TITLE: **OSMOTIC AND THERMAL CONTROL OF MAGNOCELLULAR NEUROSECRETORY NEURONS: ROLE OF AN N-TERMINAL VARIANT OF TRPV1**

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RUNNING TITLE: **ROLE OF TRPV1 IN OSMOTIC AND THERMAL SENSING**

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ABSTRACT

The release of vasopressin (antidiuretic hormone) plays a key role in the osmoregulatory response of mammals to changes in salt or water intake and in the rate of water loss through evaporation during thermoregulatory cooling. Previous work has shown that the hypothalamus encloses the sensory elements that modulate VP release during systemic changes in fluid osmolality or body temperature. These responses depend in part on a synaptic regulation of vasopressin neurons by afferent inputs arising from osmosensory and thermosensory neurons in the preoptic area. However recent studies in rats and mice have shown that vasopressin neurons in the supraoptic nucleus also display intrinsic osmosensory and thermosensory responses. Isolated vasopressin neurons exposed to increases in perfusate temperature or osmolality generate increases in nonselective cation channel activity that cause membrane depolarization and increase neuronal excitability. These channels are calcium-permeable and can be blocked by ruthenium red. Moreover, intrinsic responses to osmotic and thermal stimuli are absent in MNCs isolated from mice lacking the transient receptor potential vanilloid-1 (trpv1) gene, which encodes the capsaicin receptor. Immunostaining of vasopressin releasing neurons with anti-TRPV1 antibodies reveals the presence of amino acids present in the carboxy terminus of the protein, but not those lying in the amino terminal domain. Thus magnocellular neurosecretory neurons appear to express an N-terminal variant of trpv1 which lacks sensitivity to capsaicin, but which enables osmosensing and thermosensing.

1. INTRODUCTION

1.1 Magnocellular neurosecretory cells (MNCs) and osmoregulation.

Mammals have evolved an array of mechanisms to maintain extracellular fluid osmolality approximately constant near a value of 300 mosmol/kg . Failure to osmoregulate can lead to serious neurological consequences due to osmotically-induced brain swelling or shrinking (Ayus *et al.*, 2000; Pasantes-Morales & Tuz, 2006; Lang, 2007; Verbalis, 2010). Under normal conditions, small deviations away from a species-specific "set-point" trigger behavioral and physiological responses that work together to restore fluid osmolality at equilibrium (Bourque, 2008). Salt and water intake or abstinence are the predominant behavioral responses, whereas humoral control of renal natriuresis and diuresis provide the complementary physiological responses that sustain osmoregulation. The central mechanisms that underlie the last of these responses are addressed in this review.

Changes in renal water handling are mediated by variations in systemic release of vasopressin (VP), the antidiuretic hormone. VP is produced in the somata of MNCs located in the supraoptic and paraventricular nuclei of the hypothalamus and is released into the systemic circulation via the neurohypophysial axon terminals of these neurons through Ca^{2+} -dependent exocytosis (Bicknell, 1988; Fisher & Bourque, 2001). VP secretion is therefore triggered by the electrical activity of MNCs and varies in proportion with action potential firing rate (Dreifuss *et al.*, 1971). Experiments *in vivo* have shown that under isotonic conditions rat MNCs are spontaneously active and fire action potentials at a rate of 1-3Hz (Walters & Hatton, 1974; Brimble & Dyball, 1977; Wakerley *et al.*, 1978; Bourque, 2008). Thus VP is released tonically at rest and actively promotes basal water reabsorption by interacting with V2 receptors in the kidney (Dunn *et al.*, 1973; Robertson *et al.*, 1976; Knepper & Inoue, 1997). During hyperosmolality VP release is enhanced due to an increase in the action potential firing frequency of MNCs, which causes an adaptive increase in water conservation (Figure 1). Conversely, hypo-osmolality inhibits MNC activity, which actively promotes homeostatic water excretion (Brimble & Dyball, 1977). Interestingly, VP release is also increased during hyperthermia to oppose the loss of body water associated with thermoregulatory cooling

(Forsling *et al.*, 1976). Therefore the osmotic and thermal control of firing rate in MNCs both contribute to systemic osmoregulation.

1.2 Osmotic control of MNCs

Many factors participate in the osmotic modulation of MNC firing and VP release. For example, astrocytes in the supraoptic nucleus have been shown to release taurine as an inverse function of fluid osmolality (Deleuze *et al.*, 1998). Taurine is a potent agonist at extrasynaptic inhibitory glycine receptors on MNCs (Hussy *et al.*, 1997; Deleuze *et al.*, 2005). Therefore its release contributes to the osmotic control of firing rate via inversely proportional changes in inhibitory tone on MNCs (Hussy *et al.*, 2001). Synaptic inputs from the brain's primary osmosensing region, the *organum vasculosum laminae terminalis*, also mediate a potent osmotic regulation of MNC firing via proportional changes in glutamate release and activation of ionotropic glutamate receptors (Richard & Bourque, 1995). Inputs from other central osmosensory regions such as the organum vasculosum lamina terminalis and peripheral osmoreceptor neurons also contribute to the modulation of MNC activity and VP output (Vallet & Baertschi, 1982; Anderson *et al.*, 1990; Travis & Johnson, 1993; Andersen *et al.*, 2000; Bourque, 2008). However previous studies *in vitro* have revealed that MNCs are intrinsically osmosensitive (Mason, 1980; Bourque, 1989; Oliet & Bourque, 1992), and studies *in vivo* indicate that this intrinsic response contributes importantly to the overall osmotic modulation of firing rate (Leng, 1989). Experiments on isolated neurons have shown that acute hyperosmotic stimulation causes MNCs to shrink, which leads to a mechanically induced increase in the probability of opening of non-selective cation channels expressed in these cells (Oliet & Bourque, 1993a; 1996; Zhang *et al.*, 2007). The activation of these channels causes membrane depolarization which contributes to the increase in action potential firing rate and VP secretion observed under these conditions. Conversely, the basal activity of the channels is inhibited during hypoosmotic-swelling, which causes hyperpolarization and inhibits firing. The precise molecular identity of the mammalian osmoreceptor channel remains unknown. However recent studies suggest that it might be formed by an N-terminal variant of the transient receptor potential vanilloid 1 (*trpv1*) gene (Sharif Naeini *et al.*, 2006).

1.3 Thermal control of MNCs

Non-febrile hyperthermia (e.g., occurring during exercise or upon exposure to elevated ambient temperatures) enhances the rate of evaporative water loss (e.g. sweating, panting) as a thermoregulatory cooling mechanism (Robertshaw, 2006; Shibasaki *et al.*, 2006). Because exhaled breath condensate and sweat are both hypo-osmotic fluids (Griese *et al.*, 2003; Morgan *et al.*, 2004), prolonged exercise or exposure to heat can lead to systemic hypertonicity (Figure 1) (Shibasaki *et al.*, 2006). Interestingly rises in core body temperature as small as +1°C have been shown to induce significant VP secretion and "heat-antidiuresis" to blunt the osmotic impact of increased evaporative water loss (Azahan & Sykes, 1980; Takamata *et al.*, 1995). The initial phase of this response is *anticipatory,* because it occurs before increases in systemic osmolality can be measured (Forsling *et al.*, 1976). Conversely, hypothermia inhibits VP release and induces "cold diuresis", which may serve to minimize water accumulation under conditions where the rate of evaporative water loss is reduced (Bader *et al.*, 1952; Azahan & Sykes, 1980). Previous work in rats and pigs has shown that mild heating of the hypothalamus via implanted thermodes can excite MNCs and provoke VP release, and that VP release evoked by a rise in core body temperature can be inhibited by preventing changes in hypothalamic temperature (Forsling *et al.*, 1976; Matsumura *et al.*, 1985). Thus thermosensitive elements within the hypothalamus appear to play a key role in the thermal regulation of MNC activity and VP release.

Our understanding of the mechanisms by which increases in hypothalamic temperature modulate the activity of MNCs is incomplete. However previous studies have shown that local heating of the preoptic area can excite supraoptic nucleus MNCs in anesthetized rats (Matsumura *et al.*, 1985). Subtypes of neurons within the preoptic area are known to be intrinsically thermosensitive (Silva & Boulant, 1984; Nakashima *et al.*, 1985) and anatomical studies have shown that neurons in this part of the brain send axonal projections to the supraoptic nucleus (Anderson *et al.*, 1990). It is therefore conceivable that preoptic thermosensory neurons could modulate the activity of MNCs via synaptic connections. Direct evidence supporting this hypothesis is presently lacking. However we have recently shown that

MNCs acutely isolated from rat and mouse hypothalami are intrinsically thermosensitive, and that channels encoded by *trpv1* may play a role in this response (Sharif-Naeini *et al.*, 2008).

1.4 TRPV channels

TRPV channels represent one of the seven subfamilies of the TRP (transient receptor potential) superfamily of ion channels, the first of which was identified in *Drosophila melanogaster* (Montell & Rubin, 1989; Venkatachalam & Montell, 2007). The *trp* genes encode channel proteins that comprise 6 transmembrane segments, as well as intracellular C-terminal and N-terminal domains (Wes *et al.*, 1995; Zhu *et al.*, 1995). The first member of the TRPV subfamily to be identified was TRPV1, a heat-activated channel that forms the capsaicin receptor (Caterina *et al.*, 1997). Later, five more members of this subfamily were discovered, including three (TRPV2, TRPV3, TRPV4) that are also thermally activated. Together with TRPV1, the latter form a subgroup of channels termed "thermo-TRPVs"; (Patapoutian *et al.*, 2003). The other two TRPV channels, TRPV5 and TRPV6, are heat-insensitive and play important roles in Ca^{2+} transport (Peng *et al.*, 1999; Muller *et al.*, 2000; Peng *et al.*, 2000a; Peng *et al.*, 2000b; Wissenbach *et al.*, 2001; den Dekker *et al.*, 2003; Venkatachalam & Montell, 2007). All thermo-TRPV channels feature a nonselective cation-permeable pore that can flux monovalent cations such as Na^+ and K^+, and which also displays a high degree of permeability for Ca^{2+} compared to Na^+ (P_{Ca}:P_{Na} between 1 and 10) (Owsianik *et al.*, 2006).

1.5 Aims of the review

Osmotic and thermal signals can both regulate the activity of MNCs to modulate systemic VP levels and mediate osmoregulation. Although a number of mechanisms are involved in the modulation of MNCs *in vivo*, channels encoded by the *trpv1* gene enable intrinsic osmosensing and thermosensing by MNCs. The aim of the present review is to summarize our current understanding of how TRPV channels contribute to these processes and speculate on how MNCs might integrate osmotic and thermal signals under physiological conditions.

(Figure 1 near here)

2. TRPV CHANNELS AS PHYSIOLOGICAL OSMOSENSORS

2.1 TRPVs and osmosensing

The potential role of TRPV channels as osmosensory transducers was first suggested by the finding that *osm-9* mutants of *Caenorhabditis elegans* (*osm-9* is a homologue of mammalian TRPVs) lacked natural avoidance responses to hyperosmotic environments (Colbert *et al.*, 1997). A subsequent search for mammalian homologues of *osm-9* led to the discovery of TRPV4, the first cloned mammalian TRP channel shown to be osmotically-activated. Specifically, it was shown that TRPV4 channel activity and associated Ca^{2+} influx are increased in response to cell swelling induced by hypo-osmotic conditions (Liedtke *et al.*, 2000; Strotmann *et al.*, 2000). Shortly thereafter, TRPV2 was identified as a hypotonicity-activated channel (Muraki *et al.*, 2003). Although homomeric TRPV2 or TRPV4 channels are unlikely to mediate the osmosensory responses of MNCs (which are hypertonicity-activated), it remains possible that these channels somehow contribute to osmosensory transduction in this nucleus or elsewhere in the nervous system. Indeed, a recent study has shown that *trpv4* expression is required for hypotonicity sensing by peripheral osmoreceptors (McHugh *et al.*, 2010).

Evidence that native TRPV channels might participate in hypertonicity sensing has been provided by the demonstration that targeted expression of mammalian *trpv4* in the appropriate sensory neurons of *C. elegans* osm-9 mutants could rescue the absence of hypertonicity avoidance in these worms (Liedtke *et al.*, 2003). How TRPV4 mediates hypertonicity detection in this instance, remains to be clarified. Although wild-type TRPV1 channels are not directly activated by hypertonicity in primary sensory neurons (Figure 3A), channel responses to capsaicin are potentiated under hypertonic conditions (Liu *et al.*, 2007). The possible involvement of TRPV1 related channels as hypertonicity-sensors was also supported by the observation that expression of a chimeric construct comprising all transmembrane segments of

TRPV1 and the cytoplasmic carboxy terminus of TRPV4 can yield a hypertonicity-activated channel (Suzuki *et al.*, 1999).

2.2 TRPV1 and osmosensing in MNCs

Several observations support the hypothesis that intrinsic responses to hypertonic stimulation in rodent MNCs are mediated by channels encoded by one or more *trpv* genes. As expected from the activation of such channels, hypertonic stimulation of isolated MNCs causes an increase in membrane conductance, activation of a cation current, membrane depolarization and increased action potential firing (Oliet & Bourque, 1993a; b; Qiu *et al.*, 2004). Moreover these responses are blocked by micromolar concentrations of Gd^{3+} or ruthenium red (Oliet & Bourque, 1996; Sharif Naeini *et al.*, 2006), both non-specific inhibitors of TRPV channels (Ramsey *et al.*, 2006; Clapham, 2007). Furthermore, TRPV channels studied in expression systems and osmoreceptor channels in MNCs are both Ca^{2+} permeable (Caterina *et al.*, 1997; Zhang & Bourque, 2006) and they display similar single-channel conductances at negative membrane potentials (~35 pS for TRPV1 and ~32 pS in MNCs) (Oliet & Bourque, 1993a; Owsianik *et al.*, 2006). Finally, as illustrated in Figure 2, responses to hypertonicity-induced shrinking were found to be absent in supraoptic nucleus MNCs isolated from *trpv1*$^{-/-}$ mice (Sharif Naeini *et al.*, 2006). In agreement with an important physiological role for TRPV1 mediated hypertonicity-sensing, mice lacking *trpv1* were found to be chronically hyperosmolar and displayed attenuated VP responses to hyperosmotic stimulation *in vivo*.

(Figure 2 near here)

2.3 MNCs express an N-terminal variant of TRPV1

Although the observations summarized above support the hypothesis that *trpv1* encodes a component of the osmoreceptor channel, MNCs do not express functional capsaicin receptors. Indeed, in contrast to neurons isolated from dorsal root ganglia, which show large capsaicin-induced inward currents, we found that exogenous capsaicin fails to induce any cellular response when applied to MNCs (Figure 3A) (Sharif Naeini *et al.*, 2006). Moreover,

although MNCs can be labeled with antibodies directed against the C-terminus of TRPV1, no labeling was observed when the cells were incubated with an antibody targeting the N-terminal domain of the protein. (Figure 3B). Finally, RT-PCR analysis using primers encompassing different regions of TRPV1 mRNA confirmed the presence of mRNA coding for the C-terminus of TRPV1 and the absence of the distal N-terminus mRNA. These observations are in agreement with previous studies showing that the cytoplasmic N-terminal domain of TRPV1 is required for channel activation by capsaicin (Jordt & Julius, 2002; Jung *et al.*, 2002). MNCs therefore express a variant of TRPV1 that lacks part of the N-terminal domain.

2.4 Mechanism of osmotic activation of TRPV1 channels

Studies of recombinant homomeric channels in heterologous expression systems suggest that TRPV4 is not a mechanosensitive channel (Strotmann *et al.*, 2000). Rather, its activation in response to cell swelling appears to require formation of arachidonic acid following mobilization of phospholipase A2 activity (Vriens *et al.*, 2004). Interestingly, wild-type TRPV1 channels are not activated by hypertonicity (e.g. Figure 3A) (Liu *et al.*, 2007). This observation suggests that part of the N-terminal domain of TRPV1 that is absent in native osmoreceptor channels may somehow prevent the osmotic modulation of channel activity (e.g. by preventing a critical allosteric conformation, or by promoting the recruitment of an inhibitory subunit). However despite the lack of direct gating effects, changes in osmolality have been shown to sensitize the response of wild-type TRPV1 to capsaicin, indicating that changes in osmolality can modulate TRPV1 channel sensitivity.

As mentioned earlier, studies in isolated MNCs have shown that the osmotic modulation of the transduction channels in these cells is a mechanically-coupled process. Responses induced by hypertonicity-induced shrinking can be mimicked by reducing cell volume via pipette suction (Oliet & Bourque, 1993a). Moreover, increases in membrane conductance evoked by hyperosmolality can be reversed by inflating the cells via positive pressure applied through the recording pipette . The mechanical modulation of channel activity requires an intact actin cytoskeleton and the sensitivity of this process appears to vary as a function of subcortical actin density (Zhang *et al.*, 2007). How actin filaments regulate the activation of the

osmoreceptor channel remains to be defined. Interestingly the C-terminal domain of TRPV1 has been shown to contain specific motifs that allow binding to beta-tubulin (Goswami *et al.*, 2007). It is therefore possible that interactions with microtubules also contribute to the regulation of TRPV1. Further studies are required to elucidate the mechanisms by which mechanical forces modulate these channels.

(Figure 3 near here)

3. TRPV CHANNELS AS PHYSIOLOGICAL THERMOSENSORS

3.1 TRPVs and thermosensing

Experiments with recombinant homomeric thermo-TRPV channels expressed in heterologous transfection systems have shown that TRPV1 and TRPV2 can be activated by noxious heat (activation thresholds near 43°C and 52°C, respectively), whereas TRPV3 and TRPV4 are activated by innocuous warm temperatures (activation thresholds near 30°C) (Peier *et al.*, 2002; Caterina, 2007; Clapham, 2007; Latorre *et al.*, 2007). The dynamic activation of thermo-TRPV channels would be expected to generate an inward cation current and cause membrane depolarization and excitation. Previous work has indicated that TRPV3 and TRPV4 contribute to thermosensing *in vivo* (Lee *et al.*, 2005; Moqrich *et al.*, 2005). However few studies have explored the possible functional role of TRPV channels as thermosensors in neurons. A recent study showed that hippocampal neurons express TRPV4 and that these neurons show a significantly more depolarized resting membrane potential at 37^{o}C than at room temperature (Shibasaki *et al.*, 2007). Moreover, neurons lacking expression of *trpv4* failed to show this difference and displayed hyperpolarized membrane potentials at both temperatures, indicating that tonic thermal activation of TRPV4 channels can exert a sustained depolarizing influence at physiological temperature. It remains unclear if TRPV4 channels can mediate dynamic thermosensing in these neurons during small changes in temperature within the physiological range. However as mentioned earlier, we recently provided evidence

suggesting that expression of a channel encoded by *trpv1* enables intrinsic physiological thermosensing in MNCs.

3.2 TRPV1 and thermosensing in MNCs

The first indication that MNCs might be intrinsically thermosensitive was provided by studies showing that changes in temperature in the 33-40^{o}C range could provoke changes in the firing rate of paraventricular nucleus neurons in slices of rat hypothalamus (Inenaga *et al.*, 1987; Dewald *et al.*, 2002). Conclusive evidence that MNCs are intrinsically thermosensitive was subsequently provided by the observation that the membrane potential and firing rate of MNCs isolated from the rat supraoptic nucleus vary as a positive function of temperature in the physiological range (Figure 4)(Sharif-Naeini *et al.*, 2008). These responses were specific to MNCs, since neurons isolated from the adjacent perinuclear zone were not affected by equivalent stimuli. Further studies showed that the depolarizing effects of hyperthermia on MNCs are consistent with the involvement of thermo-TRPV channels: exposing isolated MNCs to increases in temperature in the 35-40^{o}C range caused an increase in membrane conductance and generated a ruthenium red-sensitive, Ca^{2+}-permeable non-selective cation current. Moreover two distinct lines of evidence indicate that a channel encoded by the *trpv1* gene plays a key role in this process. As illustrated in Figure 5, the thermosensitive cation current was significantly reduced in neurons obtained from *trpv1*$^{-/-}$ animals and the current recorded from wild-type neurons could be inhibited by SB366791, a specific TRPV1 channel blocker (Gunthorpe *et al.*, 2004). In agreement with these observations, animals lacking expression of *trpv1* showed attenuated VP responses to hyperthermia *in vivo* (Sharif-Naeini *et al.*, 2008).

(Figure 4 near here)

3.3 Mechanism and threshold of thermal activation of TRPV1

As mentioned earlier, homomeric TRPV1 channels studied in heterologous expression systems display activation threshold near 43^{o}C (Caterina, 2007). It therefore remains to be explained how channels encoded by the *trpv1* gene can participate in the physiological regulation of

MNCs. One possibility is that the N-terminal variant expressed in these neurons displays a lower thermal threshold than wild type TRPV1. Indeed, recent studies have shown that the thermal threshold of dorsal root ganglion neurons which express wild type TRPV1 lies above 42^{o}C (Vyklicky *et al.*, 1999; Rau *et al.*, 2007). Another possibility is that the thermal threshold of these channels can be lowered by modulatory effects that exist under natural conditions. This hypothesis is plausible because a number of studies have shown that the thermal activation threshold of TRPV1 can be reduced by various post-translational mechanisms (Huang *et al.*, 2006; Honan & McNaughton, 2007).

Changes in temperature have been reported to modulate the probability of opening of TRPV1 by affecting the voltage-dependent activation of these channels (Voets *et al.*, 2004). How this is achieved remains a mystery, but likely involves thermally-induced allosteric events. Recent studies have shown that amino acids located in the outer pore region of the TRPV1 channel play a critical role in this process (Myers *et al.*, 2008a; Myers *et al.*, 2008b). Interestingly, previous work has shown that the C-terminal domain of TRPV1 (which is retained by the variant expressed in MNCs) is also required for thermal modulation of TRPV1 (Brauchi *et al.*, 2006).

(Figure 5 near here)

4. DISCUSSION

4.1 TRPV1 and osmoregulation: unresolved issues

Interestingly, a recent study found no differences in Fos expression in the supraoptic and paraventricular nuclei of wild-type and *trpv1*$^{-/-}$ mice treated with subcutaneous hypertonic saline, implying that TRPV1 might not mediate osmotic detection in MNCs (Taylor *et al.*, 2008). However injections of hypertonic saline *in vivo* cause a significant systemic hypernatremia concurrent with hypertonicity, and previous studies have established that other types of ion channels, such as Na_X, can mediate the specific detection of hypernatremia independently of osmotic effects (Hiyama *et al.*, 2002; Hiyama *et al.*, 2004). Na_x channels are expressed in central

nuclei that send excitatory projections to MNCs, such as the organum vasculosum lamina terminalis (OVLT) and subfornical organ (Hiyama *et al.*, 2010), and the Na_x-dependent activation of neurons in these areas is clearly capable of mediating vasopressin release (Watanabe *et al.*, 2000; Hiyama *et al.*, 2004; Hiyama *et al.*, 2010). Although our electrophysiological data provide strong evidence suggesting that TRPV1 mediates cell-autonomous hypertonicity sensing in MNCs, other mechanisms including taurinergic gliotransmission and Na_x-dependent sodium detection clearly contribute to the overall response of these neurons during hypertonic saline injection *in vivo*.

We have previously reported that TRPV1 is required for intrinsic osmosensing by OVLT neurons and that mice lacking expression of trpv1 show attenuated water intake in response to intraperitoneal injection of hypertonic saline (Ciura & Bourque, 2006). However Taylor and colleagues (Taylor *et al.*, 2008) failed to observe differences in cumulative water intake and Fos expression in the OVLT following hypertonic saline injection (subcutaneous) in wild-type and *trpv1*$^{-/-}$ mice . The detection of Fos in the OVLT of knockout animals is likely to have resulted from the hypernatremia-dependent activation of Na_x channels and possibly by other mechanisms. However it is remains unclear why no effects on water intake were observed by Taylor et al (Taylor *et al.*, 2008), whereas we observed reduced intake (Ciura & Bourque, 2006). Significant technical differences characterized these studies. First, different routes were used to inject the hypertonic saline (s.c. vs i.p.). Second, Taylor et al measured cumulative water intake beginning immediately following the injection, whereas we denied access to water for 30 minutes after the injection to allow an equilibration in fluid electrolyte status prior to the assessment of water intake. It is therefore likely that these two protocols caused important differences in the time course and amplitude of changes in plasma [Na^+] and osmolality during the measurement phase of water intake. Further studies are required to resolve the basis for differences between these studies.

4.2 Convergence of osmosensory and thermal stimuli

Since osmotic and thermal signals both contribute to the regulation of firing rate in MNCs *in vivo*, it is interesting to speculate on the mechanisms that may underlie such

convergence. One possibility is that network connectivity mediates the integration of these different signals. For instance, it is possible that distinct subsets of preoptic osmosensory and thermosensory neurons send direct (or indirect) axonal projections to MNCs, and that the intensity of the respective signals encoded by the firing rate or pattern of distinct afferents is then integrated at the synaptic level. Interactions of this type are presumably important, since network contributions have already been shown to mediate part of the effects of osmolality (Richard & Bourque, 1995) and temperature on the firing rate of MNCs *in situ* (Matsumura *et al.*, 1985).

However the findings summarized above suggest that convergence could also occur at the cellular or even molecular levels because channels encoded by *trpv1* mediate both osmosensing and thermosensing in MNCs. This provokes two important questions. First, are the channels responding to osmotic and thermal stimuli in these cells physically identical? Second, do these stimuli mediate occlusive, additive, or synergistic effects on channel activation? The answer to the first question must await single channel analysis of the responses in MNCs combined with experiments involving expression of the candidate variant in heterologous cells. We have previously shown that osmoreceptor channels in MNCs are also stimulated by various G_q-coupled peptide receptors in the absence of osmotic stimulation (Chakfe & Bourque, 2000; Sharif Naeini *et al.*, 2006). Under these conditions, the activation of multiple subtypes of receptors leads to non-additive effects (Chakfe & Bourque, 2000). However G_q-mediated channel activation is likely to involve molecular mechanisms that are distinct from those engaged by heat and hyperosmolality. Indeed, we have shown recently that activation of the G_q-dependent PLC-PKC pathway by angiotensin II actually sensitizes the channel to osmotic or mechanical activation via effects on actin density (Zhang & Bourque, 2008; Prager-Khoutorsky & Bourque, 2010). Thus whereas activation of one type of G_q-coupled receptor might reduce the channel's responsiveness to another G_q-coupled receptor, it could potentially enhance its response to other forms of stimulation.

Previous studies have provided ample evidence for different forms of cross-modal sensitization in TRPV channels. For example, heat activation of wild-type TRPV1 channels can be potentiated by prostaglandin E2 via a PKC-dependent effect of EP-1 receptors (Moriyama *et al.*,

2005) and ligand activation of these channels (by capsaicin) can be sensitized by changes in osmolality (Liu *et al.*, 2007). Analogously, increases in temperature have been shown to potentiate osmotic responses in heterologous cells expressing TRPV4 channels (Liedtke *et al.*, 2000) and to enhance (left-shift) the voltage-dependence of TRPV1 channels (Voets *et al.*, 2004). We therefore speculate that the effects of heat and osmolality on MNCs might reinforce each other (synergize) under conditions where systemic hypertonicity is accompanied by hyperthermia. Indeed, previous studies in humans have shown that the VP response to hyperosmolality is enhanced during hyperthermia (Takamata *et al.*, 1995). The extent to which synergistic molecular interactions and synaptic integration contribute to this effect remains to be determined.

4.3 Future work

While the experimental data reviewed here suggest that channels encoded by the *trpv1* gene mediate cell autonomous thermosensing and osmosensing in MNCs, several questions remain to be answered. First, the exact structure of the TRPV1 variant expressed in MNCs needs to be identified. The reconstitution of a functional osmoreceptor channel in a heterologous expression system would permit important structure-function studies of the channel and ultimately explain some of the distinguishing features of this variant compared to wild-type TRPV1. Second, as highlighted by the discussion presented in section 4.1, it remains to be established to what extent TRPV1 channels expressed in MNCs contribute to the excitation of these cells during physiologically relevant osmotic and thermal stimuli *in situ*. Specifically, it remains unclear what roles, if any, are played by other types of TRPV (and other) channels during thermosensing and osmosensing. Indeed, thermally-activated currents in MNCs were not completely suppressed by the specific TRPV1 antagonist SB366791, and residual currents were inhibited by ruthenium red (Sharif-Naeini *et al.*, 2008). Moreover, it is well known that thermal changes can also modulate neuronal firing via effects on other types of channels (e.g. A-type K^+ channels) (Griffin *et al.*, 1996) which are densely expressed in MNCs (Bourque, 1988; Fisher *et al.*, 1998). Third, the relative contributions of cell-autonomous osmosensory and thermosensory responses, and presumed changes in synaptic drive need to

be explored under physiological conditions. Fourth, it remains to be established whether the mechanisms of thermosensing and osmosensing identified in supraoptic nucleus MNCs are present in other types of neurons. As mentioned above OVLT neurons also require expression of *trpv1* for hypertonicity-sensing (Ciura & Bourque, 2006). Whether OVLT neurons are also intrinsically thermosensitive remains to be established. Fifth, previous studies have shown that TRPV channels may also assemble as heteromultimers (formed by a mixture of distinct TRPV channel subunits) (Hellwig *et al.*, 2005; Rutter *et al.*, 2005; Cheng *et al.*, 2007). Since the gating properties of heteromeric channels can be different than that of homomeric channels (Cheng *et al.*, 2007), it would be of interest to determine the exact subunit composition and stoichiometry of the native osmosensory channels expressed in MNCs. Finally, a recent study has shown that a capsaicin-insensitive TRPV1 channel mediates the facilitation of glutamate release onto MNCs exposed to hypertonicity or angiotensin II (Yokoyama *et al.*, 2010). The precise location of the channel involved and the basis for this effect remain to be determined. However the study emphasizes that the *trpv1* gene might contribute to osmosensing and osmoregulation in ways that remain to be discovered.

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FIGURE LEGENDS

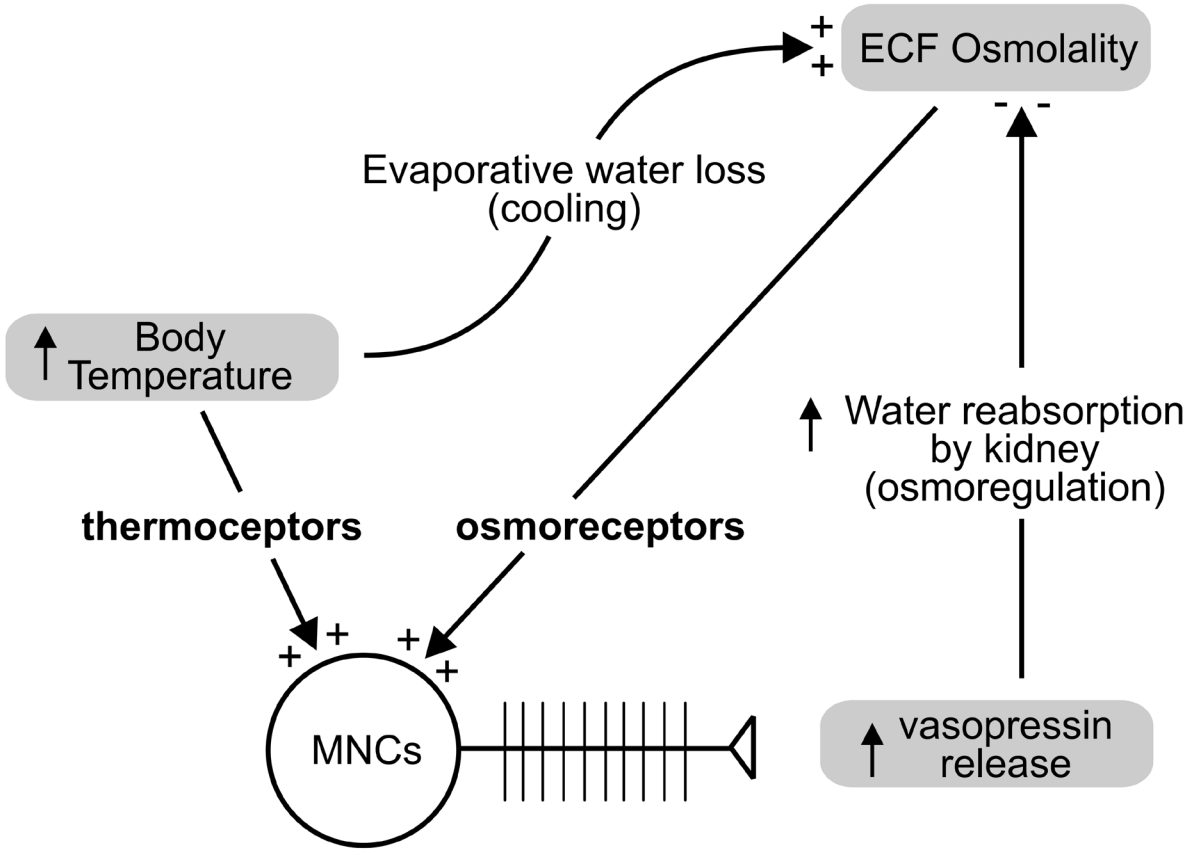

Figure 1. Thermoceptors and osmoreceptors modulate MNCs for osmoregulation. This schematic diagram illustrates the mechanisms by which increases in body temperature affect extracellular fluid (ECF) osmolality and how thermoceptors and osmoreceptors activate magnocellular neurosecretory cells (MNCs) to promote osmoregulatory water conservation.

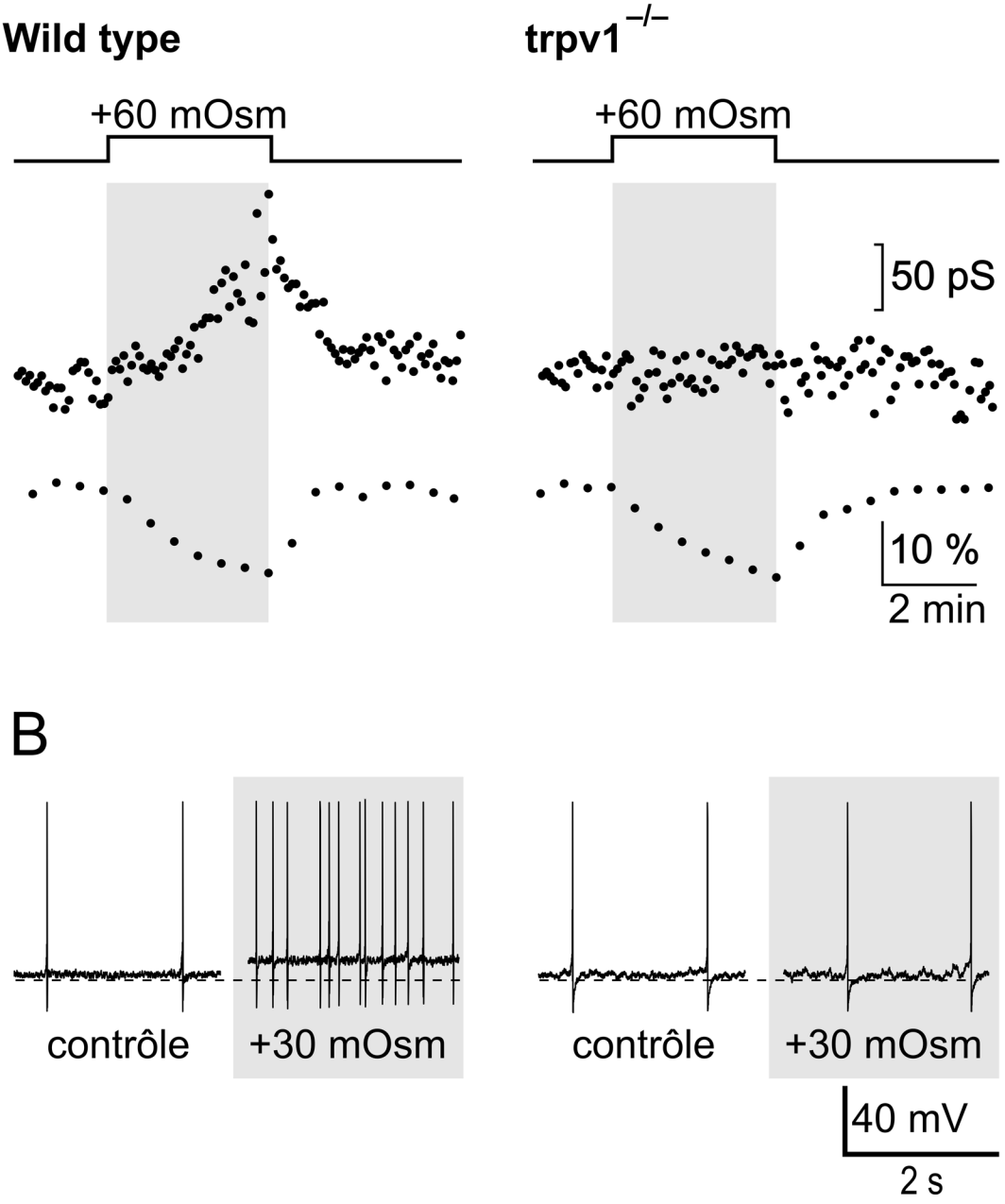

Figure 2. Lack of osmoreception in MNCs lacking expression of trpv1. The traces show simultaneous measurements of relative cell volume (lower) determined by images of cross-sectional area and changes in membrane conductance (upper) assessed by current responses to voltage steps under whole cell voltage clamp in magnocellular neurosecretory cells (MNCs) isolated from mouse supraoptic nuclei. The left panel shows the response of a wild type MNC whereas the panel on the right shows that from an MNC lacking expression of *trpv1*. Despite equivalent shrinking, the neuron lacking *trpv1* fails to generate an increase in membrane conductance. Adapted from (Sharif Naeini *et al.*, 2006).

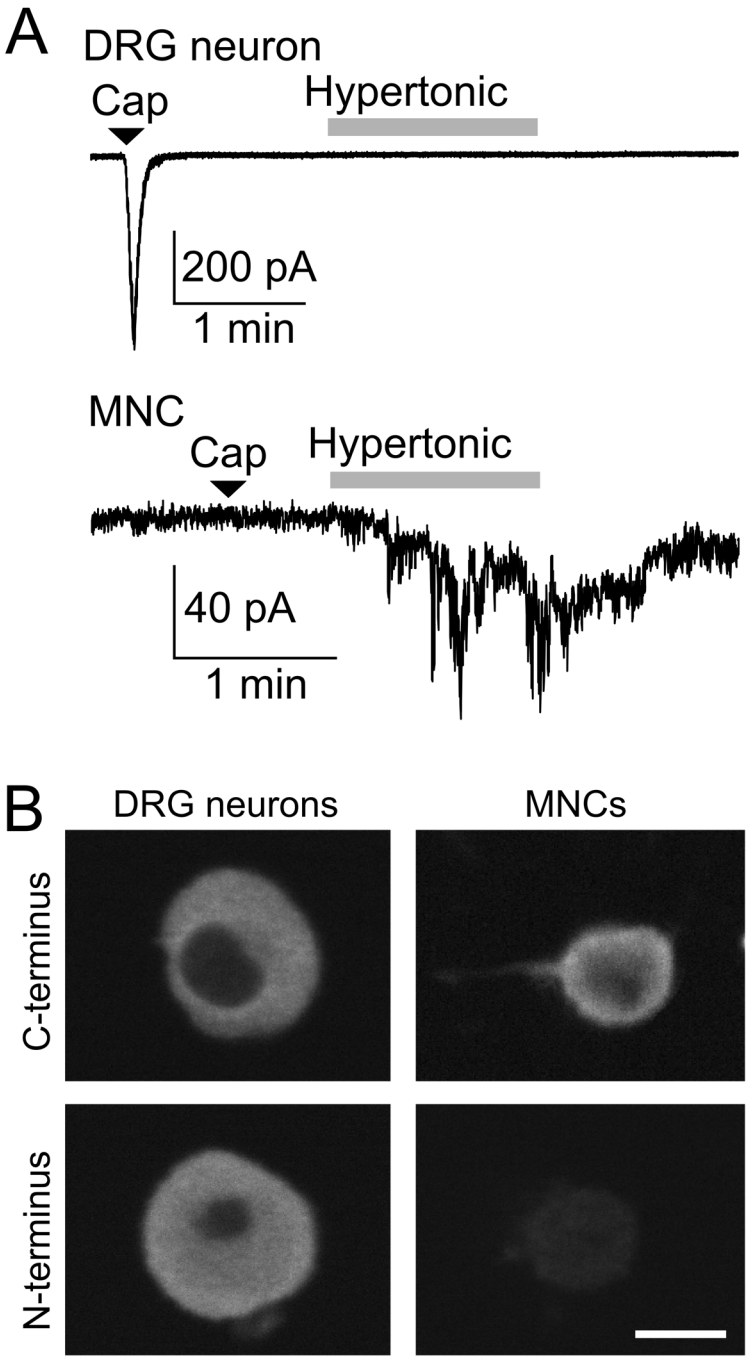

Figure 3. MNCs express an N-terminal variant of TRPV1. A, current responses to capsaicin (Cap, 10 μM, 10 s, arrowhead) and hyperosmotic stimuli (+60 mosmol/kg mannitol, bar) during whole cell voltage clamp recordings (V_H -60 mV) from a mouse dorsal root ganglion (DRG) neuron and from a mouse magnocellular neurosecretory cell (MNC) from the supraoptic nucleus. B, immunostaining of a DRG neuron (left) and MNC (right) with antibodies directed against the C-terminus (upper) and N-terminus (lower) of TRPV1. Scale bar 10 μm.

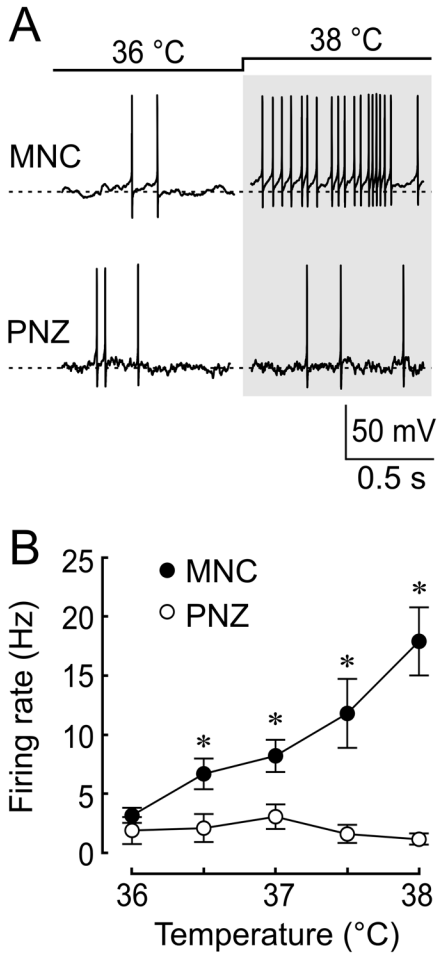

Figure 4. MNCs are intrinsically thermosensitive. A, whole cell current clamp recordings from a magnocellular neurosecretory cell isolated from rat supraoptic nucleus (MNC, upper traces) and from the adjacent perinuclear zone (PNZ, lower traces). The recordings show the effect of switching from 36°C to 38°C on membrane potential and action potential firing rate. B, Plot showing the mean (±s.e.m.) firing rate observed at different temperatures in MNCs (n= 13) and PNZ neurons (n = 5). Adapted from (Sharif-Naeini *et al.*, 2008).

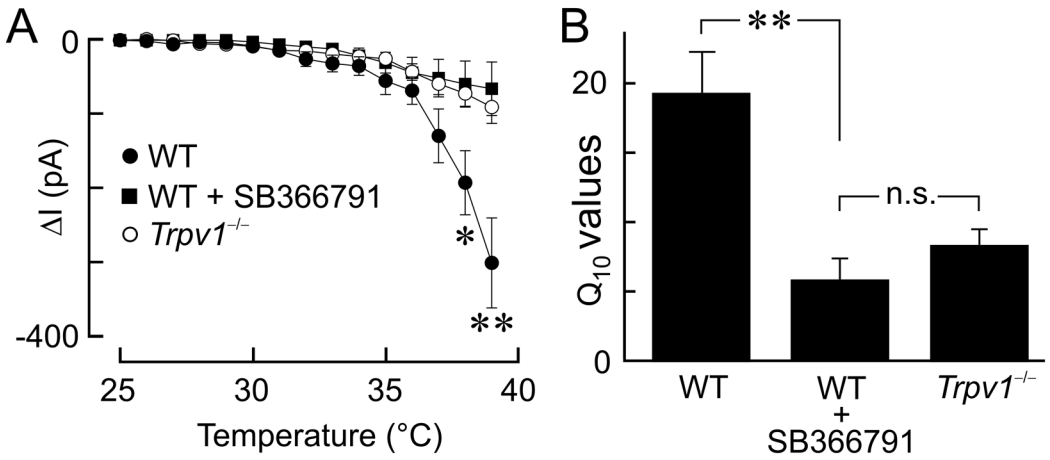

Figure 5. MNCs require *trpv1* for thermosensing. A, plots show the mean (±s.e.m.) changes in holding current recorded at different temperatures during whole cell voltage clamp recordings from supraoptic magnocellular neurosecretory cells isolated from WT mice in the absence (WT) or presence of 1.5 μM SB366791, or from *trpv1*$^{-/-}$ mice (* $P < 0.05$; ** $P < 0.01$ compared to Trpv1$^{-/-}$ group). B, Mean (±s.e.m.) Q_{10} values observed in the three different groups between 35 and 39 °C (WT: 19.3 ± 2.8, n=19; WT + SB366791: 5.8 ± 1.5, n=10; Trpv1$^{-/-}$: 8.5 ± 1.2, n=13; ** $P < 0.01$). Adapted from (Sharif-Naeini *et al.*, 2008).