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PHARMACOLOGICAL PROPERTIES OF VOLTAGE-GATED Na^+ CURRENTS IN MOTOR NEURONES FROM A HYDROZOAN JELLYFISH *POLYORCHIS PENICILLATUS*

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Summary

The Na^+ current of 'swimming motor neurones' in the hydromedusan *Polyorchis penicillatus* was tetrodotoxin-insensitive. The local anaesthetics lidocaine and procainamide caused partial, non use-dependent blockade of the Na^+ channel. Veratridine produced partial blockade of the Na^+ channel without affecting inactivation. An order of blocking potency of di- and trivalent cations was established as: $La^{3+} = Zn^{2+} = Cd^{2+} > Ni^{2+} > Mn^{2+} = Co^{2+} > Ca^{2+} > Ba^{2+} > Mg^{2+}$. All these cations, except Ba^{2+}, produced depolarizing shifts in the conductance–voltage curves. Even at relatively high concentrations, the dihydropyridines nicardipine, nitrendipine and (+)Bay K 8644 produced only weak blockade of the Na^+ current; while nimodipine, nifedipine and (−)Bay K 8644 were ineffective. Diltiazem and verapamil weakly blocked the Na^+ current in a dose-dependent manner with no evidence of use-dependence. The calmodulin inhibitors W7 and calmidazolium were ineffective blockers of Na^+ currents. Crude *Conus* venoms and the *Conus* peptides, μ-conotoxin GIIA, μO-conotoxin MrVIA, ω-conotoxin GVIA and ω-conotoxin MVIIC, were without effect. Capsaicin produced rapid, reversable blockade of Na^+ current.

It has been suggested that 'primitive' Na^+ channels could be expected to have pharmacological properties that are intermediate between those of Na^+ and Ca^{2+} channels. If such channels exist, the Na^+ channel described here is clearly not one of them.

Key words: jellyfish, *Polyorchis penicillatus*, Na^+ current, motor neurone, pharmacology, tetrodotoxin, local anaesthetics, dihydropyridines, conotoxins, evolution.

Introduction

Na^+ currents are responsible for the rapid rising phase of action potentials in many excitable cells of coelenterates (cnidarians and ctenophores), such as the hydrozoans *Polyorchis penicillatus* (Anderson, 1979; Spencer and Satterlie, 1981), *Aglantha digitale* (Meech and Mackie, 1993) and *Cladonema* sp. (Anderson and McKay, 1987), the scyphozoan *Cyanea capillata* (Anderson, 1987) and the ctenophore *Mnemiopsis* sp. (Dubas *et al.* 1988). A Na^+-dependent action potential has been reported from one protozoan species, *Actinocoryne contractilis* (Febvre-Chevalier *et al.* 1986). These Na^+ currents have unconventional pharmacological properties when compared with Na^+ channels in higher metazoans. In particular, the selective Na^+ current blocker tetrodotoxin (TTX), at 10^{-8} mol l^{-1}, blocks most neuronal Na^+ currents in higher metazoans, from flatworms to vertebrates (Hille, 1992), whereas the Na^+-dependent action potentials and Na^+ currents that have been examined to date in excitable cells of lower metazoans and protozoans are minimally affected by TTX, even at concentrations as high as 10^{-4} mol l^{-1} (Anderson, 1979, 1987; Spencer and Satterlie, 1981; Febvre-Chevalier *et al.* 1986; Anderson and McKay, 1987; Dubas *et al.* 1988; Meech and Mackie, 1993).

The TTX-insensitive Na^+ current recorded from motor neurones in the scyphozoan jellyfish *Cyanea capillata* shows a pharmacological profile that is more similar to that expected of Ca^{2+} channels (Anderson, 1987). This finding appears to support the view of Hagiwara (1983) that Ca^{2+} channels are phylogenetically more ancient, since they are diverse and are present in the most 'primitive' organisms. Hille (1984) further developed this theory by proposing that high-velocity conduction, and the associated high current density required, could not be supported by Ca^{2+} action potentials because of the resulting Ca^{2+} toxicity. Selection for Na^+-permeable channels would therefore be very strong in cells specialized for long-distance, high-frequency signalling. Domain homology

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between Ca^{2+} and Na^+ channels further demonstrates that Ca^{2+} channels were the likely immediate ancestral molecules for Na^+ channels (Strong *et al.* 1993). Here, we give an extensive description of the pharmacological properties of a 'primitive' neuronal Na^+ current that assists in determining the phylogenetic and functional relationships between channels carrying the inward currents responsible for propagation of action potentials. In particular, we were interested in determining whether a Ca^{2+}-channel-like pharmacology is common to such 'primitive' Na^+ channels. The TTX-insensitive Na^+ current that we describe from motor neurones innervating swimming muscle in *Polyorchis penicillatus* has an unusual pharmacological profile for a neuronal Na^+ current but does not exhibit any specific properties expected for Ca^{2+} currents. This is shown by the rank order of sensitivity to blockade by di- and trivalent cations ($La^{3+} = Zn^{2+} = Cd^{2+} > Ni^{2+} > Mn^{2+} = Co^{2+} > Ca^{2+} > Ba^{2+} > Mg^{2+}$) and by the low sensitivity of this current to Ca^{2+} channel antagonists (dihydropyridines, diltiazem, verapamil).

Materials and methods

Cell culture

Medium-sized jellyfish *Polyorchis penicillatus* (Eschscholtz) (diameter 20–30 mm) were collected in Bamfield and Grappler Inlets (Vancouver Island, British Columbia, Canada) and maintained at approximately 10 °C in flow-through seawater aquaria. Cells were cultured using a method from Przysiezniak and Spencer (1989) with slight modifications. Cells were exposed to collagenase for a shorter period (1 h instead of 4–5 h), with agitation. Recordings were made using cell cultures that were no more than 3 days old.

Swimming motor neurones were identified by their large size (soma 30–50 μm long, with processes up to 200 μm long), clear cytoplasm and a nucleus surrounded by membranous structures. They have a characteristic action potential that is identifiable *in vitro* and *in vivo*. Major features of the Na^+ currents, such as time-to-peak, peak current, voltage-dependence, time course of responses and pharmacological responsiveness to drugs, did not change with the age of culture.

Whole-cell recordings

Whole-cell, tight-seal recordings were made using electrodes of borosilicate glass tubing (TW-150-4, World Precision Instruments), with resistances of 1–2 MΩ when filled with electrode solutions. Recordings were made at room temperature (approximately 20 °C) with an Axopatch-1D amplifier (Axon Instruments), lowpass-filtered at 3 kHz using a four-pole Bessel filter, and digitized using a Labmaster TL-125 acquisition board (Axon Instruments).

Cultures were viewed under phase contrast with a Nikon Diaphot inverted microscope. Fine alignments of the microelectrode near the cell surface were made using a piezoelectric driver (Burleigh). Stimulus control, data acquisition and analysis were performed using pCLAMP 6.0 software (Axon Instruments) on a 486-based personal computer.

Leakage and capacitative currents were subtracted using $-P/4$ or $-P/5$ protocols (pCLAMP 6.0), from a holding potential of −80 mV, before test pulses eliciting active responses were applied. Series resistances (R_s) were optimally compensated to minimize voltage errors (R_s compensation was usually set to values of 80 % or more). Membrane capacitance (C_m) and R_s values were obtained by minimizing the capacitative transient in response to a hyperpolarizing voltage step. Only recordings that met the following criteria were used in the analysis. (1) Peak uncompensated current was >2 nA; R_s was 2–4 MΩ before compensating for R_s. (2) C_m and R_s did not change by more than 10 % before and after drug application, and recovery after drug application was >70 %. (3) Leakage resistances were in the range 100 MΩ to 0.5 GΩ. (4) After series compensation, Na^+ currents reached their peak within 800 μs using test pulses with voltage steps from −80 mV to +10 mV.

Pharmacological agents were microperfused in bath solution dispensed from a manifold with a dead volume of less than 1 μl. Gravity flow was controlled by electromechanical pinch valves and the rate of flow provided complete solution exchange in 2 s. Test pulses were applied every 30 s after drug application. As soon as a current tracing superimposed on the previous trace this was recorded as the experimental trace (saturation), and washing was started. The maximal drug effect was obtained from 30 s to 2 min after drug application.

Solutions

All culture and recording solutions were filtered through cellulose acetate membrane cartridges with a pore size of 0.2 μm before use. Artificial sea water, contained (in mmol l^{-1}): NaCl, 376; Na_2SO_4, 26; $MgCl_2$, 41.4; $CaCl_2$, 10; KCl, 8.5; Hepes hemisodium salt, 10; and gentamycin sulphate (50 mg l^{-1}). The pH of this solution was 7.5. Na^+-free electrode solutions, containing (in mmol l^{-1}): $MgCl_2$, 2; $CaCl_2$, 1; Hepes-free acid, 10; EGTA, 11; $CsCl_2$, 400; CsOH, 30; and TEA-Cl, 20, were titrated to pH 7.5 with KOH. Standard bath solutions were calcium-free (in mmol l^{-1}): NaCl, 395; $MgCl_2$, 50; *N*-methylglucamine–HCl, 30; Hepes sodium salt, 10, and were titrated to pH 7.5 with HCl. All outward currents were totally blocked within 5 min after breakthrough, and inward Ca^{2+} currents were eliminated since the external solution was Ca^{2+}-free. The total divalent ion concentration was kept constant in solutions containing Ba^{2+} or Ca^{2+} by adjusting the $MgCl_2$ concentration.

Drugs were purchased from Research Biochemicals International and were prepared daily using bath solution. Stocks of lipophilic compounds were prepared daily in fresh dimethylsulphoxide (DMSO) solution. Final working DMSO concentrations were 0.04–0.5 % (v/v). Control responses with DMSO at the above bath concentrations were subtracted from drug responses. Tetrodotoxin was prepared from a stock solution in acetate buffer at pH 4.5 (1 mg ml^{-1}). Numerical and graphical data are presented as means ± S.E.M.

Table 1. *Quantitative summary of the effects of drugs on Na^+ current in* Polyorchis penicillatus *motor neurones*

Drug	Dose (μmol l^{-1})	Effect† (%)	*N*
Sodium channel antagonists			
Tetrodotoxin	100	3.26±4.10	6
Lidocaine	1000	40.95±1.19	9
	3000	67.67±1.20	7
Procainamide	1000	23.58±1.25	4
Veratridine*	100	30.18±9.47	4
Calcium channel antagonists			
Dihydropyridines			
Nicardipine*	40	13.86±2.22	9
	70	26.38±1.15	4
	100	55.24±7.03	7
Nitrendipine*	100	16.66±0.60	5
	200	37.35±0.51	3
Nimodipine*	100	8.07±4.66	3
Nifedipine*	100	–0.40±1.79	5
	500	4.38±0.48	3
(+)Bay K 8644*	100	25.29±2.00	9
(–)Bay K 8644*	100	–4.63±0.61	9
Benzothiazepines			
Diltiazem	50	13.73±4.54	3
	100	29.26±2.64	5
	500	53.23±0.54	9
	1500	64.49±3.82	6
Phenylalkylamines			
Verapamil	100	14.97±1.31	6
	500	24.17±3.74	4
	1500	38.12±0.12	4
Calmodulin antagonists			
W7	100	17.49±1.75	4
Calmidazolium	20	15.37±1.74	9
Non-specific antagonist			
Capsaicin	1	14.70±4.43	3
	5	73.53±8.76	3
	25	89.91±7.62	3

*Control responses in the presence of DMSO were subtracted from values recorded in the presence of drugs.

†Percentage change due to drug application from peak current using voltage steps of 10 ms duration to +10 mV from a holding potential of –80 mV.

Results

The quantitative data for all the chemical agents examined are given in Table 1.

Effect of classical Na^+ channel blockers and modifiers (TTX, lidocaine, procainamide, veratridine)

The Na^+ current from 'swimming motor neurones' was insensitive to TTX at concentrations from 1 to 100 μmol l^{-1} (Fig. 1; Table 1). The local anaesthetics lidocaine and procainamide caused partial, tonic blockade of Na^+ currents at concentrations in the millimolar range and did not exhibit use-dependent blockade (Fig. 1; Table 1). Veratridine, at 100 μmol l^{-1}, produced partial blockade of the Na^+ current without affecting inactivation properties (Fig. 1; Table 1).

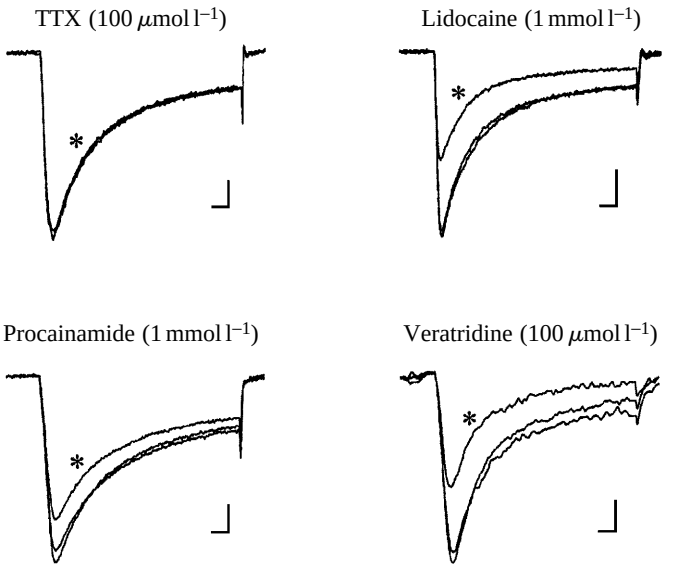

Fig. 1. Effects of Na^+ channel antagonists (TTX, lidocaine, procainamide) and a modifer (veratridine) on Na^+ current. Tracings marked with an asterisk represent drug effects. Tracings are also shown before drug application and after complete washing. In this and all other figures, where the 'before' and 'after' drug application traces do not superimpose, the trace having a smaller peak current is the trace obtained after washing. Responses were generated using voltage steps of 10 ms duration to +10 mV from a holding potential of −80 mV. Vertical and horizontal scale bars represent 1 nA and 1 ms, respectively.

Effect of di- and trivalent cations

Fig. 2 shows the effects of La^{3+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Mn^{2+} and Co^{2+} on Na^+ current at bath concentrations of 3 mmol l^{-1} and 1 mmol l^{-1}. La^{3+}, Zn^{2+} and Cd^{2+} were the most potent blockers, followed by Ni^{2+} then Mn^{2+} and Co^{2+}. All these cations produced depolarizing shifts in the conductance–voltage curves, with La^{3+}, Zn^{2+} and Cd^{2+} causing the greatest shifts of +10, +12 and +10 mV respectively (data not shown).

Ca^{2+} and Ba^{2+} were less effective cationic blockers. At concentrations of 10, 30 and 50 mmol l^{-1}, Ca^{2+} was a more potent blocker than Ba^{2+} (Fig. 3). Ca^{2+} produced a noticeable depolarizing shift in the current–voltage curve whereas Ba^{2+} had little, if any, effect. Charge screening effects (Hille *et al.* 1975) should not apply here, since the concentration of divalent cations was kept constant by substitution with Mg^{2+}. The order of blocking potency for all these cations is: $La^{3+} = Zn^{2+} = Cd^{2+} > Ni^{2+} > Mn^{2+} = Co^{2+} > Ca^{2+} > Ba^{2+} > Mg^{2+}$.

Effect of Ca^{2+} channel antagonists (dihydropyridines, verapamil, diltiazem) and an agonist (−)Bay K 8644

Of the dihydropyridines, nicardipine had the greatest

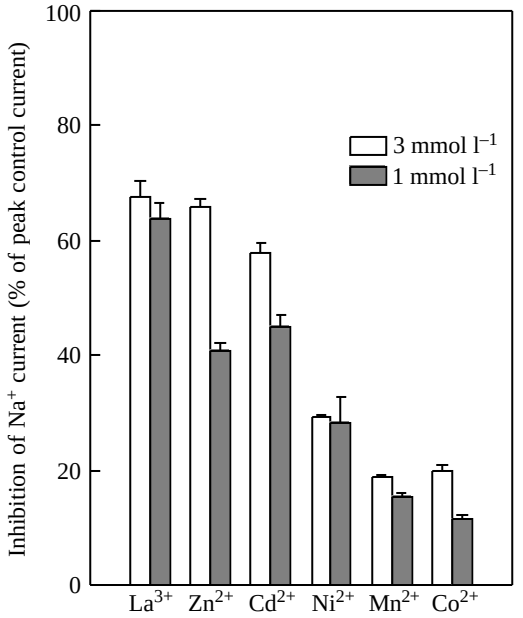

Fig. 2. The inhibitory effects of di- and trivalent cations on Na^+ current at 1 mmol l^{-1} (shaded bars) and 3 mmol l^{-1} (unshaded bars). Data were obtained from the peak of Na^+ currents generated using voltage steps of 10 ms duration to +10 mV from a holding potential of −80 mV. Bars represent S.E.M. Numbers of cells used at 1 mmol l^{-1} and 3 mmol l^{-1} respectively: La^{3+} (9, 5), Zn^{2+} (10, 12), Cd^{2+} (4, 7), Ni^{2+} (5, 4), Mn^{2+} (5, 5), Co^{2+} (6, 5).

inhibitory effect (Table 1), but even at 70 μmol l^{-1} it only produced a 26% decrease in peak current (Fig. 4; Table 1). Nitrendipine at 100 μmol l^{-1} reduced the current by 17%, while nimodipine and nifedipine at 100 μmol l^{-1} had no significant effect (when the inhibitory effect of DMSO in the vehicle solution was taken into account). The antagonistic enantiomer (+)Bay K 8644, at 100 μmol l^{-1}, inhibited peak Na^+ current by 25%, while the agonistic (−) enantiomer had no significant effect (Fig. 4; Table 1). The solubilising agent DMSO at 0.1% (v/v) also inhibited the current by approximately 10%.

Diltiazem and verapamil blocked the Na^+ current in a dose-

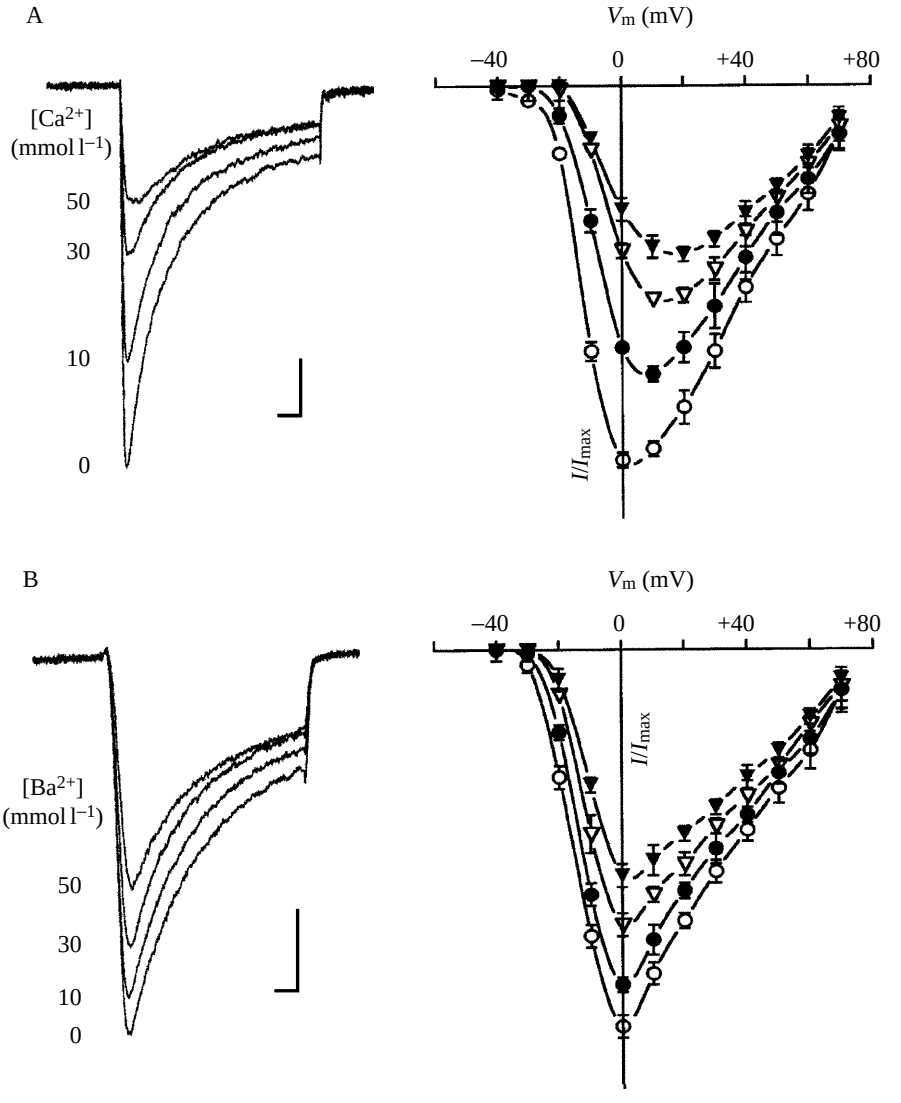

Fig. 3. Effects of 10, 30 and 50 mmol l^{-1} Ca^{2+} and Ba^{2+} on Na^+ current. Left-hand side: Na^+ current tracings in Ca^{2+} (A) and Ba^{2+} (B) solutions generated using voltage steps of 10 ms duration to +10 mV from a holding potential of −80 mV. Right-hand side: current–voltage relationships using Ca^{2+} (A) and Ba^{2+} (B) at concentrations of 0 mmol l^{-1} (open circles), 10 mmol l^{-1} (filled circles), 30 mmol l^{-1} (open triangles) and 50 mmol l^{-1} (filled triangles). Currents are normalised to peak control currents. Responses were generated using voltage steps from −40 mV to +70 mV in 10 mV increments. The holding potential was −80 mV. Vertical and horizontal scale bars represent 1 nA and 1 ms, respectively. Error bars represent S.E.M.

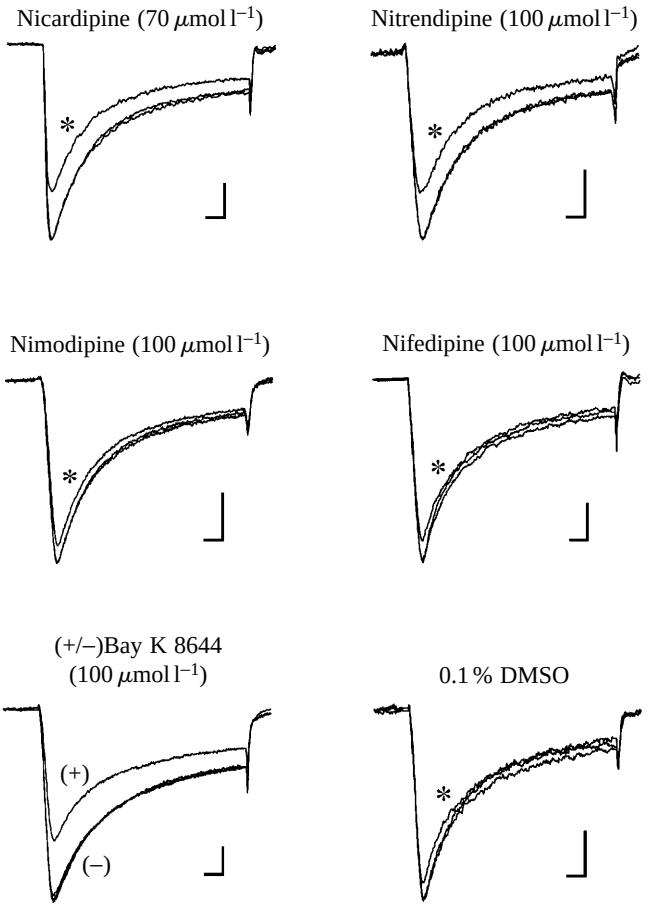

Fig. 4. Effects of dihydropyridines and the solubilizing agent dimethylsulphoxide (DMSO) on Na^+ current. Tracings marked with an asterisk represents drug effects. Tracings are also shown before drug application and after complete washing. For Bay K 8644, the + and − enantiomers are marked; note that the effect of (−)Bay K 8644 was no different from the control or wash traces for both enantiomers. Responses were generated using voltage steps of 10 ms duration to +10 mV from a holding potential of −80 mV. Vertical and horizontal scale bars represent 1 nA and 1 ms, respectively.

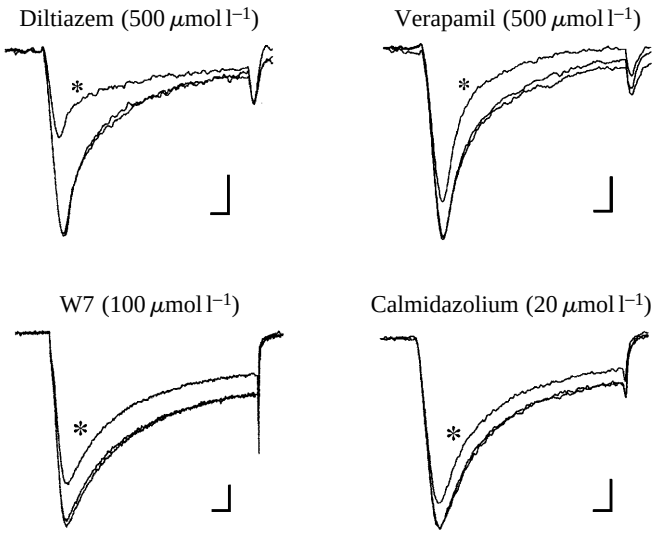

Fig. 5. Effects of antagonists of L-type Ca^{2+} channels (diltiazem, verapamil) and calmodulin antagonists (W7 and calmidazolium) on Na^+ current. Tracings marked with an asterisk represent drug effects. Tracings are also shown before drug application and after complete washing. Responses were generated using voltage steps of 10 ms duration to +10 mV from a holding potential of −80 mV. Vertical and horizontal scale bars represent 1 nA and 1 ms, respectively.

dependent manner. At 500 μmol l^{-1}, they produced 53 % and 24 % decreases, respectively, in current amplitude (Fig. 5; Table 1). There was no evidence of use-dependent blockade by verapamil.

Effect of the calmodulin inhibitors W7 and calmidazolium

Neither W7 (100 μmol l^{-1}) nor calmidazolium (20 μmol l^{-1}) was a very effective blocker of Na^+ current (Fig. 5; Table 1).

Effects of Conus *neurotoxins*

The following *Conus* venoms and peptides were applied to motor neurones: crude venom (1 mg ml^{-1}) from *C. quercinus*, *C. textile* and *C. geographicus*; purified peptides, μ-conotoxin GIIA (1 μmol l^{-1}), μO-conotoxin MrVIA (0.5 μmol l^{-1}) and the ω-conotoxins GVIA (1 μmol l^{-1}) and MVIIC (1 μmol l^{-1}). These *Conus* venoms and peptides had no effect (data not shown).

Effect of capsaicin

The only drug examined to date which was active at comparatively low concentrations was capsaicin (*trans*-8-methyl-*N*-vanillyl-6-nonemide), which produced a rapid and noticeable blockade of Na^+ current at a concentration of 5 μmol l^{-1} (Fig. 6A; Table 1). At 25 μmol l^{-1}, the current was reduced by 90 %. However, the effect of capsaicin is not specific since outward K^+ currents were also strongly inhibited at higher concentrations (300 μmol l^{-1}) (Fig. 6B).

Discussion

On the basis of their susceptibility to tetrodotoxin, three classes of Na^+ currents have been described: TTX-insensitive (>100 μmol l^{-1}), TTX-resistant (1–10 μmol l^{-1}) and TTX-sensitive (nanomolar range) (Anderson, 1987). Our data clearly show that the Na^+ current of jellyfish swimming motor neurones can be classified as TTX-insensitive since concentrations as high as 100 μmol l^{-1} did not block these currents. These data confirm the finding of Anderson (1979) that propagation of action potentials in the swimming motor neurone network of *Polyorchis penicillatus* is not affected by TTX at concentrations up to 125 μmol l^{-1}. An analogue of TTX, saxitoxin, at 25 μmol l^{-1} also failed to block Na^+ currents (Przysiezniak, 1993).

TTX-insensitive Na^+ currents have been described in other cnidarian and ctenophoran cells (Anderson, 1987; Anderson

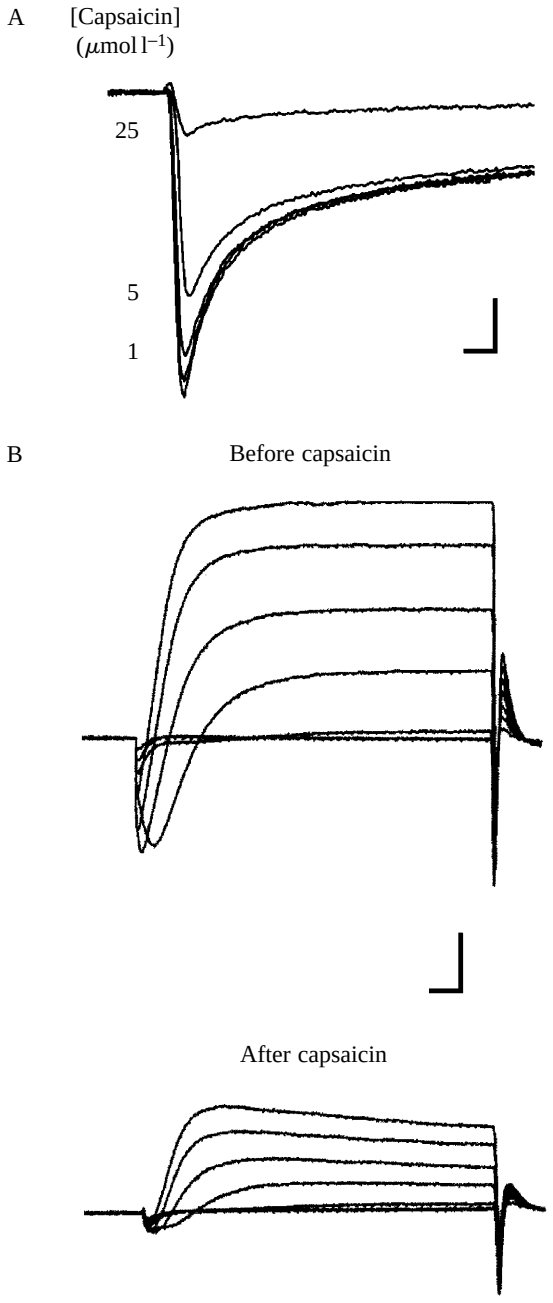

Fig. 6. Effect of capsaicin on Na^+ current. (A) Effects of 1, 5 and 25 μmol l^{-1} capsaicin on Na^+ current. Tracings are also shown before drug application and after complete washing. Responses were generated using voltage steps of 10 ms duration to +10 mV from a holding potential of −80 mV. (B) Effects of capsaicin (300 μmol l^{-1}) on total ionic current. Upper traces: before application of capsaicin. Lower traces: after application of capsaicin. Responses were generated using voltage steps from −40 mV to +60 mV in 20 mV increments. The holding potential was −80 mV. Vertical and horizontal scale bars represent 1 nA and 1 ms, respectively.

and McKay, 1987; Dubas *et al.* 1988; Meech and Mackie, 1993), while the only truly voltage-gated Na^+ current recorded from a protozoan (*Actinocoryne contractilis*) is also TTX-insensitive (Febvre-Chevalier *et al.* 1986). Thus, although TTX insensitivity or resistance is relatively rare throughout the protostome and deuterostome lineages, it appears to be a common feature of lower metazoan Na^+ channels. However, we cannot yet say whether ancestral Na^+ channels were TTX-sensitive or not.

In mammals, TTX-resistant channels have been described from cardiac muscle cells, embryonic skeletal muscle and denervated skeletal muscle (Cohen *et al.* 1981; Gonoi *et al.* 1985; Pappone, 1980). These TTX-resistant channels have a characteristic order of potency for di- and trivalent cation blockade. For example, in canine Purkinje fibre Na^+ channels this order is $Zn^{2+} > Cd^{2+} = La^{3+} > Ni^{2+} \geqslant Mn^{2+} > Co^{2+} > Ba^{2+} = Mg^{2+} = Ca^{2+}$ (Hanck and Sheets, 1992), which is very similar to the order of potency ($La^{3+} = Zn^{2+} = Cd^{2+} > Ni^{2+} > Mn^{2+} = Co^{2+} > Ca^{2+} > Ba^{2+} > Mg^{2+}$) observed in this study. The TTX-insensitive Na^+ current recorded from neurones of the *Cyanea capillata* motor nerve-net (Anderson, 1987) showed a similar Cd^{2+} sensitivity to that recorded here for *Polyorchis penicillatus* motor neurones. It should be noted that Cd^{2+}, at much lower concentrations (0.3 mmol l^{-1}) than were used in this study, completely blocks Ca^{2+} currents in the same *Polyorchis penicillatus* neurones as were used in this study (Przysiezniak and Spencer, 1992). Ca^{2+} current in *Cyanea capillata* motor neurones was blocked by 2 mmol l^{-1} Cd^{2+} (Anderson, 1989).

The high susceptibility of TTX-resistant channels to blockade by Zn^{2+} and Cd^{2+} is not apparent in mammalian brain and skeletal muscle TTX-sensitive channels (Frelin *et al.* 1986). Thus, the region that is responsible for high sensitivity to TTX also produces a lower Zn^{2+} and Cd^{2+} binding affinity. Structure/function analyses of the Na^+ channel subunit have shown that, in short segment 2 of domain I, a critical cysteine residue in cardiac Na^+ channels and a tyrosine or phenylalanine residue in a similar position in brain and skeletal Na^+ channels are critical residues of the TTX receptor site (Sather *et al.* 1994). A tyrosine to cysteine mutation in skeletal muscle Na^+ channels and a phenylalanine to cysteine mutation in brain Na^+ channels substantially lowers TTX sensitivity and creates a high-affinity binding site for Zn^{2+} and Cd^{2+} (Backx *et al.* 1992; Heinemann *et al.* 1992*a*).

Ca^{2+} blocked *Polyorchis penicillatus* neuronal Na^+ currents (Fig. 3) in a dose-dependent fashion, at concentrations similar to those reported for canine Purkinje fibre Na^+ currents (Hanck and Sheets, 1992). At 10 mmol l^{-1} Ca^{2+}, which is the normal extracellular concentration of Ca^{2+} in jellyfish, there was a 31 % blockade of Na^+ current at 0 mV (Fig. 3). Ca^{2+} blockade of Na^+ current under normal physiological conditions presumably occurs in many marine invertebrates. Despite extreme differences in the extracellular fluid concentrations of Ca^{2+} in marine (10 mmol l^{-1}) and terrestrial (1–2 mmol l^{-1}) animals, the relative affinity of Na^+ channels for Ca^{2+} appears to remain the same. Unlike Ba^{2+}, Ca^{2+} caused a significant depolarizing shift (approximately 20 mV) in the current–voltage relationship (Fig. 3A), which indicates that Ca^{2+} binds with higher affinity to a region close to the voltage sensor than does Ba^{2+} or Mg^{2+}. Under the experimental

conditions used here (constant concentration of divalent cations), this *I*/*V* shift cannot be explained by charge screening. Worley *et al.* (1992) have shown that Ca^{2+} binds to the channel itself and does not interact with the surrounding lipid. The presence of Ca^{2+} binding sites on Na^+ channels may be an evolutionary marker of the common ancestry of Ca^{2+} and Na^+ channels.

Although the local anaesthetics lidocaine and procainamide produced partial blockade of Na^+ current in *Polyorchis penicillatus* neurones, there was no evidence of the use-dependent blockade typical of the mode of action of these drugs in most Na^+ channels (Hille, 1977). This result contrasts with the finding that the Na^+ current in *Cyanea capillata* motor neurones shows use-dependent blockade by lidocaine (Anderson, 1987). Similarly, veratridine, which modifies inactivation in most Na^+ channels (Hille, 1992), produced partial blockade, but did not affect inactivation kinetics either in *Polyorchis penicillatus* neurones (this study) or in *Cyanea capillata* motor neurones (Anderson, 1987). Batrachotoxin, alpha-scorpion venom and sea anemone toxin, which are also modifiers of inactivation, were also ineffective in *Cyanea capillata* neurones (Anderson, 1987). Since all the above-mentioned drugs act at sites outside the mouth of the channel, it appears that the TTX binding region is strongly conserved but that other binding sites are less conserved. A *Conus* toxin, μO-conotoxin MrVIA (which, although being a Na^+ channel ligand has structural similarities to the Ca^{2+} channel blocking ω- and δ-conotoxins; McIntosh *et al.* 1995), was without effect. In contrast, μO-conotoxin MrVIA blocks TTX-insensitive Na^+ channels in *Aplysia californica* neurones at $350\,nmol\,l^{-1}$ (McIntosh *et al.* 1995).

The only pharmacological agent shown to have a relatively potent blocking effect on the Na^+ current was capsaicin (Fig. 6A); however, the effect was non-specific as outward currents were also affected (Fig. 6B). Capsaicin has been reported to block both Na^+ and K^+ channels in dorsal root ganglion neurones of the guinea pig and chicken where, despite its lipophilicity, it was shown to act from the outside (Petersen *et al.* 1987). Our own unpublished data also indicate that capsaicin has no effect when applied intracellularly to *Polyorchis penicillatus* motor neurones.

The Ca^{2+} channel antagonists (dihydropyridines, verapamil, diltiazem) were not effective blockers of Na^+ current (Figs 4, 5) in the concentration range ($20\,nmol\,l^{-1}$ to $50\,\mu mol\,l^{-1}$) that is normally effective for L-type Ca^{2+} channels (Hille, 1992). At higher concentrations, these antagonists are known to lose their specificity for Ca^{2+} channels and begin to block Na^+ channels (Hille, 1992). Even at the high concentrations used here, of all the dihydropyridines examined, only nicardipine, (+)Bay K 8644 and nitrendipine showed any blocking effect (Fig. 4). The related compounds nimodipine and nifedipine showed no blocking effect (Fig. 4). On the basis of the blocking action of nicardipine and verapamil on the Na^+ current in neurones of the subumbrellar motor network of *Cyanea capillata*, Anderson (1989) suggested that the Na^+ channel responsible has the pharmacology of a Ca^{2+} channel.

Drugs known to be calmodulin inhibitors, W7 and calmidozolium, have been shown to inhibit Ca^{2+} currents (Caulfield *et al.* 1991) and Na^+ currents (Ichikawa *et al.* 1991). Neither of these drugs had substantial blocking effects on *Polyorchis penicillatus* Na^+ currents (Fig. 5).

Because the majority of dihydropyridines and other specific antagonists of L-type Ca^{2+} channels were ineffective blockers of the *Polyorchis penicillatus* Na^+ channel, we are obliged to conclude that the pharmacological profile of this channel has no similarity to the profile expected for Ca^{2+} channels. Since it has been shown that a Na^+ channel can become Ca^{2+}-selective after the mutation of one amino acid in two domains of the pore region (Heinemann *et al.* 1992*b*), it is attractive to suppose that hybrid Ca^{2+}–Na^+ channels might be present in lower metazoans. Nevertheless, despite the unusual Na^+ pharmacological profile of this *Polyorchis penicillatus* motor neuronal channel, it is clear that this particular channel is not likely to have a hybrid molecular structure.

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