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1 **Adaptive remodeling of rat adrenomedullary stimulus-secretion coupling in response to**
2 **a chronic hypertensive environment**

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23 coupling; acute adrenal slices; catecholamine assays; hypertensive rats; voltage-gated ion
24 channels, nicotinic receptors

25

26 **Summary**

27 Chronic elevated blood pressure impinges on the functioning of multiple organs and therefore
28 harms body homeostasis. Elucidating the protective mechanisms whereby the organism copes
29 with sustained or repetitive blood pressure rises is therefore a topical challenge. Here we
30 address this issue in the adrenal medulla, the master neuroendocrine tissue involved in the
31 secretion of catecholamines, influential hormones in blood pressure regulation. Using acute
32 adrenal slices from spontaneously hypertensive rats, we show that chromaffin cell stimulus-
33 secretion coupling is remodeled, resulting in a less efficient secretory function primarily upon
34 sustained electrical or cholinergic challenges. The remodeling is supported by revamped
35 cellular and tissular mechanisms, including chromaffin cell excitability through voltage-gated
36 ion channel expression changes, gap junctional communication and cholinergic synaptic
37 transmission. As such, by weakening its competence to release catecholamines, the
38 ‘hypertensive medulla’ has elaborated an adaptive shielding mechanism against damaging
39 effects of redundant elevated catecholamine secretion and associated blood pressure.

40

41 **Introduction**

42 A rise in circulating catecholamine (CA) levels is a crucial step triggered by the organism to
43 cope with a stressful situation. By releasing both epinephrine (E) and norepinephrine (NE), the
44 adrenal medullary tissue crucially contributes to this response. Beyond the beneficial effect of
45 CA secretion elicited by an acute stress, sustained and/or repetitive CA secretion episodes (in
46 response to chronic stressful situations for example) can have deleterious outcomes ¹, as
47 unveiled by the elevated blood pressure observed in response to chronic infusion of E in rat ^{2,3}
48 or in chronically cold stressed rats ⁴. It has long been reported that increased blood pressure is
49 associated with a sympathetic nervous system hyperactivity leading to a raised neural tone ^{5,6}
50 and increased plasma E and NE ⁷. The adrenal medulla being the unique source of circulating
51 E, this assigns the secretory function of the medulla and more generally the sympatho-adrenal
52 axis as critical determinants of arterial hypertension pathogenesis ⁸⁻¹⁰. Reciprocally, and
53 despite it is indisputable that the adrenal medulla primarily contributes to enhance blood
54 pressure, whether and how a 'hypertensive' environment impacts the adrenal secretory function
55 itself remains elusive. We address this issue in the adult spontaneously hypertensive rat, in
56 which the circulating E levels gradually increase as arterial hypertension develops and then
57 stabilize in adult animals to become comparable to those in normotensive rats ¹¹. This suggests
58 that the adrenal gland develops shielding mechanisms dedicated to normalize blood CA
59 amounts, and to date nothing is known on the adaptive mechanisms elaborated by the
60 neuroendocrine chromaffin cells to adjust the release of CA.

61 Adrenal CA secretion and more generally the adrenomedullary tissue function are
62 controlled by the coordination of interconnected and complex pathways ¹². The initial incoming
63 command comes from the sympathetic nervous system that releases mainly acetylcholine
64 (ACh) but also neuropeptides (PACAP, VIP...) at splanchnic nerve terminals synapsing onto
65 chromaffin cells ^{13,14}. The resulting ACh-evoked chromaffin cell depolarization and subsequent

66 cytosolic Ca²⁺ rise are key processes for CA exocytosis. In addition and unraveled from studies
67 in both acute adrenal slices and *in vivo* in anaesthetized rodents, the local communication
68 mediated by gap junctions between chromaffin cells represents a functional route by which
69 biological signals propagate between adjacent cells and subsequently contribute to CA release
70 ¹⁵⁻²⁰.

71 To elucidate the feedback mechanisms elaborated by the adrenal medullary tissue to
72 manage a chronic sustained elevated blood pressure, we investigated chromaffin cell stimulus-
73 secretion coupling in acute adrenal slices of adult spontaneously hypertensive rats (SHRs) and
74 their parent age-matched normotensive Wistar Kyoto (WKY) rats. By driving numerous
75 cellular/tissular processes, chromaffin cell excitability is a major player in stimulus-secretion
76 coupling. It relies on intricate mechanisms, not only supported by ion channels expressed at the
77 plasma membrane, but also by the crosstalk between cholinergic and peptidergic innervation ²¹
78 and the gap junctional electrical coupling between chromaffin cells ^{17,19,20}. We identified relevant
79 modifications in chromaffin cell excitability through changes in voltage-gated ion channel
80 expression, cholinergic synaptic neurotransmission and gap junctional communication, that
81 argue for a less efficient stimulus-secretion coupling in hypertensive animals, markedly
82 observed upon robust challenges. We propose that this functional plasticity reflects an adaptive
83 shielding mechanism, avoiding the detrimental effects of sustained or repetitive huge CA
84 secretion episodes. More generally, this study describes novel adaptive mechanisms that take
85 place in the medullary tissue and how they act in a coordinated manner to damper CA release.

86

87 **Results**

88 **Impaired CA release evoked by high ACh concentration in hypertensive rats**

89 The competence of WKY and SHR chromaffin cells to secrete CA was investigated by assaying
90 basal and ACh-evoked epinephrine (E) and norepinephrine (NE) released from acute slices

91 (Figures 1A, 1B and 1C). We took advantage of making slices from the two adrenals to estimate
92 the medulla volume of the right and left glands for both SHRs and WKY rats, as previously
93 reported²². For each rat, whether WKY or SHR, no difference was observed between the right
94 and the left glands ($0.85 \pm 0.08 \text{ mm}^3$, $n = 4$ and $0.95 \pm 0.15 \text{ mm}^3$, $n = 4$ for the right and left
95 WKY glands, respectively, $p = 0.125$, and $1.17 \pm 0.33 \text{ mm}^3$, $n = 4$ and $1.25 \pm 0.32 \text{ mm}^3$, $n = 4$
96 for the right and left SHR glands, respectively, $p = 0.125$, Wilcoxon matched-pairs signed-rank
97 test, Figure S1D). However, medullary tissue volume was significantly greater in SHRs
98 ($1.21 \pm 0.30 \text{ mm}^3$, $n = 8$ glands for SHRs *versus* $0.90 \pm 0.12 \text{ mm}^3$, $n = 8$ glands for WKY rats,
99 $p = 0.0351$, Mann-Whitney test, Figure S1E). To make appropriate comparisons between SHRs
100 and WKY rats, CA secretion was normalized per mm^3 medulla, as previously described²².
101 Under basal conditions, SHR chromaffin cells secreted 8.6-fold less NE than WKY cells ($0.7 \pm$
102 0.7 pmol/mm^3 , $n = 11$ slices for SHRs *versus* $5.9 \pm 7.4 \text{ pmol/mm}^3$, $n = 10$ slices for WKY rats,
103 $p = 0.0003$, Mann-Whitney test, Figure 1A, right histogram). No change was observed for
104 released E amounts ($40.3 \pm 22.6 \text{ pmol/mm}^3$, $n = 11$ slices for SHRs *versus*
105 $40.0 \pm 18.3 \text{ pmol/mm}^3$, $n = 9$ slices for WKY rats, $p = 0.766$, Mann-Whitney test, Figure 1A,
106 left histogram). Likewise, a significant decrease in NE release in SHRs was observed in
107 response to ACh stimulations, both at low ($1 \text{ }\mu\text{M}$) and high ($10\text{-}100 \text{ }\mu\text{M}$) concentrations (Figure
108 1B, $p = 0.0018$, 0.0015 and 0.0002 for ACh 1 , 10 and $100 \text{ }\mu\text{M}$, respectively, Mann-Whitney
109 test). The E release amount is also significantly decreased, but only at $100 \text{ }\mu\text{M}$ ACh ($p = 0.2945$,
110 0.5269 and 0.0147 for ACh 1 , 10 and $100 \text{ }\mu\text{M}$, respectively, Mann-Whitney test). To go further,
111 we investigated the ability of chromaffin cells to be stimulated by ACh. Irrespective to ACh
112 concentrations ($1\text{-}100 \text{ }\mu\text{M}$), the stimulation ratio was similar between SHRs and WKY rats,
113 even at high ACh concentration (Figure 1C, for E: $x5.9 \pm 3.5$ ($n = 15$ slices) and $x4.0 \pm 1.5$
114 ($n = 11$ slices) for WKY rats and SHRs, respectively, $p = 0.1447$, Mann-Whitney test and for
115 NE: $x4.9 \pm 2.9$ ($n = 15$ slices) and $x5.3 \pm 2.1$ ($n = 12$ slices) for WKY rats and SHRs,

116 respectively, $p = 0.3406$, Mann-Whitney test). Strengthening a deficiency in ACh-induced E
117 secretion in SHRs, the E:NE ratio remained constant upon challenging SHR chromaffin cells
118 with increasing ACh concentrations ($p = 0.7061$, $n = 4$ rats, Kruskal-Wallis test), while it
119 gradually increased in WKY rats, as expected (Figure 1D). Altogether, this indicates that
120 despite a preserved ability to release CA, stimulus-secretion coupling of chromaffin cells is less
121 efficient in SHRs. Aiming at identifying which element(s) of the adrenal stimulus-secretion is
122 (are) altered in SHRs, we next performed a series of experiments focused on i) chromaffin cell
123 excitability, ii) the cholinergic neurotransmission at the splanchnic nerve ending-chromaffin
124 cell synapse and iii) the gap junction-mediated communication between chromaffin cells.

125

126 **Altered action potential firing in SHRs in response to sustained stimulations**

127 Regarding the passive electrical membrane properties of chromaffin cells, significant changes
128 were observed between SHRs and WKY rats for input resistance and capacitance (Table S1),
129 predicting a plausible stimulus-secretion coupling reshaping in SHRs. To investigate
130 chromaffin cell excitability under experimental conditions as close to *in situ* conditions as
131 possible, spontaneous action potential (AP) firing at resting membrane potential were recorded
132 in the loose cell-attached configuration (Figure 2A). Neither the percentage of chromaffin cells
133 exhibiting spontaneous APs (80.5 %, $n = 33/41$ cells and 81.6%, $n = 31/38$ cells for WKY and
134 SHRs, respectively, $p > 0.9999$, Fisher's exact test) nor the discharge frequency (0.69 ± 1.07 Hz,
135 $n = 32$ cells for WKY rats and 0.45 ± 0.63 Hz, $n = 31$ cells for SHRs, $p = 0.2964$, unpaired t
136 test, Figure 2B, left histogram) significantly differed between SHRs and WKY rats. Likewise,
137 the frequency distribution was not different between SHRs and WKY rats, $p > 0.9999$, Fisher's
138 exact test, Figure 2B, right histogram).

139 The loose patch configuration allowing for long-lasting recordings, we next analyzed
140 the spiking pattern of WKY and SHR chromaffin cells. Do they exhibit a regular and/or a

141 bursting pattern, as previously identified in mouse chromaffin cells²³? To discriminate between
142 the two firing patterns, the regularity of the firing discharge was investigated by calculating the
143 coefficient of variation (CV) of inter-spike interval (ISI) distribution. The analysis of the
144 discharge pattern (150-200 s recording) was performed on 24 WKY and 26 SHR chromaffin
145 cells, and shows that WKY and SHR cells both fire regularly or in bursts (Figures 3A and 3B
146 and Figure S2). As expected and consistent with previous data in mouse chromaffin cells²³, a
147 regular firing mode is associated with a $CV < 1$ and a bursting mode with a $CV > 1$. No overt
148 difference in the percentage of cells displaying a regular or a bursting firing was observed
149 between SHR and WKY rats (3 regular and 23 bursting cells ($n = 26$) for SHR and 4 regular
150 and 20 bursting cells ($n = 24$) for WKY, respectively, $p = 0.6971$, Fisher's exact test, Figure
151 3C). To determine whether the two firing patterns account for two distinct chromaffin cell
152 populations or can occur in the same cell, the spontaneous electrical activity of individual
153 chromaffin cells was recorded for a longer period of time (5-15 minutes, loose-patch
154 configuration). Interestingly, and both in WKY and SHR rats, the firing discharge can alternate
155 between the two modes, switching from a regular to a bursting mode and vice-versa (Figure
156 S3). This clearly indicates that these spiking patterns do not reflect two different cell
157 populations.

158 To further investigate chromaffin cell excitability, depolarizing steps were evoked using
159 the whole-cell configuration in cells current-clamped at their resting membrane potential
160 (between -60 and -70 mV) (Figure 4A). APs were elicited during 500 ms from a rheobase of
161 14.8 ± 9.4 pA ($n = 26$ cells) for WKY rats and 11.5 ± 5.7 pA ($n = 24$ cells) in SHRs ($p = 0.1479$,
162 unpaired t test). When plotting the AP frequency as a function of the injected current intensity,
163 no significant difference was found between SHRs and WKY rats in response to small
164 depolarizing currents (≤ 40 pA). Conversely, when injecting large current amplitudes (> 40 pA),
165 the AP accommodation increased in SHRs, leading to a significant reduction in firing frequency

166 at 60 pA (28.5 ± 4.4 Hz, $n = 13$ for WKY rats *versus* 23.0 ± 5.0 Hz, $n = 12$ for SHRs, $p = 0.0087$,
167 Mann-Whitney test, Figure 4B). This was associated with a gradual drop of the AP peak (-34%
168 between the second AP and the last AP in SHRs *versus* -25% in WKY rats) and a spike
169 widening (+20% between the second AP and the last AP in SHRs *versus* +16% in WKY rats,
170 $p < 0.001$, Figure 4C). This observation is particularly relevant in that robust depolarizations may
171 simulate the elevated peripheral sympathetic tone associated with elevated blood pressure. For
172 spontaneous APs recorded in current-clamp mode without current injection, as for evoked APs,
173 the duration was higher (+63% half-width) and the amplitude was lower in SHRs compared to
174 WKY rats (-11%, Figure 4D, mean of 30-50 successive APs). The overall features of evoked
175 and spontaneous APs in SHRs and WKY rats are summarized in Table S2. Collectively, these
176 results indicate that in response to sustained electrical stimulations, chromaffin cells of SHRs
177 are less excitable than those of WKY rats.

178 Does a decreased cell excitability occur in response to physiological stimuli? To address
179 this issue, we studied the electrical behaviour of SHR and WKY chromaffin cells in response
180 to the pituitary adenylyl cyclase-activating polypeptide (PACAP), the major neurotransmitter
181 for stress transduction at the adrenomedullary synapse^{24,25}. Cells were recorded in the loose-
182 patch cell-attached configuration and stimulated with 0.1 to 10 μ M PACAP (Figure 5).
183 Representative chart recordings in response to 10 μ M PACAP are plotted in Figure 5A. AP
184 currents were monitored before and after PACAP stimulation and the results were expressed as
185 a percentage increase in AP frequency after PACAP (Figures 5B and 5C). PACAP, at 1 and 10
186 μ M, increased the frequency of AP currents in WKY chromaffin cells, but not in SHRs. It
187 should be noted that at higher PACAP concentration (10 μ M), AP current frequency is even
188 decreased when compared to that observed before PACAP application. This result corroborates
189 the decrease in excitability reported in Figure 4. To investigate whether the change in cell
190 excitability reflects changes in the firing (regular *versus* bursting) pattern, we analysed the

191 coefficient of variation, before and after PACAP exposure (0.1 to 10 μ M, Figure S4). Although
192 there is a downward trend, no significant differences were observed in both SHRs and WKY
193 rats (2.22 ± 1.20 before PACAP and 1.89 ± 0.91 after PACAP, $n = 12$ cells for WKY rats, $p =$
194 0.2661 , Wilcoxon matched-pairs signed-rank test and 2.25 ± 1.26 before PACAP and $1.62 \pm$
195 0.50 after PACAP, $n = 10$ cells, $p = 0.1934$, Wilcoxon matched-pairs signed-rank test, Figure
196 S4A). In response to PACAP, the CV variance decreased more markedly in hypertensive than
197 in normotensive animals, suggesting that PACAP acts by homogenizing the firing pattern of
198 SHR chromaffin cells (Figure S4B). This is consistent with the lack of correlation observed in
199 SHRs, between CV after and before PACAP, in contrast to WKY rats ($\rho = 0.713$ in WKY rats,
200 $p = 0.0012$, $n = 12$ cells, Spearman's rank correlation coefficient *versus* $\rho = 0.038$ in SHRs, $p =$
201 0.916 , $n = 10$ cells, Spearman's rank correlation coefficient, Figures S4Ca and S4Cb).
202 Altogether, these data obtained in response to PACAP, the main neurotransmitter operating at
203 the 'stressed' splanchnic nerve-chromaffin cell synapse, open up a new avenue of investigation.
204 From a physiological point of view, the reduced ability of chromaffin cells to be stimulated by
205 PACAP suggests an alteration of the stress response in SHRs, likely at the catecholamine
206 secretion level.

207

208 AP waveform critically relies on the nature and the relative proportion of voltage-gated
209 ion channels. To identify the molecular determinants that could underlie the changes in AP
210 shape, we investigated the expression of transcripts encoding voltage-dependent ion channels
211 by quantitative PCR (Figure 6). We first investigated the expression level of mRNA encoding
212 the α and auxiliary β subunits of the voltage-gated sodium ($\text{Nav}1$) channel family. As
213 previously reported in mouse ²⁶, *Scn3a* gene (encoding $\text{Nav}1.3$) is the main Nav channel gene
214 expressed in the adrenal medulla of both normotensive and hypertensive rats (Figures 6A and
215 6C), with a significant upregulation in SHRs at the transcript level (1.4-fold increase,

216 p = 0.0260, Mann-Whitney test, Figure 6B), which was not observed at the protein level (Figure
217 S5). Regarding the other Nav1 isoforms, it is noteworthy that the expression level of *Scn9a*
218 mRNA (encoding Nav1.7) displayed a 11.5-fold increase in SHR s (p = 0.0049, Mann-Whitney
219 test, Figure 6B), accompanied by a 1.3-fold increase at the protein level (p = 0.0411, Mann-
220 Whitney test, Figure S5). Nav1.1, Nav1.4 and Nav1.5 transcripts (*Scn1a*, *Scn4a* and *Scn5a*
221 genes, respectively) were not detected. Regarding Nav1 channel β subunits, *Scn1b* (Nav β 1
222 subunit), *Scn2b* (Nav β 2 subunit) and *Scn3b* (Nav β 3 subunit) genes were amplified, but not
223 *Scn4b*. A significant increase was observed for *Scn2b* (2.5-fold change, p = 0.0022, Mann-
224 Whitney test, Figure S6). With respect to Ca $^{2+}$ channels, we examined both high threshold-
225 activated Cav1 (L-type) and Cav2 (N-, P/Q- and R-type) channels, highly expressed in
226 chromaffin cells and low threshold-activated Cav3 (T-type), whose expression undergoes
227 robust remodeling in response to stressful situations^{27,28}. *Cacna1d* (encoding Cav1.3) and
228 *Cacna1b* (encoding Cav2.2) genes are the principal transcripts expressed in WKY rats and
229 SHR s , with respectively a 2-fold and a 1.5-fold increase in hypertensive animals (p = 0.0022
230 for *Cacna1d* and p = 0.041 for *Cacna1b*, Mann-Whitney tests, Figures 6A and 6B). The highest
231 expression change was found for Cav2.1 channels (2.7-fold increase in SHR s , p = 0.0022,
232 Mann-Whitney test). Transcripts encoding Cav3.2 (*Cacna1h*) and Cav3.3 channels (*Cacna1i*)
233 were weakly expressed and their expression level did not change between normotensive and
234 hypertensive rats. *Cacna1g* mRNA (encoding Cav3.1) was not detected. Concerning K $^{+}$
235 channel genes, we focused on Ca $^{2+}$ -activated channels expressed in chromaffin cells²⁹, namely
236 the large (BK) KCa1.1 (*Kcnma1* gene), the intermediate (IK) KCa3.1 (*Kcnn4*) and the small
237 (SK) KCa2 conductance channels (*Kcnn1-3*). Still, the overall collection of channels expressed
238 (with a prevalent expression for KCa1.1 and KCa2.3) does not qualitatively differ between
239 WKY and SHR s (Figure 6A). Quantitatively, the expression level of all transcripts, except
240 *Kcnn2*, was significantly enhanced in SHR s (1.9-fold change for KCa1.1, 1.6-fold change for

241 KCa2.1, 1.5-fold change for KCa2.3 and 1.8-fold change for KCa3.1). KCa4.1 (*Kcnt1*) and
242 KCa4.2 (*Kcnt2*) mRNA were not detected. Altogether, these results show that i) the overall
243 repertoire of voltage-gated ion channels expressed in WKY and SHR chromaffin cells is
244 qualitatively similar (at the transcript level), except for the Nav1 channel family for which
245 Nav1.7 (*Scn9a*) channels become the second most expressed isoform in SHRs after Nav1.3
246 channels and ii) the expression ratio between WKY and SHR channels for a given channel
247 undergoes remodeling (Figure 6C).

248 Are those expression changes in voltage-gated ion channels relevant for cell electrical
249 firing and can they account for the reduced chromaffin cell excitability that occur in SHRs in
250 response to sustained depolarizations? To address this issue, the experimentally observed
251 changes in the expression of Na⁺, Ca²⁺ and Ca²⁺-dependent K⁺ channels were evaluated in a
252 numerical model of the rat chromaffin cell electrical activity ³⁰ (Figure 7). The ‘WKY’ and
253 ‘SHR’ models were implemented by the respective experimental values of resting membrane
254 potential and membrane capacitance (see Table S1). For the ‘WKY’ model, the values for G_{Na},
255 G_{Ca}, G_{BK} and G_{SK} were directly extracted from Warashina’s model. To build the ‘SHR’ model,
256 i) we assumed that the transcriptional changes equally mirror protein expression level and ii)
257 because the electrical firing pattern results from the concomitant contribution of Nav, Cav and
258 Ca²⁺-dependent K⁺ channels, we chose to change the conductance values globally, rather than
259 individually (see data in Figure S7 for individual changes in G_{Na}, G_{Ca} or G_{SK/BK}). The
260 conductance values implemented into the model were extrapolated from the changes in the
261 expression level of the most abundant transcript for Nav, Cav and Ca²⁺-dependent K⁺ channels,
262 that are Nav1.3 (1.4-fold), Cav1.3 (2-fold) and KCa1.1/KCa2.3 (1.7-fold) (Figure 6B).
263 Depolarizing voltage steps (500 ms duration) of gradually increased stimulation intensities (10
264 to 35 μA/cm²) were injected into the ‘WKY’ and ‘SHR’ models. Representative APs evoked
265 for three stimulation intensities are illustrated in Figure 7A. For low intensity stimulation

266 ($\leq 23 \mu\text{A}/\text{cm}^2$), the ‘SHR’ model generates more APs than the ‘WKY’ model, as observed in
267 electrophysiological recordings (Figure 4A, upper charts). Consistent with the data obtained in
268 the loose-patch configuration, high stimulation intensities ($>23 \mu\text{A}/\text{cm}^2$) triggered less APs in
269 the ‘SHR’ model. The AP frequency is maximal for stimulations $>30 \mu\text{A}/\text{cm}^2$ and saturates at
270 24-26 Hz for ‘WKY’ and 12 Hz for ‘SHR’ models (Figure 7B). As such, and despite the fact
271 that the ‘SHR’ model was compiled on the basis of transcriptional changes, our data suggest
272 that a coordinate increase in Nav , Ca_v and Ca^{2+} -activated K^+ channels can account for the
273 reduced excitability of SHR chromaffin cells, that occurs in response to robust depolarizations.

274

275 **Changes in cholinergic synaptic transmission in hypertensive rats**

276 Beside chromaffin cell excitability, cholinergic synaptic transmission at the splanchnic nerve-
277 chromaffin cell junction is a key element of the stimulus-secretion coupling in the adrenal
278 medulla. We therefore monitored the behaviour of cholinergic synapses by recording
279 spontaneous excitatory post-synaptic currents (sEPSCs) in chromaffin cells voltage-clamped at
280 -80 mV , as described³¹. Consistent with previous studies³²⁻³⁴ and reflecting the large number
281 of nerve fibers cut during the slicing procedure^{35,36}, few chromaffin cells exhibited sEPSCs in
282 standard 2.5 mM K^+ -containing saline (36.7%, $n = 18/49$ in WKY rats *versus* 23.1%, $n = 12/52$
283 in SHRs, $p = 0.1909$, Fisher's exact test). Figure 8A illustrates representative charts of sEPSCs
284 recorded in WKY (upper trace) and SHR (lower trace) chromaffin cells voltage-clamped at -80
285 mV , in response to a 80 mM KCl puff to increase the number of synaptic events. Mean sEPSC
286 amplitude did not significantly differ between SHRs and WKY rats ($133.1 \pm 76.3 \text{ pA}$, $n = 7$
287 cells in SHRs and $106.3 \pm 53.7 \text{ pA}$, $n = 8$ cells in WKY rats, $p = 0.4634$, Mann-Whitney test).
288 Conversely, sEPSC frequency was significantly lower in SHR chromaffin cells ($2.5 \pm 2.2 \text{ Hz}$,
289 $n = 7$ cells in SHRs *versus* $5.3 \pm 2.2 \text{ Hz}$, $n = 8$ cells in WKY rats, $p = 0.0093$, Mann-Whitney
290 test). sEPSCs kinetic parameters were also analyzed (Figure 8B). No difference was observed

291 on the activation phase (rise time of 2.04 ± 0.25 ms, $n = 8$ cells and 2.18 ± 0.30 ms, $n = 7$ cells
292 for WKY and SHR rats, respectively, $p = 0.3357$, Mann-Whitney test). By contrast, when the
293 decay phase was fitted by a single exponential curve^{32,37}, a significant increase of the time
294 constant was observed in SHRs ($\tau = 15.58 \pm 2.19$ ms, $n = 8$ cells for WKY rats *versus* $23.82 \pm$
295 5.25 ms, $n = 7$ cells for SHRs, $p = 0.0037$, Mann-Whitney test). The quantal analysis of sEPSCs
296 did not show difference between SHRs and WKY rats (16.8 ± 4.4 pA, $n = 8$ cells for WKY rats
297 and 20.5 ± 6.60 pA, $n = 7$ cells for SHRs, $p = 0.2676$, Mann-Whitney test, Figure 8C).
298 Collectively, these data argue for synaptic activity changes occurring both at pre- and
299 postsynaptic sites.

300 Excitatory postsynaptic events at the rat splanchnic nerve terminal-chromaffin cell
301 junction result from the co-activation of several nicotinic acetylcholine receptor (nAChR)
302 subtypes^{28,31,32,37}. A change in the expression and/or composition of nAChRs could account
303 for the change in sEPSC decay time constant observed in SHRs. To address this issue, the
304 expression level of transcripts encoding $\alpha 3$ (*Chrna3*), $\alpha 4$ (*Chrna4*), $\alpha 5$ (*Chrna5*), $\alpha 7$ (*Chrna7*),
305 $\beta 2$ (*Chrnb2*) and $\beta 4$ (*Chrnb4*) subunits was quantified by real-time PCR (Figure 9). The
306 expression of genes encoding $\alpha 3$ and $\alpha 7$, two subunits dominantly engaged in the synaptic
307 transmission, did not change between SHRs and WKY rats. By contrast, the transcripts
308 encoding the two auxiliary $\alpha 4$ and $\alpha 5$ subunits were differently expressed, with a significant
309 increase for $\alpha 4$ (5.7-fold, $p = 0.0043$, Mann-Whitney test) and decrease for $\alpha 5$ (0.57-fold, $p =$
310 0.0043 , Mann-Whitney test) in SHRs. For the β subunit family, the expression of $\beta 4$, the main
311 β subunit expressed in rat chromaffin cells significantly decreased in SHRs (0.48-fold, $p =$
312 0.0043 , Mann-Whitney test). *Chrnb2* expression remained unchanged. Taken together, these
313 findings indicate that nAChR subunits are transcriptionally remodeled in SHRs. Assuming
314 subsequent modifications at the protein level, this plasticity may contribute to the modifications
315 observed in sEPSC kinetics.

316

317 **Reduced gap junctional coupling between chromaffin cells in hypertensive rats**

318 In the rat adrenal medullary tissue, gap junction-mediated intercellular communication between
319 chromaffin cells is an additional pathway involved in the regulation of stimulus-secretion
320 coupling^{15,17,19}. Because adrenomedullary gap junctional coupling can remodel in
321 physiological/physiopathological conditions^{18,28,33,34,37-39}, we compared its status between
322 SHR and WKY rats. To investigate whether gap junctional coupling is modified in
323 hypertensive rats, we imaged Lucifer yellow diffusion between chromaffin cells by confocal
324 microscopy (Figure 10). As illustrated in panel 10A, the percentage of Lucifer yellow-coupled
325 cells was lower in SHRs (18%, n = 7/38 cells in SHRs *versus* 33%, n = 11/33 cells in WKY
326 rats, p = 0.1789, Fisher's exact test). Consistently, the expression of connexin 43 (Cx43), the
327 main connexin expressed in the rat adrenal medullary tissue^{15,38}, is significantly diminished in
328 SHRs, both at the transcriptional (Figure 10B, left histogram) and protein (Figure 10C) levels.
329 The expression of transcripts encoding Cx36 however did not change in hypertensive rats
330 (Figure 10B, right histogram). These results, in addition with an increased input resistance (see
331 Table S1), are consistent with a reduced gap junctional communication in SHRs. To go further
332 into the mechanisms underlying the reduced expression of Cx43 in SHRs, we addressed the
333 involvement of posttranslational changes such as stability of gap junctional plaques. Anchored
334 gap junctions at the plasma membrane bind to scaffolding proteins, such as zonula occludens-
335 1 (ZO-1). ZO-1 has been shown to interact with various connexins, including Cx43⁴⁰. As
336 shown by the densitometric analysis of immunoblots, ZO-1 expression remains unchanged in
337 SHRs (Figure 10C), indicating that the impairment in Cx43 stability at the plasma membrane
338 is unlikely the cellular mechanism whereby Cx43 expression is reduced in SHRs.

339

340

341 **Discussion**

342 This study describes the functional remodeling operated by adrenal chromaffin cells in response
343 to chronic elevated blood pressure. All observed changes converge toward a less efficient
344 stimulus-secretion coupling, especially in response to robust stimulations. This may reflect a
345 shielding mechanism dedicated to keep the hypertensive organism safe of the deleterious effects
346 on huge CA secretion episodes.

347 In addition to the functional changes unveiled between 'normotensive' and 'hypertensive'
348 chromaffin cells, this study also extends the basic knowledge on rat chromaffin cell excitability
349 *in situ*. The analysis of AP firing revealed the existence of two spiking patterns (regular and
350 bursting), as previously reported in mice^{18,23,41}. As in the mouse, regular and bursting
351 discharges coexist in a same cell. Because APs generated in bursts are more efficient than AP
352 trains fired at constant frequency to evoke CA secretion^{26,42}, identifying the cellular
353 mechanism(s) promoting the switch between the two spiking patterns would be therefore of
354 great interest. In this context, ion channels involved in setting up the resting membrane potential
355 appear as interesting targets to investigate^{23,43}. In addition, although we found no sharp
356 difference in the occurrence of regular and bursting patterns between WKY rats and SHRs,
357 further experiments are required to fully address this issue, especially in response to
358 adrenomedullary secretagogues (neurotransmitters, neuropeptides...).

359

360 **Decreased chromaffin cell excitability in response to robust depolarization/stimulation.**

361 The most functionally relevant feature of SHR chromaffin cells is their reduced AP firing in
362 response to sustained stimulations. This likely results from a coordinated upregulation of Na⁺,
363 Ca²⁺ and Ca²⁺-dependent K⁺ channels, as observed both experimentally with the enhanced
364 expression level of their respective transcripts and *in silico* in a chromaffin cell model. At the
365 single AP level, AP waveform in SHR chromaffin cells is modified as such the amplitude is

366 decreased and the half-width is increased. Because the AP waveform results from the
367 concomitant contribution of many ion channels ⁴⁴, discussing each channel separately would
368 give biased conclusions. We therefore focused the discussion on the most striking effects that
369 is the AP waveform change and reduced ability of SHR chromaffin cells to trigger sustained
370 firing during robust depolarizations. In chromaffin cells, as in most neurons, Nav channels
371 critically shape the AP waveform ⁴⁵. This is of particular importance because changes, even
372 subtle, in spike shape can drastically affect Ca²⁺ channel recruitment and therefore ensuing Ca²⁺
373 transients, and finally CA secretion. Interestingly, the more remarkable change in ion channel
374 expression in SHRs targets Nav1.7, a sodium channel involved in regulating chromaffin
375 secretory function ⁴⁶. As reported ²⁶, the reduced Nav channel availability (through Nav1.3 and
376 Nav1.7) switches cell firing from a repetitive AP firing mode to a bursting activity, boosting
377 thus hormone exocytosis. At rest, Nav channels tonically inhibit burst firing and would
378 contribute to maintain a low basal CA secretion. In SHRs, the significant increase of Nav1.7
379 would help retaining chromaffin cell to spontaneously fire in a tonic mode, preserving the gland
380 of huge CA secretion. This is consistent with our data showing that basal NE and E secretion is
381 not enhanced in SHRs (even decreased for NE). The concomitant increased expression of
382 Nav1.7, Cav1.3 and KCa channels in SHR chromaffin cells is consonant with data reporting
383 that i) L-type Ca²⁺ channel activity can upregulate Nav channels in endocrine cells ^{47,48} and ii)
384 Cav1.3 and BK/SK channels are functionally coupled in chromaffin cells ^{41,45,49}. Long-term
385 changes in Nav channel expression have been also described in endocrine tissues in response
386 to stressful situations ⁵⁰. Collectively, these findings prompt us to propose the provisional
387 sequence of events, that is an enhancement of L-type Cav channels and ensuing cytosolic
388 calcium followed by a subsequent enhancement of Nav and KCa channels as a shielding
389 mechanism to brake CA secretion, especially in response to prolonged or repetitive
390 stimulations. We have focused here on voltage-gated ion channels involved in AP firing, but

391 other ion channels, K^+/Na^+ background/leak channels operating at resting membrane potential
392 in particular, are also qualified to modify cell excitability ^{23,43,51} and would therefore be
393 interesting to investigate.

394

395 **Weakened synaptic transmission in SHRs**

396 In SHRs, the excitatory neurotransmission between splanchnic nerve and chromaffin cells is
397 pre- and postsynaptically remodeled. According to the quantal theory of neurotransmitter
398 release ⁵², the reduced number of sEPSCs in response to a sustained stimulation may relate to
399 failures eventuating on presynaptic components. Additionally, and supported by changes
400 occurring postsynaptically at nAChRs, the incoming signals would be less efficiently integrated
401 by hypertensive cells. In chromaffin cells, the prevalent nAChR compositions encompass $\alpha3\beta4$
402 and $\alpha3\beta2$ subunits with the addition of $\alpha5$ ⁵³. Because the $\beta4$ subunit modulates the density of
403 $\alpha3\beta4$ receptors at the plasma membrane ⁵⁴, the decreased expression level of $\beta4$ mRNA in
404 SHRs may reduce the membrane expression of $\alpha3\beta4$ nAChRs and attenuate the
405 neurotransmission at splanchnic nerve-chromaffin cell synapses. When incorporated in a
406 nAChR, $\alpha5$ subunit alters biophysical and biochemical properties of the channel ^{55,56}. Of a
407 particular interest for a neurosecretory cell is the less robust Ca^{2+} permeability of $\alpha3$ nAChRs
408 in the absence of $\alpha5$ ⁵⁶. If we assume that the reduced expression level of $\alpha5$ transcripts in
409 SHRs could favor $\alpha3\beta2/ \alpha3\beta4$ combinations to the detriment of $\alpha3\beta2\alpha5/\alpha3\beta4\alpha5$, it is likely
410 that the resulting Ca^{2+} entry through activated nAChRs could be less robust, and could
411 contribute to reduce hormone secretion. Both $\alpha4\beta2$ and $\alpha4\beta4$ combinations build functional
412 nAChRs, although the current properties differ between the two combinations ⁵⁷. The down-
413 regulation of $\beta4$ in SHRs suggests that the prevalent combination in hypertensive rats could be
414 $\alpha4\beta2$ rather than $\alpha4\beta4$. Assuming this, the current flowing through $\alpha4\beta2$ nAChRs would then
415 display a lower amplitude and a faster desensitization ⁵⁷. Collectively, the transcriptional

416 remodeling of nAChR subunit expression observed in SHRs converges towards a less efficient
417 cholinergic coupling between nerve endings and chromaffin cells in hypertensive animals.

418

419 **Reduced gap junctional coupling in SHRs**

420 Gap junctional signaling between chromaffin cells is a part of regulatory mechanisms involved
421 in stimulus-secretion coupling ^{16,17,19,20}. Although we did not directly assess the junctional
422 current, the reduced LY diffusion and the decreased Cx43 protein expression level strongly
423 suggest an attenuated gap junctional communication in SHRs. Changes in chromaffin cell input
424 resistance and capacitance, two parameters impacting the transmission strength and therefore
425 the propagated signals ⁵⁸, also support this statement. Given that gap junction coupling
426 contributes to CA secretion ^{15,18,20,38}, a decreased intercellular communication could be a
427 suitable mechanism whereby the adrenal medulla would dampen its secretory process,
428 especially in response to harmful challenges (*i.e.* repetitive stimulating episodes).

429

430 **Reduced CA release in SHRs in response to sustained cholinergic stimulations**

431 Our results argue for a decrease in the stimulus-secretion coupling efficiency in SHRs. E and
432 NE secretion is attenuated in SHRs, especially in response to high ACh concentrations. At rest
433 and for lower ACh concentrations, only adrenal NE outflow is decreased, as previously reported
434 ⁵⁹. This suggests that the ‘hypertensive’ environment (circulating levels of CA and other
435 adrenomedullary hormones/factors, adrenocortical secretions...) differentially impacts the
436 mechanisms involved in regulating NE and E release. However, besides their difference in
437 secretory competence, the hypertensive and normotensive medullary tissues are equally readily
438 responsive to a cholinergic stimulus, as shown by equivalent stimulation ratios. Interestingly,
439 they differ in the E:NE ratios in response to a cholinergic stimulus. Whereas in WKY rats, E:NE
440 ratio increases gradually with ACh concentration (due to enhanced E release, as expected in a

441 healthy adrenal medulla), it remains constant in SHRs, indicating a dysregulation in the
442 secretory process of E. There are many targets (secretory granules, exocytosis...), and further
443 experiments are needed to identify the impaired processes.

444 Several explanations can account for the decreased stimulus-secretion coupling
445 efficiency in SHRs. First and supported by our electrophysiological data, SHR chromaffin cell
446 less robustly fire upon strong depolarizations. This, in association with the remodeling of
447 nAChR subunits, likely contributes to lessen electrical activity- and cholinergic-evoked $[Ca^{2+}]_i$
448 rises, and subsequent CA exocytosis. Second, downstream changes may also occur, targeting
449 for example CA biosynthesis and/or intra-adrenal content. Regarding this, the literature is
450 puzzling, that is either an increase or a decrease in activity/mRNA expression level of TH, DBH
451 and PNMT in adult SHRs have been described ⁶⁰⁻⁶⁵. Similarly, the intra-adrenal CA content in
452 adult SHRs has been reported to be either decreased, increased or unchanged ^{62,65-67}. Along the
453 same line, the secretory granules could also be impacted. Of a particular interest are the granins,
454 major components of chromaffin granules critically involved in granulogenesis, granule cargo
455 and exocytosis ^{68,69}. By regulating circulating CA levels, granins and their derived peptides are
456 potent modulators of blood pressure ⁷⁰⁻⁷². In adult SHRs, the increased adrenal content of
457 chromogranin A ^{62,67} is likely one of the mechanisms contributing to elevated blood pressure.

458

459 **Physiological and/or pathological consequences of attenuated stimulus-secretion coupling**
460 **in adult SHRs: a shielding mechanism of the adrenal medullary function and/or**
461 **impairment of the stress response?**

462 The question is open to debate. From a physiological point of view, our finding of a reduced
463 stimulus-secretion coupling in the SHR adrenal medulla is functionally relevant and helps to
464 decode how chromaffin cells behave to cope with a hypertensive environment, and more
465 generally with a chronic hyperactivity as observed during a sustained splanchnic nerve firing

466 activity evoked by prolonged stress episodes. The reduced CA release would also preserve from
467 exhaustion of the secretory process, maintaining therefore the tissue still competent for further
468 stimulations. In the same context, given the importance of CA in the control of hemodynamic
469 properties (heart rate, cardiac output, blood pressure, vascular tone), the reduced NE outflow
470 observed at rest and for lower ACh concentrations could be physiologically relevant to mitigate
471 the deleterious consequences of a hypertensive environment. Indeed, by activating α -
472 adrenoceptors, NE increases peripheral arterial resistance and therefore blood pressure. E by
473 acting on both α - and β -adrenoceptors has also a hypertensive effect, but in a lesser extent
474 compared to NE. Beyond their hemodynamic properties, plasma CA, when chronically
475 elevated, can promote pathophysiological conditions including inflammation, metabolic
476 disorders, and organ failures ⁷³. In this context, any mechanism aimed at reducing plasma CA
477 levels can be considered protective.

478 In the same line, we can also propose that the remodeling of the adrenal medulla in
479 hypertensive animals could prevent excessive energy expenditure at both tissue and organism
480 levels. CA synthesis and secretion are energy-consuming processes ⁷⁴, so we speculate that
481 weakening the competence of the adrenal medulla to release catecholamines could limit this
482 energy cost. In addition, since elevated catecholamine secretion is known to induce an increase
483 of energy expenditure mediated by metabolism stimulation ⁷⁵, the remodeling we describe here
484 would be beneficial to limit catecholamine secretion.

485 Adrenal medulla-driven adaptive mechanisms are decisive for the ability of an organism
486 to cope with stress (⁷⁶ for a recent review) and any dysfunction or imbalance in one of these
487 mechanisms can jeopardise its survival. One issue, not investigated here, would be to evaluate
488 whether and how SHRs and more generally hypertensive animals adapt to stress
489 (acute/chronic). Does the 'hypertensive' adrenomedullary tissue undergo remodeling of
490 chromaffin cell excitability, synaptic cholinergic/peptidergic neurotransmission and gap

491 junctional coupling? In this context, PACAP, a major stress transmitter at the splanchnic nerve-
492 chromaffin cell synapse²⁵, is certainly an avenue to explore. Our data showing an alteration of
493 the electrical response to PACAP together with i) the previously reported effect of PACAP on
494 *Pnmt* gene expression⁷⁷ and ii) an increased expression of *Pnmt* mRNA and protein levels in
495 SHRs^{64,78} suggest altered (defective?) adrenergic transduction pathways/function in SHRs.

496 As such, the adrenal stimulus-secretion coupling is a dynamic process, continuously
497 remodeled according to physiological or pathophysiological conditions^{28,79}. Whereas acute CA
498 needs (as required during stressful situations) are associated with an increased stimulus-
499 secretion coupling efficiency^{18,34,38}, a long-lasting impregnation of the adrenal medullary tissue
500 with high circulating CA levels (as observed during hypertension development for example)
501 would correlate with a reduced stimulus-secretion coupling competence.

502

503 **Materials and Methods**

504 **Ethical approval and animals.** Animals were housed in groups of 3-4 par cages (standard
505 sizes according to the European animal welfare guidelines 2010/63/EU) and maintained in a
506 12h light/dark cycle, in stable conditions of temperature (22°C) and humidity (60%). Food and
507 water were provided *ad libitum*. All procedures in this study also conformed to the animal
508 welfare guidelines of the European Community and were approved by the French Agriculture
509 and Forestry Ministry (authorization numbers/licences 49-2011-18, 49-247, A49007002 and
510 D44015) and by the regional ethic committee (authorization CEEA.2011.12 and
511 APAFIS#2017072117413637).

512 Data were collected from 16- to 20-week-old spontaneously hypertensive male rats
513 (SHRs) and from their control age-matched normotensive male Wistar-Kyoto (WKY) rats
514 (SHR/KyoRj and WKY/KyoRj, Janvier Labs, Le Genest-St-Isle, France). The body weight did
515 not significantly differ between SHRs (401.4 ± 33.3 g, n = 38) and WKY rats (417.2 ± 43.6 g,

516 n = 47, p = 0.0689, unpaired t test, Figure S1A). As a consequence of the arterial hypertension,
517 the heart weight was statistically higher in SHR (1.51 ± 0.23 g, n = 38 *versus* 1.22 ± 0.10 g, n
518 = 47 in WKY rats, p<0.0001, unpaired t test, Figure S1B). Accordingly, the heart/body weight
519 ratio (x100) was also significantly higher in SHR (0.38 ± 0.06, n = 38 *versus* 0.29 ± 0.02, n =
520 47 in WKY rats, p<0.0001, unpaired t test, Figure S1C).

521 **Adrenal slice preparation.** Acute slices from SHR and WKY rats were prepared as described
522 ^{15,80}. Briefly, after removal, the adrenal glands were kept in ice-cold saline for 2 min. Before
523 slicing, a gland was desheathed of the surrounding fat tissue and was next glued onto an agarose
524 cube and transferred to the stage of a vibratome (HM 650V vibrating blade microtome, Microm
525 Microtech, Francheville, France). Slices (200 µm thickness for the electrophysiological
526 recordings and 150 µm thickness for CA secretion assay) were cut with a razor blade and
527 transferred to a storage chamber maintained at 37°C, containing Ringer's saline (in mM): 130
528 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, 12 glucose and buffered to
529 pH 7.4. The saline was continuously bubbled with carbogen (95% O₂/5% CO₂).

530 **Electrophysiology.** All electrophysiological recordings were performed in acute slices.
531 Adrenal slices were transferred to a recording chamber attached to the stage of a real-time
532 confocal laser scanning microscope (LSM 5Live, Zeiss) equipped with an upright microscope
533 (Axio Examiner, Zeiss, Le Pecq, France) and continuously superfused with Ringer's saline at
534 34°C. All experiments were performed using the patch-clamp technique and
535 electrophysiological signals were acquired using an EPC-10 USB Quadro patch-clamp
536 amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany) and PATCHMASTER software.
537 Signals were sampled at 10 Hz and analyzed with FITMASTER (HEKA Elektronik, Germany).
538 For whole-cell recordings, pipettes were pulled from borosilicate glass and filled with the
539 following internal solution (in mM): 140 potassium (K)-gluconate, 5 KCl, 2 MgCl₂, 1.1 EGTA,
540 5 HEPES, 4 MgATP, 0.3 NaGTP and titrated to pH 7.2 with KOH. Osmolarity was adjusted at

541 295 mOsm with K-gluconate and pipette resistance was 5-8M Ω . Voltage was corrected for the
542 junction potential of about 13 mV. Pipette and cell capacitances were fully compensated and
543 the series resistance was compensated at 75-80%. Membrane potential was recorded in the
544 current-clamp mode and filtered at 3 kHz. The AP properties were examined by injecting current
545 pulses of 500 ms duration from -50 to +60 pA at 1 Hz. The evoked AP analysis was done using
546 Mini Analysis software (Synaptosoft Inc. Fort Lee, NJ, USA). For extracellular recordings of
547 spontaneous AP currents in the loose-patch configuration, pipettes were pulled to a resistance
548 of 5-10 M Ω when filled with the following saline (in mM): 130 NaCl, 2.5 KCl, 2 CaCl₂, 1
549 MgCl₂, 10 HEPES, 10 glucose and buffered to pH 7.4 with NaOH. Osmolarity was adjusted at
550 295 mOsm with NaCl. The liquid junction potential was approximately 0 mV. Once the tip of
551 the pipette was positioned at the surface of a chromaffin cell, a minimal suction pressure was
552 applied (seal resistance <500 M Ω) and the electrical activity was recorded in the voltage-clamp
553 mode (0 mV) of the loose cell-attached configuration⁸¹. This method allows investigation of
554 membrane excitability under physiological conditions and stable recordings of firing rate can
555 therefore be obtained^{82,83}. Regarding the extracellular experiments with PACAP, after a 2-5min
556 stabilisation period, we initiated recordings for 5 minutes (control condition). Using a
557 Pressurized Drug Applicator (NPI electronic, Bauhofring, Germany) attached to a glass pipette,
558 PACAP was then puffed near the recorded cell for 5 minutes (PACAP condition). The results
559 were represented as a percentage of the AP frequency following PACAP application relative to
560 the control condition. Three different doses of PACAP were used (0.1, 1 and 10 μ M) on distinct
561 slices and chromaffin cells. Spontaneous excitatory postsynaptic currents (sEPSCs) were
562 recorded in chromaffin cells voltage-clamped at -80 mV and were filtered at 1 kHz, as
563 previously described³¹. To increase the percentage of chromaffin cells exhibiting sEPSCs,
564 recordings were performed by applying a 80 mM KCl puff at the vicinity of the recording cells
565^{32,35}. In some cells, a quantal analysis of sEPSCs was performed. Only single events were

566 selected for construction of amplitude histograms. The histograms were inspected for the
567 presence of peaks and a corresponding number of Gaussians was then fitted by nonlinear
568 regression using GraphPad Prism 5.0 software (San Diego, USA). Quantal size was estimated
569 from the mean value of the first Gaussian curve fitted to the amplitude distribution histogram.
570 Normalized sEPSC decays were fitted by a single exponential using the Simplex fit algorithm
571 based on the sum of squared errors SSE. Only fits with a $SSE < 0.001$ were taken into
572 consideration. The analysis was done using Mini Analysis software.

573 **Mathematical model of chromaffin cell electrical firing.** Chromaffin cell electrical activity
574 was modeled using the chromaffin cell simulation developed by Warashina and Ogura³⁰. The
575 numerical model was adapted to the JSim software⁸⁴, available at
576 <https://www.imagwiki.nibib.nih.gov/physiome/jsim>. The time adaptive integration using the
577 Radau method ($dt = 0.01$ ms) was used to give stable solutions to ordinary differential
578 equations. Conductance changes between SHRs and WKY rats were extrapolated from the
579 changes observed in the expression level of the most abundant transcript for Na^+ , Ca^{2+} and
580 Ca^{2+} -dependent K^+ channels, that are Nav1.3 (1.4-fold change), Cav1.3 (2-fold change) and
581 KCa1.1/KCa2.3 (1.7-fold change).

582 **Dye transfer assay.** The fluorescent dye LY (Lucifer yellow-CH, dilithium salt, 1 mM in
583 internal solution) was introduced into chromaffin cells using patch pipettes. The probability of
584 LY diffusion was expressed as a ratio corresponding to the number of injected cells that show
585 dye transfer to adjacent cells over the total number of injected cells, as previously described³⁸.
586 Cells were viewed with a 63x/1.0 NA Plan-Apochromat water immersion objective (Zeiss).
587 Dye transfer between gap junction-coupled cells was imaged with a real-time confocal laser-
588 scanning microscope (LSM 5Live, Zeiss) equipped with a diode laser 488 nm (100 mW). The
589 extent of LY diffusion was estimated by counting the number of neighboring cells that received
590 dye in 15 min.

591 **Quantification of mRNA expression levels by real-time PCR.** Total RNA was extracted from
592 macrodissected adrenal medulla using the RNeasy[®] Micro extraction kit (Qiagen, Courtaboeuf,
593 France), as previously described¹⁸. RNA (500 ng) was first reverse transcribed using the
594 QuantiTect[®] Reverse Transcription kit (Qiagen) in a final volume of 10 µl. Real-time PCR
595 analyses of the target genes and the reference genes *Gusb*, *Hprt* and *Gapdh* were performed
596 using Sybr[®] Green PCR master mix (Applied Biosystems, Foster City, CA) with 1:100 of the
597 reverse-transcription reaction, and were carried out on an ABI 7500 SDS Real-Time PCR
598 system (Applied Biosystems). After an initial denaturation step for 10 min at 95°C, the thermal
599 cycling conditions were 40 cycles at 95°C for 15 s and 60°C for 1 min. Specificity of
600 amplification was checked by melting curve analysis. Each sample value was determined from
601 duplicate measurements. Expression of the target transcripts was normalized to the mean of the
602 expression level of the three reference genes according to the formula $E = 2^{-(Ct_{mean}[Target] -$
603 $Ct_{mean}[Reference])}$, where Ct_{mean} is the mean threshold cycle. The fold change values were
604 determined according to Livak's method⁸⁵. Primer sequences of target genes are given in Table
605 S3 and the concentration used was 300 nM for all genes.

606 **Determination of Cx43 and ZO-1 protein expression.** Cx43 and ZO-1 expression levels were
607 achieved on macrodissected adrenal medulla (5 SHRs and 5 WKY rats). After decapsulation,
608 the medullary tissue was separated from the cortex, quickly frozen and pulverized in liquid
609 nitrogen. For each animal the right and the left medulla were pooled in a same sample. The
610 samples were resuspended in lysis buffer (10 mM Tris-HCl, pH 7.4, 1 mM sodium
611 orthovanadate, 10 mM NaF as phosphatase inhibitors and 1% SDS), supplemented with Mini
612 complete protease inhibitors (Roche Applied Science, Laval, Quebec). The extracts were
613 incubated 30 min at 4°C, sonicated and centrifuged for 20 min at 12000 g. The supernatant was
614 then collected, and the protein concentration was determined using the Pierce BCA Protein
615 Assays Kit (Thermo Fisher Scientific, Rockford, IL) Protein samples (25 µg) were separated by

616 10% SDS-PAGE (4-15% gradient for ZO-1 detection). Electrophoresed proteins were
617 transferred onto a nitrocellulose membrane. Blots were then blocked with 5% milk in TBS (pH
618 7.4, 0.1% Tween 20) for 1 h at room temperature with gentle agitation. Blots were next
619 incubated with polyclonal antibodies, a rabbit anti-Cx43 (1:500, Thermo Fisher Scientific) or a
620 rabbit anti-ZO-1 (1:250, Thermo Fisher Scientific) in TBS-Tween 0.1% containing 5% milk, at
621 4°C overnight. Following washout, blots were incubated with secondary antibodies peroxidase
622 conjugated for 1 h at room temperature. Proteins were visualized with a LAS-3000 imager
623 (Fuji) using an ECL-Plus Chemiluminescence kit (Thermo Fisher Scientific). To ensure equal
624 loading of protein samples, blots were stripped of connexin or ZO-1 antibodies and reprobbed
625 with an actin-specific monoclonal antibody (β -actin, 1:5000, Sigma). Intensities of Cx43 and
626 ZO-1 bands were normalized to those of actin and quantified using the Las-3000 software.

627 **CA assays from adrenal slice supernatants.** Chromaffin cell ability to secrete CA was
628 assessed by monitoring E and NE released in slice supernatant, as previously described²². After
629 a 5-min basal (B) condition, slices were challenged (S) with either ACh-containing saline or
630 Ringer saline, during 5 min. For each slice, results were expressed as the stimulation ratio S/B.
631 To calculate basal or stimulated amounts of secreted E and NE, the medulla surface was
632 estimated for each slice. To achieve this, at the end of experiments, slices were bathed for 2
633 min in hematoxyline (1 g/l). The differential staining intensity between cortex and medulla
634 allowed estimating the medulla surface, on the two slice faces. The volume was then
635 subsequently calculated. After collection, samples were kept at -20°C during 2-3 days before
636 use. E and NE were assayed by High Performance Liquid Chromatography (HPLC). A
637 benzylamine pre-column derivatization was used to generate fluorescent benzoxazole
638 derivatives⁸⁶, which were next separated by C18 reversed-phase HPLC (Vydac 218TP54
639 column, 5 mm, 4.6 mm i.d. x 250 mm; Waters Separations Module 2695; multiwavelength
640 fluorescence detector Waters 2475)⁸⁷. The derivatization reactions were carried out using 20 μ l

641 of a standard solution of CA (L-epinephrine- and L-norepinephrine-L-bitartrate salts) or
642 supernatant samples or water (blank) mixed to benzylamine (50 mM) and 3-
643 cyclohexylaminopropanesulfonic acid (3.3 mM, pH 11) and potassium hexacyanoferrate III
644 (1.7 mM). The resulting mixtures were incubated at 50°C for 20 min and 20 µl were loaded
645 onto the reverse phase C18-column. Isocratic elution was performed with a mobile phase
646 consisting of 10 mM acetate buffer (pH 5.5) and acetonitrile (65/35, v/v) run at 1 ml/min. The
647 column temperature was maintained at 23°C. The detection was monitored at an excitation
648 wavelength of 345 nm and an emission wavelength of 480 nm. To determine E and NE
649 concentrations, the areas under the peaks of samples were compared to the peaks of the standard
650 range used as external calibrator.

651 **Solutions and chemicals.** Benzylamine, 3-cyclohexylaminopropanesulfonic acid, potassium
652 hexacyanoferrate III, L-epinephrine-L-bitartrate salt, L-norepinephrine-L-bitartrate salt,
653 acetonitrile, Lucifer yellow (dilithium salt), pituitary adenylate cyclase activating polypeptide-
654 38 (PACAP-38) and acetylcholine chloride were purchased from Sigma-Aldrich (Saint-
655 Quentin Fallavier, France).

656 **Statistical analysis.** Statistics were performed with Prism 9 (version 9.4.1, GraphPad, San
657 Diego, CA). Numerical data are expressed as the mean \pm standard deviation. Differences
658 between groups were assessed by using the non-parametric Mann-Whitney test. Unpaired
659 Student's t-test was used to compare means when appropriate. The non-parametrical Wilcoxon
660 matched-pairs signed-rank test was used to compare two related samples or to compare a single
661 sample to a theoretical value (1 or 100%). For comparisons of more than two groups, the non-
662 parametric Kruskal-Wallis test was used. The Spearman's rank correlation coefficient ρ was
663 used to measure the relationship between paired data. Percentages were compared using a
664 contingency table and the chi-square test or the Fisher's exact test when appropriate. Differences

665 with $p < 0.05$ were considered significant, with *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ and ****,
666 $p < 0.0001$.

667

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960 benzylamine. *Anal Biochem* 312, 125-133. 10.1016/s0003-2697(02)00435-9.

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972 **Author contributions**

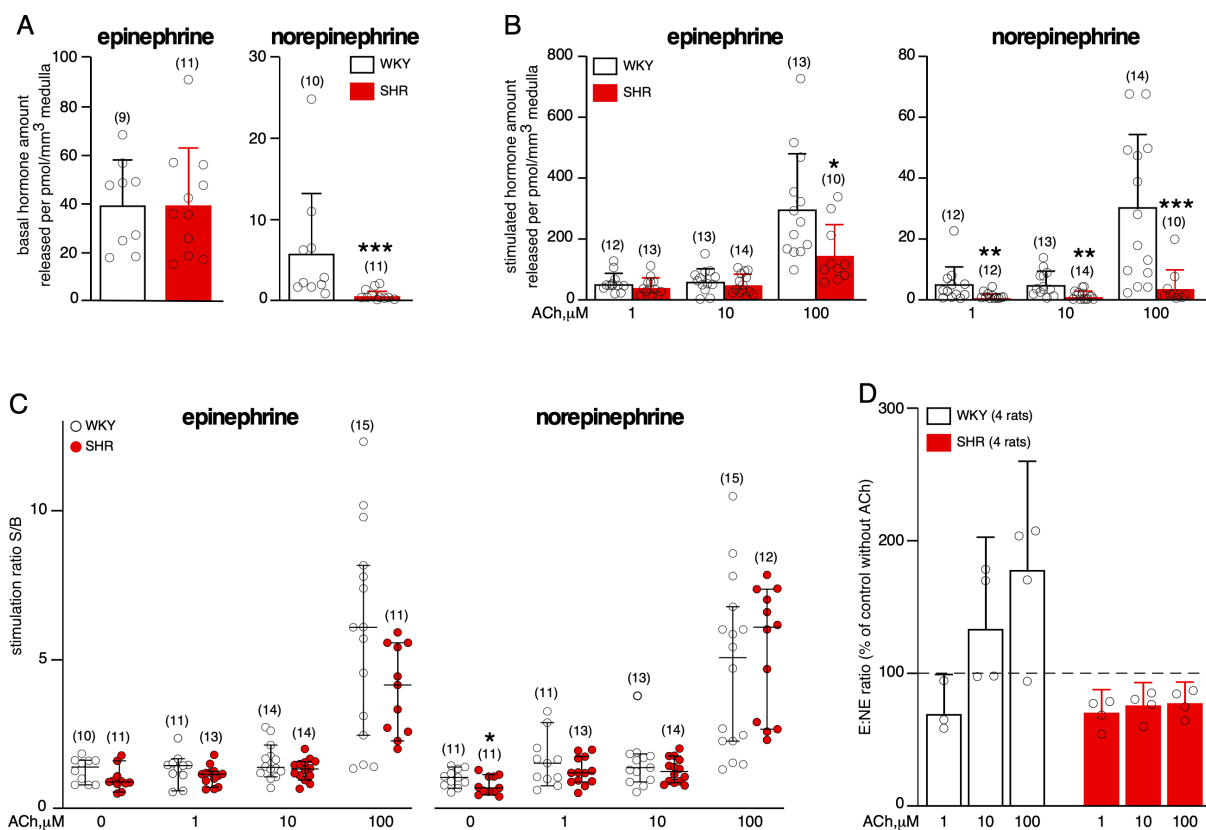
973 V.P., J.P, B.T., J.B., F.D.N., P.F., C.G.-L., C.L. and N.C.G. conducted the experiments. D.B.
974 and D.G. assisted with HPLC equipment.

975 V.P., J.P, B.T., J.B., F.D.N., C.G.-L., C.L. and N.C.G analyzed the data. N.C.G and C.L.
976 designed the experiments. N.C.G. conceived the study and wrote the paper with input from
977 D.H. and C.L.

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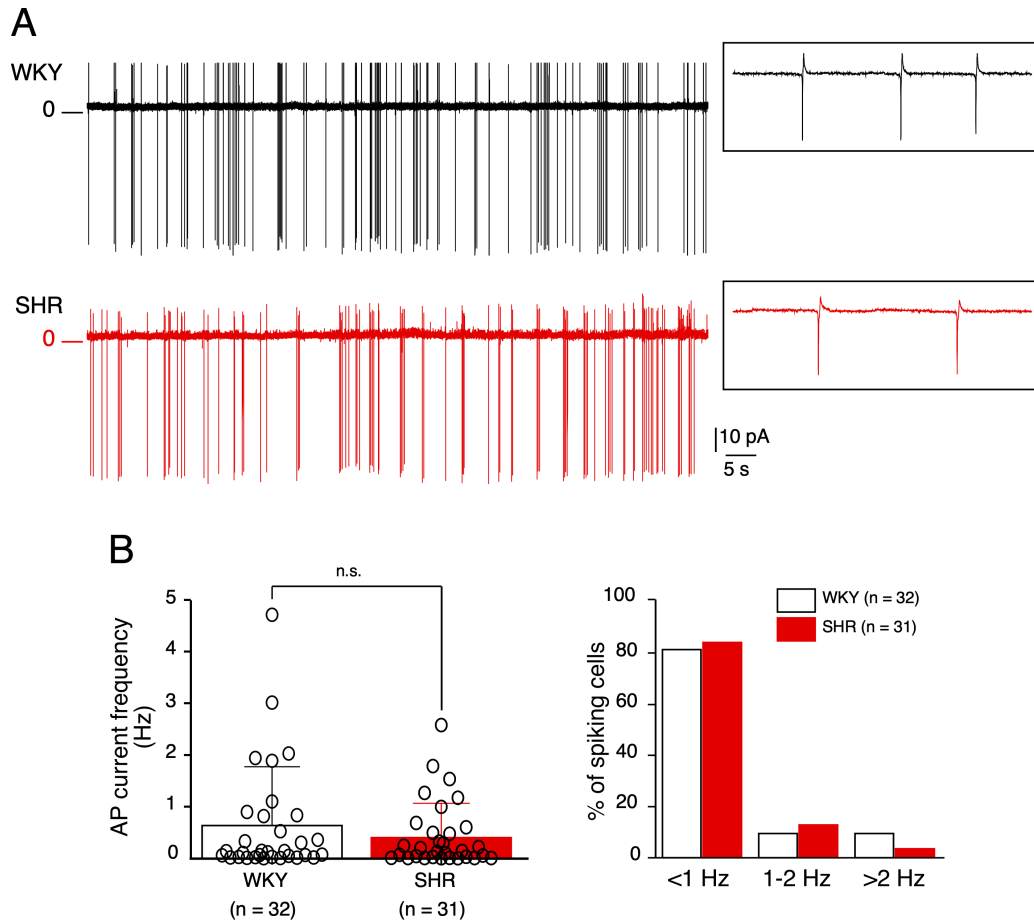
979 **Competing financial interests:** The authors declare no competing financial interests.

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Figure 1: Reduced competence to release CA in SHRs in response to robust ACh challenge. Acute adrenal slices from SHRs and WKY rats were incubated first for 5 min (basal conditions) and then challenged (stimulated (S) conditions) with either ACh-containing saline or Ringer saline, during 5 min. Secreted epinephrine (E) and norepinephrine (NE) were assayed by HPLC. **A.** Basal CA secretion. **B.** CA secretion in response to increasing ACh stimulations. Both basal and ACh-evoked NE amounts released per mm³ of medulla are significantly reduced in SHRs. Regarding E, amounts released per mm³ are decreased only in response to a high ACh concentration (100 μM)-evoked challenge. **C.** No difference in stimulation ratios S/B, indicating that the tissue responsiveness is preserved in SHRs. The number of slices is indicated in parentheses. **D.** E:NE ratios calculated from the two adrenals pooled for each rat. Constant E:NE ratios in SHRs, contrasting with a progressive increase in E:NE in WKY rats, upon exposure to increasing concentrations of ACh.

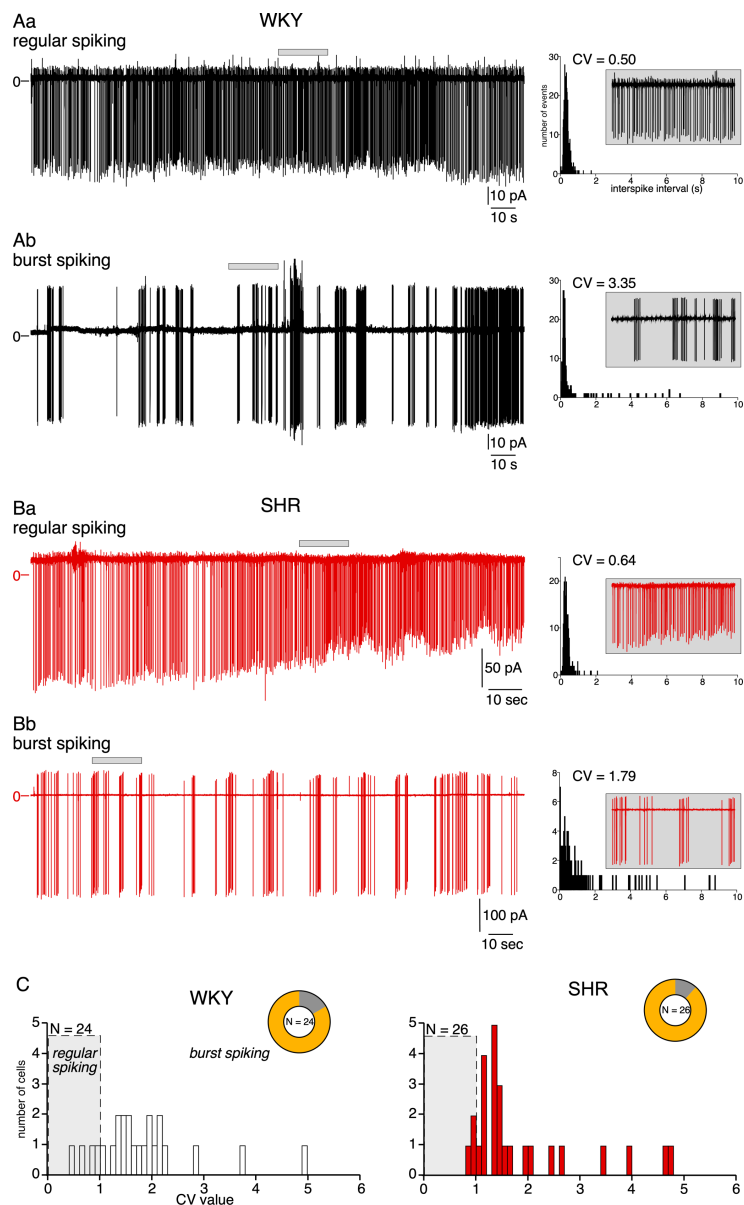


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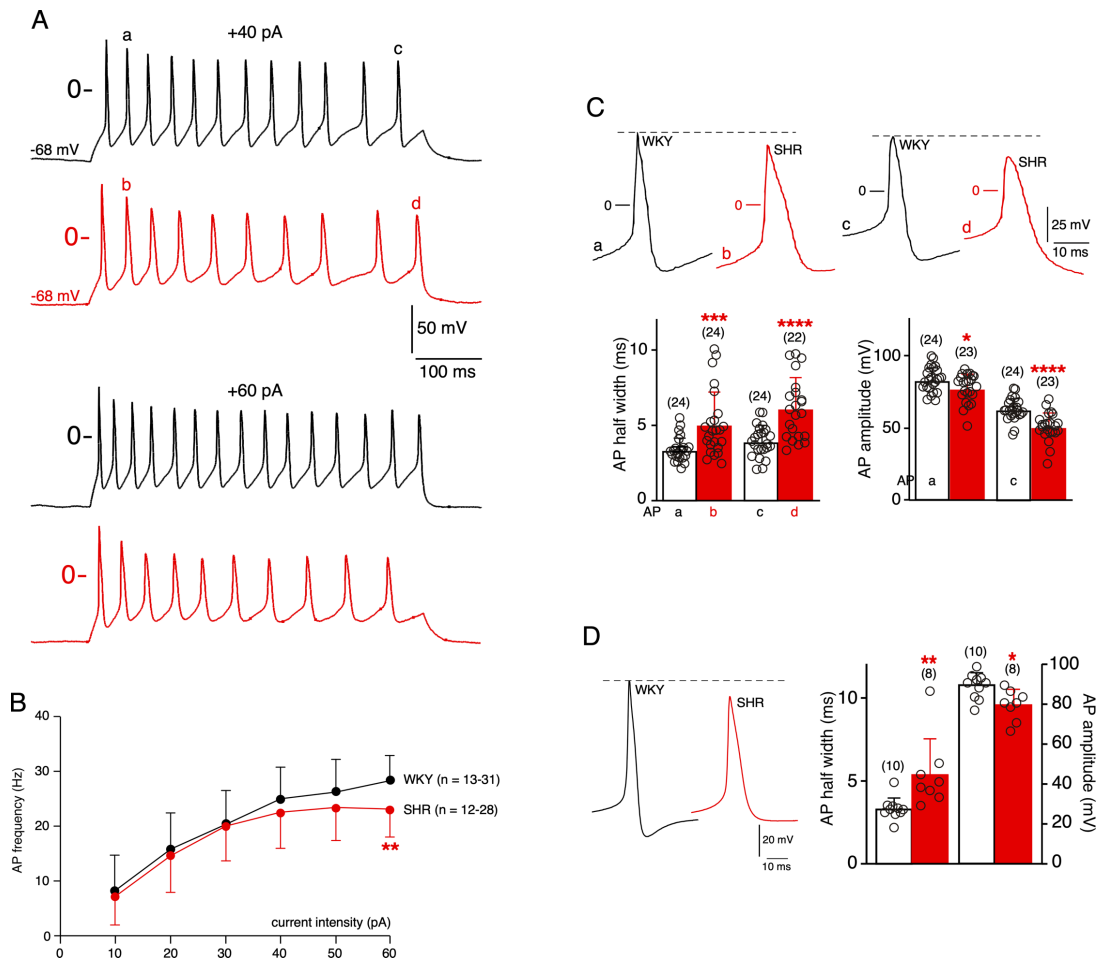
998 **Figure 2: Spontaneous electrical firing monitored in chromaffin cells in adrenal acute**
 999 **lices of WKY rats and SHRs.** Action potential (AP) currents were recorded in the voltage-
 1000 clamp mode (0 mV) of the loose cell-attached configuration. **A.** Representative chart recordings
 1001 of a WKY (upper trace) cells and a SHR (lower trace) cell. **B.** Analysis of the AP current
 1002 frequency. Neither the mean frequency nor the distribution (ranging from <1 to >2 Hz) differed
 1003 between WKY rats and SHRs.

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Figure 3: Presence of two distinct firing patterns in chromaffin cells of WKY rats and SHRs. Cells were recorded in the loose cell-attached configuration and voltage-clamped at 0 mV. **A.** Spontaneous AP currents recorded in two individual cells of WKY rats. One cell exhibits a regular spiking (**Aa**, coefficient of variation $CV = 0.5$), while the second cell displays a bursting firing pattern (**Ab**, $CV = 3.35$). The histograms on the right illustrate the distribution of the inter-spike intervals (100 ms bin), from which the coefficients of variation (CV) were calculated, as described in ²³. **B.** Spontaneous AP currents recorded in two individual cells of SHRs. One cell exhibits a regular spiking (**Ba**, coefficient of variation $CV = 0.64$), while the second cell displays a bursting firing pattern (**Bb**, $CV = 1.79$). Insets in A and B: expanded time scale illustrating a 20-s spiking period. **C.** Histograms illustrating the distribution of the mean CV values calculated in 24 WKY cells and 26 SHR cells (0.1 CV unit bin).



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1020 **Figure 4: Decreased chromaffin cell excitability in response to robust depolarizations and**

1021 **AP waveform changes in SHRs. A.** Illustrative chart recordings of evoked electrical activities

1022 in current-clamped WKY (black traces) and SHR (red traces) cells, in response to depolarizing

1023 steps (500 ms duration, +40 and +60 pA injected currents as upper and bottom traces,

1024 respectively). **B.** Analysis of the AP frequency in response to serial increasing depolarizations.

1025 Robust depolarizing steps ($I_{inj} \geq 40$ pA) elicited less APs in SHRs than in WKY rats. **C.** Single

1026 AP waveforms derived from the second (a and b traces for WKY and SHR, respectively) and

1027 the last (c and d traces for WKY and SHR, respectively) evoked APs in response to a +40 pA,

1028 500 ms duration depolarizing step. Analysis of AP half-width and amplitude changes shows an

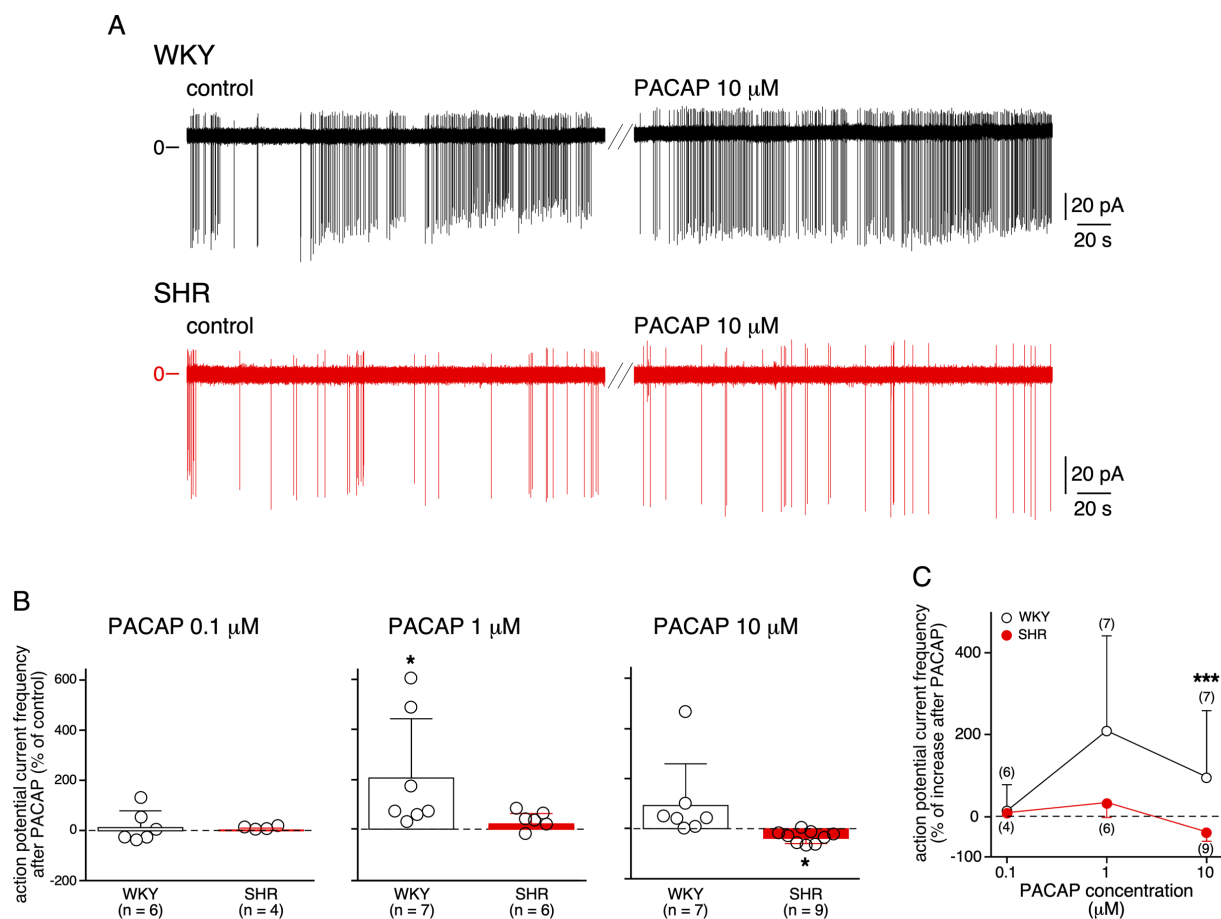
1029 increased half-width and a decreased amplitude in SHRs. **D.** Similar analysis performed for

1030 spontaneous APs (mean of 30-50 APs/cell) recorded in WKY and SHR chromaffin cells

1031 current-clamped at their resting membrane potential. AP half width and amplitude are also

1032 significantly modified in SHRs (increased and decreased, respectively).

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1036 **Figure 5: Physiological consequence of reduced excitability in SHRs: lack of electrical**

1037 **response to PACAP.** Chromaffin cells in acute adrenal slices were recorded in the loose-patch

1038 cell-attached configuration (clamped at 0 mV) and stimulated with 0.1 to 10 μ M PACAP-38.

1039 **A.** Representative chart recordings of AP currents in a WKY and SHR chromaffin cell exposed

1040 to 10 μ M PACAP. **B.** Pooled data of electrical responses to various PACAP concentration

1041 applications, illustrating the lack of response to PACAP in SHRs compared to the increase in

1042 frequency of AP currents in WKY rats. The data are presented as a percentage relative to the

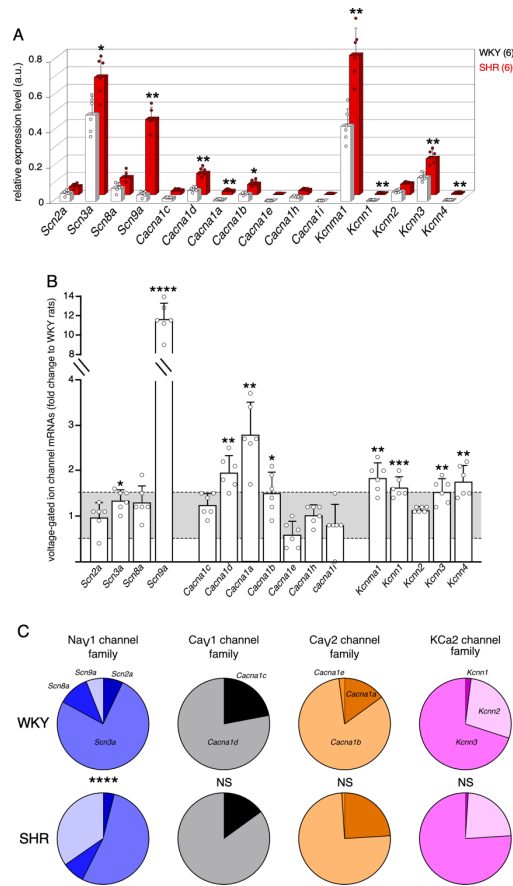
1043 firing frequency observed under control conditions. Note the decreased frequency in

1044 hypertensive animals upon a challenge with a high PACAP concentration (10 μ M). **C.** Plot

1045 summarizing the effects of PACAP and the difference between SHRs and WKY rats, in

1046 particular at high PACAP concentration.

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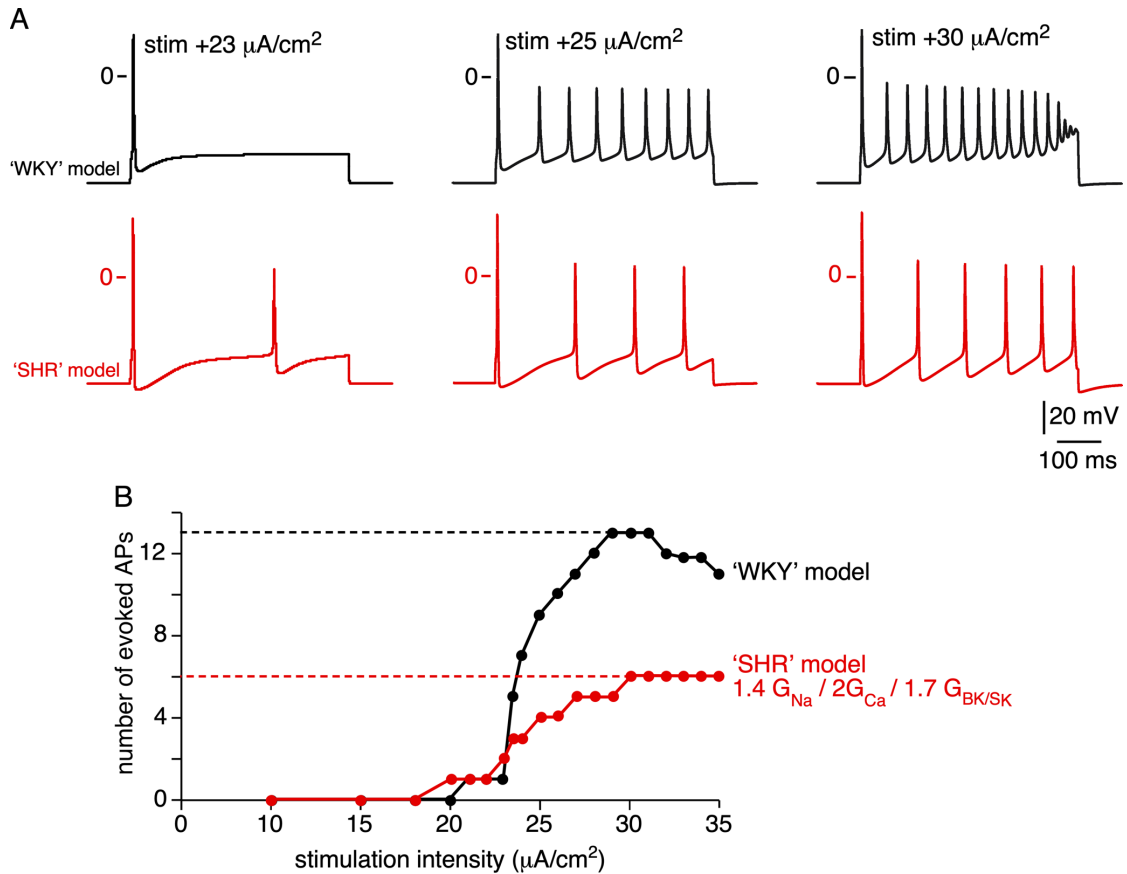
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1050 **Figure 6: Remodeling of transcripts encoding voltage-gated ion channels in SHR.**

1051 Changes in mRNA expression levels were assessed by real-time RT-PCR in macrodissected
 1052 adrenal medullary tissues from 6 WKY rats and 6 SHR. **A.** 3D-histograms illustrating the
 1053 relative expression levels in Nav, Cav and KCa channel genes. **B.** Fold changes in SHR, as
 1054 compared to WKY rat. The highest fold-change refers to *Scn9a* (encoding Nav_v1.7, 11.7-fold
 1055 increase) for the Nav family, *Cacna4a* (encoding Cav_v2.1, >2.8-fold increase) for the Cav
 1056 gene family and *Kcnma1* (encoding KCa_v1.1, 1.9-fold increase) for the KCa gene family. Fold change
 1057 values were determined according to Livak's method⁸⁵. The Shapiro-Wilk test was used to
 1058 analyze the normality of data distribution, and parametric or non-parametric unpaired tests were
 1059 used when appropriate. Fold changes between x0.5 and x1.5 (grey area) are considered
 1060 irrelevant. **C.** Distribution of the channel isoforms in WKY rats and SHR showing that the
 1061 same voltage-gated channel families are present in the two strains, but with different expression
 1062 ratios. Note the significant change for transcripts encoding Nav1 channel family, associated
 1063 with a decrease for *Scn3a* and an increase for *Scn9a*.

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1067 **Figure 7: Numerical simulation of the electrical firing of WKY and SHR chromaffin cells:**

1068 **reduced excitability in SHR model in response to sustained depolarizations. 'WKY' and**

1069 **'SHR' models were built from the rat chromaffin cell numerical simulation developed by**

1070 **Warashina and Ogura³⁰. The 'SHR' condition was simulated by added a multiplier (derived**

1071 **from the transcriptional changes) to Na^+ , Ca^{2+} and Ca^{2+} -dependent K^+ conductances, as such**

1072 **the 'SHR' model = 1.4 G_{Na} , 2 G_{Ca} and 1.7 $G_{\text{SK/BK}}$ 'WKY' model. The stimulation intensity was**

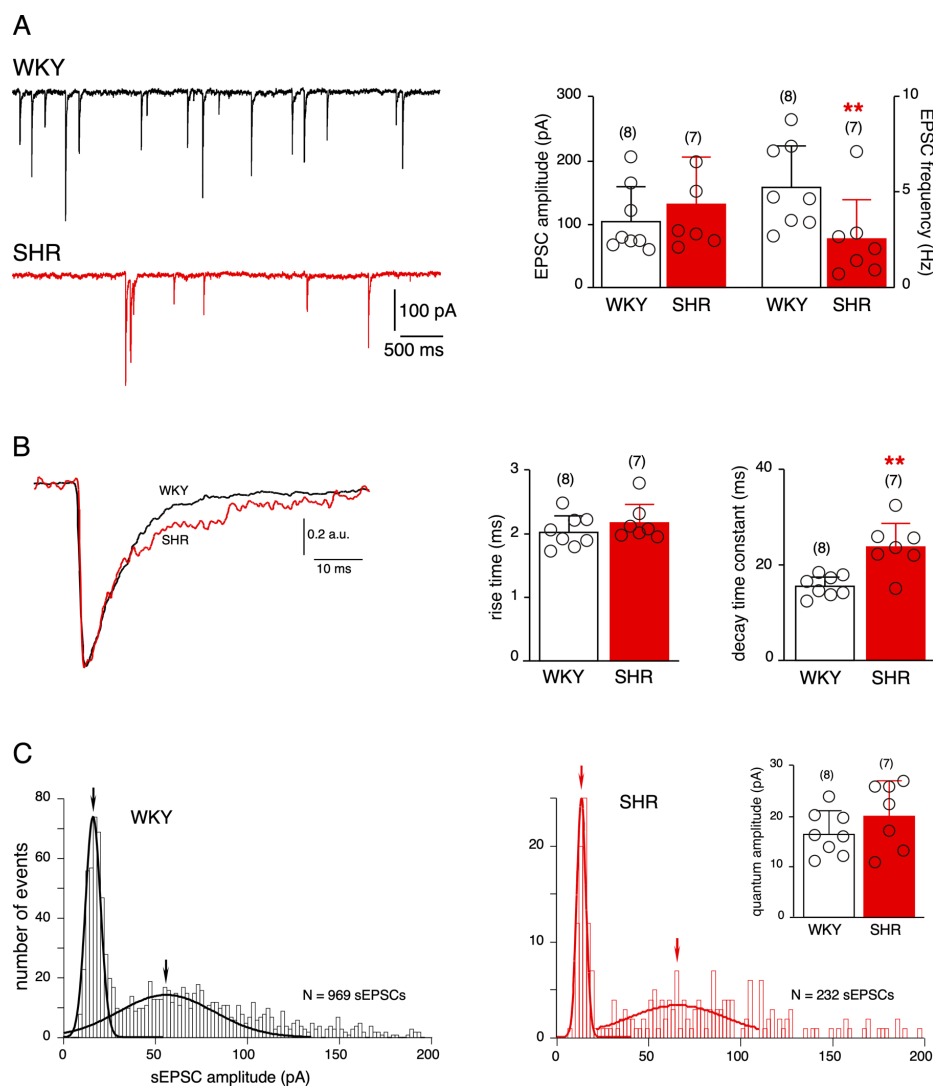
1073 **gradually increased from 10 to 35 $\mu\text{A}/\text{cm}^2$ (500 ms depolarizing steps). A. Representative APs**

1074 **extracted from 'WKY' (black traces) and 'SHR' (red traces) models plotted for three**

1075 **stimulation intensities. B. Pooled data illustrating the reduced cell excitability in the 'SHR'**

1076 **model, in response to robust depolarizations ($>23 \mu\text{A}/\text{cm}^2$).**

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1080 **Figure 8: Changes in the excitatory cholinergic synaptic neurotransmission between**

1081 **splanchnic nerve endings and chromaffin cells in SHRs. A.** Typical chart recordings of

1082 excitatory post-synaptic currents (EPSCs) recorded in WKY (upper trace) and SHR (lower

1083 trace) chromaffin cells voltage-clamped at -80 mV, in response to an 80 mM KCl puff. The

1084 analysis of synaptic transmission shows a significant decrease in EPSC frequency in SHRs

1085 (right histograms). **B.** In regard to EPSC kinetics, superimposed normalized WKY and SHR

1086 EPSCs and associated data histograms illustrate a significant elongated EPSC decay time in

1087 SHRs. **C.** Analysis of EPSC quantal size. Histograms (2 pA bin) illustrate of the distribution of

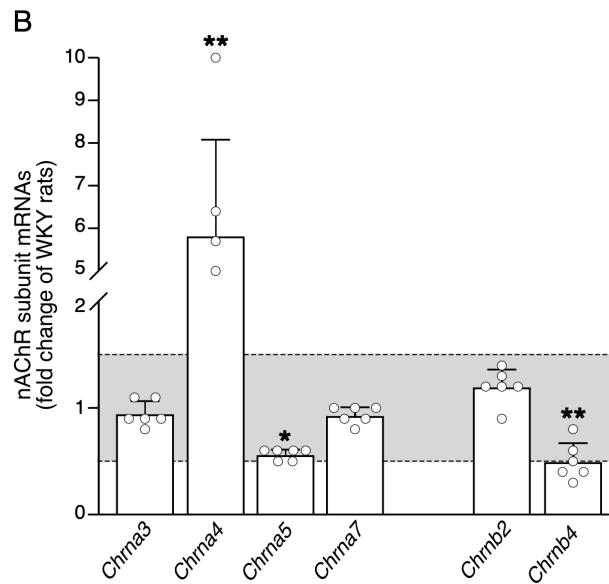
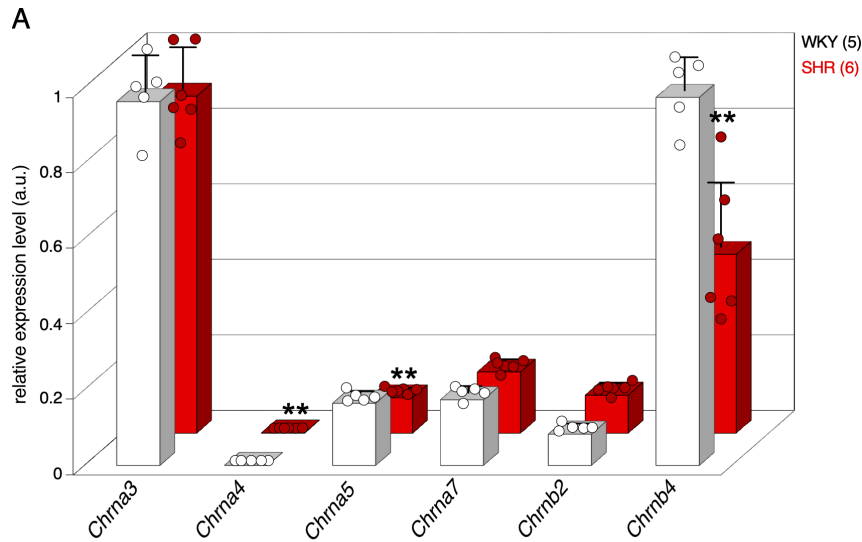
1088 sEPSC amplitudes in a WKY (left) and a SHR (right) chromaffin cells. Quantal size was

1089 estimated from the mean value of the first Gaussian curve fitted to the amplitude distribution

1090 histogram. As summarized from the 8 WKY cells and the 7 SHR cells in which the quantal

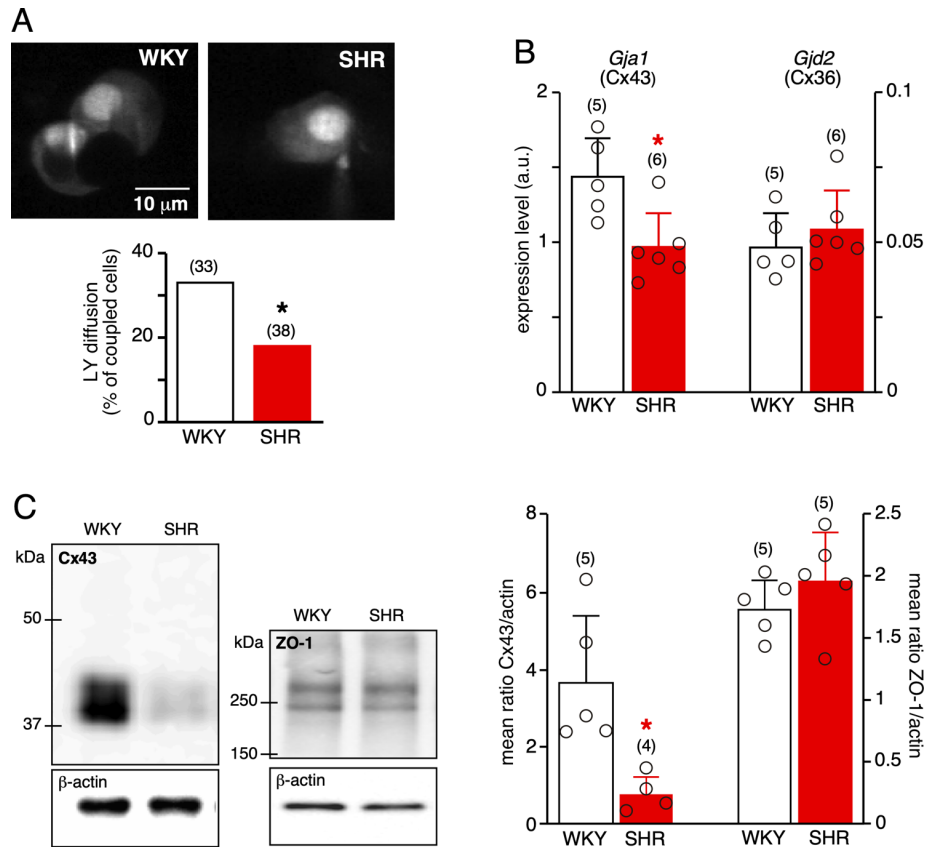
1091 analysis was performed, the EPSC quantal size does not differ between the two strains.

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1095 **Figure 9: Remodeling of transcripts encoding nAChR subunits in SHRs.** The changes in
1096 mRNA expression level of nAChR subunits were assessed by real-time RT-PCR, in
1097 macrodissected WKY (5) and SHR (6) adrenal medullary tissues. **A.** 3D-histograms illustrating
1098 the relative expression levels of four α (*Chrna3*–*5* and *Chrna7*, encoding $\alpha3$, $\alpha4$, $\alpha5$ and $\alpha7$,
1099 respectively) and two β (*Chrnb2* and *Chrnb4*, encoding $\beta2$ and $\beta4$, respectively) subunit genes,
1100 in the two rat strains. **B.** Fold changes in SHRs, as compared to WKY rat. Significant changes
1101 occur for *Chrna4* (5.8-fold), *Chrna5* (0.6-fold) and *Chrnb4* (0.5-fold). Fold change values were
1102 determined according to Livak's method⁸⁵. The Shapiro-Wilk test was used to analyze the
1103 normality of data distribution, and parametric or non-parametric unpaired tests were used when
1104 appropriate. Fold changes between $\times 0.5$ and $\times 1.5$ (grey area) are considered irrelevant.
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1108 **Figure 10: Attenuated gap junction-mediated intercellular communication in SHRs. A.**

1109 Reduced Lucifer yellow (LY) diffusion between SHR chromaffin cells. LY was introduced into

1110 chromaffin cells using patch pipettes. Dye diffusion was imaged 15 minutes after patch

1111 disruption. Less than 20% of SHR chromaffin cells are dye-coupled, as compared to more than

1112 30% in WKY rats. **B.** Decreased expression level of *Gja1* (encoding Cx43), but not *Gjd2*

1113 (encoding Cx36) in SHRs, assessed by real-time RT-PCR from macrodissected medullary

1114 tissues. **C.** Western blots and associated pooled data histograms of Cx43 and ZO-1, an

1115 associated protein. Cx43 expression, but not ZO-1, is significantly down-regulated in SHRs.

SUPPLEMENTARY INFORMATION

Adaptive remodeling of rat adrenomedullary stimulus-secretion coupling in response to a chronic hypertensive environment

Vincent Paillé, Joohee Park, Bertrand Toutain, Jennifer Bourreau, Pierre Fontanaud, Frédéric De Nardi, Claudie Gabillard-Lefort, Dimitri Bréard, David Guilet, Daniel Henrion, Christian Legros and Nathalie C. Guérineau

Table S1: Passive membrane properties of WKY and SHR chromaffin cells. Cells were recorded in the whole-cell configuration of the patch-clamp technique. The number of recorded cells is indicated in parentheses.

| | WKY | SHR | |
|--------------------------------|-----------------------|----------------------|------------------------------|
| Passive membrane properties | Mean \pm SD | Mean \pm SD | p value (unpaired t test) |
| Resting membrane potential, mV | -64.8 \pm 6.4 (67) | -64.0 \pm 7.4 (66) | 0.4951, ns |
| Input resistance, G Ω | 1.19 \pm 0.36 (68) | 1.53 \pm 0.42 (66) | <0.0001, **** |
| Membrane capacitance, pF | 8.94 \pm 0.67 (104) | 8.74 \pm 0.31 (96) | 0.009, ** |

Table S2: Action potential properties of WKY and SHR chromaffin cells. Depolarization-triggered and spontaneous action potentials were recorded in cells current-clamped at their resting potential. Evoked action potentials were triggered by a depolarizing step (+30 pA above the rheobase and 500 ms duration). For spontaneous APs, the analysis was performed in 30-50 serial APs/cell. All parameters were analyzed by Mini Analysis. The number of recorded cells is indicated in parentheses. *, p<0.05; **, p<0.01; ***, p<0.005, ****, p<0.001.

| | WKY (n = 25 cells) | SHR (n = 23 cells) | |
|--------------------------------------|------------------------------|------------------------------|---------------------------|
| PROPERTIES OF EVOKED APs | mean ± SD | mean ± SD | p value unpaired t test |
| rise time (ms) | 8.25 ± 0.17 | 8.42 ± 0.25 | 0.0086** |
| half-rise time (ms) | 1.18 ± 0.24 | 1.74 ± 0.66 | 0.0003**** |
| 10%-90% rise time (ms) | 3.41 ± 0.54 | 4.09 ± 0.83 | 0.0016** |
| 10%-90% slope (mV/ms) | 16.98 ± 4.67 | 13.44 ± 5.28 | 0.0175* |
| amplitude (mv) | 70.03 ± 7.38 | 62.56 ± 8.48 | 0.0021** |
| half width (ms) | 3.87 ± 1.01 | 5.73 ± 1.95 | 0.0001**** |
| decay time (ms) | 5.18 ± 1.74 | 7.99 ± 2.64 | p<0.0001**** |
| | | | |
| | WKY (n = 10 cells) | SHR (n = 8 cells) | |
| PROPERTIES OF SPONTANEOUS APs | mean ± SD | mean ± SD | p value Mann-Whitney test |
| rise time (ms) | 7.80 ± 0.32 | 8.23 ± 0.23 | 0.0085** |
| half-rise time (ms) | 0.64 ± 0.10 | 0.80 ± 0.10 | 0.0062** |
| 10%-90% rise time (ms) | 1.80 ± 0.33 | 2.50 ± 0.55 | 0.0014** |
| 10%-90% slope (mV/ms) | 41.95 ± 9.86 | 27.41 ± 6.61 | 0.0044** |
| amplitude (mv) | 89.51 ± 6.53 | 79.98 ± 7.78 | 0.0124* |
| half width (ms) | 3.32 ± 0.67 | 5.41 ± 2.16 | 0.0008**** |
| decay time (ms) | 6.00 ± 2.30 | 10.37 ± 6.60 | 0.0085** |

Table S3: Primer sequences used for real-time RT-PCR

| gene name | GenBank accession number | protein name | forward primer (5'-3') | reverse primer (5'-3') |
|------------------|---------------------------------|---------------------|-------------------------------|-------------------------------|
| <i>Scn1a</i> | NM_030875 | Nav1.1 | gttccgacatcgccagtt | catctcagtttcagtagttgtcca |
| <i>Scn2a</i> | NM_012647 | Nav1.2 | tggtgtccctgggttgag | ccttattctgtctcagtagttgtgc |
| <i>Scn3a</i> | NM_013119 | Nav1.3 | gcaccgtccattctaaccat | ttagcttcttgcataagaattgc |
| <i>Scn4a</i> | NM_013178 | Nav1.4 | ggcactgtctcgatttgagg | tcatgatggaggggatagc |
| <i>Scn5a</i> | NM_013125 | Nav1.5 | tgccaccaatgccttgta | catgatgagcatgctaaagagc |
| <i>Scn8a</i> | NM_019266 | Nav1.6 | ggaagttttccatcatgaatcag | gctgttatgtcgggagagga |
| <i>Scn9a</i> | NM_133289 | Nav1.7 | cagcagatgtagaccgactca | actcgtgaactcagcagcag |
| <i>Scn1b</i> | NM_017288 | Nav1b | gcgagatggtgtactgtctac | tcggaagtaatggccaggtat |
| <i>Scn2b</i> | NM_012877 | Nav2b | tggacttaccaggagttagca | ccagcttcaggtgatgatct |
| <i>Scn3b</i> | NM_139097 | Nav3b | ctgataccttgcgagtcactg | catcatgattccgagaccac |
| <i>Scn4b</i> | NM_001008880 | Navv4b | catcctgaagaagaccagagaga | agcagggcctcacactttt |
| <i>Cacna1c</i> | NM_012517 | Cav1.2 | tggctcacagaagtgaaga | agcatttctgccgtgaaaag |
| <i>Cacna1d</i> | NM_017298 | Cav1.3 | ccatgctcactgtgtccag | ctcccatcctatcgcacat |
| <i>Cacna1a</i> | NM_012918 | Cav2.1 | ctgcttgaagaggggacag | ctgacatttggtcccgttg |
| <i>Cacna1b</i> | NM_147141 | Cav2.2 | taagcgcacaccgaatg | ggccaggacaatacagttgg |
| <i>Cacna1e</i> | NM_019294 | Cav2.3 | taccgcgcctggatagac | gctgatgtcccagttttt |
| <i>Cacna1g</i> | NM_031601 | Cav3.1 | catctacttattcttctcatcatcg | aactgcgtggcaatcacc |
| <i>Cacna1h</i> | NM_153814 | Cav3.2 | acggatactctgcagacagga | gctctgtgtagctctgggatgc |
| <i>Cacna1i</i> | NM_020084 | Cav3.3 | tctgcctcaatgttgcacc | aagggtgtctctagggatgt |
| <i>Kcna1</i> | NM_031828 | KCa1.1 | aagtaattccatcaagctggtga | ggccccctgaattctccac |
| <i>Kcna2</i> | NM_019313 | KCa2.1 | tcggaacaccagcgttaagt | cttcacagtccggagcttct |
| <i>Kcna3</i> | NM_019314 | KCa2.2 | tgettgettaccggaataatg | tagcttcttgcactacgg |
| <i>Kcna4</i> | NM_019315 | KCa2.3 | cacgccaagtgcaggaaac | ccatcttgacaccctcagt |
| <i>Kcna5</i> | NM_023021 | KCa3.1 | gcaagattgtctgctgtgc | gccaccacagccaatagtaga |
| <i>Kcna6</i> | NM_021853 | KCa4.1 | ggcaagaaccgaatgaagc | tggtgttaatttgctacgtc |

| | | | | |
|---------------|--------------|-----------------|-------------------------|-------------------------|
| <i>Kcnt2</i> | NM_198762 | KCa4.2 | ccagcaaataatgagattcatgc | acctcgttctcgcctctttctt |
| <i>Chrna3</i> | NM_052805 | α 3nAChR | tccatgctgatgctggg | tcttcgaacaggtactggaaca |
| <i>Chrna4</i> | NM_024354 | α 4nAChR | ccagtacattgcagaccacct | gccacgtattccagtcctc |
| <i>Chrna5</i> | NM_017078 | α 5nAChR | cacgtcgtgaaagagaacga | ccacaaaaacatccgatcaa |
| <i>Chrna7</i> | NM_012832 | α 7nAChR | ggcaaaatgcctaagtggac | cttcatgcgcagaaacct |
| <i>Chrn2</i> | NM_019297 | β 2nAChR | caactcaatggcgctgttc | cactagccgctcctctgtgt |
| <i>Chrn4</i> | NM_052806 | β 4nAChR | tcctctcagctcatctccatc | tgatctgttctcgcctcattca |
| <i>Gjd2</i> | NM_019281 | Cx36 | cggtactgccagctctttgt | caccaccacagtcaacagga |
| <i>Gjal</i> | NM_012567 | Cx43 | cccgacgacaaccagaatg | tggtaatggctggagttc |
| <i>Tjp1</i> | NM_001106266 | ZO-1 | tcaacacatgaagatgggatc | cgcaaaccacactatctcc |
| <i>Gusb</i> | NM_017015 | Gus | ctctgggtggccttacctgat | cagactcaggtgttgcacg |
| <i>Hprt</i> | NM_012583 | Hprt | gaccggttctgtcatgtcg | acctggttcacatcactaatcac |
| <i>Gapdh</i> | NM_017008 | Gapdh | tgggaagctggatcaaac | gcatcacccttctgatgtt |

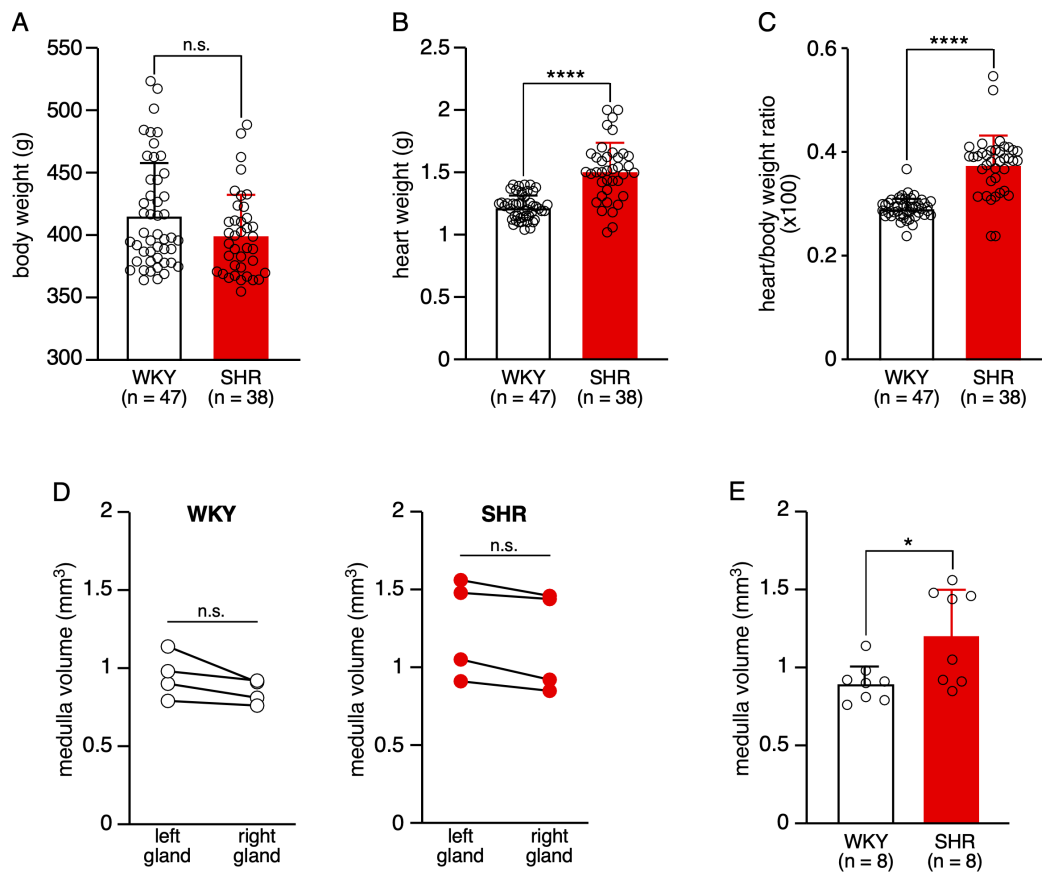


Figure S1: Validation of phenotypic traits of SHR rats compared to WKY rats. **A.** No overt difference in body weight in the two rat strains (401.4 ± 33.3 g, $n = 38$) and WKY rats (417.2 ± 43.6 g, $n = 47$, $p = 0.0689$, unpaired t test). **B.** Significant increase in heart weight in hypertensive animals compared to normotensive animals (1.51 ± 0.23 g, $n = 38$ versus 1.22 ± 0.10 g, $n = 47$ in WKY rats, $p < 0.0001$, unpaired t test). **C.** Enhanced heart/body weight ratio in SHR rats (0.38 ± 0.06 , $n = 38$ versus 0.29 ± 0.02 , $n = 47$ in WKY rats, $p < 0.0001$, unpaired t test). **D-E.** Comparison of the adrenal medulla volume between WKY rats and SHR rats. The medulla volume was calculated from acute slices (see Material and Methods). **D.** No difference between the left and the right adrenals (0.85 ± 0.08 mm³, $n = 4$ and 0.95 ± 0.15 mm³, $n = 4$ for the right and left WKY glands, respectively, $p = 0.125$, and 1.17 ± 0.33 mm³, $n = 4$ and 1.25 ± 0.32 mm³, $n = 4$ for the right and left SHR glands, respectively, $p = 0.125$, Wilcoxon matched-pairs signed-rank test) **E.** Significantly greater medulla volume in SHR rats versus WKY rats. (1.21 ± 0.30 mm³, $n = 8$ glands for SHR rats versus 0.90 ± 0.12 mm³, $n = 8$ glands for WKY rats, $p = 0.0351$, Mann-Whitney test). For each rat, the volume represents the average volume of the two glands.

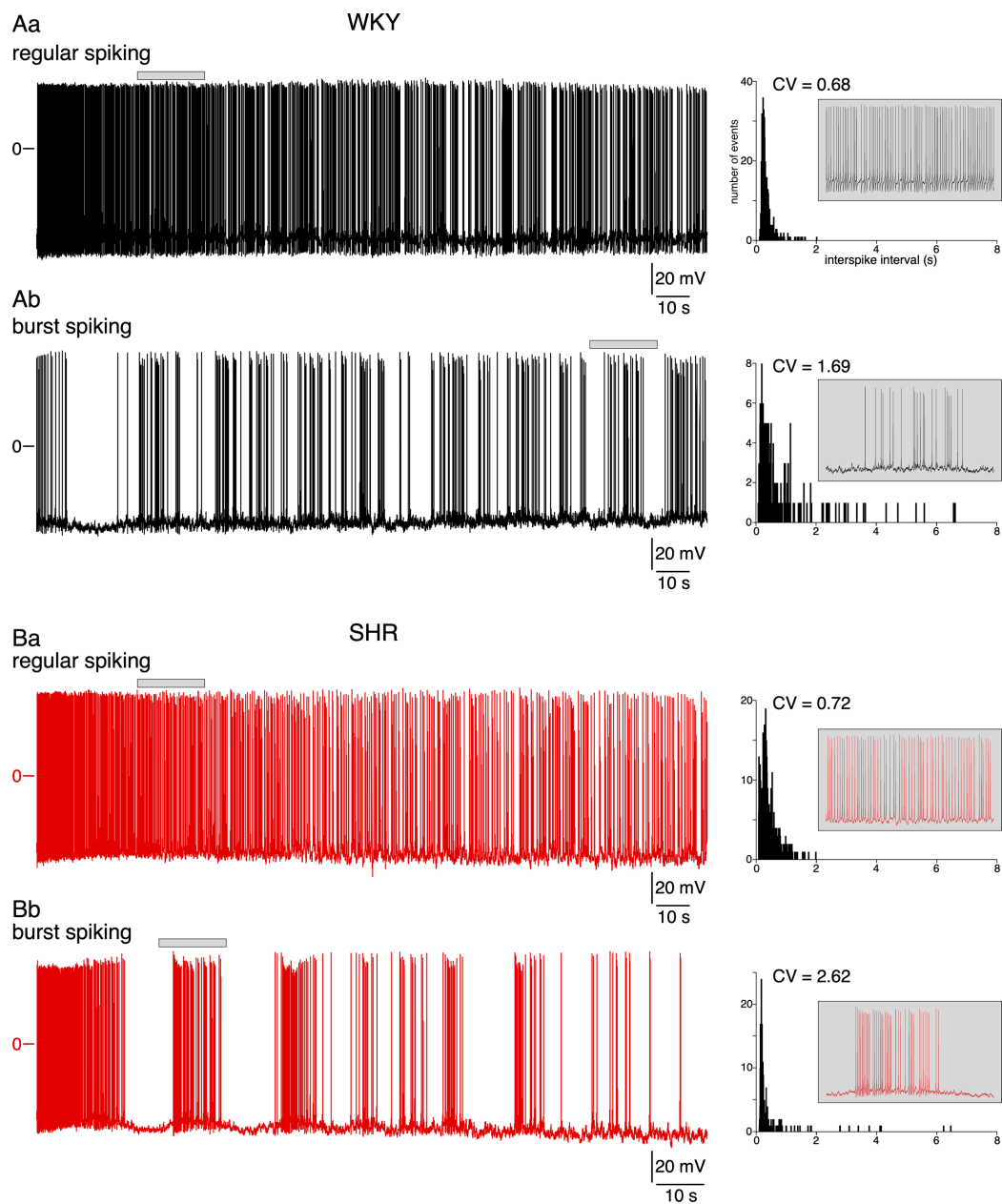


Figure S2: Whole-cell recordings of spontaneously spiking chromaffin cells in adrenal acute slices: occurrence of both regular and burst firing. Action potentials were recorded at resting membrane potential (no current injection), in normotensive (**A**) and hypertensive (**B**) rats. **A.** Representative chart recordings of two cells exhibiting a regular firing (**Aa**) or a burst firing (**Ab**). The histograms on the right illustrate the distribution of the inter-spike intervals (10 ms bin), from which the coefficients of variation (CV) were calculated. Insets: expanded time scale illustrating a 20-s spiking period. **B.** Same recording conditions and analysis performed in two SHR chromaffin cells.

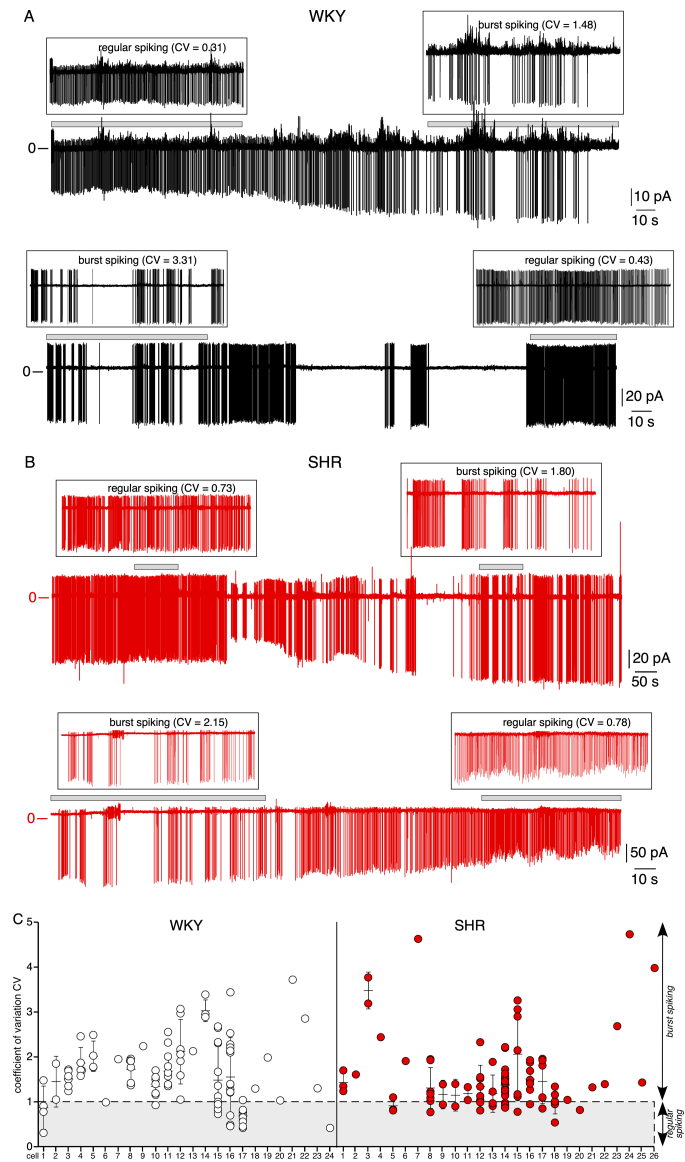


Figure S3: Dynamic switch between regular and bursting spiking patterns in a same chromaffin cell, in WKY rats and SHRs. A. Example of two WKY cells whose spontaneous electrical activity was recorded for 4-5 minutes and in which the spiking pattern switched from a regular to a bursting mode or vice versa. **B.** Same recordings in two SHR chromaffin cells, in which spontaneous action potentials were monitored for 4-20 min. Highlighted sections in A and B: expanded time scale (100 s) showing representative periods during which cells fired either regularly or in bursts. For each 100 s recording period, the distributions of inter-spike intervals were plotted and the associated CV calculated, as indicated in Figure S2. **C.** Distribution of CV values for each individual cell. All CV values were calculated from a 100 s recording (1-15 runs/cell in WKY rats and 1-21 runs/cell in SHRs). Note the wide distribution, both in normotensive and hypertensive animals, not only between cells but also between series of a same cell.

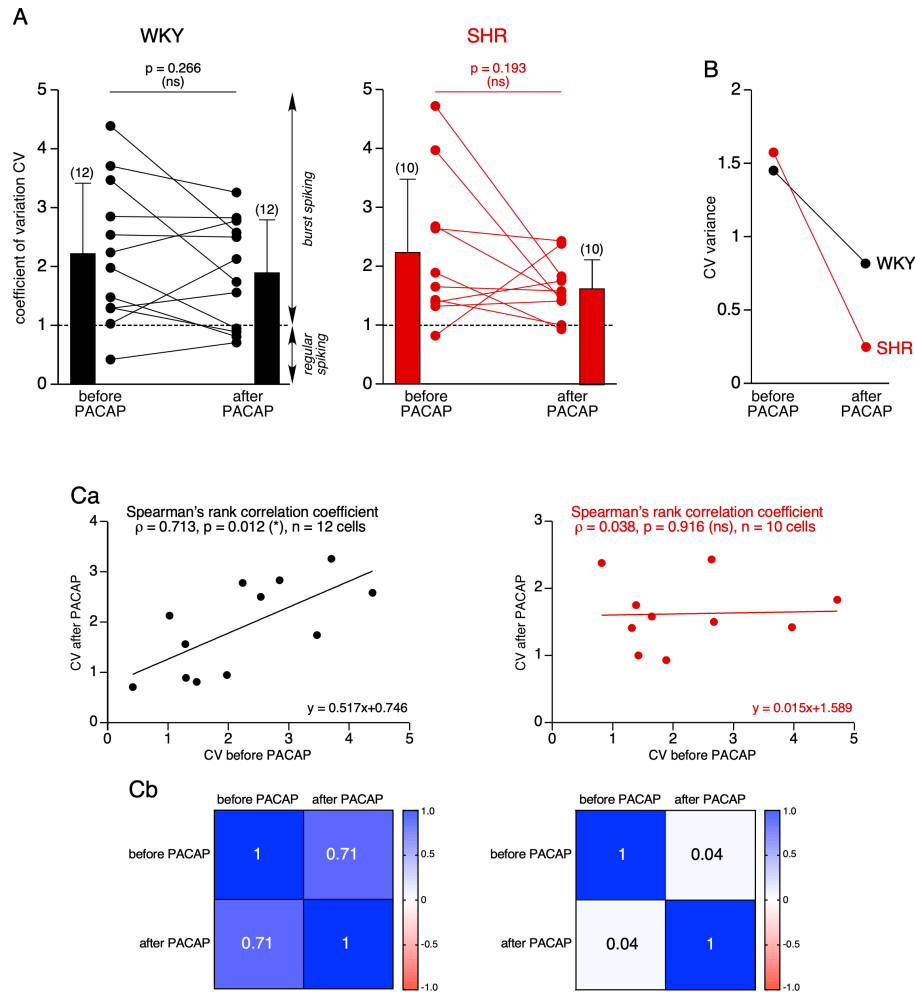


Figure S4: Analysis of CV changes in response to PACAP. Chromaffin cells from WKY rats and SHRs were exposed to 0.1-10 μ M PACAP. Basal and PACAP-stimulated electrical activity were recorded in the loose cell-attached configuration (clamped at 0 mV). **A.** For each cell (12 WKY cells and 10 SHR cells), a CV was calculated for the basal condition (before PACAP) and for PACAP-exposed condition (after PACAP). Although CV tends to decrease in response to PACAP, the result is not significant, neither in normotensive or hypertensive animals (2.22 ± 1.20 before PACAP and 1.89 ± 0.91 after PACAP, $n = 12$ cells for WKY rats, $p = 0.2661$, Wilcoxon matched-pairs signed-rank test and 2.25 ± 1.26 before PACAP and 1.62 ± 0.50 after PACAP, $n = 10$ cells, $p = 0.1934$, Wilcoxon matched-pairs signed-rank test). **B.** Reduced CV variance after PACAP exposure, in particular in SHRs, indicating that PACAP acts by homogenizing the firing pattern. **C.** Loss of the correlation between CV after PACAP and CV before PACAP in SHRs (**Ca**) ($\rho = 0.713$ in WKY rats, $p = 0.0012$, $n = 12$ cells, Spearman's rank correlation coefficient *versus* $\rho = 0.038$ in SHRs, $p = 0.916$, $n = 10$ cells, Spearman's rank correlation coefficient) and associated table of correlation (**Cb**).

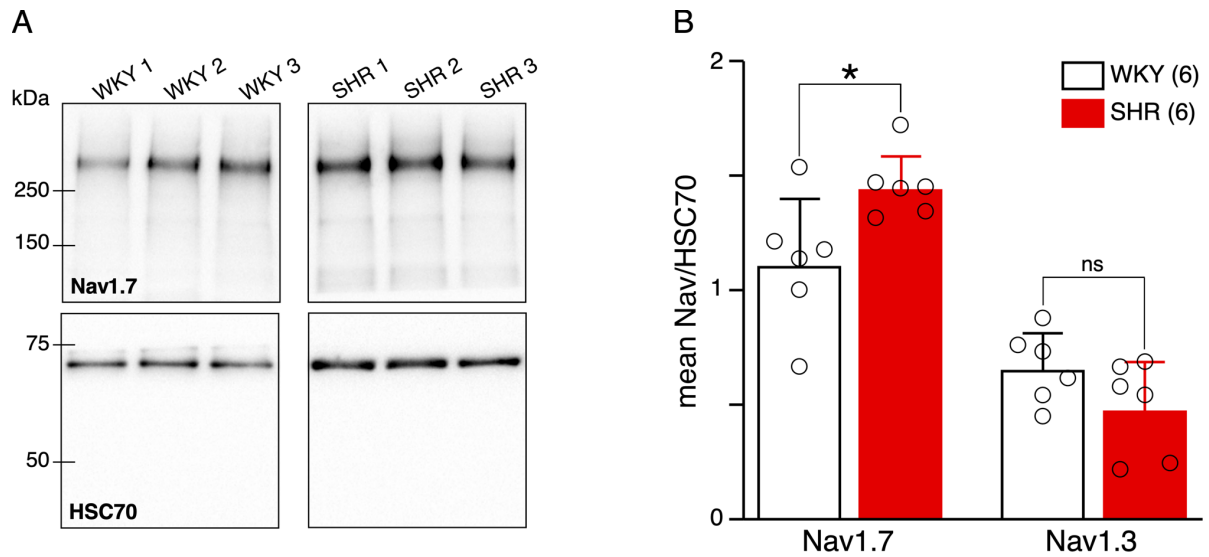


Figure S5: Increased expression of Nav_v1.7 protein in SHRs. The adrenomedullary tissue was macrodissected in 6 WKY rats and 6 SHRs. **A.** Western blot illustration of Nav_v1.7 in 3 WKY rats and 3 SHRs. Intensities of Nav_v1.7 bands were normalized to those of HSC70. **B.** Pooled data showing that Nav_v1.7 protein expression is significantly upregulated in SHRs (Nav_v1.7/HSC70 ratio: 1.11 ± 0.28 for WKY rats, n = 6 and 1.45 ± 0.14 for SHRs, n = 6, p = 0.0411, Mann-Whitney test). By contrast, the expression of Nav_v1.3 protein, the more expressed protein of the Nav family in rat chromaffin cells, is not modified in hypertensive rats compared to normotensive rats.

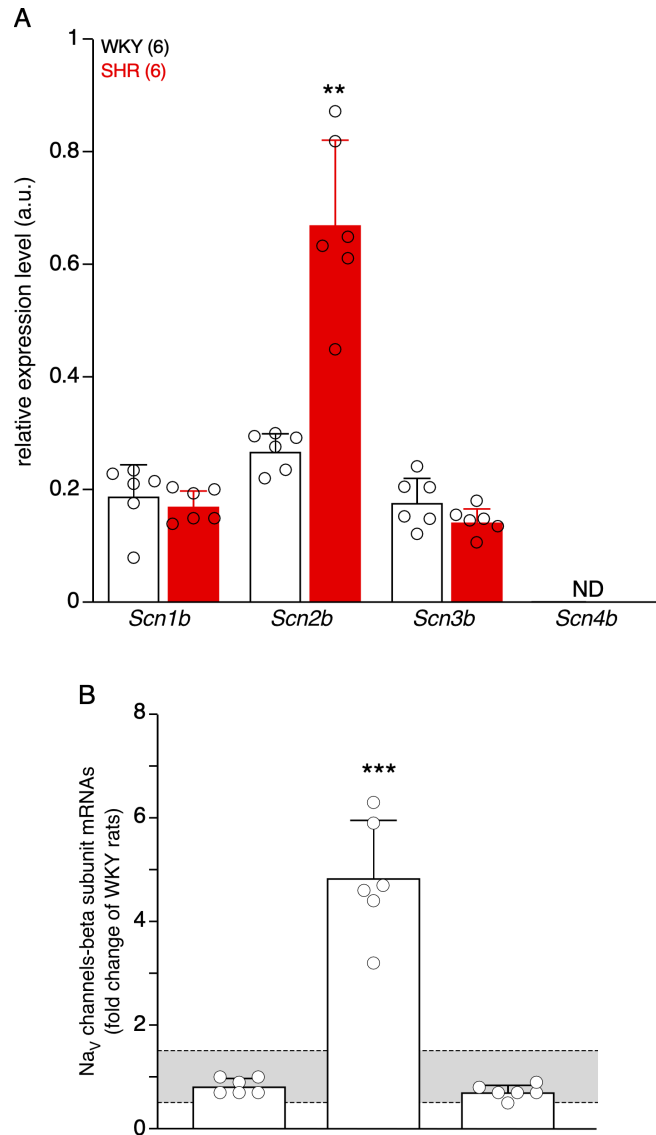


Figure S6: Expression changes in transcripts encoding auxiliary β subunits of voltage-gated Na^+ channels in SHR rats. Changes in mRNA expression levels were assessed by real-time RT-PCR in macrodissected adrenal medullary tissues from 6 WKY rats and 6 SHR rats. **A.** Of the four existing β subunits, only transcripts encoding $\beta 1$ (*Scn1b*), $\beta 2$ (*Scn2b*) and $\beta 3$ (*Scn3b*) were detected, with a significant increase for *Scn2b* (relative expression of 0.27 ± 0.03 for WKY rats, $n = 6$ and 0.67 ± 0.15 for SHR rats, $n = 6$, $p = 0.0022$, Mann-Whitney test). The *Scn4b* mRNA encoding $\beta 4$ subunit was not detected (ND). **B.** Fold changes in SHR rats, as compared to WKY rat. A significant change occurs for the gene encoding $\text{Nav}\beta 2$ subunit (4.8-fold). Fold change values were determined according to Livak's method (see Material and Methods). The Shapiro-Wilk test was used to analyze the normality of data distribution, and parametric or non-parametric unpaired tests were used when appropriate. Fold changes between $\times 0.5$ and $\times 1.5$ (grey area) are considered irrelevant.

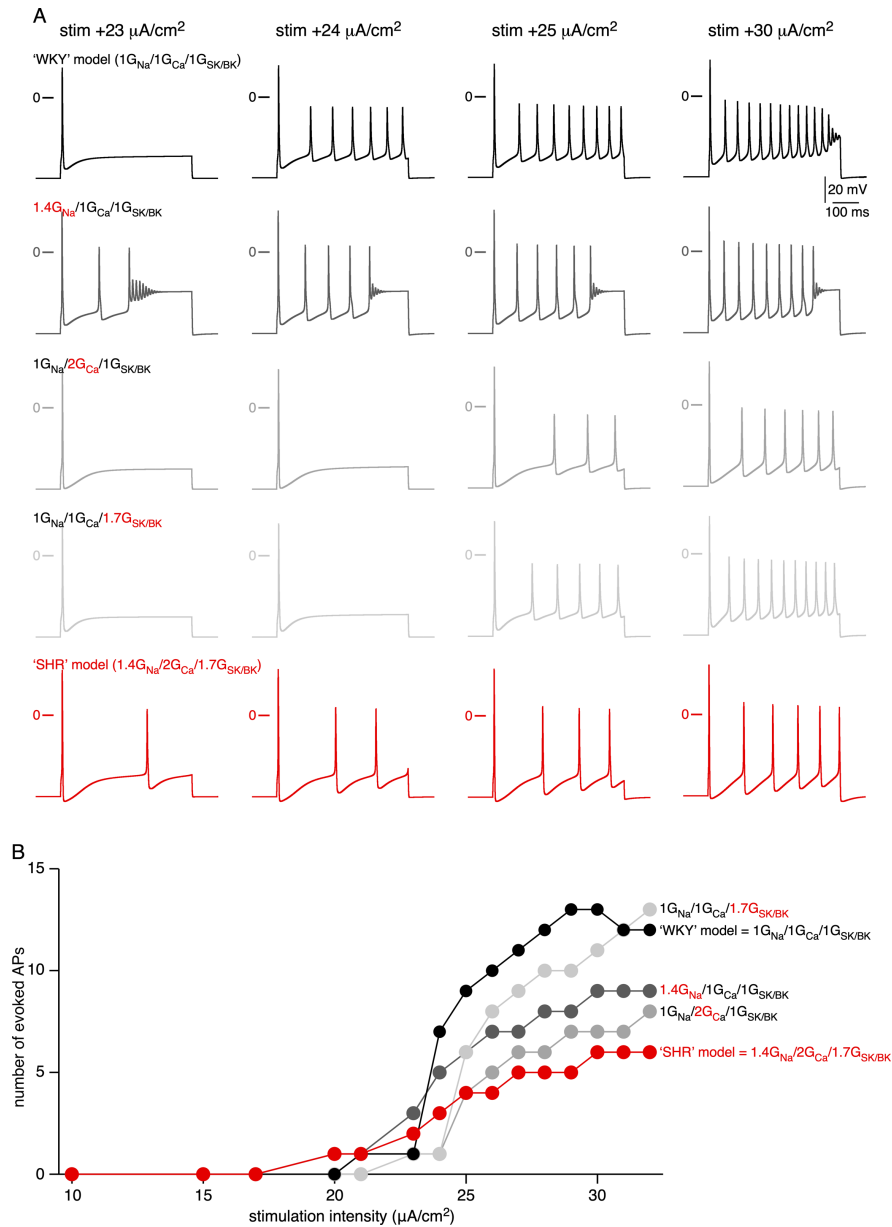


Figure S7: Impact of changes in G_{Na} , G_{Ca} and G_{SK} conductances on the electrical firing of computed rat chromaffin cells. 'WKY' and 'SHR' models were built from the rat chromaffin cell numerical simulation developed by Warashina and Ogura. **A.** Electrical firing evoked at four stimulation intensities (from 23 to 30 $\mu\text{A}/\text{cm}^2$, 500 ms duration). The 'SHR'-like condition (red traces) corresponds to $1.4G_{\text{Na}}$, $2G_{\text{Ca}}$ and $1.7G_{\text{SK/BK}}$ 'WKY' model (see Material and Methods). In the intermediate conditions (light, medium and dark grey traces), either G_{Na} , G_{Ca} or $G_{\text{SK/BK}}$ were modified by their respective multiplication coefficients, leaving the two other conductance unchanged. **B.** Pooled data showing that the most pronounced reduction in AP frequency is found when G_{Na} , G_{Ca} and $G_{\text{SK/BK}}$ are modified simultaneously, particularly at high stimulation intensities.