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Somato-dendritic vasopressin and oxytocin secretion in endocrine and autonomic regulation

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Abstract

Somato-dendritic secretion was first demonstrated over 30 years ago. However, while its existence has become widely accepted, the function of somato-dendritic secretion is still not completely understood. Hypothalamic magnocellular neurosecretory cells (MNCs) were among the first neuronal phenotypes in which somato-dendritic secretion was demonstrated and are among the neurones for which the functions of somato-dendritic secretion are best characterised. These neurones secrete the neuropeptides, vasopressin and oxytocin, in an orthograde manner from their axons in the posterior pituitary gland into the blood circulation to regulate body fluid balance and reproductive physiology. Retrograde somato-dendritic secretion of vasopressin and oxytocin modulate the activity of the neurones from which they are secreted, as well as the activity of neighbouring populations of neurones, to provide intra- and inter-population signals that coordinate the endocrine and autonomic responses for control of peripheral physiology. Somato-dendritic vasopressin and oxytocin have also been proposed to act as hormone-like signals in the brain. There is some evidence that somato-dendritic secretion from MNCs modulates the activity of neurones beyond their local environment where there are no vasopressin- or oxytocin-containing axons but, to date, there is no conclusive evidence for, or against, hormone-like signalling throughout the brain, although it is difficult to imagine that the levels of vasopressin found throughout the brain could be underpinned by release from relatively sparse axon terminal fields; the generation of data to resolve this issue remains a priority for the field.

Information transfer in the central nervous system

The classical understanding of communication in the nervous system is of synaptic transmission in a unidirectional manner within networks from presynaptic neurones to postsynaptic neurones. However, it has become clear that information transfer in the central nervous system is more complex than simple point-to-point, unidirectional transmission between neurones at synapses. Among the additional mechanisms that contribute to information transfer in the nervous system is somato-

dendritic secretion. Unlike classical synaptic transmission by neurotransmitters such as glutamate and GABA, which signals between pre- and postsynaptic neurones with spatial precision and high temporal resolution, somato-dendritic secretion causes longer-term changes than synaptic transmission that alters the overall excitability of neurones by modulating the strength of synaptic inputs and/or by modulating the baseline membrane potential. These effects can be autocrine or paracrine, on the neurone from which somato-dendritic secretion occurs or on nearby neurones, and might spread over relatively long distances to modulate the activity of neurones in brain areas distant from the site of secretion.

Somato-dendritic secretion occurs in many types of neurone and can involve many types of transmitter molecule (1). Magnocellular neurosecretory cells (MNCs) of the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN) are among those for which the mechanisms and consequences of somato-dendritic secretion are best characterised. This review focusses on studies from the authors' laboratories, some of which were presented at the 22nd International Symposium on Regulatory Peptides, which have contributed to our understanding of how somato-dendritic secretion from MNCs contributes to endocrine and autonomic regulation of peripheral physiology in health and disease.

The magnocellular neurosecretory system

The magnocellular neurosecretory system comprises MNCs that predominantly secrete either vasopressin (the antidiuretic hormone) or oxytocin into the general circulation from the posterior pituitary gland (neurohypophysis). The principal function of vasopressin is to maintain body fluid balance and blood pressure by activation of renal V_2 -receptors to increase water reabsorption from the urine and, when blood pressure/volume is decreased, by activation of vascular V_{1a} -receptors ($V_{1a}Rs$) to cause vasoconstriction (2). The best-characterised physiological functions of oxytocin are to trigger uterine contractions during birth and milk ejection during lactation (2). However, oxytocin also contributes to body fluid balance by promoting natriuresis in the kidney (3) and by stimulating atrial natriuretic peptide secretion (4).

73 The human hypothalamus contains over 100,000 MNCs (5), with ~10,000 in the rat, that are
74 principally located in the SON and PVN as well as in several accessory nuclei (6). MNCs each project
75 a single axon to the posterior pituitary gland (Figure 1) and each axon branches extensively to form
76 several thousand neurosecretory axon swellings and terminals (7) that are each tightly packed with
77 dense core vesicles containing ~85,000 molecules of vasopressin or oxytocin in rats (8). Hormone
78 secretion is triggered by action potential invasion of the neurosecretory swellings and terminals. It has
79 been estimated that each MNC contains about 10 million dense core vesicles and secretes between
80 100 and 10,000 dense core vesicles from the posterior pituitary gland every minute to maintain basal
81 hormone concentrations in the circulation (9). Hence, the sustained output of the hormones, and the
82 consequent regulation of peripheral physiology, depends on the average action potential discharge
83 from the population (2, 10).

84 MNCs also synthesise lesser amounts of other neurotransmitters and neuromodulators that can be
85 contained in the same dense core vesicles as vasopressin or oxytocin (11), as well as glutamate-
86 containing microvesicles (12). To date, the only evidence of effects of these other neurotransmitters
87 and neuromodulators on peripheral physiology is for secretin, which increases renal antidiuresis (13).
88 Rather, their principal function appears to be modulation of hormone secretion at the level of the
89 posterior pituitary gland, which is comprehensively reviewed elsewhere (14), and at the level of the
90 somata and dendrites, as we describe here.

91 Some MNCs project axon collaterals to other brain areas. Originally, these were thought to remain
92 proximal to the SON (15) and PVN (16), projecting to local interneurons as part of a proposed local
93 feedback loop. More recently, it was shown that some MNC axon collaterals project more broadly
94 throughout the brain, with oxytocin MNCs projecting to the medial amygdala (MeA), central
95 amygdala (CeA), nucleus accumbens (17) and the lateral septum (18), and vasopressin MNCs to the
96 medial and lateral preoptic area, suprachiasmatic nucleus, lateral habenula, CeA, MeA (19, 20), locus
97 coeruleus (21) and arcuate nucleus (ARC) (22). These axon collaterals have been implicated in the
98 modulation of different behaviours, but it remains to be established how secretion from axon

1
2
3 99 collaterals to modulate behaviour relates to secretion from the posterior pituitary gland to modulate
4
5 100 peripheral physiology.
6
7
8 101 MNCs possess 1 – 3 thick, varicose, aspiny dendrites of a few hundred micrometres in length. MNCs
9
10 102 of the SON extend their dendrites to the ventral surface of the nucleus, where the dendrites bundle
11
12 103 together within the ventral glial lamina (a layer of astrocytes on the ventral surface of the brain within
13
14 104 the SON) (23) and MNCs of the PVN extend their dendrites towards the subependymal region of the
15
16 105 third ventricle (24). In addition to being the site of afferent synaptic input, MNC dendrites are active
17
18 106 players in shaping MNC activity through exocytosis of vasopressin and oxytocin (as well as other
19
20 107 neurotransmitters/neuromodulators) into the extracellular space of the SON and PVN.
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23
24 108 **Somato-dendritic secretion from magnocellular neurosecretory cells**
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26
27 109 The somata and dendrites of MNCs are tightly packed with dense core vesicles containing either
28
29 110 vasopressin or oxytocin (Figure 2), which undergo exocytosis to secrete their major neuropeptides
30
31 111 (25, 26) along with lesser amounts of other co-packaged neurotransmitters and neuromodulators (11).
32
33 112 Tannic acid capture of somato-dendritic secretion reveals that the entire vesicle content is released
34
35 113 from MNCs (25).
36
37
38
39 114 Unlike synaptic transmission by classical neurotransmitters, dense core vesicle exocytosis from the
40
41 115 somata and dendrites of MNCs requires a sustained increase in intracellular calcium (27, 28,) and
42
43 116 calcium buffering limits increases in cytoplasmic calcium to restrain the activation of somato-
44
45 117 dendritic secretion from MNCs (29). MNCs express different arrays of voltage-gated calcium
46
47 118 channels in their somata and axon terminals (30). Relative to other voltage-gated calcium channels,
48
49 119 N-type calcium channels ($Ca_{v2.2}$) carry a comparatively small current in MNC somata, but
50
51 120 nevertheless contribute most significantly to somato-dendritic oxytocin secretion (31). While the
52
53 121 primary trigger for somato-dendritic secretion is the influx of extracellular calcium, intracellular
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55 122 calcium release also contributes to somato-dendritic secretion from MNCs (27, 28).
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3 123 Action potential invasion triggers exocytosis from axon terminals and MNC dendrites and appears to
4
5 124 support depolarisation-induced calcium spikes (32). Capacitance measurements from isolated MNCs
6
7 125 suggest that single action potentials trigger somato-dendritic secretion (33). However, functional
8
9 126 studies suggest that sustained intracellular calcium release is required to trigger somato-dendritic
10
11 127 secretion (27, 28). Furthermore, if every action potential fired by each MNC triggered somato-
12
13 128 dendritic secretion of a single dense core vesicle, the brain would be awash with vasopressin and
14
15 129 oxytocin (34). Exocytosis of ~6,000 dense core vesicles per second has been calculated to be
16
17 130 sufficient to maintain the concentrations of vasopressin and oxytocin measured in the rat
18
19 131 hypothalamus (34). There are ~10,000 MNCs in the rat hypothalamus (6). While ~25% of MNCs are
20
21 132 silent under basal conditions, active MNCs display a mean firing rate of ~5 Hz under basal conditions
22
23 133 (35). Hence, up to 37,500 action potentials are fired by MNCs every second, which is almost 10-fold
24
25 134 more than the number of dense core vesicles secreted. Hence, it is likely that trains of action potentials
26
27 135 that cause a more sustained depolarisation and calcium influx are required to trigger somato-dendritic
28
29 136 secretion from MNCs. Indeed, under basal conditions, some stimuli reduce the oxytocin MNC action
30
31 137 potential firing rate but increase somato-dendritic oxytocin secretion (36, 37), and it was shown
32
33 138 recently that action potential firing alone at physiological firing rates is insufficient to trigger
34
35 139 measurable somato-dendritic secretion from individual vasopressin MNCs (38).

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39
40 140 In addition to permeation through voltage-gated calcium channels, calcium influx also occurs through
41
42 141 N-methyl-D-aspartate (NMDA) receptors (NMDARs), and synaptic NMDA receptors would be
43
44 142 expected to further increase cytoplasmic calcium concentrations during action potential firing.
45
46 143 Furthermore, MNCs express extrasynaptic NMDARs (39-41). While these extrasynaptic NMDARs
47
48 144 are activated by basal glutamate levels *in vitro* (39), they are not activated under basal conditions *in*
49
50 145 *vivo*, but are activated under stimulated conditions (35) and trigger somato-dendritic peptide secretion
51
52 146 (38).

53
54
55 147 In addition to triggering somato-dendritic secretion, increased cytoplasmic calcium also promotes
56
57 148 movement of dense core vesicles from the reserve pool toward the cell surface (42), where they are
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ready for secretion in response to subsequent stimuli that raise cytoplasmic calcium. In parallel, increased intracellular calcium also promotes recruitment of N-type calcium channels (31) to make the system more sensitive to subsequent cytoplasmic calcium increases. Hence, this 'priming' increases somato-dendritic secretion triggered by subsequent signals that increase cytoplasmic calcium.

Action potential-mediated depolarisation is not the only trigger for somato-dendritic secretion from MNCs. Vasopressin and oxytocin MNCs express their respective receptors (43, 44) and activation of these receptors increases cytoplasmic calcium concentrations (45) to trigger somato-dendritic secretion (27, 28). While vasopressin and oxytocin trigger somato-dendritic secretion from vasopressin and oxytocin MNCs without a prior stimulus to prime the system, once the MNCs are primed, the peptides can trigger a much greater somato-dendritic secretion (27, 28).

There is an elaborate network of actin and microtubules in MNC somata and dendrites (46, 47). Cortical F-actin regulates somato-dendritic exocytosis; F-actin polymerization inhibits and F-actin depolymerisation increases somato-dendritic secretion from MNCs (48). Presumably, F-actin depolymerisation increases access to the plasma membrane and this process might account for the requirement for a sustained increase in intracellular calcium to trigger somato-dendritic secretion, since calcium causes F-actin depolymerisation. Unlike synaptic transmission, there does not appear to be any specific structure on the soma or dendrites that is specialised for somato-dendritic secretion (49), although it remains to be determined whether there are regions of the cortical F-actin network that are more readily depolymerised to allow dense core vesicles preferential access to the plasma membrane at specific sites for secretion.

Exocytosis can occur once the dense core vesicles reach the plasma membrane, which requires exocytotic machinery. The involvement of the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) complex in exocytosis from axon terminals is well established (50). While less well characterised, it appears that somato-dendritic secretion is also mediated by SNARE proteins. MNCs express SNARE proteins (51), however, while vesicle-associated membrane protein-2

(VAMP-2) and synaptosomal-associated protein 25 (SNAP-25) are both expressed in the axon terminals (52, 53), they are not expressed in the somata or dendrites of MNCs (54). Hence, the suite of SNARE complex proteins for somato-dendritic exocytosis likely differs from that for axon terminal secretion from MNCs.

Stimulation of somato-dendritic secretion by neurotransmitters, peptides and hormones

Noradrenergic afferents from the ventrolateral medulla (VLM) A1 cell group and the nucleus of the tractus solitarius (NTS) A2 cell group make prominent projections to MNCs (2). NTS noradrenergic afferents are activated during birth and lactation (55) as part of the Ferguson reflex (56), and noradrenaline facilitates somato-dendritic oxytocin secretion in late pregnancy and lactation (57-59). Oxytocin also increases noradrenaline secretion within the SON (60), which presumably establishes a local positive feedback loop that reinforces oxytocin MNC excitation and promotes oxytocin secretion into the circulation to trigger uterine contractions during birth and milk ejection during lactation (Figure 3).

Proopiomelanocortin (POMC) afferents from the ARC project to the SON and PVN, particularly to regions of the SON and PVN that are enriched in oxytocin MNCs (61). POMC neurones secrete α -melanocyte stimulating hormone (α -MSH), which acts on melanocortin-4 (MC4-R) receptors in the SON and PVN (62). While α -MSH inhibits oxytocin secretion into the circulation, it increases somato-dendritic oxytocin secretion (37, 63). MC4-R activation increases intracellular calcium in oxytocin MNCs to trigger somato-dendritic oxytocin secretion as well as endocannabinoid secretion, which inhibits the activity of the MNCs to reduce axonal oxytocin secretion into the blood (37, 63). Remarkably, α -MSH inhibition of oxytocin MNC activity is lost in mid-pregnancy (62), but it has yet to be determined whether this represents a switch from pre-pregnancy inhibition to stimulation during lactation, as is seen for prolactin effects on oxytocin MNCs (64). Furthermore, it is not known whether α -MSH effects on somato-dendritic oxytocin secretion change in pregnancy and lactation.

199 In addition to neurotransmitters, other hormones can also trigger somato-dendritic secretion from
200 MNCs. The orexigenic hormone, ghrelin is synthesised by oxyntic cells in the gastric mucosa, but not
201 in the brain (65). Central ghrelin administration increases vasopressin secretion into the circulation via
202 activation of neuropeptide Y neurones (66). In addition, ghrelin stimulates somato-dendritic
203 vasopressin secretion, which increases adenosine triphosphate (ATP) release from astrocytes to
204 increase presynaptic GABA release onto the vasopressin MNCs (67) (Figure 4).

205 Autocrine/paracrine modulation of vasopressin magnocellular neurosecretory cell activity

206 The effects of vasopressin, oxytocin and other neurotransmitters and neuromodulators secreted from
207 the somata and dendrites of MNCs can be broadly categorised as autocrine, regulating the activity of
208 the MNC from which secretion occurs, and paracrine, regulating the activity of neighbouring neurons,
209 including other neuronal populations.

210 $V_{1a}R$ and V_{1b} receptors ($V_{1b}R$) are expressed in the membranes of vasopressin-containing dense core
211 vesicles (68) and are presumably inserted into the plasma membrane during somato-dendritic
212 vasopressin secretion. Hence, vasopressin receptors newly trafficked to the plasma membrane will be
213 exposed to high concentrations of vasopressin to underpin activity-dependent autocrine feedback
214 regulation of vasopressin MNC activity.

215 Vasopressin MNCs express a range of activity patterns under basal conditions; some are silent
216 throughout recordings, some display irregular activity, some are continuously-active (typically at
217 ~ 6 spikes s^{-1}), and some display rhythmic 'phasic' firing (69). Phasic firing is characterised by
218 bursts of activity that last more than 15 s, after which bursts stop randomly (70). Each burst is
219 followed by inactivity for at least 10 s, after which the next burst starts randomly (70). At burst
220 onset, vasopressin MNCs can reach firing rates of $\sim 15 - 25$ spikes s^{-1} for the first 5 – 10 s, before
221 spike frequency adaptation occurs to a steady-state firing rate of ~ 6 spikes s^{-1} for the remainder of
222 the burst (71, 72).

Of the different activity patterns recorded in vasopressin MNCs, phasic bursting is the most efficient pattern for vasopressin secretion into the circulation because vasopressin secretion is maximal at ~13 spikes s^{-1} (73, 74), which is typically only achieved by phasically firing MNCs and only during the first 5 – 10 s of each phasic burst. Vasopressin secretion from the posterior pituitary gland rapidly fatigues during continuous stimulation, but this fatigue is reversed when stimulation is stopped for a few tens of seconds (75). Hence, vasopressin MNCs firing continuously at high frequency do not secrete as much vasopressin into the circulation as do phasic MNCs firing at the same frequency because the silent periods between bursts in phasic MNCs reset the system for efficient vasopressin secretion at the onset of the next burst, when the typical firing frequency is again in the range that is most efficient for vasopressin secretion. The importance of phasic activity for efficient vasopressin secretion into the circulation is highlighted by the changes in activity patterning that occur under chronically stimulated conditions, such as prolonged osmotic stimulation. While burst duration does increase during shorter periods of stimulation, prolonged osmotic stimulation leads to an increase in firing rate within bursts while the burst duration and inter-burst interval remain similar to those seen under basal conditions (76-79).

V1aR antagonists consistently increase the activity of phasic MNCs when administered into the SON (80), suggesting that somato-dendritic vasopressin mediates feedback inhibition of vasopressin MNCs via V1aR activation (Figure 5). This feedback inhibition likely involves direct autocrine actions on the MNC that secretes vasopressin because V1aR activation reduces excitatory postsynaptic potential amplitude in vasopressin MNCs (81). However, autocrine activation of V1aRs does not mediate autoregulation of vasopressin MNC activity alone because vasopressin also increases inhibitory postsynaptic potential frequency (82) via stimulation of astrocytic adenosine triphosphate (ATP) release, which acts as a gliotransmitter at P2X receptors on presynaptic GABA neurones to increase GABA release (67). Hence, somato-dendritic vasopressin secretion appears to contribute to the generation of phasic activity in vasopressin MNCs via a combination of autocrine actions on the MNC from which secretion occurs and paracrine actions on nearby cells that modulate the activity of the MNC from which secretion occurs.

250 While V1aR activation mediates autocrine and paracrine inhibition of phasic activity, local
 251 application of exogenous vasopressin was first reported to inhibit highly active phasic MNCs and
 252 stimulate weakly active phasic MNCs (83). Vasopressin MNCs also express V1bR (68), and while
 253 it has yet to be determined whether V1bR activation also contributes to autocrine regulation of
 254 vasopressin MNCs, it might underpin the excitatory effects of vasopressin evident in weakly active
 255 vasopressin MNCs. Regardless of the vasopressin excitation of weakly active phasic MNCs
 256 (perhaps via V1bR), such an action of endogenous vasopressin would presumably increase
 257 peripheral vasopressin secretion to cause robust vasoconstriction that is not present under basal
 258 conditions (84). Hence, it seems likely that any contribution of somato-dendritic vasopressin
 259 secretion to peripheral vasopressin secretion by feedback excitation is overridden by the V1aR-
 260 mediated feedback inhibition.

261 While, somato-dendritic vasopressin secretion functions as a negative feedback regulator of
 262 vasopressin MNC activity at the single cell level, the important output of the system is overall
 263 hormone secretion, which depends on the integrated activity of the MNCs at a population level
 264 (10). Some of the earliest work on somato-dendritic secretion showed that osmotic stimulation of
 265 vasopressin MNCs increases vasopressin levels in the circulation before levels increase in the SON
 266 (85), which is consistent with somato-dendritic vasopressin secretion acting as a negative feedback
 267 regulator of vasopressin MNC activity at a population level to modulate overall vasopressin
 268 secretion into the circulation.

269 **Autocrine/paracrine modulation of vasopressin magnocellular neurosecretory cell activity by** 270 **co-secreted transmitters**

271 Vasopressin MNCs also synthesise and secrete a number of other neurotransmitters and
 272 neuromodulators, including apelin (86), ATP (87), carbon monoxide (CO) (88), dynorphin (89),
 273 endocannabinoids (90-92), galanin (93), neuroendocrine regulatory peptides (NERPs) (94, 95),
 274 nitric oxide (NO) (96), PACAP (97) and secretin (13).

Most neuropeptides synthesised by MNCs are packaged within the same dense core vesicles as either vasopressin or oxytocin. However, apelin and galanin are differentially packaged in vasopressin MNCs. Apelin is packaged in dense core vesicles that do not contain vasopressin (98). While galanin is also packaged in some dense core vesicles that also contain vasopressin, it is also packaged in others that do not contain vasopressin and some dense core vesicles contain vasopressin but no galanin. Presumably, differential packaging in dense core vesicles might allow for secretion of separate pools that contain vasopressin or their co-expressed neuropeptides. Indeed, dense core vesicles containing galanin alone are trafficked to the dendrites while those that contain only vasopressin are trafficked to the axon terminals in the posterior pituitary gland (93).

Vasopressin MNCs express apelin receptors (APJ receptors) (99) and centrally-administered apelin inhibits vasopressin MNCs (86) to decrease basal vasopressin secretion (86, 100). However, systemic apelin administration increases vasopressin secretion (101) and chronic infusion of apelin into the PVN also increases vasopressin secretion (102). Furthermore, administration of apelin directly into the SON increases the activity of phasic MNCs (and presumably vasopressin secretion into the circulation) via non-specific cation channel activation, but reduces somato-dendritic vasopressin secretion (99), which presumably weakens vasopressin-mediated autoregulation to disinhibit and thus further excite vasopressin MNCs.

Vasopressin MNCs express galanin receptor-1 (Gal-R1) on their somata and dendrites (103). While centrally-administered galanin increases vasopressin secretion into the circulation *in vivo* (104, 105), it inhibits vasopressin secretion from isolated neurohypophyses or hypothalamo-neurohypophysial explants *in vitro* (106), suggesting that the direct effects of galanin are inhibitory, despite the reduced somato-dendritic vasopressin secretion. Indeed, galanin directly inhibits vasopressin MNCs *in vitro* by inducing hyperpolarization and reducing the slow afterdepolarisation (sADP) (107), which is a prominent excitatory post-spike potential in vasopressin MNCs (108). Galanin also reduces EPSC frequency (109), suggesting that it might also have paracrine effects after somato-dendritic secretion by retrograde inhibition of excitatory synaptic transmission.

Similarly to vasopressin receptors, κ -opioid receptors (KORs) are expressed in the membranes of vasopressin-containing dense core vesicles (110) and unlike apelin and galanin, the endogenous opioid peptide (EOP) ligand for KORs, dynorphin, is packaged with vasopressin in the same dense core vesicles (111). Hence, KORs newly-trafficked to the vasopressin MNC plasma membrane will be exposed to high concentrations of dynorphin upon somato-dendritic secretion of dense core vesicles. KOR agonists inhibit vasopressin MNCs *in vivo* (69, 112) and *in vitro* (113, 114). More importantly, antagonism of SON KORs increases burst duration in phasic MNCs under basal conditions *in vivo* (69, 70, 115) and *in vitro* (70, 116), showing that an endogenous KOR agonist inhibits phasic bursts. Phasic bursts are underpinned by the summation of sADPs to form a plateau potential that maintains a depolarised membrane potential to sustain further firing during bursts, and KOR activation causes activity-dependent sADP inhibition (116) to progressively decrease the plateau potential amplitude, which eventually leads to burst termination (70). Furthermore, KOR desensitisation prevents phasic activity in vasopressin MNCs, even when intensely stimulated (69). Hence, an endogenous KOR agonist inhibits phasic MNCs by autocrine inhibition of the sADP in the MNC from which dynorphin is secreted and this inhibition appears to be necessary for the expression of phasic activity by vasopressin MNCs.

In addition to sADP inhibition, KOR agonists reduce EPSP and IPSP amplitude (114, 117) and the delayed rectifier potassium current (118), while increasing the transient A-type potassium current (118) in vasopressin MNCs, although it has yet to be established whether these effects also contribute to the generation of phasic activity.

While KOR activation inhibits continuously-active vasopressin MNCs, KOR antagonism does not affect continuously-active vasopressin MNCs, even when they are strongly excited (76). Hence it appears that continuously-active vasopressin MNCs express KORs but do not release sufficient dynorphin to affect activity. Some vasopressin MNCs display irregular activity and these MNCs appear to be even more strongly excited by KOR antagonism than phasic MNCs (76). Taken together, this pattern-dependent sensitivity to KOR inhibition suggests that somato-dendritic dynorphin

secretion might determine the firing pattern of vasopressin MNCs and that transitions between firing patterns in individual vasopressin MNCs might result from changes in somato-dendritic dynorphin secretion (119).

MNCs also express receptors for pituitary adenylate cyclase-activating polypeptide (PACAP), which they also synthesise and secrete (97). PACAP increases somato-dendritic vasopressin secretion (120) by a direct depolarisation through activation of non-specific cation channels (121).

Neuroendocrine regulatory peptides (NERPs) 1 – 3 are packaged with vasopressin in the SON and PVN (94, 95). NERP-1 has paracrine effects on vasopressin MNC activity by retrograde inhibition of excitatory synaptic transmission whereas paracrine inhibition of vasopressin MNCs by NERP-2 is mediated by activation of upstream GABAergic interneurons that inhibit glutamatergic neurons that project to vasopressin MNCs (122). In contrast to NERPs 1 and 2, NERP-3 stimulates vasopressin secretion from the isolated posterior pituitary gland (95).

Vasopressin MNCs express secretin receptors and central secretin administration increases plasma vasopressin concentrations (13), suggesting that somato-dendritic secretin might stimulate systemic vasopressin secretion. However, secretin is also released from afferent inputs to the SON (123) and systemic secretin administration excites vasopressin (and oxytocin) MNCs via noradrenergic afferent inputs (124), suggesting that its actions might be mediated by afferent inputs rather than somato-dendritic secretion. While its role as a neurohypophysial hormone has not yet been definitively established, secretin is expressed in the posterior pituitary gland (13) and increases insertion of aquaporin-2 into the luminal membrane of the kidney to increase water reabsorption (125). Hence, secretin synthesised by vasopressin MNCs might act as a neurohypophysial hormone after secretion from the posterior pituitary gland rather than as an autoregulatory factor secreted from the somata and dendrites.

Vasopressin MNCs express P2X and P2Y receptors (126, 127) and injection of ATP into the SON induces antidiuresis (128). ATP is packaged in vasopressin dense core vesicles (87). ATP depolarises MNCs (129) and increases vasopressin secretion from hypothalamo-neurohypophysial explants (130).

ATP also increases glutamate and GABA release at synapses on MNCs (131). Hence, somato-dendritic ATP secretion might excite vasopressin MNCs by autocrine actions on the MNC from which it is secreted and paracrine actions on afferent inputs to the MNC from which it is secreted. However, MNCs are also excited by ATP released by astrocytes as a gliotransmitter (67, 132) as well as by ATP released from noradrenergic afferent inputs (133).

While somato-dendritic ATP secretion might modulate vasopressin MNC activity, ATP is rapidly catabolised to adenosine in the extracellular space (134). Vasopressin MNCs express adenosine A1 and A2A receptors (135) and A1 receptor antagonism excites phasic MNCs *in vivo*, but does not affect the firing rate of continuously-active vasopressin MNCs (136). A1 receptor antagonism reduces activity-dependent inhibition of EPSCs and IPSCs (137) as well as activity-dependent enhancement of the medium afterhyperpolarisation (mAHP) in vasopressin MNCs (138). mAHP activation induces spike frequency adaptation at the onset of phasic bursts (139) and so endogenous adenosine enhancement of the mAHP increases spike frequency adaptation, thereby shortening bursts in phasic MNCs (136). While A2 receptor activation depolarises MNCs to increase firing rate (135), vasopressin MNCs are inhibited when adenosine uptake is blocked. Hence, the overall effect of endogenous adenosine appears to be vasopressin MNC inhibition (140).

In addition to somato-dendritic exocytosis, vasopressin MNCs also release the gaseous transmitters, NO and carbon monoxide (CO), by diffusion after synthesis by NO synthase (NOS) and haem-oxygenase I, respectively. NO inhibits vasopressin MNCs (141, 142) by increasing IPSC amplitude and frequency (142, 143), whereas CO excites vasopressin MNCs (88).

Somato-dendritic modulation of vasopressin MNC activity appears to impact hormone secretion into the circulation through a complex interplay of excitatory (apelin, PACAP, ATP, NO and perhaps secretin) and inhibitory (galanin, dynorphin, NERPs, adenosine and CO) feedback that might fine tune the activity of individual MNCs to prevent any one MNC bearing too much of the secretory load for too long under basal conditions. It seems counter-intuitive that the autoregulatory effects of (at least some) co-secreted transmitters appear greater than that of vasopressin itself,

which is secreted in vastly greater quantities. Perhaps the autoregulatory effects of co-secreted transmitters are magnified by activation of both paracrine and autocrine mechanisms. In addition, it is possible that vasopressin's major role is paracrine inhibition of the population as a whole to prevent over-secretion of vasopressin into the circulation in response to perturbations of body fluid balance and/or blood pressure/volume.

Autocrine/paracrine modulation of oxytocin magnocellular neurosecretory cell activity

Similar to somato-dendritic vasopressin secretion, somato-dendritic oxytocin secretion also has autocrine and paracrine actions that modulate oxytocin MNC activity. In contrast to vasopressin, the autocrine and paracrine effects of oxytocin are arranged in series rather than in parallel; somato-dendritic oxytocin secretion activates oxytocin receptors (OTRs) on oxytocin MNCs to increase intracellular calcium, which has various actions including the release of endocannabinoids that cause retrograde inhibition of excitatory synaptic transmission under basal conditions (90). While the oxytocin-stimulated retrograde endocannabinoid suppression of excitatory synaptic input is expected to inhibit oxytocin MNCs (Figure 6), the best characterised effects of somato-dendritic oxytocin secretion is excitation, but only under specific (patho)physiological conditions. Hence, it has been proposed that endocannabinoid inhibition might occur over a longer timescale than autocrine effects of oxytocin to shape activity patterning in oxytocin MNCs during birth and lactation (144), but it has yet to be established whether this occurs in vivo. Alternatively, the excitatory effects of oxytocin might involve a switch from endocannabinoid inhibition to excitation during pregnancy, perhaps by enhanced expression/activation of excitatory transient receptor potential vanilloid-1 channels (145) for which the endocannabinoid, anandamide, is an endogenous ligand (146). Additionally, spillover of the endocannabinoid 2-arachidonoylglycerol from glutamate onto GABA synapses and a resulting suppression of inhibitory synaptic input has been observed in MNCs following glial retraction induced by salt loading (91). This endocannabinoid spillover could also occur with glial retraction during parturition and lactation to reduce inhibitory synaptic transmission, although this remains to be determined. Finally, it is also possible that oxytocin-stimulated endocannabinoid retrograde

modulation of excitatory and inhibitory synapses might be overridden by changes in the postsynaptic properties of oxytocin MNCs (147, 148), increased excitatory afferent inputs (149-153), or a switch in GABA signalling from inhibitory to excitatory, or less inhibitory, during pregnancy (64, 154).

Oxytocin MNCs typically exhibit continuous activity at $\sim 1 - 5$ spikes s^{-1} under basal conditions to maintain circulating oxytocin concentrations of $\sim 1 - 3$ pg ml^{-1} , with higher concentrations during sleep (2). However, oxytocin is best known for its stimulation of rhythmic uterine contraction during birth and of episodic milk ejection during suckling. Uterine contractions and mammary duct contraction each occur at intervals of several minutes and each contraction is triggered by a coordinated, high frequency burst of activity across the population of oxytocin MNCs (155) to secrete a discrete pulse of oxytocin into the circulation, which transiently increases intrauterine pressure during birth (156) and intramammary pressure during suckling (157).

Somato-dendritic oxytocin secretion increases immediately preceding each burst of activity in lactating rats (158) and bursts are blocked by OTR antagonist administration (159), as is the rise in somato-dendritic oxytocin secretion (160), suggesting that somato-dendritic oxytocin secretion is required for bursts to occur. However, the mechanisms by which somato-dendritic oxytocin secretion promotes burst firing in oxytocin MNCs are not fully understood and it is likely not the only contributor; synchronised volleys of EPSPs (161), rebound depolarization after bursts of IPSPs (162) and enhancement of the sADP (147, 163) have all been proposed to trigger or sustain firing during bursts in oxytocin MNCs.

In brain slices from male rats that do not normally fire bursts, α_1 -adrenoceptor activation in low calcium can induce bursts in oxytocin MNCs reminiscent of those seen during birth and milk ejection (164). Hence, noradrenergic inputs might trigger bursts. Consistent with this hypothesis, noradrenergic innervation of oxytocin MNCs is increased in late pregnancy (152) and these afferent inputs are activated during birth (55) to increase noradrenaline release into the SON (165).

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3 429 Noradrenergic stimulation of bursts in oxytocin MNCs might be mediated by somato-dendritic
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5 430 oxytocin release because noradrenergic receptor stimulation is required for suckling-induced somato-
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7 431 dendritic oxytocin release (58), which might be part of a positive feedback loop that builds towards
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9 432 bursts during continuous suckling. Indeed, burst-like activity can also be induced in virgin rats *in vivo*
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11 433 by coordinated activation of neighbouring oxytocin MNCs, which induces priming of somato-
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13 434 dendritic dense core vesicles for subsequent secretion (28). Once primed, high-frequency electrical
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15 435 stimulation induces bursting in oxytocin MNCs of virgin rats (28). Hence, continuous suckling might
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17 436 trigger tonic noradrenaline release onto oxytocin MNCs that triggers increasing somato-dendritic
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19 437 oxytocin secretion, which could prime further somato-dendritic oxytocin secretion until a tipping-
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21 438 point is reached to induce each burst.

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25 439 In addition to facilitating burst firing in individual oxytocin MNCs, somato-dendritic oxytocin
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27 440 secretion might also help coordinate the timing of bursts across the population of oxytocin MNCs.
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29 441 Oxytocin injection into one SON increases the frequency of milk ejection bursts in the contralateral
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31 442 SON (166). Oxytocin MNCs have 1 – 3 dendrites (148) that are normally separated from
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33 443 neighbouring dendrites by astrocytic processes. However, in late pregnancy and lactation, astrocytes
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35 444 withdraw their processes from between oxytocin MNC dendrites, which then form bundles of ~ 10
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37 445 closely apposed dendrites (167, 168). A mathematical model in which oxytocin MNCs send each
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39 446 dendrite to different dendritic bundles to form a sparse network of interactions emulates burst firing in
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41 447 which each burst is initiated randomly at any of the dendritic bundles and spreads rapidly through the
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43 448 oxytocin MNC population (144).

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46 449 However, this model does not account for coordination of bursts across the bilateral SONs and PVNs
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48 450 which might be mediated by noradrenergic inputs that project bilaterally to the SON (169). Indeed,
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50 451 sectioning the optic chiasm or mammillary body disrupts co-ordination of bursts between oxytocin
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52 452 MNCs in the left and right SON, suggesting that burst coordination across the magnocellular nuclei
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54 453 involves projections through these areas (169, 170). Furthermore, the perinuclear zone that lies
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56 454 immediately dorsal to the SON sends prominent projections to the SON (171) and PVN (172, 173)
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58 455 that might also contribute to coordination of bursts across the four main magnocellular nuclei.
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Autocrine/paracrine modulation of oxytocin magnocellular neurosecretory cell activity by co-secreted transmitters

Oxytocin MNCs also synthesise other transmitters that are likely secreted from their somata and dendrites, but the effects of these co-transmitters are not as well characterised as for those released from vasopressin MNCs.

Oxytocin MNCs express μ -opioid receptors (MORs) and KORs (174, 175), and MOR or KOR activation inhibits oxytocin MNCs (69, 176). While oxytocin MNCs synthesise μ - and κ -EOPs (177, 178), neither MOR nor KOR antagonists affect the activity of oxytocin MNCs *in vivo* (69, 179). Hence it appears that if EOPs undergo somato-dendritic secretion with oxytocin, they do not modulate oxytocin MNC activity to any appreciable extent under basal conditions. MOR-mediated EOP inhibition of somato-dendritic oxytocin secretion and oxytocin MNC activity is increased in late pregnancy (180), but this modulation is likely to be mediated by afferent inputs (181).

By contrast to the central actions of MOR activation, KOR activation appears to restrain secretion into the bloodstream at the posterior pituitary gland (182) and this effect also increases in late pregnancy (183). KOR restraint of oxytocin secretion might build up stores of oxytocin for birth and lactation and might potentiate secretion during bursts because KORs are desensitised on the day of birth (184, 185).

Oxytocin MNC dense core vesicles also contain ATP, which is presumably secreted along with oxytocin from the somata and dendrites. Co-secreted ATP does not modulate oxytocin MNC activity via adenosine receptor activation (136), it might excite oxytocin MNC via P2X receptor activation (130, 131).

Oxytocin MNCs also express NOS (96) and NO appears to restrain the activity of oxytocin MNCs, particularly under stimulated conditions (186, 187), suggesting that NO is an inhibitory autocrine/paracrine modulator of oxytocin MNC activity.

Remarkably, chronic MOR activation by the opioid alkaloid agonist, morphine, (but not EOPs (188)) induces tolerance and dependence in oxytocin MNCs (189). Tolerance is revealed as loss of inhibition to acute administration of morphine (176) and dependence is revealed by a sustained hyperexcitation upon withdrawal of chronic morphine administration (112). Somato-dendritic oxytocin secretion is increased during morphine withdrawal and OTR antagonism reduces morphine withdrawal-induced excitation of oxytocin MNCs (190). Hence, somato-dendritic oxytocin secretion appears to contribute to morphine withdrawal-induced excitation of oxytocin MNCs, although the mechanism by which it does so has yet to be identified.

Paracrine modulation of parvocellular paraventricular nucleus neurone activity by somato-dendritic secretion from magnocellular neurosecretory cells

While the SON essentially contains only MNCs (and glia and cells of the vasculature), the PVN also contains parvocellular neurones. Parvocellular neurones are sub-divided by their projections and functions: neurosecretory parvocellular neurones project to the hypothalamic median eminence, where they secrete hormones into the hypophysial portal blood vessels to control hormone secretion from the anterior pituitary gland; preautonomic parvocellular neurones project to the brainstem and spinal cord to modulate parasympathetic and sympathetic nervous system activity (191, 192); the remaining parvocellular neurones project to various brain areas to modulate behaviour.

It has long been hypothesised that somato-dendritic secretion from MNCs modulates the activity of parvocellular neurones but only recently has definitive evidence to support this hypothesis been generated for somato-dendritic vasopressin (38, 193, 194). For paracrine effects on other neuronal phenotypes to occur, first vasopressin (or oxytocin) must diffuse through the parenchyma to reach other neurones. The effective diffusion distance for somato-dendritic vasopressin was determined under basal conditions using Chinese hamster ovary cells transfected with human V1aRs and a calcium indicator to generate biosensor ‘sniffer’ cells with a threshold detection level of 0.5 nM and an EC_{50} of 7.2 nM for vasopressin (38). Using sniffer cells that were dispersed over the PVN in hypothalamic slices, it was shown that activation of an individual MNC induces sufficient somato-

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3 506 dendritic vasopressin secretion to induce intracellular calcium increases for tens of seconds in sniffer
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5 507 cells over 100 μm from the soma of the activated MNC (38). Similar results were seen using HEK-
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7 508 293 sniffer cells in the SON (194). Hence, somato-dendritic vasopressin release from an individual
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9 509 MNC diffuses through the PVN at sufficient concentration to activate V1aRs expressed on
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11 510 parvocellular neurone somata or dendrites under basal conditions (38, 193). Remarkably, astrocytes
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13 511 withdraw their processes from between MNCs when chronically stimulated (167, 168, 195, 196),
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15 512 which reduces the tortuosity of the extracellular space and likely increases the effective diffusion
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17 513 distance for somato-dendritic vasopressin and oxytocin through the parenchyma (197).
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21 514 Some preautonomic parvocellular neurones project to the rostral ventrolateral medulla (RVLM) in the
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23 515 brainstem, which projects to sympathetic ganglia to regulate sympathetic nerve activity (198).
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25 516 RVLM-projecting parvocellular neurones express V1aRs and their dendrites are intermingled with
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27 517 vasopressin MNC somata and dendrites in the PVN (193). Hence, the architecture is in place to
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29 518 provide for somato-dendritic vasopressin modulation of autonomic function by paracrine modulation
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31 519 of preautonomic neurones within the PVN (Figure 7). Activation of individual vasopressin MNCs by
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33 520 uncaging NMDA increases action potential firing in RVLM-projecting parvocellular neurones beyond
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35 521 100 μm from the activated MNC (193) and this activation is much more potent than that elicited by
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37 522 action potential firing alone (38), suggesting that dendritic NMDARs might be a main driver of
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39 523 somato-dendritic secretion. The responses of RVLM-projecting parvocellular neurones to vasopressin
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41 524 MNC activation occur after a delay of several seconds, are blocked by superfusion of a V1aR
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43 525 antagonist and are enhanced by a peptidase inhibitor (193). Most importantly, the depolarisation of
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45 526 RVLM-projecting parvocellular neurones in response to vasopressin MNC activation is not affected
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47 527 by blockade of action potential firing with tetrodotoxin. Taken together, the data demonstrate that the
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49 528 excitation of RVLM-projecting parvocellular neurones is mediated by diffusion of somato-dendritic
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51 529 vasopressin through the extracellular space of the PVN.
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56 530 Given that activation of an individual vasopressin MNC can excite preautonomic neurones within a
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58 531 radius of at least 100 μm , it appears likely that somato-dendritic secretion from the population of
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MNCs, as occurs *in vivo*, could function as a population-to-population signal to recruit preautonomic neurones as a whole. Indeed, administration of a V1aR antagonist alone reduces preautonomic neurone activity and the more active the MNC, the more effective is the antagonist at reducing preautonomic neurone activity (193), suggesting that preautonomic neurones are under tonic modulation by vasopressin MNCs to coordinate the humoral (circulating vasopressin) and neuronal (sympathetic nerve activity) responses to changes in body fluid balance and to pathophysiological conditions, such as hypertension, myocardial infarction and chronic heart failure.

Hyperosmolality increases vasopressin secretion into the circulation by MNCs and increases renal sympathetic nerve activity, both of which increase water retention to protect body fluid balance. The mechanisms that underpin these responses to hyperosmolality are thoroughly reviewed elsewhere (199, 200). While these mechanisms occur in parallel, somato-dendritic vasopressin secretion likely coordinates the responses because intracarotid infusion of hyperosmotic saline causes a dose-dependent increase in renal sympathetic nerve activity that is accompanied by increased somato-dendritic vasopressin secretion, and bilateral injection of a V1aR antagonist into the PVN abolishes the renal sympathetic nerve activation (193). Hence, it appears that somato-dendritic vasopressin secretion coordinates the humoral and neuronal responses to increased osmolality.

PVN-driven sympathoexcitation is a key pathophysiological mechanism in hypertension (201, 202), acute myocardial infarction (192, 203) and heart failure (204, 205) that contributes to morbidity and mortality (206). Vasopressin MNCs are activated in hypertension (207, 208), acute myocardial infarction (209) and heart failure (210, 211). While the increased circulating vasopressin levels contribute directly to detrimental myocardial effects (212, 213), increased vasopressin MNC activity probably also increases somato-dendritic vasopressin secretion to contribute to the pathophysiological sympathoexcitation via activation of PVN preautonomic neurones.

While less well established than for vasopressin effects on preautonomic neurones, it appears that somato-dendritic oxytocin secretion might also modulate the activity of a neighbouring population of neurones within the PVN, corticotrophin-releasing hormone (CRH) neurones. Stress-induced CRH

secretion stimulates adrenocorticotrophic hormone secretion from the anterior pituitary gland (214) that, in turn, increases adrenal corticosteroid secretion to mediate the body's response to the stressor. CRH neurones express mRNA for OTRs (215) and oxytocin inhibits EPSC frequency, but not amplitude, in CRH neurones (216). Oxytocin MNC and CRH neurone dendrites are intermingled within the PVN (216), allowing for dendro-dendritic interactions between the two populations. Hence, somato-dendritic oxytocin might suppress CRH neurone excitability by presynaptic inhibition of excitatory synaptic inputs to reduce activation of the stress axis. However, OTR antagonism has no effect on CRH neurones in brain slices (216), suggesting that, unlike vasopressin modulation of preautonomic neurone activity, there is no OTR tone on CRH neurones, at least under *in vitro* basal conditions.

Paracrine modulation of arteriolar vasoconstriction in the supraoptic nucleus by somato-dendritic secretion from magnocellular neurosecretory cells

Classically, neuronal activity is thought to dilate arterioles and thereby increase local cerebral blood flow to meet the metabolic demands of active brain areas; this 'neurovascular coupling' is generally accepted to result from glutamatergic synaptic transmission that evokes release of vasoactive substances from neurones and astrocytes to relax vascular smooth muscle cells (217). However, vascular smooth muscle cells express V1aRs, providing a target for somato-dendritic vasopressin, at least within the SON and PVN. Vasoconstriction can be elicited in SON arterioles by stimulation of an individual vasopressin MNC, an effect that is blocked by V1aR antagonism (218) (Figure 7). Consistent with its effects on preautonomic neurones, somato-dendritic vasopressin can induce responses in arterioles beyond 100 μm from the activated MNC under basal conditions (218). Importantly, this V1aR-mediated vasoconstriction is over-ridden in hyperosmotic conditions by parallel release of NO, which causes vasodilation of local arterioles (218). Presumably the NO-induced vasodilation increases blood flow through the SON when increased vasopressin MNC activity is required to protect from further fluid loss and maintain blood pressure in the general circulation through vasopressin secretion from the posterior pituitary gland.

584 Paracrine modulation of neuronal activity beyond the paraventricular nucleus by somato-
585 dendritic secretion from magnocellular neurosecretory cells

586 While paracrine modulation of parvocellular neurones within the PVN by somato-dendritic secretion
 587 from MNCs is now well characterised, it has yet to be definitively established whether neuropeptides
 588 secreted from MNC somata and dendrites can affect the activity of neurones outside the PVN.

589 Nevertheless, there is some evidence that somato-dendritic oxytocin might act on neurones in brain
 590 areas relatively close to the SON and/or PVN, particularly for brain areas that receive little or no
 591 axonal projections from MNCs or from vasopressin or oxytocin parvocellular neurones.

592 The central effects of the primary anorexigenic hormone, leptin, are mediated by oxytocin, at least in
 593 part (219). Leptin is sensed by ARC POMC neurones that, as described above, project to the SON and
 594 PVN (61), where they secrete α -MSH to activate MC4-Rs (62) and thereby increase somato-dendritic
 595 oxytocin secretion (63, 220). Oxytocin inhibition of food intake is mediated, in part, by the
 596 ventromedial hypothalamus (VMH) because oxytocin injection into the VMH decreases food intake
 597 that is driven by energy balance rather than palatability (221). While OTRs are highly expressed in the
 598 VMH (222), there are essentially no oxytocin MNC axons in the VMH (219). Given that vasopressin
 599 released from a single MNC can activate cells over 100 μ m from the MNC soma from which it is
 600 secreted (38, 193) and the VMH is situated roughly between the SON and PVN, it is possible that
 601 somato-dendritic oxytocin release from MNCs could diffuse through the parenchyma in sufficient
 602 quantities to activate OTRs in the VMH, which have nanomolar affinity for oxytocin (223). It is also
 603 possible that OTR-expressing astrocytes in the SON and PVN could expand the spatial domain of the
 604 dendritically released oxytocin signalling by relaying the signals through astrocytic networks, as has
 605 been reported for vasopressin release from vasopressin MNC dendrites (67) and CRH neurone
 606 dendrites (224).

607 While oxytocin MNCs inhibit fear responses via axon collaterals to the CeA (17), it appears likely
 608 that SON somato-dendritic oxytocin secretion enhances social recognition via actions in the MeA.

609 The CeA contains oxytocin MNC axons collaterals but there are no oxytocin (MNC or parvocellular)

neurone axons in the MeA (17). OTRs are highly expressed in the MeA (222) and OTR antagonist injection into the MeA reduces social recognition induced by SON activation (225). However, MeA OTRs are not directly activated by oxytocin secreted into the CeA from MNC axon collaterals (17). The MeA lies immediately lateral to the SON and, even if somato-dendritic oxytocin secreted within the SON does not reach the MeA, some oxytocin MNC dendrites project to the MeA (225), which might deliver sufficient oxytocin to the MeA to promote social recognition. Alternatively, OTRs might be **activated** by vasopressin MNC axon collaterals in the MeA (19) because vasopressin has appreciable activity at OTRs (223).

Hormone-like modulation of neuronal activity in distant brain areas by somato-dendritic secretion from magnocellular neurosecretory cells

It has been hypothesised that somato-dendritic vasopressin and oxytocin from MNCs are a hormone-like signal in the brain with widespread effects on distant populations of neurones (226). However, accumulating evidence of the functional impact of MNC axon collaterals on behaviour via direct projections to distant brain areas (17-21) have led to this hypothesis being challenged (227).

The half-lives of vasopressin and oxytocin are ~20 min in the cerebrospinal fluid (CSF) (228), giving time for diffusion through the ventricular system, particularly downstream. However, vasopressin (at least) has a half-life of less than 1 min in the parenchyma (229). Given that the paracrine effects of somato-dendritic vasopressin on preautonomic neurones that are only ~100 μm away is delayed by ~2 – 5 s (193), it appears unlikely that the neuropeptides could diffuse long distances through the brain to act as a hormone-like signal. However, dense core vesicle exocytosis is a slow process compared to microvesicle fusion at the synapse, with latencies of several seconds in hippocampal neurones (230), which could account for much of the latency of the preautonomic neurone response to somato-dendritic vasopressin secreted by MNCs in the PVN. Furthermore, vasopressin and oxytocin are secreted in sufficient quantities to be measured in dialysates collected from the SON and PVN (231), as well as in other brain areas (232-234) and in the cerebrospinal fluid (CSF) (235), suggesting that they diffuse through the parenchyma sufficiently to reach the CSF. Indeed, the microdialysis probes

used to measure vasopressin and oxytocin in many experiments have a recovery rate of <10% for vasopressin in the SON and PVN (236). Hence, it is likely that the actual concentrations of vasopressin and oxytocin present in the parenchyma and CSF are appreciably higher than those measured in dialysates. A further factor to be considered is retraction of astrocytic processes from around MNCs in dehydration and pregnancy (167, 168, 195, 196), which decreases tortuosity in the extracellular space, presumably allowing more ready escape of somato-dendritic vasopressin and oxytocin from the SON and PVN. It is difficult to imagine that the relatively sparse terminal fields of MNC axon collaterals and parvocellular vasopressin and oxytocin MNCs could release sufficient vasopressin and oxytocin to maintain the ambient levels of the neuropeptides found in the brain and CSF.

While there are clear examples of axon collaterals from sub-populations of MNCs affecting neuronal activity beyond the SON and PVN (237), this does not preclude the possibility that there is also long-distance inter-population signalling mediated by somato-dendritic volume transmission of vasopressin and oxytocin over a longer timescale, particularly in brain regions in which there are neuropeptide receptors but no neuropeptide axons, such as in the olfactory bulb. Nevertheless, there is still no compelling evidence for, or against, distal hormone-like signalling by somato-dendritic vasopressin and oxytocin transmission, although the levels of oxytocin and vasopressin present in the cerebrospinal fluid and in brain areas devoid of oxytocin and vasopressin axon terminals appear to be much higher than could be achieved from axon terminal release from MNC and parvocellular axon collaterals. The resolution of this debate remains an ongoing challenge for the field.

Concluding remarks

Autocrine/paracrine modulation of MNC activity by somato-dendritic vasopressin and oxytocin release has been extensively studied and is broadly accepted as a major function of somato-dendritic secretion from MNCs (238). While it is clear that co-secreted transmitters also modulate MNC activity, there is no evidence of paracrine actions on other neurones, even other MNCs. Indeed, somato-dendritic dynorphin terminates bursts in the MNC from which it is secreted (70), but there is

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3 662 no correlation between burst termination in phasic MNCs in paired recordings using a single electrode
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5 663 in which the recorded MNCs would be at most tens of micrometres apart (71), which is well within
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7 664 the range of somato-dendritic vasopressin but evidently beyond the range of somato-dendritic
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9 665 dynorphin. Hence, it is possible that co-secreted transmitters act as autocrine/paracrine modulators of
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11 666 the individual MNC from which they are secreted, whereas the much higher levels of vasopressin or
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13 667 oxytocin secreted modulate the activity of the population as a whole to regulate peripheral physiology.
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17 668 Recently, compelling evidence has emerged that somato-dendritic secretion from MNCs modulates
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19 669 arteriole diameter in the SON (218) as well as the activity of neurones, particularly RVLM-projecting
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21 670 preautonomic neurones in the parvocellular PVN (193) (and perhaps also CRH neurones (216)). This
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23 671 inter-population cross-talk between MNCs and preautonomic neurones likely coordinates the
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25 672 hormonal (vasopressin secretion into the circulation) and neural (sympathetic nerve activation)
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27 673 response to perturbations of body fluid balance and blood pressure/volume (239).
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30 674 To date, there is no definitive evidence that somato-dendritic vasopressin and oxytocin have actions
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32 675 beyond the SON and PVN and it remains to be determined whether these neuropeptides act as
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34 676 hormone-like signals after secretion from MNC somata and dendrites. Nevertheless, while much of
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36 677 the data are circumstantial, it appears likely that somato-dendritic oxytocin release modulates the
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38 678 activity of neurones in some nearby brain areas that express OTRs but do not contain oxytocin (MNC
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40 679 or parvocellular) neurone projections, specifically the VMH (219, 222) and MeA (222, 225). The
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42 680 confirmation (or refutation) of the effects of somato-dendritic oxytocin on the VMH and/or MeA is
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44 681 required to resolve this ongoing debate within the field.
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Figure legends

Figure 1. Magnocellular neurosecretory cells. Magnocellular neurosecretory cells (MNCs) of the hypothalamic supraoptic nucleus and paraventricular nucleus each possess 1 – 3 dendrites and project a single axon to the posterior pituitary gland where they secrete either oxytocin or vasopressin into the circulation. Some MNC axons project axon collaterals to other brains areas.

Figure 2. Mechanisms of somato-dendritic release of oxytocin from magnocellular neurosecretory cells. Neuropeptides are synthesized and packaged in the soma and stored in dendrites in a reserve pool containing large numbers of large dense core vesicles (LDCVs). Depolarization-induced calcium entry through voltage-gated calcium channels (VGCCs) stimulates peptide release by exocytosis of LDCVs. This requires the depolymerization of F-actin to G-actin. Furthermore, the stimulation of G-protein coupled receptors (GPR), such as the oxytocin receptor, stimulates the mobilization of Ca^{2+} from IP₃-dependent intracellular stores of the rough endoplasmic reticulum (ER) and an increase in the number of LDCVs at the plasma membrane, thus priming the exocytosis machinery for subsequent activity-dependent release. Although some members of the SNARE family are detectable by immunocytochemistry, there appears to be a lack of VAMP, SNAP-25 and synaptotagmin-1 in the somata and dendrites, with their function presumably being replaced by other SNARE proteins.

Figure 3. Autocrine modulation of burst firing in oxytocin magnocellular neurosecretory cells. Cervical stretch during birth activates stretch receptors to activate A2 noradrenergic neurones in the nucleus tractus solitarius (NTS) that, in turn, activates somato-dendritic oxytocin secretion from oxytocin magnocellular neurosecretory cells (MNCs). Oxytocin feeds back on oxytocin MNCs to increase excitability. Oxytocin also increases noradrenaline secretion within the SON to establishes a local positive feedback loop that reinforces oxytocin MNC excitation and promotes oxytocin secretion into the circulation to trigger uterine contractions during birth.

Figure 4. Ghrelin stimulation of somato-dendritic vasopressin secretion. Ghrelin activation of growth hormone secretagogue receptors (GHSR) on vasopressin magnocellular neurosecretory cells (MNCs) induces somato-dendritic vasopressin secretion, which activates V_{1a} receptors ($V_{1a}Rs$) on neighbouring astrocytes to increase intracellular calcium. Increased astrocytic calcium triggers release of the gliotransmitter, ATP, which activates ionotropic P2X receptors on GABA interneurons that project back to vasopressin MNCs.

Figure 5. Autocrine modulation of vasopressin magnocellular neurosecretory cell activity. Vasopressin magnocellular neurosecretory cells (MNCs) secrete vasopressin, ATP and dynorphin (and other transmitters) from their somata and dendrites. Endogenous AVP (2) inhibits spike discharge throughout bursts via inhibition of EPSC amplitude. Endogenous ATP is rapidly converted to adenosine (3), which enhances the medium afterhyperpolarisation (mAHP) amplitude over the first few seconds of bursts to contribute to spike frequency adaptation. Endogenous dynorphin (4) inhibition of the afterdepolarisation (ADP) increases progressively over the course of bursts, eventually resulting in burst termination. Combined, these autocrine feedback effects of somato-dendritic vasopressin and co-secreted transmitters shape phasic activity for efficient secretion of vasopressin into the circulation from the posterior pituitary gland.

Figure 6. Endocannabinoid modulation of excitatory and inhibitory synapses on MNCs.

Oxytocin activation of autocrine oxytocin receptors (OTR) on oxytocin neurons leads to a tonic basal release of the endocannabinoid anandamide (AEA) at GABA synapses, which tonically suppresses synaptic inhibitory input to oxytocin neurons by activating presynaptic CB1 receptors. Depolarization (e.g., via action potential generation) or corticosteroid (Cort) exposure (e.g., during stress) leads to a calcium-dependent release of the other main endocannabinoid, 2-arachidonoylglycerol (2-AG), at glutamate synapses, which suppresses synaptic excitation of both oxytocin and vasopressin MNCs by activating presynaptic CB1 receptors. Glial retraction induced by salt loading allows the 2-AG released at glutamate

synapses to spill over onto GABA synapses and suppress synaptic inhibition via CB1 receptor activation. Tonic AEA occupation of CB1 receptors at GABA synapses is non-saturating, allowing additional suppression of GABA release following phasic 2-AG release and synaptic spillover.

Figure 7. Paracrine actions of somato-dendritic vasopressin secretion. Activation of neurosecretory vasopressin magnocellular neurosecretory cells (MNCs) (1) triggers action potential firing (2) to release vasopressin into the circulation from the posterior pituitary gland (3). In parallel, action potentials back-propagate into the dendrites (4) to trigger somato-dendritic vasopressin secretion (5). In addition to autocrine feedback inhibition of vasopressin MNC activity via V_{1a} receptors ($V_{1a}Rs$) (6), somato-dendritic vasopressin diffuses through the extracellular space to bind to $V_{1a}Rs$ on presympathetic paraventricular nucleus neurones (7) to increase action potential firing (8) and therefore increase sympathetic outflow to peripheral organs. Somato-dendritic vasopressin also activates $V_{1a}Rs$ on local blood vessels (9) to cause vasoconstriction, which is predicted to inhibit vasopressin MNCs at a population level (10) by restricting the availability of oxygen and nutrients. Hence, somato-dendritic vasopressin secretion coordinates neurohumoral responses to (patho)physiological activation (11).

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Neuropeptides are synthesized and packaged in the soma and stored in dendrites in a reserve pool containing large numbers of large dense core vesicles (LDCVs). Depolarization-induced calcium entry through voltage-gated calcium channels (VGCCs) stimulates peptide release by exocytosis of LDCVs. This requires the depolymerization of F-actin to G-actin. Furthermore, the stimulation of G-protein coupled receptors (GPR), such as the oxytocin receptor, stimulates the mobilization of Ca^{2+} from IP_3 -dependent intracellular stores of the rough endoplasmic reticulum (ER) and an increase in the number of LDCVs at the plasma membrane, thus priming the exocytosis machinery for subsequent activity-dependent release. Although some members of the SNARE family are detectable by immunocytochemistry, there appears to be a lack of VAMP, SNAP-25 and synaptotagmin-1 in the somata and dendrites, with their function presumably being replaced by other SNARE proteins.

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