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Sodium background currents in endocrine/neuroendocrine cells: towards unraveling channel identity and contribution in hormone secretion

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Short title: Role of sodium leak currents in hormone secretion

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25 **Abstract**

26 In endocrine/neuroendocrine tissues, excitability of secretory cells is patterned by the repertoire
27 of ion channels and there is clear evidence that extracellular sodium (Na^+) ions contribute to
28 hormone secretion. While voltage-gated channels involved in action potential generation are
29 well-described, the background 'leak' channels operating near the resting membrane potential
30 are much less known, and in particular the channels supporting a background entry of Na^+ ions.
31 These background Na^+ currents (called here ' I_{Nab} ') have the ability to modulate the resting
32 membrane potential and subsequently affect action potential firing. Here we compile and
33 analyze the data collected from three endocrine/neuroendocrine tissues: the anterior pituitary
34 gland, the adrenal medulla and the endocrine pancreas. We also model how I_{Nab} can be
35 functionally involved in cellular excitability. Finally, towards deciphering the physiological
36 role of I_{Nab} in endocrine/neuroendocrine cells, its implication in hormone release is also
37 discussed.

38

39 **1. Introduction**

40 Hormone secretion is a crucial physiological process for maintaining body homeostasis and
41 preserving vital functions. To secure an optimized secretion in response to organismal demands
42 and needs, finely regulated mechanisms are required. It is therefore indisputable that any
43 alteration targeting the molecular, cellular, or tissular mechanisms involved in the regulation of
44 secretion will have functional consequences at the organism level. Hormone release is ensured
45 by endocrine/neuroendocrine cells, whose major feature is their ability to generate action
46 potentials (APs), either spontaneously or evoked in response to a stimulus. AP-mediated
47 electrical activity is one of the crucial mechanisms affiliated to cell excitability. Through the
48 opening of voltage-dependent ion channels during the depolarization phase, AP promotes a
49 transient increase in the cytosolic free calcium concentration ($[\text{Ca}^{2+}]_i$), as reported in hormone-

50 secreting pituitary cells (Schlegel et al., 1987), in insulin-secreting cells (Theler et al., 1992) or
51 in catecholamine-secreting cells (Martin et al., 2001). Because a rise in $[Ca^{2+}]_i$ is a prerequisite
52 for hormone exocytosis, any change in cell excitability, targeting for example AP waveform,
53 AP frequency or discharge pattern can likely impact on the secretory process. While plentiful
54 literature has focused on the nature of ion channels solicited during the rising and the decay
55 phases of APs in endocrine/neuroendocrine cells (reviewed in (Fletcher et al., 2018; Lingle et
56 al., 2018; Stojilkovic et al., 2010; Yang et al., 2014)), the studies dedicated to elucidating the
57 nature of ion channels contributing to the regulation of the resting membrane potential (RMP)
58 in these cells appear comparatively much less abundant. The electrical events occurring at RMP
59 are actually decisive for the subsequent triggering of APs and the patterning of the firing
60 discharges. The unique biophysical properties of ion channels operating close to RMP enable
61 them to regulate cell membrane fluctuations, which makes these channels critical components
62 of cell firing discharges and subsequent $[Ca^{2+}]_i$ signals. These channels likely act as a lever on
63 cell excitability and thus may have a crucial role in regulating cell function.

64 RMP can be defined as the difference in the electrical potential across the plasma
65 membrane when a cell is in a non-excited state, *i.e.* at rest, contrasting with the excited state got
66 during AP discharges. In the resting state, the permeability of the plasma membrane is much
67 greater for K^+ ions than for Na^+ ions, accounting for a negative RMP value, close but very
68 importantly, not equal to the K^+ equilibrium potential and far from the Na^+ equilibrium potential
69 (Enyedi & Czirjak, 2010). Maintaining a balance between inward and outward currents at RMP
70 provides a valuable mechanism for tuning cell excitability. Various membrane ionic
71 components, including channels and electrogenic pumps, work collectively to maintain an
72 elevated intracellular K^+ concentration, contributing thus to stabilize a negative RMP. The
73 Na^+/K^+ pump achieves this task by extruding 3 Na^+ ions from the cell and bringing 2 K^+ ions
74 into the cell in exchange. In that function, the Na^+/K^+ pump is assisted by specific K^+ channels

75 that are open at potentials close to RMP. These channels are responsible for a "leak" or a
76 "background" K^+ conductance. In neurons, a large fraction of resting K^+ conductances appears
77 to be due to the two-pore domain K^+ (K2P) channels, which are K^+ selective (at least under
78 physiological ionic conditions) and reverse near the K^+ equilibrium potential (Enyedi &
79 Czirjak, 2010; Feliciangeli et al., 2015). Besides the Na^+/K^+ pump and K2P channels, it appears
80 that a variety of ion channels contribute to the RMP, although not primarily involved in that
81 role, e.g. the voltage-gated Ca^{2+} channels $Ca_v1.3$, the voltage-gated K^+ channels K_v7 and the
82 Ca^{2+} -dependent K^+ channels SK and BK in adrenal chromaffin cells (Vandael et al., 2010;
83 Lingle et al., 2018), and SK/BK channels in anterior pituitary endocrine cells (Shipston, 2018).
84 Changes in the expression or the availability of those channels modify the RMP and the ability
85 of chromaffin cell to fire (Wallace et al., 2002; Marcantoni et al., 2010; Vandael et al., 2010;
86 Vandael et al., 2012; Scharinger et al., 2015). As such, ionic mechanisms involved in setting
87 the RMP (often reviewed as "leak mechanisms") are multiple, demonstrating the multifaceted
88 and challenging issue to elucidate the regulatory processes.

89 The "leak" designation is not recent, as it appeared very early, in the 50's, with the pioneer
90 works of Hodgkin and Huxley on the electrical behavior of the giant axon of *Loligo* (Hodgkin
91 & Huxley, 1952a; Hodgkin & Huxley, 1952b). Indeed, in their original model describing the
92 electrical circuit of an excitable membrane and published in 1952, Hodgkin and Huxley
93 represented the membrane electrical behavior as a network of various structural parameters,
94 that are membrane capacitance and ion conductances including sodium (g_{Na}), potassium (g_K)
95 and **leak (g_l) conductances** (Hodgkin & Huxley, 1952b). More interestingly, in the same
96 article, the authors wrote "*Our equations could easily be modified to increase the resting*
97 *potential (e.g. by reducing the leak conductance and adding a small outward current*
98 *representing metabolic extrusion of sodium ions)*". This statement clearly argues for a role of
99 the leak conductance in regulating RMP. When Hodgkin and Huxley published their series of

100 articles in *The Journal of Physiology* in 1952, the ionic nature of the "leak conductance" was
101 not fully known, although the authors anticipated "*chloride and other ions*" (Hodgkin &
102 Huxley, 1952b). As mentioned above, the ionic selectivity of the background conductances
103 contributing to maintain RMP is mainly, but not exclusively, supported by K^+ ions. The fact
104 that the RMP value is distinctively positive to the theoretical K^+ equilibrium potential argues
105 for the participation of depolarizing conductances. A plausible depolarizing ionic component
106 assisting K^+ conductances in determining RMP is the Na^+ ion. Na^+ -sensitive conductances are
107 involved at rest, as evidenced by early findings showing that changes in the extracellular Na^+
108 concentration influence the RMP value (Hodgkin & Katz, 1949; Huxley & Stampfli, 1951).

109 By taking the anterior pituitary gland, the endocrine pancreas and the adrenal medulla as
110 established examples of endocrine/neuroendocrine tissues and their respective
111 secretory/neurosecretory cells as remarkable examples of excitable cells, this review addresses
112 the role of Na^+ -permeant background ion conductances that are operating at rest in determining
113 RMP and regulating AP discharges. Contrasting with a comprehensive literature on the role of
114 K^+ background currents (*i.e.* K2P channels as molecular determinant) in cell excitability
115 (Besana et al., 2005; Enyedi & Czirjak, 2010; Feliciangeli et al., 2015) and hormone secretion
116 (Bandulik et al., 2010; Enyeart, 2005; Kim & Kang, 2015; Tse et al., 2012), little is known on
117 the contribution of Na^+ background currents to endocrine/neuroendocrine tissue function. This
118 statement is notably accurate in the adrenal gland, in which despite extensive studies, ionic
119 requirement for stimulus-secretion coupling in adrenal chromaffin cells remains open to
120 discussion.

121

122 **2. Sodium background conductances: consequences on endocrine/neuroendocrine cell**
123 **excitability**

124 Na⁺ ions are major contributors to cell excitability, not only through voltage-gated (Na_v)
125 channels which are involved in AP generation and propagation, but also through background
126 channels involved in the modulation of RMP (see Fig. 1). The mean RMP value of
127 endocrine/neuroendocrine cells (around -50 mV to -60 mV) is positive to the equilibrium
128 potential for K⁺ ions, thus suggesting the contribution of a depolarizing component. The
129 presence of a resting Na⁺ permeability in endocrine/neuroendocrine tissues has early been
130 suspected (Brandt et al., 1976; Douglas et al., 1967; Ribalet & Beigelman, 1982), but remains
131 poorly studied.

132 Indicative of the intricate role of Na_v channels in excitability of
133 endocrine/neuroendocrine tissues is the finding that the electrical firing of hormone-producing
134 pituitary cells, adrenal chromaffin cells and pancreatic beta cells is distinctively impacted by
135 tetrodotoxin (TTX), the canonical toxin used to block Na_v channels (Table 1). In endocrine
136 pituitary cells, TTX (0.3 to 300 μM) does not inhibit APs although these cells (at least
137 somatotrophs, lactotrophs and gonadotrophs) express TTX-sensitive Na_v channels (Van Goor
138 et al., 2001b). In agreement with this finding, basal release of GH, PRL and LH is insensitive
139 to TTX (Van Goor et al., 2001a). Contrasting with pituitary cells, TTX (10 nM to 6 μM) reduces
140 (or even fully blocks) AP amplitude in adrenal chromaffin cells. In pancreatic beta cells, TTX
141 effect is less manifest, showing either inhibition or no effect. In addition, it is noteworthy that
142 the effect of TTX on spontaneous or evoked AP firing is sex-independent as all cases (total
143 blockade, partial blockade or no blockade) are observed in both males and females.
144 Interestingly, in the three endocrine/neuroendocrine tissues reviewed here, the fact that AP
145 firing discharges may not be fully blocked by TTX states that Na_v channels (at least those that
146 are sensitive to TTX such as Na_v1.3 and Na_v1.7, which are especially expressed in secretory
147 cells) do not dominantly contribute to pacemaking. This indicates that other channels play this
148 role. More generally, the data collected in Table 1 attest to the differential contribution of Na⁺

149 ions in APs in endocrine/neuroendocrine cells and point to the composite nature of channels
150 involved in APs.

151 The experimental protocol used in routine during electrophysiological recordings to
152 uncover a Na⁺-sensitive background conductance consists of challenging cells (usually current-
153 clamped at RMP) with a Na⁺-deprived (or low Na⁺) extracellular medium. This is achieved by
154 replacing NaCl with an equimolar concentration of NMDG (N-Methyl-D-Glucamine)-Cl, Tris-
155 Cl, choline-Cl, lithium-Cl, TMA (tetramethylammonium)-Cl or sucrose. Tables 2-4 summarize
156 the effects of an external Na⁺ replacement on the membrane potential of secretory pituitary
157 cells, adrenal chromaffin cells and pancreatic beta cells, respectively. Collectively, the
158 replacement of extracellular Na⁺ converges toward a robust membrane hyperpolarization (>10-
159 20 mV) and subsequent abrogation of APs (highlighted lines in Tables 2-4). Interestingly, this
160 effect is found both in males (lines highlighted in blue) and females (lines highlighted in pink),
161 suggesting, at least through an overall analysis, little or no sex effect. However, this conclusion
162 should be taken with caution, as biases may occur by the fact that i) the sex of the animals is
163 not always mentioned and ii) the number of experiments carried out in males and females can
164 strongly differ (see Tables 2 and 4, in which most of the data were obtained in females). In
165 addition, most of studies carried out in females do not contain information regarding the
166 hormonal status and the phase of the estrous cycle, which precludes a detailed analysis of the
167 involvement of sex hormones in the effect on membrane potential. When analyzed individually,
168 compound by compound, tissue by tissue, cell type by cell type, the effects of an external Na⁺
169 replacement show a certain degree of variability. So many parameters, such as the species (rat,
170 mouse, calf, gerbil), the strain (Wistar vs Sprague-Dawley, C57BL/6J vs C57BL/6JN...), the
171 sex of animals, the age of animals (neonate, young, adult, aged), the hormonal status of the
172 animal (virgin, lactating), the cell phenotype (normal vs tumoral), the recording technique
173 (intracellular, conventional vs perforated patch-clamp), the composition of the intracellular

174 medium (KCl, K-acetate, K-gluconate...), the endocrine/neuroendocrine tissues (anterior
175 pituitary, adrenal gland, pancreas), the secretory cell type (pancreatic beta cells, chromaffin
176 cells, lactotrophs, somatotrophs, gonadotrophs...), the compound used to substitute Na^+
177 (NMDG, Tris, choline, lithium ...)... can account for the heterogeneity in the results, so that it
178 is illusory to draw up a summary scheme at the present time, but coming studies should certainly
179 consider these aspects.

180 Beyond this, and considering the hyperpolarizing response as the most frequently
181 observed response found upon lowering the extracellular Na^+ concentration, it is then obvious
182 that a Na^+ permeant background conductance operates at RMP and maintains a relatively
183 depolarized RMP. Representative examples of low Na^+ -induced hyperpolarization in a rat and
184 a mouse chromaffin cell are plotted in Fig. 2A. Altogether, this shows that the membrane of
185 pituitary secretory cells, adrenal chromaffin cells and pancreatic beta cells is permeant to Na^+
186 at rest and that a Na^+ -sensitive component persists at RMP and can therefore contribute to
187 establish the RMP value. Note that a persistent Na^+ -permeant conductance involved in setting-
188 up the RMP value and in the ability of cells to fire has been also described in excitable cells
189 other than endocrine/neuroendocrine cells (Atherton & Bevan, 2005; Eggermann et al., 2003;
190 Jones, 1989; Khaliq & Bean, 2010; Kiyosue et al., 1993; LeSauter et al., 2011; Noble et al.,
191 1992; Pena & Ramirez, 2004; Raman et al., 2000; Reinl et al., 2015; Spindler et al., 1998). A
192 similar Na^+ conductance is also found in invertebrates (Flourakis et al., 2015; Gao et al., 2015;
193 Gouwens & Wilson, 2009; Lu & Feng, 2011; Xie et al., 2013), clearly pointing a crucial and
194 conserved role of this conductance in regulating cell and tissue function in the animal kingdom.
195 According to the studies, the Na^+ background conductance was alternatively named I_{Nab} , I_{BNC} ,
196 $I_{\text{b,Na}}$, $I_{\text{NS,Na}}$, I_{NaP} or $I_{(\text{L-Na})}$. To designate it simply, the more frequently used " I_{Nab} " nomenclature
197 will be used in this review.

198

199 **3. Biophysical and pharmacological properties of I_{NaB}**

200 Compared to the large number of studies that have investigated the effect of Na^+ reduction
201 and/or deprivation on cell membrane potential, very few of them have characterized the
202 current/channel underlying cell hyperpolarization. To date and despite the cloning of a large
203 number of channels, the molecular characterization of channels potentially involved in Na^+
204 background conductance still remains elusive.

205

206 *3.1. Reduction of a depolarizing conductance*

207 The studies performed in cells voltage-clamped at a potential near RMP show that an
208 application of a low $[Na^+]$ - or a Na^+ free-containing extracellular saline induces a shift in the
209 holding current baseline as such it becomes more positive with the appearance of an outward
210 current. Such a change in holding current has been reported in the anterior pituitary, in both
211 tumoral (Fonteriz et al., 2002; Kucka et al., 2010; Kucka et al., 2012; Simasko, 1994; Simasko
212 & Sankaranarayanan, 1997; Tomic et al., 2011) and in normal secretory cells, including
213 lactotrophs (Kucka et al., 2010; Kucka et al., 2012; Sankaranarayanan & Simasko, 1996; Tomic
214 et al., 2011), somatotrophs (Kucka et al., 2010; Tomic et al., 2011), gonadotrophs (Kucka et
215 al., 2010; Tomic et al., 2011) and corticotrophs (Liang et al., 2011). Note that thyrotrophs have
216 not yet been investigated for the presence of a Na^+ background current. In the intermediate
217 pituitary lobe, melanotrophs also exhibit a change in holding current in response to low Na^+ -
218 containing saline (Kayano et al., 2019). Adrenal catecholamine-secreting cells also respond to
219 a bath-applied low Na^+ -containing saline by an outwardly directed shift in the holding current
220 (Milman et al., 2021). As illustrated in Fig. 2B, the shift in the holding current is found in both
221 rats (left panel) and mice (right panel), and in both males (left panel) and females (right panel).
222 From a theoretical point of view, a membrane hyperpolarization can result either from the
223 activation of a hyperpolarizing conductance or from the blockade/decrease of a depolarizing

224 one. Two experimental findings make highly plausible the explanation of the shift in baseline
225 current by the reduction of a background depolarizing conductance. First, the extracellular Na^+
226 replacement is associated with a decrease in the slope of the linear regression calculated from
227 the I/V curve data points (Milman et al., 2021) (see Fig. 2C, left panel for an example in a male
228 rat and right panel for an example in a female mouse). Second, a decrease in the noise of
229 recordings during Na^+ replacement (Kucka et al., 2012; Liang et al., 2011; Milman et al., 2021;
230 Sankaranarayanan & Simasko, 1996; Tomic et al., 2011) (see Figs. 2A and 2C) is consistent
231 with a change in the current flow through each open channel, due to the reduction in the driving
232 force.

233

234 3.2. *Voltage dependency*

235 In mouse pancreatic beta cells, a nonselective cationic conductance, mostly carried by Na^+ ions,
236 has been reported as voltage-independent and non-inactivating in the range of physiological
237 potentials (Worley et al., 1994). In agreement with this, a linear I/V relationship has been
238 reported for I_{Nab} in mouse chromaffin cells (Milman et al., 2021), in tumoral GH3 pituitary cells
239 (Simasko, 1994), in primary lactotrophs (Sankaranarayanan & Simasko, 1996) and somatrophs
240 (Fonteriz et al., 2002). When implemented in mathematical models, I_{Nab} is depicted as a linear
241 voltage-independent current (Fridlyand et al., 2009; Liang et al., 2011).

242

243 3.3 *Ionic selectivity*

244 As mentioned before, the canonical protocol used to unveil a Na^+ background conductance is
245 to reduce the extracellular concentration of Na^+ ions, which are replaced by NMDG or Li^+ ions,
246 or by Tris or choline or sucrose. Under these experimental conditions, cells are stimulated
247 through voltage ramp or voltage step commands. The subtraction of the current recorded in low
248 Na^+ -containing saline from the control current recorded in a standard Na^+ -containing reveals

249 I_{Nab} . The subsequent determination of the reversal potential (E_{rev}) of I_{Nab} is a strong indication
250 of the ionic species that could contribute to the current. In this context, a current that would be
251 purely carried by Na^+ ions would display a very positive E_{rev} , close to the theoretical
252 equilibrium potential of Na^+ ions ($\sim +60$ mV). Far from this value, the calculated E_{rev} value of
253 I_{Nab} ranges from -2 to -30 mV ((Fonteriz et al., 2002; Leech & Habener, 1997; Leech &
254 Habener, 1998; Milman et al., 2021; Worley et al., 1994) and see Fig. 2C for examples in a rat
255 and mouse adrenal chromaffin cell, in male and female). This reflects the non-selective cationic
256 nature of I_{Nab} with the sequential permeability as such $P_{\text{Na}} > P_{\text{K}} > P_{\text{Cs}} > P_{\text{Ca}}$ (Lu et al., 2007),
257 $P_{\text{Na}} > P_{\text{K}} > P_{\text{Cs}}$ (Milman et al., 2021) and $P_{\text{Na}} > P_{\text{Ca}}$ (Leech & Habener, 1997).

258

259 3.4. Sensitivity to TTX

260 Data collected from endocrine pituitary (Fonteriz et al., 2002; Kayano et al., 2019; Kucka et
261 al., 2012; Liang et al., 2011; Sankaranarayanan & Simasko, 1996; Simasko, 1994; Tomic et al.,
262 2011; Zemkova et al., 2016) and chromaffin cells (Milman et al., 2021) show that low Na^+ -
263 evoked hyperpolarization is not mimicked and not blocked by TTX, demonstrating that the
264 effect of replacing Na^+ ions is not due to loss of TTX-sensitive Na^+ current.

265

266 3.5. Sensitivity to Cs^+

267 I_{Nab} is not blocked by Cs^+ , as evidenced by the persistence of the positive shift in the holding
268 current elicited in response to extracellular Na^+ replacement (Kayano et al., 2019; Kucka et al.,
269 2012; Milman et al., 2021). In addition, the change in the E_{rev} of I_{Nab} observed when cells are
270 recorded in presence of Cs^+ (compared to standard K^+ -containing saline) indicates that I_{Nab} is
271 permeant to Cs^+ (Milman et al., 2021).

272

273 3.6. Sensitivity to Ca^{2+}

274 I_{Nab} is sensitive to changes in extracellular Ca^{2+} . While it is blocked by physiological
275 concentrations of extracellular divalent cations with $\text{Ca}^{2+} > \text{Mg}^{2+}$ and Ba^{2+} , I_{Nab} is potentiated by
276 lowering external Ca^{2+} concentration (Bouasse et al., 2019; Chua et al., 2020; Impheng et al.,
277 2021; Lee et al., 2019; Lu et al., 2010).

278

279 3.7. Sensitivity to cationic conductance blockers

280 Gadolinium ions Gd^{3+} and flufenamic acid (FFA) are currently used as nonselective blockers
281 of cationic conductances. It was also the case for the study of I_{Nab} . Bath application of Gd^{3+} or
282 FFA hyperpolarizes cell membrane and abolishes spontaneous APs in both pituitary GH3 cells
283 (Kucka et al., 2012; Tomic et al., 2011) and lactotrophs (Kucka et al., 2012). This effect
284 resembles that observed in response to bath application of a low Na^+ saline, the standard
285 protocol to unmask I_{Nab} . In the same line, Gd^{3+} and FFA induce a positive shift of the holding
286 current in pituitary melanotrophs (Kayano et al., 2019). The inhibition by cations follows the
287 rank order of potency: $\text{Gd}^{3+} > \text{Ca}^{2+} > \text{Mg}^{2+}$ and Ba^{2+} (Chua et al., 2020). It is noteworthy that Gd^{3+}
288 also blocks voltage-gated Ca^{2+} channels in many cell types, including secretory pituitary cells
289 (Biagi & Enyeart, 1990) and chromaffin cells (Bourne & Trifaro, 1982), rendering its use
290 difficult.

291

292 4. Which ion channels could contribute to I_{Nab} ?

293 As mentioned above, the investigation of the underlying current discloses biophysical
294 properties that are shared with many channels, rendering thus laborious the study and the
295 identification of channels contributing to the Na^+ background channels in a cell-specific
296 manner. Moreover, it is likely that, in a given cell, I_{Nab} is not supported by a sole conductance,
297 but rather results from a combination of several channels exhibiting a nonselective cationic
298 selectivity. We will not review here all cationic ion channels expressed in

299 endocrine/neuroendocrine cells, but rather we will focus on the most plausible candidates
300 emerging from the literature, which are the Na⁺-leak channel (NALCN) and the Transient
301 Receptor Potential (TRP) channel family. We hope to provide enough information to turn the
302 "*elusive background current*" (Henquin et al., 2009) into an "identifiable background current".
303 Note that until very recently the molecular identity of the ion channel(s) supporting the Na⁺
304 background conductance was still mentioned as "*unknown*", at least in the hormone-producing
305 cells of the anterior pituitary (Fletcher et al., 2018).

306

307 4.1. NALCN

308 Whilst it is unambiguous that NALCN is expressed in neuronal cells, its expression in
309 endocrine/neuroendocrine cells is much less documented (Table 5). *Nalcn* transcript has been
310 identified in the MIN6 pancreatic beta cell line (Swayne et al., 2009), as well as in human and
311 mouse islets (Segerstolpe et al., 2016; Swayne et al., 2009). More recently, mRNA encoding
312 NALCN has been detected in mouse CCs (Chan et al., 2019; Milman et al., 2021) and in
313 hormone-secreting pituitary cells (Cheung et al., 2018; Fletcher et al., 2019; Impheng et al.,
314 2021). The pituitary folliculostellate cells weakly express *nalcn*, as compared to endocrine cells
315 (Fletcher et al., 2019), thus corroborating what is found in glial *versus* neuronal cells. The cell
316 type-specific expression of *nalcn* in endocrine/neuroendocrine tissues is still in progress, but it
317 becomes obvious that techniques dedicated to transcriptome profiling will soon help to tackle
318 this issue, both in physiological and pathological conditions.

319 From a functional point of view, NALCN is reported to assist in determining RMP in
320 excitable cells (Lu et al., 2007; Ren, 2011), as RMP is significantly more polarized in NALCN-
321 deficient cells. This is consistent with the hyperpolarization observed in response to bath
322 application of low Na⁺-containing saline in endocrine pituitary cells, chromaffin cells, and
323 pancreatic beta cells (see chapter above). In the voltage range of RMP, NALCN conducts a

324 voltage-independent current and generates a mixed ion current with the permeability sequence
325 $P_{Na} > P_K > P_{Cs}$ (Chua et al., 2020; Lu et al., 2007). Beyond these biophysical attributes which are
326 shared with I_{Nab} in endocrine/neuroendocrine cells (Fonteriz et al., 2002; Milman et al., 2021;
327 Sankaranarayanan & Simasko, 1996; Simasko, 1994; Worley et al., 1994), some
328 pharmacological features are also common between NALCN and I_{Nab} . NALCN-mediated
329 current is i) permeant to Cs^+ (Bouasse et al., 2019; Chua et al., 2020; Lu et al., 2007), ii) not
330 blocked by TTX (Bouasse et al., 2019; Chua et al., 2020; Lu et al., 2007) and iii) blocked by
331 Gd^{3+} and FFA, two nonselective blockers of cationic conductances (Chua et al., 2020; Lu et al.,
332 2007; Lutas et al., 2016), as also found for I_{Nab} see chapter above). It is interesting to note that
333 Kucka and colleagues in their article published in 2010 cite NALCN as a possible candidate for
334 I_{Nab} in cultured secretory pituitary cells (Kucka et al., 2010).

335

336 4.2. *TRP channel family*

337 This large family of nonselective cation channels represents plausible support for I_{Nab} in
338 endocrine/neuroendocrine cells. First, TRP channels are expressed in hormone-producing
339 pituitary cells (Gotz et al., 2017; Kayano et al., 2019), in adrenal chromaffin cells (Hu et al.,
340 2009; Inoue et al., 2012; Mathar et al., 2010; Tesfai et al., 2001) and in pancreatic beta cells
341 (Colsoul et al., 2011; Jacobson & Philipson, 2007). Like for NALCN, single-cell transcriptomic
342 data are being collected to provide information on which secretory cell type expresses which
343 TRP channels and under which physiological/pathological conditions. Such recent data can be
344 now found for the anterior pituitary (Cheung et al., 2018; Fletcher et al., 2019), for the adrenal
345 medulla (Chan et al., 2019), and the endocrine pancreas (Segerstolpe et al., 2016). Second, the
346 nonselective cationic nature of the current generated upon TRP channel activation matches with
347 I_{Nab} . In pancreatic beta cells, TRP channels have been suspected to provide the inward
348 background conductance required for cells to depolarize upon K_{ATP} channel closure (Qian et

349 al., 2002). Among TRP channels, the canonical TRP channel (TRPC) family appears to be a
350 good candidate to support I_{Nab} . Accordingly, TRPC channels are widely expressed in the
351 anterior pituitary, in the adrenal medullary tissue, and the endocrine pancreas (Table 5). In the
352 pituitary, both endocrine cells and folliculostellate cells express *Trpc* gene family, with a
353 dominant expression of *Trpc1* over *Trpc2-7* (Cheung et al., 2018; Fletcher et al., 2019).
354 Accordingly, two studies, performed in the immortalized GH3 cell line and primary lactotrophs,
355 argue for a contribution of TRPC channels in supporting I_{Nab} (Kucka et al., 2012; Tomic et al.,
356 2011). In adrenal chromaffin cells and insulin-secreting beta cells, whether I_{Nab} is supported
357 by TRP/TRPC channels still remains to be studied. Another marker supporting the contribution
358 of TRPC channels to I_{Nab} is their ion permeability. Although the ion permeability sequence and
359 the permeability ratio depend on the channel expressed (TRPC1-7), TRPC channels are non-
360 selective Ca^{2+} permeable cation channels (Owsianik et al., 2006). Although the selectivity ratio
361 $P_{\text{Ca}}/P_{\text{Na}}$ varies between the TRPC family members (Gees et al., 2010), the permeability
362 sequence for Na^+ , K^+ , and Cs^+ ions matches with that found for I_{Nab} , that is $P_{\text{Na}} > P_{\text{K}} > P_{\text{Cs}}$.

363

364 **5. Mathematical models of I_{Nab}**

365 In addition to experimental findings, computed models of endocrine/neuroendocrine cell
366 excitability reveal that a faithful recapitulation of the electrical activity pattern requires the
367 implementation of a background Na^+ -sensitive component. In particular, this is the case in the
368 anterior pituitary, for modeling the regenerative electrical activity of somatotrophs (Kwiecien
369 et al., 1998) or for setting the RMP value around -55 mV in corticotrophs (Liang et al., 2011).
370 Strengthening the major role of I_{Nab} in regulating cell excitability, the removal of a small inward
371 current (possibly I_{Nab}) from the model is sufficient to hyperpolarize cells and to abolish AP
372 firing (Tsaneva-Atanasova et al., 2007).

373 Similarly, in pancreatic beta cells, the implementation of a persistent Na^+ -carrying
374 conductance is a prerequisite to model cell excitability in a reliable manner (Fridlyand et al.,
375 2009). The computed model allows to hypothesize that I_{Nab} would behave as a regulator of
376 bursting. Indeed, decreasing I_{Nab} conductance from 25 to 15 pS reduces the ability of cells to
377 burst. Interestingly, the model also predicts that the activation of I_{Nab} increases spike frequency
378 and ensuing Ca^{2+} transients and hormone secretion (Fridlyand et al., 2013). Also of note,
379 blocking I_{Nab} only is sufficient to abolish the electrical activity and to hyperpolarize cells
380 (Fridlyand et al., 2009).

381 To go further, we took advantage of the chromaffin cell numerical simulation developed
382 by Warashina and colleagues (Warashina & Ogura, 2004). We implemented Warashina's model
383 by adding a Na^+ component to the previously designed G_{leak} (see Fig. 3). As such, G_{leak} is
384 composed of both a K^+ ($G_{\text{K-leak}}$) and a Na^+ ($G_{\text{Na-leak}}$) component. To investigate the contribution
385 of $G_{\text{Na-leak}}$ to chromaffin cell excitability (RMP value and ability to generate spikes), $G_{\text{K-leak}}$
386 amplitude was maintained as a constant value of either 0.5 or 1 mS/cm^2 during the simulation
387 runs, while $G_{\text{Na-leak}}$ amplitude gradually increased (from 0 to 0.40 mS/cm^2) (see Fig. 3A). The
388 values of membrane capacitance (7.14 pF), RMP (-43.4 mV) and equilibrium potentials of K^+
389 (-106 mV) and Na^+ (+117 mV) were those reported in mouse chromaffin cells in which I_{Nab} was
390 previously reported (Milman et al., 2021). The condition in which $G_{\text{Na-leak}} = 0$ ($G_{\text{K-leak}} = 0.5$ or 1
391 mS/cm^2) sets the RMP value at E_{K} (*i.e.* -106 mV). Increases in $G_{\text{Na-leak}}$ (from 0.10 to 0.40
392 mS/cm^2) gradually depolarize the computed chromaffin cell until the threshold for generating
393 spontaneous spikes is reached. Additional $G_{\text{Na-leak}}$ increases both enhance the frequency of
394 action potentials (APs) and reduce the latency of the first AP. Note that the higher $G_{\text{K-leak}}$ is, the
395 higher should $G_{\text{Na-leak}}$ be to reach the threshold for triggering APs (see Fig. 3B). To go further,
396 RMP value was plotted as a function of $G_{\text{Na-leak}}$. To reliably calculate RMP in spiking cells, we
397 used a protocol based on the distribution of the membrane potential values during the recording,

398 as previously described (Milman et al., 2021). The distribution being unimodal, it could be
399 fitted by a single Gaussian curve. The RMP value is then defined by the peak of the Gaussian
400 curve. The analysis shows a robust correlation between RMP and $G_{\text{Na-leak}}$ (Fig. 3C, Pearson's
401 correlation coefficient $r = 0.998$ ($p < 0.01$, $n = 4$ pairs) and 0.997 ($p < 0.01$, $n = 4$ pairs) for GK-
402 leak = 0.5 and 1 mS/cm², respectively), only for membrane potentials below the AP threshold,
403 indicating thus a significant contribution of $G_{\text{Na-leak}}$ to drive membrane potential towards its
404 resting value. By contrast, the correlation is lost (Pearson's correlation coefficient = -0.132
405 ($p > 0.05$, $n = 8$ pairs) and -0.202 ($p > 0.05$, $n = 5$ pairs) for GK-leak = 0.5 and 1 mS/cm²,
406 respectively) when the cell fires, holding thus the membrane potential to its resting value.
407 Collectively, the computational implementation of I_{Nab} in *in silico* models of
408 endocrine/neuroendocrine cells strengthens the contribution of a Na⁺ leak channel to cell
409 excitability. A schematic representation of the possible contribution of I_{Nab} to RMP and spike
410 discharges in excitable cells is illustrated in Fig. 4.

411

412 **6. Physiological and pharmacological modulation of I_{Nab}**

413 *6.1. Regulation by neuropeptides and neurotransmitters*

414 Several studies, mainly performed in anterior pituitary cells, have reported the activation of a
415 Na⁺ background current by hypothalamic neuropeptides. This is in particular the case for the
416 growth hormone-releasing hormone (GHRH) in GH3 cells (Fonteriz et al., 2002), in human
417 GH-secreting adenoma cells (Takano et al., 1996a) and in primary cultured rat somatotrophs
418 (Kato & Sakuma, 1997; Kato & Suzuki, 1989; Naumov et al., 1994; Tsaneva-Atanasova et al.,
419 2007). The current activated by GHRH is i) strongly supported by a Na⁺-permeability, ii) is
420 TTX-insensitive, iii) is voltage-independent and iv) displays an E_{rev} value between -60 and -30
421 mV that suggests the contribution of (a) nonselective conductance(s). In this, it shares common

422 attributes with I_{Nab} . Activation of such a current depolarizes somatotrophs and therefore
423 controls the firing rate.

424 The corticotropin-releasing hormone CRH also activates a nonselective cation
425 conductance in human corticotrophs derived from both ACTH-secreting adenoma as well as
426 from non-adenoma tissue (Takano et al., 1996b). The current exhibits a strong Na^+ -sensitivity
427 (as it is reduced in Na^+ -deprived saline) and is TTX-insensitive. At very negative membrane
428 potential, CRH-evoked Na^+ -sensitive current shows a slight inward rectification, but it is linear
429 in the RMP potential range. The extrapolated E_{rev} was between -20 and 0 mV. All these features
430 are close to those reported for I_{Nab} . In corticotrophs isolated from mouse pituitary, exposure to
431 a cocktail of CRH + vasopressin (AVP) results from an increased AP frequency, which is
432 concomitant to a steady depolarization from RMP (Liang et al., 2011). In a Na^+ -free saline, the
433 CRH/AVP cocktail is no longer able to depolarize corticotrophs, thus indicating that
434 depolarization implies a Na^+ -carrying component.

435 In bovine chromaffin cells, the pituitary adenylate cyclase-activating peptide PACAP
436 induces a TTX-insensitive sub-threshold membrane depolarization that strongly depends on
437 external Na^+ (Tanaka et al., 1996). Similarly, PACAP stimulates a Na^+ -permeant conductance
438 in pancreatic beta cells derived from insulinoma (Leech et al., 1995). With a voltage-
439 insensitivity and a E_{rev} value of -28 mV, PACAP-evoked current resembles to I_{Nab} . As
440 mentioned before, NALCN is a possible candidate for I_{Nab} . Assuming this, any compound
441 targeting NALCN can act as a regulator for I_{Nab} . NALCN can be activated by peptides and
442 neurotransmitters, such as substance P, neurotensin or acetylcholine (Kim et al., 2012; Lu et
443 al., 2009; Lutas et al., 2016; Swayne et al., 2009). Although their effects on I_{Nab} remain to be
444 characterized, these molecules can likely regulate I_{Nab} .

445

446 *6.2. Regulation by pharmacological agents*

447 Beyond the physiological regulation of I_{NaB} by neuropeptides, others agents, with a 'non-
448 physiological' relevance, can also target I_{NaB} . In pituitary somatotrophs, the mercurial p-
449 chloromercurybenzenesulfonate (PCMBS) activates an inward current (Fonteriz et al., 2002),
450 which displays biophysical properties similar to I_{NaB} . PCMBS-evoked current is Na^+ -carrying,
451 TTX-insensitive, voltage-independent and it reverses at -35 mV. It is noteworthy that PCMBS
452 activates the 'same' I_{NaB} than that evoked in response to GHRH. PCMBS also increases a Na^+
453 permeability (possibly I_{NaB}) in pancreatic beta cells (Hellman et al., 1975). Still, in pancreatic
454 beta cells, maitotoxin (MTX), a marine toxin isolated from a dinoflagellate, is reported to
455 activate a Na^+ -carrying conductance, which is i) blocked when extracellular Na^+ is replaced by
456 NMDG, ii) TTX-insensitive, iii) voltage-independent and not inactivating and exhibits a E_{rev}
457 value between -15 and -2 mV and a $P_{Na} > P_K$ (Leech & Habener, 1998; Worley et al., 1994). This
458 conductance is presumed to contribute, at least in part, to depolarizing background conductance
459 and to regulate RMP. A similar activation by MTX of a nonselective cation (Na^+ -sensitive)
460 current has been reported in tumoral pancreatic beta TC3 cells (Roe et al., 1998). Because i)
461 MTX is an activator of TRPC1 channels (Flores et al., 2017) and ii) TRPC channels are possible
462 channels for I_{NaB} , one can reasonably hypothesize that MTX activates I_{NaB} , at least in pancreatic
463 beta cells.

464

465 **7. A role in hormone release for I_{NaB} ?**

466 In endocrine/neuroendocrine tissues, an increase in $[Ca^{2+}]_i$ is a prerequisite for hormone
467 exocytosis. Accordingly, AP-evoked transient $[Ca^{2+}]_i$ increases are a critical step for hormone
468 release (Fletcher et al., 2018; Kidokoro & Ritchie, 1980). The fact that I_{NaB} regulates RMP and
469 AP firing raises the issue of whether I_{NaB} contributes to endocrine/neuroendocrine secretion.
470 Tables 6-8 summarize the effects of external Na^+ removal on basal and stimulated hormone
471 secretion in anterior pituitary cells, adrenal chromaffin cells and pancreatic beta cells,

472 respectively. Because the most frequently observed effect of the external Na^+ removal is a
473 membrane hyperpolarization associated with AP abolition, one would expect a decrease in
474 hormone release. Although this is usually the case, experimental data still show more intricate
475 results. Several explanations can be taken into account, such as the cell type, the secretagogue
476 used to stimulate secretion, the compound used to replace external Na^+ , the complete or partial
477 Na^+ substitution, the animal species, the animal sex, the cell/tissue preparation, the technique
478 used to monitor hormone release... without forgetting a differential $I_{\text{Na}b}$ expression in the
479 various cell types. Consistent with a possible contribution of $I_{\text{Na}b}$ to hormone release,
480 compounds described to activate $I_{\text{Na}b}$ (see chapter above) also trigger secretion. This is in
481 particular the case for PCMBS that increases insulin secretion (Hellman et al., 1975; Idahl et
482 al., 1980) and MTX that increases catecholamine secretion in cultured bovine chromaffin cells
483 (Kakizaki et al., 2006) and insulin secretion in rat pancreatic islets (Lebrun et al., 1987). Also
484 supporting a functional role of $I_{\text{Na}b}$ in hormone secretion is the finding that PRL release is
485 blunted in a NALCN-knockdown GH3/B6 cell line (Impheng et al., 2021).

486

487 **8. Conclusion and perspectives**

488 Regulation of background membrane permeability to Na^+ ions in
489 endocrine/neuroendocrine cells is crucial for controlling RMP and subsequent cell excitability.
490 Because the AP discharge pattern plays a crucial role in hormone secretion, it becomes obvious
491 that $I_{\text{Na}b}$, regardless of the molecular identity of the channel(s) involved, should be considered
492 as a key actor in the regulation of endocrine/neuroendocrine stimulus-secretion coupling. In
493 addition to the functional relevance of $I_{\text{Na}b}$ *per se*, it is likely that $I_{\text{Na}b}$ influences the function of
494 other ions channels by changing local intracellular ion concentration, as reported in tumoral
495 pheochromocytoma chromaffin cells, in which a K^+ current is inhibited by nonselective cation
496 channel-mediated Na^+ entry (Strubing & Hescheler, 1996).

497 One of the next challenging issues in the study of background Na⁺ conductance(s) will
498 be i) the identification of the channels contributing to, ii) the elucidation of their regulatory
499 mechanisms and the consequences on the RMP, and iii) the discovery of pharmacological tools
500 able to selectively block or activate these channels. This latter point is now developing as
501 illustrated with the recent finding that N-benzhydryl quinuclidine compounds known as
502 tachykinin receptor antagonists potently block NALCN channels without affecting TRPC
503 channels (Hahn et al., 2020). These future research programs undoubtedly represent a
504 considerable and long-lasting challenge, as it is obvious that multiple channel candidates will
505 fit with the biophysical and pharmacological properties described for I_{Nab}. In addition, as
506 mentioned in the introduction, "leak" currents are not the sole currents to play a role in setting
507 the RMP. Their physiologically relevant action can reasonably be understood only with the
508 coordinated participation of other channels. Going towards a comprehensive elucidation of the
509 mechanisms involved in the modulation of RMP is a prerequisite to study cell physiology and
510 pathology. It will provide new tools (both experimental and conceptual) to investigate how
511 these channel disorders (channelopathies) can account for altered cell/tissue excitability. In the
512 long term, disclosing these issues will likely open new research routes in the field of hormone
513 secretion pathophysiology and more generally in other cell/tissue functions.

514

515

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524

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1089 **Figure legend**

1090 **Fig. 1: Schematic representation of Na^+ homeostasis in a secretory cell: focus on ion**
1091 **channel-mediated Na^+ entry.** The difference in Na^+ concentration between the cytoplasm (~10
1092 mM) and the surrounding extracellular medium (~140 mM) is in favor of an inward Na^+ flux
1093 when Na^+ -permeant channels open. This Na^+ entry contributes to cell depolarization and further
1094 activation of voltage-gated channels such as Na^+ and Ca^{2+} channels. At RMP, the presence of
1095 background Na^+ -permeant channels (NALCN, TRP channels...) generates a persistent Na^+
1096 influx, which counterbalances leak K^+ -permeant currents. This leads to a 'depolarized' RMP
1097 value. During spiking discharges, voltage-gated Na^+ channels take over to trigger APs.

1098 **Fig. 2: Contribution of a Na⁺-sensitive conductance to RMP in rat and mouse adrenal**
1099 **chromaffin cells. A.** Representative examples of the effect of a low [Na⁺]-containing saline on
1100 membrane potential in rodent chromaffin cells (male Wistar rat, left traces (RMP = -45.6 mV)
1101 and male C57BL/6J mouse, right traces (RMP = -43.7 mV)). **B.** Low [Na⁺]-evoked changes in
1102 the holding current in chromaffin cells voltage-clamped at -50 mV, in a male Wistar rat (left
1103 panel) and in a female OF1 mouse (right panel). Pooled data are plotted in the histograms. Note
1104 that the effect on the holding current illustrated here is similar to that recently described in male
1105 mice (Milman et al., 2021). **C.** Changes in membrane current triggered by low [Na⁺] in a male
1106 Wistar rat chromaffin cell (left panel) and in a female OF1 mouse (right panel). Cells were
1107 voltage-clamped at -50 mV and stimulated by a voltage ramp protocol (-100 to -50 mV, 3 s
1108 duration for the experiment performed in rat and -130 to -50 mV, 3 s duration for the experiment
1109 performed in mouse). Control and low Na⁺ currents were fitted by a linear regression and the
1110 reversal potential (E_{rev}) of I_{Nab} was calculated as the intercept point between the two linear
1111 regressions. The extrapolated E_{rev} unveils a value of ~ -20 mV in the male rat and ~ -17 mV in
1112 the female mouse. These values are in consistent with a non-selective conductance.

1113

1114 **Fig. 3: *In silico* modeling on the contribution of I_{Nab} to chromaffin cell excitability.** The
1115 computed model of chromaffin cells comes from the model developed by Warashina and
1116 colleagues (Warashina & Ogura, 2004). The model includes mechanisms involved in AP
1117 generation, that are Hodgkin-Huxley-based Na⁺ and K⁺ channels, voltage-dependent Ca²⁺
1118 channels, Ca²⁺-dependent K⁺ channel (SK channel) and leak channels. The latter are defined
1119 as:

1120

$$I_{leak} = G_{leak} (V - E_{leak})$$

1121 where G_{leak} represents the conductance for the leak channel and E_{leak} represents the equilibrium
1122 potential for the leak current. The numerical model developed by Warashina and Ogura

1123 (Warashina & Ogura, 2004) was adapted to the JSim software (Butterworth et al., 2013)
1124 (version 2.21, available at <https://www.physiome.org/jsim/>). To investigate the contribution of
1125 I_{Na} in cell excitability, the original I_{leak} was divided into 2 components: a I_{K-leak} and a $I_{Na-leak}$, as
1126 follows:

$$1127 \quad I_{leak} = I_{K-leak} + I_{Na-leak}$$

$$1128 \quad I_{leak} = G_{K-leak} (V - E_{leak}) + G_{Na-leak} (V - E_{leak})$$

1129 The model ran with $E_{leak} = RMP$. G_{K-leak} was maintained constant while $G_{Na-leak}$ gradually
1130 increased. The values for membrane capacitance (7.14 pF), RMP (-43.4 mV) and equilibrium
1131 potentials of K^+ (-106 mV) and Na^+ (+117 mV) are those reported in mouse chromaffin cells
1132 (Milman et al., 2021). The biophysical parameters used to trigger APs were those implemented
1133 in Warashina and Ogura's model, *i.e.* $G_{Na} = 40$ mS/cm², $G_K = 1.2$ mS/cm² and $G_{SK} = 10$ mS/cm².
1134 The Ca^{2+} permeability was set at 2 μ m/s. **A.** Spontaneous electrical activity in response to a
1135 gradual increase in $G_{Na-leak}$ in chromaffin cells implemented with a G_{K-leak} clamped at 0.5 mS/cm²
1136 (left traces) or 1 mS/cm² (right traces). As a common finding, increasing $G_{Na-leak}$ leads to cell
1137 depolarization until APs are triggered. **B.** Relationship between $G_{Na-leak}$ amplitude and the
1138 number of APs triggered. Note that G_{K-leak} and $G_{Na-leak}$ depend on each other, the greater
1139 amplitude of G_{K-leak} , the greater amplitude of $G_{Na-leak}$ must be to evoke APs (2x G_{K-leak} requires
1140 2x $G_{Na-leak}$). When G_{K-leak} is clamped at 0.5 mS/cm², the cell fires when $G_{Na-leak}$ reaches the
1141 threshold value of 0.17 mS/cm². When G_{K-leak} is clamped at 1 mS/cm², $G_{Na-leak}$ requires a
1142 threshold of 0.3 mS/cm². **C.** Relationship between $G_{Na-leak}$ amplitude and RMP. The relationship
1143 differs depending on whether the membrane potential is below the AP threshold or whether the
1144 cell spikes. Below the AP threshold, the amplitude of $G_{Na-leak}$ is significantly correlated to RMP
1145 changes (Pearson's correlation coefficient = 0.998 (**, $p < 0.01$) and 0.997 (**, $p < 0.01$) for G_{K-}
1146 $leak$ clamped at 0.5 and 1mS/cm², respectively). Conversely, when the cell starts to fire, the
1147 amplitude of $G_{Na-leak}$ is no longer correlated with RMP changes (Pearson's correlation coefficient

1148 = -0.132 (ns, $p > 0.05$) and -0.202 (*ns $p > 0.05$ for G_{K-leak} clamped at 0.5 and 1mS/cm²,
1149 respectively). Dashed lines represent the mean values of RMP and AP threshold in mouse
1150 chromaffin cells (Milman et al., 2021).

1151

1152 **Fig. 4: Schematic illustration of the contribution of I_{Nab} to RMP and cell excitability.** The
1153 addition of I_{Nab} , which acts as a depolarizing component, shifts the RMP value to more positive
1154 potentials. This, in turn, increases the spiking discharges.

Table 1

Action potential sensitivity to TTX

ANTERIOR PITUITARY – cultured cell lines					
cell type	[TTX]	AP blockade	spontaneous/evoked APs	recording techniques	references
GH3 cells (pituitary gland neoplasm, ♀ Wistar Furth rat)	2 µM	No	evoked	intracellular microelectrode (KCl)	(Kidokoro, 1975)
	1 µM	No	evoked	intracellular microelectrode (K-acetate)	(Biales et al., 1977)
	2 µM	No	spontaneous + evoked	intracellular microelectrode (KCl)	(Taraskevich & Douglas, 1980)
	0.5 µM	No	spontaneous	perforated patch (nystatin)	(Simasko, 1994)
	1 µM	No	spontaneous	perforated patch (amphotericin)	(Tomic et al., 2011)
	1 µM	No	spontaneous	perforated patch (amphotericin)	(Kucka et al., 2012)
GH4C1 cells (pituitary gland neoplasm, ♀ Wistar Furth rat)	2 µM	No	spontaneous + evoked	intracellular microelectrode (KCl)	(Taraskevich & Douglas, 1980)
GC cells (subclone of GH3 cells, ♀ Wistar Furth rat)	1 µM	No	spontaneous	perforated patch (amphotericin)	(Kwiecien et al., 1998)
AtT-20 cells (pituitary gland neoplasm, LAF1 mouse)	10 µM	No	spontaneous + evoked	intracellular microelectrode (K-acetate)	(Surprenant, 1982)
ANTERIOR PITUITARY – primary cell cultures					
somatotrophs (♂ Wistar rat)	5 µM 20 µM	No	evoked	Ca ²⁺ transients (fura-2 imaging)	(Kato et al., 1992)
somatotrophs (♀ Sprague-Dawley rat)	50 µM	No	spontaneous	Ca ²⁺ transients (fura-2 imaging)	(Tomic et al., 2011)
	1 µM	No	spontaneous	perforated patch (amphotericin)	(Tsaneva-Atanasova et al., 2007)

lactotrophs (♀ Wistar rat + lactating Wistar rat)	5 µM	No	evoked	intracellular microelectrode (K-acetate)	(Israel et al., 1987)
lactotrophs (♀ Sprague- Dawley rat)	1 µM	Yes	evoked	conventional patch-clamp (K-gluconate)	(Chen et al., 1987)
	10 µM	No	spontaneous	perforated patch (nystatin)	(Sankaranarayanan & Simasko, 1996)
	50 µM	No	spontaneous Ca ²⁺ transients	fura-2 imaging	(Tomic et al., 2011)
	1 µM	No	spontaneous	perforated patch (amphotericin)	(Kucka et al., 2012)
lactotrophs (calf)	5-30 µM	Yes	evoked	conventional patch-clamp (KCl)	(Cobbett et al., 1987)
gonadotrophs (♀ Sprague- Dawley rat)	50 µM	No	spontaneous	Ca ²⁺ transients (fura-2 imaging)	(Tomic et al., 2011)
corticotrophs ♀ mouse (mixed sv129/Bl6)	1-2 µM	No	spontaneous	perforated patch (amphotericin)	(Liang et al., 2011)
corticotrophs (♀ Wistar rat)	5 µM	No	spontaneous Ca ²⁺ transients	indo-1 imaging	(Guerineau et al., 1991)
human ACTH- secreting adenomas	300 µM	No	spontaneous	intracellular microelectrode (KCl)	(Mollard et al., 1987)
	5 µM	No	spontaneous Ca ²⁺ transients	indo-1 imaging	(Guerineau et al., 1991)
corticotrophs (♂ + ♀ POMC- DsRed C57BL/6J mouse)	0.5 µM	No	spontaneous + evoked	perforated patch (nystatin)	(Zemkova et al., 2016)
♂ rat and ♀ lactating rat	2 µM	No	spontaneous + evoked	extracellular recording	(Taraskevich & Douglas, 1977)

ADRENAL CHROMAFFIN CELLS – cultured cell lines

PC12 cells (pheochromo- cytoma, ♂ NEDH rat) NGF-treated cells	0.3 µM	Yes	evoked	intracellular microelectrode (K-acetate)	(Dichter et al., 1977)
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	0.3 μ M	Yes	evoked	intracellular microelectrode (K-acetate)	(Rudy et al., 1982)
	2 μ M	Yes	evoked	intracellular microelectrode (K-acetate)	(Rudy et al., 1987)
ADRENAL CHROMAFFIN CELLS – primary cell cultures/hemisectioned gland/acute slices					
gerbil chromaffin cells (3 days in culture)	1 μ M	Yes	spontaneous + evoked	intracellular microelectrode (K-acetate)	(Biales et al., 1976)
acute adrenal slices (adult gerbil)	1 μ M	Yes	spontaneous + evoked		
dissociated cells 1-7 days in culture (Fisher F/344 rat)	1 μ M	Yes	evoked	extracellular recording (microsuction electrode)	(Brandt et al., 1976)
dissociated cells 2-3 days in culture (σ Sprague-Dawley rat)	6 μ M	reduced amplitude and frequency	spontaneous + evoked	extracellular recording (microsuction electrode)	(Kidokoro & Ritchie, 1980)
dissociated cells 2-4 days in culture (C57BL/6N mouse)	300 nM	reduced amplitude	spontaneous	perforated patch (amphotericin)	(Vandael et al., 2010)
dissociated cells 2-6 days in culture (σ C57BL/6N mouse)	300 nM	Yes or reduced amplitude	spontaneous	perforated patch (amphotericin)	(Mahapatra et al., 2011)
dissociated cells 2-5 days in culture (σ C57BL/6J mouse)	10-300 nM	reduced amplitude	spontaneous	perforated patch (amphotericin)	(Vandael et al., 2015)
acute adrenal slices (σ Wistar rat)	0.5 μ M	Yes	spontaneous Ca^{2+} transients	Oregon Green 488 BAPTA-1 imaging	(Martin et al., 2001)
hemisectioned glands (σ mouse)	3 μ M	reduced amplitude and frequency	spontaneous	intracellular microelectrode KCl + K-citrate)	(Nassar-Gentina et al., 1988)
acute slices (σ C57BL/6J mouse)	0.5 μ M	Yes	spontaneous	conventional patch-clamp (K-gluconate)	(Milman et al., 2021)
human chromaffin cells (3 days in culture)	1 μ M	Yes	spontaneous + evoked	intracellular microelectrode (K-acetate)	(Biales et al., 1976)

human chromaffin cells (1-7 days in culture)	1 μ M	Yes	evoked (ACh)	perforated patch (amphotericin)	(Hone et al., 2017)
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PANCREATIC BETA CELLS

microdissected islets (albino mouse)	0.2-1 μ M	No	evoked	intracellular microelectrode (K-citrate)	(Meissner & Schmelz, 1974)
	1 μ M	No	evoked	intracellular microelectrode (K-citrate)	(Tarvin & Pace, 1981)
dissociated cells 4-6 days in culture (♂ Sprague-Dawley rat)	3 μ M	Yes	evoked	intracellular microelectrode (K-citrate)	(Pace, 1979)
freshly dissociated cells (human)	0.1 μ M	No	evoked	perforated patch (amphotericin)	(Braun et al., 2008)
INS-1E cells (insulinoma, ♂ NEDH rat)	0.5 μ M	Yes	evoked	perforated patch (amphotericin)	(Rizzetto et al., 2015)

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Table 2

Effect of extracellular Na⁺ substitution on anterior/intermediate pituitary cell membrane potential. The experiments in which an hyperpolarization has been reported are highlighted. Lines highlighted in blue and pink represent experiments performed in males and females, respectively and lines highlighted in gray indicate experiments for which the animal sex was not mentioned.

ANTERIOR PITUITARY – cultured cell lines					
cell type	Na⁺ substitution	effect on AP firing/membrane potential	spontaneous/evoked APs	recording techniques	references
GH3 cells (pituitary gland neoplasm, ♀ Wistar Furth rat)	Tris	AP persistence	evoked	intracellular microelectrode (KCl)	(Kidokoro, 1975)
	Tris	AP persistence	evoked	intracellular microelectrode (K-acetate)	(Biales et al., 1977)
	Tris	AP persistence	spontaneous + evoked	intracellular microelectrode (KCl)	(Taraskevich & Douglas, 1980)
	choline Tris NMDG	AP blockade hyperpolarization	spontaneous	perforated patch (nystatin)	(Simasko, 1994)
	NMDG	AP blockade hyperpolarization	spontaneous	conventional patch-clamp (KCl + K ₂ SO ₄) + perforated patch (nystatin)	(Simasko & Sankaranarayanan, 1997)
	NMDG	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Tomic et al., 2011)
	lithium	transient hyperpolarization			
	NMDG choline TMA	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Kucka et al., 2010)
	NMDG	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Kucka et al., 2012)
	NMDG	hyperpolarization	spontaneous	conventional patch-clamp (K-gluconate)	(Impheng et al., 2021)
GH4C1 cells (pituitary gland)	Tris	AP persistence	spontaneous + evoked	intracellular microelectrode (KCl)	(Taraskevich & Douglas, 1980)

neoplasm, ♀ Wistar Furth rat)					
GC cells (subclone of GH3 cells, ♀ Wistar Furth rat)	Tris	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Kwiecien et al., 1998)
AtT-20 cells (pituitary gland neoplasm, LAF1 mouse)	choline	AP persistence	evoked	intracellular microelectrode (K-acetate)	(Surprenant, 1982)
ANTERIOR PITUITARY – primary cell cultures					
somatotrophs (♂ Wistar rat)	D-mannitol NMDG Tris	reduced Ca ²⁺ response	GHRH stimulation	Ca ²⁺ transients (fura-2 imaging)	(Kato et al., 1992)
somatotrophs (♀ Sprague- Dawley rat)	NMDG	AP blockade hyperpolarization	spontaneous	conventional patch-clamp (KCl + K ₂ SO ₄) + perforated patch (nystatin)	(Simasko & Sankaranarayanan, 1997)
	NMDG	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Tsaneva-Atanasova et al., 2007)
	NMDG choline TMA	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Kucka et al., 2010)
	NMDG choline TMA	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Tomic et al., 2011)
	lithium	AP persistence	spontaneous		
lactotrophs (♀ Sprague- Dawley rat)	choline	gradual attenuation of voltage fluctuations (APs ?)	spontaneous	conventional patch-clamp (K-gluconate)	(Chen et al., 1987)
	choline NMDG Tris	AP blockade AP blockade hyperpolarization + Ca ²⁺ transient blockade	evoked spontaneous	 perforated patch (nystatin) + Ca ²⁺ transients (fura-2 imaging)	 (Sankaranarayanan & Simasko, 1996)

	NMDG choline TMA	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Kucka et al., 2010)
	NMDG choline TMA	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Tomic et al., 2011)
	lithium	AP persistence	spontaneous		
	NMDG	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Kucka et al., 2012)
gonadotrophs (♀ Sprague- Dawley rat)	NMDG choline TMA	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Kucka et al., 2010)
	NMDG choline TMA	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Tomic et al., 2011)
	lithium	AP persistence	spontaneous		
corticotrophs (♀ mouse, mixed sv129/B16)	NMDG	AP blockade hyperpolarization	spontaneous	perforated patch (amphotericin)	(Liang et al., 2011)
human ACTH- secreting adenomas	choline	AP persistence	spontaneous	intracellular microelectrode (KCl)	(Mollard et al., 1987)
corticotrophs (♂ + ♀ POMC- DsRed C57BL/6J mouse)	NMDG	AP blockade hyperpolarization + Ca ²⁺ transient blockade	spontaneous + evoked (APs, Ca ²⁺ transients)	perforated patch (nystatin) + Ca ²⁺ transients (fura-2)	(Zemkova et al., 2016)
♂ rat and ♀ lactating rat	mannitol	AP persistence	spontaneous + evoked	extracellular recording	(Taraskevich & Douglas, 1977)
INTERMEDIATE PITUITARY – primary cell cultures					
melanotrophs (♂ Wistar rat)	NMDG lithium	hyperpolarization (no spontaneous AP)	spontaneous	conventional patch-clamp (K-gluconate)	(Kayano et al., 2019)

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Table 3

Effect of extracellular Na⁺ substitution on adrenal chromaffin cell membrane potential

The experiments in which an hyperpolarization has been reported are highlighted. The line highlighted in blue represents experiments performed in males and lines highlighted in gray indicate experiments for which the animal sex was not mentioned.

cell type	Na ⁺ substitution	effect on AP firing/membrane potential	spontaneous/evoked APs	recording techniques	references
freshly dissociated cells (young gerbil)	sucrose	small hyperpolarization	spontaneous	intracellular microelectrode (KCl)	(Douglas et al., 1967)
dissociated cells 1-7 days in culture (Fisher F/344 rat)	Tris	AP blockade	evoked	intracellular microelectrode (KCl)	(Brandt et al., 1976)
thin medullary slices (calf)	sucrose choline	small hyperpolarization	spontaneous	fluorescence of a voltage-sensitive dye (DiS-C3-(5))	(Baker & Rink, 1975)
hemisectioned glands (♀ mouse)	choline	no change in membrane potential	spontaneous	intracellular microelectrode (KCl + K-citrate)	(Nassar-Gentina et al., 1988)
acute slices (♂ C57BL/6J mouse)	NMDG Tris	hyperpolarization	spontaneous	conventional patch-clamp (K-gluconate)	(Milman et al., 2021)

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Table 4

Effect of extracellular Na⁺ substitution on pancreatic beta cell membrane potential

The experiments in which an hyperpolarization has been reported are highlighted. The lines highlighted in pink represent experiments performed in females and lines highlighted in gray indicate experiments for which the animal sex was not mentioned.

cell type	Na ⁺ substitution	effect on AP firing/membrane potential	spontaneous/evoked APs	recording techniques	references
microdissected islets (albino mouse)	choline (low glucose-containing saline)	no change	no AP	intracellular microelectrode (K-citrate)	(Dean & Matthews, 1970)
	choline (11.1 mM glucose)	larger APs followed by depolarization	evoked		
microdissected islets (♂ + ♀ albino mouse)	choline (low glucose)	AP persistence	evoked	intracellular microelectrode (K-citrate)	(Matthews & Sakamoto, 1975)
microdissected islets (mouse, strain not available)	choline (glucose-free)	transient hyperpolarization followed by depolarization	no AP	intracellular microelectrode (K-citrate)	(Ribalet & Beigelman, 1982)
	choline (11.1 mM glucose)	transient hyperpolarization followed by depolarization	evoked		
	TMA (11 mM glucose)	transient hyperpolarization followed by depolarization	evoked		
perfused pieces of pancreas (♀ NMRI mouse)	choline (glucose-free saline)	hyperpolarization	no AP	intracellular microelectrode (K-citrate)	(de Miguel et al., 1988)
	lithium (glucose-free saline)	depolarization	no AP		
	choline (10 mM glucose)	transient hyperpolarization followed by depolarization	evoked		
	lithium (10 mM glucose)	AP persistence depolarization	evoked		
dissociated islets	NMDG (12 mM)	hyperpolarization	no AP	perforated patch (nystatin)	(Worley et al., 1994)

(C57BL/KsJ mouse)	glucose, Ca ²⁺ free saline)				
dissociated islets (♀ NMRI mouse)	choline (15 mM glucose)	transient hyperpolarization followed by depolarization	evoked	intracellular microelectrode (K-citrate)	(Garcia-Barrado et al., 1996)
	lithium (15 mM glucose)	transient hyperpolarization followed by depolarization	evoked		
dissociated islets (Kir6.2 ^{-/-} mouse)	choline (low glucose)	transient [Ca ²⁺] _i increase		Ca ²⁺ transients (fura-PE3 imaging)	(Henquin et al., 2009)
	choline (15 mM glucose)	marked [Ca ²⁺] _i increase			

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Table 5

Expression of NALCN and canonical TRP channels in endocrine/neuroendocrine tissues

NALCN					
ANTERIOR PITUITARY		ADRENAL GLAND		PANCREAS	
cell type species (technique)	references	cell type species (technique)	references	cell type species (technique)	references
GH3/B6 cells ♀ Wistar Furth rat (RT-qPCR)	(Impheng et al., 2021)	whole adrenal glands chromaffin cells ♂ C57BL/6J mouse (RT-qPCR) (<i>in situ</i> hybridization)	(Milman et al., 2021)	MIN6 cells mouse insulinoma (RT-qPCR, western blot)	(Swayne et al., 2009)
somatotrophs lactotrophs corticotrophs gonadotrophs thyrotrophs Sprague-Dawley rat (♂ + ♀) (single cell RNAseq)	(Fletcher et al., 2019)	macrodissected medulla ♂ Wistar rat (RT-qPCR)	Guérineau et al., unpublished data	- human tissue - pancreatic islets ♀ C57BL/6 mouse (RT-qPCR, mRNA dot blot)	(Swayne et al., 2009)
somatotrophs lactotrophs corticotrophs gonadotrophs thyrotrophs ♂ C57BL/6J mouse (single cell RNAseq)	(Cheung et al., 2018)	dissociated chromaffin cells C57BL/6J mouse (embryos E14.5 to P0)	(Chan et al., 2019)	human pancreatic beta cells (single cell RNAseq)	(Segerstolpe et al., 2016)
somatotrophs lactotrophs C57BL/6 mouse (<i>in situ</i> hybridization)	Hirbec et al., unpublished data				
TRPC family					
ANTERIOR PITUITARY		ADRENAL GLAND		PANCREAS	

<p>mixed pituitary cells ♂ Sprague-Dawley rat (RT-qPCR)</p>	(Yamashita et al., 2009)	<p>PC12 cells (pheochromocytoma, ♂ NEDH rat) (RT-PCR, western blot)</p>	(Tesfai et al., 2001)	<p>- MIN6 cells - pancreatic islets mouse (RT-PCR)</p>	(Sakura & Ashcroft, 1997)
<p>mixed pituitary cells ♀ Sprague-Dawley rat (RT-PCR)</p>	(Tomic et al., 2011)	<p>adrenal medullary cells Ossabow miniature pig (RT-qPCR, western blot)</p>	Hu et al., 2009)	<p>- betaTC3 cells - MIN6 cells - pancreatic islets C57BL/KsJ mouse (RT-PCR, Northern blot)</p>	(Roe et al., 1998)
<p>GH3 cells lactotrophs ♀ Sprague-Dawley rat (RT-qPCR)</p>	(Kucka et al., 2012)	<p>adrenal medullary cells ♂ Hartley guinea pig (RT-PCR, immunoblot)</p>	(Inoue et al., 2012)	<p>- betaTC3 cells - MIN6 cells - pancreatic islets mouse + ♂ Wistar rat (RT-PCR)</p>	(Li & Zhang, 2009; Qian et al., 2002)
<p>gonadotrophs mixed 129/SvJ and C57BL/6J mouse (♂ + ♀) (RT-qPCR)</p>	(Beck et al., 2017)	<p>dissociated chromaffin cells C57BL/6J mouse (embryos E14.5 to P0)</p>	(Chan et al., 2019)	<p>- INS-1 cells - pancreatic islets ♂ Wistar rat (RT-qPCR)</p>	(Li & Zhang, 2009)
<p>somatotrophs lactotrophs corticotrophs gonadotrophs thyrotrophs folliculostellate cells Sprague-Dawley rat (♂ + ♀) (single cell RNAseq)</p>	(Fletcher et al., 2019)			<p>purified beta cells human (RNA-seq)</p>	(Marabita & Islam, 2017)
<p>somatotrophs lactotrophs corticotrophs gonadotrophs thyrotrophs folliculostellate cells ♂ C57BL/6J mouse (single cell RNAseq)</p>	(Cheung et al., 2018)			<p>human pancreatic beta cells (single cell RNAseq)</p>	(Segerstolpe et al., 2016)

Table 6Effect of extracellular Na⁺ removal on prolactin, growth hormone and adrenocorticotropin secretion

PRL secretion					
cell type	Na⁺ substitution	basal hormone release	stimulated (X factor) hormone release	techniques	references
lactotrophs (♀ Sprague-Dawley rat)	choline	slight ↓	no effect (TRH) ↓ (VIP)	RIA	(Collu et al., 1984)
	choline	↓	↑ (TRH) ↑ (BayK8644) ↑ (A23187)	RIA	(Lafond & Collu, 1986)
	Tris	↓		reverse-hemolytic plaque assay	(Sankaranarayanan & Simasko, 1996)
	NMDG Choline TMA	↓		RIA	(Kucka et al., 2012)
adenohypophysis explants (♀ and ♂ Sprague-Dawley rat)	choline (80% substitution)	no effect	no effect (acute KCl) ↓ (prolonged KCl)	densitometry	(Parsons, 1970)
lactotrophs (♂ Sprague-Dawley rat)	choline	no effect	inhibition (veratridine)	complement fixation	(Scammell & Dannies, 1983)
bovine anterior pituitary cells	choline	no effect	no effect (TRH)	RIA	(Saith et al., 1984)
GH secretion					
adenohypophysis explants (♀ and ♂ Sprague-Dawley rat)	choline (80% substitution)		↓ (prolonged KCl)	densitometry	(Parsons, 1970)
bovine anterior pituitary cells	choline	no effect	↓ (TRH) ↓ (ACh)	RIA	(Saith et al., 1984)
dissociated cells (♂ Wistar rat)	mannitol	initial ↑ followed by a sustained ↓	inhibition (GHRH) inhibition (DBcAMP) no effect (KCl)	RIA	(Kato et al., 1988)
	Tris	no effect	inhibition (GHRH) inhibition (DBcAMP) no effect (KCl)		

human GH-secreting adenomas	TMA	↓	inhibition (GHRH)	RIA	(Takano et al., 1996a)
GH-GFP transgenic mouse	choline	↓	inhibition (GHRH)	ELISA	(Yang et al., 2008)
ACTH secretion					
human ACTH-secreting adenomas	TMA	no effect	↓ (CRH)	RIA	(Takano et al., 1996b)

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Table 7Effect of extracellular Na⁺ removal on catecholamine secretion

cell type	Na ⁺ substitution	basal hormone release	stimulated (X factor) hormone release	techniques	references
perfused cat adrenal glands	sucrose	progressive ↑	no effect (ACh 16 min) ↓ (ACh >16 min)	bioassay (contractions of rabbit's thoracic aorta)	(Douglas & Rubin, 1961)
	sucrose		↑ (ACh 14 min)	bioassay (contractions of rabbit's thoracic aorta)	(Douglas & Rubin, 1963)
perfused rabbit adrenal glands	lithium	slight ↑	no effect (ACh) no effect (KCl)	fluorimetric assay	(Sorimachi, 1968)
	choline TMA		inhibition (ACh)		
perfused bovine adrenal glands	choline		inhibition (carbamylcholine) inhibition (KCl)	colorimetric assay	(Banks et al., 1969)
	lithium	transient ↑	inhibition (carbamylcholine) inhibition (KCl)		
	sucrose		no effect (carbamylcholine)		
	sucrose	transient ↑	no effect (ACh 15-40 min) ↓ (ACh 80-100 min) no effect (KCl)	trihydroxyindole fluorometric method	(Lastowecka & Trifaro, 1974)
	choline	↑			
	lithium	no effect			
perfused bovine adrenal glands (cortex free)	sucrose	↑		trihydroxyindole fluorometric method	(Aguirre et al., 1977)
bovine chromaffin cells (24h after dissociation)	Tris	no effect	↓ (ACh) ↓ (KCl)	HPLC (fluorimetric detection)	(Lemaire et al., 1981)
	sucrose		↓ (carbamylcholine) ↓ (veratridine) no effect (KCl) no effect (A23187)		(Knight & Baker, 1983)

bovine chromaffin cells (primary culture)	sucrose		no effect (nicotine) no effect (KCl)	release of [³ H]catecholamines (liquid scintillation spectrometry)	(Kilpatrick et al., 1981)
	lithium		↓ (nicotine) ↓ (veratridine) ↑ (KCl)		
	Tris		inhibition (nicotine) inhibition (veratridine) ↑ (KCl)		
	choline ethanolamine		inhibition (nicotine) inhibition (veratridine) ↓ (KCl)		
	hydrazine	↑	inhibition (nicotine) inhibition (veratridine) inhibition (KCl)		
	sucrose	no effect	no effect (nicotine) ↓ (veratridine)	trihydroxyindole fluorometric method	(Amy & Kirshner, 1982)
	sucrose	transient ↑	inhibition nicotine)	release of [³ H]catecholamines (liquid scintillation spectrometry)	(Cena et al., 1983)
	sucrose	no effect	↓ (carbachol) ↓ (nicotine) ↓ (veratridine)	ethylene-diamine condensation method	(Wada et al., 1984)
	choline	transient ↑	no effect (nicotine)	HPLC (electrochemical detection)	(Boarder et al., 1987)
	sucrose		no effect (nicotine)		
	lithium	↑		release of [³ H]catecholamines (liquid scintillation spectrometry)	(Torok & Powis, 1990)
bovine adrenal glands (thin medullary slices)	sucrose	transient ↑		trihydroxyindole fluorometric method	(Rink, 1977)
	Tris	slow ↑			
	choline	↑			
	lithium	no effect			
perfused guinea-pig adrenal glands	sucrose	transient ↑	inhibition (veratridine)	trihydroxyindole fluorometric method	(Ito et al., 1978)
	sucrose	transient ↑		trihydroxyindole fluorometric method	(Ito et al., 1979)

	choline	transient ↑	inhibition (veratridine)		
	lithium	transient ↑	inhibition (veratridine)		
guinea-pig adrenal glands (freshly dissociated cells)	sucrose	no effect	↓ (ACh)	release of [³ H]catecholamines (liquid scintillation spectrometry)	(Role et al., 1981)

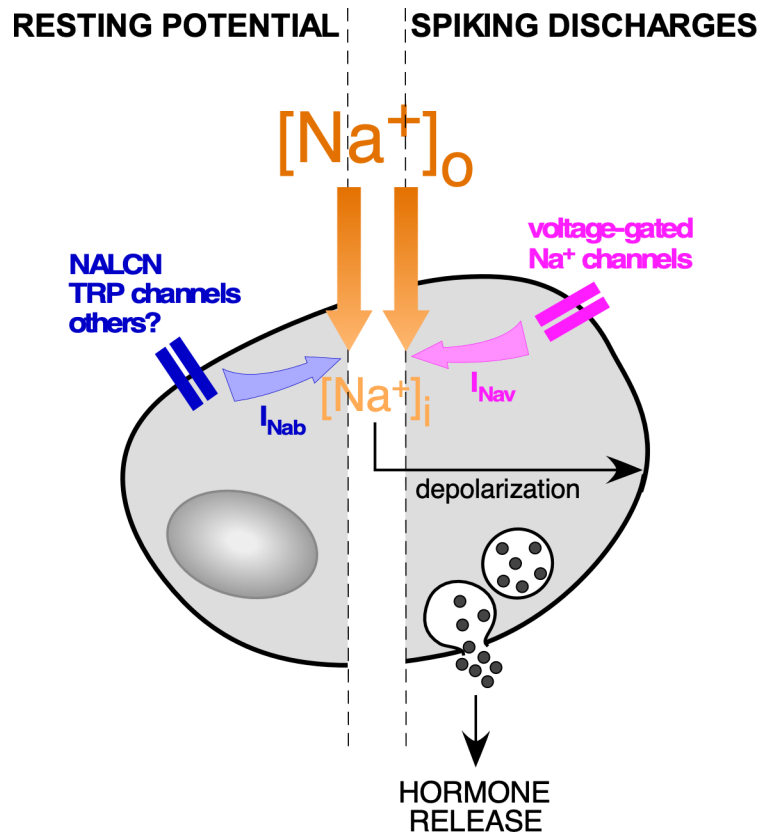
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Table 8Effect of extracellular Na⁺ removal on insulin secretion

cell type	Na ⁺ substitution	basal hormone release	stimulated (X factor) hormone release	techniques	references
rabbit (pieces of pancreas)	choline	small ↑	inhibition (glucose)	RIA	(Milner & Hales, 1967)
	choline	small ↑	↓ (glucose) inhibition (KCl) inhibition (ouabain) inhibition (L-leucine) inhibition (glucagon+glucose) inhibition (tolbutamide)	RIA	(Hales & Milner, 1968)
	lithium	small ↑	↓ (glucose) inhibition (KCl) inhibition (ouabain) inhibition (L-leucine) inhibition (glucagon + glucose) inhibition (tolbutamide)		
freshly microdissected islets (adult ♀ ob/ob mouse)	choline	↑	transient ↑ followed by ↓ (glucose)	RIA	(Hellman et al., 1974)
isolated islets (♀ NMRI mouse)	choline	no effect	↑ (glucose)	RIA	(de Miguel et al., 1988)
	lithium	transient ↑	↑ (glucose)		
	choline		transient ↑ (glucose)	RIA	(Garcia-Barrado et al., 1996)
	sucrose		transient ↑ (glucose)		
	lithium		transient ↑ (glucose)		
perfused islets (♂ albino rat)	choline	transient ↑	transient ↑ followed by ↓ (glucose)	RIA	(Lambert et al., 1974)
	lithium	transient ↑	↓ (glucose)		
	choline	transient ↑	↓ (veratridine)	RIA	(Donatsch et al., 1977)
perfused islets (♀ albino rat)	sucrose	no effect	transient ↑ followed by ↓ (glucose)	RIA	(Herchuelz et al., 1980)
pieces of pancreatic tissue (rat)	sucrose		↓ (KCl)	RIA	(Malaisse et al., 1971)
	choline		↑ (glucose)		
	Tris, lithium		↓ (glucose)		

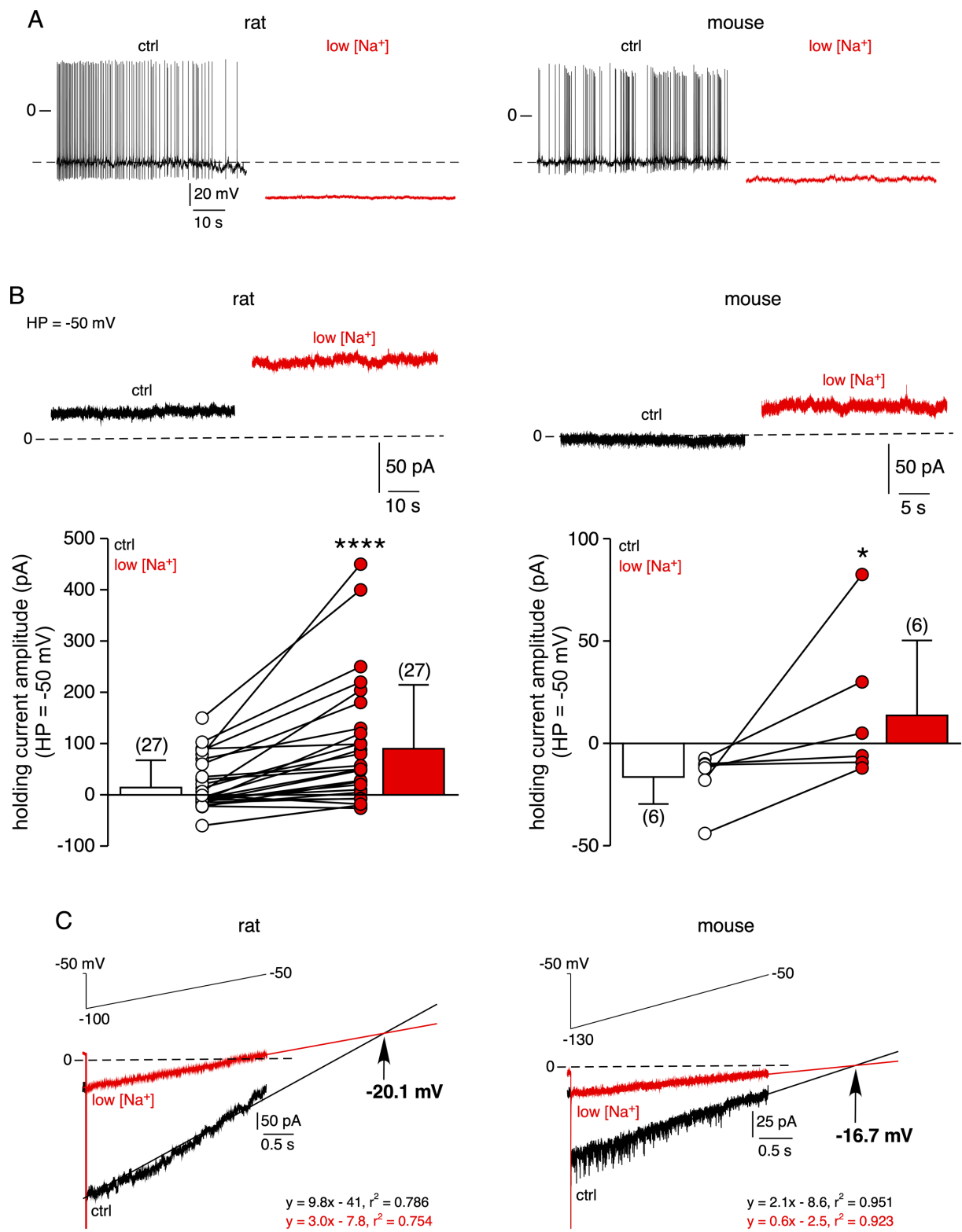
cultured fetal pancreatic explants (Wistar rat)	choline	no effect (KCl) ↑ (glucose + caffeine)	RIA	(Lambert et al., 1969)
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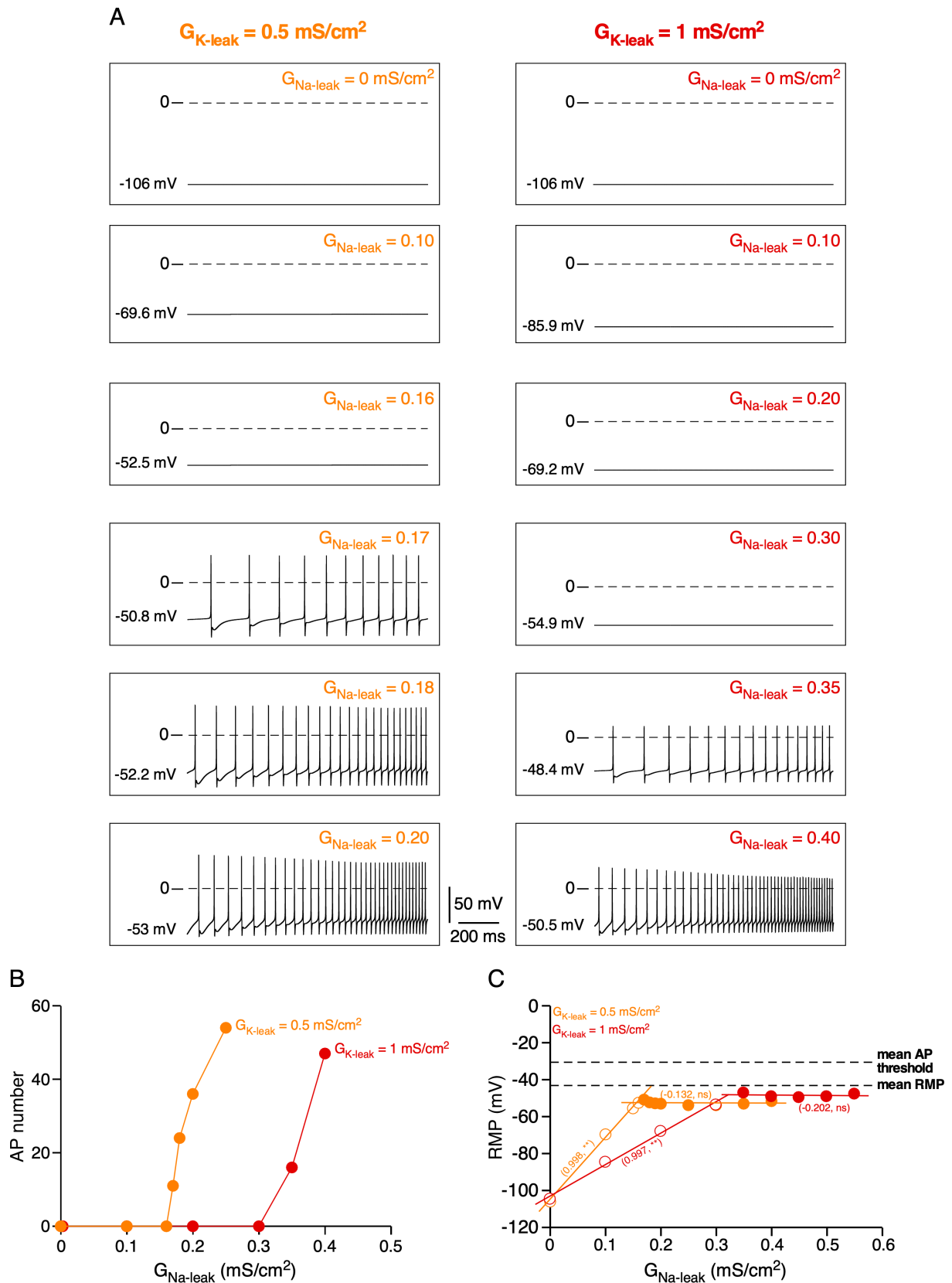
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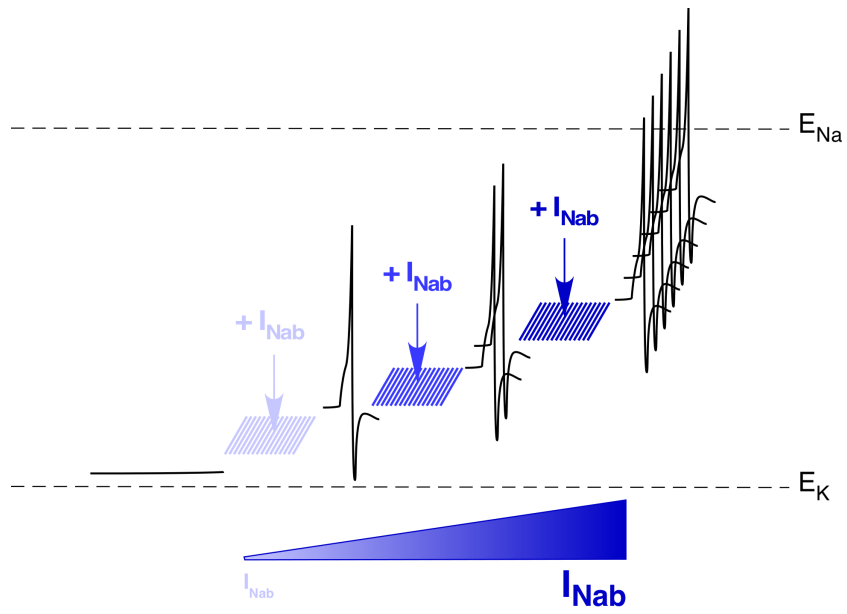
FIGURE 1



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FIGURE 2





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FIGURE 4