Journal of Physiology (1989), **417**, *pp.* 263–277
With 13 *text-figures*
Printed in Great Britain

IONIC BASIS FOR THE INTRINSIC ACTIVATION OF RAT SUPRAOPTIC NEURONES BY HYPEROSMOTIC STIMULI

BY CHARLES W. BOURQUE

From the Centre for Research in Neuroscience, McGill University, and Division of Neurology, Montreal General Hospital, 1650 Cedar Avenue, Montreal, Quebec, Canada H3G 1A4

(*Received 3 October 1988*)

SUMMARY

1. Magnocellular neurosecretory cells (MNCs) were impaled in the supraoptic nucleus of rat hypothalamic explants maintained *in vitro*. Current- and voltage-clamp analysis of the osmotically induced response was performed at 34 °C.

2. Addition of mannitol or NaCl to cause a rise in fluid osmolarity ($> +6$ mosM) caused a membrane depolarization whose amplitude increased when elicited from more hyperpolarized levels. Changes in temperature (34–28 °C), addition of TTX, or superfusion with Na^+-free or Ca^{2+}-free solutions did not block the osmotically induced depolarization. In control solutions the response was consistently accompanied by an increase in the frequency of spontaneous postsynaptic potentials. Thus, osmotic stimuli have a direct effect on MNCs, and they also apparently activate other neurones which are presynaptic to these cells.

3. Under voltage-clamp, hyperosmotic stimuli induced an inward current (I_o) accompanied by an increase in membrane conductance. The current was unaffected or slightly enhanced by doubling the external K^+ concentration. I_o was also characterized by a linear I–V relation (between -100 and -50 mV) and an extrapolated reversal potential near -10 mV. I_o presumably results from the activation of a voltage-independent and non-selective cationic conductance.

4. Hyperosmotic stimuli did not affect the depolarizing after-current (I_{DAP}) responsible for the production of phasic bursts. However, the inward shift of the post-spike I–V curve caused by I_o could reduce or eliminate the region of net outward current which lies negative to spike threshold in silent neurones. Thus in MNCs displaying I_{DAP}, activation of I_o by a rise in osmotic pressure can induce or enhance phasic bursting activity.

5. Application of hyperosmotic stimuli sufficient to excite most MNCs ($+20$ to $+80$ mosM) did not elicit a response from any of seventeen neurones impaled in areas lateral and caudal to the supraoptic nucleus. Recordings obtained from three CA1 neurones in slices of rat hippocampus revealed that stimuli in excess of $+100$ mosM are required to evoke appreciable non-specific depolarizations.

6. These studies indicate that the specific endogenous osmosensitivity of MNCs results from the activation of the intrinsic current I_o. Furthermore, interactions between I_o and I_{DAP} explain how osmotic stimuli can lead to the induction of phasic

bursting activity, a response which is known to potentiate the secretion of vasopressin from the neural lobe.

INTRODUCTION

The potency of a rise in plasma osmolarity as a stimulus for vasopressin (VP) release in mammals has been recognized since the pioneering studies of Verney (1947). In rats, the osmotic induction of VP release results from an increase in firing rate or the appearance of phasic bursting activity from the hypothalamic magnocellular neurosecretory cells (MNCs) which synthesize the peptide and release it from their axonal terminals in the neurohypophysis (see Poulain & Wakerley, 1982 for review). The 'osmoreceptor' which mediates the activation of VP-secreting MNCs is believed to comprise several groups of central and peripheral osmosensitive neurones whose information ultimately converges upon hypothalamic MNCs (Johnson, 1985; Sladek & Armstrong, 1985). In addition, as originally suggested by Jewell & Verney (1957), an important component of the osmotically evoked VP response results from the endogenous osmosensitivity of MNCs themselves (Mason, 1980; Abe & Ogata, 1982; Bourque & Renaud, 1984). Hypothalamic MNCs are therefore an integral part of what has been termed 'the osmoreceptor complex' (Leng, Mason & Dyer, 1982).

In this study, current- and voltage-clamp techniques were used to examine the ionic basis of the osmotically induced depolarization of rat supraoptic MNCs *in vitro*. The analysis reveals how hyperosmotic stimuli can induce or intensify phasic bursting activity, responses which are known to potentiate VP release in the neurohypophysis (Dutton & Dyball, 1979; Bicknell & Leng, 1981). Preliminary results have appeared (Bourque, 1987).

METHODS

Preparation of superfused rat hypothalamic explants.

Explants of basal hypothalamus (8 × 8 × 2 mm) were obtained from ether-anaesthetized Wistar rats (100–300 g) of either sex (Bourque & Renaud, 1983) and pinned to the Sylgard base of a humidified and temperature-regulated (34 °C) superfusion chamber. Within 3 min of decapitation, an open-ended silicone tube (2 mm o.d.) delivering artificial cerebrospinal fluid (ACSF; 0·7–1·2 ml min^{-1}) was positioned near the caudal end of the median eminence and the oxygenated (95% O_2; 5% CO_2) solution began to flow over the surface of the explant. The covering membranes were then removed and cotton wicks placed near the cut end of the optic nerves to improve drainage and minimize the volume of fluid overlying the supraoptic nucleus.

Solutions

The composition of the standard ACSF (pH = 7·4) was (mM): NaCl, 126; KCl, 3; $MgCl_2$, 1·3; $NaHCO_3$, 25·9; $CaCl_2$, 2–3; glucose, 10. For low-Na^+ solutions, NaCl was replaced by choline chloride, but retained 25·9 mM-$NaHCO_3$ as buffer (pH = 7·4). Sodium-free solutions were prepared by replacing both NaCl and $NaHCO_3$ with Tris hydroxymethyl aminomethane (Tris; pH = 7·4). Nominally Ca^{2+}-free solutions were prepared by replacing $CaCl_2$ with $MnCl_2$. The osmolarity of all solutions was adjusted to 294 ± 1 mosM using the appropriate salt. Tetrodotoxin (TTX) was directly added to solutions (0·5 μM) as required. Measurements of fluid osmolarity were obtained with a freezing point osmometer (Advanced Instruments Inc.).

Hyperosmotic stimulation

Osmotic stimulation of MNCs was performed using either of the two following methods. For sustained stimulation of fixed intensity, solutions containing an excess of mannitol (5–40 mM) or NaCl (2–20 mM) were simply bath applied for the desired period of time. Alternatively, 5–30 μl injections of ACSF supplemented with 1 M-mannitol or 0·5 M-NaCl were made into the silicone

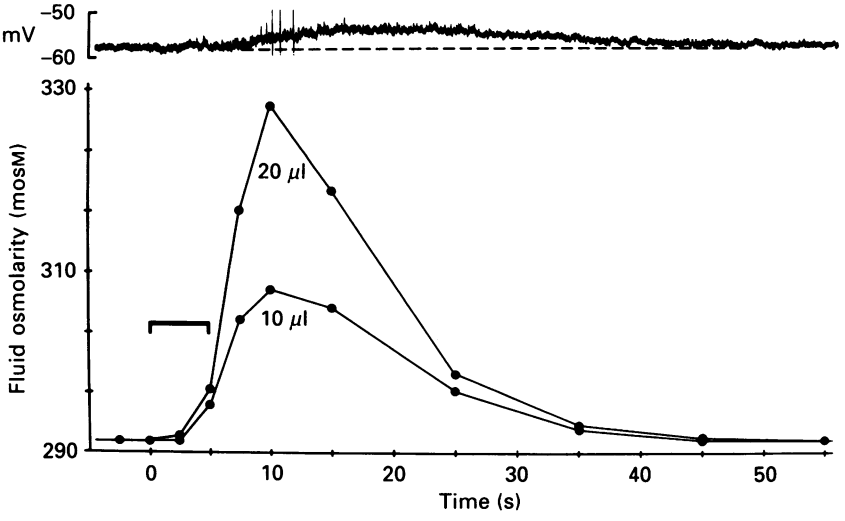


Fig. 1. Time course and amplitude of the change in fluid osmolarity following 10 and 20 μl infusions of 1 M-mannitol (bar) into the silicone delivery tube (see text). Each point represents the average of three to eight measurements. For each measurement, a single 20 μl sample was taken (and assayed) from the fluid lying immediately over the area of the supraoptic nucleus before, during or after an individual infusion. The top trace shows at high gain (action potentials clipped) the response of an MNC to a 20 μl infusion recorded from another explant superfused at the same rate (0·8 ml min^{-1}). Note the slight lag and persistence of the cell's response compared to the time course of the osmotic challenge.

delivery tube about 0·2 ml upstream from its cut end lying over the explant. These injections were made gradually and uniformly over a period of 2–10 s. This allowed reproducible and reasonably short-lasting (< 2 min) responses to be obtained at frequent intervals. In contrast to bath-applied stimuli which were sustained, the osmolarity increase achieved by the injection method was characterized by a swift rise and a slow decay. The peak amplitude of the stimulus depended on the precise rate of flow as well as the volume of fluid dwelling over the explant. Figure 1 illustrates how the amplitude and time course of a particular stimulus could be determined at the end of an experiment. Peak stimuli ranging between +10 and +60 mosM were typically used in these experiments. The numbers indicated in the text and figures refer to peak osmotic stimuli only.

Electrophysiological recordings

Cells were impaled with micropipettes pulled on a horizontal puller (Sutter Instruments) and filled with 4 M-potassium acetate–0·15 M-KCl or 4 M-KCl (tip resistances 30–100 MΩ). The reference electrode consisted of a chloride-treated silver wire embedded in agar gel and interfaced with the recording solution. This arrangement avoided drift problems and changes in junction potential during changes of superfusing solutions. An Axoclamp 2A amplifier (Axon Instruments) was used for intracellular recording of membrane voltage or current from neurones impaled with a single microelectrode. Membrane currents were measured under voltage clamp with the amplifier switching at a rate of 2–6 kHz depending on electrode capacitance. The data acquired were taped using an FM recorder (Racal) and displayed on a chart recorder (Gould RS 3200). The noise level

of membrane current recording is inherently large when using the single microelectrode switching voltage-clamp technique. Because many of the signals recorded from MNCs are of the order of tens of pA, conservative filtering of the data is often not sufficient to optimize the signal-to-noise ratio. Thus, when necessary (e.g. Figs 9 and 10), membrane currents were measured using high gain originals and a transparent graph overlay as displacements of the middle portion of the membrane noise (estimated by eye).

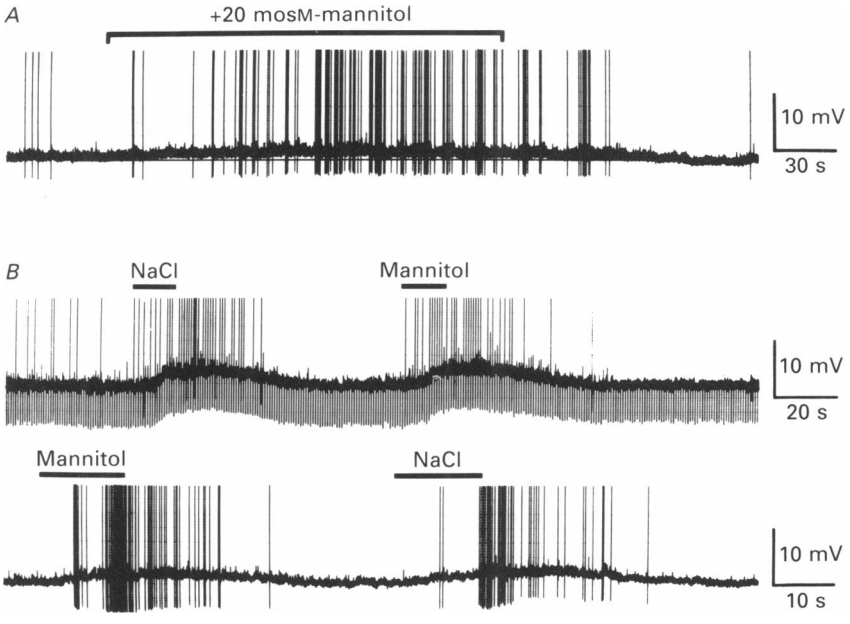


Fig. 2. In *A*, bath application of a solution containing excess mannitol induced a progressive sustained and reversible depolarization of the membrane potential (continuous horizontal line drawn through −62 mV). *B* shows the responses of two other MNCs to hyperosmotic stimuli (+40 mosM) induced by infusing excess mannitol or NaCl into the superfusion line (see Methods). Negative deflections (top trace in *B*) are electrotonic responses to 80 ms hyperpolarizing current pulses (50 pA). Note that both NaCl and mannitol (+40 mosM) elicit comparable responses. Spike amplitudes were 81, 70 and 88 mV respectively.

Recordings from hippocampal slices

To emphasize the specific nature of the endogenous osmosensitivity of MNCs, several recordings were made of cells lying outside the supraoptic nucleus in hypothalamic explants (see results). In addition, to examine possible non-specific effects of large hyperosmotic stimuli, more recordings were made from CA1 hippocampal neurones. For this purpose, transverse slices (400–500 μm thick) of rat hippocampus were made using a falling razor blade strip. The maintenance of slices as well as the recording procedure were as described by Halliwell & Adams (1982; see acknowledgments).

RESULTS

The data presented below were obtained from forty-six magnocellular neurosecretory cells (MNCs) impaled in the supraoptic nucleus of thirty-one explants of rat hypothalamus. These neurones displayed resting membrane potentials negative to −55 mV and input resistances between 90 and 310 MΩ. In all cells, spike

amplitudes exceeded 70 mV. In control solutions depolarizing current injection evoked sustained repetitive firing and caused a frequency-dependent broadening of the action potential. These combined characteristics are typical of rat supraoptic MNCs (Renaud, 1987).

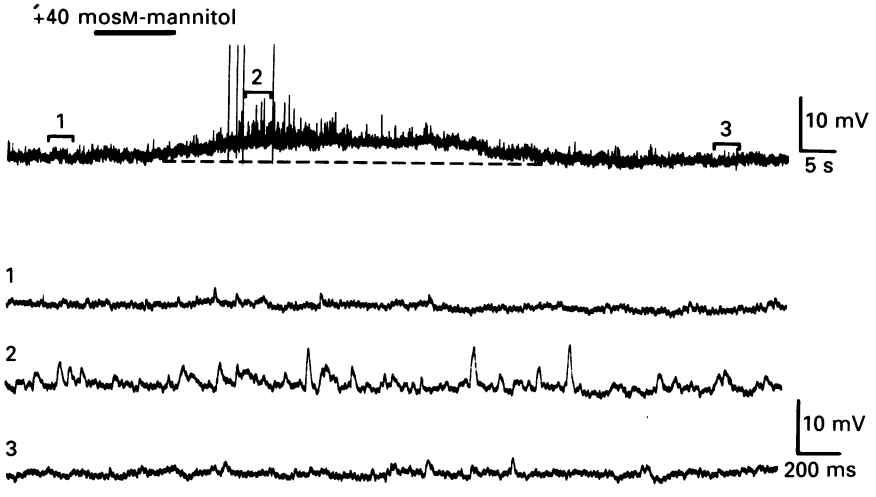


Fig. 3. Chart recording of an MNC recorded under current-clamp. The top trace shows a reversible depolarization induced by an infusion of hypertonic mannitol. Action potential amplitude (78 mV) not shown. Selected segments of the record are expanded below to illustrate the induction of excitatory postsynaptic potentials during (2) but not before (1) or after (3) the hyperosmotic stimulus.

General features of the osmotically induced response

In each of eight cells, bath application of solutions made hypertonic (10–40 mosM) with mannitol or excess NaCl evoked sustained (2–15 mV) depolarizations that could elicit or accelerate spike discharge (Fig. 2*A*).Osmotic stimuli ranging between +4 and +8 mosM were far less effective and only three of seven cells tested showed a clear depolarizing response to bath-applied osmotic stimuli of +6 to +8 mosM. In response to briefer infusions (see Methods) of hypertonic mannitol ($n = 24$ cells) or NaCl ($n = 17$ cells) all MNCs showed reversible, reproducible, and dose-dependent depolarizations when exposed to stimuli ranging between +15 and +60 mosM (Fig. 2*B* and *C*). In addition to a membrane depolarization, hyperosmotic stimuli consistently induced an increase in baseline noise. While part of this effect may have resulted from the appearance of non-synaptic depolarizing potentials, which accompany all near-threshold depolarizations in MNCs (see Bourque, Randle & Renaud, 1986), an important component of the noise increase was caused by a rise in the frequency (or the apparent induction) of spontaneous excitatory postsynaptic potentials (Fig. 3).

Ionic basis of the osmotically induced depolarization

In each of six MNCs hyperpolarization of the initial membrane potential by sustained current injection increased the amplitude of the osmotically induced depolarization compared to that recorded at control potentials (Fig. 4). In two cells,

progressive cooling of the tissue from 34 to 27 °C did not affect the amplitude of depolarizing responses to fixed hyperosmotic stimuli. An electrogenic pump, therefore, is presumably not involved in the generation of these responses.

Under voltage-clamp, hyperosmotic stimuli (+15 to +60 mosM) elicited a

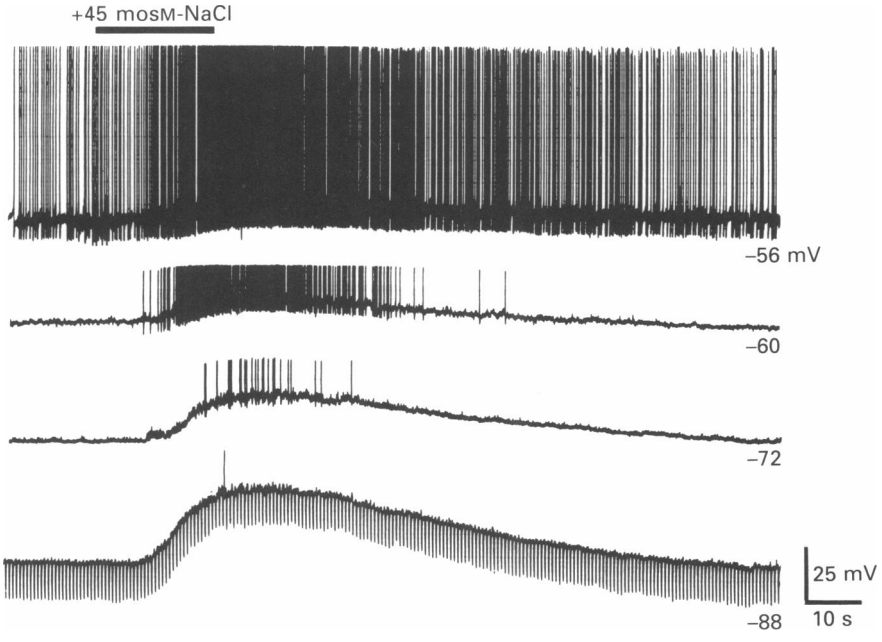


Fig. 4. Current-clamp responses of a non-phasic MNC to the infusion of a fixed (NaCl) hyperosmotic stimulus (bar). Hyperpolarization of the initial membrane potential (as indicated) by sustained current injection causes an increase in amplitude of the osmotically induced depolarization. Negative deflections on the bottom trace are electrotonic responses to constant current pulses (0·1 nA; 80 ms duration). Note that input resistance does not appear to change appreciably when measured in this way (but see Fig. 5). Spike amplitude not shown in the bottom three traces.

reversible inward current accompanied with an increase of input conductance (Fig. 5). In response to an osmotic stimulus of fixed intensity, the amplitude of the osmotically evoked current (I_o) became greater as the holding potential was made more negative and smaller at more positive voltages. Figure 6*A* shows the effect of an increase in osmolarity on the steady-state current–voltage (*I–V*) relation of an MNC measured in the presence of TTX. Subtraction of the two curves (Fig. 6*B*) reveals that I_o is characterized by a linear *I–V* relationship and an extrapolated reversal potential E_o) near −4 mV. Similar data obtained from seven cells provided E_o values ranging between −24 and −3 mV with an average of -10 ± 3 mV (mean ± S.E.M.). The osmotic response was unaffected ($n = 3$ cells) by superfusion with Ca^{2+}-free, Mn^{2+}-containing solutions (Fig. 7). Superfusion with low-Na^+ ($n = 2$ cells) or 0-Na^+ solutions ($n = 4$ cells) also failed to affect the amplitude of the osmotically induced depolarization (Fig. 8). Doubling the external K^+ concentration either had no effect or caused a slight enhancement of the osmotically induced

inward current (Fig. 9). These results suggest that I_o results from the opening of non-selective cation-permeable channels.

Osmotic induction of phasic bursting activity

The response of MNCs to hyperosmotic challenges *in vivo* is often characterized by the appearance of phasic bursting activity (Brimble & Dyball, 1977). This firing

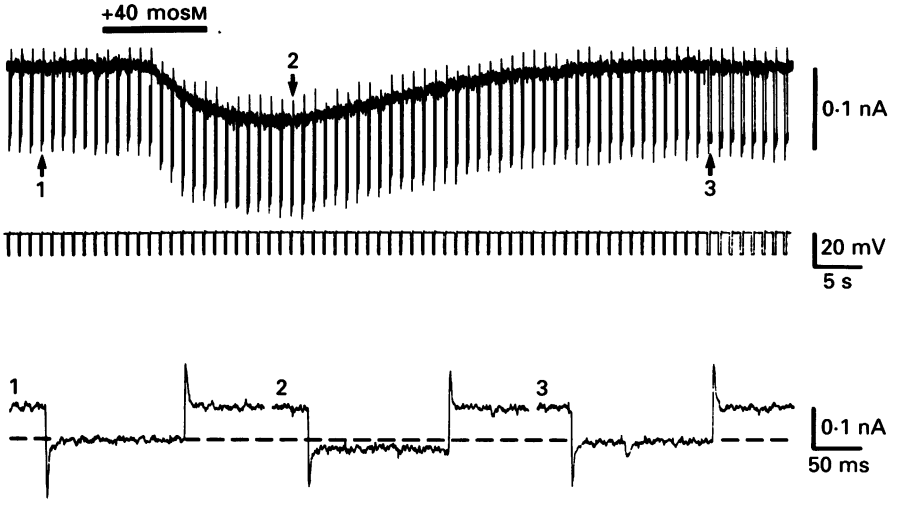


Fig. 5. Response of an MNC to an infused hyperosmotic stimulus (bar) applied under voltage-clamp, in the presence of 0·5 μM-TTX. The holding potential was −65 mV and voltage steps to −80 mV were applied at 1 Hz. The evoked response consists of a reversible inward current (top trace) accompanied by an increase of membrane conductance. The bottom records show expanded segments of the top trace as indicated by the numbered arrows. The holding current was lined up for these records and a dashed line was drawn through the steady-state current level of the control and wash pulses. Note that the amplitude of the current pulse recorded near the peak of the response (2) is larger than the others, indicating a state of increased conductance.

pattern is known to result from a summation of the depolarizing after-potentials (DAPs) which follow individual spikes in bursting MNCs (Andrew & Dudek, 1983). In each of four cells tested, the current underlying DAPs (I_{DAP}) measured under voltage-clamp (see Bourque, 1986) was not significantly affected by hyperosmotic (10–50 mosM) stimulation with mannitol or NaCl (Fig. 10 *A*). As a result, the inward shift of the steady-state *I–V* relationship, which is due to the activation of I_o (e.g. Fig. 6*A*), also causes a net inward displacement of the transient post-spike *I–V* curve measured at the peak of I_{DAP} (Fig. 10*B*). Thus depending on the prevailing resting membrane potential, activation of I_o will reduce or eliminate the region of net outward current lying between the existing zero-current potential and spike threshold in the MNC's post-spike *I–V* relationship (* in Fig. 10*B*). In current-clamp, the progressive reduction of this 'barrier' of outward current will gradually raise the probability that the membrane potential will spontaneously enter the zone of negative resistance and generate a subsequent spike. Because the onset and maintenance of firing within phasic bursts depends on the regenerative depolarizing

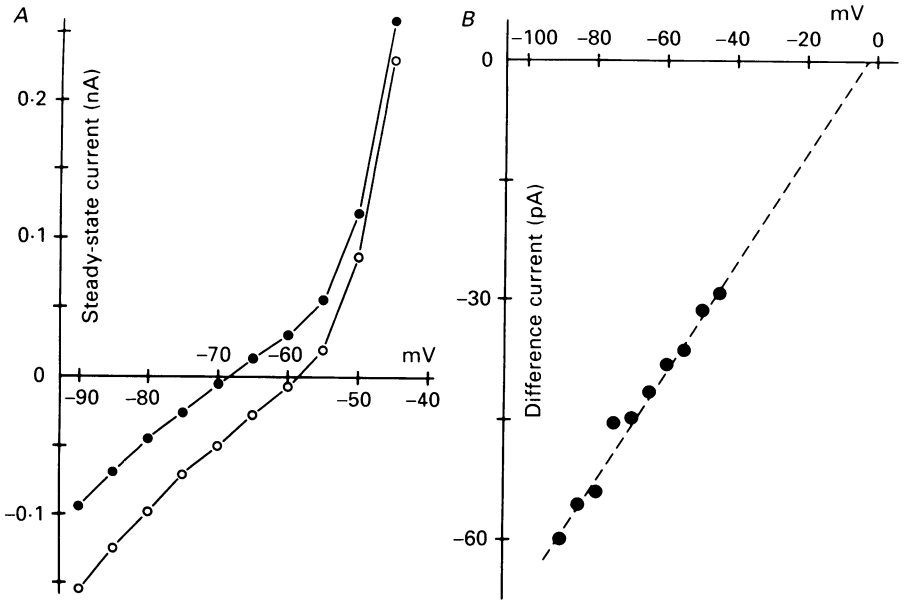


Fig. 6. The graph in *A* shows steady-state *I–V* relations of an MNC measured near the end of 2 s voltage-clamp steps applied from a holding potential of −70 mV. ●, *I–V* of the cell measured in control ACSF; ○, *I–V* obtained during a bath-applied hyperosmotic (+26 mosM-mannitol) stimulus. In *B*, the difference in steady-state currents shown in *A* is replotted yielding the *I–V* relation of the osmotically induced current (I_o). The dashed line was fitted by eye and shows an extrapolated reversal potential near −4 mV.

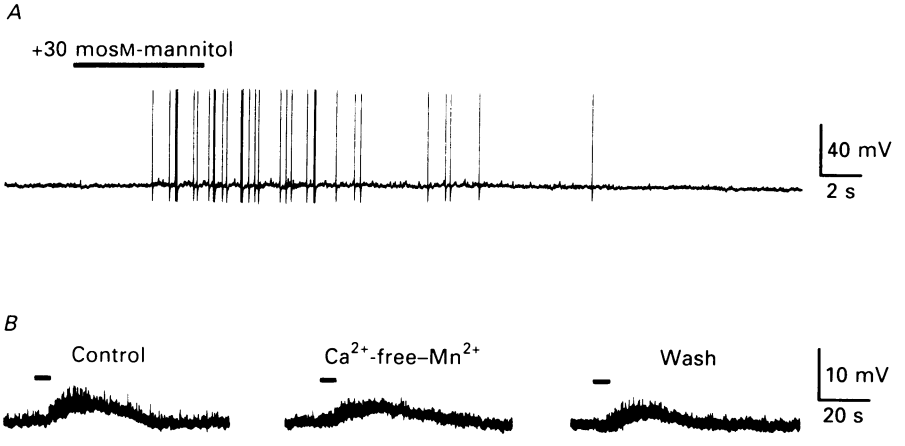


Fig. 7. *A* shows the response of an MNC to an infused hyperosmotic stimulus (mannitol). In *B*, responses to identical stimuli (bars) were recorded from the same cell (initial potential −75 mV) in the presence and absence of external Ca^{2+} in the recording solution. Note that while the depolarizing response is largely unaffected, a reduction of baseline (postsynaptic) noise is apparent on the rising phase of the response recorded in Ca^{2+}-free medium.

effect of the negative resistance induced by I_{DAP} (Bourque, 1986), the inward shift of the I–V relationship induced by I_o will induce the appearance, or favour the production of, longer lasting and more intense bursts of action potentials (Fig. 11). By analogy, increases in I_o amplitude evoked by progressively larger osmotic stimuli also elicited longer and more intense bursts of action potentials.

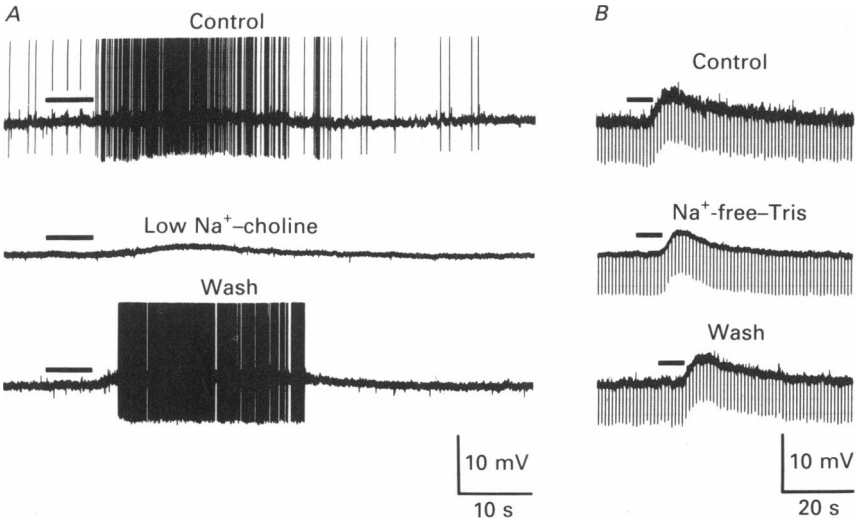


Fig. 8. Chart recordings of osmotically induced depolarizations in rat MNCs. In *A*, superfusion of the explant with low Na^+, choline-containing solution (20 min) blocked the increase in baseline noise, but failed to abolish the depolarization evoked by an infused (+40 mosM) osmotic stimulus (bar) from an initial membrane potential of −60 mV (spike amplitude 80 mV). *B* shows a similar result when another MNC was exposed (15 min) to a Na^+-free Tris-containing solution (initial V_m = −68 mV).

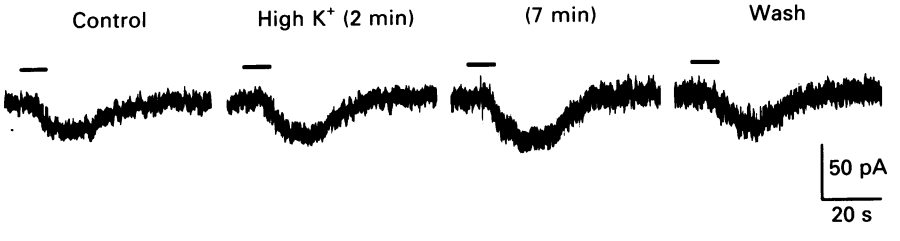


Fig. 9. Membrane current responses recorded from an MNC voltage clamped at a holding potential of −65 mV. Note that the inward current (I_o) induced by an infused hypertonic mannitol (+30 mosM) stimulus (bar) is unaffected or slightly enhanced by increasing the external K^+ concentration from 3 to 6 mM.

Effects of hyperosmotic stimulation on other neurones

While depolarizing responses could be recorded from all supraoptic MNCs using osmotic stimuli ranging between +15 and +60 mosM, none of fourteen neurones impaled in areas outside the immediate lateral and caudal borders of the supraoptic

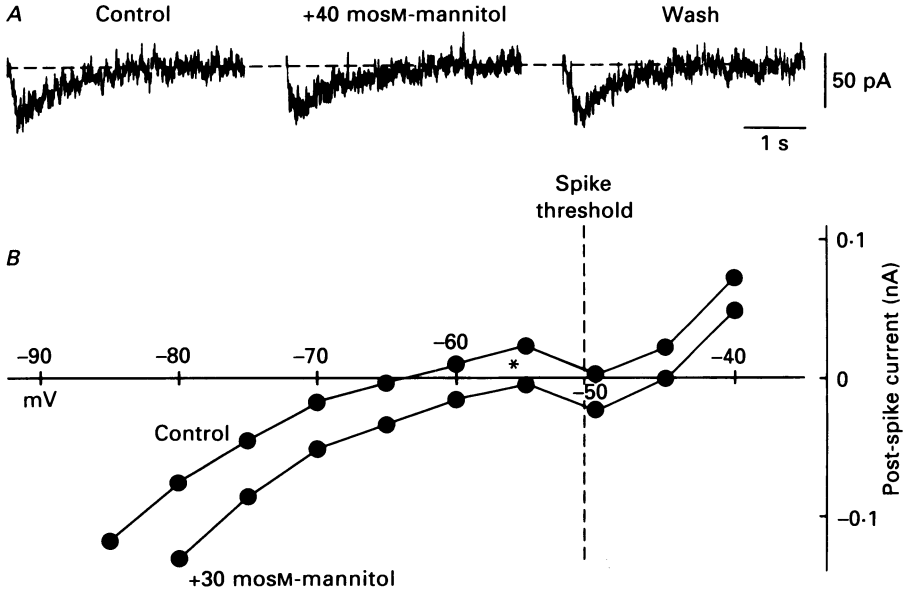


Fig. 10. In A, the post-spike inward current I_{DAP} was measured by switching the amplifier to the voltage-clamp mode ($V_h = -65$ mV) immediately following a pair of current-evoked action potentials. Note that a hypertonic (mannitol) stimulus has no significant effect on I_{DAP}. In B, post-spike I–V curves were measured by plotting the net membrane current flowing 200 ms after the onset of the clamp (time of peak DAP) as a function of various test potentials. Note that in the presence of a bath-applied hyperosmotic (mannitol) stimulus the inward shift of the post-spike I–V eliminates the outward current region (*) which normally lies negative to spike threshold.

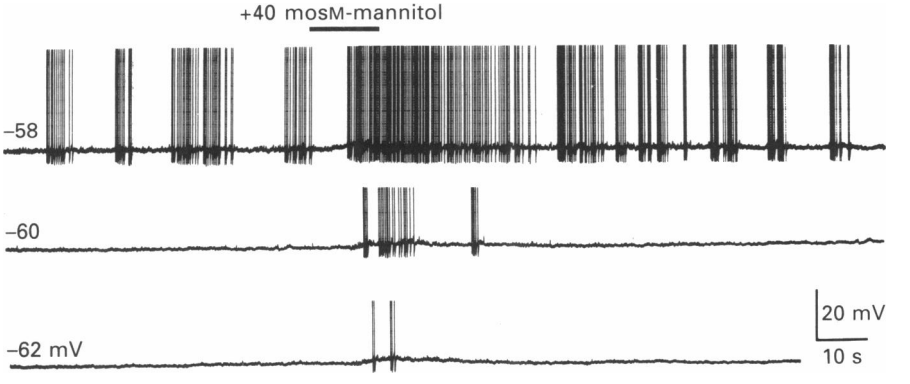
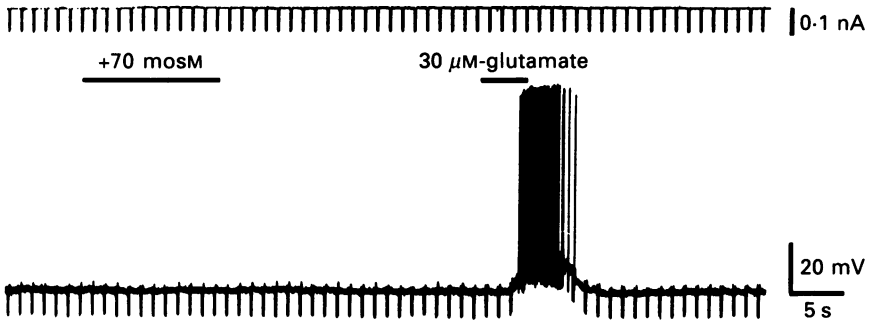
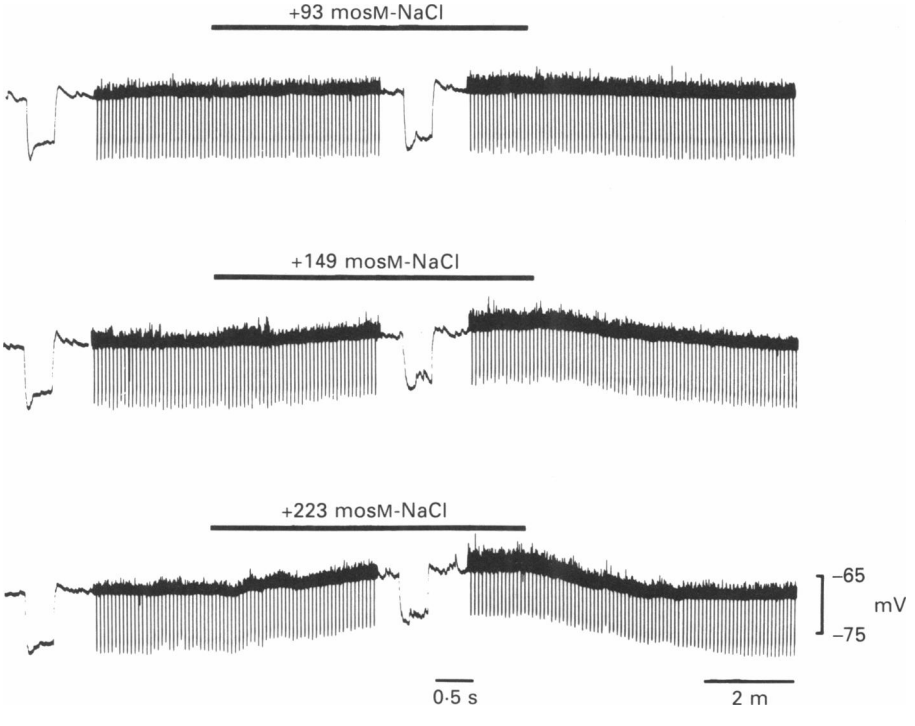


Fig. 11. Current-clamp responses of a phasic MNC to the application of a fixed hyperosmotic (mannitol) stimulus (bar). Slight changes in initial membrane potential (as indicated) by sustained current injection modify the qualitative aspects of the osmotically induced response. Note that from progressively more depolarized initial membrane potentials the osmotic stimulus induces longer and more intense bursts of activity. Spike amplitude was 74 mV.



'ig. 12. Chart recording of a neurone impaled in the medial amygdaloid area of an explant f rat hypothalamus. This cell failed to respond to osmotic stimuli ranging between +10 nd +70 mosM. The lack of response to one such challenge is shown followed by a positive esponse to glutamate administered via the same route. Therefore, lack of access, was not esponsible for the absence of an osmotically evoked response.



'ig. 13. Chart recordings obtained from a CA1 hippocampal neurone impaled in a slice of at hippocampus. Negative deflections are electrotonic responses to constant current ulses (0·2 nA) applied at 0·2 Hz. Note that bath application of hyperosmotic stimuli NaCl) only cause an obvious depolarization when stimuli in excess of +100 mosM are sed.

nucleus displayed any response to osmotic stimuli of similar magnitude (Fig. 12). To further examine whether 'non-osmosensitive' neurones could respond, at least non-specifically, to hyperosmotic solutions, additional experiments were performed on CA1 neurones impaled in transverse slices of hippocampus. In each of three cells, bath application (5–10 min) of solutions containing an excess of NaCl amounting to +80 mosM failed to induce any measurable depolarization or change of membrane resistance. Larger stimuli, however, ranging between +100 and +250 mosM, induced dose-dependent depolarizations and decreases of membrane resistance in each of the three cells tested (Fig. 13).

DISCUSSION

Intrinsic components of the osmotic response

The experiments described in this study indicate that MNCs of the rat supraoptic nucleus are depolarized by hyperosmotic stimuli which do not affect other neighbouring hypothalamic or CA1 hippocampal neurones. These findings mirror results obtained *in vivo* which show that neurones outside the immediate area of the supraoptic nucleus respond weakly and inconsistently, if at all, to osmotic stimuli (Brimble & Dyball, 1977; Leng, 1980). In addition, the data shows that supraoptic neurones can be depolarized by an excess of either mannitol or NaCl, and that this response is unaffected by TTX, Na^+-free or Ca^{2+}-free solutions. MNCs are therefore intrinsically osmosensitive rather than simple detectors of extracellular [Na^+]. The activation of non-synaptic depolarizing potentials (NSDPs) by membrane depolarization (Bourque *et al.* 1986) can also contribute to the endogenous component of the hyperosmotic activation of MNCs. Because the amplitude of the osmotic response is dose dependent, and because NSDPs can serve as pre-potentials to trigger action potentials, it is not surprising that large osmotic stimuli can evoke firing in the absence of synaptic transmission (e.g. Bourque & Renaud, 1984).

Synaptic component of the osmotic response

In addition to non-synaptic potentials, the osmotically induced depolarization of MNCs was accompanied by an increase in the frequency of occurrence of spontaneous excitatory postsynaptic potentials (EPSPs). Mono- or polysynaptic inputs impinging upon these cells must therefore originate from other osmosensitive neurones located within the confines of the explanted tissue. Preliminary studies indicate that neurones of the organum vasculosum of the lamina terminalis (OVLT), which are retained in hypothalamic explants, are also osmosensitive and may contribute to this response (Renaud, Nissen & Bourque, 1988). While the osmotically induced depolarization was robust and sufficient to elicit or accelerate the firing of MNCs, this response required a minimum increase of the osmotic pressure of 2% or more ($\geqslant +6$ mosM). The existence of additional osmosensitive inputs to MNCs must therefore be of importance for providing the greater sensitivity (threshold $< +1\%$ increase in osmolarity) of the VP response measured *in vivo* (Dunn, Brennan, Nelson & Robertson, 1973).

Ionic basis of the osmotically induced depolarization

The results presented here indicate that the depolarizing response results from the activation of a voltage-independent inward current (I_o) which is triggered by a rise in osmotic pressure. While the transduction mechanism responsible for the activation of I_o remains unknown, the ion channels involved appear to be permeable to a variety of cations including Tris and choline and do not require Ca^{2+} *influx* to be opened. In these respects the activated conductance is somewhat reminiscent of that activated by acetylcholine at the motor endplate (Dwyer, Adams & Hille, 1980).

In contrast to the present finding that a conductance increase underlies the osmotic effect in the rat, a previous report by Abe & Ogata (1982) suggested that the osmotically induced depolarization of guinea-pig supraoptic neurones results from a suppression of K^+ conductance. While a species-related difference is possible, it should be noted that the osmotic stimuli used in that study were large (up to +205 mosM) and likely to have produced non-specific effects. Furthermore, their current-clamp observations may have been complicated by non-linearities in the *I–V* relation of the cells recorded. For instance, the osmotic depolarization may have suppressed an intrinsic inward rectifier; this would have increased the membrane resistance, thereby obscuring the true conductance change underlying the osmotic response. Such problems were avoided here by the use of the voltage-clamp technique.

The supraoptic nucleus of the rat contains both oxytocin (Oxy) and vasopressin (VP) secreting MNCs. Presumably, therefore, both Oxy and VP neurones were recorded in this study. While both cell types are known to respond to osmotic stimuli *in vivo*, only VP-secreting MNCs adopt phasic bursting activity (Brimble & Dyball, 1977; Yamashita, Inenaga, Kawata & Sano, 1983). Thus, during *in vitro* recordings it is reasonable to characterize phasically active MNCs as putative VP-secreting neurones. But what about other MNCs? It has been our experience that any supraoptic cell displaying a depolarizing after-potential (DAP) has the *capacity* to fire phasic bursts (Bourque, 1986). It can be assumed, therefore, that DAP-positive supraoptic neurones are VP-secreting MNCs. It would appear therefore that a specific property of Oxy cells is that they lack the ionic channels which mediate the DAP. In practice, however, MNCs which display no DAP cannot be categorized because expression of the DAP is extremely sensitive to a variety of factors, including the quality of the cellular impalement (Andrew & Dudek, 1983). Nevertheless, because the membrane current response induced by hyperosmotic stimuli (I_o) was recorded from all MNCs, whether or not they displayed a DAP, it is likely that osmotic pressure affects both Oxy and VP-secreting MNCs through the same type of membrane conductance. However, the interactions between I_o and I_{DAP} which lead to phasic bursting activity are presumably specific to VP-secreting MNCs.

Osmotic induction of phasic bursting activity

A well-known feature of the osmotically induced response of MNCs *in vivo* (Brimble & Dyball, 1977; Leng, 1980) and *in vitro* (Bourque & Renaud, 1984) is the appearance of phasic bursting activity or an increase of intraburst firing rate and prolongation of burst duration from phasically active neurones. This particular aspect of the response is significant since the appearance or intensification of phasic bursting activity strongly potentiates the secretion of vasopressin in the rat neurohypophysis (Dutton & Dyball, 1979; Bicknell & Leng, 1981). The results obtained here show that a net inward shift of the MNC's post-spike *I–V* curve can promote burst firing by raising the probability that the membrane voltage will enter

the region of negative resistance temporarily imparted to the I–V relationship by the activation of I_{DAP} (Bourque, 1986). Thus while synaptic inputs from other osmosensitive cells may be required to maintain the exquisite sensitivity of the whole animal VP response to small osmotic challenges, many of the qualitative features of the *in vivo* response of MNCs depend on the activation of an endogenous inward current (I_0) and its interaction with a Ca^{2+}- and voltage-dependent current (I_{DAP}) which is essential for the generation of bursting activity.

This work was supported by FCAR, FRSQ and the MRC of Canada. I thank L. Renaud, R. Nissen and C. Jarvis for helpful comments and J. V. Halliwell for help in performing the experiments on hippocampal slices. The author is a recipient of a scholarship from the MRC of Canada.

REFERENCES

ABE, H. & OGATA, N. (1982). Ionic mechanism for the osmotically-induced depolarization in neurones of the guinea-pig supraoptic nucleus *in vitro*. *Journal of Physiology* **327**, 157–171.

ANDREW, R. D. & DUDEK, F. E. (1983). Burst discharge in mammalian neuroendocrine cells involves an intrinsic regenerative mechanism. *Science* **221**, 1050–1052.

BICKNELL, R. J. & LENG, G. (1981). Relative efficiency of neural firing patterns for vasopressin release *in-vitro*. *Neuroendocrinology* **33**, 295–299.

BOURQUE, C. W. (1986). Calcium-dependent spike after-current induces burst firing in magnocellular neurosecretory cells. *Neuroscience Letters* **70**, 204–209.

BOURQUE, C. W. (1987). Osmotic induction of burst firing in magnocellular neuroendocrine cells: *In vitro* analysis using perfused hypothalamic explants. *Neuroscience Letters Supplement* **29**, S16.

BOURQUE, C. W., RANDLE, J. C. R. & RENAUD, L. P. (1986). Non-synaptic depolarizing potentials in rat supraoptic neurones recorded *in vitro*. *Journal of Physiology* **376**, 493–505.

BOURQUE, C. W. & RENAUD, L. P. (1983). A perfused *in-vitro* preparation of rat hypothalamus for electrophysiological studies on neurosecretory neurones. *Journal of Neuroscience Methods* **7**, 203–214.

BOURQUE, C. W. & RENAUD, L. P. (1984). Activity patterns and osmosensitivity of rat supraoptic neurones in perfused hypothalamic explants. *Journal of Physiology* **349**, 631–647.

BRIMBLE, M. J. & DYBALL, R. E. J. (1977). Characterization of the responses of oxytocin- and vasopressin-secreting neurones in the supraoptic nucleus to osmotic stimulation. *Journal of Physiology* **271**, 253–271.

DUNN, F. L., BRENNAN, T. J., NELSON, A. E. & ROBERTSON, G. L. (1973). The role of blood osmolality and volume in regulating vasopressin secretion in the rat. *Journal of Clinical Investigation* **52**, 3212–3219.

DUTTON, A. & DYBALL, R. E. J. (1979). Phasic firing enhances vasopressin release from the rat neurohypophysis. *Journal of Physiology* **290**, 433–440.

DWYER, T. M., ADAMS, D. J. & HILLE, B. (1980). The permeability of the endplate channel to organic cations in frog muscle. *Journal of General Physiology* **75**, 469–492.

HALLIWELL, J. V. & ADAMS, P. R. (1982). Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Research* **250**, 71–92.

JEWELL, P. A. & VERNEY, E. B. (1957). An experimental attempt to determine the site of neurohypophyseal osmoreceptors in the dog. *Philosophical Transactions of the Royal Society* B **240**, 197–324.

JOHNSON, A. K. (1985). Role of the periventricular tissue surrounding the anteroventral third ventricle (AV3V) in the regulation of body fluid homeostasis. In *Vasopressin*, ed. SCHRIER, R. W., pp. 319–331. New York: Raven Press.

LENG, G. (1980). Rat supraoptic neurones: the effect of locally applied hypertonic saline. *Journal of Physiology* **304**, 405–414.

LENG, G., MASON, W. T. & DYER, R. G. (1982). The supraoptic nucleus as an osmoreceptor. *Neuroendocrinology* **34**, 75–82.

MASON, W. T. (1980). Supraoptic neurones of rat hypothalamus are osmosensitive. *Nature* **287**, 154–157.

POULAIN, D. A. & WAKERLEY, J. B. (1982). Electrophysiology of hypothalamic magnocellular neurones secreting oxytocin and vasopressin. *Neuroscience* **7**, 773–808.

RENAUD, L. P. (1987). Magnocellular neuroendocrine neurons: update on intrinsic properties, synaptic inputs and neuropharmacology. *Trends in Neurosciences* **10**, 498–501.

RENAUD, L. P., NISSEN, R. & BOURQUE, C. W. (1988). Properties of organum vasculosum lamina terminalis (OVLT) neurons in the rat. *Society for Neuroscience Abstracts* **14**, 1179p.

SLADEK, C. D. & ARMSTRONG, W. E. (1985). Neural pathways subserving osmotic control of vasopressin release. In *Vasopressin*, ed. SCHRIER, R. W., pp. 435–441. New York: Raven Press.

VERNEY, E. B. (1947). The antidiuretic hormone and the factors which determine its release. *Proceedings of the Royal Society* B **135**, 25–106.

YAMASHITA, H., INENAGA, K., KAWATA, M. & SANO, Y. (1983). Phasically firing neurons in the supraoptic nucleus of the rat hypothalamus: immunocytochemical and electrophysiological studies. *Neuroscience Letters* **37**, 87–92.