrenal medulla (21-23). More recently, gap junctions have also been found in human medulla (24). Table 1 summarizes the available data concerning the presence of connexin-related gap junctions in the adrenal medulla of different species.

Connexin subtypes expressed in the adrenal medulla

Since the cloning of the first members at the end of the 1980s, the connexin family has considerably expanded, and there are about 20 identified genes (14). To date, six connexins (Cx26, Cx29, Cx32, Cx36, Cx43 and Cx50) have been found in the adrenal medulla (Table 2). Their expression not only varies between species but also differs between normal and tumoral tissue. Cx43 appears to have the highest expression, in rat (19,21-23), in mouse (19,25-27), in guinea-pig (19) and in human (24). By contrast, expression of Cx50 seems to be restricted to human, both in normal medulla and in pheochromocytomas (24). Not surprisingly, Cx36, a connexin present in neuronal tissues (28) is also expressed in the adrenal medulla, which originates from the neural crest, and in the rat tumoral cell line PC-12 (29). Interestingly, acquisition of a tumoral phenotype appears to be accompanied by a change in connexin expression. Indeed, Cx26 and Cx32, mainly identified in exocrine but not in endocrine glands (21), are present in human pheochromocytomas while they are absent from normal medulla (24).

Brief focus on gap junction hemichannels

The formation of a gap junction channel results from the apposition of two gap junction hemichannels, which are delivered to the plasma membrane (30,31) where they diffuse laterally into cell-contact regions to dock head-to-head with partner connexins present on the neighboring cell. Hemichannels have long been considered as mere structural precursors of

gap junctions. This concept of hemichannels being only the halves of gap junction channels began to be questioned after the finding that they can generate ionic currents and modulate cell activity (32). Functional hemichannels have been described in various tissues such as glial cells, retina or heart (33-35) and more recently in PC-12 cells (36). They have been reported to contribute to paracrine communication or to the integration of the incoming information (37-39). When open, hemichannels that are traveling on the plasma membrane prior to incorporation into gap junction plaques allow diffusion of ions and small molecules across the plasma membrane (37,40,41). It is noteworthy that some results are still discussed (42). Various factors can be released from a cell in the extracellular medium through hemichannels, such as ATP from Cx36- and Cx43-built hemichannels (43,44,45), or glutamate from Cx32-built hemichannels (46). As mentioned above, adrenal chromaffin cells express both Cx36 and Cx43 and although the expression of connexin-built hemichannels has not yet been reported in adrenal medullary tissue, one could reasonably propose that gap junction hemichannels might be potent modulators of the adrenal medulla physiology. If this hypothesis is confirmed, connexins would contribute to two modes of communication in the adrenal medulla : i) direct cell-cell communication between chromaffin cells coupled by gap junctional channels and ii) diffuse paracrine communication, targeting several cell types, through hemichannels. Because gap junction channels and hemichannels can be differently regulated by a same molecule (47,48), both communication modes likely contribute to specific and distinct functions in a tissue.

Adrenal medullary cell types expressing connexin-built gap junctions

Since the adrenal medullary tissue is mainly composed of neuroendocrine chromaffin cells, gap junctional communication was first investigated at the chromaffin cell level. Suspicion that chromaffin cells may be electrically coupled *in situ* first came from

electrophysiological recordings of membrane potentials in hemi-sectioned gland (49,50). Later on, comparison of the membrane properties of chromaffin cells measured in thin acute tissues slices, to those of isolated cultured cells, suggested that chromaffin cells are electrically coupled (22,51). Confirming this hypothesis, dual patch-clamp recordings from chromaffin cell pairs showed that electrical signals can propagate from the stimulated to the non-stimulated cell (22,23,52,53). Regarding the molecular determinants supporting gap junctional communication between chromaffin cells, transcripts encoding Cx36 and Cx43 were detected in single chromaffin cells by using RT-PCR (22) and related proteins were next detected by immunoblot and immunohistofluorescence techniques (23). An unsolved issue deals with the specificity of gap junctional coupling between chromaffin cells, in particular with regards to the hormonal nature of coupled cells. Chromaffin cells can be divided into two separate populations, depending on the hormone secreted (54). To date, we do not know whether gap junctions connect only adrenaline-containing cells or only noradrenaline-containing cells or both. Because i) these two populations of chromaffin cells are innervated and regulated by morphologically different nerve terminals originating from distinct spinal cord and brain regions (55-58) and ii) gap junctional coupling can be modulated by the cholinergic synaptic activity (52), elucidating this question would help understanding the contribution of gap junctions to hormone secretion.

Although chromaffin cells are the predominantly expressed cellular constituents of the adrenal medulla, it also contains non neuroendocrine cells including sustentacular cells, homologous in nature to Schwann cells (59,60). As extensively documented in other tissues including both neuronal and endocrine tissues, glial cells or glial-like cells are highly coupled by gap junctions (61-63). Sustentacular cells in the adrenal medulla are likely gap junction-coupled, as evidenced by the expression of Cx29 (27). It is noteworthy that the presence of gap junctions in the other medulla tissue components, *i.e.* ganglion cells, nerve fibers

originated from pre-ganglionic sympathetic axons, connective-vascular tissue, small intensely fluorescent (SIF) cells and pluripotent stem cells (60,64,65) has not been reported yet.

Functional roles of gap junction-mediated cell-to-cell communication in the adrenal medulla: the stimulus-secretion coupling revisited

While gap junctions in the adrenal cortex have been extensively studied, very few works have been devoted to the functional characterization of gap junctional coupling in the medulla. Until the beginning of the two thousand-years, the current view with regards to stimulus-secretion coupling in the adrenal medulla was that there is no need for a direct coupling between chromaffin cells to ensure hormone release since each chromaffin cell receives its own synaptic input. However, a series of recently obtained data allows confronting this view.

Gap junctional communication between chromaffin cells: a tool for synchronizing their secretory activity

The hypothesis of a direct cell-to-cell communication between adrenal chromaffin cells amplifying the secretory signal in the intact gland has been initially proposed by Ceña and collaborators (66). At the same time, the publication of a series of works illustrating the influence of chromaffin cell-cell contacts on levels of tyrosine hydroxylase, an enzyme involved in catecholamine biosynthesis, strongly supported this hypothesis (67-69), although the nature of the intrinsic membrane protein involved was not characterized. A decade later, a junctional pathway coupling mouse chromaffin cells *in situ* has been reported to potentially underlie the simultaneous firing of a cell cluster (51). This contrasts with a study performed in rat and published one year before reporting that the spreading of electrical activity between rat chromaffin cells induced by transmural stimulation of the splanchnic nerve would be mostly

dependent on nerve activity rather than on cell-to-cell coupling (70). More recently, the presence of functional gap junction-mediated electrical coupling between rat chromaffin cells has been reported in acute adrenal slices (22). It is noteworthy that the percentage of coupled chromaffin cells is species- and gender-dependent with a higher percentage in female versus male, and also differs as a function of the physiological state of the animals (Fig. 2A). In addition, simultaneous recordings from chromaffin cell pairs allowing to monitor the junctional current flowing between two cells revealed two populations of coupled chromaffin cells: a weakly coupled cell population and a robustly coupled cell population (22,23). Again, the proportion of the two populations differs between male and female rats and also differs as a function of the physiological state of the rats (normal *versus* stressed, 23; adult *versus* neonate 53; Fig. 2B). As remarkable differences, one can point out i) the dramatic up-regulation of electrical coupling in stressed rats that is associated with the appearance of robust coupling in half of cell pairs and ii) the increased electrical coupling in neonate rats when compared to adults. These results will be discussed later.

Strengthening the role of gap junctions in adrenal catecholamine release, it is noteworthy that the higher level of gap junctional communication in rat female parallels i) higher basal circulating adrenaline and noradrenaline concentrations, as compared to male (71), and ii) an increased catecholaminergic activity of the adrenal medulla in female (72,73). The underlying mechanisms still remain to be elucidated. One attractive hypothesis is that the expression level of connexins may be higher in female. This would be especially efficient for Cx43, which exhibits a higher unitary conductance than Cx36 (74,75). This hypothesis is strengthened both by steroid hormone-induced regulation of Cx36 and Cx43 expression levels and by a sexually dimorphic regulation of Cx43 (76-79). Although hormonal and gender-specific regulation of Cx36 and Cx43 has not been yet reported in the adrenal medulla, this

could likely be part of the explanation for different gap junctional coupling strength and extent between male and female chromaffin cells.

The contribution of Cx36- and/or Cx43-built channels to weak *versus* robust coupling is unknown but, since Cx36 and Cx43 channels exhibit different voltage sensitivities (80), their roles in propagating electrical signals between adjacent cells likely differ. The weak voltage dependence of Cx36 channels suggests that electrical coupling may not be disrupted during secretagogue-mediated action potential discharges. By contrast, Cx43 channels that are highly voltage-sensitive may predominantly be regulated during firing. Through the coupling of electrical activities, gap junctional communication provides the basis for coordinating spontaneous and agonist-induced synchronous multicellular $[Ca^{2+}]_i$ increases (22,23,81). The synchrony of $[Ca^{2+}]_i$ changes amplifies catecholamine release in response to excitation of a single chromaffin cell (22), and could therefore constitute an efficient complement to synaptic neurotransmission. This complementary action of gap junctional coupling and neuronal command is particularly obvious in situations in which the neuronal control of catecholamine release is not fully competent, that is at birth, in splanchnectomized adrenals or in response to pharmacological blockade of postsynaptic nicotinic receptors (52,53,82). In summary, it is now clear that gap junction-mediated communication between chromaffin cells complements the nerve command, or even relays it in some cases. Both signals contribute to the synchronization of electrical activity, ensuing $[Ca^{2+}]_i$ transients and hormone secretion.

Although the function of gap junctional communication to help synapses is of major interest for the adrenal secretory tissue, one could reasonably think that gap junctions between chromaffin cells might be involved in other functions, based in particular on the fact that chromaffin cells do not represent a homogeneous cell population. Chromaffin cells can be distinguished by the peptidergic content of their secretory granules (83,84), their localization within the lobule (at the periphery *versus* at the center), their nearness with the cortex or their

vicinity with blood vessels. A specific coupling of cells belonging to one of these populations may be relevant in physiological conditions (such as during embryogenesis, postnatal development, ...) or in pathological conditions (such as during inflammation, infectious diseases, ...). The exhaustive study of gap junctional communication (in terms of connexin subtypes expressed, coupling strength, coupling extent, ...) between these different chromaffin cell populations would likely unmask not yet anticipated roles of gap junctions and connexins in the medullary tissue function. Indeed, gap junction channels are permeant for several second messengers including Ca^{2+} , cAMP and cGMP which regulate numerous cell activities. Additionally and independently of their gap junctional function, connexins also directly interact with a wide variety of signaling intracellular and membrane-associated proteins and can regulate gene expression and cell function (85,86). Gap junctions are now perceived, not only as channels between neighboring cells, but also as signaling complexes that regulate cell function and transformation. Subsequently, it should be considered in further studies that, beyond a tool for synchronizing catecholamine release, connexins may also be involved in many chromaffin cell functions such as differentiation, growth, division, repair, adhesion.

Gap junctional communication between sustentacular cells

To date, a unique study describes the presence of connexin-built gap junctions between adrenal sustentacular cells, mediated by Cx29 channels (27), which were recently reported as a novel marker for cells of the defined Schwann cell lineage (87). Nevertheless, based on other reports on sustentacular cells (88) and other glial-like cell types (63,89), we propose that gap junction-coupled sustentacular cells may form a long-distance communication route, as described for the anterior pituitary folliculostellate cell network (90). This hypothesis is consistent with a recent study suggesting that adrenal medulla sustentacular

cells take an active part in Ca^{2+} metabolism, regulating indirectly the synthesis and release of catecholamines from chromaffin cells (91).

Contribution of gap junctions to interactions between chromaffin cells and other elements of the adrenal tissue

In the medulla, hormone secretion from chromaffin cells results from the precise coordination and integration of cellular and intercellular signals arising from distinct inputs. Not only can catecholamine secretion of a single chromaffin cell be modulated by neighboring chromaffin cells if gap junction-coupled (22), but secretion can also be regulated by other tissue components. First, splanchnic nerve endings synapsing onto chromaffin cells are major inputs controlling intracellular signaling and ensuing catecholamine release (2,92,93). Is gap junctional communication between chromaffin cells able to interact with synaptic cholinergic neurotransmission? Although the underlying mechanisms are not totally elucidated, it is clear that gap junction-mediated cell-to-cell communication is under a tonic inhibitory control exerted by synaptic activity, as also reported in other neuronal tissues (94,95). Indeed, studies performed under experimental conditions leading to impairment of synaptic transmission (pharmacological blockade of post-synaptic nicotinic receptors, unilateral splanchnectomy, immature synaptic transmission in newborn rats) showed an up-regulation of gap junctional coupling between chromaffin cells (52,53). Second, sustentacular cells that are commonly observed in close proximity to chromaffin cells and that elongate their thin processes between secretory cells (60) are also good candidates to modulate signaling pathways in adjacent chromaffin cells and therefore catecholamine release. Assuming that sustentacular cells can form a functional circuitry intermingled between chromaffin cells, they may also have a privileged role in coordinating the biological activities

of distant chromaffin cells in both physiological and pathological conditions, and thus may modulate hormone release.

At the level of the whole adrenal gland, that is cortex and medulla, it is likely that gap junctional communication between medullary cells may influence activities of cortical cells and further release of steroids and sex hormones. Although no data ascertain this hypothesis yet, several evidences support it: i) morphological data show close appositions of chromaffin cells with cortical cells (96,97) in particular with dehydroepiandrosterone (DHEA)-producing cells (98), and ii) functional interaction mediated by paracrine factors between chromaffin and cortical cells have been extensively reported (99). In particular, the release of catecholamines locally supports influence of chromaffin on neighboring cortical cells (100). Thus, we propose that, by modulating catecholamine secretion, gap junctional coupling may indirectly affect cortical cell function. Reciprocally, as previously reported for induction of catecholamine enzymes in chromaffin cells by glucocorticoids (101), steroids and sex hormones released from the cortex may also influence junctional coupling in the medulla. Because adrenocortical factors are involved in many cell functions (differentiation, growth, apoptosis, hormone release, ...), their action on chromaffin gap junctional communication may be particularly relevant in physiological/pathological situations such as development or tumorigenesis.

Gap junctional communication in the developing adrenal medulla: towards the identification of the mechanism responsible for adrenal catecholamine secretion in response to birth-induced stress

In developing neuronal tissues, a finely tuned coordination between gap junction-mediated electrical coupling and synaptic neurotransmission (102,103) is crucial for synapse formation. At birth, the synaptic transmission is immature in the rat adrenal medulla (53) and it becomes fully competent during the first post-natal week (104,105). Maturation of

cholinergic synaptic transmission results from a 2-fold increase in synaptic current amplitude without significant change in synaptic current frequency (53). In parallel, the gap junction-mediated coupling is prominent at birth (52; Fig. 3A). With the help of the extracellular matrix protein agrin (53,82), electrical coupling then decreases, in coincidence with the establishment of functional and mature chemical synapses. What can be the role of gap junctions between chromaffin cells in newborn rats and can they be involved in catecholamine secretion? During birth, neonate rats are subjected to various stresses, including hypoxia, hypoglycemia or glycopenia. During this period, the adrenal medulla exerts a very specific protective function, in particular against hypoxia, involving a variety of respiratory, cardiovascular and metabolic adjustments (105). In the absence of functional synapses originating from the splanchnic nerve, this function is handled directly by chromaffin cells via their ability to respond to hypoxia by a non-neurogenic catecholamine release (106). It is likely that cell-to-cell communication mediated by extracellular factors is not appropriate for the massive catecholamine mobilization which is needed at that time. Gap junction-mediated fast signal propagation would therefore become physiologically relevant. The contribution of gap junctional communication to the non-neurogenic response is also indicated by the fact that both the high occurrence of electrical coupling and the non-neurogenic response observed in neonates decrease during the first postnatal week and are inhibited by establishment of functional splanchnic synapses (52,53,104). In addition, the non-neurogenic response to hypoxia involves calcium ions and reactive oxygen species (107,108), two messengers that are diffusible through gap junctions (13,109-111). Since only a proportion of chromaffin cells are sensitive to hypoxia in late embryos and neonates (106), we propose that gap junctional communication facilitates the spreading of the response from hypoxia-sensitive cells to the whole population of chromaffin cells and thus plays a major role in the extent of the response of the adrenal medulla to the birth-related hypoxic stress. In view of the impact of this non-

neurogenic response on the adaptation of respiratory and cardiac function to the extrauterine life (104), this would provide gap junctions with a major role in early neonatal life.

Beyond the role of junctional communication as support to catecholamine secretion in neonates, it is likely that gap junctions also play important functions during adrenal embryogenesis, in particular with regards to acquisition of a chromaffin cell fate and subsequent acquisition of an adrenergic or a noradrenergic phenotype. In addition, the acquisition of a specific neuropeptidergic phenotype during development (112) may also be helped by gap junctional communication. By promoting factor exchange between cells, gap junctions may contribute to a harmonious exposure of sympathoadrenal progenitors to morphogenetic factor-induced instructive signals (65,113).

Gap junctional communication: involvement in physiopathology of the adrenal gland

The “stressed” adrenal medulla

A rise in catecholamine plasma levels is a key event in response to stressors. Consistent with a crucial role of the adrenal gland in stress-induced catecholamine secretion, the "stressed" medulla is significantly remodeled when compared to an "unstressed" gland (Fig. 3B). In particular, the gap junction-mediated metabolic and electrical coupling between chromaffin cells is dramatically up-regulated (23,114). Regarding electrical coupling, the most striking change is the appearance of robustly coupled chromaffin cells, leading to the transmission of action potentials and ensuing $[Ca^{2+}]_i$ transients between coupled cells. We propose that the up-regulated gap junctional communication between chromaffin cells, similarly to what has been observed in pancreatic beta-cells (115,116), might serve as a general model to better understand how secretory tissues dynamically adapt to an increased hormonal demand. In addition to an up-regulation of gap junctional communication, synaptic transmission between splanchnic nerve terminals and chromaffin cells is also enhanced in

stressed rats (114). We propose that all these changes contribute to optimize the stimulus-secretion coupling efficiency in the adrenal gland. From a mechanistic view-point, the “stressed” medulla represents an interesting model in which interactions between gap junction-mediated coupling and synaptic transmission are differently regulated from the “unstressed” medulla. In particular, the tonic inhibitory control exerted by the synaptic neurotransmission on gap junctional communication is masked in stressed animals at variance with what is observed in unstressed animals (52). The underlying mechanisms have not been investigated yet. One plausible possibility is the synaptic release of noncholinergic factors (ATP, nitric oxide, ...) or neurotransmitters (VIP, PACAP, ...) by presynaptic terminals (117-121). Supporting our hypothesis, the relative amplitude of their release depends on the pattern of electrical activity in the incoming nerve (118,122). Some of these transmitters are known to regulate gap junctional cell-cell communication (123-125). Although not yet investigated in the adrenal medulla, one of their functions would be to counteract acetylcholine-mediated regulation of gap junctional communication between chromaffin cells.

The tumoral adrenal medulla

Tumor development is a process resulting from the disturbance of various cellular functions including cell proliferation, adhesion and motility, in which gap junctional communication is strongly implicated. Thus, there is growing evidence supporting the etiologic implication of gap junctional communication disorders in tumorigenesis (126,127). In addition, some genes encoding proteins involved in cell-to-cell communication, including connexin genes, appear to form a family of tumor-suppressor genes (128,129). More recently, the finding of aberrant connexin phosphorylation in tumoral cells (130) has strengthened the emergence of gap junctions as attractive targets for anti-tumoral therapy (131,132). Regarding adrenal medulla, pheochromocytomas are catecholamine-secreting tumors that arise from

chromaffin tissue within adrenal and extra-adrenal sites (133). With regards to gap junctions, pheochromocytomas express Cx26 and Cx32, contrasting with the normal human medulla (24). Whether this change in connexin expression pattern originates or results from tumorigenesis still remains to be elucidated. In addition, connexin50-built gap junctions have been found in human intra-adrenal pheochromocytomas with a predominant expression in benign *versus* malign tumors (24). Mice deficient for Cx32 are more sensitive to radiation-induced tumorigenesis and develop pheochromocytomas more frequently, suggesting that connexins and gap junctional intercellular communication may function as tumor suppressors/modulators (129,134). Although gap junction-mediated cell-to-cell communication has not been extensively studied in the tumoral adrenal medulla, it is likely that gap junction expression is altered, as observed in numerous tumoral tissues, including endocrine tissues (135-137).

Gap junctional communication between chromaffin cells: which added value for the adrenal medulla?

Keeping in mind that each chromaffin cell receives several synaptic inputs (8), the finding of a functional gap junction-mediated communication between chromaffin cells raises the question of the added value for the physiology and/or pathology of the gland. This issue is of great interest more especially as we have previously demonstrated that gap junctional communication and synaptic transmission interact with each other. In particular, when the cholinergic synaptic transmission is not competent, the junctional coupling is significantly enhanced and thus relays neurotransmission in catecholamine secretion (52,53,82). In stressful conditions, these two pathways undergo profound remodeling (23,114) and seem to act in synergy, allowing the adrenal medulla to respond to an increased catecholamine demand. However, this finding calls into question the relevance of the up-regulated gap

junctional coupling since synaptic neurotransmission, the major stimulus triggering catecholamine release, is also enhanced and could on its own trigger increased hormone secretion. Attempting to address this issue, we hypothesize that gap junctional communication between chromaffin cells may have either a synergistic or an opposite action to synaptic transmission on catecholamine secretion, depending on both splanchnic nerve discharge frequency and gap junctional coupling strength (Fig. 4). In basal conditions when the hormonal need is weak, the firing nerve discharge is low (≤ 1 Hz) and chromaffin cells exhibit an action potential firing matching the sympathetic tone and release catecholamines at a modest rate (138). Under these conditions, gap junction-mediated coupling may help chromaffin cells to coordinate their activities (electrical events and ensuing calcium changes) and may therefore represent an efficient complement to synaptic transmission to amplify catecholamine release, as previously proposed (22; Fig. 4A). Gap junctional intercellular communication would also facilitate catecholamine release in case of a sudden increase in splanchnic nerve discharge frequency, as observed in response to an acute stress (Fig. 4B). When the firing discharge of the splanchnic nerve is high (>10 Hz) and the hormonal need is large and sustained as observed in response to chronic stress, increased strength of gap junctional coupling (23,114) could also have a synergistic effect with synaptic transmission (Fig. 4C). The occurrence of such a synergy would need the inhibitory control of synaptic transmission on junctional communication to be impaired. To date, the cellular mechanisms shutting down this inhibitory control remain to be elucidated. Several hypothesis, not mutually exclusive, would be that in stressed animals, i) the junctional communication is facilitated by synaptic factors (139) co-released with acetylcholine during intense discharges of sympathetic preganglionic nerve fibers (140) or by factors released from peptidergic sensory afferents that innervate the adrenal medulla (141) and/or ii) the gap junctional coupling is positively regulated by other factors, originating from the medulla itself or from

the cortex, such as the adrenocorticotropin hormone ACTH, which is a potent modulator of gap junction channels (142).

In case of very robust coupling combined with a high nerve firing frequency, gap junctional communication would not act in synergy with synaptic activity, but conversely would counteract synaptic transmission and have a buffering and filtering action on signal propagation (Fig. 4D). This would avoid a huge catecholamine release potentially harmful for the organism or even lethal. Indeed, as stated by Aunis and Langley (58), catecholamine exocytosis from chromaffin cells must be strictly regulated to avoid excessive release of potentially toxic molecules. We based our hypothesis on a concept arising from mathematical modeling of gap junctional coupling in pancreatic beta-cells, called the "channel sharing" theory (143). The "channel sharing" process acts as a protective effect by quantitatively filtering signal propagation between coupled cells, in so that the coupled cells become less excitable, thus preventing any deleterious signal propagation between cells.

Concluding remarks and future perspectives

Since the pioneering work of W. W. Douglas in the sixties (88,144-147), the study of stimulus-secretion coupling in the adrenal medulla has been continuously pursued. This stimulus-secretion coupling now appears far more intricate than was previously envisioned and its deciphering still represents a major domain of interest for neurobiologists engaged in the study of the regulation of body homeostasis.

The finding that chromaffin cells are electrically coupled *in situ* and that this coupling is remodeled under physiological/pathological conditions is, in our view, one of the most significant results obtained over the last decade in the field of adrenal stimulus-secretion coupling. Nevertheless, whether this gap junction-mediated communication between chromaffin cells is relevant for the regulation of catecholamine secretion in the entire animal

still remains to be determined. Although *in vivo* measurements of catecholamine secretion in response to splanchnic nerve stimulation have been performed (148), the contribution of gap junctions was not investigated. One experimental approach allowing to solve this latter issue would be to conduct *in vivo* pharmacological manipulation of gap junctions during electrophysiological recordings of the adrenal medulla combined with the blood measurement of catecholamine secretion. Through the constant development of new innovative strategies, this issue will be likely addressed in the next future.

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Figure legends

Fig. 1. Traditional picture of the stimulus-secretion coupling in the adrenal medulla: the vertical view

Catecholamine secretion from adrenal chromaffin cells is considered to be under the major, if not exclusive, control of synaptically released acetylcholine at the splanchnic nerve-chromaffin cell synapses. Post-synaptic binding of ACh to cholinergic receptors triggers excitatory synaptic currents that are subsequently responsible for calcium-dependent catecholamine exocytosis. ACh, acetylcholine; APs, action potentials; sEPSPs, spontaneous excitatory post-synaptic potentials.

Fig. 2. Gap junction-mediated electrical coupling between rodent adrenal chromaffin cells: a highly plastic cell-to-cell communication pathway

(A) Histogram illustrating the differential proportion of electrically coupled cells between rat and mouse, between male and female and as a function of the physiological state (untressed *versus* stressed rats, neonates *versus* adults). **(B)** Gender- and physiological state-dependent percentage of weakly coupled and robustly coupled rat chromaffin cell pairs.

(data collected from Refs 22,23,46-48).

Fig. 3. Morpho-functional remodeling of the adrenal medulla in various physiological/pathological states

(A) A robust gap junction-mediated chromaffin cell coupling and an immature cholinergic synaptic transmission in the adrenal medulla of newborn rats contrasting with a weak junctional coupling and a robust synaptic transmission in adult animals. Insets: examples of electrical gap junctional intercellular communication (GJIC) monitored by dual current-clamp recordings in chromaffin cell pairs. The upper trace refers to the stimulated cell (1) and the

bottom trace to the non-stimulated cell (2). The developmental switch from a predominant gap junctional communication to a chemical neurotransmission contributes to the postnatal acquisition of the neurogenic control of catecholamine secretion. **(B)** In response to stressors, the rat adrenal medulla displays anatomical and functional changes, which converge to improve the efficiency of the stimulus-secretion coupling, likely to ensure a catecholamine secretion optimum for the organism demand. These changes are observed i) at the chromaffin cell level by an increased excitability, ii) at the synaptic input level by a denser innervation associated with an increased frequency of spontaneous excitatory synaptic currents and iii) at the gap junctional communication level by the appearance of a robust electrical coupling between chromaffin cells, that allows transmission of action potentials between coupled cells.

Fig. 4. Cartoons illustrating the hypothesis that intercellular communication (GJIC) would exert two opposite actions on signal propagation between adult chromaffin cells

In each case, the strength of the GJIC is illustrated in insets by the propagation of an electrically evoked signal from the stimulated cell (1) to a non-stimulated cell (2). When the catecholamine demand is low, GJIC between chromaffin cells is weak but helps the modest splanchnic nerve discharge to release catecholamines **(A)**. Similarly, when the nerve firing increases (in response to an acute stress, for example), the weak GJIC between chromaffin still acts in synergy with synaptic transmission to secrete catecholamines **(B)**. A further GJIC increase leading to a robust coupling between chromaffin cells may then have two opposite actions on signal propagation and ensuing hormone release. First, by acting in synergy with synaptic activity, GJIC would contribute to a robust and sustained catecholamine secretion, as observed in chronic stressful situations for example **(C)**. Alternatively, a huge GJIC increase between chromaffin cells would counteract synaptic transmission by triggering a "channel sharing" effect **(D)**. In this case, GJIC exerts a buffering and filtering action on cell signaling,

leading to a shunt of signal propagation between secretory cells. This "channel sharing" process would therefore function to prevent an excessive stimulation of the adrenal tissue that would be harmful for the organism.

Table 1: Gap junctions in the adrenal medulla

Data collected from the combination of freeze-fracture (FF), western blot (WB) immunohistological staining (IHS), dye transfer (DT) and electrophysiological (E) techniques.

	no detection	weak amounts	large amounts
rat	Grynszpan-Wynograd et al., 1980 (FF)	Murray and Pharrams, 1997 (IHS)	Meda et al., 1993 (IHS) Martin et al., 2001 (DT, E) Martin et al., 2003 (DT, E) Martin et al., 2005 (DT, E) Colomer et al., 2008a (DT, E, WB, IHS) Colomer et al., 2008b (DT)
mouse		Murray and Pharrams, 1997 (IHS)	Grynszpan-Wynograd et al., 1980 (FF) Moser, 1998 (E) Li et al., 2004 (WB, IHS) Degen et al., 2004 (IHS)
guinea-pig		Murray and Pharrams, 1997 (IHS)	Grynszpan-Wynograd et al., 1980 (FF)
hamster			Grynszpan-Wynograd et al., 1980 (FF)
rabbit			Grynszpan-Wynograd et al., 1980 (FF)
human			Willenberg et al., 2006 (WB, IHS)

Table 2: Summary of the connexin protein subtypes expressed in normal and tumoral adrenal medulla

	Cx26	Cx29	Cx32	Cx36	Cx43	Cx50	References
rat	-		-	+ +	+ +		Meda et al., 1993 Murray and Pharrams, 1997 Martin et al., 2001 Colomer et al., 2008a
mouse		++		+ ++	+		Murray and Pharrams, 1997 Li et al., 2004 Degen et al., 2004 Eiberger et al., 2006
guinea-pig					+		Murray and Pharrams, 1997
human	-		-		+	++	Willenberg et al., 2006
benign pheochromocytoma (human)	++		+		+	++	Willenberg et al., 2006
malign pheochromocytoma (human)	++		++		+	+	Willenberg et al., 2006
PC-12 cells (rat pheochromocytoma cell line)				+			Lu et al., 2007

(-, not expressed; +, modestly expressed; ++, highly expressed)

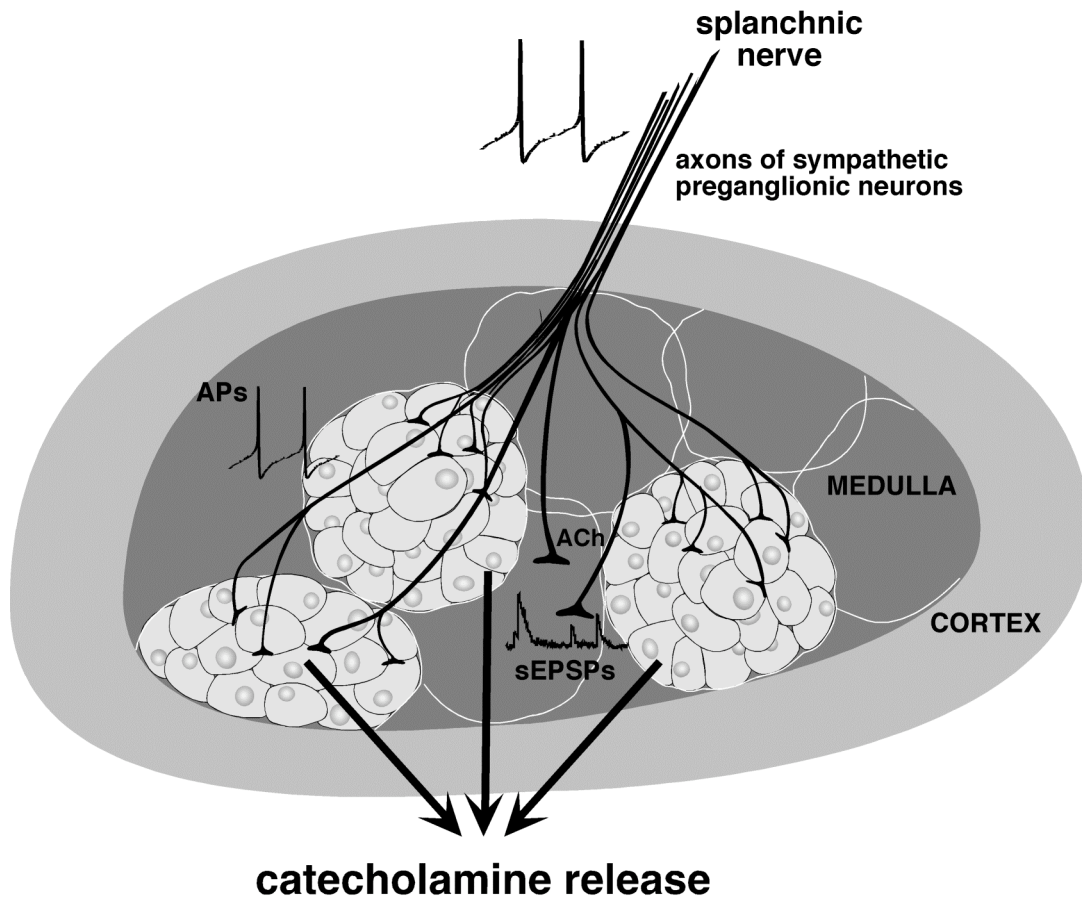


Figure 1

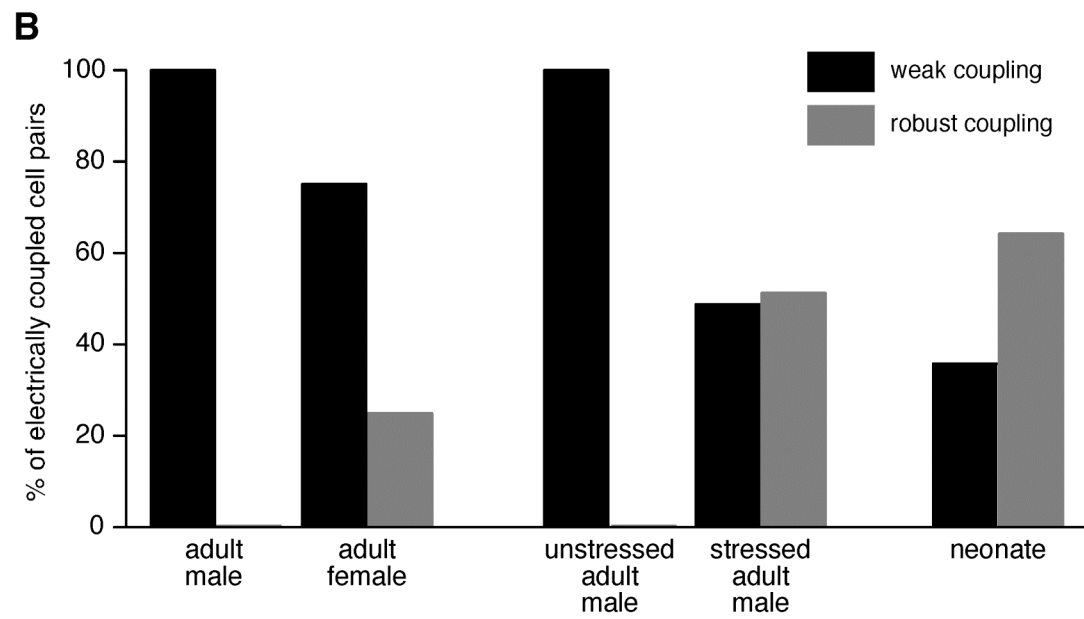
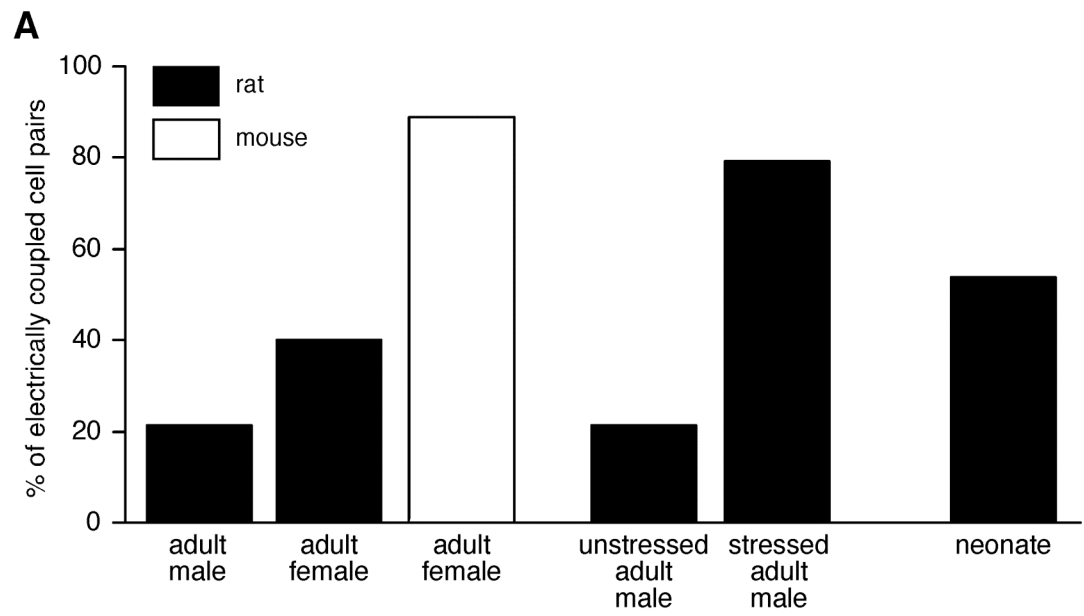


Figure 2

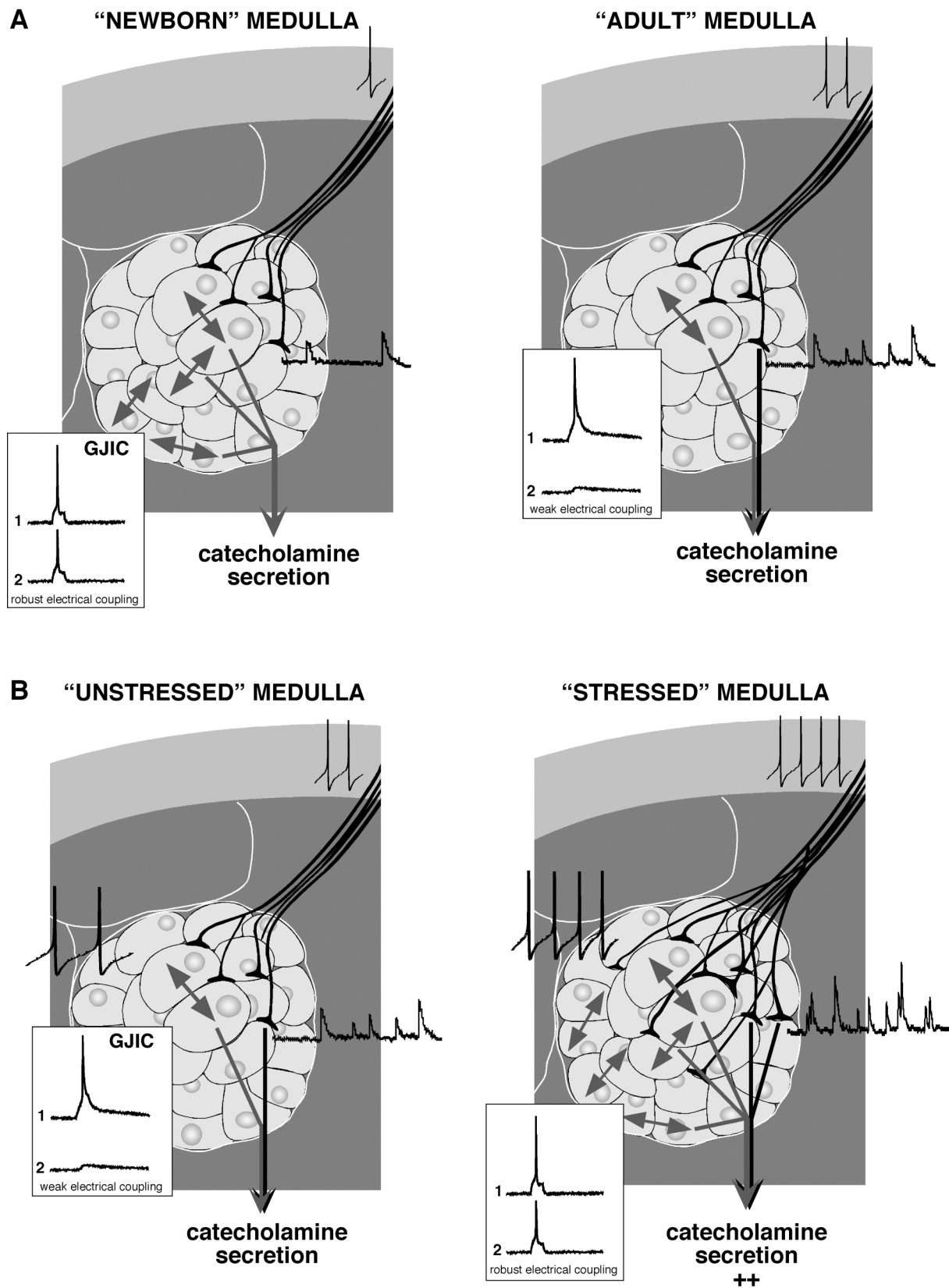


Figure 3

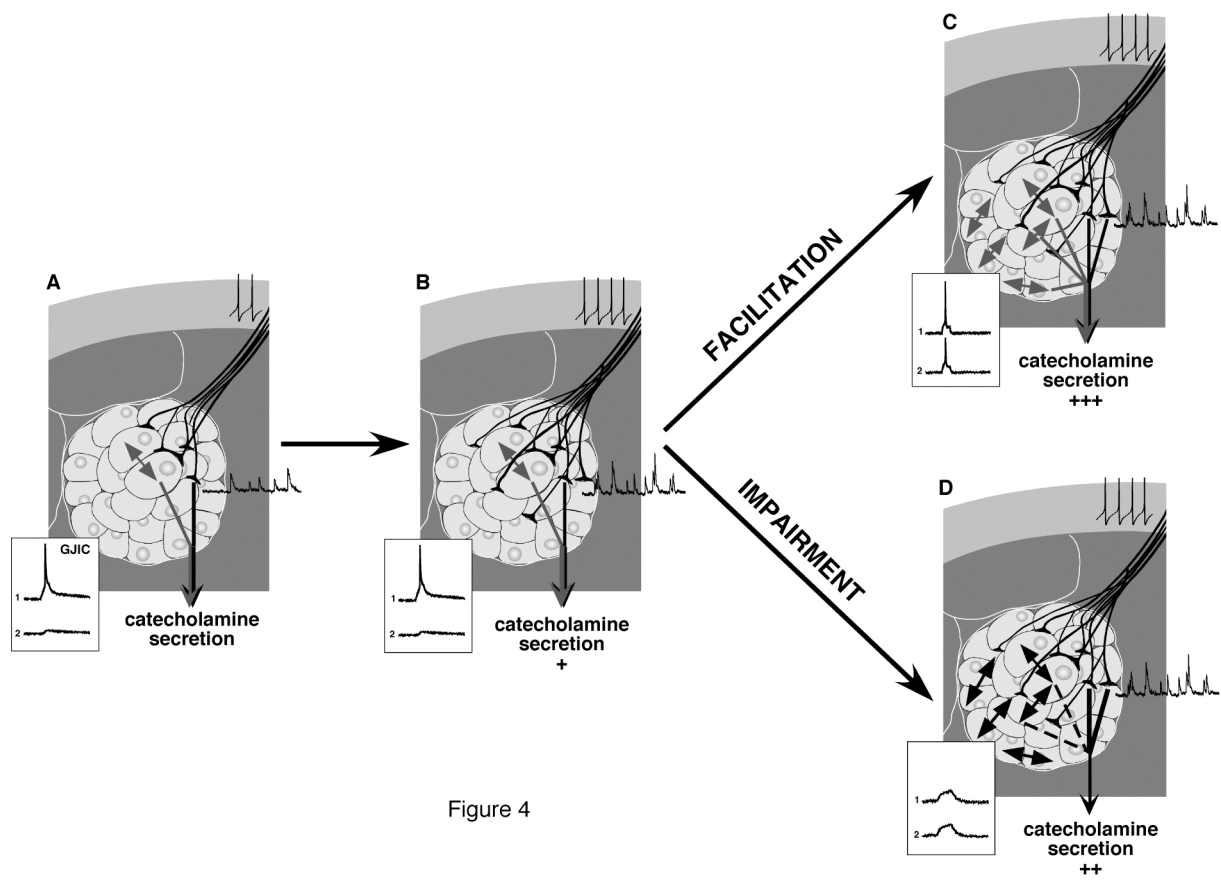


Figure 4