CLASSICAL PERSPECTIVES

The neurophysiology of neurosecretory cells

William E. Armstrong
Department of Anatomy and Neurobiology and Neuroscience Institute, University of Tennessee Health Science Center, 855 Monroe Avenue, Memphis, TN 38163, USA

Email: warmstrong@utmem.edu

With the current trend in neurobiology of increasingly molecular/genetic studies, coupled with the faster payoff and feasibility of *in vitro* preparations, it is well to reflect on the advances from, and continued need for, the often more difficult systems research in neuroendocrinology. A huge leap in understanding the relationship between the electrical discharge of oxytocin (OT) and vasopressin (VP) neurons and hormone release occurred courtesy of the discovery that the milk ejection known to result from OT release during lactation in mammals could be obtained under anaesthesia (Lincoln *et al.* 1973). This preparation paved the way for correlating the discharge patterns of antidromically identified neurosecretory neurons with the selective OT release *in vivo*, and led to an important series of papers by Wakerley, Lincoln and co-workers that set the bar in this field for decades to come. The obvious power of this preparation lay in the ability to precisely record each milk ejection by recording intramammary pressure, whilst simultaneously registering the spike trains of neurosecretory neurons. Two papers from *The Journal of Physiology* considered here (Lincoln & Wakerley, 1974, 1975) are part of a remarkable series of studies from the same lab during the 1970s. Collectively, these are testaments to the lasting rewards of *in vivo* electrical recording when combined with appropriate experimental conditions that allow expression of the system's physiological behaviour. They also punctuate the evolution in our understanding of the hypothalamo-neurohypophysial system – from the discovery of the hypothalamic origin and peptidergic nature of VP and OT (Bargmann & Scharrer, 1951), to the acceptance of the parent, large cells (magnocellular) of the supraoptic (SON) and paraventricular nuclei (PVN) as fully neuronal in character.

Although electrical stimulation of the neural stalk was long known to evoke neurohypophysial hormone release (Cross & Harris, 1950), the excitable membrane properties characteristic of neurons were not demonstrated in convincing fashion in neurosecretory cells until the elegant, pioneering work of Kandel (1964), from the Nobel Prize winner's early scientific years. Kandel made intracellular recordings from neurosecretory neurons of the preoptic nucleus of goldfish (the SON and PVN homologue), demonstrated their ability to generate action potentials, antidromically identified the neurons with neural stalk stimulation, and produced orthodromic, synaptic responses with olfactory bulb stimulation. Antidromic recordings from the rat SON soon followed (Yagi *et al.* 1966). These recording studies were complemented by the contemporary, now classical experiments of Douglas & Poisner (1964), which demonstrated unequivocally that calcium–secretion coupling of vasopressin release in the neural lobe was like that governing acetylcholine release at neuromuscular junction. With this information, scientists moved to determine the relationship of the electrical behaviour of SON and PVN neurons to hormone release, with the understanding that it was the action potentials generated from the somatic-dendritic region of SON and PVN neurons that invaded and depolarized nerve terminals in the neural lobe, thereby opening calcium channels and instigating the biochemical cascade underlying vesicle exocytosis.

The conventional wisdom in the early 1970s was that the SON was primarily responsible for VP release, and the PVN for OT release, conclusions drawn mostly from the effects of hypothalamic lesion and stimulation studies. In truth, many inconsistencies in the literature challenged this dogma. Wakerley and Lincoln (1973) found that about half the antidromically identified PVN neurons fired a brief (2–4 s) burst of activity associated with each milk ejection (confirmed by measuring intramammary pressure, and occurring every few minutes). However, when the same experiment was applied to the SON (Lincoln & Wakerley, 1974), a similar result was obtained – i.e. about half the antidromically identified SON neurons were associated with milk ejection. The authors found of the SON 'milk-ejection' neurons to be largely indistinguishable in their behaviour from those in the PVN, and concluded that both nuclei must participate in OT release. The study also marked the first report of the synchronous activation of OT neurons as shown from dual recordings on the same electrode. The association of VP and OT with both the SON and PVN was soon verified with the seminal immunochemical studies of VP and OT neurons in the hypothalamus (Swaab *et al.* 1975; Vandesande & Dierickx, 1975).

A logical conclusion from Lincoln & Wakerley (1974) was not only that the SON participated in OT release, but that its larger size (i.e. greater number of neurons) relative to the PVN made it quantitatively the more important of the two nuclei for release of *both* hormones. This is not to say that the PVN is nothing more than a diminutive SON (cytoarchitectonically, the PVN is more complicated and heterogeneous), but simply that more cells mean more hormone to be released. Cell counts have verified that the rat SON contains 3–4 times as many OT *and* VP neurons as does the PVN, and retrograde tracing studies indicate that virtually all SON neurons project to the neurohypophysis. There are similar proportions of OT to VP neurons in the two nuclei, but slightly more OT than VP neurons are found in PVN, and the reverse is true in the SON.

While successfully determining that the periodic release of OT during suckling was a direct result of the synchronous, explosive bursting activity of OT neurons, these studies also unveiled a still unresolved mystery – how is the continuous stimulation of the attached pups translated into a relatively slow, periodic expression? In the following year, Lincoln & Wakerley (1975) focused on the extent to which milk ejection bursts were influenced by the number of suckling pups. The experiment and its results were simple but the implications profound: an increased number of suckling pups (once a threshold of ~5 pups was reached) was correlated with an increased amount of OT released, and also with the amplitude of the milk ejection burst – but the frequency of milk ejections was unaffected. Thus the continuous stimulation appeared to activate a central pattern generator whose peak responses were dependent upon the intensity of

 DOI: 10.1113/jphysiol.2007.145755

stimulation. Given the additional fact that the neurosecretory response was not temporally locked to suckling (pups suckled most vigorously when first attached, yet milk ejection first occurred several minutes after pups had been first attached; also, the most intense suckling occurred *after*, not before, each increase in intramammary pressure), the authors speculated that a gating mechanism sculpted this relatively constant frequency, and that afferent summation controlled response magnitude.

Exploiting the *in vivo* milk-ejection preparation in another series of landmark studies, Moos and coworkers confirmed the massive synchronization of OT neurons across the SON and PVN (e.g. Moos & Richard, 1989). More importantly, these workers discovered that the *somato-dendritic* release of OT during suckling was critical to the recruitment of OT neurons in the burst. Indeed, local OT release in response to the suckling stimulus, probably mediated *via* noradrenergic and glutamatergic inputs, is the most likely candidate mechanism regulating burst amplitude and recruiting OT neurons into the milk ejection reflex. Further *in vitro* studies confirmed that OT neurons possessed OT receptors, and VP neurons VP (particularly V1a) receptors (Lambert *et al.* 1994). Activation of the OT receptors releases Ca^{2+} from internal stores, and this event is now thought to prime dense core vesicles for further OT release and the facilitation of bursting (however, dendritic OT release can also be associated with an *inhibition* of firing, and this disparity is likely to reflect the possibility of different processes coupled to discrete sites, or amounts, of intracellular Ca^{2+} release). This somato-dendritic release of neuropeptide, first documented in this system with electron microscopy, is further known to modulate afferent inputs to OT and VP neurons (Kombian *et al.* 1997), and may account for a significant amount of the OT and VP present in the cerebrospinal fluid (Ludwig & Leng, 2006).

The pattern generator responsible for the frequency of OT bursting during lactation is still not well understood. Simplistically, the milk ejection response behaves as an electrical capacitor that takes minutes to charge (with nipple attachment), then explosively discharges in seconds. In recent *in vitro* studies investigators have been able to pharmacologically induce asynchronous milk-ejection-like bursts in hypothalamic slices with prolonged applications of OT or noradrenaline (Wang & Hatton, 2004). In organotypic cultures of the magnocellular neurons (taken from early postnatal pups), OT induces *synchronous* bursting, suggesting again that the pattern generator may be hypothalamic (Jourdain *et al.* 1998). Interestingly, in both preparations, the response to OT was dependent on intact glutamatergic afferents, as is milk ejection *in vivo*. Thus one might speculate that continuous release of OT during nipple attachment eventually reaches a threshold to allow bursts driven primarily by afferent input, but modulated by the intrinsic properties of OT neurons (Stern & Armstrong, 1996).

As hinted above, the SON also contained a large number of 'non-milk ejection' cells, as did the PVN, and these later were confirmed as VP neurons. These neurons displayed phasic bursting activity, and initially were suspected to be OT neurons. However, this bursting was quite distinct from that determined for milk-ejection neurons – the bursts lasted tens of seconds, even minutes on occasion, with interburst intervals of a similarly long duration. This pattern of phasic bursting has been found since to be asynchronous, to be promoted by hypovolaemia, hypotension and hyperosmolality, and to underlie enhanced VP release. The behaviour is easily observed *in vitro* in identified VP neurons, and from intense study we know it to be largely an intrinsic, Ca^{2+}-dependent property, but one whose expression *in vivo* is actuated primarily by synaptic, glutamatergic excitation (Nissen *et al.* 1995; Brown *et al.* 2004). The precise quantitative relationship of this pattern with hormone release has been intensely examined, expanding the original description of frequency-dependent stimulation to encompass facilitation, fatigue and recovery from fatigue, in terms of Ca^{2+} handling (Cazalis *et al.* 1985). Like with OT neurons, the local release of VP and cosecreted peptides such as dynorphin auto-regulates phasic bursting activity (Ludwig & Leng, 2006). The study of the origin and control of phasic bursting activity has lately become rich with the application of modern molecular, anatomical, computational and electrophysiological techniques to a phenomenon originally described using metal electrodes and a pen recorder. Nowhere is this more evident than our recent understanding that OT and VP neurons also behave as osmoreceptors, with an identified molecular mechanism in the expression of a transient receptor potential vanilloid type-1 channel (Sharif Naeini *et al.* 2006).

There is significant irony now when reflecting on the slow acceptance of mammalian SON and PVN cells as fully neuronal. After motor neurons, these neurons were the first for which an unambiguous physiological function was understood. Furthermore, the majority of neurons, peripheral and central, are undoubtedly peptidergic, regardless of what other neurotransmitters they possess. Students of the hypothalamo-neurohypophysial system have led our current understanding of the mechanisms and functions of central, and particularly dendritic, peptide release and its consequent retrograde synaptic actions, a phenomenon of growing importance to understanding synaptic plasticity and local cerebral blood flow.

As a graduate student working with Glenn Hatton in the late 1970s, I heard Jonathan Wakerley give a lecture that described, with the same clarity as the aforementioned papers, the electrical activity of antidromically identified SON and PVN neurons during lactation, and all its ramifications. At its end, someone asked with grave sincerity: But what does this electrical activity really have to do with hormone secretion? Dr Wakerley patiently explained, and here I paraphrase from imperfect memory: The action potentials propagate down the axons to the neural lobe, where they invade neurosecretory terminals. This depolarizes the terminals, increases Ca^{2+} influx, which then drives exocytosis of hormone, just as it does acetylcholine release at the frog neuromuscular junction. They are neurons, after all.

References

Brown CH, Bull PM & Bourque CW (2004). Phasic bursts in rat magnocellular neurosecretory cells are not intrinsically regenerative in vivo. *Eur J Neurosci* **19**, 2977–2983.

Cazalis M, Dayanithi G & Nordmann JJ (1985). The role of patterned burst and interburst interval on the excitation-coupling mechanism in the isolated rat neural lobe. *J Physiol* **369**, 45–60.

Cross BA & Harris GW (1950). Milk ejection following electrical stimulation of the pituitary stalk in rabbits. *Nature* **166**, 994–995.

Douglas W & Poisner A (1964). Stimulus-secretion coupling in a neurosecretory organ: the role of calcium in the release of vasopressin from the neurohypophysis. *J Physiol* **172**, 1–18.

Jourdain P, Israel JM, Dupouy B, Oliet SHR, Allard M, Vitiello S, Theodosis DT & Poulain DA (1998). Evidence for a hypothalamic oxytocin-sensitive pattern-generating network governing oxytocin neurons in vitro. *J Neurosci* **18**, 6641–6649.

Kandel ER (1964). Electrical properties of hypothalamic neuroendocrine cells. *J Gen Physiol* **47**, 691–717.

Kombian SB, Mouginot D & Pittman QJ (1997). Dendritically released peptides act as retrograde modulators of afferent excitation in the supraoptic nucleus in vitro. *Neuron* **19**, 903–912.

Lambert RC, Dayanithi G, Moos FC & Richard P (1994). A rise in the intracellular Ca^{2+} concentration of isolated rat supraoptic cells in response to oxytocin. *J Physiol* **478**, 275–287.

Lincoln DW, Hill A & Wakerley JB (1973). The milk-ejection reflex of the rat: an intermittent function not abolished by surgical levels of anesthesia. *J Endocrinol* **57**, 459–476.

Lincoln DW & Wakerley JB (1974). Electrophysiological evidence for the activation of supraoptic neurones during the release of oxytocin. *J Physiol* **242**, 533–554.

Lincoln DW & Wakerley JB (1975). Factors governing the periodic activation of supraoptic and paraventricular neurosecretory cells during suckling in the rat. *J Physiol* **250**, 443–461.

Ludwig M & Leng G (2006). Dendritic peptide release and peptide-dependent behaviours. *Nat Rev Neurosci* **7**, 126–136.

Moos F & Richard P (1989). Paraventricular and supraoptic bursting oxytocin cells in rat are locally regulated by oxytocin and functionally related. *J Physiol* **408**, 1–18.

Nissen R, Hu B & Renaud LP (1995). Regulation of spontaneous phasic firing of rat supraoptic vasopressin neurones in vivo by glutamate receptors. *J Physiol* **484**, 415–424.

Sharif Naeini R, Witty MF, Seguela P & Bourque CW (2006). An N-terminal variant of Trpv1 channel is required for osmosensory transduction. *Nat Neurosci* **9**, 93–98.

Stern JE & Armstrong WE (1996). Changes in the electrical properties of supraoptic nucleus oxytocin and vasopressin neurons during lactation. *J Neurosci* **16**, 4861–4871.

Swaab DF, Nijveldt F & Pool CW (1975). Distribution of oxytocin and vasopressin in the rat supraoptic and paraventricular nucleus. *J Endocrinol* **67**, 461–462.

Vandesande F & Dierickx K (1975). Identification of the vasopressin producing and of the oxytocin producing neurons in the hypothalamic magnocellular neurosecretory system of the rat. *Cell Tiss Res* **164**, 153–162.

Wakerley JB & Lincoln DW (1973). The milk-ejection reflex of the rat: a 20- to 40-fold acceleration in the firing of paraventricular neurones during oxytocin release. *J Endocrinol* **57**, 477–493.

Wang YF & Hatton GI (2004). Milk ejection burst-like electrical activity evoked in supraoptic oxytocin neurons in slices from lactating rats. *J Neurophysiol* **91**, 2312–2321.

Yagi K, Azuma T & Matsuda K (1966). Neurosecretory cell: capable of conducting impulse in rats. *Science* **154**, 778–779.