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

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16 **Abstract**

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

33

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

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

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

76 What evidence, even indirect, do we have? The fraction of gap junction-coupled  
77 chromaffin cells, the connexin composition of junctional channels and the robustness of the  
78 electrical coupling vary by species, gender, age, cholinergic activity and stress conditions  
79 ([5,7] for reviews) (discussed below). Of particular interest is that all these situations are  
80 associated with distinct catecholamine needs and demands, arguing for a possible contribution  
81 of gap junctions to hormone release. Along the same lines, the cumulative observations that i)  
82 rat chromaffin cells are more frequently and more robustly coupled in females [24,8,7], ii)

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

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

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

118

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

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

129

130 *1- Prevalent gap junctional communication between chromaffin cells in neonates: cell-cell*  
131 *coupling for survival*

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

140

141 *2- Gradual reduction of gap junctional communication between chromaffin cells in postnatal*  
142 *animals: a crucial mechanism for acquiring the neurogenic control of catecholamine*  
143 *secretion*

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



157 *3- A deficient neurogenic control of catecholamine secretion.... and gap junctions re-emerge!*  
158 The crosstalk between gap junctional coupling and synaptic communication is not restricted  
159 to the perinatal period. Conditions leading to an acute or a chronic decrease in the cholinergic  
160 neurotransmission between splanchnic nerve terminals and chromaffin cells favor the re-  
161 expression of functional gap junctions between the secretory cells (Figure 2). This was  
162 reported in response to both a pharmacological blockade of postsynaptic nicotinic receptors  
163 [25,9] or a surgical splanchnectomy [25]. Interestingly and illustrating the guardian role of  
164 chromaffin cell gap junctions on adrenal medulla function is the fact that the functional gap  
165 junction signaling takes place less than 30 min after nAChR inhibition. At present, the  
166 underlying mechanism is not fully elucidated but connexin/connexon trafficking seems to be  
167 involved [25]. The fast kinetic of the re-appearance of functional gap junctions indicates that,  
168 in conditions of normal synaptic transmission, the junctional communication between  
169 chromaffin cells is silenced rather than suppressed.

170

171 *4- Tonic brake of the cholinergic system on gap junctional coupling under low sympathetic*  
172 *tone: a mechanism contributing to the maintenance of basal catecholamine secretion*

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

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

196

#### 197 *5- Up-regulation of gap junctional communication between chromaffin cells: a functional* 198 *remodeling to boost catecholamine secretion in response to stress*

199 In its early phase, the stress response consists of a heightened sympathetic tone and increased  
200 catecholamine secretion. Do gap junctions between chromaffin cells contribute to this, and if  
201 so, how? The few studies available to date were performed both *ex vivo* in acute adrenal slice  
202 [6,8,15] and *in vivo* in anaesthetized animals [10], and they unequivocally converge toward an  
203 increased gap junctional communication between secretory cells (Figure 1). The underlying  
204 mechanisms still remain largely unexplored, and the facts that i) acetylcholine (ACh) released  
205 at the splanchnic nerve-chromaffin cell synapse can bind both nicotinic and muscarinic  
206 receptors and ii) non-cholinergic neurotransmitters are co-released with ACh upon robust

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

222         Mechanistically, the stress condition correlates with up-regulation of Cx36 and Cx43  
223 expression [8,15], the two main gap junction proteins expressed in the rat adrenal medulla  
224 [24], and perhaps more importantly with the appearance of a robust and extended electrical  
225 coupling between coupled cells [8]. Does this enhanced coupling impact hormone secretion?  
226 The answer is yes, but with some distinctions. Indeed, the *in vivo* gap junction blockade in  
227 unstressed animals reduces only NE secretion evoked by high frequency splanchnic nerve  
228 stimulation, whereas the same experiments conducted in stressed animals lessens both E and  
229 NE release [10]. Nevertheless and even though the remodeling of gap junction signaling  
230 significantly contributes to boost catecholamine secretion upon stress conditions, it is likely  
231 that additional mechanisms also contribute to this rise in hormone secretion.

232 *6- Down-regulation of gap junctional communication between chromaffin cells: an adaptive*  
233 *mechanism to protect body homeostasis against the damaging effects of chronic*  
234 *catecholamine elevation*

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

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

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

286

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

297

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

301 Although their role has long been remained elusive, it is now indisputable that gap junctions  
302 contribute to secretory output, the primary function of the adrenal medulla. In this context,  
303 gap junctions between adrenal chromaffin cells underlie a remarkable degree of plasticity, as  
304 they continuously remodel to adjust the number of coupled cells, and the strength of the  
305 intercellular coupling, to the physiological/pathological context. The continuous and  
306 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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457

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.

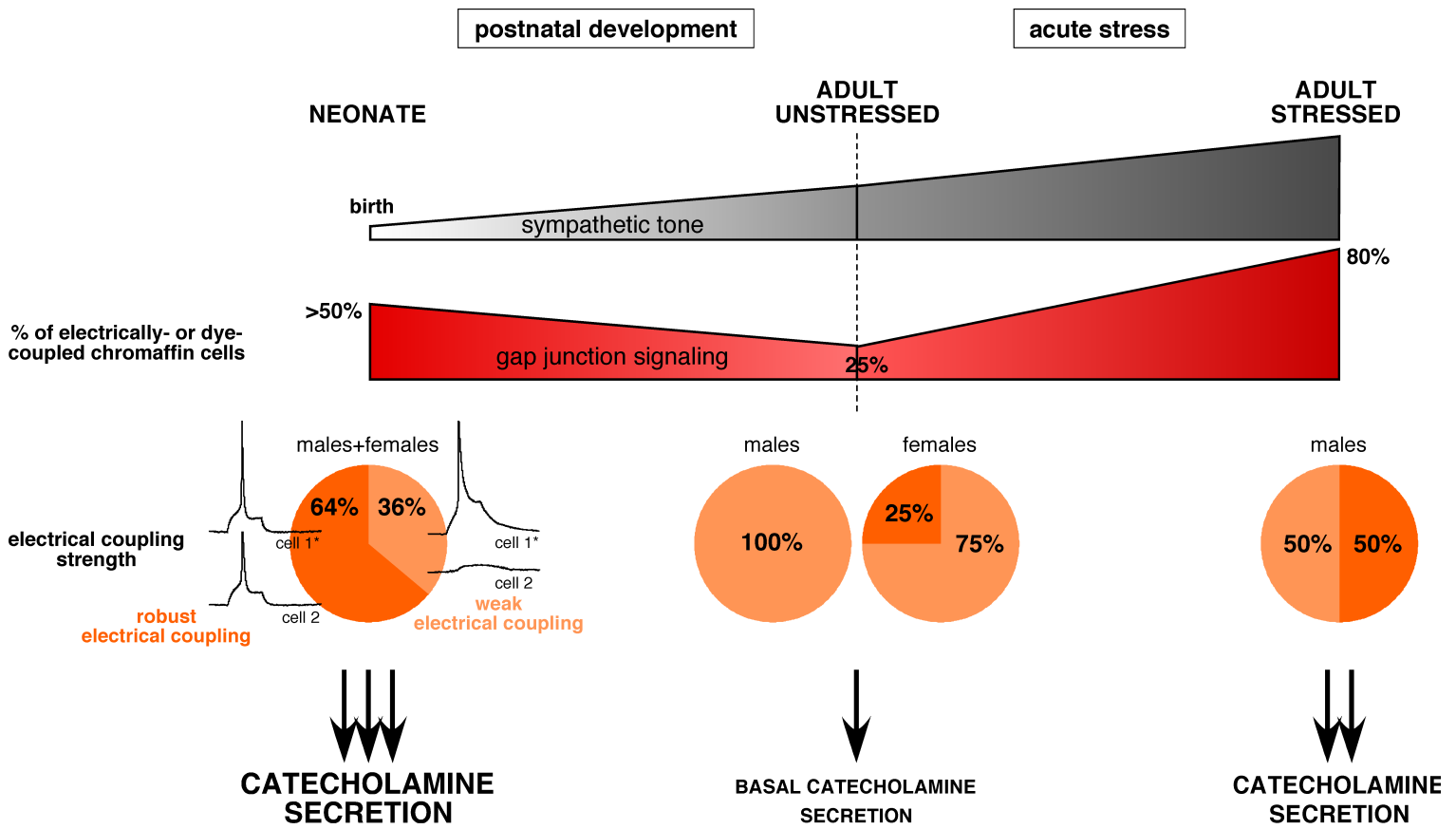


FIGURE 1

# PATHOLOGICAL CONDITIONS

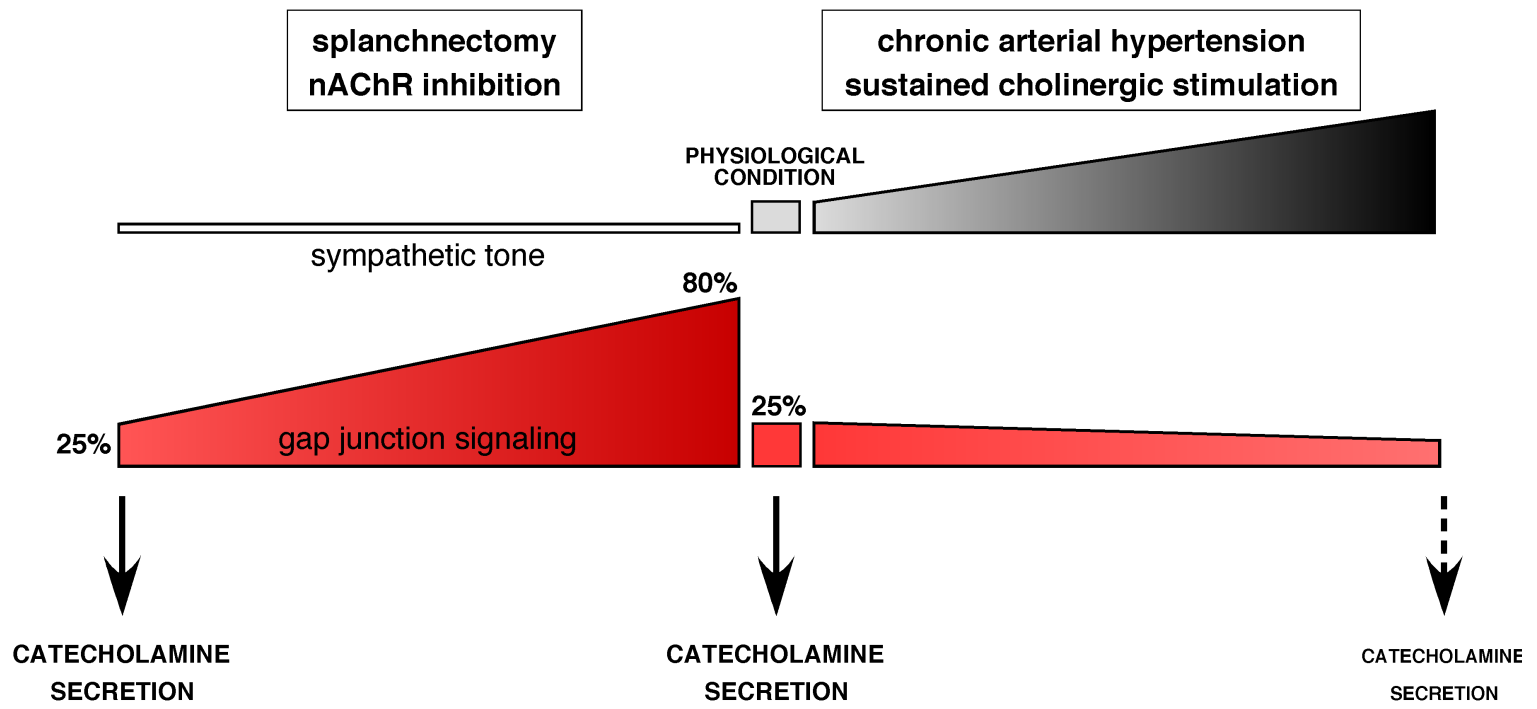


FIGURE 2