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Modulation/physiology of calcium channel sub-types in neurosecretory terminals

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ABSTRACT

The hypothalamic-neurohypophysial system (HNS) controls diuresis and parturition through the release of arginine-vasopressin (AVP) and oxytocin (OT). These neuropeptides are chiefly synthesized in hypothalamic magnocellular somata in the supraoptic and paraventricular nuclei and are released into the blood stream from terminals in the neurohypophysis. These HNS neurons develop specific electrical activity (bursts) in response to various physiological stimuli. The release of AVP and OT at the level of neurohypophysis is directly linked not only to their different burst patterns, but is also regulated by the activity of a number of voltage-dependent channels present in the HNS nerve terminals and by feedback modulators. We found that there is a different complement of voltage-gated Ca^{2+} channels (VGCC) in the two types of HNS terminals: L, N, and Q in vasopressinergic terminals *vs.* L, N, and R in oxytocinergic terminals. These channels, however, do not have sufficiently distinct properties to explain the differences in release efficacy of the specific burst patterns. However, feedback by both opioids and ATP specifically modulate different types of VGCC and hence the amount of AVP and/or OT being released. Opioid receptors have been identified in both AVP and OT terminals. In OT terminals, μ-receptor agonists inhibit all VGCC (particularly R-type), whereas, they induce a limited block of L-, and P/Q-type channels, coupled to an unusual potentiation of the N-type Ca^{2+} current in the AVP terminals. In contrast, the N-type Ca^{2+} current can be inhibited by adenosine *via* A_1 receptors leading to the decreased release of both AVP and OT. Furthermore, ATP evokes an inactivating Ca^{2+}/Na^{+}-current in HNS terminals able to potentiate AVP release through the activation of P2X2, P2X3, P2X4 and P2X7 receptors. In OT terminals, however, only the latter receptor type is probably present. We conclude by proposing a model that can explain how purinergic and/or opioid feedback modulation during bursts can mediate differences in the control of neurohypophysial AVP *vs.* OT release.

1. The hypothalamic-neurohypophysial system

Depolarization–secretion coupling is the primary mechanism [1–3] for transforming electrical activity into chemical signals [4]. It is particularly important to study this mechanism at nerve terminals, which are specifically differentiated for release, rather than just at neuronal cell bodies [5]. Our primary goal is to understand how specific patterns of electrical activity generated at somata regulate Ca^{2+}-entry and subsequent transmitter release at nerve terminals.

1.1. Bursting patterns of activity

At the neuromuscular junction level, there is no simple linear relationship between the amount of acetylcholine (ACh) released and the number of action potentials (AP) stimulating the motor ending [4]. Other systems, in particular peptide-releasing neurons, maximally release transmitter only in response to specific "bursting" patterns of activity [6–12]. In the bullfrog sympathetic ganglia bursts of APs are necessary to specifically elicit peptide (such as LHRH) release. While single APs do not release LHRH, they do release ACh [13]. Similarly, in chromaffin cells, trains of APs (bursts)

Abbreviations: HNS, hypothalamic-neurohypophysial system; ATP, adenosine tri-phosphate; AVP, arginine-vasopressin; OT, oxytocin; VGCC, voltage-gated Ca^{+2} channels; MCN, magnocellular neurons; AP, action potentials; SON, supraoptic nuclei; PVN, paraventricular nuclei; BK, calcium-activated K^{+}; TTX, tetrodotoxin; CNS, central nervous system; GVIA, ω-conotoxin GVIA; ω-AgaIVA, ω-agatoxin IVA; DAMGO, D-Ala2, MePhe4, Glyol[5]enkephalin; rKO, receptor knockout, μOR, κOR.

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are needed to elicit the "fight or flight" response, *i.e.* large amounts of norepinephrine release, while a single AP only releases ACh [14].

Much is known about the physiological bursting patterns of the HNS (reviews: [15,16]). OT neurons are characterized by a high frequency (up to 200 Hz) discharge during suckling which leads to the pulsatile release of OT into the blood and to subsequent milk-ejection [17]. AVP neurons are characterized by their asynchronous (10–60 Hz) phasic activity (bursting) during maintained AVP release and the subsequent regulation of water balance. In both cases, it is the clustering of spikes, albeit with different time courses for each peptide, which facilitates hormone release [7,8,18]. A "typical" AVP burst [8,19,20] has a low frequency (<5 Hz) for the first 2–3 s, then high to low frequency (60–10 Hz) for the next 20–60 s. Interestingly, interburst silent periods of greater than 21 s have been shown to be critical for maximal peptide release [7,8].

1.2. Hypothalamic-neurohypophysial system

The two neuropeptides, arginine vasopressin (AVP) and oxytocin (OT) are mainly synthesized in magnocellular neurons (MCN) located in the hypothalamus. AVP is a vasoconstrictor and an antidiuretic and, thus, is involved in fluid homeostasis. OT has recognized functions in parturition and lactation [21], and has an emerging role as a natriuretic agent [12]. Both hormones may also be central neurotransmitters and have been implicated in sexual behavior [22], stress, learning, memory processes [23,24], the development and maintenance of tolerance to ethanol [25], and also in several pathophysiological functions [24].

Neuroendocrine MCNs are located principally in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus [26–28]. Almost all the neurons within this system project to the neurohypophysis [29]. The NH nerve terminals have a mean diameter of about 2 μm, although much larger (*ca.* 5–10 μm) terminals can be observed [19].

1.3. Electrophysiology of terminals in situ

Direct electrophysiological measurements of individual nerve terminals have been very difficult. Even though loose patch-clamp recordings from intact, whole rat HNS have become possible [30,31], only in the large sized squid giant, rat NH, and crab sinus gland terminals has it been possible to record endogenous electrical activity *via* intracellular electrodes [5,9,11,33,34]. Optical recording techniques have yielded much data on murine NH terminals, but not at the individual terminal level [35,36]. Neither intracellular nor optical recordings have allowed specific characterization and localization of the ionic channels responsible for individual nerve terminal electrical activity in the intact HNS. Thus, these questions remain: how and where does Ca^{2+} enter each type of terminal in response to the specific patterns of electrical stimulation and by what process(es) does Ca^{2+} entry lead to the regulated exocytotic release of the different peptide transmitters *in situ*?

1.4. Isolated neurohypophysial terminals

In order to address such questions, we have developed a preparation of isolated neurohypophysial terminals [19,37–40]. Electron microscopy [29] and immunocytochemistry [19,20] have been used to characterize this preparation. The integrity and purity of the isolated NH terminal preparation is important, and in the intact NH, blood vessels, pituicytes, and other cells are distinguishable from the terminals [20]. It is certain that this preparation contains only nerve terminals with minimal contamination from the other components and numerous studies have confirmed that the physiological functioning of isolated terminals is the same as in the intact NH [29,41–44]. In addition, we can confirm by immunoblotting [42,45], or specific ELISAs [32] after patch-clamp recordings and Ca^{2+} measurements, whether the individual terminal is vasopressinergic or oxcytocinergic. Recently, transgenic rats tagged by a visible fluorescent protein have been developed and successfully employed to study the physiology of AVP (enhanced green fluorescent protein), OT (enhanced cyan fluorescent protein) and OT (monomeric red fluorescent protein) in neurons and terminals [24,47–51]

1.5. Intraterminal $[Ca^{2+}]_i$

It is possible to show, using fluorescent probes, that depolarization of populations of isolated nerve terminals is associated with an increased Ca^{2+} concentration in their cytoplasm [52]. It has also become feasible to measure $[Ca^{2+}]_i$ levels in individual terminals to determine the role of Ca^{2+} in various physiological functions in rats [46,53–65], as well as in mice [60,66,67] neurohypophyses. These techniques are complementary and confirm findings made using electrophysiological characterization of Ca^{2+} effects in these terminals.

2. Ca^{2+} currents in terminals

There are two main voltage-dependent outward currents in NH terminals. One is a fast, inactivating current that can be blocked with 4-aminopyridine [68], and the other is a non-inactivating current which can be blocked with tetrandrine [69]. The pharmacology and kinetics of the initial transient outward current identify it as an A-current. The second, slower developing outward current, which is dependent upon increase in $[Ca^{2+}]_i$, shows no steady-state inactivation [42,70]. Interestingly, this Ca^{2+}-activated K^+ (BK) current can be blocked by extracellular ATP [71]. There are fast, voltage-dependent inward currents that are blocked by tetrodotoxin (TTX), as well as slow, inward currents that are not blocked by TTX but disappear in low Ca^{2+} buffer suggesting that they are, respectively, voltage-dependent Na^+ and Ca^{2+} currents [71,74,75].

2.1. Different Ca^{2+}-channel subtypes

There are two kinetic components to the slower inward Ca^{2+} current, which are differentially inactivated depending upon the holding potential [71]. The two Ca^{2+} current kinetic components appear to be composed of distinct subtypes of voltage-gated Ca^{2+} channels (VGCC) in NH terminals. VGCCs, classified as L-, N-, P-, Q-, R-, and T-type based on their functional and pharmacological properties, are critical for many cellular functions including muscular contraction, neurotransmitter release, and excitability. To date, nine neuronal Ca^{2+} channel genes have been identified and termed α_{1A} through α_{1I} [76].

2.2. VGCC subtypes in AVP vs. OT terminals

Our previous findings show that there is heterogeneity of Ca^{2+} channel types in the NH terminals [71–75]. Perforated patch-clamp measurements of Ba^{2+} currents in NH terminals (see Fig. 1) classify them into two groups: one in which the GVIA- and nicardipine-resistant component is sensitive to ω-AgaIVA/SNX-230 (Fig. 1A) and one in which it is insensitive (Fig. 1B). The former channel is not found, or not functional, in about half of the terminals. Immunohistochemistry of isolated NH terminals labeled with AVP or OT *vs.* P/Q subtype VGCC (Fig. 2) indicates that the P/Q channel is found only in AVP terminals. Thus, Q-type Ca^{2+} channels are preferentially located on AVP-containing NH terminals [56].

The biophysical properties of the GVIA- and nicardipine-resistant component (Fig. 1B) are that of a high-voltage activated,