Journal of Physiology (1988), **397**, *pp.* 331–347
With 12 text-figures
Printed in Great Britain

TRANSIENT CALCIUM-DEPENDENT POTASSIUM CURRENT IN MAGNOCELLULAR NEUROSECRETORY CELLS OF THE RAT SUPRAOPTIC NUCLEUS

BY CHARLES W. BOURQUE

From the MRC Neuropharmacology Research Group, Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX

(*Received 8 January 1987*)

SUMMARY

1. Magnocellular neurosecretory neurones were impaled in the supraoptic nucleus of perfused explants of rat hypothalamus. Membrane currents were studied at 35 °C using the single-microelectrode voltage-clamp technique.

2. Depolarizing voltage steps applied from −100 mV evoked a transient outward current (TOC) from a threshold of −75 mV. From this potential, the amplitude of the current increased non-linearly with voltage.

3. Following its activation TOC reached a peak within 7 ms and subsequently decayed monotonically with a time constant of 30 ms. This time constant did not vary significantly with voltage between −75 and −55 mV.

4. The TOC showed complete steady-state inactivation at potentials positive to −55 mV. Inactivation was removed by hyperpolarization, with a mid-point near −80 mV. The removal of inactivation followed a complex time course with distinct fast (tens of milliseconds) and slow (hundreds of milliseconds) components.

5. Tail current measurements revealed that the TOC equilibrium potential (E_{TOC}) lies near −97 mV in the presence of 3 mM $[K^+]_o$. Increasing $[K^+]_o$ caused a decrease of TOC amplitude and a shift in E_{TOC} of 57 mV/log $[K^+]_o$. The TOC is therefore predominantly a K^+ current.

6. The TOC was unaffected by tetraethylammonium (up to 12 mM) but was reversibly reduced by 4-aminopyridine (*ca.* 50% block at 1·0 mM) and dendrotoxin (*ca.* 50% block at 4 nM).

7. The TOC was strongly inhibited (> 70%) by adding Co^{2+} or Mn^{2+} (1–3 mM) or Cd^{2+} (50–400 μM) to Ca-containing solutions, or by removal of Ca^{2+} from the perfusate. These effects were not accompanied by detectable changes in threshold voltage. The amplitude of TOC was also depressed by the organic Ca^{2+} channel blocker methoxyverapamil (D600). Finally replacement of Ca^{2+} by Ba^{2+} in the perfusate completely and reversibly abolished the TOC.

8. These findings suggest that neurosecretory neurones of the rat supraoptic nucleus display a transient K^+ current which is strongly dependent on the presence of external Ca^{2+}. The possible role of this current is briefly discussed.

Present address: Neuroscience Unit, Montreal General Hospital and McGill University, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4, Canada.

INTRODUCTION

The release of oxytocin and vasopressin in the neurohypophysis is controlled by the electrical activity of magnocellular neurosecretory cells (MNCs) located in the hypothalamic supraoptic and paraventricular nuclei. In the rat, MNCs display changes in spontaneous firing rate which are proportional to the intensity of relevant activating physiological stimuli. In addition, both cell types adopt specific and distinct bursting discharge patterns under conditions demanding maximal hormone release (see Poulain & Wakerley, 1982; Renaud, Bourque, Day, Ferguson & Randle, 1985, for review). These responses of MNCs are extremely significant since the amount of neuropeptide release evoked by each of their action potentials is enhanced by an increase in firing rate and is even further potentiated by the appearance of bursting activity (Dreifuss, Kalnins, Kelly & Ruf, 1971; Dutton & Dyball, 1979; Bicknell & Leng, 1981; Cazalis, Dayanithi & Nordmann, 1985).

Recent intracellular recordings obtained *in vitro* have revealed that intrinsic membrane properties account for much of the electrical behaviour of MNCs, including the generation of phasic bursts (Mason, 1980, 1983; Abe & Ogata, 1982; Andrew & Dudek, 1983, 1984; Bourque, 1984, 1986; Bourque & Renaud, 1985; Bourque, Randle & Renaud, 1985, 1986*b*; Bourque, Brown & Renaud, 1986*a*). Direct measurement of their membrane currents under voltage-clamp conditions has also recently become practicable (Bourque, 1986; Bourque & Brown, 1986), and should allow a more detailed examination of their individual conductance systems. Such information will help clarify the role ionic currents play in controlling excitability, as well as how they may interact with various neurotransmitters to effect changes in the discharge characteristics of MNCs.

In this report, the single-electrode voltage-clamp technique was used to examine the properties and ionic basis of a fast-activating transient outward current in MNCs of the rat supraoptic nucleus. This current is remarkably similar to the well-studied K^+ current termed I_A (see Rogawski, 1985, for review) which is found in many excitable cells and plays a role in controlling their near-threshold behaviour. The current observed in MNCs however is strongly calcium dependent and displays other properties which differ from those generally reported for I_A. This current may play an important role in the control of spike duration and membrane potential, as well as firing rate and pattern in MNCs. A portion of these results have appeared in preliminary form (Bourque & Brown, 1986; Bourque, 1987).

METHODS

Preparation and maintenance of perfused hypothalamic explants

Explants of basal hypothalamus (8 × 8 × 2 mm) were removed from ether-anaesthetized Wistar rats (150–300 g) of either sex and pinned to the Sylgard base of a temperature-regulated (34–35 °C) perfusion chamber. Within 4 min of decapitation, the right anterior cerebral artery was cannulated as previously described (Bourque & Renaud, 1983) and perfused with oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF). Gravity-fed solutions flowed at a rate of 0·9–1·3 ml/min through the intact capillary networks supplied by the circle of Willis and out through the open-ended arteries and veins. The fluid emerging from the explant provided a secondary superfusion of the tissue before leaving the chamber. To minimize electrode capacitance, the level of this superfusing layer was adjusted with cotton wicks such that the area of the

supraoptic nucleus was not submerged by more than 0·3 mm. All recordings were obtained within 7 h following cannulation.

Solutions

The composition of the standard ACSF (pH = 7·4) was (mM): NaCl, 126; KCl, 3·0; $MgCl_2$, 1·3; NaH_2PO_4, 1·2; $NaHCO_3$, 25·9; glucose, 10; $CaCl_2$, 2·0–3·0. All voltage-clamp records were obtained in the presence of 0·3–1 μM-tetrodotoxin (TTX). The ACSF was modified (as specified in the text) by the addition of KCl, dendrotoxin (DTX), tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), $BaCl_2$, $CoCl_2$, $MnCl_2$, $MgCl_2$, $CdCl_2$, methoxyverapamil (D600) or ethyleneglycol-bis-(β-aminoethylether)*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA).

Microelectrodes and impalement

Individual cells were impaled with micropipettes prepared by pulling thick-walled glass capillary tubing (1·0 mm o.d., Clark Electromedical Instruments) on a Livingston-type horizontal puller. When filled with 3 M-potassium acetate the tip resistance of these microelectrodes ranged between 70 and 110 MΩ. Filling similar micropipettes with 3 M-KCl yielded tip resistances of 55–90 MΩ. Such electrodes could pass as much as 2–5 nA of current and, with adequate capacitance neutralization, display settling times of less than 100 μs.

The cells were impaled by continuously advancing an electrode with a 'Jena' manipulator (Zeiss Ltd) and applying 25 ms saturating pulses of depolarizing current. Good quality impalements obtained from supraoptic MNCs lasted up to 90 min. These cells displayed an input resistance of 80–400 MΩ and resting potentials of −50 to −73 mV. In the absence of TTX, all cells sustained repetitive firing (> 10 Hz) during strong membrane depolarization and showed a frequency-dependent broadening of their action potentials (amplitude 70–110 mV). These combined characteristics are typical of MNCs impaled in the supraoptic nucleus of the rat (Bourque, 1984).

Voltage clamp and data acquisition

A home-built (see Acknowledgements) 'switching' voltage-clamp amplifier (IVAN-2) based on the design of Wilson & Goldner (1975) was used for intracellular recording of membrane voltage or current in MNCs impaled with a single electrode. In all cases the switching frequency (50% duty cycle) was maximized while being kept sufficiently slow to allow a complete settling of the continuously monitored input voltage. The range of operational frequencies utilized in these experiments was 1–6 kHz with most cases between 2·5 and 4 kHz. Following correct adjustment, the electrotonic voltage responses to repeated current pulses recorded in bridge and switched current-clamp were always identical and superimposed each other when going from one mode to the other (Fig. 1*A*). In most cases, command steps applied under voltage clamp settled within 2–3 ms and capacity currents 1–3 ms thereafter (Fig. 1*B*). Membrane currents activated at potentials above *ca.* −30 mV were usually unreliably clamped and not examined in this report.

The data acquired during these experiments were digitized (44·1 kHz) by a pulse-code modulator (Sony Ltd) and stored on videotape (Sony-Betamax). The pulse-code modulator was modified to convert two analog and four digital tracks, which along with an audio signal were recorded onto tape. All traces shown are original records photographed from a storage oscilloscope. Leakage currents have not been subtracted. Records were measured using a ruler or transparent graph overlay. Averaged values are expressed in terms of mean ± S.E. of mean.

Space-clamp considerations

An assumption of critical importance in the interpretation of any voltage-clamp measurement is that the area of membrane under study is subject to good voltage control, i.e. isopotential. Serial reconstruction of dye-filled MNCs have revealed their simple morphology (Randle, Bourque & Renaud, 1986*b*). From their elongated somata (mean: 25 × 13 μm) emerge 1–3 virtually unbranched dendrites (0·4 branch/dendrite) coursing for an average distance of 377 μm. During the injection of a small current pulse, the recorded electrotonic voltage response of an MNC (when coursing through the linear part of their current–voltage relationship) displays an exponential time course for which only one time constant can be resolved (Fig. 1*A*). Thus, according to the lumped-soma, finite length equivalent cylinder model of Rall (1977), this finding suggests that the MNC behaves as a single, nearly isopotential compartment. In accordance with previous observations (Bourque,

1984) the somatic and dendritic membrane of most MNCs should be under reasonably good spatial control of voltage during command steps applied near the soma.

RESULTS

The data presented below were obtained from eighty-three magnocellular neurosecretory cells (MNCs) impaled in the supraoptic nucleus of seventy-one

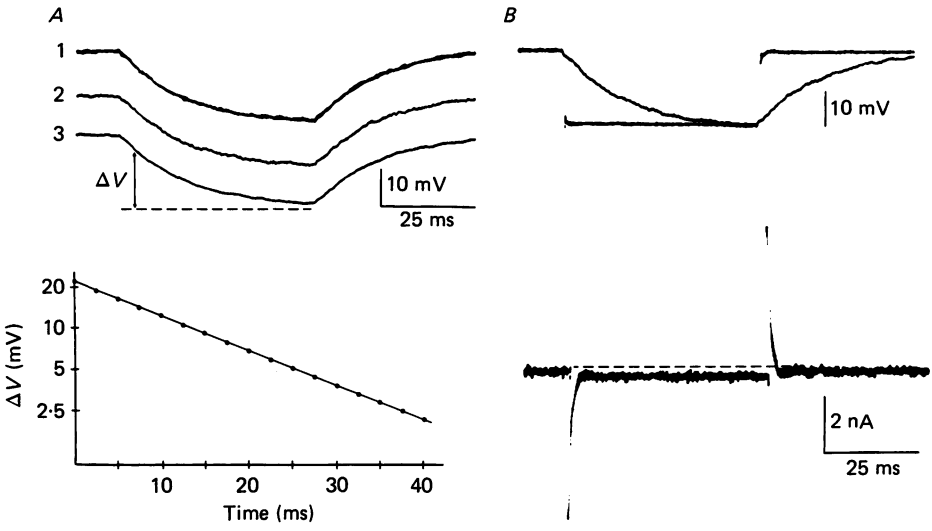

Fig. 1. *A*, electrotonic voltage responses (V) to a hyperpolarizing current pulse are shown for an MNC impaled with a 75 MΩ, KCl-filled microelectrode. The responses recorded in the bridge (trace 3) and discontinuous (trace 2) current-clamp modes superimpose each other when the amplifier is switched from one mode to the other (trace 1). The bottom panel shows, as a semilogarithmic plot, the time course of the electrotonic relaxation shown above. *B*, the top trace compares the electrotonic voltage response recorded in *A* with a hyperpolarizing command of similar amplitude applied under voltage clamp. Note that the voltage trace settles within 2–3 ms. The bottom trace is the corresponding membrane current response recorded in voltage clamp. Note that the large capacity currents are complete within 3–4 ms.

perfused explants of rat hypothalamus. These cells displayed an average resting membrane potential of $-60{\cdot}1 \pm 0{\cdot}7$ mV and an input conductance of $5{\cdot}3 \pm 0{\cdot}2$ nS (189 ± 5 MΩ). Spike amplitudes ranged between 70 and 110 mV. These values are comparable to previous observations (Mason, 1983; Andrews & Dudek, 1983, 1984; Bourque & Renaud, 1985; Bourque *et al*, 1985, 1986*a*, *b*) and suggest that the use of relatively low-resistance microelectrodes did not cause significant injury to the impaled neurones.

Observations in current clamp

As previously described (Mason, 1983) MNCs displayed a linear voltage–current relationship between -60 and -100 mV when measured from an initial (DC) potential near -60 mV (Fig. 2). However, when this initial membrane potential was artificially hyperpolarized by sustained current injection, the voltage–current

relationship of the cells displayed an outward rectification at potentials positive to *ca.* −75 mV. The amplitude of this rectification increased as the initial membrane voltage was made more negative and at potentials of −100 mV the slope conductance of the cell measured at −60 mV was increased by as much as 500% compared to that measured with an initial potential of −60 mV (Fig. 2*B*, inset). The characteristics of the membrane current underlying this behaviour are presented in the remainder of this section.

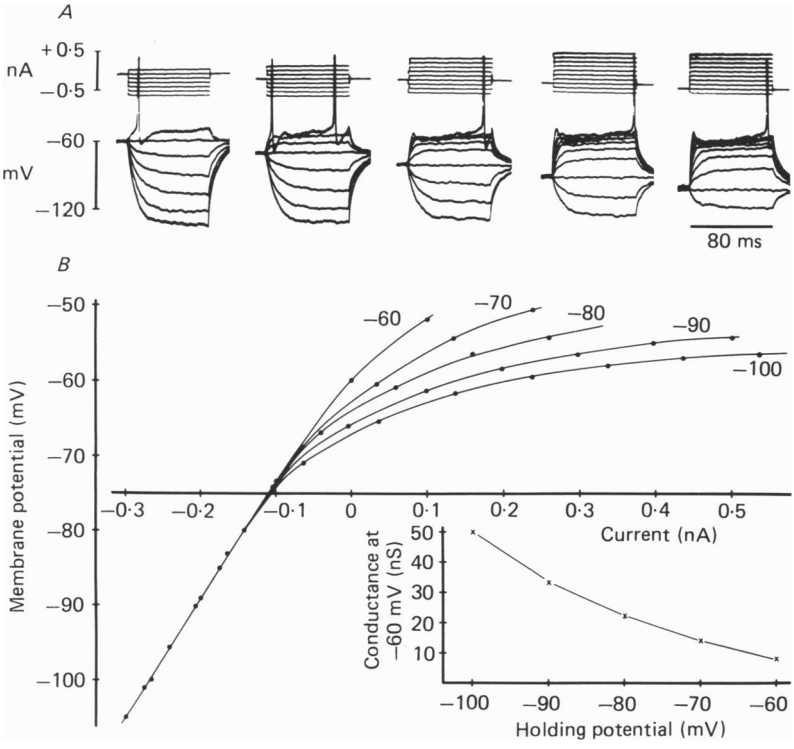

Fig. 2. *A*, voltage responses (lower traces) to current pulses (upper traces) applied to an MNC held at 'initial' potentials of −60, −70, −80, −90 and −100 mV. Note that at hyperpolarized initial potentials a negative-going notch appears on the depolarizing responses and that a larger abolute depolarizing current is required to achieve spike threshold. *B*, membrane voltage responses measured 40 ms after pulse onset are plotted as a function of the corresponding absolute current. As the initial potential is made progressively more negative an increasing amount of outward rectification is observed at voltages positive to *ca.* −75 mV. The inset shows the effect of changes in initial potential on the membrane conductance (reciprocal of the slope) measured at −60 mV.

General observations in voltage clamp

In a typical experiment a neurone was impaled in the control solution and its behaviour was examined in current clamp. Subsequent addition of 0·3–1·0 μM-TTX abolished the Na^+-dependent component of the action potential but did not affect the input resistance or the transient outward rectifier of each of forty-seven MNCs tested (Fig. 3). Under voltage-clamp conditions, depolarizing clamp commands could elicit a transient outward current (TOC) when the holding potential was made more

negative than −60 mV (Fig. 3*C*). In most MNCs the late part of the rising phase of TOC could be detected and a clear peak recorded. The activation voltage threshold measured in fifty-seven cells was −74·5±4·2 mV and the amplitude of the current increased non-linearly with voltage (see Fig. 6). While the rate of onset of TOC could

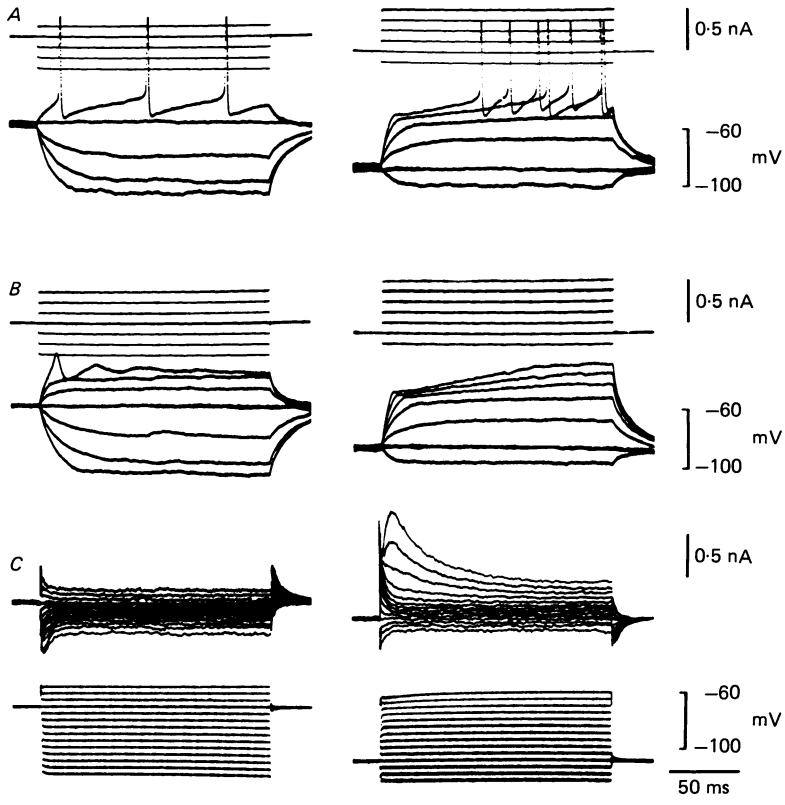

Fig. 3. *A* and *B*, current-clamp recordings obtained from an MNC illustrate that the outwardly rectifying behaviour which appears during depolarizing pulses applied from negative initial potentials in control solutions (*A*) is not blocked by the addition of 0·5 μM-TTX (*B*). This treatment, however, clearly abolishes the low-threshold Na^+-dependent action potentials. *C*, the membrane current (upper) responses of the same cell to voltage-clamp (lower) commands spanning a similar range of potentials. Note that a fast-activating transient outward current (TOC) is elicited during depolarizing commands applied from negative, but not positive, holding potentials.

not be reliably analysed, activation of the current was rapid, reaching a peak within 5–7 ms. From this peak value the current decayed exponentially with a time constant of 30·1±0·9 ms ($n = 17$ cells). This value was not strongly affected by voltage between −75 and −55 mV (Fig. 4).

Charge carrier for the transient outward current

The reversal potential of TOC (E_{TOC}) was determined using the experimental protocol shown in Fig. 5*A*. The current was activated by a step to −40 mV from a holding potential near −100 mV. A few milliseconds after TOC had reached a peak,

the membrane potential was clamped to a variety of voltages between −130 and −40 mV. The amplitude of the resulting current tails was plotted as a function of the test potential as shown in Fig. 5*B*. From this procedure, the average value of E_{TOC} obtained from seven cells was −96·7 ± 1·8 mV. Upon raising $[K^+]_o$, the amplitude of TOC was decreased and the value of E_{TOC} changed to a more positive level ($n = 3$

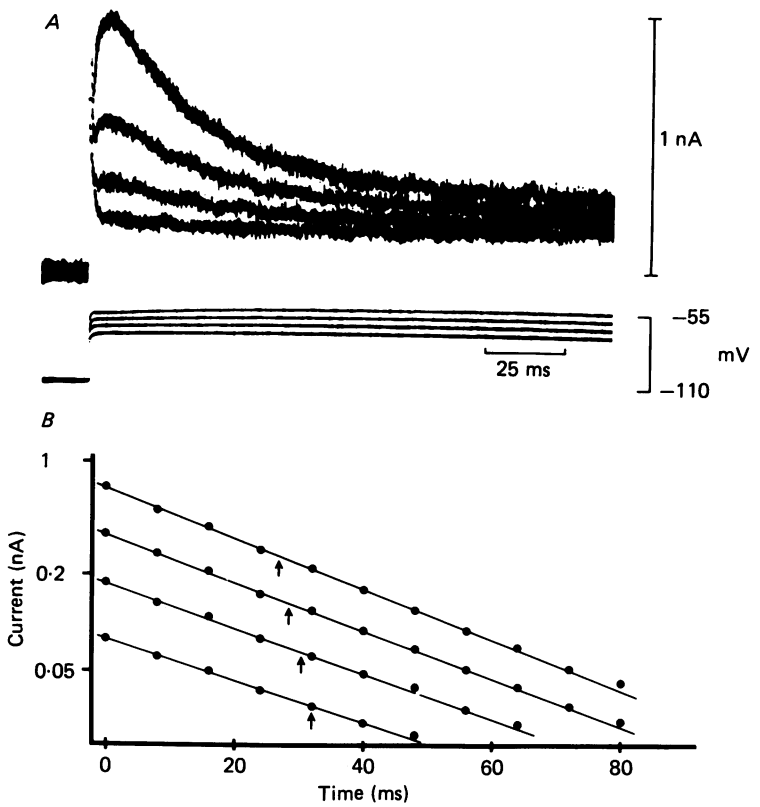

Fig. 4. *A*, the membrane current responses of an MNC to depolarizing voltage-clamp commands applied from −110 mV. The TOC first appears during clamp commands to −75 mV (lowest trace) and in all cases reaches a peak within 7 ms of the onset of the voltage command. *B*, the decay phase of each response is plotted semilogarithmically. Note that at all potentials TOC decays mono-exponentially and that the time constant of this decay (arrows) is not strongly affected by voltage.

cells). This shift amounted to 57·3 ± 2·9 mV/log $[K^+]_o$ and approximates that predicted for E_K by the Nernst equation (59·9 mV/log $[K^+]_o$) at 34 °C. These observations suggest that TOC is almost exclusively carried by K^+.

Removal of transient outward current inactivation

The removal of TOC inactivation during a period of hyperpolarization was both time and voltage dependent. The voltage dependence of the process was examined in twenty-three cells by clamping the membrane potential near −45 mV and measuring the amplitude of TOC triggered at this potential following 1–5 s steps to more

negative potentials. As seen in Fig. 6, TOC was fully inactivated at potentials positive to −55 mV, with the steady-state inactivation being removed as a sigmoid function of voltage with half-removal at $-82{\cdot}4 \pm 1{\cdot}1$ mV.

The time dependence for the removal of TOC inactivation was examined in ten cells using the protocol illustrated in Fig. 7*A*. In all cases the removal of inactivation

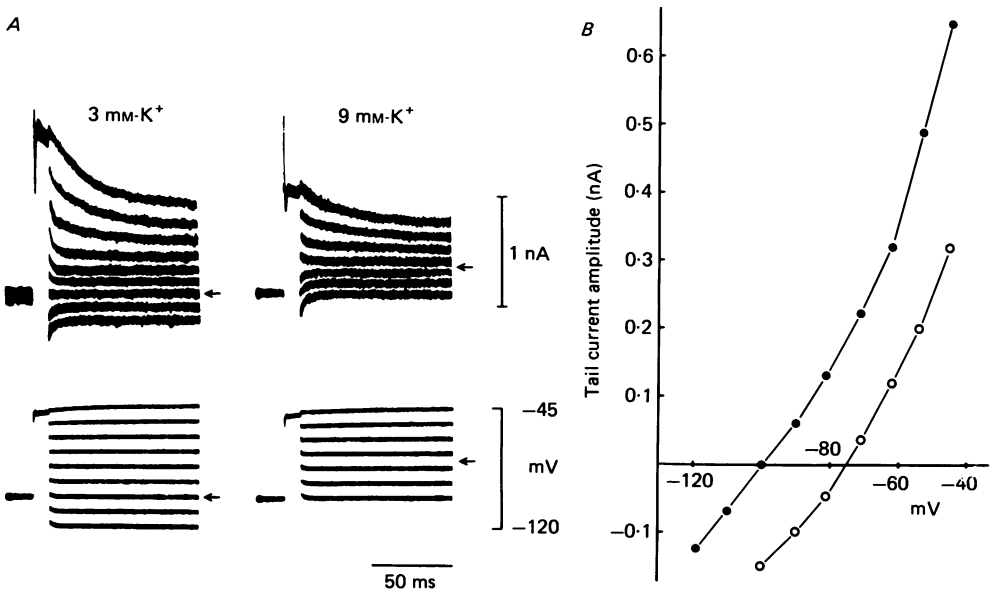

Fig. 5. Measurement of the reversal or equilibrium potential for TOC (E_{TOC}) by tail current analysis. *A*, TOC was triggered by a depolarizing command to −45 mV from a holding potential near −100 mV. Shortly after TOC had reached a peak the membrane voltage was clamped to a variety of test potentials and the resulting current tails examined. Leakage currents were not subtracted for this analysis since, except for the very brief capacity transients (not shown), no other time-dependent currents were observed between −60 and −120 mV or activated by the depolarizing pulse in the cells studied. Note that raising $[K^+]_o$ causes a decrease of TOC amplitude during the activating step and a positive shift of E_{TOC} (arrows). *B*, the amplitude of the tails (peak minus steady-state current) are plotted against test potential. The measured values of E_{TOC} in 3 and 9 mM $[K^+]_o$ were −100 and −75 mV, respectively.

followed a complex time course for which at least two time constants could be distinctly resolved (Fig. 7*C*). In individual cells the slower component (τ range 280–600 ms) did not appear to vary significantly with conditioning voltage, but the time constant of the faster component (range 12–50 ms at −100 mV) became longer as the conditioning potential was made more positive.

Pharmacology of the transient outward current in magnocellular neurosecretory cells

The effects of various K^+ channel blockers on TOC were examined in thirty-one MNCs. Addition of 2–12 mM-TEA to the perfusate did not reduce the amplitude of TOC measured in any of fifteen cells examined. In fact, at potentials positive to −55 mV, TEA increased the peak-minus-steady-state measurement of TOC. This effect resulted from the inhibition of a slower-rising, sustained current activated at

potentials more positive than −55 mV (Fig. 8*B*). In contrast, TOC was blocked in a reversible, dose-dependent manner by 4-AP (51·7 % at 1 mM). In voltage clamp the effects of 4-AP consistently became apparent at a concentration of 0·5 mM and were virtually maximal at 5 mM (Fig. 8*A*). Furthermore, similar to the transient K^+ current (I_A) of hippocampal pyramidal cells (Halliwell, Othman, Pelchen-Matthews & Dolly, 1986), TOC in MNCs was also potently affected by dendrotoxin (DTX). At concentrations of 4–20 nM, DTX caused more than a 50 % decrease in TOC with little

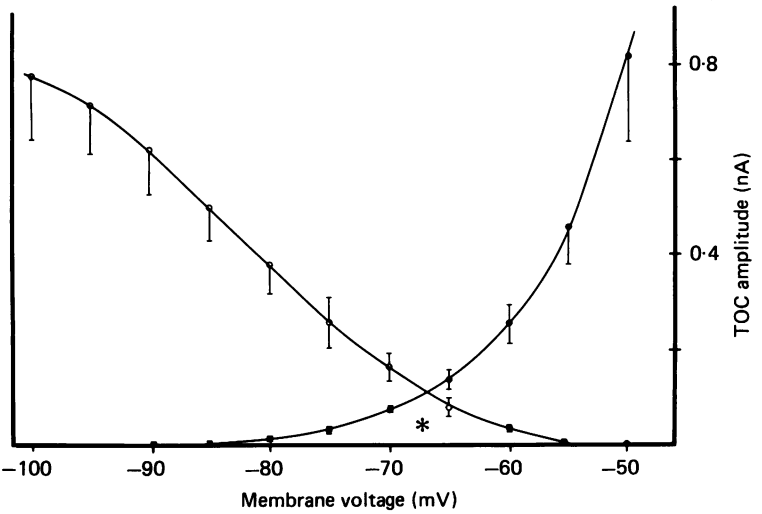

Fig. 6. Voltage dependence of TOC activation (●) and inactivation (○) averaged (mean ± S.E.M.) from thirteen MNCs. The filled circles plot the amplitude of TOC (peak minus steady-state current) as a function of the voltage of clamp commands applied from −100 mV. The open circles illustrate the amplitude of TOC measured at −50 mV (in the same cells) as a function of the voltage achieved during a conditioning hyperpolarizing pulse lasting 1–5 s. Note the wide overlap (*) between the activation and inactivation curves of TOC in MNCs.

change in leak conductance (Fig. 8*C*). During current-clamp observations both DTX ($n = 4$ cells) and 4-AP ($n = 8$ cells) reduced the outward rectification observed from negative initial potentials and increased the duration of action potentials triggered from potentials negative to −55 mV (see Fig. 12*A*).

Ca^{2+} dependence of transient outward current

The effects of Ca^{2+} channel blockers were examined in twenty-three MNCs. Addition of 1–2 mM-Co^{2+} or Mn^{2+} to Ca^{2+}-containing solutions (Fig. 9*A*) or isomolar replacement of Ca^{2+} by Mg^{2+} in the perfusate (Fig. 9*B*) consistently and reversibly reduced TOC amplitude by more than 70 %. These effects took place without detectable changes in threshold voltage for TOC activation. Similarly, the TOC was reversibly reduced in a dose-dependent manner by the addition of Cd^{2+} to Ca^{2+}-containing solutions (Fig. 10). Experiments using various concentrations of Cd^{2+} revealed that most (> 70 %) of TOC can be blocked before a positive shift in activation threshold or in the inactivation curve can be detected (Fig. 10*B*).

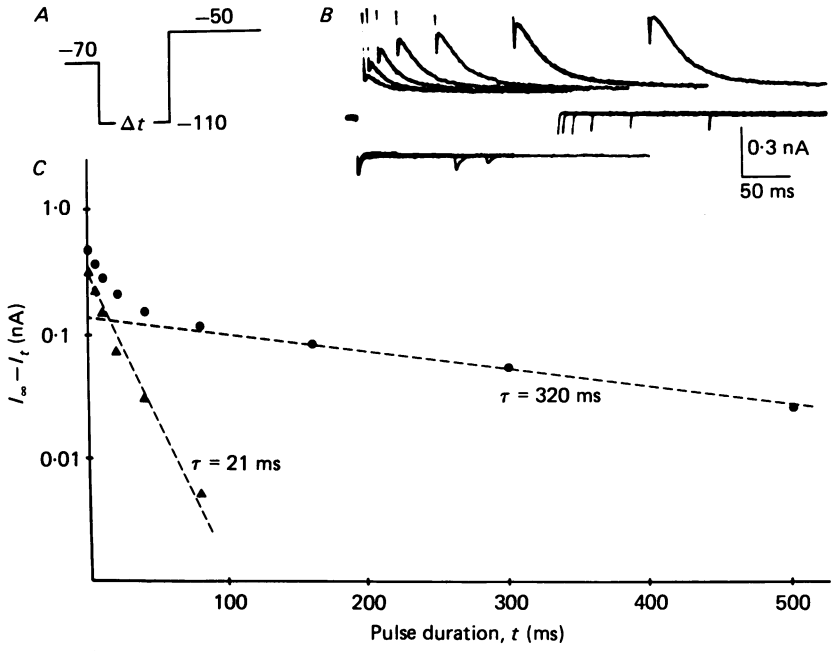

Fig. 7. Time dependence of the removal of TOC inactivation. *A*, a schematic representation; the cell was clamped at −70 mV (resting potential) and TOC amplitude was measured at −50 mV following a conditioning hyperpolarizing pulse of variable duration. *B*, superimposed membrane current responses for conditioning pulses lasting 5, 10, 20, 40, 80, 160 and 300 ms. *C*, the filled circles plot the residual steady-state inactivation of TOC ($I_\infty - I_t$, where I_∞ is the amplitude of TOC measured following a 10 s hyperpolarizing pulse) as a function of pulse duration (t). From this record the long time constant (τ) of 320 ms was obtained by drawing a line (fitted by eye) through the terminal decay. Note that a second briefer time constant (21 ms) was also 'peeled' by replotting the difference between the early data points and the extrapolated exponential function underlying the slow process (▲).

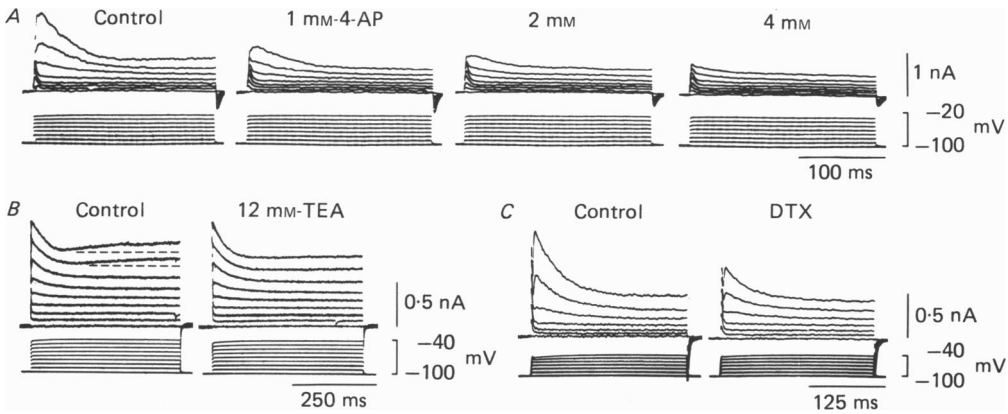

Fig. 8. Effects of K^+ channel blockers on TOC. *A*, a dose-dependent reduction in TOC amplitude following addition of 4-AP to the perfusate (pH adjusted to 7·4). *B*, longer-lasting voltage-clamp commands were used to reveal the appearance of a slower-rising and sustained outward current at potentials positive to −55 mV (top two traces). Note that adding 12 mM-TEA to the solution does not affect the peak amplitude of TOC, but strongly depresses the 'late' outward current. *C*, the traces illustrate the effects of a 3 min application of DTX (4 nM).

Furthermore, in two of three cells, the TOC was reduced by the addition of 14 and 26 μM-methoxyverapamil (D600) (Fig. 9*C*). Addition of 2 μM-D600 failed to affect the TOC examined in a third cell (not shown). Finally, isomolar replacement of Ca^{2+} by Ba^{2+} completely and reversibly abolished TOC in each of four cells tested (Fig. 11).

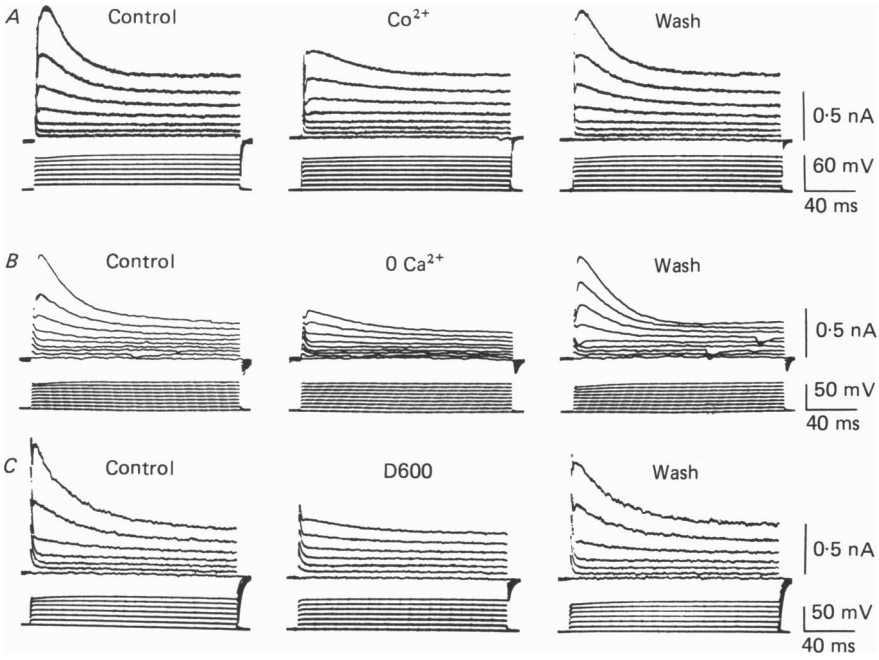

Fig. 9. Calcium dependence of TOC. *A*, addition of 2 mM-Co^{2+} to perfusate already containing 2·5 mM-Ca^{2+} caused a 70% reduction of TOC amplitude. *B*, removal of the Ca^{2+} from the solution (and addition of 2 mM-Mg^{2+}) led to a gradual reduction of TOC amplitude. The middle trace in *B* was recorded 35 min after removing Ca^{2+} and represents a 73% decrease from control. Recovery from this treatment occurred within 4 min of returning to the Ca^{2+}-containing solution. *C*, addition of 14 μM-D600 caused an 80% decrease in TOC amplitude which was gradually reversed during a 10 min wash with normal solution. Note that each effect took place without changing the threshold voltage for the activation of TOC.

DISCUSSION

Voltage dependence and kinetics of transient outward current

The present results indicate that MNCs of the rat supraoptic nucleus display a prominent inactivating K^+ current which is highly reminiscent of the current termed I_A (see Rogawski, 1985, for review). Similar to I_A in other vertebrate neurones, TOC in MNCs reaches a peak within 7 ms and subsequently decays monotonically with a time constant of *ca.* 30 ms. Furthermore, TOC in MNCs is completely inactivated at potentials positive to -55 mV and this inactivation is removed in a time- and voltage-dependent manner by membrane hyperpolarization. Unlike I_A, however, removal of TOC inactivation at a given voltage was found to show distinct fast and slow components. In addition, the activation threshold for TOC was near -75 mV,

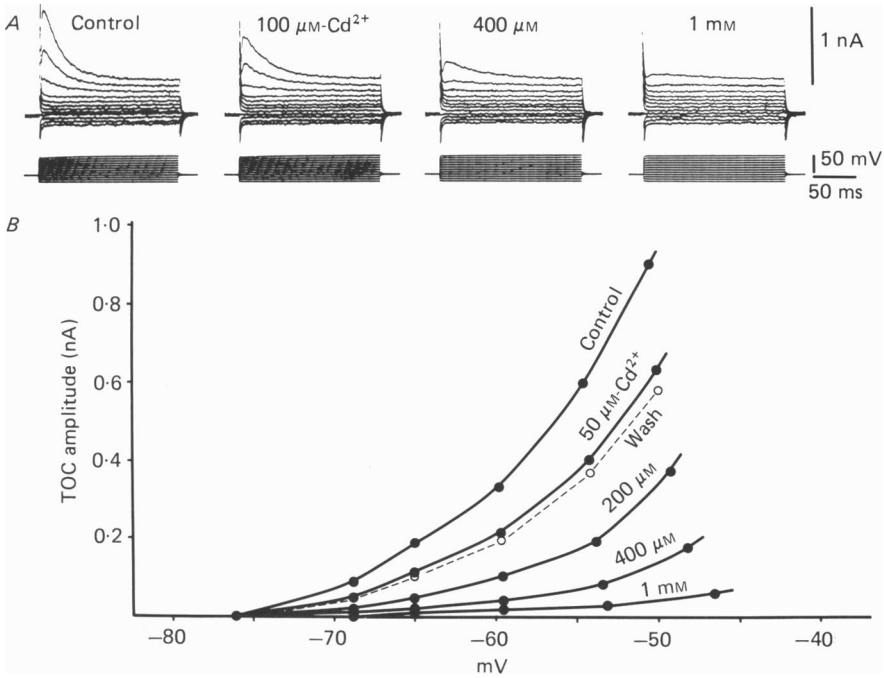

Fig. 10. Effects of Cd^{2+} on TOC. *A* shows the dose-dependent effects of different concentrations of Cd^{2+} added to a Ca^{2+}-containing perfusate. *B* shows the activation curves for TOC measured in another cell during a similar experiment. Note that the TOC is strongly reduced by Cd^{2+} and that at concentrations of up to 400 μM this effect occurs without a positive shift in the threshold voltage of the activation curve. The mid-point (−83 mV) of the steady-state inactivation curve was unaffected by 200 μM-Cd^{2+} (not shown).

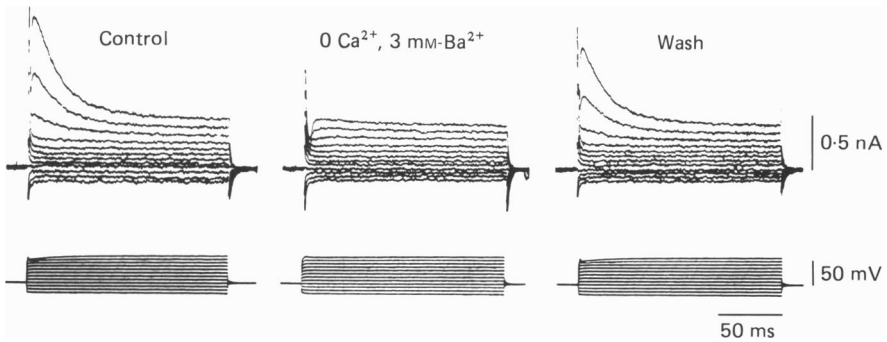

Fig. 11. In this experiment the Ca^{2+} (3 mM) was removed from the solution and replaced by Ba^{2+} (3 mM). This procedure rapidly (< 2 min) and reversibly abolished the TOC.

a value which is considerably more negative than those previously reported for I_A in mammalian neurones (Gustafsson, Galvan, Grafe & Wigstrom, 1982; Brown, Adams & Constanti, 1982; Galvan & Sedlmeir, 1984; Segal & Barker, 1984; Aghajanian, 1985; Belluzzi, Sacchi & Wanke, 1985; Cooper & Schrier, 1985; Zbicz & Weight, 1985).

Calcium dependence of transient outward current

Perhaps the most outstanding feature of TOC recorded in MNCs is its dependence on the presence of extracellular Ca^{2+}. The role played by extracellular Ca^{2+} in the control of inactivating K^+ currents has received an increasing amount of attention. In most cases however it would appear that only a small component of I_A is Ca^{2+} dependent (Mounier & Vassort, 1975; Thompson, 1977; Galvan & Sedlmeir, 1984; Segal & Barker, 1984; Junge, 1985) or that an altogether different type of membrane current is involved (Siegelbaum & Tsien, 1980; MacDermott & Weight, 1982; Brown, Constanti & Adams, 1983; Salkoff, 1983; Zbicz & Weight, 1985; Mallart, 1985; Gho & Mallart, 1986). Whether a 'conventional' or non-Ca^{2+}-dependent I_A is also present in MNCs is difficult to assess from the available data. If present however it would presumably only contribute a small proportion of the total current since up to 90% of TOC could be blocked by Cd^{2+} at concentrations which did not affect its voltage threshold and which are without effect on I_A in other neurones (Gustafsson *et al.* 1982; Halliwell *et al.* 1986).

The implication of these findings is that Ca^{2+} influx into MNCs strongly influences the gating of TOC channels. It is worth noting that a low-threshold Ca^{2+} current found in rat sensory neurones (Bossu, Feltz & Thomann, 1985) has been reported to display an activation threshold as negative as -75 mV (Fedulova, Kostyuk & Veselovsky, 1985). The presence of such a current in MNCs could therefore participate in the activation of TOC. Preliminary experiments using Ba^{2+} as a charge carrier suggest that this type of conductance may indeed exist in MNCs (Bourque, 1987). Interestingly, the low-threshold Ca^{2+} current of sensory neurones recovers from inactivation in a complex time-dependent manner which requires up to several seconds (Bossu *et al.* 1985). The slow time-dependent component of the removal of TOC inactivation in MNCs could presumably reflect the occurrence of such a process. These problems clearly require further analysis.

Pharmacological sensitivity of transient outward current

A possible clue to the nature of the channel 'type' underlying TOC in MNCs may reside in its sensitivity to pharmacological blockers. In contrast to other 'transient Ca^{2+}-dependent K^+ currents' (e.g. Mounier & Vassort, 1975; MacDermott & Weight, 1982; Mallart, 1985; Zbicz & Weight, 1985; Gho & Mallart, 1986) TOC in MNCs was completely unaffected by TEA at concentrations of up to 12 mM. In fact TOC became easier to examine in the presence of TEA, particularly at potentials above -55 mV where TEA abolished a slower-rising but overlapping current. In contrast, TOC was markedly sensitive to agents known to block I_A in other neurones (i.e. 4-AP and DTX). The TOC of MNCs may therefore represent a genuinely Ca^{2+}-dependent form of I_A.

Role of transient outward current in magnocellular neurosecretory cells

The kinetic and voltage-dependent characteristics of TOC measured in this study suggest that this current could play several important functions in MNCs. First, because a substantial portion of the channels is available at high resting potentials, activation of these channels by depolarization and Ca^{2+} influx during a spike may contribute to its repolarization, as well as to the peak and initial phase of the

hyperpolarizing after-potential. This possibility is supported by current-clamp recordings which show that spike duration is decreased by raising $[Ca^{2+}]_o$ (unpublished observation) or increased by adding 4-AP to the perfusate (Fig. 12). Furthermore, a portion of the inactivation of TOC may be removed during the hyperpolarization associated with the hyperpolarizing after-potential. This would allow some TOC channels to be opened during the depolarizing drift associated with the decay of hyperpolarizing after-potential, thus prolonging the interspike interval (Connor & Stevens, 1971). The TOC may therefore play an important role in

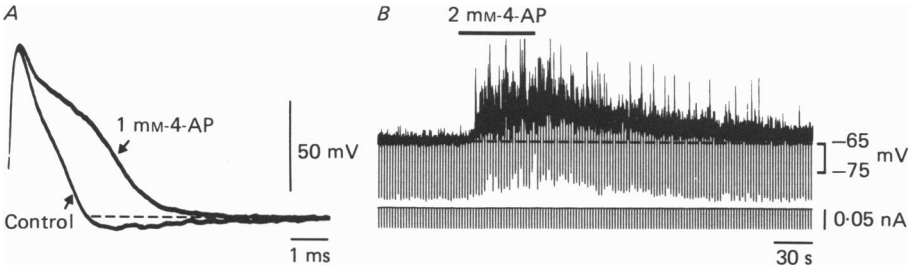

Fig. 12. *A*, addition of 4-AP to the perfusate prolonged the duration of the action potentials triggered by depolarizing current pulses applied from an initial potential of −70 mV (0·5 Hz). *B*, addition of 4-AP to the perfusate caused a small depolarization and a 9% increase in membrane resistance.

controlling the firing rate of MNCs. Finally, the activation and inactivation curves of TOC (Fig. 6) overlap each other over the precise range of resting potentials normally reported to occur in MNCs (e.g. Mason, 1983; Andrew & Dudek, 1984; Bourque & Renaud, 1985). It is therefore possible that a small steady-state current is carried through TOC channels. While such a current may be very small, given the very low resting conductance of MNCs (2–10 nS), a sustained current as small as 10 pA could contribute up to 5 mV of their resting potential. Addition of 1–4 mM-4-AP to the perfusate consistently increased membrane noise and depolarized MNCs with initial membrane potentials near −65 mV (Fig. 12*B*). This effect was accompanied by a small increase of membrane resistance and could therefore reflect the inhibition of a small (4-AP-sensitive) steady-state outward current. Lower concentrations of 4-AP (20–100 μM) consistently caused a large increase in membrane noise (not shown) but an accompanying membrane depolarization only appeared at concentrations capable of blocking the TOC (i.e. 0·5–5 mM). The depolarizing action of 4-AP was therefore presumably of postsynaptic origin. It should be noted that many of the parameters involved in the generation of bursting activity in MNCs are strongly voltage dependent over this precise range of potentials (Bourque, 1986; Bourque *et al.* 1986*a*, *b*). The TOC is therefore poised to influence both the *rate* and *pattern* of firing in MNCs. Modulation of this intrinsic membrane current by neurotransmitters could therefore mediate part of the afferent neural control of the electrical behaviour of these cells. Depression of I_A by α-adrenergic agonists (Alkon, 1984; Aghajanian, 1985) or by serotonin (Wu & Farley, 1984) has already been implicated in the control of electrical excitability. Current-clamp recordings have

recently suggested this may also be the case in MNCs (Randle, Bourque & Renaud, 1986*a*).

This research was supported by a programme grant to Professor D. A. Brown. I thank Professor D. A. Brown and Dr J. V. Halliwell for their helpful comments, C. J. Courtice for building much of the electronic apparatus and Miss K. Marwa for typing the manucript. The author is a Post-doctoral Fellow of 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.

AGHAJANIAN, G. K. (1985). Modulation of a transient outward current in serotonergic neurones by α_1-adrenoreceptors. *Nature* **315**, 501–503.

ALKON, D. L. (1984). Calcium-mediated reduction of ionic currents: A biophysical memory trace. *Science* **226**, 1037–1045.

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

ANDREW, R. D. & DUDEK, F. E. (1984). Intrinsic inhibition in magnocellular neuroendocrine cells of rat hypothalamus. *Journal of Physiology* **353**, 171–185.

BELLUZZI, O., SACCHI, O. & WANKE, E. (1985). A fast transient outward current in the rat sympathetic neurone studied under voltage-clamp conditions. *Journal of Physiology* **358**, 91–108.

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

BOSSU, J. L., FELTZ, A. & THOMANN, J. M. (1985). Depolarization elicits two distinct calcium currents in vertebrate sensory neurones. *Pflügers Archiv* **403**, 360–368.

BOURQUE, C. W. (1984). Membrane properties of rat supraoptic nucleus neurons *in vitro*. Ph.D. Thesis, McGill University, Montreal, p. 323.

BOURQUE, C. W. (1986). Calcium-dependent spike after-current induces burst firing in magnocellular neurosecretory cells. *Neuroscience Letters* **709**, 204–209.

BOURQUE, C. W. (1987). Calcium-dependence of a transient potassium current in magnocellular neurosecretory cells of the rat supraoptic nucleus. *Neuroscience Letters Supplement* **29**, S18.

BOURQUE, C. W. & BROWN, D. A. (1986). A transient outward current in voltage-clamped supraoptic nucleus neurosecretory neurons. *Proceedings of the International Union of Physiological Sciences* **16**, 550.

BOURQUE, C. W., BROWN, D. A. & RENAUD, L. P. (1986*a*). Barium ions induce prolonged plateau depolarizations in neurosecretory neurones of the adult rat supraoptic nucleus. *Journal of Physiology* **375**, 573–586.

BOURQUE, C. W., RANDLE, J. C. R. & RENAUD, L. P. (1985). Calcium-dependent potassium conductance in rat supraoptic nucleus neurosecretory neurons. *Journal of Neurophysiology* **54**, 1375–1382.

BOURQUE, C. W., RANDLE, J. C. R. & RENAUD, L. P. (1986*b*). 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 hypothalamus for electrophysiological studies on neurosecretory neurons. *Journal of Neuroscience Methods* **7**, 203–214.

BOURQUE, C. W. & RENAUD, L. P. (1985). Calcium-dependent action potentials in rat supraoptic neurosecretory neurones recorded *in vitro*. *Journal of Physiology* **363**, 419–428.

BROWN, D. A., ADAMS, P. R. & CONSTANTI, A. (1982). Voltage-sensitive K-currents in sympathetic neurons and their modulation by neurotransmitters. *Journal of the Autonomic Nervous System* **6**, 23–35.

BROWN, D. A., CONSTANTI, A. & ADAMS, P. R. (1983). Ca-activated potassium current in vertebrate sympathetic neurones. *Cell Calcium* **4**, 407–420.

CAZALIS, M., DAYANITHI, G. & NORDMANN, J. J. (1985). The role of patterned burst and interburst interval on the excitation-coupling mechanism in the isolated rat neural lobe. *Journal of Physiology* **369**, 45–60.

CONNOR, J. A. & STEVENS, C. F. (1971). Prediction of repetitive firing behaviour from voltage-clamp data on an isolated neurone soma. *Journal of Physiology* **212**, 31–52.

COOPER, E. & SCHRIER, A. (1985). Single-channel analysis of fast transient potassium currents from rat nodose neurones. *Journal of Physiology* **369**, 199–208.

DREIFUSS, J. J., KALNINS, I., KELLY, J. S. & RUF, K. B. (1971). Action potentials and release of neurohypophysial hormones *in vitro*. *Journal of Physiology* **215**, 805–817.

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

FEDULOVA, S. A., KOSTYUK, P. G. & VESELOVSKY, N. S. (1985). Two types of calcium channels in the somatic membrane of new-born rat dorsal root ganglion neurones. *Journal of Physiology* **359**, 431–446.

GALVAN, M. & SEDLMEIR, C. (1984). Outward currents in voltage-clamped rat sympathetic neurones. *Journal of Physiology* **356**, 115–133.

GHO, M. & MALLART, A. (1986). Two distinct calcium-activated potassium currents in larval muscle fibres of *Drosophila melanogaster*. *Pflügers Archiv* **407**, 526–533.

GUSTAFSSON, B., GALVAN, M., GRAFE, P. & WIGSTROM, H. (1982). A transient outward current in a mammalian central neurone blocked by 4-aminopyridine. *Nature* **299**, 252–254.

HALLIWELL, J. V., OTHMAN, I. B., PELCHEN-MATTHEWS, A. & DOLLY, J. O. (1986). Central action of dendrotoxin: Selective reduction of a transient K conductance in hippocampus and binding to localized acceptors. *Proceedings of the National Academy of Sciences of the U.S.A.* **83**, 493–497.

JUNGE, D. (1985). Calcium-dependence of A-currents in perfused Aplysia neurons. *Brain Research* **346**, 294–300.

MACDERMOTT, A. B. & WEIGHT, F. F. (1982). Action potential repolarization may involve a transient, Ca-sensitive outward current in a vertebrate neurone. *Nature* **300**, 185–188.

MALLART, A. (1985). A calcium-activated potassium current in motor nerve terminals of the mouse. *Journal of Physiology* **368**, 577–591.

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

MASON, W. T. (1983). Electrical properties of neurones recorded from the rat supraoptic nucleus *in vitro*. *Proceedings of the Royal Society* B **217**, 141–161.

MOUNIER, Y. & VASSORT, G. (1975). Evidence for a transient potassium membrane current dependent on calcium influx in crab muscle fibre. *Journal of Physiology* **251**, 609–625.

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

RALL, W. (1977). Core conductor theory and cable properties of neurons. In *Handbook of Physiology*, section 1, *The Nervous System*, ed. KANDEL, E. R., pp. 39–97. Baltimore, MD, U.S.A.: Williams & Wilkins.

RANDLE, J. C. R., BOURQUE, C. W. & RENAUD, L. P. (1986*a*). α_1-Adrenergic receptor activation depolarizes rat supraoptic neurosecretory neurons *in vitro*. *American Journal of Physiology* **251**, R569–574.

RANDLE, J. C. R., BOURQUE, C. W. & RENAUD, L. P. (1986*b*). Serial reconstruction of Lucifer Yellow-labeled supraoptic nucleus neurons in perfused rat hypothalamic explants. *Neuroscience* **17**, 453–467.

RENAUD, L. P., BOURQUE, C. W., DAY, T., FERGUSON, A. V. & RANDLE, J. C. R. (1985). Electrophysiology of mammalian hypothalamic supraoptic and paraventricular neurosecretory cells. In *The Electrophysiology of the Secretory Cell*, ed. POISNER, A. M. & TRIFARO, J., pp. 165–194. Amsterdam: Elsevier Science Publishers B.V.

ROGAWSKI, M. A. (1985). The A-current: how ubiquitous a feature of excitable cells is it? *Trends in Neurosciences* **8**, 214–219.

SALKOFF, L. (1983). Drosophila mutants reveal two components of fast outward current. *Nature* **302**, 249–251.

SEGAL, M. & BARKER, J. L. (1984). Rat hippocampal neurons in culture: potassium conductances. *Journal of Neurophysiology* **51**, 1409–1433.

SIEGELBAUM, S. A. & TSIEN, R. W. (1980). Calcium-activated transient outward current in calf cardiac Purkinje fibres. *Journal of Physiology* **299**, 485–506.

THOMPSON, S. H. (1977). Three pharmacologically distinct potassium channels in molluscan neurones. *Journal of Physiology* **265**, 465–488.

WILSON, W. A. & GOLDNER, M. M. (1975). Voltage-clamping with a single electrode. *Journal of Neurobiology* **6**, 411–422.

WU, R. & FARLEY, J. (1984). Serotonin reduces K currents and enhances a Ca^{2+} current in Hermissenda type B photoreceptors. *Society for Neuroscience Abstracts* **10**, 620 p.

ZBICZ, K. L. & WEIGHT, F. F. (1985). Transient voltage and calcium-dependent outward currents in hippocampal CA3 pyramidal neurons. *Journal of Neurophysiology* **53**, 1038–1058.