

SAXITOXIN DIFFERENTIATES BETWEEN TWO TYPES OF Na^+ -DEPENDENT POTENTIALS IN THE RETZIUS CELL OF HIRUDINID LEECHES

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SUMMARY

The effects of tetrodotoxin (TTX) and saxitoxin (STX) on the action potentials recorded in Ca^{2+} -free solution in the absence of Ca^{2+} and K^+ currents were investigated in the Retzius cell of three hirudinid leech species (*Hirudo medicinalis*, *Macrobdella decora* and *Poecilobdella granulosa*) and in the glossiphoniid leech *Haementeria ghilianii*.

In the four leech species, stimulation of the Retzius cell in the presence of 25 mmol l^{-1} tetraethylammonium chloride (TEA), 3 mmol l^{-1} 4-aminopyridine (4-AP) and 2 mmol l^{-1} Mn^{2+} evoked prolonged action potentials consisting of an initial fast-rising spike followed by a plateau lasting several hundreds of milliseconds. The amplitude and duration of both components of action potentials recorded under these conditions were dependent on $[\text{Na}^+]_o$.

In the hirudinid leeches the initial spike was unaffected by TTX and STX whereas the plateau was selectively blocked by micromolar concentration of STX. In *Haementeria* both the initial spike and the subsequent plateau were sensitive to nanomolar concentrations of STX and TTX with an estimated ED_{50} of approximately 20 nmol l^{-1} for inhibition of \dot{V}_{max} of the fast spike.

The results suggest that there are two types of ionic currents mediating the two distinct components of Na^+ -dependent action potentials in the Retzius cell: (1) a fast-inactivating one, presumably underlying the normal spike which is TTX- and STX-resistant in hirudinid leeches but sensitive to both agents in *Haementeria* and (2) a low-threshold, prolonged current which underlies the plateau recorded from these cells in the absence of Ca^{2+} and K^+ currents and which is selectively blocked by STX in hirudinid leeches but sensitive to both STX and TTX in *Haementeria*.

It is likely that the variable kinetic and pharmacological properties that characterize the various Na^+ potentials in these identified homologous neurones may be of functional significance and result from differences in the molecular structure of their Na^+ channels.

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INTRODUCTION

Previous work from this laboratory has shown that excitation mechanisms in identified leech neurones generally rely on the sequence of permeability changes to Na^+ and K^+ described for other excitable membranes (Kleinhaus & Prichard, 1975, 1976). However, variable sensitivity to tetrodotoxin among leech neurones with Na^+ -dependent action potentials suggested that their Na^+ conductances are not homogeneous but may be structurally and functionally different (Kleinhaus & Prichard, 1976, 1983; Johansen & Kleinhaus, 1986c). Recent findings indicate that while the Na^+ conductances of some neurones are completely resistant to TTX, homologous cells from different leech species have Na^+ conductances that respond to nanomolar doses of the drug (Johansen & Kleinhaus, 1986c). In some cases, as in the lateral nociceptive cells, TTX-resistant and TTX-sensitive channels coexist on the same membrane (Johansen & Kleinhaus, 1986c).

Among the TTX-resistant neurones with a Na^+ -dependent action potential in hirudinids is the large serotonin-containing Retzius cell (Kleinhaus & Prichard, 1976, 1983; Kleinhaus, Yang & Johansen, 1986; Lent, 1982). In normal Ringer, the action potential of the Retzius cell rises with a maximum rate of depolarization (\dot{V}_{max}) of about $20\text{--}40\text{ V s}^{-1}$ and lasts for about $2\text{--}3\text{ ms}$ (Kleinhaus & Prichard, 1979). Under certain experimental conditions, however, stimulation of these neurones evoked prolonged action potentials consisting of an initial Na^+ -dependent spike of normal rise time followed by Na^+ -dependent plateaus that could last for several seconds. Such Na^+ -dependent plateaus have also been observed following the application of barbiturates (Kleinhaus & Prichard, 1977a, 1979), physostigmine (King, Yang & Lent, 1984) and verapamil (Osmanovic, Beleslin & Lekic, 1985).

In the present paper we show that the appearance of these prolonged action potentials is dependent on the blockage of Ca^{2+} channels and outward K^+ currents. Furthermore, our findings suggest that in the Retzius cell of *Macrobdella* and other hirudinid species there are two types of Na^+ currents with different pharmacological and kinetic properties: (1) a fast-inactivating Na^+ current insensitive to the classical blockers STX and TTX and (2) a low-threshold prolonged Na^+ current that is blocked by STX but not TTX. The latter is the current underlying the Na^+ -dependent plateau when counteracting K^+ currents are blocked. Experiments with acutely ligated cells *in situ* and with cultured neurones suggest that the low-threshold prolonged Na^+ current is preferentially distributed in the axon of the neurone.

MATERIALS AND METHODS

The experiments were performed on Retzius cells in isolated leech segmental ganglia. These are large neurones which are easily identified by their size, position and electrical parameters (as described by Nicholls & Baylor, 1968; Keyzer & Lent, 1977; Kramer & Goldman, 1981).

Leeches of the species *Macrobdella decora* were obtained from local commercial suppliers; *Hirudo medicinalis* and *Poecilobdella granulosa* were purchased from Biopharm Ltd, Swansea UK, and kept in spring water at 15°C . Specimens of the

glossiphoniid leech *Haementeria ghilianii* were the generous gift of Dr E. M. Bureson (Virginia Institute of Marine Science) and were maintained at room temperature (22–24°C). The dissection and experimental procedure, adapted from Nicholls & Baylor (1968), were similar to those described in previous reports (Kleinhaus, 1976, 1980). Individual ganglia were dissected and pinned out in Ringer solution containing (in mmol l⁻¹) NaCl, 110; KCl, 4; CaCl₂, 2; glucose, 10; Tris-HCl, 10; pH 7.4, before being transferred to a small chamber through which solutions flowed at room temperature. The K⁺ channel blockers tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP) were added directly to the experimental Ringer. Tetrodotoxin (TTX) (Sanyo) was dissolved daily from the dry powder. Saxitoxin (STX) was the generous gift of Dr C. Y. Kao; it was diluted to final concentration from a frozen stock solution. Ca²⁺-free Ringer was made by substituting Mn²⁺ for Ca²⁺. Variations in [Na⁺]_o were made by substitutions with sucrose or choline chloride. Both methods gave identical results. The connective capsule overlying the ganglia was surgically removed with specially sharpened forceps to facilitate electrode penetration and to remove possible diffusion barriers; this procedure has no effect on cell properties (Kuffler & Potter, 1964). In some experiments, after the cell body had been exposed by splitting the capsule, the soma was ligated with a fine nylon thread to separate it from its ramifications (Fuchs, Nicholls & Ready, 1981). In others, ganglia were desheathed as described above and then exposed to 2 mg ml⁻¹ collagenase/dispase in L 15 medium for 1 h. The enzyme-containing solution was then replaced by a fresh L 15 solution and the cells were removed by suction applied through a fire-polished microelectrode. They were then plated in polylysine-coated culture dishes (Falcon no. 3034) containing L 15 with 5% foetal calf serum (Gibco) (P. Drapeau & J. G. Nicholls, personal communication). Cultured cells were kept at room temperature in a humid atmosphere and remained viable for more than a week. These cells maintain their electrophysiological characteristics as is shown in Fig. 5A. Microelectrodes were pulled from thin-walled tubing (Haer, o.d. 1 mm) and were filled with either 4 mol l⁻¹ potassium acetate or 3 mol l⁻¹ KCl and bevelled (Anderson, Kleinhaus, Manuelidis & Prichard, 1974) to d.c. resistances of 15–20 MΩ. Whenever possible we used each ganglion as its own control by impaling it in normal Ringer, passing the experimental solution over it after it had become stable and reversing the effect by washing it in control Ringer. In cases where it was impossible to maintain the impalement for prolonged periods, we recorded 3–5 responses in control solution, withdrawing the electrode and re-impaling the same cells after equilibration in the experimental solution. The same procedure was repeated after a prolonged (15- to 30-min) period of washout. The maximal rates of depolarization of the action potential were determined before (\dot{V}_{\max}) and after (\dot{V}'_{\max}) exposure to the drug. The ratio $\dot{V}'_{\max}/\dot{V}_{\max}$ for each cell was used as a measure of the level of TTX inhibition. Only cells in which \dot{V}_{\max} had recovered to about 80% of control value were used in the final analysis. Data were acquired with an IBM-PC computer system equipped with a Tecmar Labmaster a.-d. converter which digitized the traces at 32 kHz and stored them on floppy disks for subsequent

reproduction. The stored data were plotted on a digital (Hewlett-Packard 7470A) plotter off-line with the aid of appropriate software (PCLamp, Axon Instruments).

RESULTS

Effect of STX on the prolonged action potential in Retzius cells of hirudinid leeches

Fig. 1A illustrates the type of record that is typical for the action potential of a Retzius cell bathed in Na^+ -Ringer containing the K^+ channel blockers TEA (25 mmol l^{-1}) and 4-AP (3 mmol l^{-1}) as well as $2 \text{ mmol l}^{-1} \text{ Mn}^{2+}$ substituting for Ca^{2+} . The Ca^{2+} current in leech neurones is effectively blocked by $2 \text{ mmol l}^{-1} \text{ Mn}^{2+}$ (Kleinhaus, 1976; Johansen & Kleinhaus, 1985) and voltage-clamp experiments have shown that with the Ca^{2+} channels blocked and in the presence of TEA and 4-AP all outward current is eliminated in the Retzius cell (Johansen & Kleinhaus, 1986b). The action potential evoked by a single 40-ms stimulus had an initial peak clearly separated from the subsequent plateau. The prolonged action potential lasted approximately 300 ms in this record but could be several seconds long. Under these conditions, the neurone frequently became hyperexcitable, presumably due to the block of the transient K^+ current by 4-AP together with the incomplete repolarization following the plateau and cross-excitation between the two electrically coupled Retzius cells. The plateau duration exceeded that of the stimulus, which is indicative of the slow closing kinetics of the underlying conductance. Addition of $50 \mu\text{mol l}^{-1}$ TTX changed neither the duration nor the amplitude of either component of the action potential (Fig. 1B). However, the plateau was rapidly and selectively abolished by $50 \mu\text{mol l}^{-1}$ STX while the initial spike remained unaffected (Fig. 1C).

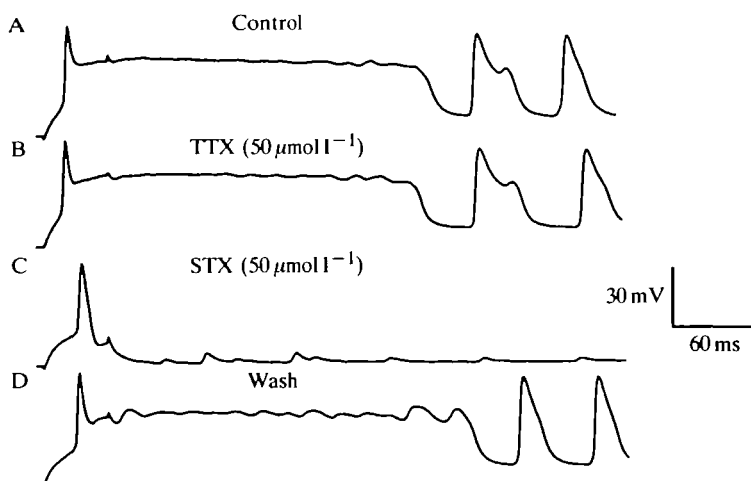


Fig. 1. STX-sensitivity and TTX-insensitivity of prolonged action potentials in the Retzius cell of *Macrobdella*. Traces show action potentials recorded in Ca^{2+} -free Ringer in the presence of 25 mmol l^{-1} TEA, 3 mmol l^{-1} 4-AP and $2 \text{ mmol l}^{-1} \text{ Mn}^{2+}$ and are reproduced from magnetic media on a digital plotter. Membrane potential -42 mV . (A) Control; (B) both the initial spike and the plateau are resistant to $50 \mu\text{mol l}^{-1}$ TTX; (C, D) the plateau is selectively and reversibly abolished by $50 \mu\text{mol l}^{-1}$ STX.

The action of STX was entirely reversible and occurred within minutes of removal of the drug (Fig. 1D).

The effect of STX on the plateau of the prolonged action potential was dose-dependent (Fig. 2). In this experiment, 10 $\mu\text{mol l}^{-1}$ STX (Fig. 2B) reduced the duration of the action potential to about 50% of what it had been in the absence of drug (Fig. 2A) while 50 $\mu\text{mol l}^{-1}$ STX entirely abolished it (Fig. 2C). Since the threshold for the STX inhibition of the plateau in hirudinids was below 10 $\mu\text{mol l}^{-1}$, whereas TTX had no effect at concentrations as high as 200 $\mu\text{mol l}^{-1}$, it is unlikely that the selective STX-sensitivity of the prolonged plateau resulted from a simple displacement of the dose-response curves for the two drugs. The inhibition of the plateau of the prolonged action potential by STX was not the result of a decrease in the input resistance of the cell (Fig. 3). In fact, STX increased the threshold for spike generation, suggesting that the STX-sensitive, prolonged Na⁺ potential has a lower threshold of activation than that of the spike, as has also been reported for the 'slow' Na⁺ potential in cockroach axons (Yawo, Kojima & Kuna, 1985). Since virtually identical results were obtained in the two other hirudinid leech species (*Hirudo medicinalis* and *Poecilobdella granulosa*), the data shown here for *Macrobdella decora* are considered representative for all three hirudinid leech species.

Na⁺ dependence of the action potential

We have previously reported that the action potential evoked by intracellular stimulation of Retzius cells in hirudinid leeches is dependent on [Na⁺]_o (Kleinhaus & Prichard, 1976). Decreasing [Na⁺]_o greatly reduces the amplitude and duration of the initial spike and plateau components of the prolonged action potential revealed in Ca²⁺-free Ringer containing 25 mmol l⁻¹ TEA, 3 mmol l⁻¹ 4-AP and 2 mmol l⁻¹

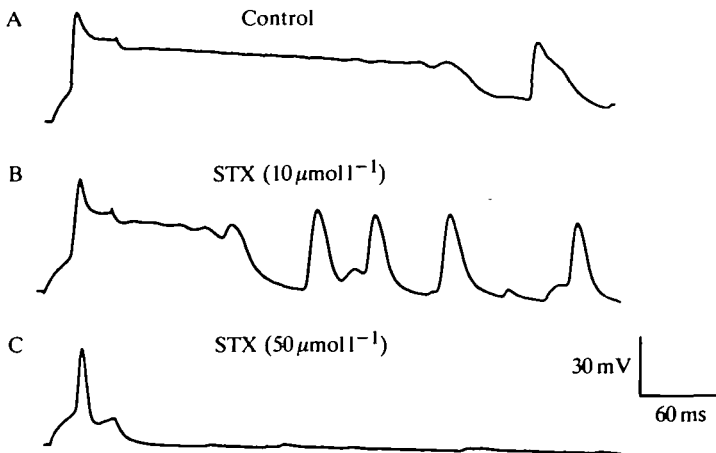


Fig. 2. Dose-dependent block by STX of the plateau of the prolonged action potential in the Retzius cell of *Macrobdella*. Prolonged action potential recorded in Ca²⁺-free Ringer containing 2 mmol l⁻¹ Mn²⁺, 25 mmol l⁻¹ TEA and 3 mmol l⁻¹ 4-AP (A) is progressively reduced by increasing concentrations of STX (B,C). The initial peak was unaffected. Membrane potential -44 mV.

Mn^{2+} (Fig. 4). Replacing all the external Na^+ with sucrose or choline chloride rendered the Retzius cell completely inexcitable, indicating that both components of the prolonged action potential recorded under these conditions were Na^+ -dependent. Comparable results were obtained in all four species of leeches examined in this study. These results, taken together with reports on similar distinct Na^+ -dependent potentials and conductances in other preparations (Gilly & Armstrong, 1984; Davis & Stuart, 1985; Yawo *et al.* 1985) including mammalian neurones (Llinas & Sugimori, 1980; Connors, Gutnick & Prince, 1982; Jahnsen & Llinas, 1984; Stafstrom, Schwindt, Flatman & Crill, 1984), are consistent with the hypothesis that the action potentials recorded in the absence of Ca^{2+} and K^+ currents in the Retzius cell of several leech species consist of Na^+ current flowing through two distinct types

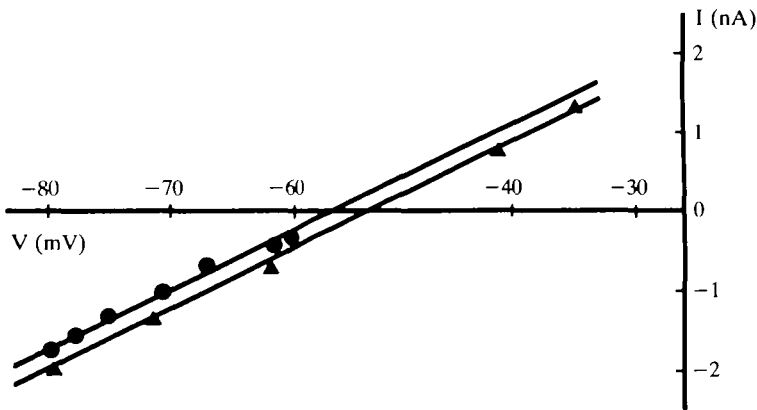


Fig. 3. The effect of STX on input resistance in the Retzius cell of *Macrobdella*. The slope resistance of the current-voltage relationship in a Retzius cell of *Macrobdella* in normal Ringer (●) is identical to that obtained in the presence of $50 \mu\text{mol l}^{-1}$ STX (▲). The threshold for initiation of the action potential is very near to the resting potential in the absence of drug, whereas STX increases it so that points on the curve could be obtained in the depolarization direction. The 3 mV shift in membrane potential between the measurements was not considered significant since such shifts frequently occurred when the perfusion solution was changed regardless of its composition.

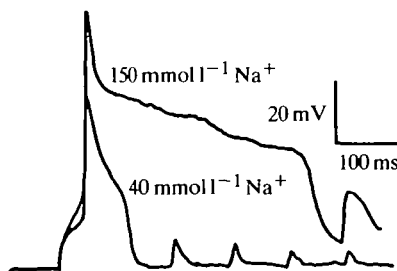


Fig. 4. Na^+ dependence of both components of the prolonged action potential in the Retzius cell of *Hirudo*. Both the amplitude and the duration of the action potential recorded in Ca^{2+} -free Ringer containing 150 mmol l^{-1} NaCl, 2 mmol l^{-1} Mn^{2+} , 25 mmol l^{-1} TEA and 3 mmol l^{-1} 4-AP decrease when $[Na^+]_o$ is lowered to 40 mmol l^{-1} . Membrane potential -58 mV .

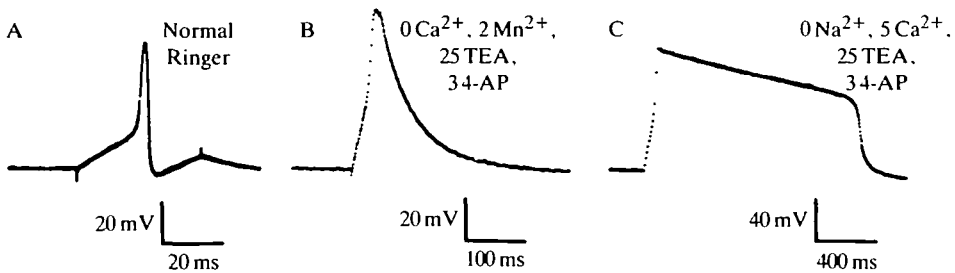


Fig. 5. The isolated soma of the Retzium cell in *Hirudo* lacks plateau Na⁺ conductance but has Ca²⁺ channels. (A) Action potential recorded in normal Ringer from a Retzium cell soma in culture is identical to the control *in vivo*. Membrane potential -42 mV. (B) In Ca²⁺-free Ringer containing $2 \text{ mmol l}^{-1} \text{ Mn}^{2+}$ with repolarizing K⁺ currents blocked by TEA and 4-AP, the action potential in a cultured neurone is prolonged but does not have the plateau seen under comparable conditions in neurones *in situ* (Figs 1–3). Membrane potential -45 mV. (C) In Na⁺-free Ringer and with the K⁺ current blockers TEA and 4-AP, the Retzium cell soma exhibits a long Ca²⁺-dependent action potential with a plateau, as it does before separation from its ramifications. Membrane potential -49 mV.

of Na⁺-dependent conductances: (1) a fast, TTX- and STX-resistant inactivating Na⁺ conductance and (2) a low-threshold, slowly or non-inactivating Na⁺ conductance which underlies the plateau and is selectively sensitive to STX.

Localization of the Na⁺-dependent plateau

The existence of two types of Na⁺ potentials in the same neurones raises the issue of whether they are distributed equally in all parts of the neuronal membrane. To resolve this question we compared the action potentials obtained under conditions known to elicit prolonged action potentials in intact cells (Fig. 1A) with those recorded from isolated somata in culture. As has been previously described (Fuchs *et al.* 1981), action potentials recorded in such cells in normal Ringer retain their characteristic cell-specific shapes and are indistinguishable from those recorded in intact cells. This was corroborated in normal Ringer for a cultured Retzium cell from *Hirudo medicinalis* (Fig. 5A). In contrast, the action potential evoked in another cultured Retzium cell in Ca²⁺-free Ringer containing 25 mmol l^{-1} TEA, 3 mmol l^{-1} 4-AP and $2 \text{ mmol l}^{-1} \text{ Mn}^{2+}$ (Fig. 5B), was quite different from that obtained from intact Retzium cells in the same solution (Fig. 1A). The action potential in the cultured Retzium cell was only slightly prolonged and did not have a plateau. The duration of the action potential recorded from isolated Retzium cells was variable but in the 10 cells examined we never observed a plateau. Failure to produce a plateau was not the consequence of non-specific damage resulting from the isolation or culture procedure, for prolonged Ca²⁺-dependent action potentials were recorded in Na⁺-free Ringer containing TEA and 4-AP from an isolated Retzium soma maintained in culture (Fig. 5C), a response identical with that recorded from such neurones *in situ* (Kleinhaus & Prichard, 1975, 1977b; Kleinhaus, 1980). Furthermore, similar results were obtained with acutely ligated somata from neurones *in situ*, excluding changes in density and distribution of ion channels resulting from

axotomy and culture conditions. It therefore seems that the density of Ca^{2+} channels on the isolated soma is sufficient to account for the ability of the Retzius cell to sustain action potentials carried by divalent cations, while the density of Na^+ channels responsible for the prolonged Na^+ -dependent action potential is not. These experiments therefore suggest that the conductance underlying the prolonged Na^+ potential in the Retzius cell of the leech is not uniformly distributed along the neurone but may be primarily distributed in the axon and dendrites.

TTX-sensitivity of the Retzius cell action potential in Haementeria

We have recently reported that the Na^+ -dependent action potential in the nociceptive neurone in the glossiphoniid leech *Haementeria ghilianii* is much more sensitive to TTX (nanomolar concentrations) than the homologous neurones from the hirudinid species (micromolar concentrations) (Johansen & Kleinhaus, 1986c). We therefore wanted to compare the TTX-sensitivities of the Retzius cells of the two orders of leeches. Fig. 6 illustrates the dose-dependent reduction of \dot{V}_{\max} produced by TTX for the Na^+ -dependent action potential recorded in normal Ringer in the Retzius cell of *Haementeria*. Like the N cell in this leech species, the 'normal' action potential was sensitive to nanomolar doses of the drug with an ED_{50} of about 20 nmol l^{-1} (Johansen & Kleinhaus, 1986c). The homologous neurones in the hirudinid leeches *Hirudo medicinalis* and *Macrobdella decora* are completely resistant to TTX even at concentrations in excess of $200 \mu\text{mol l}^{-1}$ (Kleinhaus &

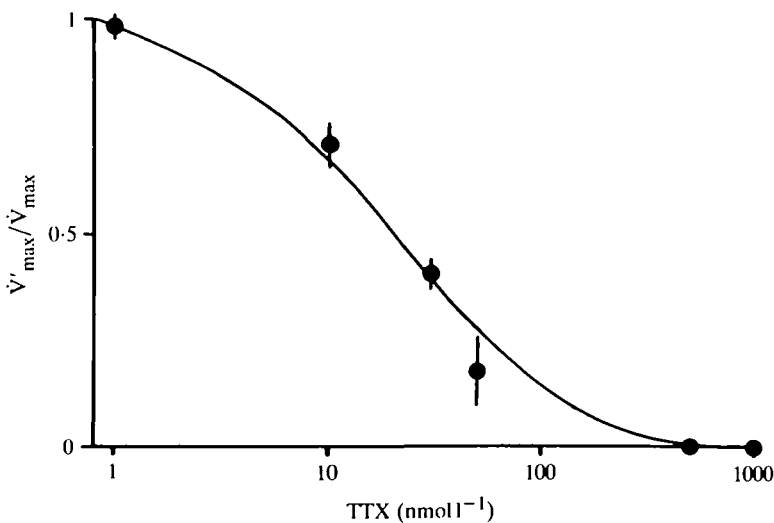


Fig. 6. Dose-response curve for inhibition by TTX of \dot{V}_{\max} of the action potential in a Retzius cell of *Haementeria*. Each data point represents the mean \pm s.e.m. of three determinations from three different cells at each concentration. The ED_{50} for TTX inhibition of \dot{V}_{\max} of the action potential was estimated from the data points to be approximately 20 nmol l^{-1} . From this value the theoretical Langmuir curve for a bimolecular reaction was computed using the equation: $\dot{V}_{\max}/\dot{V}_{\max} = 1/[1 + ([\text{TTX}]/\text{ED}_{50})]$ (Johansen & Kleinhaus, 1986c).

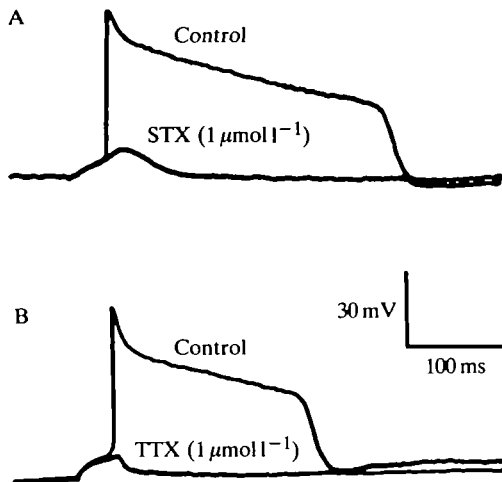


Fig. 7. The two types of Na⁺ conductances in the Retzium cell of *Haementeria* are sensitive to TTX and STX. Top traces in A and B are prolonged action potentials recorded in a Retzium cell of *Haementeria* in Ca²⁺-free Ringer containing 2 mmol l⁻¹ Mn²⁺, 25 mmol l⁻¹ TEA and 3 mmol l⁻¹ 4-AP. In the presence of 1 μmol l⁻¹ STX (A) or TTX (B) the initial spike and plateau are completely abolished. Membrane potentials -51 mV (A) and -49 mV (B).

Prichard, 1976, 1983), suggesting that their Na⁺ channels may be structurally different from those in *Haementeria*.

TTX-sensitivity of the Na⁺-dependent plateau in the Retzium cell of Haementeria

Our data so far indicate that the Na⁺ channels responsible for the rising phase of the normally occurring action potential in several neurones of *Haementeria* are uniformly sensitive to nanomolar doses of TTX, in contrast to the situation found in other leech species (Johansen & Kleinhaus, 1986c). This would suggest that a lesser degree of heterogeneity of Na⁺ conductances is present in this species. However, exposure of Retzium cells of *Haementeria* to Ca²⁺-free Ringer containing TEA and 4-AP does induce prolonged action potentials with Na⁺-dependent plateaus as shown in Fig. 7. The prolonged action potential recorded in *Haementeria* appears to be identical with that recorded in the homologous neurones in the hirudinid leeches under the same conditions, suggesting that two types of Na⁺ potentials with differing properties are present in this cell as well. However, the two types of Na⁺ potentials in the Retzium cell of *Haementeria* are not distinguishable by their TTX- or STX-sensitivity. The initial rising phase as well as the consequent plateau are completely abolished by the application of 1 μmol l⁻¹ of either drug (Fig. 7).

DISCUSSION

The results described in this paper show that Na⁺-dependent action potentials, with plateaus lasting for several hundred milliseconds, can be evoked in the Retzium cells of four different leech species, provided that the normally counteracting K⁺

currents are blocked by TEA and 4-AP (Johansen & Kleinhaus, 1986b). The potentials were Na^+ -dependent and persisted in Ca^{2+} -free solutions when Ca^{2+} channels were blocked by Mn^{2+} . In hirudinid leeches these prolonged action potentials consisted of a TTX- and STX-resistant initial spike followed by a plateau which could be selectively eliminated by STX but not by TTX. The findings strongly suggest that two separate populations of Na^+ conductances, which can be distinguished by their sensitivity to STX and by their different time courses, coexist in the membrane of this cell type. One conductance is presumably responsible for the rising phase of the normally occurring action potential and inactivates with a relatively fast time course. The other conductance, which underlies the prolonged plateau revealed in the absence of Ca^{2+} and K^+ currents, appears to have slow kinetics and lasts for hundreds of milliseconds. Our experiments do not allow us to distinguish whether the current underlying the plateau inactivates slowly or not at all. Experiments with acutely ligated cells *in situ* and cells maintained in culture strongly suggest that the two types of Na^+ conductances are non-uniformly distributed, and that the conductance underlying the plateau is preferentially located in the axon and dendritic branches. This distribution, however, complicates the biophysical characterization of the long-lasting conductance since adequate spatial clamp has only been attainable in ligated or isolated somata. The Na^+ dependence of both components of the action potential in all four leech species examined was confirmed. In addition, sensitivity of the prolonged action potential to TTX and STX in *Haementeria* provides good evidence for the role played by Na^+ in generating the plateau of the action potential. The selective sensitivity to STX of the plateau in hirudinid leeches further supports the notion that a completely different type of Na^+ conductance is responsible for the Na^+ -dependent plateau phase of the action potential.

Prolonged action potentials similar to those described above can be unmasked in leech *Retzius* cells by a number of drugs [barbiturates (Kleinhaus, 1982), procaine (Kleinhaus *et al.* 1986), physostigmine (King *et al.* 1984), verapamil (Osmanovic *et al.* 1985) and benzodiazepines (J. Johansen & A. L. Kleinhaus, unpublished results)]. Although the Na^+ dependence of these responses was demonstrated in each case, neither the nature of the underlying conductance nor the exact mechanism responsible for the appearance of the plateau were rigorously determined. Our results in this study suggest that blocking of the Ca^{2+} current and all outward K^+ currents are necessary conditions for the unmasking of the prolonged current flow responsible for the Na^+ -dependent plateau evoked in the *Retzius* cell. It is, therefore, likely that all the drugs used in the previous experiments are prolonging the action potential through this common mechanism. This theory is corroborated by: (1) the block of leech neuronal Ca^{2+} currents by barbiturates (Johansen & Kleinhaus, 1986a), procaine (Johansen & Kleinhaus, 1985; Kleinhaus *et al.* 1986) and benzodiazepines (Johansen *et al.* 1985) and (2) our recent findings under voltage-clamp which show that the barbiturates and benzodiazepines effectively block all outward current in leech *Retzius* cells (J. Johansen & A. L. Kleinhaus, in

preparation), mimicking the combined actions of TEA and 4-AP (Johansen & Kleinhaus, 1986b).

Two distinct types of Na⁺ currents with differing time courses have recently been described in invertebrate axons [squid (Gilly & Armstrong, 1984) and cockroach (Yawo *et al.* 1985)] as well as in a putative neurosecretory neurone in the barnacle (Davis & Stuart, 1985). In addition to the rapidly inactivating classical Na⁺ conductance, these workers discovered a 'slow' non-inactivating Na⁺ conductance which activated at subthreshold voltages. This slow Na⁺ conductance found in the invertebrates appears to be very similar to the Na⁺ conductance underlying long-duration action potentials in several types of mammalian neurones *in vitro* (Connors *et al.* 1982; Jahnsen & Llinas, 1984; Llinas & Sugimori, 1980; Stafstrom *et al.* 1984). In these invertebrate and mammalian preparations, both types of Na⁺ conductances were equally sensitive to TTX. The slow, non-inactivating Na⁺ conductance is activated at voltages below those for spike initiation and hence, along with other currents operative in this voltage range, may be involved in the regulation of repetitive or oscillatory firing patterns. However, a definitive functional role for this conductance has yet to be determined.

It has been widely accepted that functional similarities of Na⁺ channels in most excitable membranes were based on their structural uniformity across wide phylogenetic boundaries (Hille, 1984). It is, however, becoming increasingly apparent that the diversity in pharmacological differentiation of the Na⁺ channel parallels that reported for K⁺ and Ca²⁺ channels in various tissues and can best be explained by assuming the existence of channel subtypes with different molecular structures (Catterall, 1984; Cruz *et al.* 1985; Moczydlowski, Olivera, Gray & Strichartz, 1986). For example, hybridization experiments with poly(A)⁺ RNA from adult rat brain, and skeletal and cardiac muscle with cDNAs from rat brain Na⁺ channels suggest that structurally distinct Na⁺ channels may be encoded by different mRNAs in various kinds of excitable tissues (Noda *et al.* 1986). In the leech we have recently shown that functionally different neurones, as well as those sharing a sensory modality, possess different types of Na⁺ conductances (Kleinhaus & Prichard, 1976, 1983; Johansen & Kleinhaus, 1986c; Kleinhaus *et al.* 1986) and that two kinds of Na⁺ conductances coexist in some cell types (Johansen & Kleinhaus, 1986c).

The Retzius cell is a spontaneously active cell which has been implicated in a number of behavioural functions. It is important for peripheral mucus secretion (Lent, 1973) and is thought to play a pivotal role in complex behaviour such as swimming (Willard, 1981) and feeding (Lent, 1985). A more detailed analysis of the basis for this specialized low-threshold prolonged Na⁺ potential in the Retzius cell may further our understanding of how the cell regulates this behaviour.

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