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Frequency-dependent depletion of secretory vesicle pools modulates bursting in vasopressin neurones of the rat supraoptic nucleus

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Abstract

When stimulated, vasopressin neurones discharge lengthy, repeating bursts of action potentials. An increase in the stimulus strength causes both a lengthening of the bursts' active phase and an increase in the intra-burst firing frequency. Here we extend our earlier model (P. Roper, J. Callaway, W. Armstrong. *J. Neurosci.* 24(20) (2004) 4831.) for phasic bursting at a constant stimulus. We show that an increase in burst length could be due to a reduction of the co-secretion of an inhibitory factor, dynorphin, and we propose this to be caused by a frequency-dependent depletion of the pool of secretory vesicles.

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1. Introduction

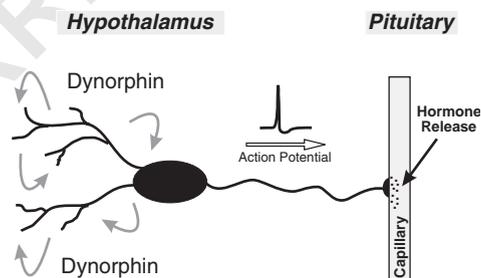
Vasopressin (VP) neurons project from the hypothalamus to the pituitary, where they secrete a hormone (VP), rather than a neurotransmitter, into the blood from their axonal terminals. These cells are activated by the physiological stress of dehydration and discharge lengthy (>20 s) bursts of action potentials (APs), each of

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1 which rides on a plateau-potential. The cells steadily depolarize as dehydration
 2 progresses, and this increases both the length of the burst's active phase, T , and the
 3 mean intra-burst firing rate, ν (typically < 10 Hz) [10]. The consequence of each of
 4 these effects is to increase the secretion of the hormone VP. The same modulation of
 5 the discharge pattern can be reproduced in vitro by direct depolarization [1].
 6 However, T can be significantly reduced if the cell is induced to fire at higher
 7 frequencies (~ 30 Hz) than the physiological range (< 20 Hz), e.g., by pharmaco-
 8 logical block of spike frequency adaptation (SFA) [5]. Thus T depends non-
 9 monotonically on ν : increasing at low, physiologically relevant, ν and decreasing
 10 at high ν .

11 The plateau is thought to be caused by the Ca^{2+} -dependent inhibition of a resting
 12 K^+ current [6], (but see also [4]), and is triggered by the Ca^{2+} influx that follows
 13 several proximal, synaptically evoked, APs. We have proposed [8] that the burst is
 14 terminated when the plateau becomes progressively desensitized to Ca^{2+} , and that
 15 this in turn is mediated by the progressive accumulation of the opioid dynorphin.
 16 Dynorphin is an autocrine messenger secreted from the somato-dendritic region of
 17 VP cells (Fig. 1) by dense-core granule (DCG) exocytosis, and is known to modulate
 18 firing: its agonists inhibit bursting, while antagonists prolong burst duration [2].
 19 Naïvely one should expect that if dynorphin secretion followed every spike, then
 20 faster firing should result in more rapid accumulation. Thus depolarization should
 21 cause bursts to terminate earlier, rather than later and hence dehydration should
 22 cause faster firing but shorter bursts, which is in contrast to what occurs
 23 experimentally. Here we propose that the releasable pool of dynorphin vesicles
 24 becomes depleted as ν increases. In consequence, the amount of dynorphin available
 25 for secretion diminishes, and this in turn slows accumulation and prolongs the active
 26 phase despite the increase in ν . We further conjecture that the effects of SFA
 27 blockade are due to residual release from a non-primed pool.



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43 Fig. 1. VP cells project from the hypothalamus to the pituitary where they secrete the hormone
 44 vasopressin into the bloodstream. The cells are auto-regulated by secretion of dynorphin, an opioid, from
 45 their soma and dendrites. Dynorphin is secreted via dense-core granule exocytosis and acts through κ -
 receptors to shift the K_d of the plateau-potential, and terminate the burst.

1 2. The model

3 We quantify the effect on the plateau of the transduction of the κ -opioid receptor
 5 activation by dynorphin with the parameter D . D therefore quantifies both the
 7 extracellular concentration of dynorphin, and also the κ -receptor density in the cell
 9 wall. The receptor density is not constant, but can be increased in a stimulus-
 11 dependent manner [9]. This upregulation is also driven by DCG exocytosis since the
 vesicular membrane carries κ -receptors and hence fusion of the granule also
 upregulates the receptor. Once bound, the dynorphin– κ -receptor complex is cleared
 by internalization and de-phosphorylation, and both are then recycled into new
 granules. The secretory mechanism is discussed more fully in [8].

13 In [8] we proposed that D is zero when the cell is at rest, is augmented by an
 amount Δ after every spike, and decays exponentially when the cell is quiet, so that

$$15 \quad \frac{d}{dt}D = -\frac{1}{\tau_D}D \quad \text{and} \quad D = D + \Delta \iff V = V_{\text{thresh}}^+, \quad (1)$$

17 where the decay constant $\tau_D = 5$ s, and we choose the threshold $V_{\text{thresh}} = 0$. To
 19 include the effects of receptor upregulation and to account for known properties of
 the phasic pattern we further proposed that D facilitated its own action, so that Δ
 depends directly on D ,

$$21 \quad \Delta = \alpha + \beta D, \quad (2)$$

23 α is a small constant bias term that breaks the symmetry of the $D = 0$ state, and β is
 25 proportional to the amount of dynorphin secreted by a single AP. β can be
 interpreted as being the quantity of dynorphin secreted when a κ -receptor is
 activated.

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29 3. Results

31 In the full model [8], the plateau carries the cell above spike threshold into a
 33 repetitive spiking mode, and is sustained by spike-driven Ca^{2+} -influx. Within the
 active phase, firing goes through a brief period of spike-frequency adaptation, but
 35 then remains steady until the burst abruptly terminates. Concurrently, $[\text{Ca}^{2+}]_i$
 rapidly attains a steady state where influx and efflux balance, and only decays at the
 37 end of the burst when spiking has ceased. Our main finding in [8] was that D must act
 by shifting the K_d of the plateau current, and so progressively desensitizing the
 plateau to calcium until it no longer supports repetitive spiking and collapses, thus
 39 terminating the active phase. A mechanism for desensitization has yet to be
 discerned but it is likely to involve a second messenger pathway, since resting
 41 K^+ currents such as TASK-1 are not thought to be directly Ca^{2+} -sensitive but can be
 inhibited by phospholipase C [3].

43 We simplify the model first by assuming that the active phase terminates when D
 reaches some threshold, say \bar{D} , and second by replacing the full spiking model of Eq.
 45 (1) with a firing-rate model. Eq. (1) can then be solved for constant v to give the

1 mean trajectory for $D(t)$ (see [8])

$$3 \quad D(t) = \frac{\alpha v \tau_D}{1 - \beta v \tau_D} \left[1 - \exp\left(-\frac{(1 - \beta v \tau_D)t}{\tau_D}\right) \right]. \quad (3)$$

5 T can then be found as a function of β for a particular firing rate, v , by setting
7 $D(T) = \bar{D}$ and inverting Eq. (3), and is plotted in Fig. 2. Note that T increases with
decreasing β as expected, and increases asymptotically for small β .

9 Bursts can therefore become longer with increasing dehydration/depolarization if
 β decreases as the cell fires faster.

11 Dynorphin is secreted by dense-core granule exocytosis, and in other neuroendo-
crine cells the recovery of the releasable pool of vesicles is known to be slow
13 following a secretory event. If refilling after one spike is so slow that it becomes
interrupted by the next, then the number of vesicles available for secretion will be
15 significantly smaller than the maximum size of the pool. Thus, since β is a measure of
the size of the releasable pool, it can become progressively depleted as v increases.

17 The releasable pool is known to be a small subset (typically $< 10\%$) of the docked
pool of granules, and movement of granules from the docked to the releasable pool is
19 termed priming. Priming does not involve a physical translocation, but is a chemical
step that depends on both calcium and ATP.

21 The data can be best fit by assuming that β has two components: secretion from a
dynamic, primed, pool that empties and then slowly replenishes, and a small
23 constant term, which may correspond to direct secretion from the docked pool. We
further find that priming in this model must be a two-stage process, possibly

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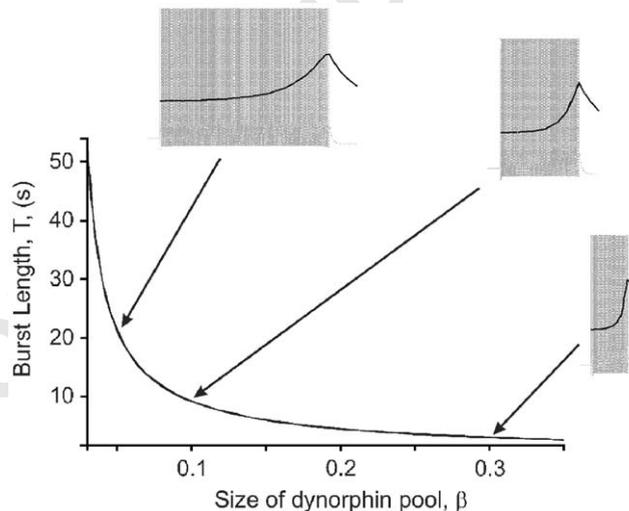
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43 Fig. 2. Length of active phase, T , as a function of the quantity of dynorphin secreted, β , for fixed firing
frequency, v . Note that T increases with decreasing β , and increases asymptotically for small β . Voltage
traces for three representative bursts are shown (greyscale) with superimposed trajectories for D (black
45 line). The effect of increasing v is to shift the asymptote leftward (data not shown).

1 representing transitions from the docked pool (DP) first to a readily releasable pool
 2 (RRP) and subsequently to an immediately releasable pool (IRP). Let I be the
 3 concentration in the IRP, and let R , the concentration in the RRP, be filled from an
 4 infinite docked pool. The simplest model is then that the pool is emptied ($I = 0$)
 5 following a spike, and refills according to

$$7 \quad \frac{d}{dt}R = \frac{I_\infty - I}{\tau_R} - \frac{R}{\tau_I}, \quad (4)$$

$$9 \quad \frac{d}{dt}I = \frac{R}{\tau_I}. \quad (5)$$

11 The time constants τ_R and τ_I are unknown, and so for concreteness we have set
 12 $\tau_R = 0.5$ s and $\tau_I = 0.2$ s, but the model is stable for a range of parameters.

13 Solving for $I(t)$ we plot the fractional filling of the pool, $I(t)/I_\infty$, as a function of
 14 time in Fig. 3(a). Note that as the firing frequency, ν , increases, the concentration in
 15 the IRP at each spike will be $I(\nu^{-1})$.

16 We now set $\beta(\nu) = I(\nu) + \delta$, where δ represents direct secretion from the docked
 17 pool. We assume δ to be constant since the docked pool is typically much larger than
 18 the releasable pool. Following our earlier prescription we plot T versus ν in Fig. 3(b).
 19 The model clearly reproduces both the increase in T seen at low frequencies, and its
 20 subsequent decrease at high ν . Note also that T increases asymptotically at very low
 21 ν , and in fact phasic activity only occurs in vivo for $\nu > \sim 1.5$ Hz [7]. The increase in
 22 T for intermediate frequencies marks the progressive depletion of the IRP with
 23 increasing ν . However, the IRP becomes almost fully depleted at high frequency
 24 ($I(\nu) \rightarrow 0$ for $\nu > 15$ Hz) and instead the residual component δ starts to dominate,
 25 and so secretion from the releasable pool becomes less effectual at high ν . Although δ
 26 is small, the burst length starts to decrease for frequencies above $\nu > 20$ Hz since the
 27 total rate of secretion depends not only on the secretion-per-spike, but also on the
 28 firing frequency.

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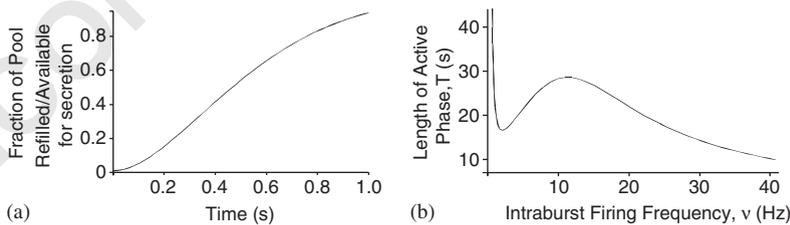


Fig. 3. (a) The fractional filling of the Immediately Releasable Pool, $I(t)/I_\infty$, as a function of time; see Eq. (5). (b) Length of active phase, T , as function of firing frequency, ν . Here β is dynamic and decreases as ν increases.

1 4. Conclusions

3 We have proposed that the concomitant increase of firing rate (ν) and lengthening
 4 of the active phase (T) observed in vasopressin cells during increasing dehydration/
 5 depolarization is caused by depletion of the releasable pool of dense-core granules.
 6 We further find that the subsequent decrease in T at high ν is due to a small, residual
 7 secretion directly from the docked pool and not from the releasable pool. This model
 8 extends our earlier analysis [8] in which we proposed that a slow priming step could
 9 account for a transient response that occurs following the sudden onset of a stress.
 10 We have assumed that secretion directly from the docked pool, δ , is constant due to
 11 its relative size, but a more realistic model should also account for depletion of the
 12 docked pool. However, as a consequence of its size, depletion of the docked pool
 13 should occur at discharge frequencies much higher than those that occur
 14 physiologically.

15 In essence this model is similar to earlier models of synaptic depression, except
 16 that DCG exocytotic dynamics are known to be significantly slower than those of
 17 synaptic vesicles. The model could be tested by a manipulation of the priming
 18 process, either at the ATP-dependent step, or via the use of caged calcium.

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