

## Role of Q-type $\text{Ca}^{2+}$ channels in vasopressin secretion from neurohypophysial terminals of the rat

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1. The nerve endings of rat neurohypophyses were acutely dissociated and a combination of pharmacological, biophysical and biochemical techniques was used to determine which classes of  $\text{Ca}^{2+}$  channels on these central nervous system (CNS) terminals contribute functionally to arginine vasopressin (AVP) and oxytocin (OT) secretion.
2. Purified neurohypophysial plasma membranes not only had a single high-affinity binding site for the N-channel-specific  $\omega$ -conopeptide MVIIA, but also a distinct high-affinity site for another  $\omega$ -conopeptide (MVIIC), which affects both N- and P/Q-channels.
3. Neurohypophysial terminals exhibited, besides L- and N-type currents, another component of the  $\text{Ca}^{2+}$  current that was only blocked by low concentrations of MVIIC or by high concentrations of  $\omega$ -AgaIVA, a P/Q-channel-selective spider toxin.
4. This  $\text{Ca}^{2+}$  current component had pharmacological and biophysical properties similar to those described for the fast-inactivating form of the P/Q-channel class, suggesting that in the neurohypophysial terminals this current is mediated by a 'Q'-type channel.
5. Pharmacological additivity studies showed that this Q-component contributed to rises in intraterminal  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) in *only* half of the terminals tested.
6. Furthermore, the non-L- and non-N-component of  $\text{Ca}^{2+}$ -dependent AVP release, but *not* OT release, was effectively abolished by the same blockers of Q-type current.
7. Thus Q-channels are present on a subset of the neurohypophysial terminals where, in combination with N- and L-channels, they control AVP but *not* OT peptide neurosecretion.

The regulated release of bioactive compounds from neurons is controlled by  $\text{Ca}^{2+}$ , and an understanding of chemical signalling by neurons thus requires an analysis of how the various types of  $\text{Ca}^{2+}$  channels interact to control synaptic transmission and neurosecretion (for review, see Dunlap, Luebke & Turner, 1995).

Voltage-dependent  $\text{Ca}^{2+}$  channels are involved in neurotransmitter release, but the existence and specific role at central nervous system (CNS) terminals of many of the different types of  $\text{Ca}^{2+}$  channels is unclear. A number of studies have defined several electrophysiologically distinct voltage-activated  $\text{Ca}^{2+}$  channels on neuronal cell bodies (for

reviews, see Bean, 1989; Tsien, Ellinor & Horne, 1991; Llinás, Sugimori, Hillman & Cherksey, 1992). The L-, N-, T- and P-type channels are the best characterized (Fox, Nowycky & Tsien, 1987; Tsien *et al.* 1991; Llinás *et al.* 1992), but other classes of channels, such as the Q- and R-types, have been revealed by molecular cloning (Snutch & Reiner, 1992; Ellinor *et al.* 1993; Sather, Tanabe, Zhang, Mori, Adams & Tsien, 1993) and the use of polypeptide  $\text{Ca}^{2+}$  channel antagonists (Olivera, McIntosh, Cruz, Luque & Grey, 1984; Hillyard *et al.* 1992; Ramachandran *et al.* 1993). The N-type  $\text{Ca}^{2+}$  channel is involved in the release of many 'classical' neurotransmitters, while the L-type is known to regulate the secretion of certain peptides

(Cazalis, Dayanithi & Nordmann, 1987; Dunlap *et al.* 1995). The class A ( $\alpha_{1A}$ )  $\text{Ca}^{2+}$  channel has recently been localized at central nerve terminals by both immunohistochemical studies (Westenbroek *et al.* 1995) and pharmacological studies of transmitter release (Miljanich & Ramachandran, 1995). However, the phenotype of the expressed class A channel can resemble that of native channels described as either P or Q (Llinás *et al.* 1992; Sather *et al.* 1993; Stea *et al.* 1994; Randall & Tsien, 1995), and the biophysical properties of the class A channel in CNS terminals remain to be determined.

In order to determine which  $\text{Ca}^{2+}$  channels are functionally involved in peptide secretion, we studied the identified nerve terminals of the rat neurohypophysis, a population of relatively homogeneous peptidergic nerve endings that allow comparative study by a number of different techniques. This has been a very useful model system for identification and characterization of nerve terminal  $\text{Ca}^{2+}$  channels (Lemos & Nowycky, 1989; Wang, Treistman & Lemos, 1992; Wang & Lemos, 1994; Fisher & Bourque, 1995) and for examination of the mechanisms underlying depolarization–secretion coupling (Cazalis *et al.* 1987; Lim, Nowycky & Bookman, 1990; Lindau, Stuenkel & Nordmann, 1992; Wang, Treistman, Wilson, Nordmann & Lemos, 1993*b*). At these terminals, certain dihydropyridines (DHP) can block a long-lasting ('L')  $\text{Ca}^{2+}$  channel (Wang, Treistman & Lemos, 1993*a*) and a substantial portion of depolarization-stimulated peptide release (Cazalis *et al.* 1987). Furthermore, omega ( $\omega$ )-conopeptide GVIA at low concentrations can block a large proportion of the transient component (' $\text{N}_T$ ') of the  $\text{Ca}^{2+}$  current (Wang *et al.* 1992) and of stimulated peptide release (Dayanithi *et al.* 1988). Nonetheless, neither channel antagonist, even when added together at saturating concentrations, can block all of the terminal  $\text{Ca}^{2+}$  current or all of the  $\text{Ca}^{2+}$ -dependent neuropeptide release (Wang *et al.* 1993*b*). This led us to examine, using a combination of pharmacological, biophysical and biochemical techniques, whether another class of  $\text{Ca}^{2+}$  channels might also co-exist on these CNS terminals and functionally contribute to neurosecretion. Part of this work has appeared previously in abstract form (Nordmann, Dayanithi, Stuenkel, Kristipati, Lemos & Newcomb, 1994; Wang, Newcomb, Hom, Mezo, Ramachandran & Lemos, 1994).

## METHODS

### Channel blocking peptides

The  $\omega$ -conopeptides used in this study were the synthetic versions, prepared at Neurex Corp. (Ramachandran *et al.* 1993). These were termed SNX-111, the synthetic version of  $\omega$ -conopeptide MVIIA (Olivera, Miljanich, Ramachandran & Adams, 1994), SNX-124, the synthetic version of  $\omega$ -GVIA (Olivera *et al.* 1984), and SNX-230, the synthetic version of  $\omega$ -MVIIC (Hillyard *et al.* 1992). The synthetic version of  $\omega$ -AgaIVA (Mintz, Venema, Swiderek, Lee, Bean & Adams, 1992) was purchased from Peptides Intl (Louisville, KY, USA) or synthesized as described by Gaur *et al.* (1994). In the text the synthetic peptides are referred to by their original names instead of the Neurex terms, except occasionally following in parenthesis.

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### Binding studies

Plasma membranes were prepared from bovine (Arena Bros Slaughterhouse, Hopkington, MA, USA) neurohypophyses by differential centrifugation and Percoll gradients as described for whole rat brain (Reinhart, Chung & Levitan, 1989). Binding experiments were performed by filtration over polyethyleneimine-coated glass fibre filters, as described previously (Kristipati *et al.* 1994), with an incubation volume of 0.5 ml. Saturation experiments used membranes containing 1.3  $\mu\text{g}$  protein for MVIIA, and 0.8  $\mu\text{g}$  protein for MVIIC. Displacement experiments used 0.8  $\mu\text{g}$  protein, and 2.4 (with MVIIA) or 5.8 pM (with MVIIC) of the radiiodinated conopeptide; concentrations were chosen such that the highest affinity binding sites were predominantly occupied. Consistent with its selectivity for N-type channels,  $\omega$ -conopeptide-GVIA also had a low affinity ( $\text{IC}_{50}$ , 480 nM) in the displacement of  $^{125}\text{I}$ -labelled MVIIC. Binding studies have shown that AgaIVA binds to rat brain membranes (Adams, Myers, Imperial & Olivera, 1993). Displacement by AgaIVA of radiolabelled MVIIC from neurohypophysial membranes was observed, with an  $\text{IC}_{50}$  of approximately 300 nM (data not shown).

### Electrophysiological recordings

After sedation by  $\text{CO}_2$ , male rats were killed by decapitation using a guillotine. The neurohypophysis was then excised, following previously described protocols (Cazalis *et al.* 1987), and homogenized in a solution containing (mM): 270 sucrose, 10 Hepes and 0.01  $\text{K}_2$ -EGTA; pH 7. The isolated neurohypophysial nerve terminals could be identified using an inverted microscope or by using immunoblotting following patch-clamp recordings. Normal Locke saline (LS; Cazalis *et al.* 1987) was then used to perfuse the terminals. Before recording, the terminals (usually 5–8  $\mu\text{m}$  in diameter) were perfused with 5 mM  $\text{Ba}^{2+}$  LS containing (mM): 145 NaCl, 5 KCl, 5  $\text{BaCl}_2$ , 1  $\text{MgCl}_2$ , 10 Hepes, 15 glucose; and 1  $\mu\text{M}$  TTX; 0.02% bovine serum albumin (BSA); pH 7.3. To obtain 'whole-terminal' perforated (Rae, Cooper, Gates & Watsky, 1991)-patch recordings (Hamill, Marty, Neher, Sakmann & Sigworth, 1981), freshly made amphoterin B (240  $\mu\text{g ml}^{-1}$ ) was applied in a pipette solution that contained (mM): 135 caesium glutamate, 10 Hepes, 5 glucose, 2  $\text{CaCl}_2$ , 1  $\text{MgCl}_2$  and 20 TEA; pH 7.3.

It is important to note that the experiments reported here were performed using the perforated-patch (Rae *et al.* 1991) recording configuration and thus there was no problem with the run-down that complicated former studies (Lemos & Nowycky, 1989; Wang *et al.* 1992; Wang & Lemos, 1994; Fisher & Bourque, 1995). Only perforated terminals with access resistances of 3–5  $\text{M}\Omega$  were chosen for further recordings. The  $\text{Ba}^{2+}$  current ( $I_{\text{Ba}}$ ), which was activated by depolarizing from –80 to +10 mV and demonstrated both transient and long-lasting components, could be maintained for more than 1 h without any run-down.  $I_{\text{Ba}}$  was filtered at 3 kHz and sampled at 10 kHz. pCLAMP (Axon Instruments) was used for acquisition and analysis of data.

### Intraterminal [ $\text{Ca}^{2+}$ ]

Fura-2 was loaded into isolated nerve terminals of the rat neurohypophysis (see Electrophysiological recordings) that had adhered to the glass coverslip forming the bottom of the recording chamber by perfusion of the chamber (0.5 ml  $\text{min}^{-1}$ ) for 20 min at 37 °C with normal saline containing 1  $\mu\text{M}$  fura-2 AM and consisting of (mM): 100 *N*-methyl-D-glucamine (NMG)-Cl, 40 NaCl, 1  $\text{MgCl}_2$ , 2.2 (or 1)  $\text{CaCl}_2$ , 5  $\text{KHCO}_3$ , 10 glucose and 10 Hepes; pH 7.2. Following loading, extracellular indicator was removed and time allowed for accumulated fura-2 AM to be cleaved by changing the perfusing solution to the same saline without the indicator. Monitoring of the intraterminal  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) in

individual nerve endings (generally 5–10  $\mu\text{m}$  in diameter) was performed, following optical masking of surrounding regions, by dual wavelength microspectrofluorometry similar to that described previously (Stuenkel, 1990). Alternating excitation wavelengths of 340 and 380 nm were applied and photons quantified at an emission wavelength of  $510 \pm 10$  or  $500 \pm 20$  using a photon counting photomultiplier-based SPEX Industries AR-CM system. The fluorescence ratio (340 nm/380 nm) was converted to  $[Ca^{2+}]_i$  by the use of the converting equation of Grynkiewicz, Poenie & Tsien (1985). The constants for this converting equation were determined using an external standard calibration and a  $K_d$  value for fura-2 of 224 nM. During experimental protocols, saline or saline containing the appropriate antagonist or antagonist plus elevated  $K^+$  was perfused at  $1.5 \text{ ml min}^{-1}$  through a chamber volume of 65  $\mu\text{l}$ . Antagonists were generally applied for 2–4 min prior to application of the antagonist plus elevated  $K^+$ . High  $K^+$  solution was prepared by equimolar substitutions of 45 mM KCl for NMG-Cl.

### Peptide release

Rat neurohypophyses (see Electrophysiological recordings) were homogenized as described previously (Dayanithi *et al.* 1988). The homogenate was centrifuged at 2400  $g$  for 6 min. The resulting pellet contains highly purified nerve terminals. The nerve endings were equally loaded onto four filters (0.45  $\mu\text{m}$  Acro disc, Gelman Sci., Ann Arbor, MI, USA) and perfused at 37 °C with normal LS. Control experiments were always run in parallel with the toxin experiments. Four minute fractions of perfusate were collected and the evoked release was triggered by an 8 min duration pulse of a depolarizing concentration (50 mM) of  $K^+$  (indicated by the arrows in Figs 6 and 7). The results are given as arginine vasopressin (AVP) or oxytocin (OT) release per fraction using specific radioimmunoassays (Cazalis *et al.* 1987; see also below). The medium before and after the depolarizing period contained (mM): 40 NaCl, 5  $\text{KHCO}_3$ , 100 NMG-Cl, 1  $\text{MgCl}_2$ , 2  $\text{CaCl}_2$ , 10 glucose, 10 Tris-Hepes and 0.02% BSA; pH 7.25. Depolarization medium contained 50 mM  $K^+$ , in which NMG-Cl was reduced to maintain the osmolarity (300–310 mosmol  $\text{l}^{-1}$ ).

The released AVP and OT were assayed by radioimmunoassays using specific antibodies (kindly supplied by Dr R. John Bicknell, AFRC Institute of Animal Physiology, Babraham, Cambridge, UK). AVP and OT antisera were raised in sheep, the latter by Dr Sheldric and Dr Flint. The final dilutions of antibodies used here were 1 : 120 000 for AVP and 1 : 90 000 for OT. The cross-reactivity of the OT antiserum with AVP was 0.015%, whereas that of the AVP antiserum with OT was 0.001%. The limits of sensitivity of the assays were 0.5 pg (AVP) and 1 pg (OT). The inter- and intra-assay coefficients of variation were 5–7% for AVP and 7–9% for OT. AVP and OT standards were purchased from Calbiochem-Novabiochem Co. (La Jolla, CA, USA).  $^{125}\text{I}$ -labelled AVP and OT were purchased from Amersham, France.

All results are given as means  $\pm$  standard error of the mean (S.E.M.) and significance of differences was analysed using SigmaStat (Jandel Scientific, San Rafael, CA, USA) by using Student's or Tukey's  $t$  tests.

## RESULTS

### Binding

In order to determine the types and pharmacology of neurohypophysial  $Ca^{2+}$  channels, saturation radioligand binding experiments were first conducted (Kristipati *et al.* 1994) using conopeptides with different selectivities (Fig. 1).

Preliminary experiments on crude membranes prepared from rat neurohypophyses showed primarily low-affinity non-saturable binding of the radioiodinated conopeptides (data not shown). Subsequent binding experiments used purified plasma membranes, and, because of the larger amounts of material required, bovine neurohypophyses were used. Previous studies (Kristipati *et al.* 1994) have shown that  $K_d$  values for binding of radioiodinated MVIIA and MVIIC are, within experimental error, identical on membranes prepared from rat and cow brain.

With the isolated plasma membranes prepared from bovine neurohypophyses,  $^{125}\text{I}$ -labelled synthetic MVIIA (SNX-111, a specific N-type channel blocker) was found to have a single class of high-affinity binding sites (Fig. 1*Aa*;  $K_d$ ,  $1.6 \pm 0.4 \text{ pM}$ ,  $n = 3$  independent experiments,  $B_{\text{max}}$  (maximal number of binding sites),  $1.1 \pm 0.3 \text{ pmol (mg protein)}^{-1}$ ). These results are consistent with previous data obtained for the rat neurohypophysis using GVIA (Dayanithi *et al.* 1988), a conopeptide of different binding kinetics from but similar specificity to MVIIA (Kristipati *et al.* 1994; Olivera *et al.* 1994). The  $K_d$  obtained here for MVIIA is similar to that ( $3.2 \pm 0.7 \text{ pM}$ ) obtained with central synaptosomes from the rat (Kristipati *et al.* 1994; Olivera *et al.* 1994), while the  $B_{\text{max}}$  value obtained with the peptidergic nerve terminals is slightly higher than that obtained ( $0.54 \text{ pmol mg}^{-1}$ ) with CNS synaptosomes (Kristipati *et al.* 1994; Olivera *et al.* 1994).

Displacement of radiolabelled MVIIA by unlabelled MVIIA or MVIIC (Fig. 1*Ba*) gave  $K_i$  values of  $0.47 \pm 0.4$  ( $n = 3$ ) and  $30 \pm 7 \text{ pM}$  ( $n = 3$ ), respectively. In comparison, the corresponding values for central synaptosomes are  $0.39 \pm 0.02$  ( $n = 3$ ) and  $12.6 \text{ pM}$  ( $n = 1$ ), respectively. It is concluded that there is a single high-affinity binding site for the N-type  $Ca^{2+}$  channel-selective conotoxins on the isolated neurohypophysial plasma membranes, and that this site is similar or identical to that on CNS synaptic membranes.

In contrast, saturation binding experiments with the less selective synthetic toxin MVIIC (SNX-230) showed evidence for multiple binding sites (Fig. 1*Ab*). The binding isotherm was fitted to logistic equations with one to three binding sites, and in all cases the  $K_d$  value obtained for the highest affinity binding site was within the range obtained with membranes prepared from central synaptosomes (5–10  $\text{pM}$ , data reflect three independent experiments). The  $B_{\text{max}}$  values estimated for the highest affinity binding site were also within the range obtained with central synaptosomes (from 1 to  $1.5 \text{ pmol mg protein}^{-1}$  for the highest affinity site). However, the data are different from those obtained from CNS synaptic membranes in that the latter show only a single high-affinity site for MVIIC, which is attributed to a P/Q class of  $Ca^{2+}$  channel (Kristipati *et al.* 1994; Olivera *et al.* 1994).

Displacement of radioiodinated MVIIC from the neurohypophysial membranes (Fig. 1*Bb*) showed that, unlike CNS synaptic membranes (Hillyard *et al.* 1992; Ramachandran *et al.*

al. 1993; Kristipati *et al.* 1994; Gaur *et al.* 1994; Olivera *et al.* 1994), multiple binding components are observed with displacements by both MVIIA and MVIIC. However, with both displacing ligands, the values of  $IC_{50}$  for the major component are indistinguishable from those obtained with CNS synaptosomes at comparable radioligand concentrations (30–100 nM with MVIIA, 10–50 pM with MVIIC, values bound the data from three experiments). In CNS synaptosomes this displacement is thought to reflect occupancy by MVIIC of the P/Q class of  $Ca^{2+}$  channels (Kristipati *et al.* 1994; Olivera *et al.* 1994). Thus this additional high-affinity MVIIC site on the posterior pituitary membranes led us to test whether a functional P- or Q-type  $Ca^{2+}$  channel also existed on these nerve terminals.

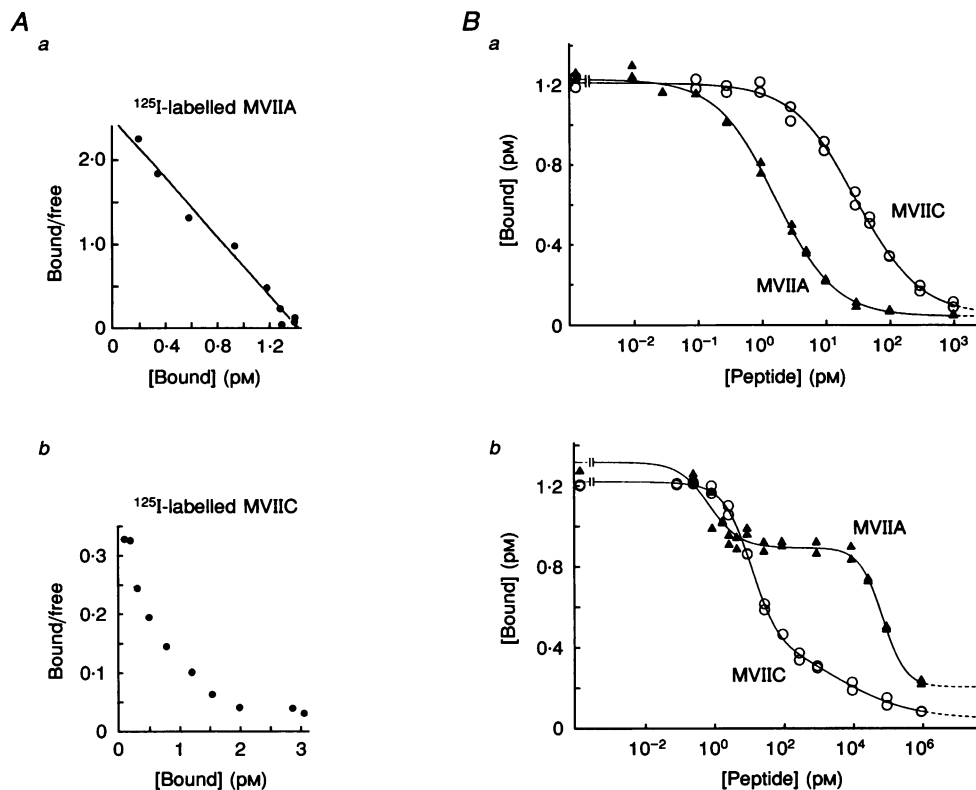
### $Ca^{2+}$ channel currents

The use of the DHP  $Ca^{2+}$  channel antagonist nifedipine (at 2.5  $\mu M$ ) selectively inhibited the long-lasting component of the  $Ba^{2+}$  current (Fig. 2) in these isolated neurohypophysial terminals. This confirmed experiments at the single-channel level, indicating selective blockade of the L-type  $Ca^{2+}$  channel (Lemos & Nowycky, 1989; Wang *et al.* 1993a). As can be seen in Fig. 2A and B, this block reached plateau values within 3 min, and remained steady for up to 1 h

(data not shown). Further addition of 800 nM GVIA (SNX-124) or MVIIA (SNX-111) led to rapid inhibition of a large proportion ( $39.8 \pm 5\%$ ,  $n = 8$ ) of the isolated transient component of the  $Ba^{2+}$  current (Fig. 2A and B). This concentration had been previously shown to maximally block the transient component (Wang *et al.* 1992), which had an  $IC_{50}$  of 50 nM for GVIA. However, in agreement with previous results, not all of the transient component could be blocked by this or even higher (3–4  $\mu M$ ) concentrations of this toxin (Fig. 2C).

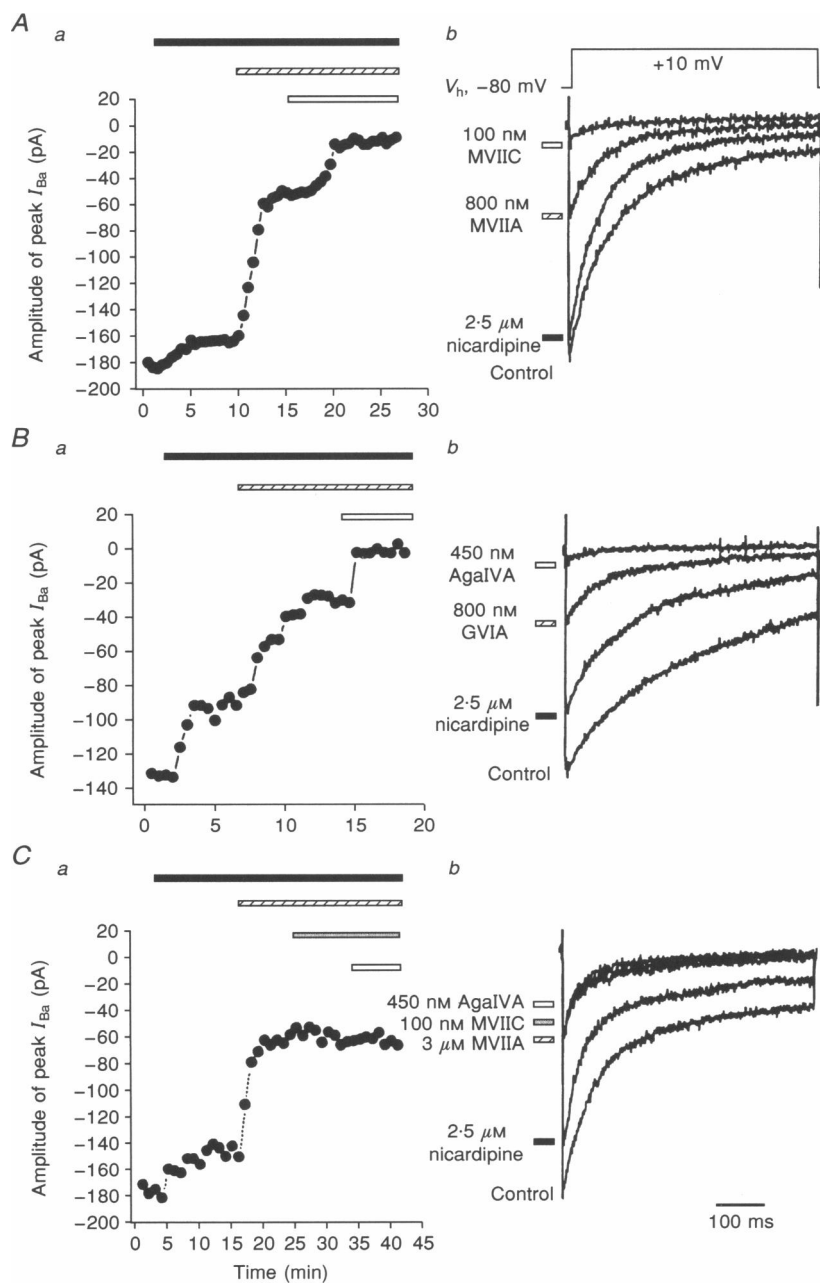
In a majority (57%; see group 1 of Table 1) of neurohypophysial terminals, subsequent addition of low (36 nM) concentrations of MVIIC inhibited (by  $31.4 \pm 9\%$ ,  $n = 3$ ) this resistant component and higher (150 nM) concentrations almost completely abolished it (Fig. 2A). The  $IC_{50}$  using MVIIC for the resistant component was 40.8 nM (Fig. 3A). Higher concentrations (1  $\mu M$ ) could also inhibit the N-type  $Ba^{2+}$  current (data not shown).

In order to test whether the resistant component could be a P/Q-type channel, the same protocol was repeated (Fig. 2B), but instead of MVIIC the funnel-web spider toxin AgaIVA (Mintz *et al.* 1992) was subsequently added. Even with long-term incubations, concentrations (< 30 nM) at which this



**Figure 1. Saturation and displacement binding of synthetic  $\omega$ -conopeptides to the plasma membranes of the neurohypophysial terminals**

A, saturation experiments for MVIIA (a) and MVIIC (b). The line indicates linear Scatchard relationship for MVIIA binding. B, displacement experiments, with MVIIA (a) or with MVIIC (b), using the  $^{125}I$ -labelled conopeptide. Note the different scales. Binding experiments were performed on plasma membranes prepared from bovine neurohypophyses. Displacement data are fitted with logistic equations for 1 and 2 site models (Kristipati *et al.* 1994; Olivera *et al.* 1994).



**Figure 2.** Subpopulations of the macroscopic  $I_{\text{Ba}}$  in the neurohypophysial terminals can be pharmacologically identified by applying different  $\text{Ca}^{2+}$  channel blockers

$I_{\text{Ba}}$ , elicited with 5 mM  $\text{Ba}^{2+}$  as the charge carrier, was recorded using the perforated-patch-clamp method on individual isolated neurohypophysial nerve terminals of the rat. *Ab*, a series of representative (typical of group 1; see Table 1) traces of macroscopic  $I_{\text{Ba}}$  elicited by depolarizations from  $-80$  (holding potential,  $V_h$ ) to  $+10$  mV. The largest current was recorded under control conditions and the others after subsequent applications of the L-type blocker nicardipine, the N-type  $\text{Ca}^{2+}$  channel blocker MVIIA, and the P/Q-type blocker MVIIC. *Aa*, time–response curve of the peak values of  $I_{\text{Ba}}$  with the corresponding traces in *b*, shown by the different bars, indicating each maximal effect. *Bb*, a series of representative traces (again typical of group 1) of macroscopic  $I_{\text{Ba}}$  elicited by depolarizations to  $+10$  mV from a holding potential of  $-80$  mV. The largest  $I_{\text{Ba}}$  was recorded under control conditions and the others after subsequent applications of nicardipine, then GVIA at saturating concentrations, and, finally, another P/Q-type  $\text{Ca}^{2+}$  channel blocker, AgaIVA. *Ba*, time–response curve of the peak values of  $I_{\text{Ba}}$  with the corresponding traces (*b*), shown by the different bars, indicating each maximal effect. *Cb*, representative (typical of group 2; see Table 1) traces of macroscopic  $I_{\text{Ba}}$  in the isolated rat neurohypophysial terminal that was not pharmacologically sensitive to the P/Q-type  $\text{Ca}^{2+}$  channel blockers AgaIVA or MVIIC. Using the same protocols as described in *A* and *B*,  $I_{\text{Ba}}$  was recorded under control conditions and the others after subsequent applications of the L-type blocker nicardipine, the N-type  $\text{Ca}^{2+}$  channel blocker MVIIA (in order to ensure that all of this type of current was blocked), and the P/Q-type blockers MVIIC and AgaIVA. Unlike the results shown in *A* and *B*, there was a residual  $I_{\text{Ba}}$  that could not be inhibited by either AgaIVA or MVIIC. *Ca*, time–response curve of the peak values of  $I_{\text{Ba}}$  with the corresponding traces in *b*, shown by the different bars, indicating each maximal effect.

**Table 1. Relative effects of different Ca<sup>2+</sup> channel blockers on neurohypophysial terminals**

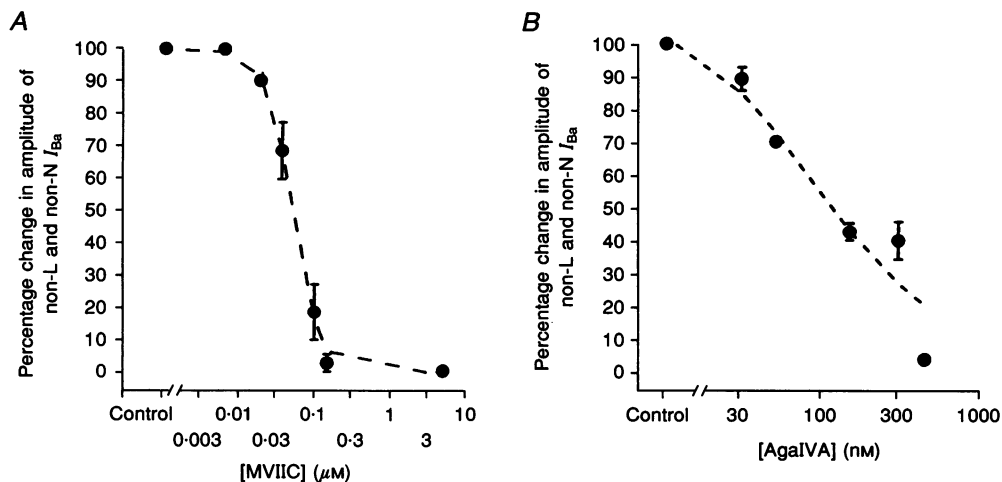
Channel blocker	$I_{Ca}$ (group 1)	$I_{Ca}$ (group 2)	$[Ca^{2+}]_i$ (group 1)	$[Ca^{2+}]_i$ (group 2)	Release of AVP	Release of OT
Per cent of total	57	43	47	53	—	—
Nic (L)	28.6 ± 3.7	24.9 ± 6.7	28.5 ± 4.4	25.4 ± 6.7	30.7 ± 2.5	29.5 ± 3.3
GVIA (N)	35.1 ± 1.8	51.1 ± 4.5	33.5 ± 3.7	67.6 ± 8.25	31.8 ± 1.8	31.5 ± 5.1
MVIIC (Q)	31.4 ± 2.2	7.9 ± 3.2	30.0 ± 5.1	-3.3 ± 8.2	24.5 ± 1.1	-3.5 ± 8.2
None	4.9 ± 1.9	16.1 ± 2.5	8.0 ± 1.4	10.3 ± 3.7	13.0 ± 1.4	35.5 ± 5.1

The relative contributions of the different Ca<sup>2+</sup> channel types to macroscopic Ca<sup>2+</sup> current ( $I_{Ca}$ ), intraterminal calcium ( $[Ca^{2+}]_i$ ), vasopressin (AVP) and oxytocin (OT) release were assessed by blocking with the appropriate concentrations of blockers known to be relatively specific for each type (L, N and Q) of Ca<sup>2+</sup> channel. Nicardipine (Nic; 2.5–5 μM) was used to block the L-type, 800 nM GVIA or MVIIA to block the N-type, and 100–300 nM MVIIC to block the putative Q-type Ca<sup>2+</sup> component. The neurohypophysial terminals tested ( $n = 69$ ) were sorted into two groups depending on whether they were (group 1) or were not (group 2) affected by low concentrations of MVIIC (or high concentrations of AgaIVA) after application of nicardipine and MVIIA/GVIA. All data are expressed as percentages of total ± s.e.m.

toxin is reported to be specific for P-type channels (Mintz *et al.* 1992) had no significant effect on the resistant Ba<sup>2+</sup> current component ( $10.2 ± 3.9\%$ ,  $n = 5$ ,  $P > 0.05$ ), but at higher concentrations ( $IC_{50}$ , 116.5 nM; Fig. 3B) AgaIVA completely, but reversibly, blocked the remaining Ba<sup>2+</sup> current (Fig. 2B). The block of the resistant Ba<sup>2+</sup> current by AgaIVA was much faster than that by MVIIC (Fig. 2), even when the latter was applied at higher (> 450 nM) concentrations. Similar inhibition was observed using FTX, another P-channel selective toxin from the funnel-web spider (Wang & Lemos, 1994). In some terminals, in contrast, the resistant current was not blocked by either AgaIVA or MVIIC (see Fig. 2C and group 2 in Table 1). For this

experiment MVIIA was used because while both GVIA and MVIIA have similar specificities and potencies, MVIIA acts more quickly and its action is more easily reversible.

The inhibition of the resistant Ca<sup>2+</sup> current component, in about half of the neurohypophysial terminals, by both low concentrations of MVIIC and high concentrations of AgaIVA led to the conclusion that this channel most closely resembles the  $\alpha_{1A}$  Ca<sup>2+</sup> channel subunit expressed in *Xenopus* oocytes (Sather *et al.* 1993) and/or the Q-type channel reported in cerebellar granule neurons (Randall & Tsien, 1995). Biophysical characterization of the GVIA- and DHP-resistant component of the neurohypophysial terminal  $I_{Ca}$  also favours



**Figure 3. Dose–response curves for both MVIIC and AgaIVA inhibition of the nicardipine- and GVIA-resistant, macroscopic  $I_{Ba}$  of neurohypophysial nerve terminals**

A, dose–response curve for the effect of MVIIC on the non-L and non-N  $I_{Ba}$  in a total of eleven nerve terminals (each concentration is presented as the mean ± s.e.m.). B, dose–response curve for the effect of AgaIVA on the non-L and non-N  $I_{Ba}$  in a total of fourteen nerve terminals (means ± s.e.m.). The  $IC_{50}$  of each curve was obtained from fitting with the equation:  $I = I_{max}(1 - (x/(IC_{50} + x)))$ , where  $I$  is the current amplitude at a given voltage,  $I_{max}$  is the maximum current and  $x$  is the concentration of the blocker.

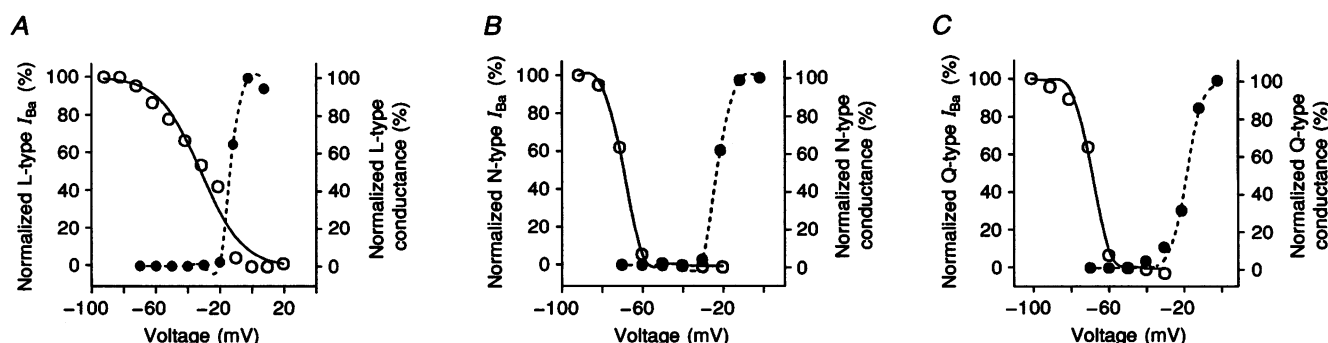
a Q-type  $\text{Ca}^{2+}$  channel classification. This component of the current is a transient, high-voltage-activated  $\text{Ba}^{2+}$  current with a rate of inactivation of  $22.7 \pm 2.9$  ms ( $n = 10$ ; see also Fig. 2) during a step to 0 mV. Figure 4 compares the activation and steady-state inactivation curves of the L-, N- and Q-type components of the nerve terminal  $\text{Ca}^{2+}$  current. The activation ( $V_{1/2}$ ,  $-17.1$  mV) and steady-state inactivation ( $V_{1/2}$ ,  $-68.2$  mV) characteristics of this neurohypophysial MVIIC- and AgaIVA-sensitive current in this group (1) of terminals are also most consistent with those of the Q-type  $\text{Ca}^{2+}$  channel. The DHP- and GVIA-resistant  $\text{Ca}^{2+}$  channel component of group 2 appears to have similar biophysical properties, but it has not yet been well characterized.

### Intraterminal $[\text{Ca}^{2+}]_i$

As previous experiments had effectively shown roles for the N-like and L-like  $\text{Ca}^{2+}$  currents in the release of AVP (Cazalis *et al.* 1987; Dayanithi *et al.* 1988), subsequent experiments were directed towards demonstrating a role for the Q-like current in depolarization–secretion coupling. In order to investigate its contribution to depolarization-induced rises in bulk  $[\text{Ca}^{2+}]_i$ , terminals were loaded with fura-2 and perfused with 15–20 s duration pulses of 50 mM  $\text{K}^+$ .

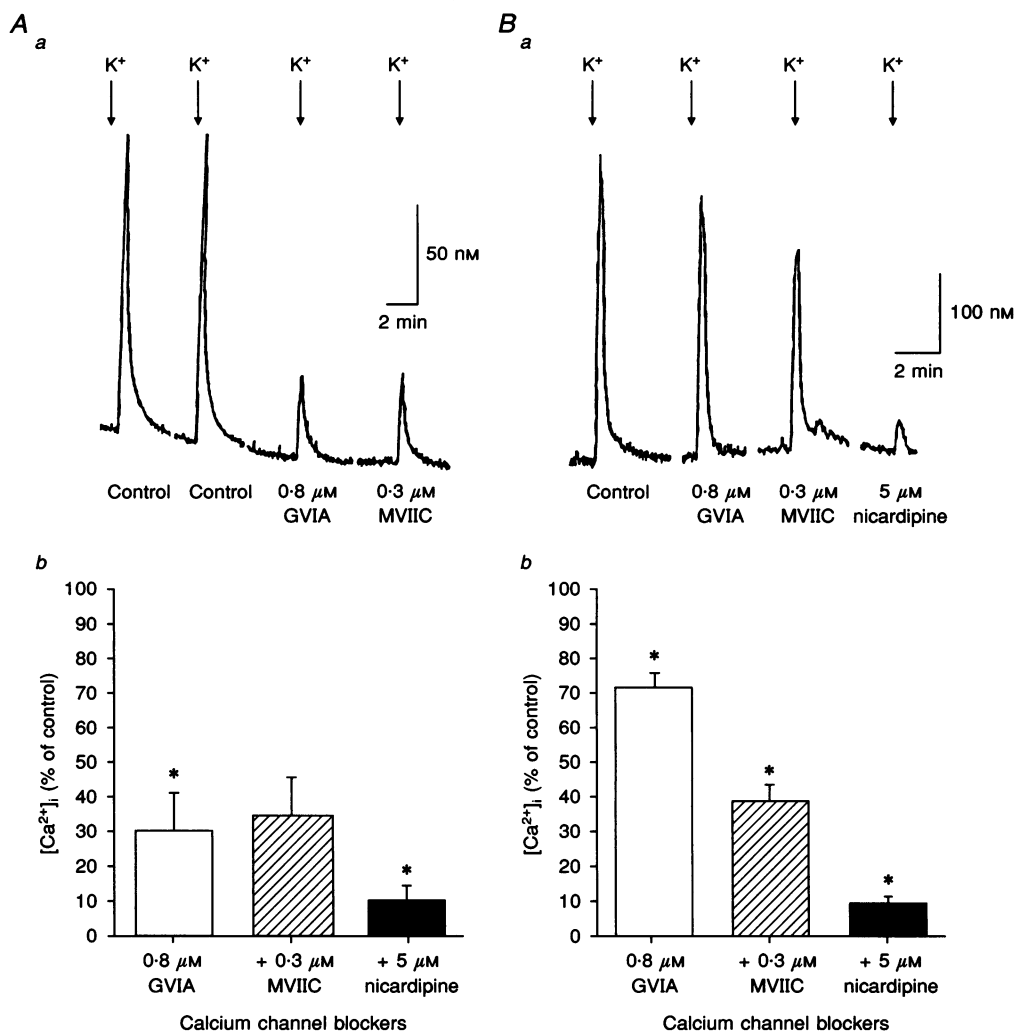
Toxin additivity experiments were performed with concentrations of GVIA (800 nM) and MVIIC (300 nM) that produced maximal block of the fura-2 signal for each

peptide. Under these conditions, two separate populations of nerve terminals were observed (Fig. 5A and B), based on whether or not MVIIC, after GVIA, had any significant effect on depolarization-induced rises in bulk  $[\text{Ca}^{2+}]_i$ . In about half (53%) of the terminals tested ( $n = 33$ ), MVIIC substantially inhibited (by 34%) the GVIA-resistant increase in  $[\text{Ca}^{2+}]_i$  (Fig. 5B). Thus the two conotoxins showed substantial additivity in their inhibitions. Interestingly, in this group GVIA showed only a modest (33.5%) inhibition, while in the other group of terminals (group 2), GVIA showed a more significant (67.6%) inhibition, but, most importantly, subsequent applications of MVIIC did not have any statistically significant effect (Fig. 5Ab). As illustrated in Fig. 5Aa for a single terminal, addition of 800 nM GVIA greatly inhibited the rise in intraterminal  $[\text{Ca}^{2+}]_i$  in response to 50 mM  $\text{K}^+$ . Subsequent addition of 300 nM MVIIC (together with GVIA), however, gave no further reduction of the rise in  $[\text{Ca}^{2+}]_i$ . Note that there is no significant rundown in the fura-2 response, as indicated by the almost identical responses to the two control (no added toxins) stimulations given initially. In Fig. 5Ba, in contrast, is a representative group 1 neurohypophysial terminal, where MVIIC had a significant inhibition and GVIA showed a more modest inhibition of the rise in  $[\text{Ca}^{2+}]_i$ . The remaining increase in  $[\text{Ca}^{2+}]_i$  was nearly eliminated by subsequent applications of 5  $\mu\text{M}$  nifedipine. In Fig. 5Ab and Bb these



**Figure 4.** Voltage dependence of  $\text{Ba}^{2+}$  currents in the rat neurohypophysial terminals

Activation (●) and steady-state inactivation (○) curves for the three pharmacologically isolated, macroscopic  $\text{Ba}^{2+}$  currents are illustrated. These representative curves were obtained from isolated  $\text{Ba}^{2+}$  currents recorded from three different single nerve terminals. The peak  $\text{Ba}^{2+}$  responses were normalized to the maximal currents (for the steady-state inactivation curve) or conductances (for the activation curve) and plotted *vs.* the holding potentials or *vs.* the depolarizing potentials. Data for activation and steady-state inactivation were fitted using appropriate forms of the Boltzmann equations. For activation, the equation is  $G = G_{\text{max}}(1 + \exp((V_{1/2} - V_s)/k))^{-1}$ , whereas for steady-state inactivation, the equation is  $I = I_{\text{max}}(1 + \exp((V_s - V_{1/2})/k))^{-1}$ , where  $G$  is the conductance at a given voltage,  $G_{\text{max}}$  is the maximum conductance,  $I$  is the current amplitude at a given voltage,  $I_{\text{max}}$  is the maximum current,  $V_s$  is the voltage step,  $V_{1/2}$  is the mid-point potential and  $k$  is the slope parameter. *A*, in order to isolate the L-type  $I_{\text{Ba}}$ , the terminal was pretreated with 800 nM MVIIC and 100 nM MVIIC. The fitting of the two curves for the isolated, nifedipine-sensitive, long-lasting  $I_{\text{Ba}}$  gives  $V_{1/2}$  values of  $-11.4$  mV ( $k = 2.3$ ) and  $-30.3$  mV ( $k = 12.3$ ) for activation and inactivation, respectively. *B*, the isolated N-type  $\text{Ba}^{2+}$  current was obtained by applying the L-type  $\text{Ca}^{2+}$  blocker nifedipine (2.5  $\mu\text{M}$ ) and the P/Q channel blocker AgaIVA (450 nM). The fitting of the two curves for the isolated, GVIA-sensitive (N-type)  $I_{\text{Ba}}$  gives  $V_{1/2}$  values of  $-21.2$  mV ( $k = 2.6$ ) and  $-68.5$  mV ( $k = 3.2$ ) for activation and inactivation, respectively. *C*, using the same protocols as described in Fig. 2A and B, the MVIIC/AgaIVA-sensitive, transient  $I_{\text{Ba}}$  was isolated. The fitting of the two curves for the isolated Q-type  $I_{\text{Ba}}$  gives  $V_{1/2}$  values of  $-17.1$  mV ( $k = 4.4$ ) and  $-68.2$  mV ( $k = 3.8$ ) for activation and inactivation, respectively.



**Figure 5. Sensitivity of depolarization-induced  $[Ca^{2+}]_i$  transients to  $Ca^{2+}$  channel blockers**

*Aa*, representative traces, typical of group 2 (see Table 1) neurohypophysial terminals, of elevated (50 mM)  $K^+$ -induced changes in  $[Ca^{2+}]_i$ . A single nerve terminal was sequentially depolarized, with the two initial stimulations in the absence of any channel blockers, then in the presence, as indicated, of 0.8 μM GVIA, and, finally, together with 0.3 μM MVIIC. Each blocker was present for > 2 min prior to  $K^+$ -induced (arrows) membrane depolarizations (each for 20 s) with each treatment containing the blocker in combination with those used in the prior  $K^+$  stimulations. Breaks in the trace omit recovery periods (> 4 min) following  $K^+$ -induced depolarization. *Ab*, summarized data for group 2 (53%) of terminals tested. The bar graph shows the percentage (%) of control  $[Ca^{2+}]_i$  evoked by high (50 mM)  $K^+$  after treatment for 3–5 min with subsequent applications of 0.8 μM GVIA, plus 0.3 μM MVIIC, and plus 5 μM nicardipine. *Ba*, representative recording, typical of group 1 (see Table 1) neurohypophysial terminals, of elevated (50 mM)  $K^+$ -induced changes in  $[Ca^{2+}]_i$ . A single nerve terminal was sequentially stimulated, initially in the absence of any channel blockers, then in the presence, as indicated, of 0.8 μM GVIA, 0.3 μM MVIIC and 5 μM nicardipine. Each blocker was present for > 2 min prior to  $K^+$ -induced (arrows) membrane depolarizations (each for 20 s) with each treatment containing the blocker in combination with those used in the prior  $K^+$  stimulations. Breaks in the trace omit recovery periods (> 4 min) following  $K^+$ -induced depolarization. *Bb*, summarized data for group 1 (47%) of terminals tested ( $n = 33$ ). The bar graph shows the percentage (%) of control  $[Ca^{2+}]_i$  evoked by high (50 mM)  $K^+$  after treatment for 3–5 min (see *Ba*) with subsequent applications of 0.8 μM GVIA, plus 0.3 μM MVIIC, and plus 5 μM nicardipine. Note s.e.m. bars and significant effects (\*) at the  $P < 0.05$  level above each relevant bar.

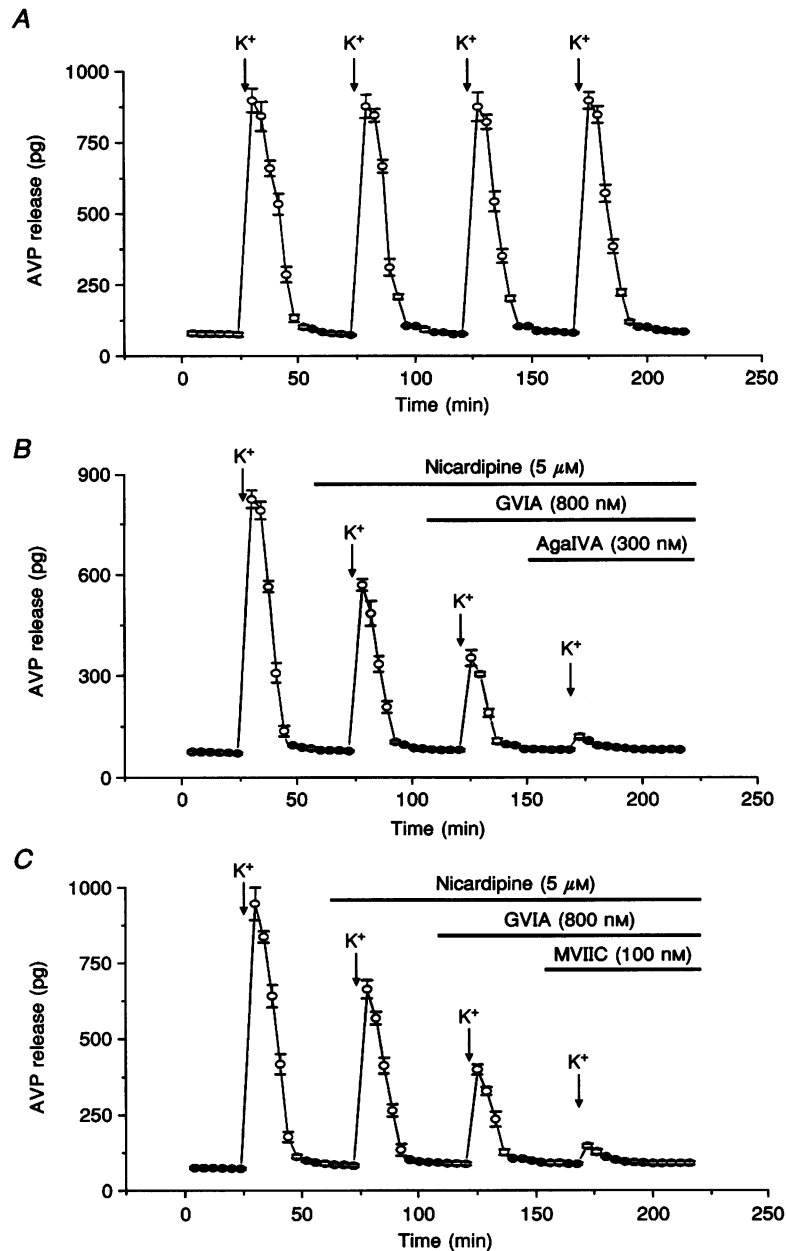


results are summarized, and in Table 1 are correlated with the electrophysiological data.

### Peptide release

In order to determine whether the Q-type  $Ca^{2+}$  channel could play a role in neurosecretion, AVP and OT release from perfused populations of isolated nerve terminals was

measured using peptide-specific radioimmunoassays (see Methods, and also Cazalis *et al.* 1987). Figure 6A shows that repeated stimulation with high (50 mM)  $K^+$  gave, each time, essentially identical AVP release profiles. Utilizing the same pharmacological protocol used to isolate the Q-component electrophysiologically (see Fig. 2), a similar



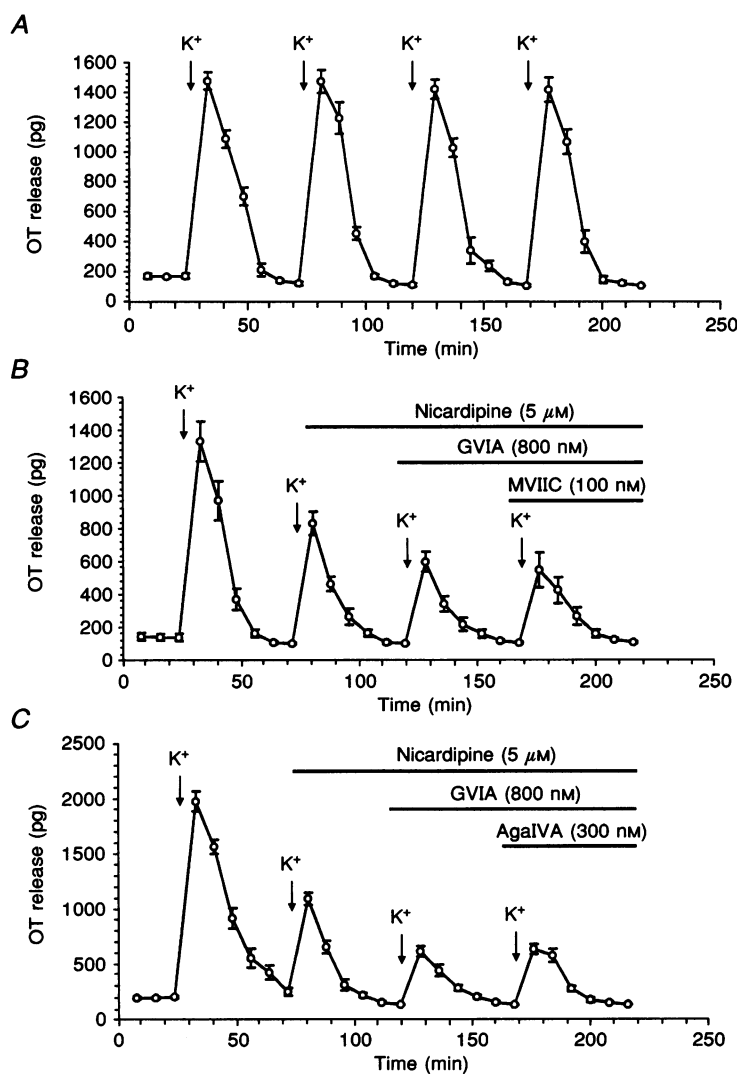
**Figure 6. Effects of  $Ca^{2+}$  channel blockers on the release of AVP from isolated rat neurohypophysial nerve terminals**

A, AVP release stimulated by 50 mM  $K^+$  (arrows) repeatedly (S1, S2, S3 and S4) at regular time intervals. B, using a similar experimental protocol, the nerve terminals were challenged with elevated  $K^+$  either in the absence of channel blockers (S1) or in the presence of 5  $\mu$ M nicardipine (S2), 800 nM GVIA (S3) and 300 nM AgaIVA (S4), as indicated by bars. All these drugs were present for at least 20 min before, during, and after  $K^+$  challenge. C, similarly, in order to further characterize the latter component of  $Ca^{2+}$ -dependent release, 100 nM MVIIC was added 20 min before and during the S4 stimulus. All data points represent the mean  $\pm$  s.e.m. of 4–6 experiments.

resistant component of  $\text{Ca}^{2+}$ -dependent AVP release was revealed (Fig. 6*B* and *C*). This confirmed earlier experiments (Dayanithi *et al.* 1988), which showed that even saturating concentrations of DHP and GVIA when added together could not abolish all of  $\text{Ca}^{2+}$ -dependent peptide release (Wang *et al.* 1993*b*). More importantly, when either high (300 nM) concentrations of AgaIVA (Fig. 6*B*) or low (100 nM) concentrations of MVIIC (SNX-230; Fig. 6*C*) were subsequently added to the isolated terminals, this resistant component of AVP release was essentially abolished ( $P < 0.001$ , Student's *t* test). This was also true even if the order of drugs was reversed or scrambled (data not shown). Furthermore, stimulated release was stable during prolonged

applications of each of the  $\text{Ca}^{2+}$  channel blockers, indicating that steady-state effects had been established.

In contrast, complete inhibition of release was *not* obtained with OT. Utilizing the same pharmacological protocol used to isolate the Q-component electrophysiologically (see Fig. 2) and for AVP release (Fig. 6), a similar resistant component of  $\text{Ca}^{2+}$ -dependent OT release was revealed (Fig. 7). In contrast to AVP release, however, when either low (100 nM) concentrations of MVIIC (Fig. 7*B*) or high (300 nM) concentrations of AgaIVA (Fig. 7*C*) were subsequently added to the isolated terminals, this resistant component of OT release was essentially unchanged



**Figure 7.** Effects of  $\text{Ca}^{2+}$  channel blockers on the release of OT from isolated rat neurohypophysial nerve terminals

*A*, OT release stimulated by 50 mM  $\text{K}^+$  (arrows) repeatedly (S1, S2, S3 and S4) at regular time intervals. *B*, using a similar experimental protocol, the nerve terminals were challenged with elevated  $\text{K}^+$  either in the absence of channel blockers or in the presence of 5  $\mu\text{M}$  nicardipine, 800 nM GVIA and 100 nM MVIIC, as indicated by bars (see Fig. 6). All these blockers were present for at least 20 min before, during and after  $\text{K}^+$  challenge. *C*, similarly, in order to further characterize the latter component of  $\text{Ca}^{2+}$ -dependent release, 300 nM AgaIVA was added 20 min before and during the S4 stimulus. All data points represent the mean  $\pm$  s.e.m. of 4–6 experiments.

( $P = 0.11$ ). Furthermore, the lack of effects of the Q-channel blockers on OT release was significantly ( $P < 0.001$ ; Tukey's test) different from their effects on AVP release (Table 1).

## DISCUSSION

By using purified plasma membranes from neurohypophyses, we were able to obtain saturable high-affinity binding of radiolabelled synthetic MVIIA, and complex (i.e.  $\geq$  two sites) but high-affinity binding of radiolabelled synthetic MVIIC (Fig. 1). The high-affinity binding of MVIIA behaves identically on neurohypophysial plasma membranes and central synaptosomes and almost certainly reflects binding to the N-type  $\text{Ca}^{2+}$  channel (Kristipati *et al.* 1994; Olivera *et al.* 1994). Neurohypophysial membranes are different from CNS synaptosomal membranes in that the latter only show evidence for a single predominant binding site for MVIIC (Kristipati *et al.* 1994), while the former show clear evidence for multiple binding sites (Fig. 1*Ab* and *Bb*). Despite this, the majority of high-affinity MVIIC binding on the neurohypophysial terminals was indistinguishable from that on CNS synaptosomes in being displaced with high affinity by MVIIC and with low affinity by MVIIA or GVIA. Since dihydropyridine-sensitive  $\text{Ca}^{2+}$  channels are thought to be absent from CNS synaptosomes, this binding should represent the P/Q-type of  $\text{Ca}^{2+}$  channel (Kristipati *et al.* 1994; Olivera *et al.* 1994). In contrast to CNS synaptosomes, which contain a high abundance of P/Q-type  $\text{Ca}^{2+}$  channels, the neurohypophysial terminals apparently contain nearly equal amounts of N- and P/Q-type  $\text{Ca}^{2+}$  channels, not to mention DHP-binding sites (Dayanithi *et al.* 1988) corresponding to the L-type  $\text{Ca}^{2+}$  channel. The complexity of MVIIC binding on neurohypophysial membranes probably reflects these differences. Furthermore, because of species differences between rat and cow, as well as the existence of nerve endings of varying diameters, the relative proportions of N- and P/Q-type channels may be different between the binding and the functional studies.

There appear to be four pharmacologically distinct components in terms of relative contributions to the total  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ), intraterminal  $[\text{Ca}^{2+}]_i$ , and peptide release from neurohypophysial terminals (summarized in Table 1). All terminals exhibit an MVIIA/GVIA-sensitive component, but the proportion of the MVIIA/GVIA-sensitive  $I_{\text{Ca}}$  (Fig. 2) and intraterminal  $[\text{Ca}^{2+}]_i$  (Fig. 5) components is larger than its contribution to AVP/OT release (Figs 6 and 7; Table 1). In contrast, the proportions are nearly the same for the nicardipine-sensitive component.

As stated previously, the terminals appear to fall into two groups: those in which the MVIIA/GVIA- and DHP-resistant component was sensitive to MVIIC (group 1) and those that were insensitive (group 2). In group 1 (see Table 1), of the terminals tested, the proportions of each  $\text{Ca}^{2+}$  current component were approximately equal. The MVIIC-sensitive component in this subset of terminals accounts for almost a third of the  $I_{\text{Ca}}$ , which is quite

comparable to its effects on AVP, but not OT, peptide release, but smaller than the inhibition of increases in  $[\text{Ca}^{2+}]_i$  by MVIIC (Fig. 5*B*).

Group 2 terminals (see Table 1), however, did not exhibit a significant MVIIC-sensitive  $I_{\text{Ca}}$  component after application of nicardipine and MVIIA/GVIA. This was also true for about half of the terminals tested for depolarization-induced increases in  $[\text{Ca}^{2+}]_i$  (Fig. 5*A*). Most interestingly, OT release showed no sensitivity to either MVIIC or AgaIVA (Fig. 7), again in the presence of nicardipine and GVIA. At present we do not know which  $\text{Ca}^{2+}$  channel type underlies this resistant component of OT release, but a likely candidate would be the 'R'-type.

The inactivation rate of the MVIIC/AgaIVA-sensitive component of the group 1 neurohypophysial terminal  $\text{Ca}^{2+}$  current is much faster than any reported P-type (Llinás *et al.* 1992), N-type (Tsien *et al.* 1991), or L-type (Bean, 1989) channel and, instead, most closely resembles either the R-type (Zhang *et al.* 1993), T-type (Bean, 1989; Tsien *et al.* 1991), or the fastest component of the  $\alpha_{1A}$ /Q-type (Sather *et al.* 1993; Randall & Tsien, 1995) channels. The T-type, however, is a low-voltage-activated channel, while the R-type is not affected by either MVIIC or AgaIVA, thus a Q-type classification seems most appropriate.

The affinity differences between the binding and electrophysiological studies may be due to a number of reasons, such as different divalent cation concentrations or differences in channel tertiary structure caused by biochemical procedures or by membrane potential gradients. Nevertheless, we find that, as reported for the expressed class A  $\alpha_1$ -subunit in oocytes (Sather *et al.* 1993; Stea *et al.* 1994), concentrations of AgaIVA in the 100–450 nM range are necessary for effective blockade of the Q-currents in the neurohypophysial terminals. In contrast, the P-type  $\text{Ca}^{2+}$  channel is sensitive to block by  $< 30$  nM AgaIVA (Mintz *et al.* 1992). Differential localization of the P, Q, or other type variants of the class A  $\text{Ca}^{2+}$  channel may explain these differences (Stea *et al.* 1994; Westenbroek *et al.* 1995). In further support of this, the hypothalamic cell bodies of these neurohypophysial terminals appear to express a P-, rather than a Q-type variant  $\text{Ca}^{2+}$  channel (Fisher & Bourque, 1995).

A heterogenous localization can also explain the lack of contribution by the Q-type  $\text{Ca}^{2+}$  channel to  $\text{Ca}^{2+}$  intraterminal levels measured by fura-2 (Fig. 5*B*) or to  $I_{\text{Ba}}$  by perforated-patch recordings in about half of the terminals tested. The  $[\text{Ca}^{2+}]_i$  data suggest that in the group 2 terminals (see Table 1), GVIA was more substantial in its inhibition, indicating that the proportion of N-channels was greater here than in the group 1 terminals, which exhibited Q-channels. Since GVIA is specific for the N-type  $\text{Ca}^{2+}$  channel while MVIIC blocks both N and Q, it is likely that both these channels contribute to rises in intraterminal calcium, but that the Q-channel is not homogeneously distributed among the nerve endings. The total block of

AVP release by MVIIC and AgaIVA argues that the Q-type channels can contribute substantially to AVP peptide release (Fig. 6). Together with the lack of any significant (see Table 1 and Fig. 7) effect by these same blockers on DHP- and GVIA-insensitive OT release, these results lead to the hypothesis that these channels are preferentially localized on AVP peptide-containing nerve terminals, and thus do not affect intraterminal  $\text{Ca}^{2+}$  levels or currents in oxytocinergic terminals. Q-type pharmacological heterogeneity has recently been reported in hippocampal synaptic boutons (Reuter, 1995). Since those terminals are homogenous in terms of the transmitter released, it cannot be ruled out that the heterogeneity in peptidergic terminals is instead functional, i.e. Q-type channels can be found in both vaso-pressinergic and oxytocinergic terminals but are not always expressed (i.e. functionally regulated) or are, perhaps, not coupled to the release machinery in oxytocinergic terminals.

In summary, the isolated neurohypophysial terminals are uniquely useful for studying the biochemical, biophysical, and functional properties of  $\text{Ca}^{2+}$  channels at the site of secretion. The use of ligands with different selectivities for the L, N and P/Q type  $\text{Ca}^{2+}$  channels has allowed us to show that these three types of  $\text{Ca}^{2+}$  channels co-exist on these peptidergic CNS terminals, and that they contribute to depolarization–secretion coupling. The electrophysiological results strongly suggest that the high-affinity MVIIA/GVIA binding site on these nerve endings corresponds to a large proportion of the transient (originally termed  $\text{N}_T$ -type) calcium current, while the additional high-affinity binding site that uniquely interacts with MVIIC corresponds to the MVIIA/GVIA-resistant component of the transient  $\text{Ca}^{2+}$  current. Although it has been recently shown that the P- or Q-channel can exist in nerve terminals (Westenbroek *et al.* 1995) where it appears to be involved in neurotransmission (Miljanich & Ramachandran, 1995), the biophysical properties of the P/Q-current at nerve terminals had not previously been defined. We show here that the fast-inactivating, or Q-form of the  $\alpha_{1A}$  class of  $\text{Ca}^{2+}$  currents exists on these peptidergic nerve terminals, where it participates in the control of neurohormonal secretion. Furthermore, these Q-type channels are not homogeneously distributed in all neurohypophysial terminals. Rather they appear to be functional in only half of the terminals, while the L- and N-type  $\text{Ca}^{2+}$  channels are co-localized in all terminals. The release results suggest that the Q-type component plays an important role in AVP, but not OT, neurosecretion from these CNS terminals. Thus there appear to be three components to  $\text{Ca}^{2+}$ -dependent AVP release from these nerve terminals: corresponding to 'L', 'N', and, now, 'Q'-type  $\text{Ca}^{2+}$  channels.

In conclusion, Q-type  $\text{Ca}^{2+}$  channels appear to exist in at least one type of CNS terminal and are involved in depolarization–secretion coupling. This lends support to the idea that Q-type channels may play a role in synaptic transmission in other CNS synapses (Wheeler, Randall & Tsien, 1994; Reuter, 1995). The data presented here verify

the specific identities and importance of the  $\text{Ca}^{2+}$  channels actually located at nerve terminals, but point out that one type (Q), at least, may be heterogeneously functional. This could have widespread ramifications in terms of CNS synaptic plasticity.

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#### Acknowledgements

Portions of this work were supported by Neurex Corp. and we would like to thank members of their synthetic chemistry group for providing the synthetic peptides. We also thank G. Miljanich for support and advice. We would also like to acknowledge support by NSF (National Science Foundation) grant IBN 9410834 (E. L. S.) and NIH grant NS29470 (J. R. L.).

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Received 7 October 1996; accepted 18 April 1997.