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OSMORECEPTORS IN THE CENTRAL NERVOUS SYSTEM

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ABSTRACT

Osmoreceptors regulate sodium and water balance in a manner that maintains the osmotic pressure of the extracellular fluid (ECF) near an ideal set point. In rats, the concerted release of oxytocin and vasopressin, which is determined by the firing rate of magnocellular neurosecretory cells (MNCs), plays a key role in osmoregulation through the effects of natriuresis and diuresis. Changes in excitatory synaptic drive, derived from osmosensitive neurons in the organum vasculosum lamina terminalis (OVLT), combine with endogenously generated osmoreceptor potentials to modulate the firing rate of MNCs. The cellular basis for osmoreceptor potentials has been characterized using patch-clamp recordings and morphometric analysis in MNCs isolated from the supraoptic nucleus of the adult rat. In these cells, stretch-inactivated cationic channels transduce osmotically evoked changes in cell volume into functionally relevant changes in membrane potential. The experimental details of these mechanisms are reviewed in their physiological context.

INTRODUCTION

Among the physiological parameters whose short-term stability is most aggressively defended by vertebrates is the osmotic pressure of the extracellular fluid (ECF). Terrestrial mammals, in particular, have long been known to maintain a relatively constant ECF osmolality despite pronounced variations in salt and water intake (18). This observation implies that osmoregulatory responses present in these animals are subject to control by osmoreceptors, sensors specialized for the detection of small changes in fluid osmolality. In this review, we

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focus on recent advances concerning the cellular and molecular basis for signal transduction in osmoreceptors of the central nervous system (CNS). The significance of the findings is best emphasized if considered in a relevant physiological context. We begin, therefore, with a brief historical overview of osmoreceptors and of their functional role in the regulation of body fluids.

Osmoreceptors and Body Fluid Homeostasis

In mammals, CNS osmoreceptors are known to contribute to the concerted regulation of sodium and water balance in a manner that maintains the osmotic pressure of the ECF near an ideal set point (12). Thus the maintenance of an equilibrium between water intake and excretion is achieved largely through osmoreceptor-driven changes in thirst and diuresis. By analogy, sodium balance is maintained, in part, through osmotically regulated changes in sodium appetite and natriuresis (68). Not surprisingly, acute disturbances in either sodium or water balance can produce rapid shifts in ECF osmolality. The clinical consequences of such perturbations are mainly neurological (3, 4, 5) and include confusion, paralysis, coma, and convulsive seizures. When particularly severe, osmotically evoked brain swelling or shrinking can have traumatic or even lethal consequences (4, 5, 29, 46). Mechanisms underlying osmoreception and osmoregulation, therefore, are of considerable pathophysiological interest.

Discovery of Central Osmoreceptors

In 1937, Gilman (26) observed that drinking in dogs could be provoked by systemic infusion of hypertonic sodium chloride, but not by equivalent osmotic loads of membrane-permeant urea. He concluded, therefore, that the sensation of thirst must arise as a consequence of cellular dehydration; the reduction of cell volume that results from water efflux upon increases in external osmotic pressure. At that time, however, investigators did not know whether the sensation of thirst resulted from a generalized decrease in the volume of all cells, or from the activation of specialized receptor cells or tissues.

The term osmoreceptor was first introduced by EB Verney in the discussion of his now classic series of experiments on the release of vasopressin (88), the antidiuretic hormone. Similar to Gilman's findings on thirst (26), Verney discovered that vasopressin release in dogs can only be evoked by hypertonic solutions comprising membrane-impermeant solutes, such as sodium chloride, sodium sulfate, or sucrose, but not by hypertonic urea. Further, by studying the effects of acute infusions of hyperosmotic solutions into different arteries and veins, Verney concluded that osmoreceptors regulating vasopressin secretion were located within the brain. In subsequent experiments featuring intracranial lesions and arterial ligations, Jewell & Verney established that central

osmoreceptors controlling vasopressin release were located in the anterior region of the hypothalamus (31).

Osmoreceptors Comprise Neuronal Elements

As indicated above, osmoreceptors in the CNS contribute not only to the regulation of water balance, but to sodium homeostasis as well (12). Moreover, most of the responses regulated by osmoreceptors are either directly or indirectly mediated by the brain. The osmotic regulation of sodium and water intake, for example, is achieved by changes in ingestive behavior brought about by variations in perceived thirst (2, 23, 67, 79–82) and appetite for salt (9, 75, 83, 92). Osmotic control of renal diuresis and natriuresis is also centrally mediated via the release of hormones by neurosecretory neurons (6, 13, 20, 42, 44, 61, 67). Because signals derived from osmoreceptors must ultimately be relayed to neurons mediating each of these responses, osmoreceptors themselves must comprise neuronal elements.

Location of Central Osmoreceptors

There is no reason a priori why the brain should house only one osmoreceptor. In fact, signals derived from a variety of central and peripheral receptors may eventually converge on each of the osmoregulatory output systems (12), thereby providing useful redundancy in the osmotic control of body fluid balance. Moreover, because all cells in the brain are exposed to variations in ECF osmolality, neurons responsible for the activation of various osmoregulatory reflexes might themselves be intrinsically sensitive to changes in osmolality (12).

Lesion experiments by many investigators have suggested that structures near the anterior part of the hypothalamus are important not only for vasopressin secretion (25, 32, 34, 37, 38, 42, 49, 62, 69, 72, 80–82), as found by Jewell & Verney (88), but also for the osmotic regulation of thirst (25, 32, 36–38, 43, 80–82), sodium appetite (83), and natriuresis (6, 7, 41, 45, 78). Indeed, the results of these and other studies (12) suggest that osmotic control of most osmoregulatory responses is achieved through central pathways originating from neurons located in three structures associated with the lamina terminalis: the subfornical organ (SFO), the median preoptic nucleus (MnPO), and the organum vasculosum lamina terminalis (OVLT). Neurons in these areas, therefore, may effectively operate as cellular osmoreceptors. In agreement with this hypothesis, electrophysiological experiments in vivo have demonstrated the presence of osmoresponsive neurons in each of these loci (12). Moreover, recordings obtained in vitro under conditions blocking synaptic transmission have demonstrated that at least some of the neurons located in the SFO (12, 71), MnPO (65), and OVLT (65, 89) are intrinsically sensitive to changes in fluid osmolality.

Osmosensitive neurons, however, can only be recognized as functional osmoreceptors if they also can be shown to innervate and regulate neuronal elements responsible for the genesis of specific osmoregulatory responses. Because the neural circuits mediating thirst and sodium appetite are unknown, definitive proof concerning the location of cellular osmoreceptors regulating these responses cannot be obtained presently. In contrast, pathways involved in the osmotic control of neurohypophysial hormone release are well characterized (12) and offer a unique opportunity to investigate the cellular basis for osmoreception.

OSMOTIC CONTROL OF NEUROHYPOPHYSIAL HORMONE RELEASE

Neurohypophysial Hormones and Osmoregulation

Vasopressin, the antidiuretic hormone, is one of the two neurohypophysial peptides synthesized by distinct populations of magnocellular neurosecretory cells (MNCs) in the mammalian hypothalamus (76, 85). The other product of MNCs, oxytocin, is well known for its role as a humoral trigger for uterine contractions at birth and for milk ejection during lactation (13, 61). In the rat, however, oxytocin also contributes to sodium balance and osmoregulation because it is potently natriuretic (17, 87) and because its release is regulated by changes in osmolality (49, 86). Indeed, in this species, systemic concentrations of both hormones increase as a function of plasma osmolality (20, 67, 86), from an apparent threshold near 280 mosmol/kg. At the physiological osmotic set point ($\approx$295 mosmol/kg in rats), both oxytocin and vasopressin are detectable in blood at concentrations sufficient to contribute to basal natriuresis (17, 87) and antidiuresis (20, 67), respectively. By suppressing neurohypophysial hormone release, therefore, hypotonic stimuli simultaneously promote compensatory sodium retention and water excretion. Systemic hypertonicity, in contrast, enhances the release of both hormones, which promotes sodium excretion and water retention. Thus the concerted osmotic control of vasopressin and oxytocin secretion represents a powerful osmoregulatory response in the rat (12).

Functional Organization of the Hypothalamo-Neurohypophysial System

Somata of MNCs are located in the paraventricular and supraoptic nuclei of the hypothalamus (76, 85) from which they emit axons projecting toward the median eminence (13, 61). Collectively, these axons emerge from the base of the brain, forming the infundibular stalk, and enter the neurohypophysis where they individually branch into thousands of neurosecretory terminals (52). Peptides released at this location reach the general circulation through the fenestrated

capillaries of the neurohypophysis (28). Experiments with the isolated neurointermediate lobe of the rat pituitary have shown that secretion of oxytocin and vasopressin from the secretory terminals depends on action potential discharge and that the rate of secretion increases as a function of firing frequency in the neurohypophysial axons (19). Because the axons and terminals of MNCs cannot sustain firing in the absence of somatic drive (11), hormone secretion is primarily determined by the rate at which MNC somata generate action potentials (11, 13, 61).

Plasma Osmolality and Electrical Activity in MNCs

Electrophysiological recordings in anesthetized rats have revealed that the rates at which action potentials are discharged by the somata of both types of MNCs vary as positive functions of plasma osmolality (90, 91). Moreover, at the osmotic set point, both types of MNCs tend to display slow (2–3 Hz) irregular firing (61), consistent with the presence of low, but measurable, concentrations of plasma vasopressin and oxytocin under resting conditions. Intraperitoneal injection of water, which reduces plasma osmolality, inhibits MNCs (14), whereas injection of hypertonic solutions provokes increases in firing rate (12, 13, 30, 35, 61). Functionally, therefore, osmoreceptor-mediated control of the rat hypothalamo-neurohypophysial system is achieved primarily via changes in the rate at which action potentials are discharged by the somata of MNCs.

OSMORECEPTORS FOR NEUROHYPOPHYSIAL HORMONE RELEASE

A large number of central and peripheral sites may contribute to the osmotic regulation of neurohypophysial hormone release (12). Cellular osmoreceptors, however, are defined not only by their intrinsic sensitivity to changes in fluid osmolality, but also by their ability to regulate neuronal systems responsible for the production of specific osmoregulatory responses. With respect to such criteria, only two brain regions can presently be identified as comprising cellular osmoreceptors controlling neurohypophysial hormone secretion: the OVLT and the supraoptic nucleus.

Osmoreceptors in the OVLT

Electrolytic lesions encompassing the OVLT have been found to impair osmotic control of neurohypophysial hormone release (62, 79–82). Moreover, anatomical studies have indicated that neurons in the OVLT send projections to hypothalamic nuclei containing MNCs (15, 63, 84, 93, 94). Because some OVLT neurons are intrinsically osmosensitive (65, 89), it is likely that these cells represent cellular osmoreceptors contributing to the osmotic control of

neurohypophysial hormone release. Three additional experimental approaches support this hypothesis: First, OVLT neurons anatomically identified as projecting toward the supraoptic nucleus express the protein product of the activity-dependent immediate early gene *fos* following systemic hypertonic stimulation (53). Second, OVLT neurons antidromically identified as projecting to the supraoptic nucleus are excited by hypertonic stimuli in vitro (51). Third, localized hypertonic stimulation of the region of the OVLT in vivo (30) or in vitro (64, 66) excites hypothalamic MNCs.

Intracellular recordings (66) from MNCs maintained at a potential just below spike threshold by current injection revealed that changes in firing rate provoked by osmotic stimulation of the OVLT result from a selective modulation in the frequency of glutamatergic excitatory postsynaptic potentials (EPSPs). This observation is consistent with electrophysiological data indicating that axonal projections from the OVLT mediate glutamatergic excitation of MNCs in the rat supraoptic nucleus (63, 66, 96). Quantitative analysis in vitro (66) has shown that the frequency of spontaneous EPSPs recorded in MNCs increases as a function of the osmotic pressure of the solution superfusing the OVLT, with an apparent threshold near 280 mosmol/kg. Osmoreceptor neurons in the OVLT, therefore, contribute to the osmotic control of the hypothalamo-neurohypophysial system via changes in the synaptic excitation of hypothalamic MNCs (12, 64, 66). Based on these in vitro observations, the electrical activity of the OVLT neurons involved can be presumed to vary as a positive function of fluid osmolality with a threshold near 280 mosmol/kg, a value corresponding to the osmotic threshold for neurohypophysial hormone release in vivo (67, 86).

Intrinsic Osmosensitivity of Hypothalamic MNCs

It was Verney himself who first suggested that MNCs might operate as osmoreceptors (88). The intrinsic osmosensitivity of MNCs, however, was first demonstrated by Mason (39) in a report revealing that neurons in the supraoptic nucleus are depolarized by hypertonic stimulation in the absence of chemical synaptic transmission. Surprisingly, the functional importance of this response has not been universally endorsed (62, 70, 81). The main argument against a physiological role for the intrinsic osmosensitivity of MNCs rests on the fact that whereas urea readily permeates cell membranes, it has only limited permeability across the blood-brain barrier (98). Thus systemic infusion of urea should cause water to move from the brain parenchyma to the circulation faster than urea can permeate in the opposite direction, thereby increasing the osmotic pressure on the neural side of the blood-brain barrier (62). Because systemic urea is ineffective as a stimulus for vasopressin release (88), it is argued that the osmoreceptors controlling release must lie outside the blood-brain barrier (62, 70,

81). Although the lack of effect of systemic urea provides indirect support for the involvement of the OVLT and other circumventricular organs (which lack a blood-brain barrier) in osmoregulation (70, 81), it does not prove that these sites comprise osmoreceptors. Furthermore, the observation does not exclude the possibility that the intrinsic osmosensitivity of MNCs represents an important, if not essential, aspect of osmotic control. Indeed, intrinsically generated changes in membrane potential in MNCs may be subthreshold for spike discharge during physiological osmotic stimulation (12, 35). If this is the case, functional excitation in response to systemic hypertonicity would necessitate that excitatory synaptic inputs (EPSPs) also be present to trigger the action potentials (12). Lack of vasopressin release in response to urea (88) or to hypertonic stimulation in OVLT-lesioned rats (80–82), therefore, simply could be due to the absence or loss of excitatory synaptic drive under the conditions of these experiments. This hypothesis is supported by the demonstration that in OVLT-lesioned rats, osmotically evoked increases in firing rate in MNCs can be restored by providing a constant excitatory drive through the sustained application of glutamate (34). By analogy, the total number of impulses discharged during individual milk-ejection bursts in oxytocinergic MNCs of lactating female rats can be enhanced by hypertonic stimulation (50). Finally, it is possible that the special properties of capillaries in the supraoptic nucleus (see below) allow a relatively high permeability to urea in this region of the CNS. Considering these results and those discussed below, it is likely that the intrinsic osmosensitivity of MNCs combines with osmotically regulated excitatory inputs, derived from the OVLT, to ensure adequate osmotic control of neurohypophysial hormone release in situ (12).

FUNCTIONAL ANATOMY OF OSMORECEPTORS

The Supraoptic Nucleus

The supraoptic nucleus comprises a compact array of MNC somata, glial cells, and capillaries (60, 77). Electron microscopic analysis (28) reveals that the vasculature of the nucleus bears the hallmark features of a functional blood-brain barrier. Thus the capillary endothelium is not fenestrated and features few cytoplasmic pits and vesicles. Moreover, the processes of glial cells are interposed between MNC somata and the endothelial cells of local capillaries. In agreement with these morphological features, the permeability of endothelial cells and *trans*-endothelial flux of some solutes (per unit area) are as low in the supraoptic nucleus as they are in other parts of the cerebral gray matter (28). It has long been recognized that the magnocellular nuclei of the hypothalamus feature an unusually dense vascularization (22). Indeed, morphometric analysis reveals that the density, volume fraction, and surface area of the capillary bed

are four times greater in the rat supraoptic nucleus than in hypothalamic nuclei lacking MNCs (28). Furthermore, the average capillary diameter within the supraoptic nucleus is significantly smaller than in other regions. Thus while solute flux per unit area may be slow across the capillary endothelium of the supraoptic nucleus (28), the overall permeability of the tissue to water and various solutes (e.g. urea) may be relatively higher here than in other parts of the brain. Enhanced permeability to water, in fact, is suggested by the recent observation that this region expresses a high density of mRNA coding for aquaporin-4 (33), a member of the family of transmembrane water channels (21). The combination of a large capillary surface area, facilitated water flux, and low permeability to solutes, suggests that the supraoptic nucleus is optimized to promote rapid changes in tissue volume and parenchymal osmotic pressure during variations in systemic osmolality.

The OVLT

The OVLT is a member of the specialized group of brain nuclei termed circumventricular organs. These structures are characterized by both the presence of a dense vascularization (95) and the absence of a functional blood-brain barrier (27). Indeed, the capillary endothelium in circumventricular organs is highly fenestrated and features numerous pits and vesicular profiles. The high rate of tissue perfusion supported by such features presumably facilitates solute exchange between the vascular and neuronal compartments of the tissue. A common feature of both osmoreceptors is the facilitated exposure of local neurons to changes in ECF osmolality.

CELLULAR BASIS FOR OSMORECEPTION

Because the first cells identified as intrinsically osmosensitive were supraoptic neurons (39), and because such neurons are easily identifiable in various in vitro preparations from rat brain (13), most of what is currently known about the ionic basis for osmoreception has come from experiments in rat MNCs. The main features of the mechanism underlying the osmosensitivity of these cells are discussed below.

Effects of Osmotic Stimuli on Membrane Voltage and Conductance

Intracellular recordings from supraoptic neurons in hypothalamic slices (1, 39) or explants (10) have revealed that MNCs become depolarized when exposed to hypertonic solutions. In the rat, this response is associated with a reduction of input resistance (10) and persists in the presence of tetrodotoxin or in Ca^{2+}-free solutions, suggesting a postsynaptic origin. Voltage-clamp measurements

with sharp electrodes (10) reveal that at potentials near −60 mV, hypertonic stimuli provoke an inward current associated with an increase of membrane conductance. Although current reversal was not observed in those experiments, extrapolation of current voltage relations recorded in the presence and absence of osmotic stimuli suggested a null potential lying between spike threshold (≈−50 mV) and 0 mV. Because responses were not affected by chloride injections sufficient to invert the polarity of Cl^--mediated inhibitory synaptic potentials, activation of a nonselective cationic conductance was suggested as underlying the effects of hypertonic solutions (10).

A procedure permitting the acute isolation of viable and identifiable MNCs from adult rats has provided the definitive demonstration that these cells behave as intrinsic osmoreceptors (55). Thus in the absence of any synaptic input, MNCs remain sensitive to small changes in fluid osmolality, a response that cannot be observed in neighboring non-neuroendocrine cells or in cortical neurons (56). A significant observation, first made in isolated neurons (56, 57), was that individual MNCs respond not only to increases in fluid osmolality, but to hypotonicity as well. Thus whereas hypertonic solutions provoke membrane depolarization, hypotonic stimuli elicit hyperpolarizing responses. Under whole-cell voltage clamp (56, 57), brief hypertonic stimulation of MNCs held at −60 mV elicited a reversible inward current. Current-voltage analysis indicates that this response results from an increase of membrane conductance and that the activated current reverses near −40 mV. In the same cells, application of hypotonic stimuli reduces membrane conductance, suppressing a current that also reverses near −40 mV. These observations suggest that a single membrane conductance might be differentially regulated by increases and decreases in fluid osmolality (58).

Ionic Permeability of the Osmotically Regulated Conductance

During whole-cell recording experiments on isolated MNCs, changes in the concentration of extracellular or intracellular chloride did not noticeably affect the reversal potential of the current modulated by osmotic stimuli (54, 56, 57). However, removal of external Na^+ caused this potential to shift toward the equilibrium potential for K^+ (≈−100 mV) and raising the concentration of external K^+ ions caused the reversal potential to shift toward E_{Na}. Cationic channels permeable to both Na^+ and K^+ ions, therefore, mediate the responses of MNCs to changes in fluid osmolality. Under near-physiological conditions ($[K^+]_O$ ≈3 mM), the cationic conductance was found to favor K^+ over Na^+ with a ratio of about 5:1 (54). Because the resting potential of MNCs lies near −65 mV under resting osmotic conditions (54, 56), activation of this conductance tends to depolarize the cells toward spike threshold, whereas its suppression hyperpolarizes the cell and reduces membrane excitability (58).

Role of Cell Volume in Osmoreception

As described above, centrally controlled osmoregulatory reflexes are believed to be activated as a consequence of the cellular dehydration that results from efflux of cytoplasmic water upon increases in external osmolality (26). In order to determine whether changes in cellular hydration are associated with the responses of MNCs to osmotic stimuli, we compared the time course of changes in cationic conductance with that of cell volume in response to the application of a brief (90 s) hypertonic stimulus. Morphometric analysis using confocal laser scanning microscopy, and whole-cell patch-clamp measurements revealed that the osmotically evoked decrease in cell volume occurred simultaneously with the changes in conductance provoked by the hypertonic stimulus (54, 56), supporting the notion that changes in cell volume play a key role in osmoreception. The close temporal relationship between changes in cell volume and cationic conductance further implied that generation of a long-lived second messenger was unlikely to participate in the production of these responses. Given such constraints, two mechanisms became apparent as possible candidates for the regulation of cationic conductance during changes in fluid osmolality. First, because variations in cell volume associated with osmotic stimulation result from transmembrane water flux, changes in solute concentration could play a role in signal transduction. Alternatively, changes in physical strain could mediate the osmotic regulation of a cationic conductance by modulating mechanosensitive channels.

Effects of Isotonic Volume Changes on Membrane Conductance

To determine if changes in solute concentration are required for coupling changes in cell volume to the modulation of cationic conductance, the effects of cell swelling or shrinking were examined in the absence of osmotic stimulation (79, 82). Under whole-cell recording conditions, this was accomplished readily by modifying the pressure inside the recording pipette while monitoring cell size through the microscope. Current clamp recordings from potentials near rest revealed that a decrease in cell volume provoked by pipette suction elicits a depolarization (56), whereas pressure-evoked cell swelling results in a hyperpolarization (54). Although these results are consistent with those obtained during osmotically evoked changes in cell volume, the nature of the conductance underlying responses to variations in pipette pressure might have been different from that modulated by osmotic stimuli. Examination of current-voltage relations recorded during isotonic shrinking and swelling indicates that depolarizing and hyperpolarizing responses are associated, respectively, with increases and decreases in membrane conductance, and that the currents underlying both responses reverse near -40 mV (54, 56). Moreover, increases in external $[K^+]$ provoked identical changes in the reversal

potential of the currents modulated by each of the four treatments (56). Finally, responses to hypertonic stimuli could be reversed by inflating the cells via increased pressure in the recording pipette, and responses to hypotonic stimuli could be reversed by applying suction to the pipette (54). The results of these experiments indicate that changes in cell volume, rather than variations in cytosolic solute concentration, are required for the osmotic modulation of cationic conductance.

PROPERTIES OF SINGLE CHANNELS UNDERLYING OSMORECEPTION

The importance of changes in cell volume for osmoreception suggests that the activity of the channels responsible for variations in macroscopic conductance is physically regulated by cell swelling or shrinkage. Parameters involved in the regulation of individual cationic channels, therefore, were examined using single-channel recording techniques.

Supraoptic MNCs Express Mechanosensitive Channels

Under cell-attached conditions, patch-clamp recordings obtained from acutely isolated MNCs revealed inward single-channel currents when membrane patches were held at −100 mV (56). Current-voltage analysis revealed a reversal potential near −40 mV and an open channel conductance of ≈32 pS. The activity of these cationic channels could be reversibly modulated by changes in pipette pressure, or by modifying the osmolality of the solution surrounding the cell, thereby identifying these channels as possible mechanotransducers for osmoreception (56).

Is Channel Mechanosensitivity Required for Osmoreception?

Although these observations suggest that the channels underlying osmoreception are mechanosensitive, they did not demonstrate that mechanical gating is involved in transducing osmoreception. As indicated by Morris (47), a number of criteria must be fulfilled to establish that single-channel mechanosensitivity is responsible for the generation of a macroscopic mechanoreceptor response. In the context of osmoreception, demonstration of the following items would be particularly important. First, the physical sensitivity of the channels should be consistent with the changes in tension that occur during the volume-dependent regulation of macroscopic conductance. Second, the kinetic features associated with the direct mechanical gating of channel activity should be similar to those provoked by osmotic stimulation of the cell. Third, the sensitivity of single channels to a pharmacological blocker should be comparable to that of the macroscopic osmoreceptor current. Fourth, the dynamic range

of single-channel responsiveness should agree with that of the physiological response.

Channel Mechanosensitivity Is Consistent with Macroscopic Osmoreception

Ideally, the assessment of physical parameters regulating a mechanosensitive channel would involve correlation of variations in measured channel activity with observed changes in membrane structure (74). Unfortunately, unless simultaneous high-resolution imaging can be performed (73), the precise geometry of a membrane patch cannot be determined during a cell-attached recording experiment. Moreover, while controlled changes in pipette pressure can be applied through a variety of devices (40), the absolute residual pressure on the pipette side of a patch is difficult to ascertain, even when the pipette is open to the atmosphere (48). Under resting conditions, therefore, the orientation of a patch (i.e. whether convex or concave) and its radius of curvature are unknown. Fortunately, these problems can be partly overcome by examining channel responses to a series of changes in pipette pressure (48). Thus assuming that application of a large negative pipette pressure establishes patch convexity with respect to the cell, stepwise removal of this pressure, followed by stepwise increases in positive pressure, should gradually flatten the patch and subsequently provoke increasing patch concavity.

In MNCs, the average opening probability (P_O) of the mechanosensitive channels was found to be maximal near zero pressure and to decrease in response to either increases or decreases in pipette pressure (54, 56, 58, 59). The existence of a symmetric relationship between channel activity and pipette pressure suggests that the channels expressed in MNCs are not regulated by forces perpendicular to the membrane, which would vary as a monotonic function of pipette pressure. Rather, the channels appear to be regulated by changes in force tangential to the plasma membrane, which would be minimal when the patch is flat and increase as a function of either patch convexity or concavity. Because their activity is inhibited by increases or decreases in pipette pressure, the channels expressed in MNCs are identified as stretch inactivated (SI) (48).

As illustrated in Figure 1, the presence of SI cationic channels in MNCs is consistent with the osmotic regulation of cell volume, macroscopic conductance, and membrane potential. Thus by increasing tangential force, hypotonic cell swelling suppresses SI channel activity, reduces whole-cell cationic conductance, and provokes membrane hyperpolarization. Conversely, relaxation of tangential forces upon hypertonic shrinkage increases channel activity and membrane cationic conductance, thereby depolarizing the cell (58).

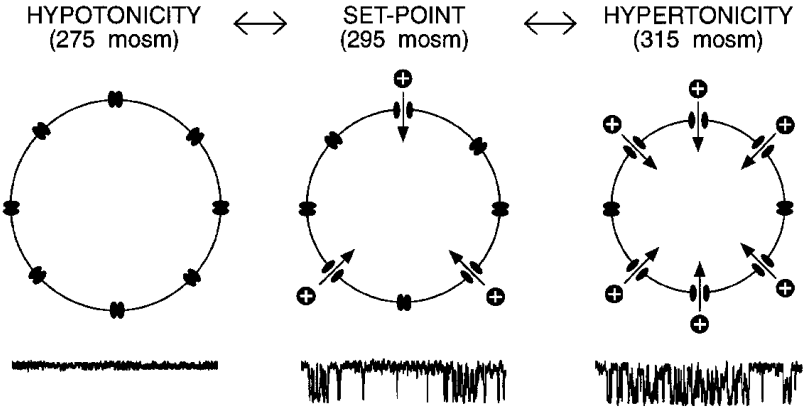

Figure 1 Stretch inactivated (SI) cationic channels transduce osmoreception. Under resting osmotic conditions (*middle panel*) a portion of the SI cationic channels is active and allows the influx of positive charge (diagram). Hypotonic stimulation (*left*) provokes cell swelling and inhibits channel activity, thereby hyperpolarizing the cell. In contrast, hypertonic stimulation (*right*) causes cell shrinking. Activation of an increased number of channels under this condition augments charge influx and results in membrane depolarization. Traces representing changes in the activity of a single SI channel are shown below.

Common Changes in Channel Kinetics During Mechanical and Osmotic Stimulation

Kinetic analysis of recordings made from cell-attached patches containing single channels reveals that the distribution of channel open times recorded from SI cationic channels at rest can be described as an exponential function, with a mean time constant of 1.6 ± 0.2 ms (59). This value is not affected by changes in channel activity provoked either by osmotic stimulation of the cell or by modification of pipette pressure (59). Changes in channel P_O, therefore, are not mediated by variations in the duration of individual channel openings. Channel closed times observed at rest are distributed more broadly, with dwell time histograms approximating the sum of two or three exponentials (54, 59). Because the slowest components of such distributions have time constants exceeding 1000 ms, information concerning evoked changes in specific components has not been obtained. By simplifying the analysis to an examination of the mean channel closed time (16), however, we have observed that changes in channel closed time can explain the totality of changes in channel P_O evoked by either variations of pipette pressure or osmotic stimulation (59). Although further analysis will be required to confirm that the kinetic schemes underlying changes in channel P_O provoked by both types of stimuli are identical,

available results support the hypothesis that mechanical and osmotic stimuli regulate channel gating through a common mechanism.

Gadolinium Blocks Single SI Channels and Macroscopic Osmoreceptor Current

Previous studies have demonstrated that the trivalent inorganic cation gadolinium (Gd^{3+}) causes open channel blockade of many types of cationic mechanosensitive channels (8, 24, 97). In agreement with these reports, addition of varying concentrations of Gd^{3+} to recording pipettes was found to cause a dose-dependent decrease in the mean open time of SI cationic channels in isolated MNCs (59). The concentration at which the mean duration of SI channel openings was reduced by 50% was $\approx$30 μM. In order to determine whether Gd^{3+} interferes with other aspects of osmo-mechanical transduction, we examined whether high concentrations (100–300 μM) of the blocker affect osmotically evoked volume changes or the mechanical sensitivity of the SI channels themselves. Laser scanning microscopic imaging experiments revealed that neither the time course nor the amplitude of the volume decrease provoked by a 60 mosmol/kg stimulus was altered by the presence of 100 μM Gd^{3+} in the external medium (54, 59). Moreover, recording of SI channels in cell-attached membrane patches, using pipettes containing 100 μM Gd^{3+}, revealed that the bell-shaped relationship between normalized channel P_O and changes in pipette pressure was identical in the presence and absence of Gd^{3+} (54, 59). At a concentration of 30 μM, Gd^{3+} would be expected to reduce the mean duration of channel openings by 50% without affecting osmotically evoked changes in cell volume or mechanically evoked changes in channel closed time and relative P_O. Therefore, if SI channels underlie osmoreception, macroscopic osmoreceptor currents should be inhibited by comparable concentrations of Gd^{3+}. In agreement with this prediction, macroscopic current responses to hypertonic stimuli were inhibited with an IC_{50} of $\approx$40 μM (54, 59). Moreover, under current clamp, depolarizing osmoreceptor potentials evoked by hypertonic stimuli were reversibly inhibited by adding 100–250 μM Gd^{3+} to the external solution. The pharmacological effects of Gd^{3+} on macroscopic responses, therefore, are consistent with those observed on single SI cationic channels.

Dynamic Range of SI Channel Activity and Macroscopic Responses

Single-channel analysis has revealed that the activity of SI cationic channels in MNCs can be abolished by changes in pipette pressure as small as +3 cm H_2O (54, 56, 58, 59). It is conceivable that complete suppression of channel activity might occur during modest hypotonic stimulation. Indeed, under whole-cell recording conditions, application of solutions of decreasing osmotic pressures has revealed that membrane conductance reaches a minimum

at ≈275 mosmol/kg (57). Based on the changes in whole-cell conductance observed during osmotic stimulation (54), it is likely that under resting osmotic conditions (i.e. at 295 mosmol/kg) approximately 60% of the input conductance of MNCs is mediated through SI-cationic channels. In support of this proposal, Gd^{3+} has been found to inhibit a current reversing near −40 mV when applied to MNCs bathed in a solution adjusted to 295 mosmol/kg (54). Application of Gd^{3+} to MNCs under hypotonic conditions (275 mosmol/kg), however, was without effect (59). The apparent osmotic threshold for SI channel activity in vitro, therefore, corresponds to the threshold for oxytocin and vasopressin release in vivo (67, 86). Indeed, during hypertonic stimulation, increases in membrane conductance are first detected at 280 mosmol/kg (57).

CONCLUDING REMARKS

Experiments on MNCs of the rat supraoptic nucleus have provided a unique opportunity to examine the mechanism underlying signal transduction in a CNS osmoreceptor. Our analysis reveals that cationic channels whose activity is reduced by membrane stretch may be the molecular mechanotransducers responsible for converting osmotically evoked changes in cell volume into functionally relevant changes in membrane potential (Figure 1). Although mechanisms underlying signal transduction may be different in osmoreceptors located elsewhere in the CNS or in the periphery (12), preliminary observations suggest that the intrinsic osmosensitivity of some cells in the SFO (12), MnPO (65), and OVLT (65) depends on a mechanism similar to that found in MNCs. However, a more detailed understanding of the biophysical aspects of osmo-mechanical transduction must await the identification of the molecular structure of the ion channels involved and the cytoskeletal molecules that presumably regulate their gating apparatus.

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Literature Cited

1. Abe H, Ogata N. 1982. Ionic mechanism for the osmotically induced depolarization in neurones of the guinea-pig supraoptic nucleus in vitro. *J. Physiol.* 327:157–71
2. Andersson B. 1978. Regulation of water intake. *Physiol. Rev.* 58:582–603
3. Andrew RD. 1991. Seizure and acute os-

motic change: clinical and neurophysiological aspects. *J. Neurol. Sci.* 101:7–18
4. Arieff AI. 1986. Hyponatremia, convulsions, respiratory arrest, and permanent brain damage after elective surgery in healthy women. *N. Engl. J. Med.* 314:1529–35
5. Arieff AI, Guisado R. 1976. Effects on the central nervous system of hypernatremic and hyponatremic states. *Kidney Int.* 10:104–16
6. Bealer SL. 1983. Hemodynamic mechanisms in CNS-induced natriuresis in the conscious rat. *Am. J. Physiol.* 244:F376–82
7. Bealer SL, Haywood JR, Gruber KA, Buckalew VM Jr, Fink GD, et al. 1983. Preoptic-hypothalamic periventricular lesions reduce natriuresis to volume expansion. *Am. J. Physiol.* 244:R51–57
8. Berrier C, Coulombe A, Szabo I, Zoratti M, Ghazi A. 1992. Gadolinium ion inhibits loss of metabolites by osmotic shock and large stretch-activated channels in bacteria. *Eur. J. Biochem.* 206:559–65
9. Blackburn RE, Samson WK, Fulton RJ, Stricker EM, Verbalis JG. 1993. Central oxytocin inhibition of salt appetite in rats: evidence for differential sensing of plasma sodium and osmolality. *Proc. Natl. Acad. Sci. USA* 90:10380–84
10. Bourque CW. 1989. Ionic basis for the intrinsic activation of rat supraoptic neurones by hyperosmotic stimuli. *J. Physiol.* 417:265–78
11. Bourque CW. 1990. Intraterminal recordings from the rat neurohypophysis in vitro. *J. Physiol.* 421:247–62
12. Bourque CW, Oliet SHR, Richard D. 1994. Osmoreceptors, osmoreception, and osmoregulation. *Front. Neuroendocrinol.* 15:231–74
13. Bourque CW, Renaud LP. 1990. Electrophysiology of mammalian magnocellular vasopressin and oxytocin neurosecretory neurons. *Front. Neuroendocrinol.* 11:183–12
14. Brimble MJ, Dyball REJ. 1977. Characterization of the responses of oxytocin- and vasopressin-secreting neurones in the supraoptic nucleus to osmotic stimulation. *J. Physiol.* 271:253–71
15. Camacho A, Phillips MI. 1981. Horseradish peroxydase study in rat of the neural connections of the organum vasculosum of the lamina terminalis. *Neurosci. Lett.* 25:201–4
16. Colquhoun D, Sigworth F. 1995. Fitting and statistical analysis of single channel records. In *Single Channel Recording*, ed. B Sakmann, E Neher, pp. 483–87. New York: Plenum. 2nd ed.
17. Conrad KP, Gellai M, North WG, Valtin H. 1986. Influence of oxytocin on renal hemodynamics and sodium excretion. *Ann. NY Acad. Sci.* 689:346–62
18. Darrow DC, Yannet H. 1935. The changes in the distribution of body water accompanying increase and decrease in extracellular electrolyte. *J. Clin. Invest.* 14:266–75
19. Dreifuss JJ, Kalnins I, Kelly JS, Ruf KB. 1971. Action potentials and release of neurohypophysial hormones in vitro. *J. Physiol.* 215:805–17
20. Dunn FL, Brennan TJ, Nelson AE, Robertson GL. 1973. The role of blood osmolality and volume in regulating vasopressin secretion in the rat. *J. Clin. Invest.* 52:3212–19
21. Engel A, Walz T, Agre P. 1994. The aquaporin family of membrane water channels. *Curr. Opin. Struct. Biol.* 4:545–53
22. Finley K. 1939. The capillary beds of the paraventricular and supra-optic nuclei of the hypothalamus. *J. Comp. Neurol.* 71:1–19
23. Fitzsimons JT. 1976. The physiological basis of thirst. *Kidney Int.* 10:3–11
24. Franco A Jr, Winegar BD, Lansman JB. 1991. Open channel block by gadolinium ion of the stretch-inactivated ion channel in mdx myotubes. *Biophys. J.* 59:1164–70
25. Gardiner TW, Stricker EM. 1985. Hyperdipsia in rats after electrolytic lesions of nucleus medianus. *Am. J. Physiol.* 248:R214–23
26. Gilman A. 1937. The relation between blood osmotic pressure, fluid distribution, and voluntary water intake. *Am. J. Physiol.* 120:323–28
27. Gross PM, Sposito NM, Pettersen SE, Fenstermacher JD. 1986. Differences in function and structure of the capillary endothelium in gray matter, white matter and a circumventricular organ of rat brain. *Blood Ves.* 23:261–70
28. Gross PM, Sposito NM, Pettersen SE, Fenstermacher JD. 1986. Differences in function and structure of the capillary endothelium in the supraoptic nucleus and pituitary neural lobe of rats: evidence for the supraoptic nucleus as an osmometer. *Neuroendocrinology* 44:401–7
29. Gullans SR, Verbalis JG. 1993. Control of brain volume during hyperosmolar and hypoosmolar conditions. *Annu. Rev. Med.* 44:289–301
30. Honda K, Negoro H, Higuchi T, Tadokoro Y. 1987. Activation of neurosecretory cells by osmotic stimulation of anteroventral third ventricle. *Am. J. Physiol.* 252:R1039–45

31. Jewell PA, Verney EB. 1957. An experimental attempt to determine the site of the neurohypophyseal osmoreceptors in the dog. *Philos. Trans. R. Soc. London Ser. B* 240:197–324
32. Johnson AK. 1985. Role of the periventricular tissue surrounding the anteroventral third ventricule (AV3V) in the regulation of body fluid homeostasis. See Ref. 70a, pp. 319–31
33. Jung JS, Bhat RV, Preston GM, Guggino WB, Baraban JM, Agre P. 1994. Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance. *Proc. Natl. Acad. Sci. USA* 91:13052–56
34. Leng G, Blackburn RE, Dyball REJ, Russel JA. 1989. Role of anterior peri-third ventricular structures in the regulation of supraoptic neuronal activity and neurohypophyseal hormone secretion in the rat. *J. Neuroendocrinol.* 1:35–46
35. Leng G, Dyball REJ, Mason WT. 1985. Electrophysiology of osmoreceptors. See Ref. 70a, pp. 333–4
36. Lind RW, Thunhorst RL, Johnson AK. 1984. The subfornical organ and the integration of multiple factors in thirst. *Physiol. Behav.* 32:69–74
37. Mangiapane ML, Thrasher TN, Keil LC, Simpson JB, Ganong WF. 1983. Deficits in drinking and vasopressin secretion after lesions of the nucleus medianus. *Neuroendocrinology* 37:73–77
38. Mangiapane ML, Thrasher TN, Keil LC, Simpson JB, Ganong WF. 1984. Roles for the subfornical organ in vasopressin release. *Brain Res. Bull.* 13:43–48
39. Mason WT. 1980. Supraoptic neurones of rat hypothalamus are osmosensitive. *Nature* 287:154–57
40. McBride DW Jr, Hamill OP. 1995. A fast pressure-clamp technique for studying mechanogated channels. In *Single Channel Recording,* ed. B Sakmann, E Neher, pp. 329–40. New York: Plenum. 2nd ed.
41. McKinley MJ. 1992. Common aspects of the cerebral regulation of thirst and renal sodium excretion. *Kidney Int.* 41(Suppl. 37):S102–6
42. McKinley MJ, Denton DA, Coghlan JP, Harvey RB, McDougall JG, et al. 1987. Cerebral osmoregulation of renal sodium excretion—A response analogous to thirst and vasopressin release. *Can. J. Physiol. Pharmacol.* 65:1724–29
43. McKinley MJ, Denton DA, Leskell LG, Mouw DR, Scoggins BA, et al. 1982. Osmoregulatory thirst in sheep is disrupted by ablation of the anterior wall of the optic recess. *Brain Res.* 236:210–15
44. McKinley MJ, Denton DA, Park RG, Weisinger RS. 1983. Cerebral involvement in dehydration-induced natriuresis. *Brain Res.* 263:340–43
45. McKinley MJ, Lichardus B, McDougall JG, Weisinger RS. 1992. Periventricular lesions block natriuresis to hypertonic but not isotonic NaCl loads. *Am. J. Physiol.* 262:F98–107
46. McManus ML, Churchwell KB. 1994. Clinical significance of cellular osmoregulation. In *Cellular and Molecular Physiology of Cell Volume Regulation,* ed. K Strange, pp. 63–77. Boca Raton, FL: CRC Press
47. Morris CE. 1992. Are stretch-sensitive channels in molluscan cells and elsewhere physiological mechanotransducers? *Experientia* 48:852–58
48. Morris CE, Sigurdson WJ. 1989. Stretch-inactivated ion channels coexist with stretch-activated ion channels. *Science* 243:807–9
49. Negoro H, Higuchi T, Tadokoro Y, Honda K. 1988. Osmoreceptor mechanism for oxytocin release in the rat. *Jpn. J. Physiol.* 38:19–31
50. Negoro H, Honda K, Uchide K, Higuchi T. 1987. Facilitation of milk ejection-related activation of oxytocin-secreting neurones by osmotic stimulation in the rat. *Exp. Brain Res.* 65:312–16
51. Nissen N, Bourque CW, Renaud LP. 1993. Membrane properties of organum vasculosum lamina terminalis neurons recorded in vitro. *Am. J. Physiol.* 264:R811–15
52. Nordmann JJ. 1977. Ultrastructural morphometry of the rat neurohypophysis. *J. Anat.* 123:213–18
53. Oldfield BJ, Badoer E, Hards DK, McKinley MJ. 1994. Fos production in retrogradely labelled neurons of the lamina terminalis following intravenous infusion of either hypertonic saline or angiotensin II. *Neuroscience* 60:255–62
54. Oliet SHR. 1994. *Osmoreception in rat supraoptic neurons.* PhD thesis. McGill Univ., Montreal. 169 pp.
55. Oliet SHR, Bourque CW. 1992. Properties of supraoptic magnocellular neurones isolated from the adult rat. *J. Physiol.* 455:291–306
56. Oliet SHR, Bourque CW. 1993. Mechanosensitive channels transduce osmosensitivity in supraoptic neurons. *Nature* 364:341–43
57. Oliet SHR, Bourque CW. 1993. Steady-state osmotic modulation of cationic conductance in neurons of rat supraoptic nucleus. *Am. J. Physiol.* 265:R1475–79

58. Oliet SHR, Bourque CW. 1994. Osmoreception in magnocellular neurosecretory cells: from single channels to secretion. *Trends Neurosci.* 17:340–44
59. Oliet SHR, Bourque CW. 1996. Gadolinium uncouples mechanical detection and osmoreceptor potential in supraoptic neurons. *Neuron* 16:175–81
60. Perlmutter LS, Tweedle CD, Hatton GI. 1984. Neuronal/glial plasticity in the supraoptic dendritic zone: dendritic bundling and double synapse formation at parturition. *Neuroscience* 13:769–79
61. Poulain DA, Wakerley JB. 1982. Electrophysiology of hypothalamic magnocellular neurones secreting oxytocin and vasopressin. *Neuroscience* 7:773–808
62. Ramsay DJ, Thrasher TN, Keil LC. 1983. The organum vasculosum lamina terminalis: a critical area for osmoreception. In *The Neurohypophysis: Structure, Function and Control*, ed. BA Cross, G Leng, pp. 91–98. Amsterdam/Lausanne: Elsevier
63. Renaud LP, Cunningham JT, Nissen R, Yang CR. 1993. Electrophysiology of central pathways controlling release of neurohypophysial hormones: focus on the lamina terminalis and diagonal band inputs to the supraoptic nucleus. *Ann. NY Acad. Sci.* 689:122–32
64. Richard D, Bourque CW. 1992. Synaptic activation of rat supraoptic neurons by osmotic stimulation of the organum vasculosum lamina terminalis. *Neuroendocrinology* 55:609–11
65. Richard D, Bourque CW. 1994. Osmotic activation of neurons dissociated from the OVLT and median preoptic nucleus (MnPO). *Soc. Neurosci. Abstr.* 20:1567
66. Richard D, Bourque CW. 1995. Synaptic control of rat supraoptic neurones during osmotic stimulation of the organum vasculosum lamina terminalis in vitro. *J. Physiol.* 489:567–77
67. Robertson GL. 1985. Osmoregulation of thirst and vasopressin secretion: functional properties and their relationship to water balance. See Ref. 70a, pp. 203–12.
68. Rose BD. 1989. *Clinical Physiology of Acid-Base and Electrolyte Disorders*, pp. 153–208. New York: McGraw-Hill. 853 pp. 3rd ed.
69. Russel JA, Blackburn RE, Leng G. 1988. The role of the AV3V region in the control of magnocellular oxytocin neurons. *Brain Res. Bull.* 20:803–10
70. Share L. 1996. Control of vasopressin release: an old but continuing story. *News Physiol. Sci.* 11:7–13
70a. Shrier RW, ed. 1985. *Vasopressin.* New York: Raven. 506 pp.
71. Sibbald JR, Hubbard JE, Sirrett NE. 1988. Responses from osmosensitive neurons of the rat subfornical organ in vitro. *Brain Res.* 461:205–14
72. Sladek CD, Johnson AK. 1983. Effect of anteroventral third ventricle lesions on vasopressin release by organ-cultured hypothalamo-neurohypophyseal explants. *Neuroendocrinology* 37:78–84
73. Sokabe M, Sachs F. 1990. The structure and dynamics of patch-clamped membranes: a study using differential interference contrast light microscopy. *J. Cell. Biol.* 111:599–606
74. Sokabe M, Sachs F. 1992. Towards molecular mechanism of activation in mechanosensitive ion channels. In *Advances in Comparative and Environmental Physiology*, ed. F Ito, pp. 55–77. Berlin Heidelberg: Springer-Verlag
75. Stricker EM, Verbalis JG. 1988. Hormones and behavior: biological basis of thirst and sodium appetite. *Am. Sci.* 76:261–67
76. Swaab DF, Pool CW, Nijveldt F. 1975. Immunofluorescence of vasopressin and oxytocin in the rat hypothalamo-neurohypophyseal system. *J. Neural Trans.* 36:195–215
77. Theodosis DT, Poulain DA. 1984. Evidence for structural plasticity in the supraoptic nucleus of the rat hypothalamus in relation to gestation and lactation. *Neuroscience* 11:183–93
78. Thornborough JR, Passo SS, Rothballer AB. 1973. Forebrain lesion blockade of the natriuretic response to elevated carotic blood sodium. *Brain Res.* 58:355–63
79. Thrasher TN. 1985. Circumventricular organs, thirst and vasopressin secretion. See Ref. 70a. pp 311–18
80. Thrasher TN. 1989. Role of forebrain circumventricular organs in body fluid balance. *Acta Physiol. Scand.* 136(Suppl. 583):141–50
81. Thrasher TN, Keil LC. 1987. Regulation of drinking and vasopressin secretion: role of organum vasculosum lamina terminalis. *Am. J. Physiol.* 253:R108–20
82. Thrasher TN, Keil LC, Ramsay DJ. 1982. Lesions of the organum vasculosum of the lamina terminalis (OVLT) attenuate osmotically induced drinking and vasopressin secretion in the dog. *Endocrinology* 110:1837–39
83. Thunhorst RL, Ehrlrich KJ, Simpson JB. 1990. Subfornical organ participates in salt appetite. *Behav. Neurosci.* 104:637–42

84. Tribollet E, Amstrong WE, Dubois-Dauphin M. 1985. Extra-hypothalamic afferent inputs to the supraoptic area of the rat as determined by retrograde and anterograde tracing techniques. *Neuroscience* 15:135–48
85. Vandersande F, Dierickx K. 1975. Identification of the vasopressin producing and of the oxytocin producing neurons in the hypothalamic neurosecretory system of the rat. *Cell Tissue Res.* 164:153–62
86. Verbalis JG, Baldwin EF, Robinson AG. 1986. Osmotic regulation of plasma vasopressin and oxytocin after sustained hyponatremia. *Am. J. Physiol.* 250:R444–51
87. Verbalis JG, Mangione MP, Stricker EM. 1991. Oxytocin produces natriuresis in rats at physiological plasma concentration. *Endocrinology* 128:1317–22
88. Verney EB. 1947. The antidiuretic hormone and the factors which determine its release. *Proc. R. Soc. London Ser. B* 135:25–26
89. Vivas L, Chiaraviglio E, Carrer HF. 1990. Rat organum vasculosum lamina terminalis in vitro: responses to changes in sodium concentration. *Brain Res.* 519:294–300
90. Wakerley JB, Poulain DA, Brown D. 1978. Comparison of firing patterns in oxytocin and vasopressin-releasing neurones during progressive dehydration. *Brain Res.* 148:425–40
91. Walters JK, Hatton GI. 1974. Supraoptic neuronal activity in rats during five days of water deprivation. *Physiol. Behav.* 13:661–67
92. Weisinger RS, Considine P, Denton DA, McKinley MJ. 1979. Rapid effect of change in cerebrospinal fluid sodium concentration on salt appetite. *Nature* 280:490–91
93. Weiss ML, Hatton GL. 1990. Collateral input to the paraventricular and supraoptic nuclei in the rat. I. Afferents from the subfornical organ and anteroventral third ventricle region. *Brain Res. Bull.* 24:231–38
94. Wilkin LD, Mitchell LD, Ganten D, Johnson AK. 1989. The supraoptic nucleus: afferents from areas involved in control of body fluid homeostasis. *Neuroscience* 28:573–84
95. Yamaguchi K, Morimoto A, Murakami N. 1993. Organum vasculosum lamina terminalis (OVLT) in rabbit and rat: topographical studies. *J. Comp. Neurol.* 330:352–62
96. Yang CR, Senatorov VV, Renaud LP. 1994. Organum vasculosum lamina terminalis-evoked postsynaptic responses in rat supraoptic neurones in vitro. *J. Physiol.* 477:59–74
97. Yang XC, Sachs F. 1989. Block of stretch-activated ion channels in *Xenopus* oocytes by gadolinium and caesium ions. *Science* 243:1068–71
98. Yudilevich DL, de Rose N. 1971. Blood-brain transfer of glucose and other molecules measured by rapid indicator dilution. *Am. J. Physiol.* 220:841–46