Endogenous modulators of synaptic transmission: cannabinoid regulation in the supraoptic nucleus

Neil A. McDonald^{1,*}, J. Brent Kuzmiski¹, Nima Naderi², Yannick Schwab³, and Quentin J. Pittman¹

¹Hotchkiss Brain Institute, Department of Physiology and Biophysics, Faculty of Medicine, University of Calgary, Calgary, AB, Canada

²Neuroscience Research Centre, Shaheed Beheshti University of Medical Sciences, Tehran, Iran

³IGBMC-Centre d'Imagerie, Microscopie Electronique, Illkirch Cedex, France

Abstract

The magnocellular neurons of the hypothalamic supraoptic nucleus (SON) are a major source of both systemic and central release of the neurohypophyseal peptides, oxytocin (OXT) and arginine-vasopressin (AVP). Both OXT and AVP are released from the somatodendritic compartment of magnocellular neurons and act within the SON to modulate the electrophysiological function of these cells. Cannabinoids (CBs) affect hormonal output and the SON may represent a neural substrate through which CBs exert specific physiological and behavioural effects. Dynamic modulation of synaptic inputs is a fundamental mechanism through which neuronal output is controlled. Dendritically released OXT acts on autoreceptors to generate endocannabinoids (eCBs) which modify both excitatory and inhibitory inputs to OXT neurons through actions on presynaptic CB receptors. As such, OXT and eCBs cooperate to shape the electrophysiological properties of magnocellular OXT neurons, regulating the physiological function of this nucleus. Further study of eCB signalling in the SON, including its interaction with AVP neurons, promises to extend our understanding of the synaptic regulation of SON physiological function.

Keywords

hypothalamus; oxytocin; magnocellular neurons; retrograde messengers

Introduction

The hypothalamic-neurohypophyseal system consists of two nuclei, the supraoptic nucleus (SON), situated lateral to the optic chiasm, and the paraventricular nucleus (PVN), on each side of the third ventricle. Magnocellular neurons in these nuclei synthesize either oxytocin (OXT) or arginine-vasopressin (AVP) and send axonal projections to the posterior pituitary. Neuronal activity stimulates release of the hormones into the blood, regulating a number of important physiological functions, most notably lactation/parturition (OXT; Neumann et al., 1993, 1994; Leng et al., 2005) and body-fluid homeostasis (AVP; Landgraf et al., 1988;

*Corresponding author. Tel.: +403 220 4497; Fax: +403 283 2700; nmcdona@ucalgary.ca.

Leng et al., 1999). The somatodendritic regions of magnocellular neurons are also a major site of OXT and AVP release within the central nervous system (CNS) and contain many peptidergic large dense core vesicles (LDCVs). These vesicles release their contents in response to elevated calcium, which can be elevated independent of electrical activity in the neuron (Pow and Morris, 1989; Ludwig et al., 2002). It has been suggested that OXT and AVP may diffuse to affect remote brain areas that do not receive direct OXT/AVP innervation (Ludwig and Leng, 2006).

Central neuropeptide release impacts on systemic hormone output and the role of somatodendritic neuropeptide release in shaping electrical activity of magnocellular neurons has been an area of investigation for many years (Ludwig and Pittman, 2003). Although somatodendritic and axonal release often respond to the same stimuli, there is evidence that the release can be independently regulated from each compartment. For example, during lactation and parturition, levels of OXT increase within the SON before systemic levels rise (Neumann et al., 1993). This may be important because OXT neurons are excited by OXT itself and injection of OXT into the SON facilitates its own release (Moos et al., 1984; Yamashita et al., 1987). In vivo, central AVP, which is also found to be regulated by known stimuli (e.g. osmotic), acts within the SON to bring the spiking activity of AVP neurons to an intermediate level (Gouzenes et al., 1998) which maximizes the effectiveness of hormonal output at the neurohypophysis (Dutton and Dyball, 1979). AVP also facilitates its own release through action on AVP receptors within the nucleus (Wotjak et al., 1994).

Functional disparities between AVP and OXT neurons may reflect, to a degree, differing regulation by somatodendritically released neuropeptides (Oliet et al., 2007). Recent reports have highlighted the interaction of the endogenous cannabinoid (CB) system with central hormone release in the modulation of magnocellular neuron synaptic physiology (Hirasawa et al., 2004; Sabatier and Leng, 2006).

The cannabinoid system in the SON

Cooperative signalling of OXT and the cannabinoid system

Two subtypes of CB receptor, CB₁ and CB₂, have been identified and cloned (Matsuda et al., 1990; Munro et al., 1993) although there is evidence for further subtypes (Hajos et al., 2001). The CB₁ receptor is the predominant CNS subtype (Freund et al., 2003), whereas the CB₂ receptor is primarily expressed in immune cells, although it is also found in the CNS (Van Sickle et al., 2005). CB receptors are believed to be primarily associated with presynaptic terminals (Freund et al., 2003) and studies on recombinant receptors expressed in cultured neurons agree with in vitro reports (Katona et al., 1999; Egertova and Elphick, 2000) indicating the selective expression of CB₁ receptors at presynaptic sites (Leterrier et al., 2006; McDonald et al., 2007). Depolarization of the postsynaptic cell generates endocannabinoids (eCBs) which act retrogradely to inhibit subsequent neurotransmitter release; depending on whether the presynaptic input is excitatory or inhibitory, this phenomenon is termed depolarization-induced suppression of excitation or inhibition (DSE/DSI) respectively (Kreitzer and Regehr, 2001; Wilson and Nicoll, 2001).

Dynamic modulation of synaptic inputs constitutes a fundamental mechanism through which neuronal output is regulated. A number of neuro-modulatory transmitters postsynaptically influence, or presynaptically regulate excitatory and inhibitory inputs to SON magnocellular neurons; a non-exclusive list includes glutamate (Schrader and Tasker, 1997), noradrenaline (Wang et al., 1998), dopamine (Price and Pittman, 2001), acetylcholine (Li and Pan, 2001; Li et al., 2001; Hatton and Yang, 2002), histamine (Hatton and Yang, 2001), adenosine (Oliet and Poulain, 1999), nitric oxide (Stern and Ludwig, 2001; Gillard et al., 2007) and a number of peptides (e.g. Galanin, Papas and Bourque, 1997; Kozoriz et al., 2006; pituitary adenylate cyclase activating polypeptide (PACAP), Gillard et al., 2006).

OXT itself modulates excitatory neurotransmission onto magnocellular neurons through what were thought to be presynaptic receptors (Kombian et al., 1997; de Kock et al., 2003). However, OXT receptor expression in the SON is largely localized postsynaptically (Freund-Mercier et al., 1994; Adan et al., 1995). The link between OXT action and presynaptic glutamate release became apparent when it was subsequently found that exogenously applied CB agonists also presynaptically inhibit neurotransmission in the SON (Hirasawa et al., 2004; Soya et al., 2005; Oliet et al., 2007). Immuno-reactivity for the CB₁ receptor within the SON is consistent with a presynaptic localization, with electron microscopy confirming that expression of the CB₁ receptor is restricted to axons (Fig. 1). The apparent presynaptic action of OXT was blocked by a CB antagonist, suggesting that somatodendritically released OXT activates OXT autoreceptors and triggers eCB synthesis to presynaptically inhibit glutamatergic transmission (Fig. 2); DSE in this nucleus is also blocked by OXT antagonists (Hirasawa et al., 2004). Metabotropic regulation of eCB production is well documented; in the hippocampus, for example, muscarinic activation can potentiate, and may occlude, DSE (Varma et al., 2001; Straiker and Mackie, 2007). However, it appears that in the SON activation of OXT autoreceptors is a prerequisite for eCB release with depolarization alone being insufficient (Hirasawa et al., 2004).

The role of tonic eCB production in determining distinct functional characteristics of OXT and AVP neurons

There is evidence for a basal eCB tone in the SON (Di et al., 2005; Oliet et al., 2007). GABAergic neurotransmission onto OXT neurons is tonically inhibited by eCBs. Since this tonic eCB generation is dependent on constitutive OXT release, this underlies the distinct characteristics of inhibitory inputs onto OXT and AVP neurons. The phenotype of the postsynaptic cell determines the strength of presynaptic GABAergic inputs. The tonic, concerted action of OXT and eCBs selectively maintains a low probability of release at GABAergic synapses onto OXT cells, allowing facilitation of GABAergic transmission under repetitive stimulation and the effective termination of bursting (Oliet et al., 2007). Furthermore, GABAergic inputs appear to be important in determining an irregularity in basal firing of OXT neurons (Li et al., 2007), which is believed to facilitate their probability of bursting (Moos et al., 2004). Therefore, it is likely that the tonic regulation of these inputs by basal eCB production contributes to maintaining this conducive state.

Role of cannabinoids in the independent control of central and systemic OXT release

Neurotransmitters which elevate intracellular calcium can potentially stimulate somatodendritic neuropeptide release. As such, somatodendritic OXT/AVP release is not dependent on action potential firing and can be stimulated, in the absence of electrical activity, without a corresponding rise in systemic levels (Ludwig et al., 2002). It has recently been reported that somatodendritic release of OXT is stimulated by α -melanocyte-stimulating hormone whilst concurrent inhibition of excitatory input onto OXT cells through eCB release, which may be secondary to somatodendritic OXT release, reduces peripheral secretion of the hormone (Sabatier, 2006). Interestingly, administration of either OXT or α -melanocyte-stimulating hormone within the CNS leads to similar effects on appetite and sexual behaviour. It is proposed that it is the increase of OXT within the SON induced by α -melanocyte-stimulating hormone that mediates these behavioural outcomes.

Cannabinoids and AVP neuron function

Studies examining the role of the CB system in magnocellular neuron electrophysiology have primarily concentrated on the relationship between eCB and OXT signalling. AVP preferentially inhibits excitatory over inhibitory inputs to magnocellular neurons (Kombian et al., 2000) with the locus and mechanism of this effect remaining undetermined. Cannabis usage is associated with diuresis, indicative of reduced systemic AVP release, consistent with CBs selectively inhibiting excitatory inputs to AVP neurons. Although both exogenously applied CB agonists and postsynaptic depolarization presynaptically inhibit excitatory and inhibitory transmission regardless of magnocellular phenotype (Hirasawa et al., 2004; Soya et al., 2005), Olier et al. (2007) report that tonic CB production selectively inhibits GABAergic inputs onto OXT neurons.

Central AVP release in response to dehydration is dependent on PACAP receptor activation. Administration of PACAP is associated with decreased glutamate release within the SON. The similarity of this response profile to that of OXT action on OXT neurons raises the possibility that eCBs may be released and mediate the reduction in glutamate release (Gillard et al., 2006). However, nitric oxide, which stimulates central whilst reducing systemic AVP release, stimulates glutamate release within the SON (Gillard et al., 2007). Thus, perhaps it is possible to override eCB tone with other secretagogues.

Future perspectives

It has been suggested that the functional organization of the eCB system in the SON may be unique compared with what has been reported for other areas of the CNS. In magnocellular neurons of the PVN, glucocorticoid-induced eCB synthesis is dependent on G_s -stimulated cAMP production (Malcher-Lopes et al., 2006); perhaps protein kinase A-activated, calcium-insensitive isoforms of CB synthesis enzymes are expressed in magnocellular neurons. Another unresolved question is why eCBs do not appear to be generated by depolarization-induced calcium influx alone. Calcium released from ryanodine sensitive stores has been shown to play a role in eCB production (Isokawa and Alger, 2006). In fact, in an autaptic preparation of hippocampal neurons, DSE was found to require calcium release from stores (Straiker and Mackie, 2005). Coupling eCB generation selectively to

calcium release from stores rather than calcium-influx resulting from depolarization could result from a tighter control of calcium-induced calcium release in OXT neurons. In the SON, OXT actions on autoreceptors might conceivably increase the sensitivity of the CB system, amplifying eCB signalling. Furthermore, it is conceivable that OXT may diffuse further than eCBs, and thus, allow eCB action on many cells to co-ordinate activity, transforming a local effect to a global one.

The further study of eCB signalling in the SON promises to illuminate many more aspects of the physiological function of this nucleus. What is still lacking is an understanding of how the eCB system works in a functional sense. We are still faced with the conundrum that OXT is *facilitory* in promoting OXT release from neurohypophyseal axons during lactation and delivery of pups. Yet our electrophysiological studies indicate an inhibitory action, mediated by eCBs. In resolving this apparent contradiction, it may be appropriate to recall that magnocellular neurons receive many different inputs, in addition to the glutamatergic and GABAergic ones discussed here. Some of these, such as the noradrenergic, histaminergic, cholinergic and various peptidergic afferents may be resistant to eCB retrograde inhibition (due to lack of presynaptic CB receptors). Thus signals important in lactation and birth may influence the OXT neuron, whereas extraneous inputs from other sources (mediating stress inputs for example) will be suppressed at this time (Fig. 2). Alternatively, retrograde signalling may be important during lactation to regulate the activity level of the neuron to enable efficient peptide release from the neurohypophysis. It is also possible that there may be gender-specific effects of OXT since *in vivo* studies have largely been conducted on lactating rats whereas much of the *in vitro* work uses male rats.

To resolve the role of eCBs in regulating magnocellular activity *in vivo*, it may be useful to consider that the CB₁-knockout mouse is able to deliver pups and to provide them with milk (Fride et al., 2003 and unpublished observations). Although it has been reported that CB₁-knockout pups are somewhat compromised in feeding during the first couple of days after delivery, this is thought to be due to a problem in the orofacial musculature functioning of the pups (Fride et al., 2005). If the pattern and delivery of milk by the mother is normal in these mice (something that has not been investigated properly to the best of our knowledge), this would argue that the eCBs are not of major importance in the regulation of OXT neuronal activity during lactation. Future studies might also be profitably directed at understanding how these animals respond to other stimuli known to activate magnocellular neurons.

Abbreviations

AVP	arginine–vasopressin
CB	cannabinoid
CNS	central nervous system
DSE/DSI	depolarization-induced suppression of excitation or inhibition
eCBs	endocannabinoids

LDCV	large dense core vesicle
OXT	oxytocin
PACAP	pituitary adenylate cyclase activating polypeptide
PVN	paraventricular nucleus
SON	supraoptic nucleus

References

- Adan RA, Van Leeuwen FW, Sonnemans MA, Brouns M, Hoffman G, Verbalis JG, Burbach JP. Rat oxytocin receptor in brain, pituitary, mammary gland, and uterus: partial sequence and immunocytochemical localization. *Endocrinology*. 1995; 136:4022–4028. [PubMed: 7649111]
- de Kock CP, Wierda KD, Bosman LW, Min R, Koksmma JJ, Mansvelder HD, Verhage M, Brussaard AB. Somatodendritic secretion in oxytocin neurons is upregulated during the female reproductive cycle. *J Neurosci*. 2003; 23:2726–2734. [PubMed: 12684458]
- Di S, Boudaba C, Popescu IR, Weng FJ, Harris C, Marcheselli VL, Bazan NG, Tasker JG. Activity-dependent release and actions of endocannabinoids in the rat hypothalamic supraoptic nucleus. *J Physiol*. 2005; 569:751–760. [PubMed: 16239276]
- Dutton A, Dyball RE. Phasic firing enhances vasopressin release from the rat neurohypophysis. *J Physiol*. 1979; 290:433–440. [PubMed: 469785]
- Egertova M, Elphick MR. Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. *J Comp Neurol*. 2000; 422:159–171. [PubMed: 10842224]
- Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev*. 2003; 83:1017–1066. [PubMed: 12843414]
- Freund-Mercier MJ, Stoeckel ME, Klein MJ. Oxytocin receptors on oxytocin neurones: histoautoradiographic detection in the lactating rat. *J Physiol*. 1994; 480(Pt 1):155–161. [PubMed: 7853219]
- Fride E, Bregman T, Kirkham TC. Endocannabinoids and food intake: newborn suckling and appetite regulation in adulthood. *Exp Biol Med* (Maywood). 2005; 230:225–234. [PubMed: 15792943]
- Fride E, Foox A, Rosenberg E, Faigenboim M, Cohen V, Barda L, Blau H, Mechoulam R. Milk intake and survival in newborn cannabinoid CB1 receptor knockout mice: evidence for a “CB3” receptor. *Eur J Pharmacol*. 2003; 461:27–34. [PubMed: 12568912]
- Gillard ER, Coburn CG, de Leon A, Snissarenko EP, Bause LG, Pittman QJ, Hou B, Curras-Collazo MC. Vasopressin autoreceptors and nitric oxide-dependent glutamate release are required for somatodendritic vasopressin release from rat magnocellular neuroendocrine cells responding to osmotic stimuli. *Endocrinology*. 2007; 148:479–489. [PubMed: 17082256]
- Gillard ER, Leon-Olea M, Mucio-Ramirez S, Coburn CG, Sanchez-Islas E, de Leon A, Mussenden H, Bause LG, Pittman QJ, Curras-Collazo MC. A novel role for endogenous pituitary adenylate cyclase activating polypeptide in the magnocellular neuroendocrine system. *Endocrinology*. 2006; 147:791–803. [PubMed: 16282358]
- Gouzenes L, Desarmenien MG, Hussy N, Richard P, Moos FC. Vasopressin regularizes the phasic firing pattern of rat hypothalamic magnocellular vasopressin neurons. *J Neurosci*. 1998; 18:1879–1885. [PubMed: 9465012]
- Hajos N, Ledent C, Freund TF. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience*. 2001; 106:1–4.
- Hatton GI, Yang QZ. Ionotropic histamine receptors and H2 receptors modulate supraoptic oxytocin neuronal excitability and dye coupling. *J Neurosci*. 2001; 21:2974–2982. [PubMed: 11312281]
- Hatton GI, Yang QZ. Synaptic potentials mediated by alpha 7 nicotinic acetylcholine receptors in supraoptic nucleus. *J Neurosci*. 2002; 22:29–37. [PubMed: 11756485]

- Hirasawa M, Schwab Y, Natah S, Hillard CJ, Mackie K, Sharkey KA, Pittman QJ. Dendritically released transmitters cooperate via autocrine and retrograde actions to inhibit afferent excitation in rat brain. *J Physiol.* 2004; 559:611–624. [PubMed: 15254151]
- Isokawa M, Alger BE. Ryanodine receptor regulates endogenous cannabinoid mobilization in the hippocampus. *J Neurophysiol.* 2006; 95:3001–3011. [PubMed: 16467427]
- Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K, Freund TF. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci.* 1999; 19:4544–4558. [PubMed: 10341254]
- Kombian SB, Mougnot D, Hirasawa M, Pittman QJ. Vasopressin preferentially depresses excitatory over inhibitory synaptic transmission in the rat supraoptic nucleus in vitro. *J Neuroendocrinol.* 2000; 12:361–367. [PubMed: 10718934]
- Kombian SB, Mougnot D, Pittman QJ. Dendritically released peptides act as retrograde modulators of afferent excitation in the supraoptic nucleus in vitro. *Neuron.* 1997; 19:903–912. [PubMed: 9354336]
- Kozoriz MG, Kuzmiski JB, Hirasawa M, Pittman QJ. Galanin modulates neuronal and synaptic properties in the rat supraoptic nucleus in a use and state dependent manner. *J Neurophysiol.* 2006; 96:154–164. [PubMed: 16611841]
- Kreitzer AC, Regehr WG. Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. *J Neurosci.* 2001; 21:RC174, 1–5. [PubMed: 11588204]
- Landgraf R, Neumann I, Schwarzborg H. Central and peripheral release of vasopressin and oxytocin in the conscious rat after osmotic stimulation. *Brain Res.* 1988; 457:219–225. [PubMed: 3219551]
- Leng G, Brown CH, Russell JA. Physiological pathways regulating the activity of magnocellular neurosecretory cells. *Prog Neurobiol.* 1999; 57:625–655. [PubMed: 10221785]
- Leng G, Caquineau C, Sabatier N. Regulation of oxytocin secretion. *Vitam Horm.* 2005; 71:27–58. [PubMed: 16112264]
- Leterrier C, Laine J, Darmon M, Boudin H, Rossier J, Lenkei Z. Constitutive activation drives compartment-selective endocytosis and axonal targeting of type 1 cannabinoid receptors. *J Neurosci.* 2006; 26:3141–3153. [PubMed: 16554465]
- Li C, Tripathi PK, Armstrong WE. Differences in spike train variability in rat vasopressin and oxytocin neurons and their relationship to synaptic activity. *J Physiol.* 2007; 581:221–240. [PubMed: 17332000]
- Li DP, Pan HL. Potentiation of glutamatergic synaptic input to supraoptic neurons by presynaptic nicotinic receptors. *Am J Physiol Regul Integr Comp Physiol.* 2001; 281:R1105–R1113. [PubMed: 11557616]
- Li DP, Pan YZ, Pan HL. Acetylcholine attenuates synaptic GABA release to supraoptic neurons through presynaptic nicotinic receptors. *Brain Res.* 2001; 920:151–158. [PubMed: 11716821]
- Ludwig M, Leng G. Dendritic peptide release and peptide-dependent behaviours. *Nat Rev Neurosci.* 2006; 7:126–136. [PubMed: 16429122]
- Ludwig M, Pittman QJ. Talking back: dendritic neurotransmitter release. *Trends Neurosci.* 2003; 26:255–261. [PubMed: 12744842]
- Ludwig M, Sabatier N, Bull PM, Landgraf R, Dayanithi G, Leng G. Intracellular calcium stores regulate activity-dependent neuropeptide release from dendrites. *Nature.* 2002; 418:85–89. [PubMed: 12097911]
- Malcher-Lopes R, Di S, Marcheselli VS, Weng FJ, Stuart CT, Bazan NG, Tasker JG. Opposing crosstalk between leptin and glucocorticoids rapidly modulates synaptic excitation via endocannabinoid release. *J Neurosci.* 2006; 26:6643–6650. [PubMed: 16775153]
- Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature.* 1990; 346:561–564. [PubMed: 2165569]
- McDonald NA, Henstridge CM, Connolly CN, Irving AJ. An essential role for constitutive endocytosis, but not activity, in the axonal targeting of the CB1 cannabinoid receptor. *Mol Pharmacol.* 2007; 71:976–984. [PubMed: 17182888]
- Moos F, Fontanaud P, Mekaouche M, Brown D. Oxytocin neurones are recruited into co-ordinated fluctuations of firing before bursting in the rat. *Neuroscience.* 2004; 125:391–410. [PubMed: 15062982]

- Moos F, Freund-Mercier MJ, Guerne Y, Guerne JM, Stoeckel ME, Richard P. Release of oxytocin and vasopressin by magnocellular nuclei in vitro: specific facilitatory effect of oxytocin on its own release. *J Endocrinol.* 1984; 102:63–72. [PubMed: 6539805]
- Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993; 365:61–65. [PubMed: 7689702]
- Neumann I, Koehler E, Landgraf R, Summy-Long J. An oxytocin receptor antagonist infused into the supraoptic nucleus attenuates intranuclear and peripheral release of oxytocin during suckling in conscious rats. *Endocrinology.* 1994; 134:141–148. [PubMed: 8275928]
- Neumann I, Russell JA, Landgraf R. Oxytocin and vasopressin release within the supraoptic and paraventricular nuclei of pregnant, parturient and lactating rats: a microdialysis study. *Neuroscience.* 1993; 53:65–75. [PubMed: 8469313]
- Oliet SH, Baimoukhametova DV, Piet R, Bains JS. Retrograde regulation of GABA transmission by the tonic release of oxytocin and endocannabinoids governs postsynaptic firing. *J Neurosci.* 2007; 27:1325–1333. [PubMed: 17287507]
- Oliet SH, Poulain DA. Adenosine-induced pre-synaptic inhibition of IPSCs and EPSCs in rat hypothalamic supraoptic nucleus neurons. *J Physiol.* 1999; 520(Pt 3):815–825. [PubMed: 10545146]
- Papas S, Bourque CW. Galanin inhibits continuous and phasic firing in rat hypothalamic magnocellular neurosecretory cells. *J Neurosci.* 1997; 17:6048–6056. [PubMed: 9236216]
- Pow DV, Morris JF. Dendrites of hypothalamic magnocellular neurons release neurohypophysial peptides by exocytosis. *Neuroscience.* 1989; 32:435–439. [PubMed: 2586758]
- Price CJ, Pittman QJ. Dopamine D4 receptor activation inhibits presynaptically glutamatergic neurotransmission in the rat supraoptic nucleus. *J Neurophysiol.* 2001; 86:1149–1155. [PubMed: 11535665]
- Sabatier N. Alpha-melanocyte-stimulating hormone and oxytocin: a peptide signalling cascade in the hypothalamus. *J Neuroendocrinol.* 2006; 18:703–710. [PubMed: 16879169]
- Sabatier N, Leng G. Presynaptic actions of endocannabinoids mediate alpha-MSH-induced inhibition of oxytocin cells. *Am J Physiol Regul Integr Comp Physiol.* 2006; 290:R577–R584. [PubMed: 16269571]
- Schrader LA, Tasker JG. Presynaptic modulation by metabotropic glutamate receptors of excitatory and inhibitory synaptic inputs to hypothalamic magnocellular neurons. *J Neurophysiol.* 1997; 77:527–536. [PubMed: 9065826]
- Soya A, Serino F, Fujihara H, Onaka T, Ozaki Y, Saito T, Nakamura J, Ueta Y. Cannabinoids modulate synaptic activity in the rat supraoptic nucleus. *J Neuroendocrinol.* 2005; 17:609–615. [PubMed: 16101900]
- Stern JE, Ludwig M. NO inhibits supraoptic oxytocin and vasopressin neurons via activation of GABAergic synaptic inputs. *Am J Physiol Regul Integr Comp Physiol.* 2001; 280:R1815–R1822. [PubMed: 11353687]
- Straiker A, Mackie K. Depolarization-induced suppression of excitation in murine autaptic hippocampal neurons. *J Physiol.* 2005; 569:501–517. [PubMed: 16179366]
- Straiker A, Mackie K. Metabotropic suppression of excitation in murine autaptic hippocampal neurons. *J Physiol.* 2007; 578:773–785. [PubMed: 17110416]
- Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di MV, Pittman QJ, Patel KD, Sharkey KA. Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science.* 2005; 310:329–332. [PubMed: 16224028]
- Varma N, Carlson GC, Ledent C, Alger BE. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J Neurosci.* 2001; 21:RC188. [PubMed: 11734603]
- Wang YF, Shibuya I, Kabashima N, Setiadji VS, Isse T, Ueta Y, Yamashita H. Inhibition of spontaneous inhibitory postsynaptic currents (IPSC) by noradrenaline in rat supraoptic neurons through presynaptic alpha2-adrenoceptors. *Brain Res.* 1998; 807:61–69. [PubMed: 9756995]
- Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature.* 2001; 410:588–592. [PubMed: 11279497]

- Wotjak CT, Ludwig M, Landgraf R. Vasopressin facilitates its own release within the rat supraoptic nucleus in vivo. *Neuroreport*. 1994; 5:1181–1184. [PubMed: 7919160]
- Yamashita H, Okuya S, Inenaga K, Kasai M, Uesugi S, Kannan H, Kaneko T. Oxytocin predominantly excites putative oxytocin neurons in the rat supraoptic nucleus in vitro. *Brain Res*. 1987; 416:364–368. [PubMed: 3620965]

Fig. 1.

The subcellular distribution of the CB₁ receptor is restricted to presynaptic processes in the SON. (A, B) Electron micrograph showing a synaptic terminal (ST) labelled for CB₁ (dark deposit), contacting a dendritic shaft. Note that the expression of the CB₁ receptor within the SON is restricted to presynaptic processes. (B) Magnified area of micrograph shown in A.

Fig. 2.

Retrograde signalling by eCBs in the SON. Schematic illustrating the proposed mechanism of cooperative signalling by OXT and eCBs in the modulation of synaptic inputs to magnocellular neurons in the SON. (1) Excitatory stimulation and depolarization of OXT magnocellular neurons causes the somatodendritic release of OXT which acts on OXT autoreceptors, (2) leading to calcium release from stores and the generation of eCBs. OXT may diffuse to neighbouring OXT neurons to initiate the temporal and spatial spread of CB signalling. (3) Acting at presynaptic CB receptors, eCBs inhibit both glutamatergic and GABAergic afferents onto magnocellular neurons. (4) Specific afferents, lacking CB₁ receptors and perhaps, for example, related to suckling, may be unaffected by eCBs.