L-, N- and T- but neither P- nor Q-type Ca^{2+} channels control vasopressin-induced Ca^{2+} influx in magnocellular vasopressin neurones isolated from the rat supraoptic nucleus

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1. The role of voltage-dependent Ca^{2+} channels during vasopressin and oxytocin actions on their respective neurones has been analysed by measuring intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in individual, freshly dissociated magnocellular neurones from rat supraoptic nucleus (SO) using microspectrofluorimetry.

2. Pre-incubation of vasopressin-sensitive neurones with Cd^{2+} (100 μM), a non-discriminatory high-voltage-activated Ca^{2+} channel antagonist, or Ni^{2+} (50 μM), a blocker of T-type Ca^{2+} current, reduced $[Ca^{2+}]_i$ responses by 77 and 19%, respectively. When Cd^{2+} was given together with Ni^{2+}, the response was blocked by 92%. Similarly, when Ni^{2+} was pre-incubated with Cd^{2+}, the response was blocked by ~84%.

3. Exposure of vasopressin-sensitive neurones to a specific Ca^{2+} channel blocker, nicardipine (L-type) reduced vasopressin responses by 48% at 1 μM and 62% at 5 μM. Similarly, ω-conotoxin GVIA (ω-CgTX, N-type; 500 nM) inhibited the response by 46% with a stronger inhibition (75%) at 800 nM. By contrast, neither ω-agatoxin IVA (ω-Aga IVA; 300 nM), which blocks both P- and Q-type channels, nor synthetic ω-conotoxin MVIIC (ω-MVIIC; 100 or 500 nM), a Q-type blocker, affected vasopressin-induced $[Ca^{2+}]_i$ responses. These antagonists, given together (nicardipine 5 μM + ω-CgTX 800 nM + ω-Aga IVA 300 nM), decreased vasopressin-induced $[Ca^{2+}]_i$ responses by 76%.

4. In vasopressin-sensitive neurones, the presence of both nicardipine and ω-CgTX, reduced the K^+-evoked $[Ca^{2+}]_i$ increase by 61%. This blockade was increased by a further 21% with ω-Aga IVA, suggesting that N-, L- and P-type channels contribute to the depolarization-induced $[Ca^{2+}]_i$ rise. In addition, ω-MVIIC alone reduced the K^+-evoked $[Ca^{2+}]_i$ release by 24%. Also the remaining K^+ responses were further reduced by 60% when pre-incubated with L- N- and P-type blockers, suggesting the involvement of Q-type channels .

5. In oxytocin-sensitive neurones, the peak amplitude of the $[Ca^{2+}]_i$ response was not affected by Cd^{2+} alone, by combined Cd^{2+} and Ni^{2+}, or by the mixture of nicardipine, ω-CgTX and ω-Aga IVA. By contrast, the responses evoked by depolarization with K^+ were blocked by Cd^{2+}. Both nicardipine and ω-CgTX blocked 65% of K^+ response and an additional block of ~18% was obtained with ω-Aga IVA, suggesting the involvement of L-, N- and P-type channels. In combination, these antagonists strongly inhibited (~80% reduction) the K^+ responses. Further reduction to 18% was made by the Q-type blocker ω-MVIIC. Pre-incubation with L-, N- and P-type blockers caused an additional block of 71%.

6. Some supraoptic neurones (5–10%) responded to both vasopressin and oxytocin, with only the $[Ca^{2+}]_i$ responses induced by vasopressin blocked (>90% inhibition) by the mixture of Ca^{2+} channel antagonists.

7. In conclusion, both vasopressin and oxytocin magnocellular SO neurones have been shown to express T-, L-, N-, P-, Q- and R-type Ca^{2+} channels in their somata. Our results show that the vasopressin-induced $[Ca^{2+}]_i$ increase in vasopressin-sensitive neurones is mediated by L-, N- and T-type Ca^{2+} channels and not by P- and Q-type channels; Ca^{2+} channels are not involved in oxytocin action on oxytocin-sensitive neurones and L-, N-, P- and Q-type channels control the K^+-induced $[Ca^{2+}]_i$ increase in SO neurones.

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Since the pioneering work of Wakerley & Lincoln (1973), it is well established that the magnocellular neurones of the supraoptic (SO) and paraventricular (PV) nuclei of the hypothalamus show characteristic firing patterns depending on whether they synthesize and secrete either oxytocin or vasopressin (see, for instance, review in Poulain & Wakerley, 1982; Richard, Moos & Freund-Mercier, 1991). Oxytocin neurones show high frequency, synchronized bursts of activity associated with suckling-induced milk ejection, whilst vasopressin neurones fire in a phasic pattern. The results of Freund-Mercier & Richard (1981, 1984) showing that intracerebroventricular administration of oxytocin dramatically enhances bursting in oxytocin neurones initiated the concept of autocontrol of magnocellular neurone firing. More recently, this idea was extended to vasopressin neurones although the autocontrol exerted by vasopressin on phasic activity is not so well defined, being facilitatory or inhibitory according to different authors (Leng & Mason, 1982; Abe, Inoue, Matsuo & Ogata, 1983; Inenaga & Yamashita, 1986; Dayanithi, Moos & Richard, 1995). Data supporting the existence of autocontrol by oxytocin and vasopressin have been reviewed in our previous papers; each peptide exerts a direct and specific action on its respective neurones (identified by immunocytochemistry) via an increase in intracellular free calcium concentration ($[Ca^{2+}]_i$) (Lambert, Dayanithi, Moos & Richard, 1994; Dayanithi, Widmer & Richard, 1996). The autocontrols of oxytocin and vasopressin neurones are distinctly different mechanisms: oxytocin-induced $[Ca^{2+}]_i$ responses are independent of external Ca^{2+}, oxytocin mobilizing Ca^{2+} mainly from thapsigargin-sensitive intracellular Ca^{2+} stores, whereas responses induced by vasopressin usually require an influx of external Ca^{2+}, suggesting that membrane Ca^{2+} channels could play an important role in the responses of vasopressin-sensitive neurones.

Using whole-cell patch clamp techniques, Fisher & Bourque (1995) have demonstrated that somata of both oxytocin and vasopressin neurones express T-, L-, N- and P-type Ca^{2+} currents, plus a low threshold, nifedipine-sensitive current and an unidentified inactivating component. In addition, they demonstrated distinct ω-agatoxin IVA (ω-Aga IV)-sensitive Ca^{2+} currents between axon terminals and somata (Fisher & Bourque, 1996*a*, *b*). In another report, the presence of L-, N-, P- as well as Q- and R-type Ca^{2+} currents has also been demonstrated recently in the same preparations (Foehring & Armstrong, 1996). Thus, although the magnocellular neurosecretory cells (MNCs) appear to express almost all types of Ca^{2+} current, we still do not know their specific role and physiological implications.

To investigate the function of various Ca^{2+} channel types, the effects of non-discriminative Ca^{2+} channel antagonists (Cd^{2+} for high-voltage-activated (HVA); Ni^{2+} for low-voltage-activated (LVA) T-type Ca^{2+} channel) and selective antagonists (nicardipine for L-type; ω-conotoxin GVIA (ω-CgTX) for N-type; ω-Aga IVA for P- and/or Q-type and synthetic ω-conotoxin MVIIC-SNX 230 (ω-MVIIC) for Q-type) were tested on the $[Ca^{2+}]_i$ responses to vasopressin or oxytocin in vasopressin- and oxytocin-sensitive neurones. In addition, to check the presence of different Ca^{2+} channel types in vasopressin- and oxytocin-sensitive neurones, the effects of channel blockers were studied on $[Ca^{2+}]_i$ responses induced by depolarization with high K^+. In addition, it has been shown that a subpopulation (5–10%) of SO neurones (hereafter abbreviated as 'AVP–OT-sensitive neurones') responds to both vasopressin and oxytocin by a rise in $[Ca^{2+}]_i$ (Dayanithi *et al.* 1996; Sabatier, Richard & Dayanithi, 1996*b*). Although no direct proof exists, this subpopulation could be related to that co-expressing messenger RNA for both oxytocin and vasopressin (Kiyama & Emson, 1990), or containing both peptides (Mezey & Kiss, 1991) or to that showing simultaneous phasic activity and suckling-induced bursting (Moos & Ingram, 1995) or to some immunocytochemically identified oxytocin neurone displaying phasic activity *in vitro*, in male rats (Armstrong, Smith & Tian, 1994). This type of neurone could be an interesting model with which to compare the receptor-selective mechanisms of action of oxytocin and vasopressin which differ so considerably on specific oxytocin or vasopressin neurones. Therefore, we have also analysed AVP–OP-sensitive neurones for the effect of Ca^{2+} channel antagonists on $[Ca^{2+}]_i$ responses induced by both peptides.

We report that the vasopressin-induced intracellular $[Ca^{2+}]_i$ rise in vasopressin neurones is mediated via L-, N- and T- but not P- or Q-type Ca^{2+} channels, whereas no Ca^{2+} channels are required in oxytocin action on oxytocin neurones. Similar results were obtained from the AVP–OT-sensitive neurones which are sensitive to both peptides. Preliminary accounts of this work have appeared in abstract form (Sabatier *et al.* 1996*b*; Sabatier, Richard & Dayanithi, 1996*a*).

METHODS

Dissociation of SO neurones

Supraoptic nucleus neurones were isolated as previously described (Lambert *et al.* 1994; Dayanithi *et al.* 1996) with modifications. Briefly, adult male Wistar rats (100–200 g) were killed by decapitation with a guillotine. After dissection of the SO, tissue pieces were transferred to Locke buffer containing (mM): NaCl, 140; KCl, 5; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $CaCl_2$, 1·8; glucose, 10; Hepes–NaOH, 10; pH 7·2, the osmolarity being 290–310 mosmol l^{-1}. The neurones were enzymatically dissociated by incubation in Locke buffer supplemented with 650 units ml^{-1} deoxyribonuclease I, proteases X and XIV (each 1 mg ml^{-1}; Sigma) and 0·025% bovine serum albumin (tissue culture grade; Miles Laboratories, Kankake, IL, USA) for 30 min at 34 °C in a shaking water bath. After incubation, the tissue pieces were rinsed three times with Locke buffer, dissociated mechanically by gentle trituration and then the cell suspension was used for dye loading. In contrast to dissociation with trypsin (Lambert *et al.* 1994) a large majority of the cells possessed long dendritic processes when dissociated with deoxyribonuclease I, proteases X and XIV enzymes. Only cells with a soma diameter greater than 18 μm were used for the $[Ca^{2+}]_i$ measurements. It has been demonstrated that these larger diameter MNCs with dendritic processes contain either

vasopressin- or oxytocin-neurophysin (Oliet & Bourque, 1992; Lambert *et al.* 1994).

Dye loading and $[Ca^{2+}]_i$ measurement

Cells were loaded by incubation with 2·5 μM fura-2 AM plus 0·02% (w/v) Pluronic F-127 (trademark of BASF Wyandotte; Molecular Probes Inc., Eugene, OR, USA) in Locke buffer and plated onto a polylysine-coated chambered coverglass (Lab-tek no. 178565; Nunc, Inc., Naperville, IL, USA). Dye loading was carried out for 40 min at 37 °C in a humidified atmosphere. $[Ca^{2+}]_i$ in single cells were measured as described by Dayanithi *et al.* (1996). Briefly, fluorescence measurements were performed with the fast fluorescence photometer system (FFP; Zeiss, Oberkochen, Germany) which is based on an inverted microscope (Axiovert 100, Zeiss) equipped with epi-fluorescence objectives (Plan-Neofluar ×100 oil immersion lens; 1·30 numerical aperture). With fluorescence values corrected for background and dark current, $[Ca^{2+}]_i$ are calculated from the ratio between 340 and 380 nm recordings, in accordance with the equation given by Grynkiewicz, Poenie & Tsien (1985). Fura-2 calibration was performed as described in our previous papers (Lambert *et al.* 1994; Dayanithi *et al.* 1996).

Solutions and drug application

The Locke buffer described above was the control medium. Stock solutions of vasopressin and oxytocin (Boehringer Mannheim) were diluted in the control buffer. The Ca^{2+} channel blocker nicardipine (Sigma) was dissolved first in DMSO (final concentration, 0·01%) and further diluted in Locke buffer at appropriate concentrations. Other blockers such as ω-conotoxin GIVA (ω-CgTX; Alomone labs, Jerusalem, Israel), ω-Agatoxin IVA (ω-Aga IVA; kindly supplied by Dr N. A. Saccomano, Pfizer Inc., CT, USA) and ω-conotoxin (ω-MVIIC-SNX-230; supplied by the Neurex Corporation, Menlo Park, CA, USA) were prepared as concentrated stocks in distilled water, stored at −70 °C and diluted to working concentrations before use. Cadmium and nickel chloride (Sigma) were first prepared as stock solutions in distilled water and dissolved in measured buffer at appropriate concentrations before use. High-K^+ solution contained (mM) : NaCl, 97; KCl, 48; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $CaCl_2$, 1·8; glucose, 10; Hepes–NaOH, 10; pH 7·2, the osmolarity being 290–310 mosmol l^{-1}.

The test substance (50 μl; vasopressin or oxytocin or high K^+) was added close to the cell under investigation using a motor-driven syringe (Hamilton Microlab 900, Bonaduz, Switzerland) and the drug equilibrated within the bath containing 450 μl buffer. After application, the test substance was maintained in the bath throughout the experimental period. After each measurement, (a period of 5 min unless otherwise stated), the cells were washed two or three times with 1 ml of the control buffer (normal Locke) before retesting. This method allowed rapid and highly reproducible changes of the medium surrounding a selected cell.

Results are given as means ± S.E.M.

RESULTS

All the cells tested were subjected to successive applications of oxytocin and vasopressin. The experiments were performed on thirty-four vasopressin-, fourteen oxytocin- and six AVP–OT-sensitive neurones. Application of 100 nM vasopressin to neurones which did not respond to 100 nM oxytocin induced an increase in $[Ca^{2+}]_i$ from 84 ± 10 to 770 ± 60 nM ($n = 34$). This response was transient in 27/34 (79%) cells and sustained in the remaining 7 (21%) cells. In neurones which did not respond to vasopressin, oxytocin (100 nM) induced an increase in $[Ca^{2+}]_i$ from 81 ± 10 to 453 ± 32 nM ($n = 14$). A sustained response was observed in 11/14 (80%) of these neurones and a transient response was observed in 3/14 (21%) cells. These values are similar to those previously reported (Lambert *et al.* 1994; Dayanithi *et al.* 1996). In the AVP–OT-sensitive neurones (responding to both peptides) the $[Ca^{2+}]_i$ increase induced by either of the peptides was smaller than those from neurones which responded to only one peptide. The resting $[Ca^{2+}]_i$ level was 80 ± 12 nM. Application of AVP (100 nM) then OT (100 nM) and vice versa, without any order of preference, evoked an increase in $[Ca^{2+}]_i$ of 455 ± 46 and 283 ± 45 nM ($n = 20$), respectively. The great majority (18/20) of AVP–OT neurones displayed $[Ca^{2+}]_i$ responses to AVP that were greater by 38% than those to OT.

Vasopressin-sensitive neurones

Effects of non-discriminative inorganic Ca^{2+} channel antagonists

The effects of two successive applications of vasopressin were used as control. The resting $[Ca^{2+}]_i$ was 70 ± 6 nM. The first and the second vasopressin application evoked an increase of 618 ± 60 and 551 ± 41 nM ($n = 6$), respectively. Pre-incubation with Cd^{2+} (100 μM) reduced vasopressin-induced $[Ca^{2+}]_i$ responses by 77% (Fig. 1*A*; control, 821 ± 170 nM; Cd^{2+}, 188 ± 33 nM; $n = 7$). As complete inhibition was not observed after incubation with Cd^{2+}, we tested the T-type channel blocker Ni^{2+} at 50 μM, separately or in combination with 100 μM Cd^{2+}. Figure 1*B* shows that $[Ca^{2+}]_i$ rise due to vasopressin was almost completely abolished by these blockers (control, 523 ± 145 nM; Cd^{2+} plus Ni^{2+}, 44 ± 5 nM; $n = 3$). The resting $[Ca^{2+}]_i$ in these neurones in the absence or presence of Cd^{2+} and Ni^{2+} were 73 ± 16 and 72 ± 6 nM, respectively. Ni^{2+} (50 μM) alone inhibited the vasopressin-induced $[Ca^{2+}]_i$ response by 19%, or 84% when given in combination with 100 μM Cd^{2+} (control, 613 ± 110 nM; Ni^{2+}, 493 ± 62 nM; Ni^{2+} plus Cd^{2+}, 96 ± 12 nM; $n = 5$; Fig. 1*C*).

Effects of selective organic Ca^{2+} channel antagonists

L- and N-type blockers. The above results indicate an involvement of both high-voltage-activated (HVA) and low-voltage-activated (LVA) Ca^{2+} channels in the actions of vasopressin. In order to investigate the involvement of HVA channels further, the effects of different Ca^{2+} channel blockers such as nicardipine and ω-CgTX were tested on vasopressin-induced $[Ca^{2+}]_i$ responses. Figure 2*A* shows that when applied separately, nicardipine (5 μM) and ω-CgTX (800 nM) reduced vasopressin responses by 62 and 75%, respectively (nicardipine, 308 ± 64 nM *versus* 802 ± 126 nM for control, $n = 7$; ω-CgTX, 265 ± 83 nM *versus* 1038 ± 123 nM for control, $n = 5$). Since a strong inhibition was observed in the presence of 5 μM nicardipine or 800 nM ω-CgTX, the vasopressin-induced $[Ca^{2+}]_i$ responses were tested in the presence of lower concentrations of toxin. Nicardipine given at 200 nM did

not modify the vasopressin responses (nicardipine, 299 ± 87 nM *versus* 329 ± 98 nM for control, $n = 3$) while the responses were reduced by 48% in the presence of 1 μM nicardipine (Fig. 2*B*; nicardipine, 298 ± 63 nM *versus* 570 ± 103 nM for control, $n = 3$). This inhibition was additive when the cells were further exposed to 500 nM ω-CgTX (130 ± 11 nM; 77% inhibition). To confirm the specific involvement of L- and N-type channels in the action of vasopressin, the order of application of the toxins was reversed. Figure 2*C* shows that 500 nM ω-CgTX reduced the vasopressin responses within vasopressin-sensitive neurones by 46% and in its continued presence, 1 μM nicardipine further decreased the responses by 66% compared with control (control, 672 ± 153 nM; ω-CgTX, 393 ± 106 nM; ω-CgTX plus nicardipine, 228 ± 33 nM; $n = 3$).

P- and Q-type blockers. It is shown in the literature that ω-Aga IVA at 30 nM blocks P-type channels and at 300 nM blocks both P- and Q-type channels. Our results show that at none of the concentrations used did ω-Aga IVA affect vasopressin-induced $[Ca^{2+}]_i$ responses, (Fig. 3*A* and *B*; control, 865 ± 105 nM; ω-Aga IVA, 936 ± 165 nM; $n = 5$). Another Ca^{2+} channel toxin, more selective for Q-type channels was ω-MVIIC at 100 nM, which did not affect the vasopressin responses (Fig. 3*C*). Vasopressin responses were also unaffected by higher concentrations of ω-MVIIC (500 nM), even after 45 min of prolonged incubation (control vasopressin, 578 ± 77 nM; plus ω-MVIIC, 513 ± 83 nM; $n = 5$). However, in the continued presence of this toxin, vasopressin responses were strongly inhibited only when the cells were pre-incubated with the combination of ω-CgTX and nicardipine, suggesting that Q-type channels are not involved in the actions of vasopressin (control vasopressin, 714 ± 45 nM; plus ω-MVIIC, 692 ± 33 nM; ω-MVIIC plus ω-CgTX and nicardipine, 129 ± 14 nM; $n = 4$).

Figure 4 shows the responses obtained in SO neurones pre-incubated with a combination of specific Ca^{2+} channel blockers (5 μM nicardipine, 800 nM ω-CgTX and 300 nM ω-Aga IVA). The $[Ca^{2+}]_i$ increase induced by vasopressin was decreased to only 76% of the control vasopressin response (control, 574 ± 141 nM; antagonists, 136 ± 37 nM; $n = 5$) and was observed after the application of L- and

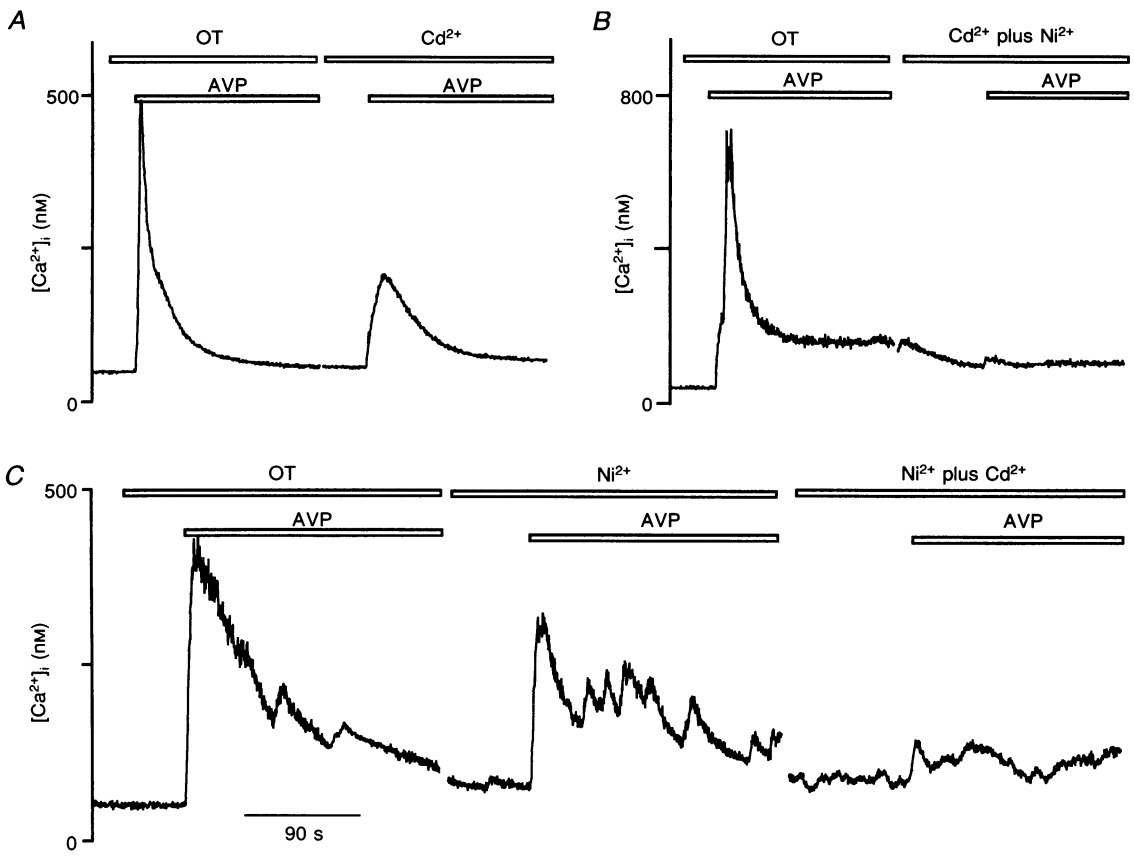

Figure 1. Effects of the divalent Ca^{2+} channel blockers Cd^{2+} and Ni^{2+} on AVP-induced $[Ca^{2+}]_i$ rise in SO neurones

A, traces represent a typical vasopressin- (100 nM) induced $[Ca^{2+}]_i$ increase in a vasopressin-sensitive (oxytocin-insensitive) neurone maintained in Locke buffer. After washing, the same neurone was pre-incubated with 100 μM Cd^{2+} for 5 min and then subjected to vasopressin. *B*, profiles show the $[Ca^{2+}]_i$ increase induced by vasopressin in an oxytocin-insensitive neurone. The effect of vasopressin was completely abrogated when pre-incubated with a mixture of Cd^{2+} (100 μM) and Ni^{2+} (50 μM). *C*, the effect of Ni^{2+} was tested alone or incombination with Cd^{2+}.

N-blockers only (see Fig. 2), i.e. there was no potentiation of the effects of L- and N-blockers by the P- and Q-blockers.

Specific Ca^{2+} channel blockers affect K^+-induced $[Ca^{2+}]_i$ increase

The above results indicated that L-, N- and T-type channels are involved in the action of vasopressin. Obviously, to investigate the role for P- and Q-type channels, which are shown to be present in SO cells, we first monitored $[Ca^{2+}]_i$ responses to vasopressin and then subjected the same neurones to high K^+ followed by pre-incubation with the Ca^{2+} channel blockers. The resting $[Ca^{2+}]_i$ (74 ± 13 nM) was not affected in the presence of Cd^{2+} (76 ± 16 nM). Repeated applications of K^+ evoked a constant rise in $[Ca^{2+}]_i$ (e.g. see Fig. 7*A*) in SO neurones without any desensitization (peak response values: 1st, 728 ± 124 nM; 2nd, 696 ± 96 nM; 3rd, 716 ± 110 nM and 4th, 680 ± 82 nM; $n = 3$). An interval of 12–15 min was maintained between

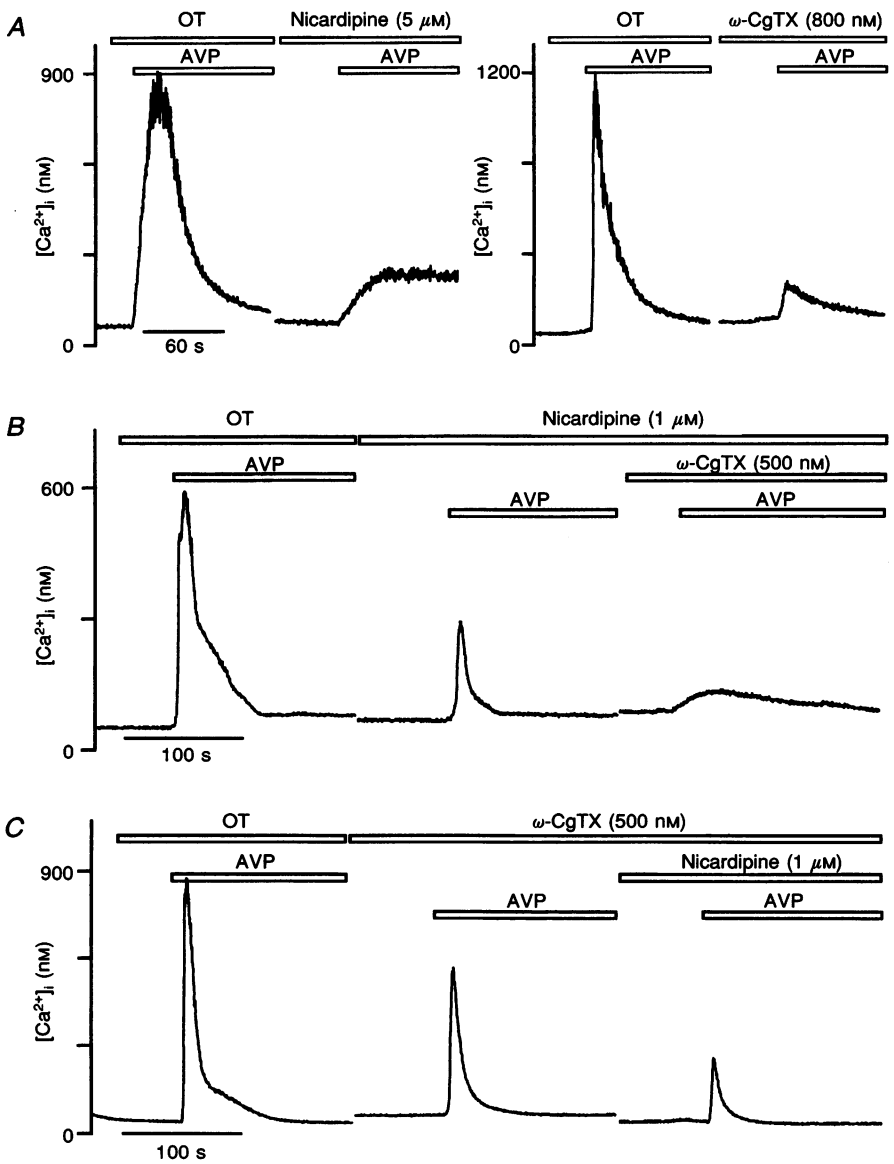

Figure 2. Specific actions of L- or N-type Ca^{2+} channel blockers on vasopressin-sensitive neurones

All traces represent $[Ca^{2+}]_i$ responses monitored in vasopressin-sensitive neurones which were all insensitive to oxytocin. *A* (left panel), a neurone pre-incubated with 5 μM nicardipine, a selective L-type channel blocker, for 5 min and then exposed to vasopressin (100 nM). In a different series of experiments, neurones were tested for vasopressin-induced $[Ca^{2+}]_i$ responses in the presence of 800 nM ω-CgTX, a selective N-type channel blocker (right panel). *B*, selected neurone responding to 100 nM vasopressin (left trace) was pre-incubated with 1 μM nicardipine (middle trace) followed by 500 nM ω-CgTX (right trace) *C*, using a similar approach another representative cell tested for vasopressin responses, first, in the presence of ω-CgTX (middle trace) and then in combination with nicardipine (right trace).

subsequent high-K^+ solution application. Figure 5 shows that depolarization of vasopressin-sensitive neurones with high K^+ (48 mM) results in an substantial increase in $[Ca^{2+}]_i$ and the resulting $[Ca^{2+}]_i$ responses were decreased by 66% in the presence of Cd^{2+}. Peak values were: K^+, 574 ± 78 nM; plus Cd^{2+}, 197 ± 36 nM ($n = 4$).

In order to study the specific participation of each channel type, the K^+-induced $[Ca^{2+}]_i$ responses were monitored using the blockers separately or together. Figure 6 shows the specific effects of nicardipine (1 μM), ω-CgTX (500 nM) and ω-Aga IVA (30 nM) on $[Ca^{2+}]_i$ rise evoked by high K^+ in vasopressin-sensitive neurones (mean peak value, 643 ± 63 nM). Following combined pre-incubation with nicardipine and ω-CgTX, the $[Ca^{2+}]_i$ responses were inhibited by 61% (249 ± 37 nM) and this blockade was additive (21%; 115 ± 5 nM, $n = 5$) when the same neurones were incubated with ω-Aga IVA (Fig. 6*A*). The percentage of inhibition was calculated by considering the mean values obtained with the K^+ stimulus as 100%. These results were confirmed by reversing the order of presentation of the P-type channel blocker (Fig. 6*B*). When pre-incubated first with ω-Aga IVA, the $[Ca^{2+}]_i$ rise evoked by K^+ was reduced by 23%. In the continued presence of ω-Aga IVA, a further 47% reduction was obtained with nicardipine and ω-CgTX (mean peak values: K^+, 606 ± 77 nM; with ω-Aga IVA, 467 ± 58 nM; with all three blockers, 181 ± 26 nM; $n = 5$).

Similarly, specific blockers of L-, N- and P- and/or Q-type channels (5 μM nicardipine, 800 nM ω-CgTX and 300 nM ω-Aga IVA), given in combination, strongly inhibited (85% reduction) the K^+-induced responses (Fig. 6*C*). Peak values obtained within vasopressin-sensitive neurones were: K^+, 663 ± 82 nM; plus blockers, 97 ± 6 nM ($n = 5$). The resting $[Ca^{2+}]_i$ levels were not modified by the blockers: 108 ± 19 nM without blockers *versus* 98 ± 17 nM with blockers, $n = 3$). These results clearly show that L- and N-type, as well as P- and/or Q-type, channels control K^+-depolarization-induced $[Ca^{2+}]_i$ increase in vasopressin neurones.

Figure 7 shows the specific involvement of Q-type channels during the K^+-induced response in vasopressin-sensitive neurones. To characterize the Q-type channel, we used ω-MVIIC at 500 nM (see Randall & Tsien, 1995). The toxin was present in the incubation medium for about 45 min before the application of the K^+ stimulus. Interestingly,

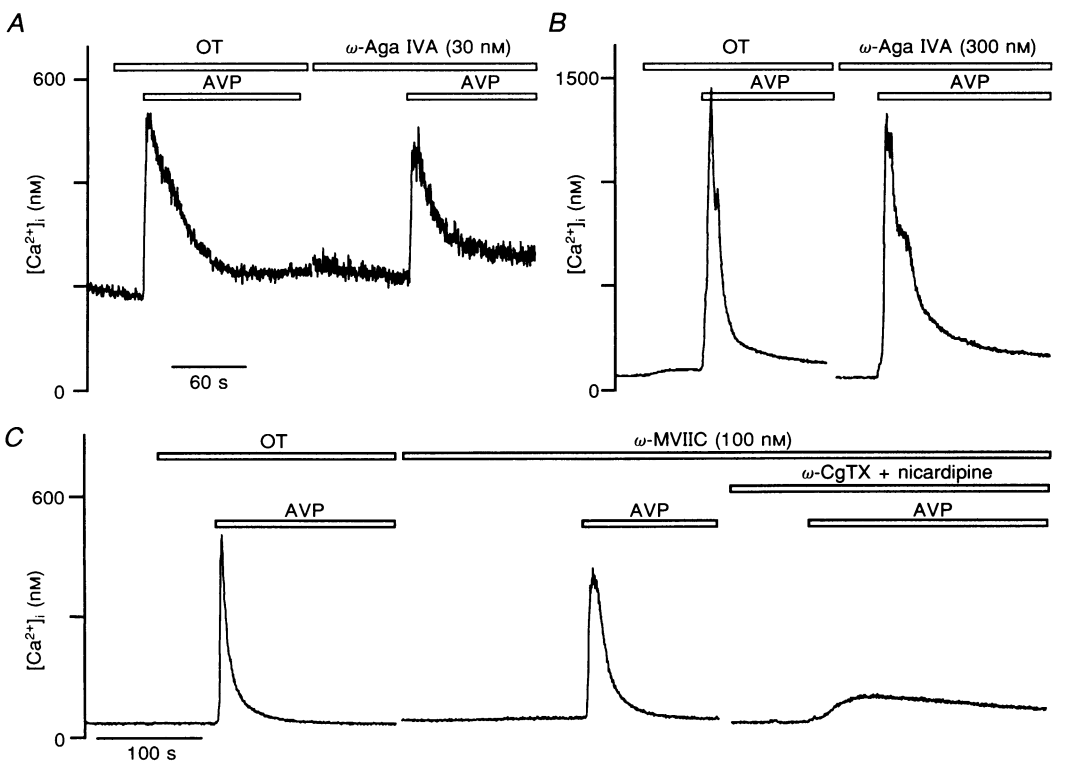

Figure 3. Effects of P- or Q-type blockers on vasopressin neurones

A vasopressin-sensitive neurone was pre-incubated with ω-Aga IVA at concentrations at which it acts as a P-type channel blocker (30 nM; *A*) or a Q-type channel blocker (300 nM; *B*). Note that the $[Ca^{2+}]_i$ responses induced by vasopressin were unaffected by ω-Aga IVA. *C*, a vasopressin-sensitive neurone (left trace) was pre-incubated with ω-MVIIC (SNX-230), a blocker of Q-type channels at 100 nM (middle trace), followed by a combination of 500 nM ω-CgTX and 1 μM nicardipine (right trace), and $[Ca^{2+}]_i$ responses were recorded after vasopressin application. Note that the Q-type blocker, ω-MVIIC (SNX-230), a relatively specific blocker, does not modify vasopressin-induced $[Ca^{2+}]_i$ rise.

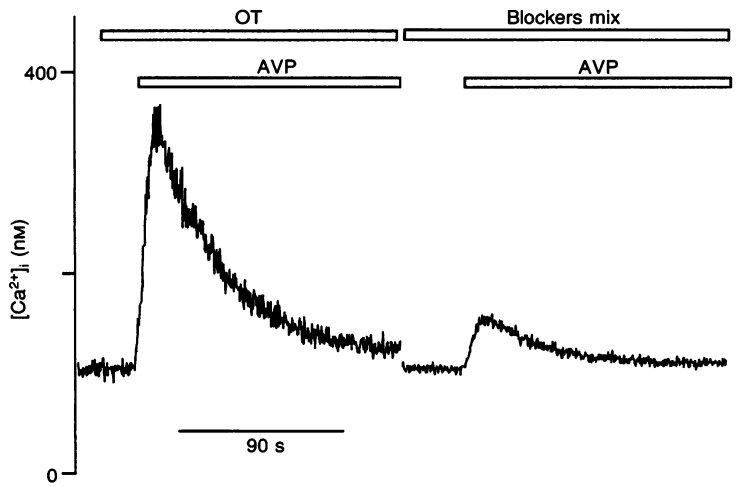

Figure 4. Effects of a mixture of specific Ca^{2+} channel blockers on $[Ca^{2+}]_i$ rise

$[Ca^{2+}]_i$ profiles observed after 100 nM vasopressin. In this neurone oxytocin did not induce any $[Ca^{2+}]_i$ response. The same neurone, after washing was pre-incubated with a mixture of specific Ca^{2+} channel blockers (nicardipine (5 μM), ω-CgTX (800 nM) and ω-Aga IVA (300 nM)) for 5 min and then subjected to vasopressin. Note that this combination of blockers significantly reduced the $[Ca^{2+}]_i$ response induced by vasopressin (right panel).

ω-MVIIC alone inhibited the K^+ response by 24% and a further block of 60% was obtained when it was given in combination with other antagonists. The values were: control K^+, 918 ± 69 nM; ω-MVIIC, 697 ± 58 nM; and ω-MVIIC plus 1 μM nicardipine, 500 nM ω-CgTX and 30 nM ω-Aga IVA together, 147 ± 26 nM ($n = 4$). The block of K^+-induced responses by ω-MVIIC was further confirmed by reversing the order of presentation of these toxins, i.e. the neurones were first pre-incubated with the mixture of L-, N- and P-blockers and then followed with ω-MVIIC (45 min pre-incubation). In these experiments, the K^+-induced $[Ca^{2+}]_i$ increase (750 ± 72 nM) was strongly inhibited by L-, N- and P-blockers (67% reduction; 244 ± 30 nM) and was further reduced by ω-MVIIC (to 20% of control; 99 ± 6 nM; $n = 4$), suggesting the involvement of Q-type channels in a K^+-induced $[Ca^{2+}]_i$ rise.

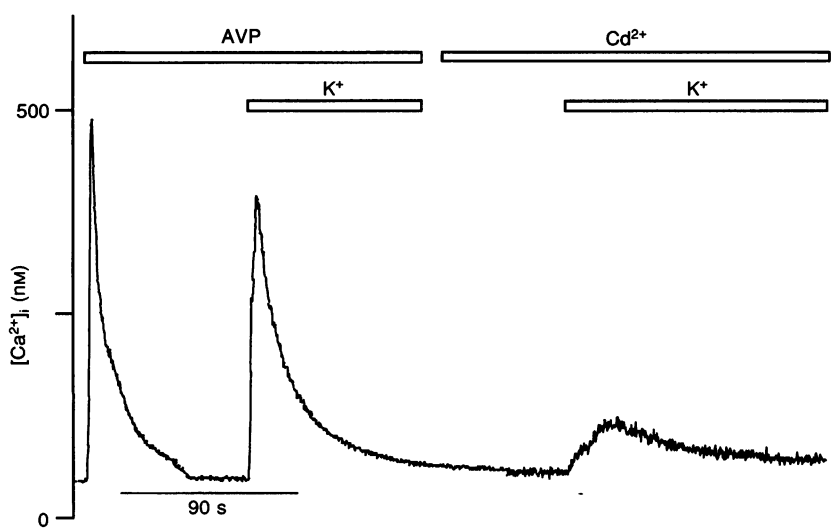

Figure 5. Action of Cd^{2+} on K^+-induced $[Ca^{2+}]_i$ increase

Vasopressin-induced $[Ca^{2+}]_i$ increase observed in a neurone where oxytocin had no effect on $[Ca^{2+}]_i$ transients; the same neurone was subjected to 48 mM K^+-induced depolarization. After recording, the neurone was pre-incubated for 5 min in the presence of Cd^{2+}. Note that Cd^{2+} strongly inhibited the K^+ response.

Oxytocin-sensitive neurones

Effects of Ca^{2+} channel antagonists

As oxytocin-induced $[Ca^{2+}]_i$ responses are independent of external Ca^{2+}, it is not surprising to observe that none of the Ca^{2+} channel antagonists affected the peak amplitude of the oxytocin response. Figure 8*A* shows that the peak amplitude of oxytocin responses was not affected by Cd^{2+} (control, 336 ± 52 nM; Cd^{2+}, 280 ± 24 nM; $n = 4$). Similarly, the peak $[Ca^{2+}]_i$ increase induced by oxytocin was not altered by pre-incubation of oxytocin-sensitive neurones with both Cd^{2+} and Ni^{2+} (Fig. 8*B*; control, 419 ± 45 nM; Cd^{2+} plus Ni^{2+}, 441 ± 22 nM; $n = 4$; basal $[Ca^{2+}]_i$ were, 109 ± 28 and

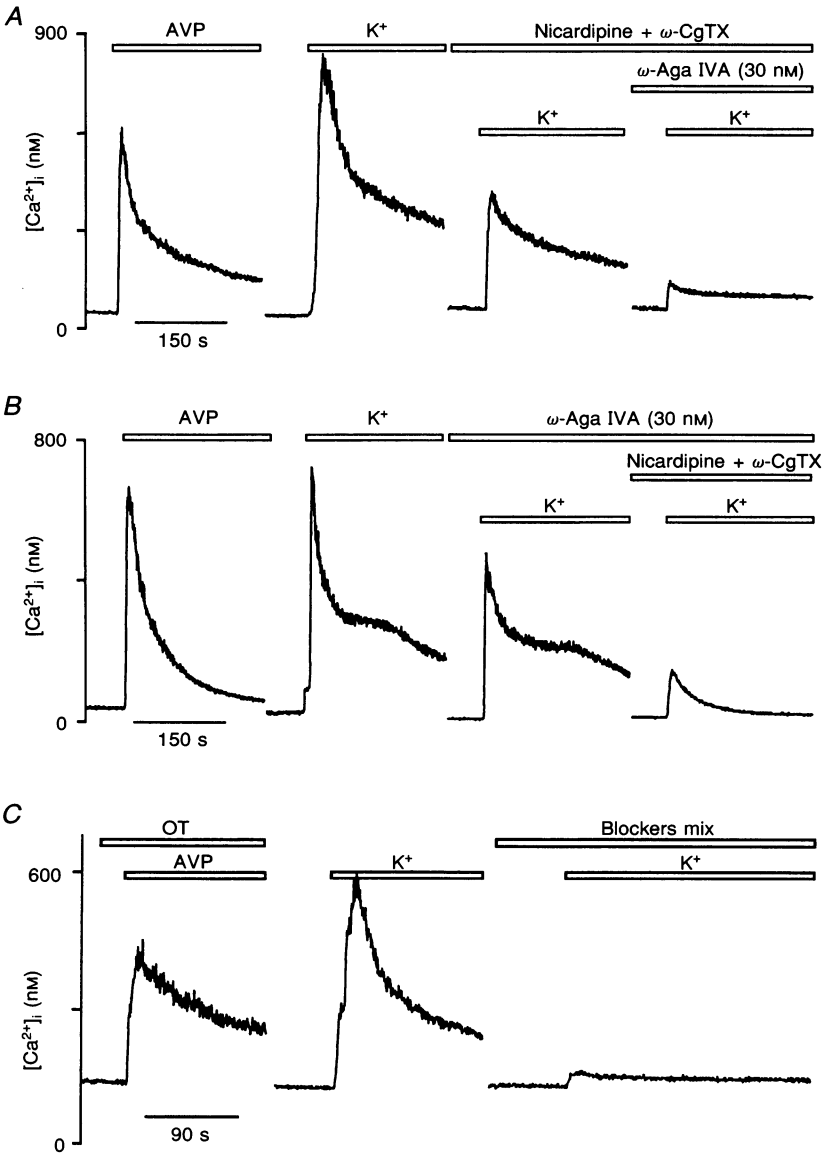

Figure 6. Block of K^+ responses by specific Ca^{2+} channel antagonists in vasopressin-sensitive neurones

All traces were obtained from the same neurone (*A*). A typical vasopressin-sensitive neurone (left trace, obtained with 100 nM vasopressin) was subjected to 48 mM K^+. This neurone did not respond to oxytocin (trace not shown). After washing, the cells were incubated with 1 μM nicardipine in combination with 500 nM ω-CgTX and then challenged with K^+. After recording, the cells were further incubated for 5 min with 30 nM ω-Aga IVA in combination with other antagonists. Note that additional inhibition was observed with the P-type channel blocker. *B*, in a similar type of experiment, another neurone sensitive to vasopressin was first tested for the effect of the P-type channel blocker followed by other blockers. *C*, vasopressin-induced $[Ca^{2+}]_i$ increase observed in an oxytocin-insensitive neurone and after washing, the same neurone was subjected to 48 mM K^+. After recording, the neurone was pre-incubated for 5 min with the mixture of blockers (see text for Fig. 4), then challenged with K^+ (right panel). Note that the $[Ca^{2+}]_i$ rise after K^+ depolarization was almost completely blocked in the presence of the blockers mix.

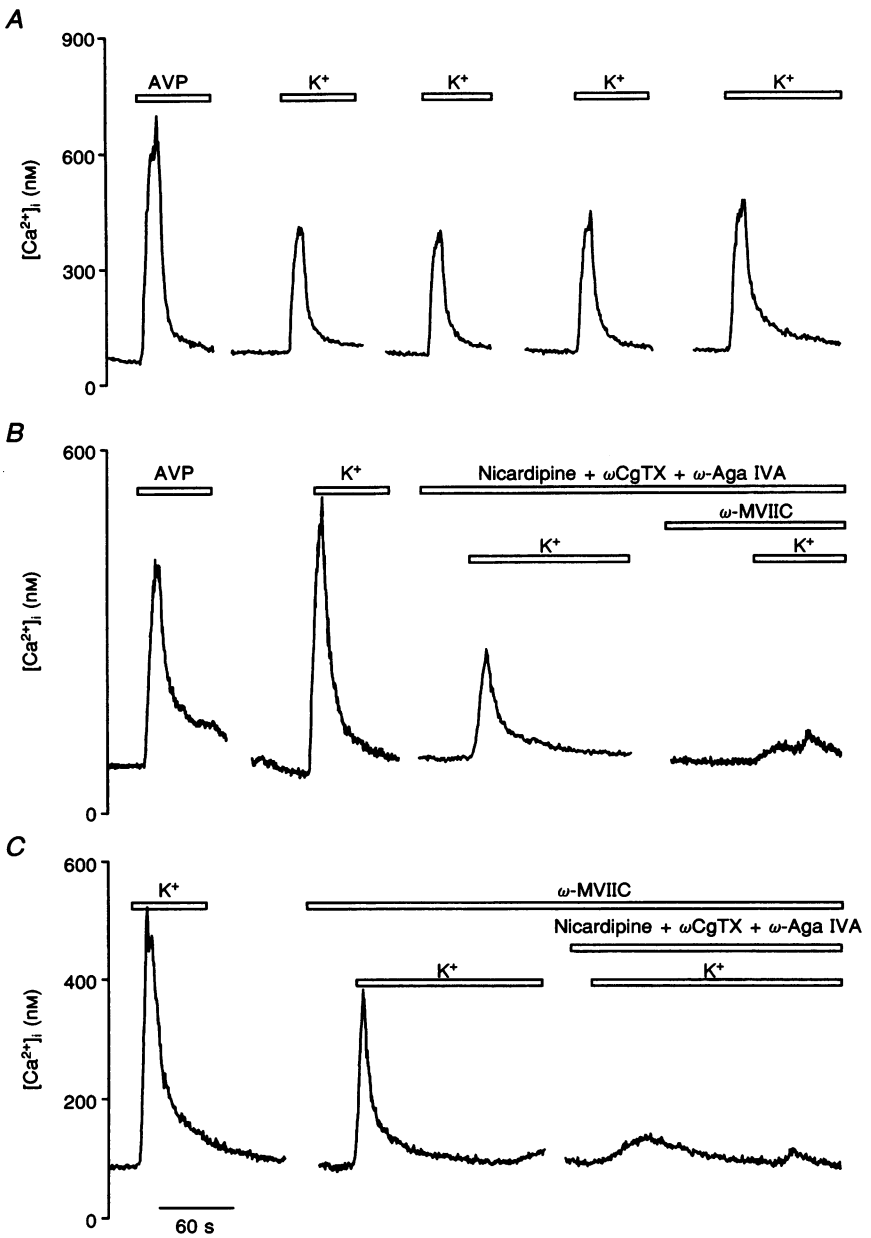

Figure 7. Sensitivity of K^+ depolarization-induced $[Ca^{2+}]_i$ transients to a Q-type blocker in vasopressin-sensitive neurones

Control traces, typical of vasopressin-sensitive neurones, of $[Ca^{2+}]_i$ responses to sequential application of 48 mM K^+ (*A*). Cells were washed after each stimulus. Note that there is no desensitization after several applications of high K^+. *B*, a neurone responding to vasopressin was first subjected to 48 mM K^+. After washout, the same neurone was pre-incubated with mixture of nicardipine (5 μM), ω-CgTX (500 nM) and ω-Aga IVA (30 nM) for 5 min before challenge with 48 mM K^+. After recording, the cells were washed and further pre-incubated for about 45 min with medium containing the above mixture of Ca^{2+} channel antagonists plus ω-MVIIC (500 nM) and then stimulated with 48 mM K^+. Note that ω-MVIIC, applied after L-, N- and P-blockers, reduced the K^+-induced responses. *C*, the involvement of Q-type channels in K^+-induced responses was further confirmed by pre-incubating the cells first with ω-MVIIC (500 nM) for 45 min before subjecting them to K^+ stimulus. After washout, the same neurone was further pre-incubated with other blockers (see above) in addition to ω-MVIIC. Left trace shows the control K^+ response in this selected neurone.

115 ± 30 nM, respectively) or by the mixture of specific channel blockers (Fig. 8*C*; control, 356 ± 57 nM; antagonists, 321 ± 16 nM; $n = 5$). Although the transient peak amplitude of the $[Ca^{2+}]_i$ response induced by oxytocin was not affected by any of these blockers tested, we observed a decrease in the sustained responses (plateau phase) due to blockers; i.e. the values returned to basal levels more rapidly.

By contrast, Ca^{2+} channel types are involved in K^+-induced $[Ca^{2+}]_i$ rise in oxytocin-sensitive neurones as is also found in vasopressin-sensitive neurones. Following pre-incubation with 100 μM Cd^{2+}, the $[Ca^{2+}]_i$ responses to 48 mM K^+ depolarization were decreased by 79% (results not shown). The mixture (nicardipine (5 μM), ω-CgTX (800 nM) and ω-Aga IVA (300 nM)) of specific antagonists, given in combination also strongly (80% reduction) inhibited the K^+ responses in oxytocin-sensitive neurones. Peak values were: K^+, 655 ± 76 nM; K^+ plus blockers, 132 ± 18 nM ($n = 3$); basal $[Ca^{2+}]_i$ were 80 ± 5 and 80 ± 7 nM, respectively. When applied separately (Fig. 9*A*), nicardipine (1 μM) and ω-CgTX (500 nM) reduced the K^+-induced $[Ca^{2+}]_i$ responses by 65% and ω-Aga IVA (30 nM) enhanced this inhibitory effect to an 83% reduction (i.e. 18% more inhibition; the mean peak values above basal were: K^+, 559 ± 76 nM; with nicardipine and ω-CgTX, 192 ± 24 nM; with all blockers, 95 ± 6 nM; $n = 4$). The effects of the blockers were further confirmed by using the P-type channel blocker first followed by L- and N-type channel blockers (Fig. 9*B*). ω-Aga IVA alone reduced the K^+-evoked $[Ca^{2+}]_i$ increase by about 27% and a mixture of the other blockers increased this blockade by a further 54% (peak values above basal were K^+, 611 ± 126 nM; with ω-Aga IVA, 448 ± 124 nM; with all blockers, 118 ± 19 nM; $n = 4$).

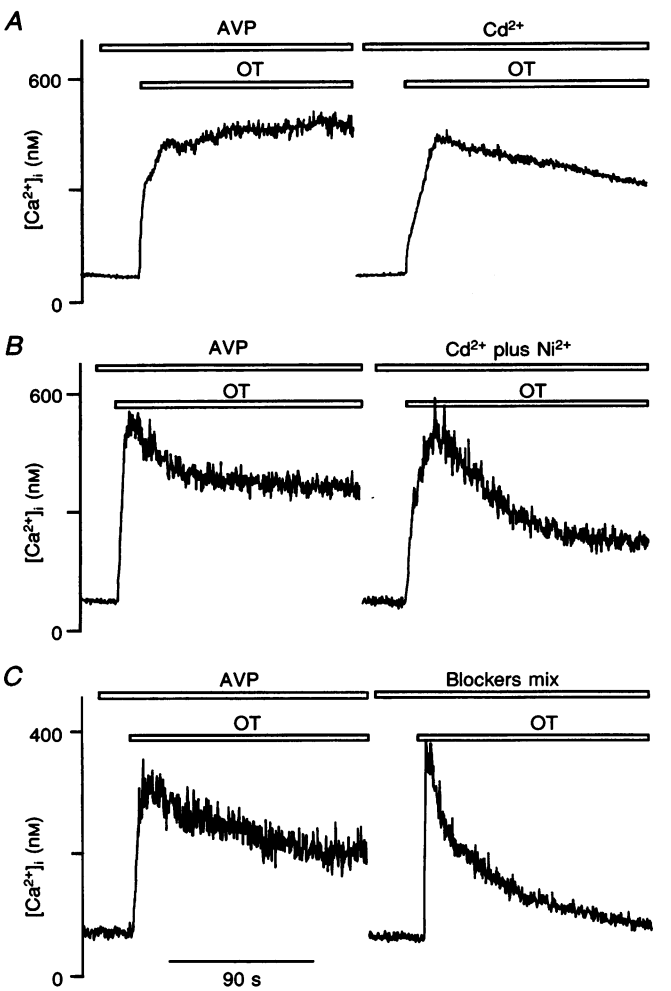

Figure 8. Effects of non-specific and specific Ca^{2+} channel antagonists on oxytocin-induced $[Ca^{2+}]_i$ rise
A, profiles show oxytocin responses in an AVP-insensitive neurone subjected to 100 μM Cd^{2+}. *B*, an oxytocin-sensitive neurone was treated with both Cd^{2+} and Ni^{2+} (50 μM) 5 min before applying oxytocin for second time. *C*, similarly, the oxytocin response was tested in the presence of specific Ca^{2+} channel blockers in combination as has been described in Fig. 4. Three to five neurones were tested in a similar manner and displayed similar responses.

The specific involvement of Q-type channels during K^+-induced responses in oxytocin-sensitive neurones was tested using ω-MVIIC (500 nM). The toxin was present in the incubation buffer for about 45 min before the application of the K^+ stimulus. ω-MVIIC alone inhibited the K^+ response by 18% and a further block of 71% was obtained when given in combination with other antagonists. The mean peak values were: control K^+, 862 ± 85 nM; ω-MVIIC, 707 ± 80 nM; ω-MVIIC plus other blockers (L, N and P), 124 ± 11 nM ($n = 4$).

AVP and OT-sensitive neurones

From a series of control experiments, we have observed that 20/295 SO neurones responded to both vasopressin (100 nM) and oxytocin (100 nM). In these cells the resting $[Ca^{2+}]_i$ level was 80 ± 12 nM. Application of vasopressin followed by oxytocin and vice versa, without any order of preference, evoked an increase in $[Ca^{2+}]_i$ of 455 ± 46 and 283 ± 45 nM, respectively. The great majority (18/20) of AVP–OT-sensitive neurones displayed $[Ca^{2+}]_i$ responses to vasopressin that were 38% greater than those to oxytocin. In AVP–OT-sensitive neurones, the $[Ca^{2+}]_i$ response induced by vasopressin was completely abolished in the absence of external Ca^{2+} whereas the oxytocin response was unaffected (data not shown) as has been previously described for vasopressin actions on specific vasopressin-sensitive neurones and for oxytocin on oxytocin-sensitive neurones (Lambert *et al.* 1994; Dayanithi *et al.* 1996).

In six of these AVP–OT-sensitive neurones, the mixture of specific Ca^{2+} channel antagonists blocked the $[Ca^{2+}]_i$ responses (Fig. 10) induced by vasopressin (control, 523 nM; plus blockers, 146 nM). In contrast, the oxytocin-induced peak $[Ca^{2+}]_i$ rise was unaffected (control, 305 nM; plus antagonists, 369 nM) whereas the duration of the $[Ca^{2+}]_i$ response (plateau phase) observed after incubation with the blockers was reduced (Fig. 10, right trace) compared with that of the control oxytocin stimulus (Fig. 10, middle trace). Since the percentage of AVP–OT-sensitive neurones is small, further pharmacological characterization was limited in this study.

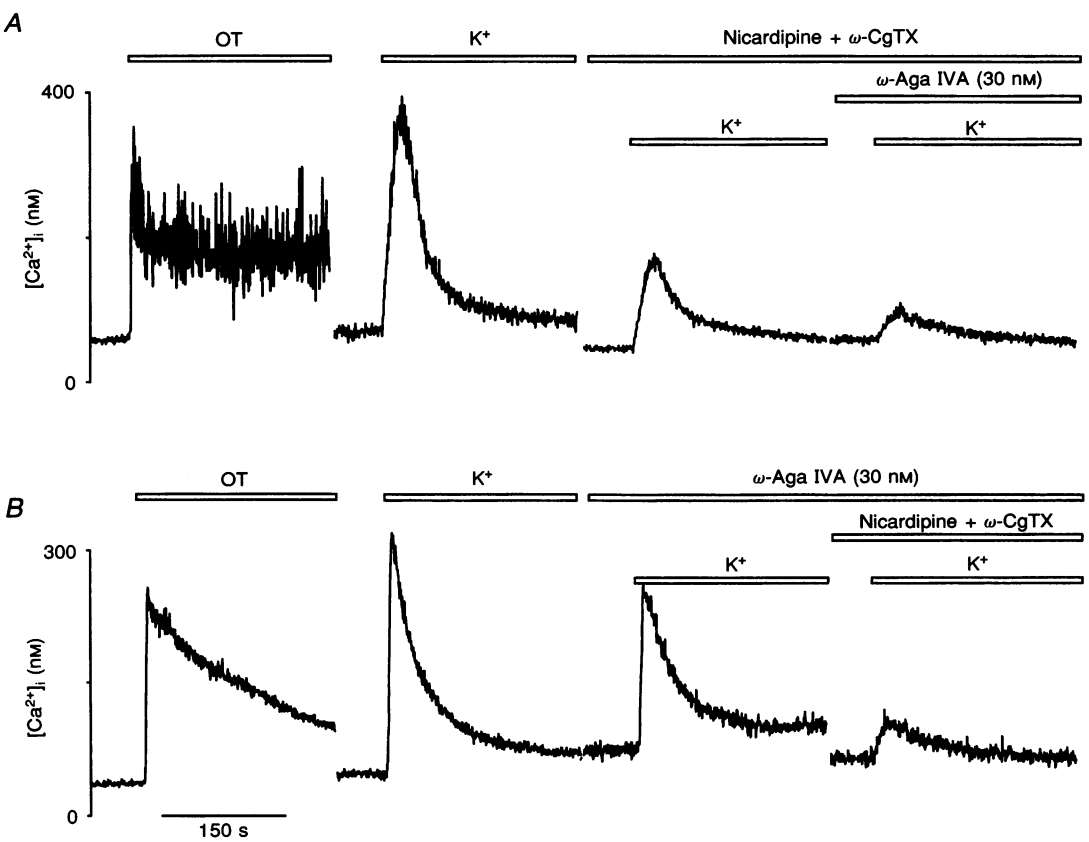

Figure 9. Blockade of K^+ responses by specific Ca^{2+} channel antagonists in oxytocin-sensitive neurones

To test the specific involvement of L-, N- and P-type channel antagonists during K^+ depolarizations in oxytocin-neurones, similar experiments were performed as described above (Fig. 6). *A* shows a characteristic $[Ca^{2+}]_i$ response to 100 nM oxytocin. This neurone was insensitive to vasopressin (trace not shown). K^+-induced $[Ca^{2+}]_i$ responses were first monitored in the presence of both nicardipine (1 μM) and ω-CgTX (500 nM) and followed with ω-Aga IVA (30 nM) in the continued presence of other blockers. *B*, similarly, the specific effect of ω-Aga IVA was tested first, followed by L- and N-type blockers.

DISCUSSION

Using specific Ca^{2+} channel blockers, we have shown here that the actions of vasopressin are mediated via specific Ca^{2+} channel types such as L, N and T both in vasopressin and AVP–OT neurones. No channel blockers prevented the response to oxytocin, in either oxytocin- or AVP–OT-sensitive neurones. This confirms our previous observations that oxytocin responses are independent of external Ca^{2+} and that oxytocin mobilizes Ca^{2+} mainly from intracellular stores. Finally, in both cell types, the $[Ca^{2+}]_i$ increase in response to K^+ depolarization is not only mediated by L- and N-, but also by P- and Q-channel types, indicating that they are expressed on the soma membrane of magnocellular neurones. Moreover, the effects of oxytocin and vasopressin on $[Ca^{2+}]_i$ increases recorded in AVP–OT-sensitive neurones are similar to those observed in specifically oxytocin-sensitive and vasopressin-sensitive neurones, that is to say oxytocin-induced $[Ca^{2+}]_i$ increases by are independent of, and vasopressin-induced increases are dependent on, external Ca^{2+}.

Limits of the pharmacological classification of voltage-dependent Ca^{2+} channels

Many types of Ca^{2+} channels exist in mammalian neurones and high-affinity blockers are now available for almost all these. The divalent cations Cd^{2+} and Ni^{2+} are considered as non-discriminative inorganic Ca^{2+} channel blockers. At the concentrations used in this study (50 μM for Ni^{2+} and 100 μM for Cd^{2+}), Ni^{2+} preferentially blocks T-type currents, whereas Cd^{2+} is more effective in blocking high-voltage-activated calcium currents (Fox, Nowycky & Tsien, 1987; Fisher & Bourque, 1995). The dihydropyridine Ca^{2+} channel antagonist, nicardipine (5–10 μM), has been shown to block L-type Ca^{2+} channels in a variety of cells where the antagonist inhibited both slowly inactivating and non-inactivating components of the Ca^{2+} current (Fox *et al.* 1987). It has been reported that 1,4-dihydropyridine produces a selective inhibition of L-type channels at low concentrations but may also partially block N-type Ca^{2+} channels at high concentrations (see Scott, Pearson & Dolphin, 1991). The N-type channels, which are activated by relatively large depolarizations and inactivate at a moderate rate, are targeted by ω-conotoxin. Another class of high-voltage-activated currents is supported by P-type channels, which are targeted by a spider toxin, ω-Aga IVA. This toxin is selective at very low concentrations (up to 10 nM) for P-type channels and at higher concentrations (over 30 nM). It als blocks Q-type channels (Randall & Tsien, 1995 and see review by Saccomano & Ahlijanian, 1994). These Q-type channels are also more sensitive to another toxin, ω-MVIIC (Zhang *et al.* 1993; Wheeler, Randall & Tsien, 1994). Given the complexity of using the Ca^{2+} channel antagonists, we have chosen concentrations which fall within the range used by others and we believe that we have selectively dissected the involvement of T-, N-, L-, P- and Q-type channels by the respective use of Ni^{2+} (50 μM), nicardipine (1 and 5 μM), ω-CgTX (200, 500 and 800 nM), ω-Aga IVA (30 and 300 nM), and ω-MVIIC (SNX-230; 100 and 500 nM).

Expression of Ca^{2+} channels on the soma and axon terminals of magnocellular hypothalamic neurones

Complete blockade of vasopressin- or K^+-induced $[Ca^{2+}]_i$ rise required pre-incubation with a mixture of both Cd^{2+} and Ni^{2+}, demonstrating that both LVA and HVA Ca^{2+} channels are involved in these responses. The fact that Cd^{2+}

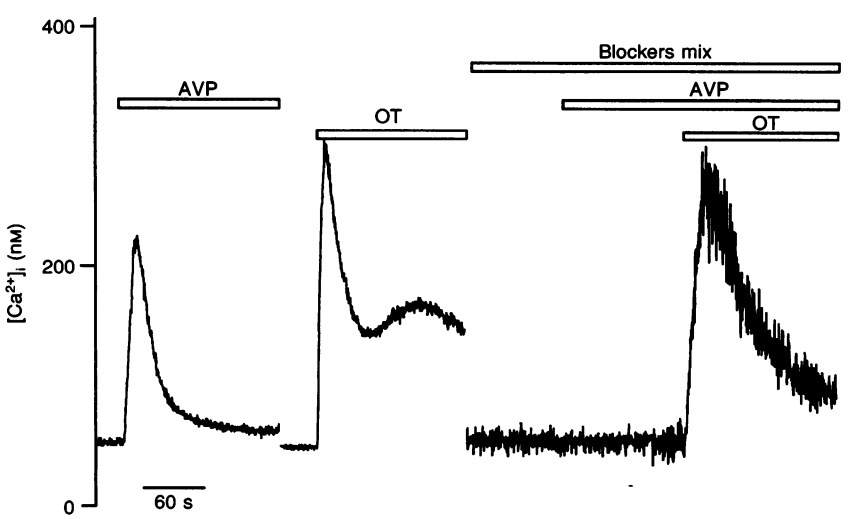

Figure 10. Effects of mixed blockers on neurones responding to both vasopressin and oxytocin

A selected neurone was first subjected to 100 nM vasopressin (left trace) followed by 100 nM oxytocin (middle trace). After washing, the same neurone was pre-incubated for 5 min with the mixture of Ca^{2+} channel blockers (5 μM nicardipine, 800 nM ω-CgTX and 300 nM ω-Aga IVA) and then exposed to vasopressin and oxytocin (right trace). These blockers specifically abolished vasopressin, but not oxytocin, responses.

alone reduced the vasopressin- and K^+-induced $[Ca^{2+}]_i$ rise by 77 and 66%, respectively, indicates that HVA channels support most of the Ca^{2+} currents involved in these responses. This is in agreement with the observation of the presence of both LVA and HVA currents, and variation of their relative amplitude during postnatal development, performed on a very similar preparation in our laboratory (Widmer, Amerdeil, Fontanaud & Desarménien, 1997). Extensive pharmacological dissection of both HVA and LVA currents in MNCs have been published and, although somewhat controversial, the results show that T-, L-, N-, P-, Q- and R-type channels can be co-expressed on the soma of SO neurones (Fisher & Bourque, 1995; Foehring & Armstrong, 1996). Interestingly, the proportion of current blocked by N- and L-specific blockers reported in these studies is in agreement with the actions of these blockers on vasopressin responses.

In the presence of nicardipine (1 μM), the vasopressin-induced $[Ca^{2+}]_i$ response was reduced by about 48%. Our results could be related to the observation of Fisher & Bourque (1995) that a proportion (23%) of activating Ca^{2+} currents in SO neurones was sensitive to 10 μM nifedipine. Similarly, using 5 μM nifidipine, Foehring & Armstrong (1996) observed an approximately 28% inhibition of L-type currents in the same model. The vasopressin-induced $[Ca^{2+}]_i$ response was also strongly inhibited (75% reduction) by the N-type blocker ω-CgTX given at 800 nM; and at 500 nM, we observed an approximate 46% inhibition, suggesting that N-type channels could be activated by vasopressin. This result supports the observation that in SO neurones, ω-CgTX at 500 nM (Fisher & Bourque, 1995) or at 1 μM (Foehring & Armstrong, 1996) inhibited 39 and 26%, respectively, of N-type current.

ω-Aga IVA toxin and ω-MVIIC had no effect on the vasopressin response, suggesting that P- and Q-type channels are not involved in the actions of vasopressin. However, we observed that K^+-induced responses were significantly inhibited by ω-Aga IVA as well as by ω-MVIIC in both vasopressin- and oxytocin-sensitive neurones. In the same preparation, ω-Aga IVA at 50 nM blocked (20% reduction) P-type currents (Fisher & Bourque, 1995, 1996*b*). It is of interest to note that Foehring & Armstrong (1996) observed an inhibition of 17, 23 and 37% with 30, 100 and 400 nM toxin, respectively, suggesting the presence of Q-type currents, and their presence has been further confirmed by using 2 μM ω-MVIIC (21% inhibition).

In summary, a large variety of HVA Ca^{2+} channels have been reported on the soma of MNCs, whether studied with electrophysiological techniques or by visualizing Ca^{2+} entry. The reasons for such a diversity are not clear, and the specific role of each channel type needs to be clarified. Indeed, our present results show that all HVA channels are not equivalent since, although they all respond to K^+, P- and Q-type channels are not activated by vasopressin and none of the channels is activated by oxytocin.

A similar situation is found at nerve endings in the neurohypophysis, where several Ca^{2+} channels are expressed as shown by direct recordings: L- and N-types (Lemos & Nowycky, 1989; Wang, Triestman & Lemos, 1992, 1993), and Q-types (Wang, Newcomb, Hom, Mezo, Ramachandran & Lemos, 1994; Fisher & Bourque, 1996) are present but play different roles. In these terminals, certain dihydropyridines block L-type Ca^{2+} channels (Wang *et al.* 1993) as well as partially blocking the K^+-induced vasopressin and oxytocin release. Furthermore, a low concentration of ω-CgTX inhibits a large proportion of the transient component (N_t) of the Ca^{2+} current (Wang *et al.* 1992) and of the K^+-stimulated peptide release (Dayanithi *et al.* 1988). Interestingly, L- N- and Q-type channels control vasopressin release, whereas only L- and N-type channels control oxytocin release (Wang *et al.* 1997).

Mechanisms underlying vasopressin- or oxytocin-induced $[Ca^{2+}]_i$ rise

The mechanisms by which vasopressin activates the previously identified Ca^{2+} channels in MNCs are not yet known. If this activation of voltage-gated channels resulted simply from a depolarization or an activation of electrical activity, all channel types would be activated, as observed with K^+, which induced a $[Ca^{2+}]_i$ rise sensitive to all channel blockers used in the present study. One possibility is that vasopressin may selectively inhibit P- and Q-type channels through a G protein and thus turn off P- and Q-type channels activated by depolarization.

Vasopressin has been shown to activate various types of Ca^{2+} channels, in several cell types including neurones (see review by Chen, Brinton, Shors & Thompson, 1993). On the other hand, in hippocampal neurones, V_1 vasopressin-receptor stimulation leads to activation of the phosphatidylinositol signalling pathway and mobilization of intracellular Ca^{2+} (Brinton, Gonzalez & Cheung, 1994). Such a mechanism is more reminiscent of the action of oxytocin on SO neurones. The diversity of vasopressin action on neuronal $[Ca^{2+}]_i$ may result from the presence of various receptor types. Further studies, using a combination of electrophysiological and microspectrofluorimetric techniques, together with pharmacological analysis, are required for a better understanding of these actions.

In our experimental conditions, non-descriminatory or specific Ca^{2+} channel blockers did not affect the peak amplitude of the oxytocin-induced $[Ca^{2+}]_i$ response but reduced the duration of the plateau response. The mechanisms by which the plateau responses are affected by the channel blockers in oxytocin-sensitive neurones are yet to be determined. It is important to note that oxytocin was shown to depolarize the membrane of the SO neurones, but this effect was at least 100 times less potent than that of vasopressin (Abe *et al.* 1983). Ca^{2+}-dependence experiments were performed with the buffer containing 100–150 nM external free Ca^{2+}. If 100 nM oxytocin depolarizes the membrane it may be possible for an influx of a small amount

of external Ca^{2+} sufficient to initiate calcium-induced calcium release (CICR) mechanisms. Whether Ca^{2+} channel blockers affect CICR mechanisms remains to be elucidated.

We found that 5–10% of SO neurones responded to both peptides by a rise in $[Ca^{2+}]_i$ (Lambert *et al.* 1994; Dayanithi *et al.* 1996; Sabatier *et al.* 1996*a*,*b*). As has also been suggested previously, these AVP–OT-sensitive neurones are similar to those SO neurones expressing mRNAs for both peptides or displaying both vasopressin- and oxytocin-related electrical activities (see Introduction). In AVP–OT-sensitive neurones, only vasopressin responses are dependent on external Ca^{2+}. In addition, only the $[Ca^{2+}]_i$ increase induced by vasopressin was blocked by the mixture of specific Ca^{2+} channel antagonists (Sabatier *et al.* 1996*b*). These results also indicate that sensitivity of oxytocin and vasopressin responses arises from receptor-specific properties rather than from cell-specific mechanisms.

Physiological implications

In addition to their peripheral actions, oxytocin and vasopressin play a role as neurotransmitters or neuromodulators in different areas of the CNS where they are released at axon terminals as in neurohypophysis (see Richard *et al.* 1991). In the magnocellular nuclei, oxytocin and vasopressin are released locally and may control the firing patterns of oxytocin–vasopressin neurones. By contrast to the release mechanisms in CNS or neurohypophysis, intranuclear release is TTX independent but Ca^{2+} dependent, suggesting the possibility of dendritic release (Di Scala-Guénot, Strosser & Richard, 1987; Ludwig, Callaham & Morris, 1995). This notion has been further supported by morphological evidence (Pow & Morris, 1989). Moreover, the intranuclear release of oxytocin (Moos, Freund-Mercier, Guerné, Guerné, Stoeckel & Richard, 1984; Morris, Pow, Sokol & Ward, 1993) and vasopressin (Ludwig *et al.* 1995) are the factors mediating autocontrol in these systems. Although the involvement of voltage-dependent Ca^{2+} channels in somato-dendritic release is quite obvious, the type of channels involved remains to be determined. This information may enhance our understanding of the importance of the diversity of Ca^{2+} channels expressed by one single MNC.

Concerning the control of the firing pattern of MNCs, oxytocin enhances the bursting activity of oxytocin neurones (Freund-Mercier & Richard, 1984). This autocontrol action of oxytocin on oxytocin neurones may be related to the prolonged increase in $[Ca^{2+}]_i$ which we have previously described in detail (Lambert *et al.* 1994), and is independent of external Ca^{2+}. On the other hand, vasopressin induces a transient, external Ca^{2+}-dependent increase in $[Ca^{2+}]_i$, which may affect the phasic firing of vasopressin neurones. Vasopressin has been shown to depolarize the membrane of the neurones, suggesting that it could modulate the excitability of parvocellular neurones (Abe *et al.* 1983). At the same time, vasopressin was reported to have mixed effects on the spontaneous firing rate of vasopressin neurones with decreases (Abe *et al.* 1983), increases (Inenaga & Yamashita, 1986), no effects (Poulain & Carette, 1987), or inhibition of firing by reducing the length of the bursts (Leng & Mason, 1982; Dayanithi *et al.* 1995). These mixed effects of vasopressin could be due to the state of depolarization, which in turn influences the firing pattern (Andrew & Dudek, 1984). The activation of Ca^{2+} currents by vasopressin could contribute to the activation of a Ca^{2+}-dependent K^+ current, which would hyperpolarize the neurone and stop phasic bursts (Bourque, Randle & Renaud, 1985). Both plateau potentials underlying the bursting activity and depolarizing after-potentials following each action potential, have been shown to be dependent on Ca^{2+} (Andrew & Dudek, 1984), and voltage-dependent Ca^{2+} currents control the burst spike frequency (Mason & Leng, 1984). Of interest, Kirkpatrick & Bourque (1996) show that the Ca^{2+}-dependent K^+ current underlying the after-hyperpolarization potential does not stop the bursts, but rather contributes to the control of interburst firing rate. In addition, blocking the current with apamin actually decreases the burst length.

Furthermore, it has recently been observed, using whole-cell recordings from SO neurones, that depolarizing after-potentials (DAPs) and plateau potentials (PPs) were either abrogated or reduced by 10 μM nifedipine or the absence of external Ca^{2+}, suggesting that initiation of both DAPs and PPs is dependent on an increase in $[Ca^{2+}]_i$ after spikes (Li & Hatton, 1995). Their results also indicate that Ca^{2+} entry via HVA Ca^{2+} channels could induce the release of Ca^{2+} from intracellular compartments, and thereby generate DAPs and PPs which could initiate firing activity in SO neurones.

In conclusion, our results demonstrate that vasopressin-induced Ca^{2+} entry is mediated via voltage-dependent Ca^{2+} channels, mainly of the L-, N- and T-types. P- and Q-type channels are not involved in the actions of vasopressin on vasopressin neurones. Although all Ca^{2+} currents are also expressed in oxytocin neurones, no Ca^{2+} channels are required for oxytocin-induced $[Ca^{2+}]_i$ rise. Nevertheless, L-, N-, P- and Q-type channels participate in the K^+-induced $[Ca^{2+}]_i$ increase in both vasopressin- and oxytocin-sensitive neurones.

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