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Gap junction communication between chromaffin cells: the hidden face of adrenal stimulus-secretion coupling

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Abstract

From birth to death, catecholamine secretion undergoes continuous adjustments, allowing the organism to adapt to homeostasis changes. To cope with these stressful conditions, the neuroendocrine cells of the adrenal medulla play an immediate and crucial role. Chromaffin cell-driven catecholamine release is chiefly controlled by a neurogenic command that arises from the sympathetic nervous system, which releases acetylcholine at the splanchnic nerve terminal-chromaffin cell synapses. In addition to receiving several synaptic inputs individually, chromaffin cells are coupled by gap junctions. This raises interesting questions about the usefulness and the role of the gap junctional coupling within the chromaffin tissue, considering that secretory function is efficiently completed by the neurogenic pathway. The findings that gap junctions contribute to catecholamine secretion, both *ex vivo* and *in vivo*, provide some early answers, but their involvement in other cellular functions still remains unexplored. This review summarizes the molecular and physiological evidence that gap junctions can act either as an accelerator or a brake of stimulus-secretion coupling, and discusses this functional plasticity in the context of specific needs in circulating catecholamine levels. It introduces the concept of gap junctions as sympathetic activity sensors and guardians of the functional integrity of the chromaffin tissue.

33 Intimate crosstalk between cell populations crucially contributes to the maintenance of tissue
34 syncytial function within an organ, and more generally body homeostasis. In this context, by
35 promoting the exchange of biological signals between adjacent cells [1], gap junction-
36 mediated intercellular communication plays a key role in many cell functions. Hormone
37 secretion from endocrine/neuroendocrine tissues is one of those functions, and as extensively
38 reviewed in two recent articles [16,27], it is unequivocal that gap junctional signaling between
39 secretory cells contributes to control hormone release. This article focuses on the adrenal
40 chromaffin tissue, which is responsible for the secretion of catecholamines. Rather than being
41 a narrative review on adrenomedullary gap junctions, the article addresses the author's
42 personal opinion on the benefit for chromaffin cells to be both synaptically connected to an
43 external neuronal command and locally coupled by gap junctions.
44 What is the added value for an adrenal chromaffin cell to be coupled by gap junctions
45 to other chromaffin cells? Is this coupling relevant for hormone secretion? If yes, how does
46 gap junctional communication between chromaffin cells interact with the synaptic control of
47 catecholamine secretion? Many questions are raised by the presence of gap junctions in the
48 adrenal medullary tissue, and in chromaffin cells in particular. And finally, do we have
49 answers?
50 As a preamble to this review, the author draws the reader's attention to the paucity of
51 studies dedicated to gap junction-mediated communication in the adrenal medulla, although
52 medullary gap junctions were anatomically identified more than thirty-five years ago [12]!
53
54 **The secretory role of gap junctions between chromaffin cells: for a long time suspected,**
55 **only recently disclosed**
56 Attempting to elucidate the enigmatic role of gap junctions in the adrenal medullary tissue is
57 not a recent issue. In a seminal study describing the presence of ultrastructurally-identified

gap junction plaques in the adrenal medulla of various species [12], the authors stated that "… *Since the triggering of hormonal release is achieved at synaptic junctions, there is no need of coupling between chromaffin cells to allow the exchange of a stimulating agent. It seems likely that gap junctions are involved in another process…".* At the same time, a study performed in rat pancreatic islets, an endocrine tissue sharing multiple features with the adrenal chromaffin tissue, reported an increase in gap junction number between beta-cells in response to stimulation of insulin secretion [28]. This opened a wide research field on the contribution of gap junctional communication between endocrine/neuroendocrine cells to their secretory function.

Do adrenal medullary gap junctions contribute to catecholamine secretion? A simple question in theory, but with more than one answer, thus demonstrating the intricate contribution of gap junctional communication to the adrenal stimulus-secretion coupling. Mechanistically, by favoring the diffusion of instructive signals (ions, second messengers, cell metabolites…) between cells [1], gap junction channels are decisive determinants engaged in cell network function through the collective organization of cell activity, and more generally in the regulation of physiological functions. It was thus rational to envision that adrenal medullary gap junctions could be involved in the regulation of catecholamine secretion.

What evidence, even indirect, do we have? The fraction of gap junction-coupled chromaffin cells, the connexin composition of junctional channels and the robustness of the electrical coupling vary by species, gender, age, cholinergic activity and stress conditions ([5,7] for reviews) (discussed below). Of particular interest is that all these situations are associated with distinct catecholamine needs and demands, arguing for a possible contribution of gap junctions to hormone release. Along the same lines, the cumulative observations that i) rat chromaffin cells are more frequently and more robustly coupled in females [24,8,7], ii)

Cx36 and Cx43 proteins are more expressed in the adrenal medulla of female mice [15], iii) stress responses (increased E plasma levels) are higher in females than in males rats [22,42], and iv) basal catecholamine amounts assayed in adrenal venous blood are enhanced upon gap junction blockade [10] indirectly plead for a possible role of adrenal gap junctions in catecholamine release.

The hypothesis that gap junctions might be conduits allowing propagation of electrical signals between chromaffin cells has been early proposed [4,17]. Many years later, the experimental demonstration came from recording of either electrical "echoes", which correspond to action potentials in neighbouring cells evoked by the depolarization of the patch-clamped cell [30,15] or junctional currents in dual-patched chromaffin cell pairs [24]. Whether this electrical communication between chromaffin cells can support catecholamine release was initially disclosed *ex vivo* by records of exocytotic currents in cells neighboring a stimulated cell in rat acute adrenal slices [24], and more recently *in vivo* in response to a high frequency splanchnic nerve stimulation in anaesthetized mice [10]. Nevertheless, in another study performed in mouse adrenal slices, catecholamine secretion evoked by a transmural electrical stimulation of the chromaffin tissue was reported to be independent on gap junctional pathway [32]. At first glance, this could appear as an inconsistency, but further examination of the data reveals a subtle and complex involvement of gap junction in the hormone secretion. Whether or not gap junctions contribute to the secretory process seems largely dictated by the degree of sympathetic activation of the adrenal medullary tissue, although this is difficult to compare between studies. Upon modest activation of chromaffin cells (mimicking the basal sympathetic tone), gap junction coupling does not significantly contribute to hormone release [32,10]. Conversely, in response to a robust stimulation of the chromaffin tissue (mimicking an enhanced sympathetic tone as, for example, found under stress conditions), the expression of medullary gap junctions is upregulated [6,8,10] and the

biological signals exchanged between coupled cells unequivocally participate in catecholamine release [10]. Providing further support for this is the demonstration that pituitary adenylate cyclase-activating polypeptide (PACAP), a splanchnic-derived peptide transmitter that is mainly released during elevated nerve firing [36], enhances gap junction-mediated electrical coupling in the mouse adrenal medulla [15]. Together, these studies unveil the sympathetic activity-dependent contribution of adrenal medullary gap junctional communication in catecholamine secretion. This later point may be decisive in decoding the functional role of gap junctions in the adrenal medullary tissue, and it deserves to be kept in mind not only when reading this review but more generally when engaging experiments on the role of gap junctions in hormone release from neuroendocrine tissues.

Adrenal medullary gap junctions: a highly and finely regulated cell-cell communication route devoted to continuously adjusting catecholamine secretion to body needs

Deciphering the molecular, cellular and tissue mechanisms regulating hormone secretion is still a topical challenge for scientists engaged in the field of endocrinology/neuroendocrinology. Regarding the adrenal medullary tissue, it has been long reported that catecholamine release primarily relies on synaptic neurotransmission between splanchnic nerve endings and chromaffin cells [39]. Since functional gap junctions were described between secretory cells [24], the situation became more complicated and it is no longer tenable to investigate the mechanisms of hormone release without considering the intricate interactions between chemical and electrical communication.

1- Prevalent gap junctional communication between chromaffin cells in neonates: cell-cell coupling for survival

In adrenal medulla, the prevalence of gap junctional communication between chromaffin cells during the perinatal period is remarkable from a physiological point of view. To cope with hypoxic stress triggered during parturition, the newborn organism secretes massive amounts of epinephrine (E) and norepinephrine (NE). Because the neurogenic control of the adrenal catecholamine secretion is not competent at birth, at least in the rat and mouse [35,18], the prevalent gap junction coupling observed in rodent neonates [25,23], by supporting the propagation of signaling molecules between the secretory cell network, likely sustains the hormone secretion that is essential to survival (Figure 1).

2- Gradual reduction of gap junctional communication between chromaffin cells in postnatal animals: a crucial mechanism for acquiring the neurogenic control of catecholamine secretion

After experiencing a secretory role in neonates, gap junctions between chromaffin cells decline in parallel with the development of neuronal synaptic connections during the first postnatal weeks (Figure 1). In this context, we identified the proteoglycan agrin as a primary factor promoting the switch from electrical to chemical synapses [23]. Indicating how helpful, if not indispensable the contribution of gap junctions is to the regulation of catecholamine secretion in early life, this study shows that, in the adrenal medullary tissue, like in the central nervous system [20], electrical (gap junction-mediated) and chemical (synapse-mediated) communication pathways are functionally interconnected and exhibit coordinated plasticity mechanisms. This mutual and reciprocal interaction actively contributes to set the respective tone of the neurogenic and non-neurogenic control of chromaffin cell function. This crosstalk is of a particular relevance not only in young animals for the establishment of the neurogenic control of catecholamine secretion, but also, as discussed below, in adults in response to physiological (acute stress) or pathological (chronic stress and related diseases) episodes.

3- A deficient neurogenic control of catecholamine secretion.... and gap junctions re-emerge!

The crosstalk between gap junctional coupling and synaptic communication is not restricted to the perinatal period. Conditions leading to an acute or a chronic decrease in the cholinergic neurotransmission between splanchnic nerve terminals and chromaffin cells favor the re-expression of functional gap junctions between the secretory cells (Figure 2). This was reported in response to both a pharmacological blockade of postsynaptic nicotinic receptors [25,9] or a surgical splanchnectomy [25]. Interestingly and illustrating the guardian role of chromaffin cell gap junctions on adrenal medulla function is the fact that the functional gap junction signaling takes place less that 30 min after nAChR inhibition. At present, the underlying mechanism is not fully elucidated but connexin/connexon trafficking seems to be involved [25]. The fast kinetic of the re-appearance of functional gap junctions indicates that, in conditions of normal synaptic transmission, the junctional communication between chromaffin cells is silenced rather than suppressed.

4- Tonic brake of the cholinergic system on gap junctional coupling under low sympathetic tone: a mechanism contributing to the maintenance of basal catecholamine secretion

In vivo, basal catecholamine release is associated with low frequency splanchnic nerve discharges. The blockade of adrenal gap junctional communication by the uncoupling agent carbenoxolone (CBX) does not impact the amounts of E and NE released in adrenal venous blood in response to low frequency splanchnic nerve stimulation in anaesthetized mice [10]. This suggests the existence of inhibitory mechanisms that silence gap junction channel function, an issue largely overlooked. Our group addressed it a decade ago in rat acute adrenal slices, and to the best of our knowledge, no new findings have contributed to this research field to date. As mentioned in the previous chapter, the gap junctional communication between chromaffin cells is up-regulated (increased number of dye- or electrically-coupled

chromaffin cells) in response to the acute pharmacological blockade of post-synaptic nicotinic acetylcholine receptors [25,9] or in a denervated gland [25] (figure 2). This led us to identify the cholinergic system, via sympathetic tone, as one of the determinants operating a tonic brake over chromaffin cell gap junctions, as reported at the same time for the glutamatergic system over the spinal motor neuron gap junctions [29]. What could be the physiological relevance of such an inhibitory control? The establishment of a low catecholamine secretory rate under basal condition is crucial to maintain body homeostasis. Therefore, dampening the gap junctional communication between secretory cells would finely tune basal catecholamine secretion by reducing chromaffin tissue excitation, thus contributing to the low secretion rate associated with the 'rest and digest" state of energy storage. Reflecting how intricate is the contribution of gap junctions to hormone secretion is blockade of gap junctional communication (either pharmacologically or genetically by silencing connexin-encoding genes) tends to enhance basal catecholamine release [10], as also observed for insulin release from ß-cell in pancreatic islets [3,33].

5- Up-regulation of gap junctional communication between chromaffin cells: a functional remodeling to boost catecholamine secretion in response to stress

In its early phase, the stress response consists of a heightened sympathetic tone and increased catecholamine secretion. Do gap junctions between chromaffin cells contribute to this, and if so, how? The few studies available to date were performed both *ex vivo* in acute adrenal slice [6,8,15] and *in vivo* in anaesthetized animals [10], and they unequivocally converge toward an increased gap junctional communication between secretory cells (Figure 1). The underlying mechanisms still remain largely unexplored, and the facts that i) acetylcholine (ACh) released at the splanchnic nerve-chromaffin cell synapse can bind both nicotinic and muscarinic receptors and ii) non-cholinergic neurotransmitters are co-released with ACh upon robust

splanchnic nerve discharges [40] complicate the situation. The current view on the respective roles of nAChRs and mAChRs states that the acute activation of nAChRs, as occurs during episodes of stress, co-operates with gap junctional signaling to promote the spread of excitation within the chromaffin tissue. Concerning mAChRs, the muscarinic regulation of chromaffin cell gap junctions has been proposed, with the hypothesis that muscarine could increase gap junction coupling between chromaffin cells [31]. As discussed in [14], mAChRs, by their extrasynaptic distribution, require intense and/or prolonged synaptic activity (as observed during stress episodes) to be activated, and thus may act in concert with other neurotransmitters released upon sustained sympathetic discharges to modulate gap junctional communication between secretory cells and to enhance catecholamine secretion. Among the non-cholinergic neurotransmitters co-secreted with ACh, PACAP is undoubtedly one of the most relevant factors from a physiological point of view [36,37]. Beyond its secretagogue action on catecholamine secretion [41,13,21], PACAP enhances gap junction-mediated electrical coupling and the subsequent spread of electrical excitation between mouse chromaffin cells [15].

Mechanistically, the stress condition correlates with up-regulation of Cx36 and Cx43 expression [8,15], the two main gap junction proteins expressed in the rat adrenal medulla [24], and perhaps more importantly with the appearance of a robust and extended electrical coupling between coupled cells [8]. Does this enhanced coupling impact hormone secretion? The answer is yes, but with some distinctions. Indeed, the *in vivo* gap junction blockade in unstressed animals reduces only NE secretion evoked by high frequency splanchnic nerve stimulation, whereas the same experiments conducted in stressed animals lessens both E and NE release [10]. Nevertheless and even though the remodeling of gap junction signaling significantly contributes to boost catecholamine secretion upon stress conditions, it is likely that additional mechanisms also contribute to this rise in hormone secretion.

6- Down-regulation of gap junctional communication between chromaffin cells: an adaptive mechanism to protect body homeostasis against the damaging effects of chronic catecholamine elevation

Beyond the beneficial effect of catecholamine secretion elicited in response to acute stress episodes, sustained and/or repetitive catecholamine rises (in response to chronic stressful situations for example) can have deleterious outcomes [38] and are well-known risk factors for chronic diseases. This is particularly the case for arterial hypertension, as unveiled by the elevated blood pressure observed in response to chronic infusion of E in rat [19,34] or in chronically cold stressed rats [11]. Because gap junctions between chromaffin cells contribute to catecholamine secretion in physiological conditions, it is likely that they are also involved in pathological circumstances. But again, this research field is largely unexplored.

Consistent with the vision of a multifaceted intercellular communication route, we recently provide evidence that a chronic hypertensive environment remodels the gap junctional coupling between chromaffin cells. In the spontaneously hypertensive rat (SHR) used as a model of chronic elevated blood pressure, the number of coupled chromaffin cells is reduced (Figure 2). What could be the relevance of such a decrease in gap junctional coupling? A heightened sympathetic tone is a hallmark of chronic arterial hypertension. The resulting phasic activation of the splanchnic nerve endings increases the cholinergic stimulation weight onto chromaffin cells, with potentially damaging consequences. We therefore propose that the dampening of the gap junctional communication between secretory cells would be an adaptive mechanism devoted to the protection of adrenal medullary tissue, and body homeostasis, against the deleterious effects of redundant elevated catecholamine secretion and associated blood pressure elevation. Although experienced in a different context, our observation that the gap junction coupling probability between chromaffin cells decreases in response to a pharmacologically enhanced cholinergic activity [7] reinforces our

257 hypothesis. Interestingly, it is of note that in insulin-secreting pancreatic tissue, Cx36
258 expression between beta-cells decreases in response to chronic glucose elevation [2].
259 Together, we propose that, more generally, under pathological conditions associated with
260 redundant elevated hormone levels (arterial hypertension, hyperinsulinemia associated with
261 type 2 diabetes, with obesity…), gap junctions between endocrine/neuroendocrine cells
262 contribute to damper intercellular communication, aiming at protecting body homeostasis and
263 helping the organism to cope with a chronic deleterious environment. Beyond secretory
264 function, this working hypothesis might be extended to other cell functions (contraction,
265 neural signal propagation…).
266
267 **Adrenal medullary gap junctions: other functions and open questions**
268 As stated in the introduction, very few studies have been focused on gap junctions between
269 chromaffin cells, and therefore there is to date more unanswered than answered questions.
270 Rather than providing an exhaustive list of open questions, I chose to emphasize some
271 stimulating issues relevant for people engaged in the study of gap junctions in
272 endocrine/neuroendocrine tissues.
273 A first set of queries relates to gap junction/connexin expression. Do gap junctions
274 differently couple E- and NE-secreting chromaffin cells? And if so, how? Given that i) the
275 gap junction blocker carbenoxolone distinctively affects E and NE secretion, both under basal
276 and stimulated conditions [10], ii) gap junctional coupling is distinctly regulated by nicotinic
277 and muscarinic agonists and iii) mAChRs are more concentrated in NE cells than in E cells
278 [43], unraveling this issue will certainly contribute to the better understanding of the
279 individual role of gap junctions in E and NE secretion. Elucidating the respective role of
280 Cx36 and Cx43, two connexins expressed in the chromaffin tissue, is also a challenging issue.
281 Bearing in mind that when engaged in a cell-cell communication pathway, the gap junction

channels display distinct biophysical properties in terms of voltage dependency, unitary conductance, charge selectivity and regulatory mechanisms, it is likely that chromaffin cell clusters coupled by Cx36 or Cx43 will not have the same ability to modulate the secretory process.

A second set of still unsolved questions deals with the involvement of connexin in gap junction channel- and hemichannel-independent functions, a rapidly developing topic in the gap junction research field. As postulated by Grynszpan-Wynograd and Nicolas in a paper published in 1980 [12], "*it seems likely that gap junctions are involved in another process, e.g. recycling of the plasma membrane after exocytosis, or synthesis procedure of catecholamines or chromogranins…*". In light of recent studies, connexins via channel-independent mechanisms can contribute to cell motility and migration by regulating cell-cell adhesion, interactions with the cytoskeleton, and activation of intracellular signaling pathways [26,44]. This stimulating topic at the border between Cell Biology and Neurophysiology remains to be addressed in the adrenal medulla.

Concluding remarks: Chromaffin cell gap junctional coupling is an intra-adrenal guardian devoted to finely tune catecholamine secretion and to secure body homeostasis, but not exclusively….

Although their role has long been remained elusive, it is now indisputable that gap junctions contribute to secretory output, the primary function of the adrenal medulla. In this context, gap junctions between adrenal chromaffin cells underlie a remarkable degree of plasticity, as they continuously remodel to adjust the number of coupled cells, and the strength of the intercellular coupling, to the physiological/pathological context. The continuous and reciprocal dialogue between sympathetic synaptic activity and gap junction-mediated

307 intercellular communication guarantees maintenance of chromaffin tissue syncytial function.
308 Notably, such interplay allows catecholamine secretion to be finely tuned to body needs.
309 However, and even if gap junctions between chromaffin cells appear to be primarily involved
310 in secretory function, the intercellular coupling does not necessarily relate to the secretory
311 function. This issue opens up a very exciting research field, with wide-ranging implications
312 for endocrine/neuroendocrine function.
313
314
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458
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461
462

463 **Figure legends**

464 **Figure 1: Physiological remodeling of gap junctional signaling between chromaffin cells**

465 During adrenal postnatal development (left panel), the gap junctional coupling that is
466 prevalent at birth gradually decreases, as the sympathetic tone matures. This is associated
467 with a reduction in both the number of coupled chromaffin cells (from >50% to 25%) and the
468 strength of the electrical coupling, thus contributing to the maintenance of basal
469 catecholamine secretion. By contrast, in response to a stress episode (right panel), when a
470 huge catecholamine release is needed, the gap junctional signaling between chromaffin cells
471 is upregulated, leading to increased coupling strength and coupled cell number (from 25% to
472 80%). Data collected from [24,25,23,8,5,7,15,10].

473

474 **Figure 2: Remodeling of gap junctional signaling in response to pathological conditions**
475 **associated with sustained changes in the sympathetic tone**

476 When sympathetic tone is impaired, as experienced by a splanchnectomy or the
477 pharmacological blockade of nAChRs (left panel), the gap junctional communication between
478 chromaffin cells increases. Conversely, upon a chronically heightened sympathetic tone, as
479 observed in hypertensive animals or mimicked by a sustained cholinergic stimulation (right
480 panel), the gap junctional coupling between chromaffin cells downregulates. Data collected
481 from [25,7] and unpublished results.

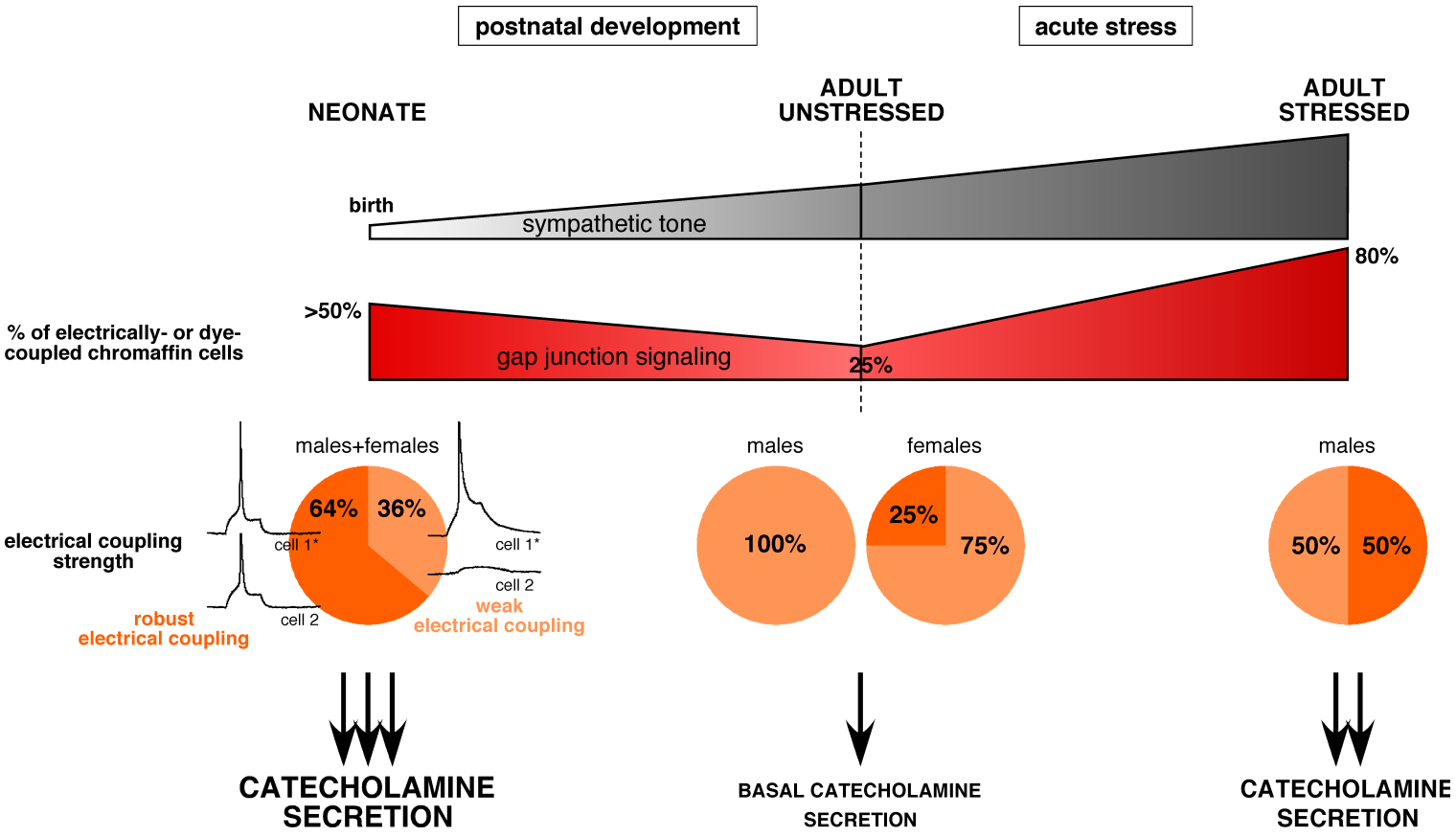
postnatal development
acute stress
NEONATE
ADULT UNSTRESSED
ADULT STRESSED
birth
sympathetic tone
80%
>50%
% of electrically- or dye-coupled chromaffin cells
gap junction signaling
25%
males+females
males
females
males
electrical coupling strength
64%
36%
cell 1*
cell 2
cell 1*
cell 2
robust electrical coupling
weak electrical coupling
100%
25%
75%
50%
50%
CATECHOLAMINE SECRETION
BASAL CATECHOLAMINE SECRETION
CATECHOLAMINE SECRETION

FIGURE 1

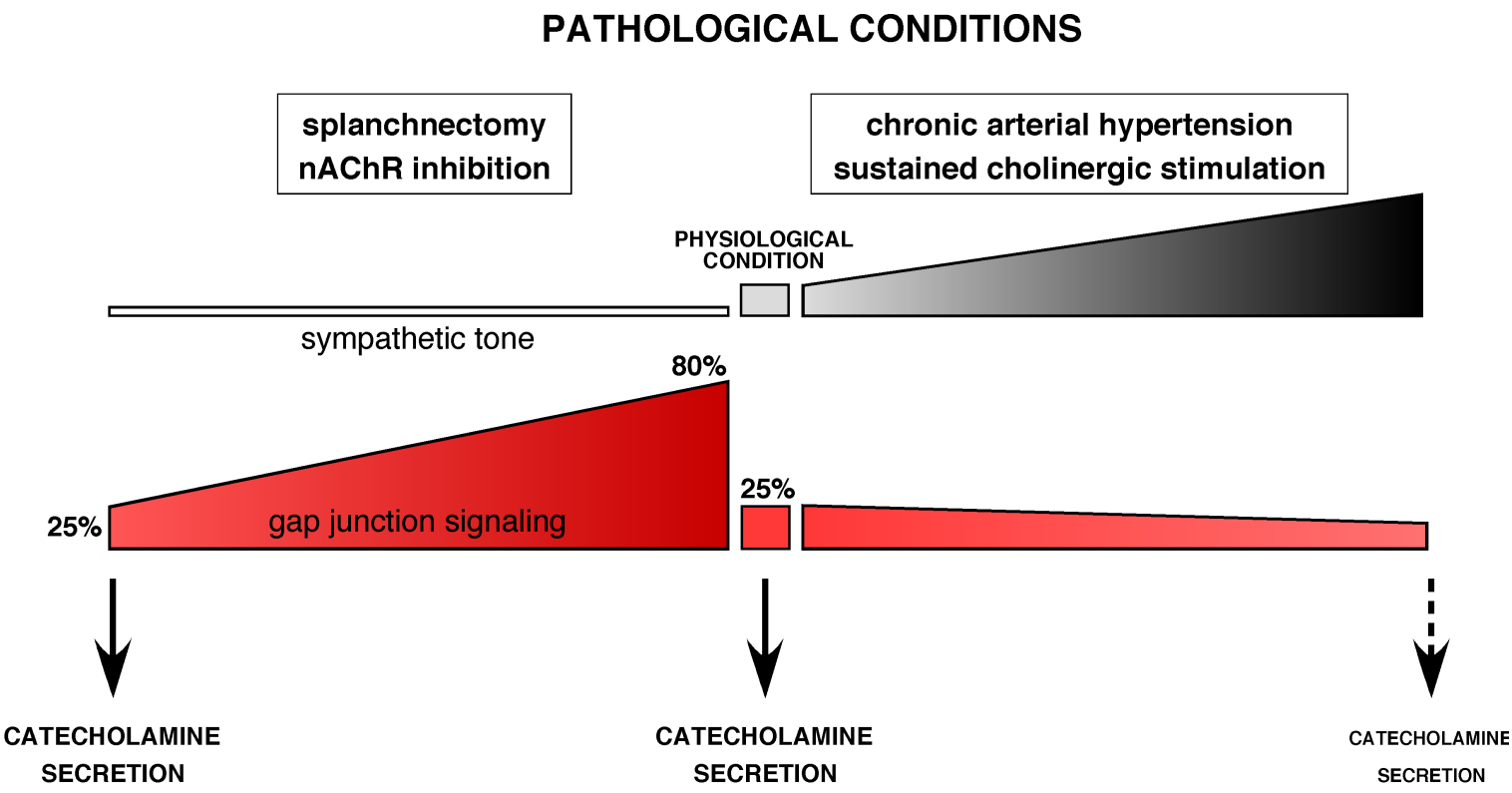
PATHOLOGICAL CONDITIONS
splanchnectomy
nAChR inhibition
chronic arterial hypertension
sustained cholinergic stimulation
PHYSIOLOGICAL CONDITION
sympathetic tone
80%
25%
gap junction signaling
25%
CATECHOLAMINE SECRETION
CATECHOLAMINE SECRETION
CATECHOLAMINE SECRETION

FIGURE 2