J. Physiol. (1983), **340**, *pp.* 19–45
With 13 *text-figures*
Printed in Great Britain

A VOLTAGE-CLAMP ANALYSIS OF INWARD (ANOMALOUS) RECTIFICATION IN MOUSE SPINAL SENSORY GANGLION NEURONES

BY MARK L. MAYER* AND GARY L. WESTBROOK

From the Laboratory of Developmental Neurobiology, N.I.C.H.D., Building 36, Room 2A21, National Institutes of Health, Bethesda, MD 20205, U.S.A.

(*Received 8 October 1982*)

SUMMARY

1. Mouse embryo dorsal root ganglion neurones were grown in tissue culture and voltage-clamped with two micro-electrodes. Hyperpolarizing voltage commands from holding potentials of −50 to −60 mV evoked slow inward current relaxations which were followed by inward tail currents on repolarization to the holding potential. These relaxations are due to the presence of a time- and voltage-dependent conductance provisionally termed G_h.

2. G_h activates over the membrane potential range −60 to −120 mV. The presence of G_h causes time-dependent rectification in the current–voltage relationship measured between −60 and −120 mV. G_h does not inactivate within this range and thus generates a steady inward current at hyperpolarized membrane potentials.

3. The current carried by G_h increases when the extracellular K^+ concentration is raised, and is greatly reduced in Na^+-free solutions. Current–voltage plots show considerably less inward rectification in Na^+-free solution; conversely inward rectification is markedly enhanced when the extracellular K^+ concentration is raised. The reversal potential of I_h is close to −30 mV in media of physiological composition. Tail-current measurement suggests that I_h is a mixed Na^+–K^+ current.

4. Low concentrations of Cs^+ reversibly block I_h and produce outward rectification in the steady-state current–voltage relationship recorded between membrane potentials of −60 and −120 mV. Cs^+ also reversibly abolishes the sag and depolarizing overshoot that distort hyperpolarizing electrotonic potentials recorded in current-clamp experiments.

5. Impermeant anion substitutes reversibly block I_h; this block is different from that produced by Cs^+ or Na^+-free solutions: Cs^+ produces outward rectification in the steady-state current–voltage relationship recorded over the I_h activation range; in Na^+-free solutions inward rectification, of reduced amplitude, can still be recorded since I_h is a mixed Na^+–K^+ current; in anion-substituted solutions the current–voltage relationship becomes approximately linear.

6. It is concluded that in dorsal root ganglion neurones anomalous rectification is generated by the time-and voltage-dependent current I_h. The possible function of I_h in sensory neurones is discussed.

* Address for correspondence: Department of Pharmacology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.

INTRODUCTION

The term anomalous rectification has been used to describe the increase and decrease in slope conductance that occur close to the resting potential in a number of vertebrate and invertebrate neurones as the membrane potential is either hyperpolarized or depolarized (metacerebral giant neurones of *Helix aspersa*: Kandel & Tauc, 1966; motoneurones: Nelson & Frank, 1967; hippocampal pyramidal cells *in vivo*: Purpura, Prelevic & Santini, 1968; hippocampal pyramidal cells *in vitro*: Hotson, Prince & Schwartzkroin, 1979). As noted by Nelson & Lux (1970) slope-conductance measurements provide little information on the nature or ionic mechanism of the rectifying process.

Originally the term anomalous rectification was introduced by Katz (1949) following his discovery of an inward rectifier present in muscle but not axonal membranes. The properties of the inward rectifier in muscle have since been examined in some detail (Hodgkin & Horowicz, 1959; Adrian & Freygang, 1962*a*, *b*; Nakajima, Iwasaki & Obata, 1962; Almers, 1972; Standen & Stanfield, 1978*a*, *b*, 1980; Hestrin, 1981) and it is known that the underlying conductance, which is relatively specific for K^+ ions, increases with membrane potential hyperpolarization and varies with the extracellular K^+ concentration as a function of the difference between the membrane and K^+ equilibrium potentials (Hestrin, 1981; Leech & Stanfield, 1981). A similar conductance is present in a number of marine egg cell membranes (starfish: Hagiwara & Takahashi, 1974; sea-squirt: Miyazaki, Takahashi, Tsuda & Yoshii, 1974).

The mechanisms responsible for anomalous rectification in neuronal membranes have not yet been identified unambiguously. The experimental accessibility offered by dissociated cell culture has enabled us to investigate, with a two-electrode voltage clamp, the mechanism which produces anomalous rectification in mouse dorsal root ganglion neurones. Our findings suggest the presence of a time- and voltage-dependent conductance activated by membrane potential hyperpolarization which qualitatively explains the features of *anomalous* rectification recorded under current clamp. This conductance, which we have labelled G_h, has a reversal potential depolarized to the K^+ equilibrium and resting potentials, and is strikingly similar to the conductance carrying one of the pace-maker currents in the cardiac sinoatrial node and labelled I_f (Brown & DiFrancesco, 1980) or I_h (Yanagihara & Irisawa, 1980). Preliminary reports of this work have been communicated to the Physiological Society (Mayer & Westbrook, 1982, 1983).

METHODS

Tissue culture

The spinal cord and dorsal root ganglia of 13-day-old mouse embryos (*C57/BL6*) were dissociated and grown in antibiotic-free media according to methods previously developed in this laboratory (Ransom, Neale, Henkart, Bullock & Nelson, 1977), with the exception that nerve growth factor (30 ng/ml.) was added to the growth medium. After 3–8 weeks the dorsal root ganglion neurones (20–30 μm) were impaled with two independent micro-electrodes. The dorsal root ganglion neurones were unambiguously identified morphologically under phase contrast microscopy by their smooth round phase-bright somas. In a few cases neurones were stained intracellularly with horseradish peroxidase or Lucifer Yellow. The stained cells were approximately spherical, had one or two axonal processes and lacked dendrites, and should thus be electrically compact (see Brown, Perkel, Norris & Peacock, 1981) and suitable for voltage clamp. In contrast spinal cord neurones had an extensive dendritic field, a darker phase appearance and an irregular cell body morphology.

Electrophysiological recording

Intracellular recordings were made at room temperature (23–28 °C) on the stage of a Leitz inverted phase-contrast microscope, using two micro-electrodes filled with 3 M-K acetate titrated to pH 7·0 with glacial acetic acid (resistance 18–100 MΩ). Dorsal root ganglion neurones selected for study on morphological criteria were electrophysiologically distinguishable from spinal cord cells by their broad action potentials (2–4 msec), lack of spontaneous post-synaptic potential activity and their rapid accommodation to a depolarizing current pulse. Voltage-clamp experiments were performed on neurones only if after impalement with two micro-electrodes the resting membrane potential stabilized to a value more negative than −50 mV, and action potentials of amplitude greater than 80 mV were evoked by the passage of depolarizing current pulses through one of the electrodes. A number of twin-impaled neurones had resting potentials of −60 mV and action potential amplitudes in excess of 100 mV.

Membrane potential was controlled using a two-electrode voltage-clamp amplifier with a maximum closed-loop d.c. gain of 2,500; in a few experiments this was increased to 12,500. With a grounded shield placed between the electrodes the clamp settling time to step changes of membrane potential was 2–10 msec, which was adequate for the slow relaxations described here. Membrane current was recorded by a virtual ground current to voltage converter (3 dB point: 1·6 kHz). The series resistance was not estimated, but was unlikely to introduce serious error in voltage control since the maximum currents recorded were normally less than 5 nA. The membrane potential and clamp current were recorded at several gains on a six-channel Gould Brush recorder, and on a storage oscilloscope. The experimental records shown were photographed directly from the experimental chart traces, or from the oscilloscope screen.

Composition of media

The normal recording medium contained 135 mM-NaCl; 4·5 mM-KCl; 5 mM-$CaCl_2$; 5 mM-$MgCl_2$; 5 mM-HEPES; 10 mM-glucose; 0·01 mg Phenol Red/ml. and was titrated to pH 7·3. The osmolarity of batches varied between the limits of 325 and 340 mosM. After the growth medium was changed for recording medium a layer of mineral oil was added to reduce evaporation. In later experiments no oil was used and the medium was changed every 90 min or so.

When the extracellular K^+ concentration was varied equimolar amounts of KCl were substituted for NaCl. Na^+-free solutions were made by substituting either choline chloride or Tris base (titrated with HCl) for NaCl. A solution containing 20 mM-Cl^- was made by substituting Na isethionate for NaCl and K methylsulphate for KCl. Equimolar quantities of $BaCl_2$ (1 mM) and $MnCl_2$ (3 mM) were substituted for $CaCl_2$. CsCl (0·2–10 mM), and RbCl (1 mM) were added to the recording medium from 1 M-aqueous stock solutions. Tetrodotoxin (1 or 10 μM) was added to the recording medium from a stock solution (50 μg/ml.).

Extracellular perfusion technique

During experiments in which the extracellular environment of single neurones was changed by perfusion of medium from a pressure micropipette, the osmotic strength of the perfusing medium was adjusted to be equal to or less than 5 mosM *hyperosmotic* to that of the recording medium by adding small quantities of the principle salt (e.g. NaCl or choline Cl) until the required osmotic strength was achieved, since perfusion with even weakly hypo-osmotic solutions irreversibly depolarized neurones. Pressure pipettes had tip diameters of 5–8 μm and pressure from a nitrogen source was applied to the pipette at 0·5–1·5 lb./sq. in. via a valve switching between atmospheric pressure and the nitrogen source. In some experiments a blunt pipette (10–20 μm) was used to apply CsCl or KCl by diffusion. In several experiments the effects of a pressure application of recording medium was examined. In addition to a small hyperpolarizing artifact, recorded as an outward current under clamp (see Choi & Fischbach, 1981; Dunlap & Fischbach, 1981), such applications occasionally irreversibly abolished the currents described in this paper. This effect was traced to osmotic imbalance between the recording and perfusing media (see above). In addition, the health of twin-impaled neurones usually deteriorated during the course of long experiments, especially when the membrane potential was frequently stepped over a large range of values. This deterioration was recorded as a gradual increase in the leak conductance, and a progressive fall in the amplitude of current relaxations evoked by step changes of membrane potential. To avoid these issues we relied on obtaining reversible effects in perfusion experiments which were repeated many times on neurones in several culture preparations.

RESULTS

The results of this study are based on observations made in 176 voltage-clamped dorsal root ganglion neurones, all of which showed inward (anomalous) rectification at voltages negative to the resting membrane potential.

Time-dependent rectification and the dorsal root ganglion neurone current–voltage relationship

All of the dorsal root ganglion neurones examined in the present study showed a time-dependent sag in hyperpolarizing electrotonic potentials (Fig. 1*A*) similar to those observed in dorsal root ganglion neurones prepared from adult animals, of a variety of species, for recording *in vitro* (Ito, 1957; Görke & Pierau, 1980) or *in vivo* (Czéh, Kudo & Kuno, 1977). It is this phenomenon that others have described as anomalous rectification.

In normal recording medium containing 4·5 mM-K^+ the resting membrane potential of twin-impaled dorsal root ganglion neurones was $-56{\cdot}0 \pm 5{\cdot}6$ mV (mean ± S.D., $n = 96$). Hyperpolarizing electrotonic potentials larger than 15–20 mV normally showed some sag, suggesting a threshold for activation of the rectifying process of around −70 mV, and were followed by a depolarizing overshoot on termination of the current pulse. Depolarizing electrotonic potentials never showed evidence of anomalous rectification of the type recorded in hippocampal neurones and described by Hotson *et al.* (1979), but were instead reduced in amplitude by an outward rectifying process operating close to the resting potential (Fig. 1*A*). Under voltage clamp, hyperpolarizing commands from a holding potential of −60 mV evoked slow inward relaxations over the same membrane potential range as that producing sags and depolarizing overshoots in hyperpolarizing electrotonic potentials (Fig. 1*B*).

The steady-state current–voltage plot of most dorsal root ganglion neurones did not show obvious inward rectification over the range from −120 to −25 mV (Fig. 1*C*), but was instead dominated by large currents flowing through outward rectifiers operating close to the resting potential. However when carefully examined over a smaller potential range (−60 to −120 mV) current–voltage plots frequently showed a shoulder at around −70 mV, with an increase in slope in the hyperpolarizing direction; such inward rectification was particularly obvious in media containing an elevated K^+ concentration (see Figs. 3 and 7).

Conductance changes during membrane potential hyperpolarization

In the majority of experiments dorsal root ganglion neurones were initially voltage-clamped at −60 mV, at which potential the holding current was normally close to zero. Hyperpolarizing commands evoked an instantaneous inward current followed by a slow inward relaxation and an inward tail current on repolarization to −60 mV (Fig. 2). The amplitude of the inward relaxation and tail current increased as the command potential was made more negative (Fig. 3). Such inward relaxations were consistent with a membrane-conductance increase since the instantaneous current flowing at the end of the hyperpolarizing command, on repolarization to the holding potential, was larger than that recorded on first jumping to the command potential (Fig. 2). The application of short conductance testing pulses during

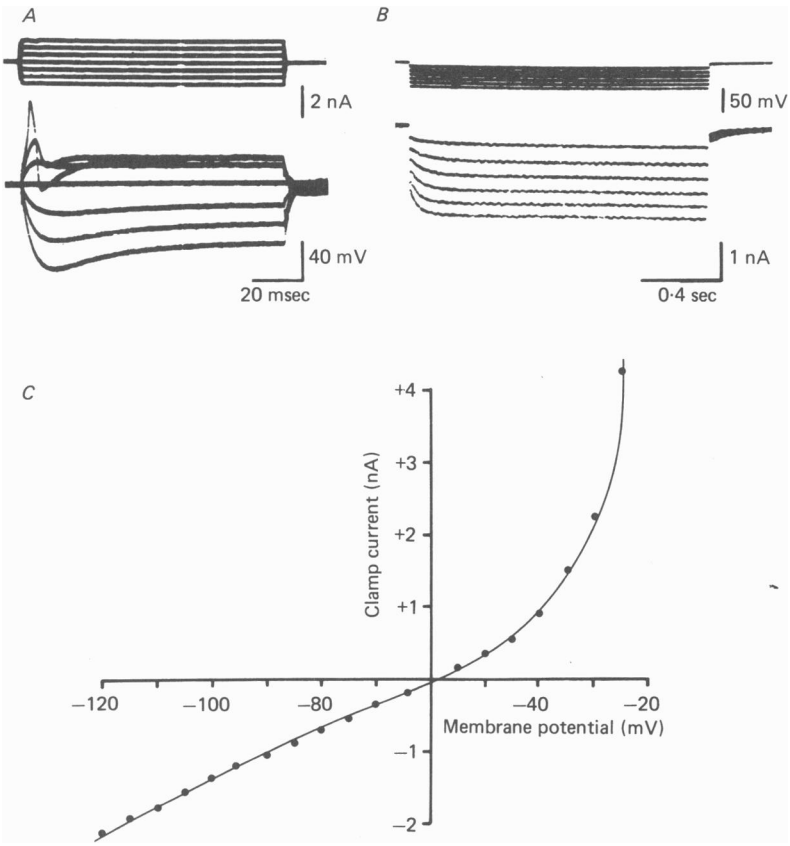


Fig. 1. *A*, time- and voltage-dependent rectification evoked by the passage of constant current pulses across the membrane of a dorsal root ganglion neurone impaled with separate current-passing and voltage-recording electrodes, and bathed in a solution containing 1 μM-tetrodotoxin (resting potential −55 mV). Depolarizing pulses activate a tetrodotoxin-resistant action potential followed by delayed (outward) rectification; note that outward rectification occurs at membrane potentials below threshold for action potential generation. Hyperpolarizing pulses evoke anomalous (inward) rectification. *B* shows slow inward current relaxations evoked under voltage clamp by 1·5 sec, 10 mV incrementing hyperpolarizing step command potentials from a holding potential of −60 mV. *C* shows the steady-state current–voltage relationship between −25 and −120 mV recorded at the end of 1·5 sec step commands from a holding potential of −60 mV under voltage clamp. All data from one neurone.

hyperpolarizing commands (see DiFrancesco 1981*a*; DiFrancesco & Ojeda, 1980) confirmed that the inward relaxation was produced by a slow time-dependent membrane conductance increase. In contrast to inward relaxations associated with closure of a K^+ conductance open at the holding potential (Brown & Adams, 1980; Adams, Brown & Constanti, 1982), no reversal of the inward relaxation occurred at command potentials as negative as −160 mV, even when the external K^+ concentration was raised from 4·5 to 25 mM.

At a holding potential of −90 or −100 mV a steady inward current flowed (Fig. 2). Hyperpolarizing commands no longer evoked large inward relaxations, and the

instantaneous current resulting from such step commands was larger than when recorded at a holding potential of −60 mV. In contrast, depolarizing commands from a holding potential of −90 or −100 mV evoked small outward relaxations followed by large inward activating tail currents (Figs. 2 and 3). The instantaneous current flowing at the start of such depolarizing commands was larger than that recorded on repolarization to the holding potential, consistent with the occurrence of a conductance decrease during membrane potential depolarization. Measurement of the instantaneous (chord) membrane conductance of fifteen neurones gave mean values

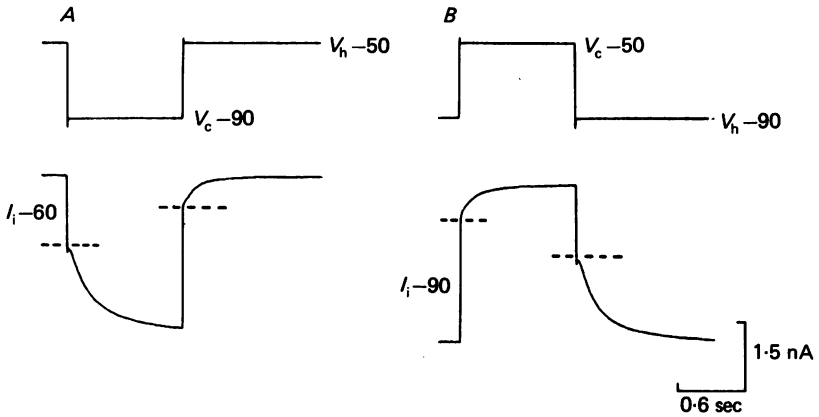


Fig. 2. Relaxation currents recorded from one dorsal root ganglion neurone bathed in a solution containing 14·5 mM-K^+ and 116 mM-Na^+ during *A*, a 1 sec 40 mV *hyperpolarizing* voltage command from a holding potential (V_h) of −50 mV to a command potential (V_c) of −90 mV, and *B*, a 40 mV *depolarizing* voltage command from a holding potential of −90 mV to a command potential of −50 mV. Upper trace, voltage; lower trace, current (outward current upwards). The slow inward and outward relaxations following the instantaneous currents reflect the activation and de-activation of I_h respectively. The instantaneous conductance calculated from the magnitude of the instantaneous current (I_i) recorded on jumping from −50 to −90 mV is 42 nS; the instantaneous conductance calculated from the current recorded on jumping from −90 to −50 mV is 81 nS. The holding current at −90 mV (4·4 nA) is nearly equal to the inward current flowing at the end of the 1 sec command from −50 to −90 mV (4·1 nA); the current values recorded at a holding potential of −50 mV and at the end of the command from −90 to −50 mV are similarly matched.

of 44·9 nS (range 17–82) at a holding potential of −60 mV, and 71·5 nS (range 26–132) at a holding potential of −90 mV, as shown for one neurone in Fig. 3.

These results suggest the presence in the dorsal root ganglion cell body membrane of a conductance activated by membrane hyperpolarization beyond the resting potential. We have named this conductance G_h and the current it carries I_h.

Reversal potential of I_h

At a membrane potential of −90 or −100 mV G_h is strongly activated (Fig. 4) and does not show time-dependent inactivation. This provides a convenient method for estimating the reversal potential of I_h which is obtained from the intersection of the instantaneous (chord) current–voltage relationships recorded at holding potentials of −60 mV and −90 or −100 mV (Fig. 3), i.e. in the absence and presence of I_h.

In fifteen neurones the mean value obtained for the reversal potential was −34 mV (range −8 to −42). The classical and more accurate method for estimating the reversal potential of a voltage sensitive conductance, i.e. tail current reversal, could not be usefully applied in this case due to the activation of several outward rectifiers in the membrane potential range over which reversal was expected (M. L. Mayer & G. L. Westbrook, unpublished observations; see also Kostyuk, Veselovsky, Fedulova & Tsyndrenko, 1981).

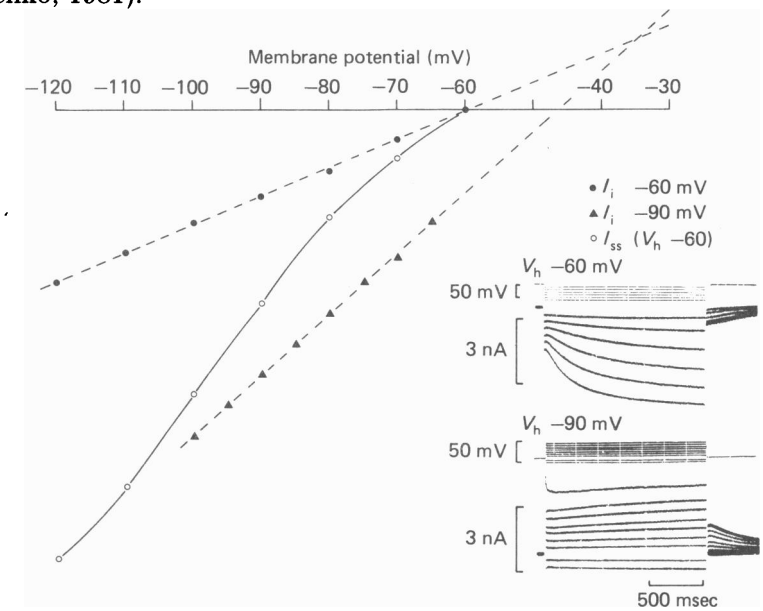


Fig. 3. Instantaneous (chord) and 'steady-state' current–voltage relationships of a dorsal root ganglion neurone voltage-clamped at holding potentials of −60 mV (chord conductance 32 nS) and −90 mV (chord conductance 60 nS). Note that the chord conductance plots are approximately linear at both holding potentials despite the presence of strong inward rectification in the 'steady-state' current–voltage relationship measured at the end of 1·5 sec hyperpolarizing voltage jumps from a holding potential of −60 mV. Oscillographic traces in the lower right quadrant show the command potentials and resulting slow current relaxations recorded at holding potentials of −60 and −90 mV. Inward rectification in the 'steady-state' current–voltage relationship is entirely accounted for by these slow relaxations.

G_h activation curve

The activation curve of G_h was estimated from the amplitude of the tail currents recorded at −50 or −60 mV following a series of hyperpolarizing prepulses. In ten neurones in which the tail current amplitude, normalized with respect to the maximum recorded, was plotted against the membrane potential during the hyperpolarizing prepulse, the data points were well fitted by activation curves generated by an equation of the form

$$I/I_{max} = \left(1 + \exp \frac{V - V_0}{k}\right)^{-1} \quad (1)$$

where V is the membrane potential, V_0 the value of membrane potential at which the conductance is half-activated, k a slope factor, and I the amplitude of the tail

current recorded after a prepulse to a membrane potential V and I_{max} is the maximum tail current recorded after a prepulse to −120 mV.

In four neurones bathed in medium containing 4·5 mM-K^+ the mean value of V_0 was −81·1 mV; in medium containing 25 mM-K^+ estimates for V_0 obtained from six neurones had a mean value of −77·6 mV. The slope factor k had mean values of 3·64 and 4·33 in 4·5 and 25 mM-K^+ respectively. The significance of the difference is not known, but may be due in part to experimental error. No strong dependence of the activation variable on the K^+ equilibrium potential is apparent from the data obtained, despite a depolarizing shift of the K^+ equilibrium potential by approximately 40 mV.

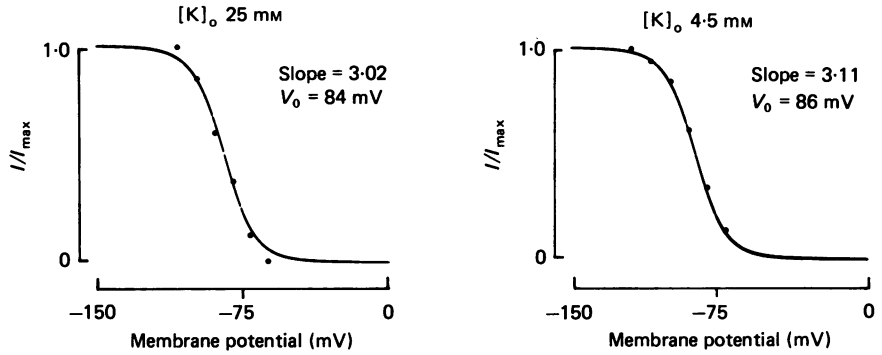


Fig. 4. Activation curves for the hyperpolarization activated conductance recorded in two dorsal root ganglion neurones, one bathed in medium containing 25 mM-K^+ (holding potential −50 mV), the other bathed in medium containing 4·5 mM-K^+ (holding potential −60 mV). The ordinate scale shows the inward tail current amplitude recorded on repolarization to the holding potential divided by the maximum tail current amplitude recorded plotted against the membrane potential during a 1·5 sec hyperpolarizing prepulse. The activation curves shown are computer-generated best fits of the curve generated by eqn. (1) to the data points. For this analysis it was assumed that the tail current recorded at −120 mV was produced by a fully activated conductance.

The activation variable approaches unity at −120 mV (Fig. 4), and this was the most negative command potential used in the majority of experiments. The activation curves illustrated in Fig. 4 were generated assuming that the conductance was fully activated at −120 mV. This was not easy to confirm experimentally since when more negative command potentials to between −130 and −160 mV were used the slope of the steady-state current–voltage curve was reduced at hyperpolarized potentials. The tail current recorded on return to the holding potential from −160 mV was also reduced compared to that recorded on return from −120 mV, suggesting block of the inward rectifier during strong membrane hyperpolarization. We were unable to investigate this in detail due to the frequent occurrence of membrane breakdown during attempts to further hyperpolarize the membrane. It is of interest that the inward rectifier of skeletal muscle also shows block during extreme hyperpolarization (Standen & Stanfield, 1978*a*, 1979). The explanation proposed by these authors, channel block by the impermeant cation Na^+, cannot apply to the inward rectifier present in sensory ganglion neurones since Na^+ ions are highly permeable through this hyperpolarization-activated conductance (see below).

Kinetic behaviour of I_h

Hodgkin–Huxley theory predicts that the relaxation time constants of voltage-sensitive conductances, the activation of which is described by curves of the type shown in Fig. 4, should be bell-shaped functions of membrane potential with a peak at V_0 (Hodgkin & Huxley, 1952; Ehrenstein & Lecar, 1977). Examination of the relaxations illustrated in Figs. 1, 3, 7, 8, 10 and 12 clearly indicates voltage sensitivity, the relaxations becoming faster as the membrane potential is hyperpolarized. The off-relaxation time constant, determined from envelope tests close to the reversal potential of I_h, also becomes faster as the membrane potential is depolarized (Fig. 5). However the time constant of the on-relaxations was not adequately described by a single exponential (Fig. 5), and we were unable to improve the fit convincingly by attempting to peel two exponentials. This was true of all neurones examined.

It is possible that such non-single exponential kinetics reflect co-operativity of the gating process as occurs with the delayed rectifier present in axonal membranes (Hodgkin & Huxley, 1952), so named because of its sigmoidal activation time course. Alternatively the relaxations due to I_h could be contaminated by the presence of an additional time- and voltage-sensitive conductance, or by K^+ depletion from the immediate extracellular environment, and a consequent shift of the K^+ equilibrium potential. However such depletion is associated with an outward relaxation (Di-Francesco, 1981*a*), and in contrast the relaxations recorded in the present study showed a second slow inward component that could still be recorded on stepping from -90 to -120 mV, at which potentials G_h is nearly fully activated. We did not attempt to characterize the nature of this second slow inward rectifier. It is of interest that non-single exponential activation kinetics have also been observed for the inward rectifier present in photoreceptors (C. R. Bader, personal communication).

The apparently single time constant of the off-relaxation, determined by an envelope test using a twin-pulse command (Fig. 5), may be fortuitous, although theory predicts that this should occur. Without more detailed kinetic measurements no estimate of the values of the on and off rate constants (and hence derivation of the activation curve by an alternative approach) is possible at present.

Potassium equilibrium potential and the contribution of I_K to I_h

The anomalous rectifier in muscle and egg membranes is a K^+-specific conductance (Hagiwara & Takahashi, 1974; Standen & Stanfield, 1979). The reversal potential of I_h is around -30 mV (Fig. 3) and this is considerably depolarized to the value expected for E_K. Over its activation curve, which is likely to cross E_K (see Fig. 4), I_h is recorded as an inward current, suggesting that Na^+ or Cl^- ions in addition to K^+ may carry inward current.

An estimate of E_K was made by recording the spike afterhyperpolarization amplitude with separate current and voltage electrodes as the membrane potential was changed by d.c. current injection (Fig. 6). The mean afterhyperpolarization reversal potential of -83 mV ($n = 5$) is close to the value of E_K calculated from the Nernst equation assuming an intracellular K^+ concentration of 120 mM ($E_K = -84$ mV with 4·5 mM-K^+), and this is considerably hyperpolarized to the reversal potential of I_h.

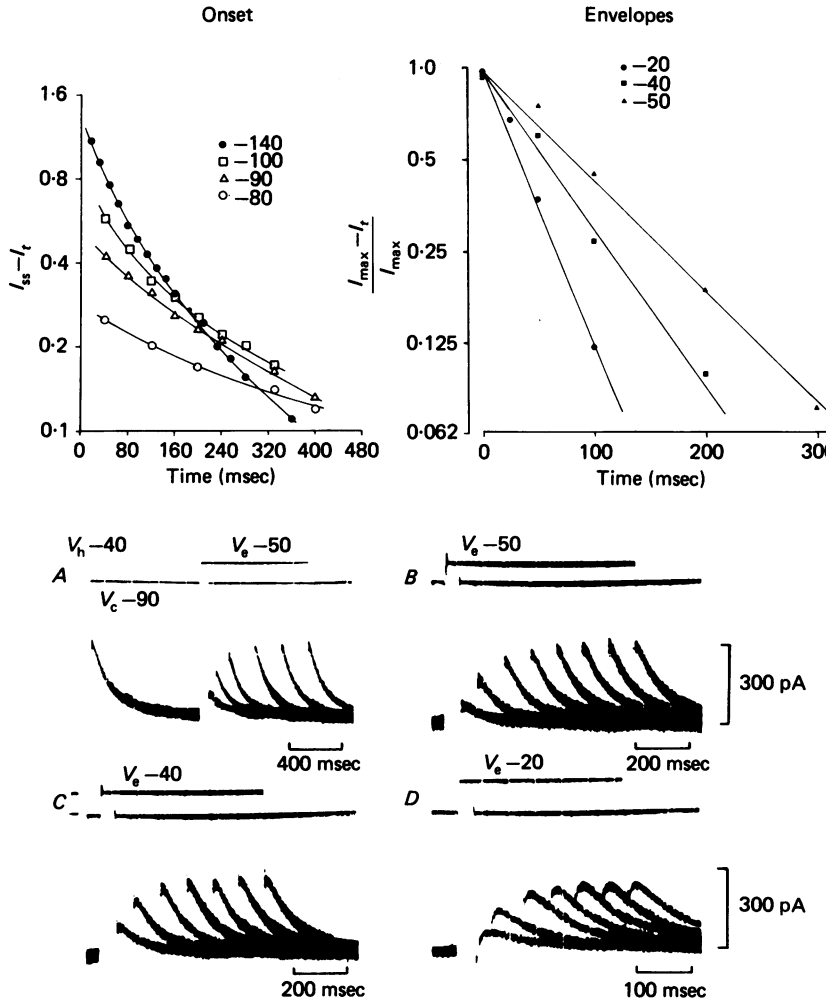


Fig. 5. Kinetics of relaxation currents. The semilogarithmic plots of the on-relaxations recorded at command potentials of −80, −90, −100 and −140 mV are not fitted by single exponential plots (graph on left of steady-state inward current (I_{ss})−inward current at time t (I_t), plotted on a log scale against time in msec elapsed since stepping to the command potential). The envelopes of the off-relaxations recorded at −20, −40 and −50 mV are fitted by single exponential plots (graph on right of the maximum inward relaxation amplitude (I_{max}) recorded on stepping from potential V_e to −90 mV − the value of the inward relaxation amplitude recorded at time t (I_t) divided by I_{max}, plotted on a log scale against the time for which the membrane potential was commanded to V_e. The oscillographic records shown in *A–D* illustrate the envelope technique used to measure the off-relaxation rate near the reversal potential. The panel in *A* shows the start of the inward relaxation produced by stepping from −50 to −90 mV and the envelope of inward relaxations recorded on stepping back to −90 mV after short depolarizing commands of increasing duration to the potential V_e. The panels shown in *B–D* are expanded sweeps of the envelopes recorded on stepping for short intervals to V_e values of −50, −40 and −20 mV. All data obtained from one neurone.

When the external K^+ concentration was increased from 4·5 to 25 mM the afterhyperpolarization reversal potential shifted to a more depolarized value and the amplitude of the inward relaxations and tail currents recorded over the I_h activation range was dramatically increased (Fig. 7). In such high $[K^+]$ media the steady-state current–voltage relationship between −120 and −50 mV was characteristically sigmoid, and a clear increase in slope conductance occurred as the membrane potential was hyperpolarized over the range −60 to −90 mV (Fig. 7). However no obvious shift of the I_h activation curve occurred (Fig. 4), in contrast to results

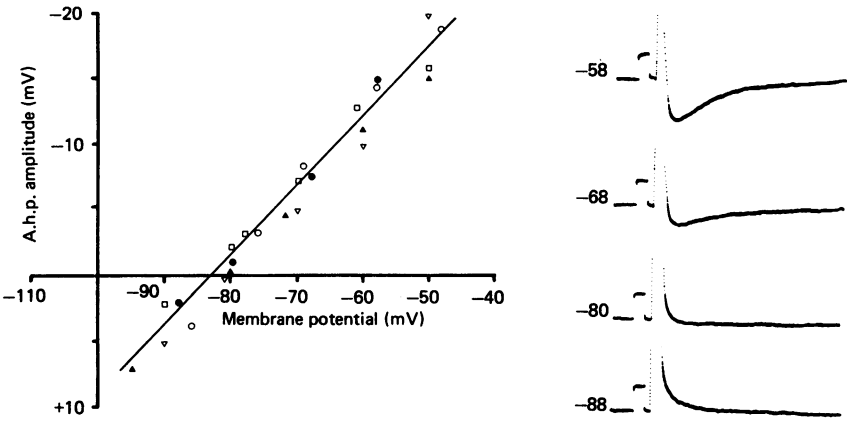


Fig. 6. Reversal potential of the dorsal root ganglion neurone spike afterhyperpolarization (a.h.p.) in medium containing 4·5 mM-external K^+. Oscillographic traces to the right show the spike a.h.p. recorded in one dorsal root ganglion neurone as the membrane potential was shifted by injection of d.c. hyperpolarizing current through a second intracellular electrode. Action potentials were evoked by the passage of brief depolarizing current pulses. The square deflexion at the start of each record is a 10 mV, 5 msec calibration pulse. Data points for this neurone (filled circles) and for other neurones (open circles, filled and open triangles and open squares) are plotted as the a.h.p. amplitude (ordinate) against the steady-state membrane potential (abscissa).

obtained in muscle and egg membranes. Part of the potentiating action of K^+ undoubtedly occurs as a result of an increase in the driving force for inward K^+ current, since the K^+ equilibrium potential shifts in the depolarizing direction. It is possible that K^+ also has a direct potentiating action on current flow through the inward rectifier, as occurs in skeletal muscle (Standen & Stanfield, 1978*c*; Hestrin, 1981; Leech & Stanfield, 1981) and cardiac Purkinje fibres (DiFrancesco, 1982).

The time constant of the relaxations recorded in 25 mM-K^+ was markedly slowed (Fig. 7). Similar slowing of the kinetics of the inward rectifier of hippocampal pyradmidal neurones also occurs on elevating the extracellular K^+ concentration (Halliwell & Adams, 1982). It is possible that this reflects an action of the permeating ion species on the ionophore properties, as has been suggested to occur in other systems (Marchais & Marty, 1979; Stanfield, Ashcroft & Plant, 1981). An alternative explanation could be that K^+ ions bind to an additional external site, modulating the lifetime or conductance of inward rectifier channels. Clear evidence for the existence of such an activatory site has been found in experiments on the inward rectifier in Purkinje fibres (DiFrancesco, 1982).

Effect of Na^+ removal in normal and high K^+ solutions

Since I_h is recorded as an inward current with a reversal potential depolarized to E_K, the contribution of Na^+ to I_h was examined by replacing Na^+ with either choline or Tris. This was achieved by local extracellular perfusion of individual neurones with

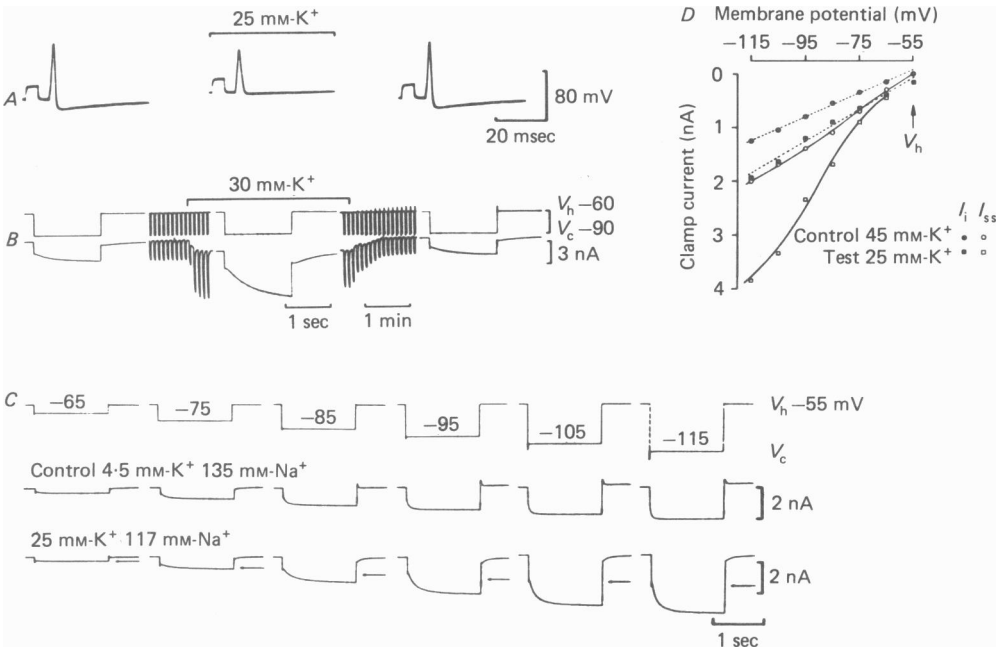


Fig. 7. *A*, raising $[K^+]_o$ from 4·5 to 25 mM reversibly depolarizes the membrane potential from −56 to −44 mV, and abolishes the spike afterhyperpolarization. *B* shows the effect of increasing $[K^+]_o$ from 4·5 to 30 mM on the amplitude of inward relaxations recorded during 1·5 sec step hyperpolarizations from a holding potential (V_h) of −60 mV to a command potential (V_c) of −90 mV. Note that both the inward relaxation and tail current amplitude increase dramatically, and that a small inward holding current develops. A 6 min break in the record occurs between the expanded trace, shown in the middle, and the slow trace on the right showing reversal of the effect of increasing $[K^+]_o$; during this time some inactivation of the hyperpolarization activated conductance occurred due to deterioration of the neurone. *C* shows the inward relaxations recorded from another neurone during 1·5 sec step hyperpolarizations from a holding potential of −55 mV to command potentials of −65 to −115 mV, in 4·5 and 25 mM-K^+. Arrows in the lower row of current traces indicate the steady-state current level recorded at equivalent command potentials in 4·5 mM-K^+. Note the reduced time constant of relaxations recorded in 25 mM-K^+. *D* shows the instantaneous (I_i) and steady-state (I_{ss}) current–voltage relationships plotted from the data shown in *C*. Note the marked increase in inward rectification recorded in 25 mM-K^+.

Na^+-free solutions which rapidly and reversibly abolished the fast Na^+ component (see Ransom & Holz, 1977) of the dorsal root ganglion neurone action potential (Fig. 8). Under voltage clamp, with either 4·5 or 25 mM-external K^+, perfusion with Na^+-free solutions rapidly and reversibly reduced the amplitude of inward relaxations produced by hyperpolarizing commands (Fig. 8). At holding potentials of −50 to −60 mV Na^+-free solutions produced minimal changes in the holding current. This

effect was observed with both choline (nine neurones bathed in 4·5 mM-K^+, six neurones bathed in 25 mM-K^+) and Tris (two neurones bathed in 4·5 mM-K^+, ten neurones bathed in 25 mM-K^+).

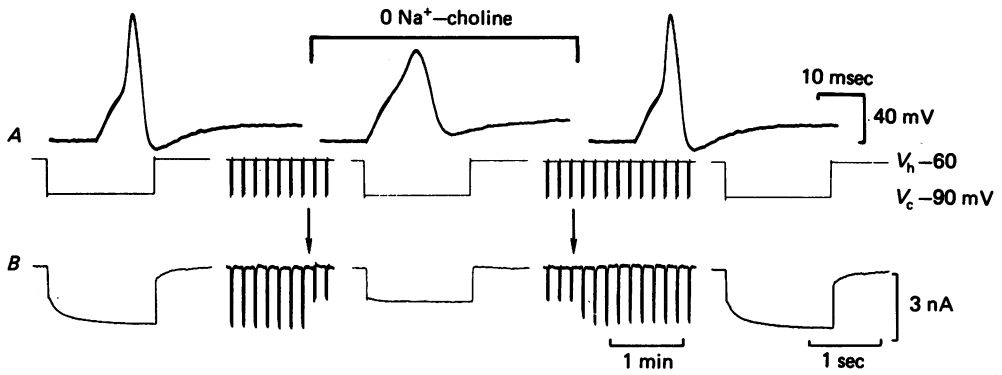
When neurones were clamped at −90 mV, depolarizing commands evoked outward *deactivating* relaxations associated with a conductance decrease. In media containing 4·5 mM-K perfusion with Na^+-free solutions caused an outward shift of the holding current and considerably reduced the instantaneous conductance at −90 mV. The steady-state current, at the end of depolarizing commands to −60 mV, remained relatively unaltered, while the previously outward relaxations were reversed.

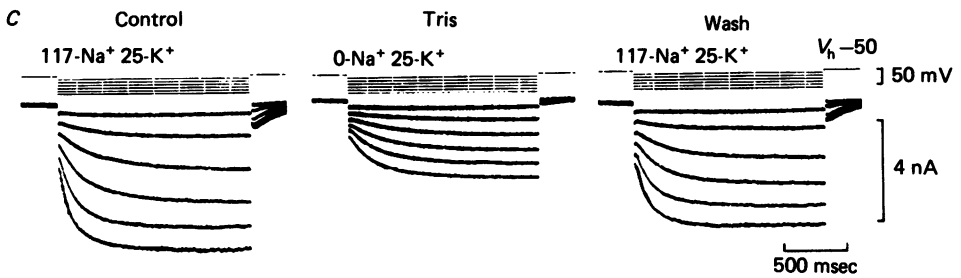
When the external K^+ concentration was increased to 25 mM, shifting E_K to around −45 mV, the reduction of I_h by Na^+-free media was less pronounced than when the external K^+ concentration was 4·5 mM (Fig. 8). This is to be expected since in 25 mM-K^+ an *inward* K current will contribute to I_h throughout its activation range, whereas in 4·5 mM-K^+ the activation curve will cross E_K, and an *outward* K^+ current will contribute to I_h at membrane potentials depolarized to E_K, effectively reducing the amplitude of relaxations produced by activation of an inward Na^+ current. Thus with 25 mM-K^+, perfusion with Na^+-free medium reduced the amplitude of but did not invert the outward *deactivating* relaxations produced by depolarizing commands from a holding potential of −90 mV, since E_K is now depolarized to the membrane potential range over which I_h activates.

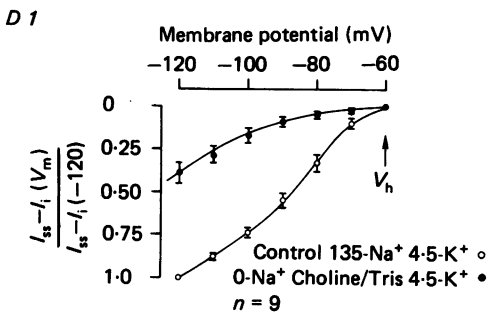
The probable existence of sites separate from the ion channel, at which ions bind to modulate single channel lifetime or conductance, complicates the interpretation of experiments in which changes in the external solution composition alter the amplitude of current relaxations produced by step changes of membrane potential. For example Na^+ ions increase the single-channel conductance of the inward rectifier in tunicate egg cell membranes (Fukushima, 1982) but are not themselves permeant. Potassium ions increase the conductance of the inward rectifier in skeletal muscle (Hestrin, 1981; Leech & Stanfield, 1981) and Purkinje fibres (DiFrancesco, 1982) and are also permeable. Thus although the data presented strongly suggest that both Na^+ and K^+ contribute directly to I_h, the results are not unambiguous.

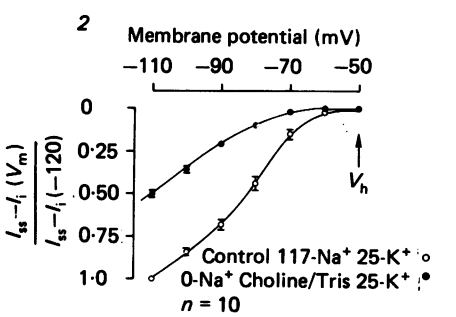
Clear evidence that I_h is indeed a mixed Na^+–K^+ current is shown in Fig. 9, where tail currents recorded in the presence and absence of Na^+, in media containing 25 and 4·5 mM-K^+, are illustrated. In 25 mM-K^+ the tail currents, recorded at −50 mV, are strongly inward in the presence of Na^+, and essentially flat in Na^+-free medium; since the holding potential (−50 mV) is close to E_K the driving force for a K^+ current is close to zero. In 4·5 mM-K^+ the tail currents, recorded at −60 mV, are inward in the presence of Na^+ and outward in Na^+-free medium, since at a holding potential of −60 mV an *outward* K^+ current contributes to the tail.

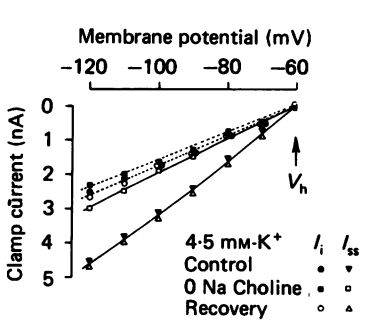
Tetrodotoxin (1 or 10 μM, seven neurones) and Mn^{2+} (3 mM, twenty-five neurones) were not effective blockers of I_h. Barium (1 mM, five neurones) produced only a small block of I_h. All of these substances strongly reduce anomalous rectification in hippocampal neurones (Hotson *et al.* 1979), and Ba^{2+} is an effective blocker of the inward rectifier in muscle (Standen & Stanfield, 1978*b*) and egg (Hagiwara, Miyazaki, Moody & Patlak, 1978) membranes at concentrations of less than 1 mM.

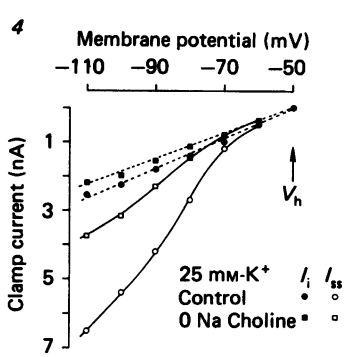

0 Na+–choline
10 msec
40 mV
A
Vh –60
Vc –90 mV
B
3 nA
1 min
1 sec


C
Control
117-Na+ 25-K+
Tris
0-Na+ 25-K+
Wash
117-Na+ 25-K+
Vh –50
50 mV
4 nA
500 msec


D 1
Membrane potential (mV)
–120 –100 –80 –60
0
0·25
0·50
0·75
1·0
Vh
Control 135-Na+ 4·5-K+
0-Na+ Choline/Tris 4·5-K+
n = 9


2
Membrane potential (mV)
–110 –90 –70 –50
0
0·25
0·50
0·75
1·0
Vh
Control 117-Na+ 25-K+
0-Na+ Choline/Tris 25-K+
n = 10


3
Membrane potential (mV)
–120 –100 –80 –60
Clamp current (nA)
0
1
2
3
4
5
Vh
4·5 mM-K+
Control
0 Na Choline
Recovery


4
Membrane potential (mV)
–110 –90 –70 –50
Clamp current (nA)
1
3
5
7
Vh
25 mM-K+
Control
0 Na Choline

Effect of Cs^+ on anomalous rectification and I_h

There is considerable evidence that low concentrations of Cs^+ block the anomalous rectifier in marine egg and muscle membranes (Gay & Stanfield, 1977; Hagiwara, Miyazaki & Rosenthal, 1976). In current-clamp experiments 1 mM-Cs^+ produced a striking increase in the dorsal root ganglion neurone input resistance and abolished the sag and depolarizing overshoot present in hyperpolarizing electrotonic potentials.

Under voltage clamp Cs^+ (0·2–10 mM) reversibly blocked the inward relaxation due to I_h in thirty-one neurones, as shown in Fig. 10. Rubidium (1 mM; three neurones) was much less effective than an equivalent concentration of Cs^+. At a holding potential of -50 or -60 mV Cs^+ did not alter the holding current (Fig. 10), but at holding potentials within the I_h activation range Cs^+ produced an outward shift of the holding current (see Mayer & Westbrook, 1982).

I_h was not blocked when dorsal root ganglion neurones were loaded with Cs^+ following impalement with two CsCl electrodes. This procedure completely blocked outward currents produced by depolarizing commands. Thus Cs^+ selectively blocks I_h at a site accessible from the outer face of the membrane. Addition of a sufficient concentration of tetraethylammonium (TEA, 25 mM) to the bathing medium to greatly reduce the amplitude of outward currents activated by membrane depolarization also failed to block I_h. It is worth noting that in TEA the I_h on-relaxation retained its complex activation kinetics, indicating that inward relaxations produced by deactivation of a K^+ conductance (see DiFrancesco & Ojeda, 1980) were not contaminating the relaxations due to I_h.

At 1 and 10 mM the block by Cs^+ of the inward relaxations due to I_h was complete (Figs. 10 and 11); at 200 μM-Cs^+, the inward relaxations were reduced in amplitude but were not blocked, the reduction being voltage-dependent and greatest at hyperpolarized membrane potentials (Fig. 11). Control current–voltage plots between

---

Fig. 8. *A* shows the reversible reduction of the rate of rise and amplitude of the action potential of a dorsal root ganglion neurone that occurs on perfusion with choline-substituted Na^+-free solution. *B* shows the effect of perfusion with the same solution on inward current relaxations recorded during 1·5 sec step hyperpolarizing commands from a holding potential (V_h) of -60 mV to a command potential (V_c) of -90 mV. The inward relaxations recorded at -90 mV are virtually abolished and the inward tail currents recorded on repolarization to -60 mV become outward in Na^+-free solution. *C* shows oscillographic records of the inward relaxations produced by 10 mV incrementing hyperpolarizing voltage jumps from a holding potential of -50 mV in a medium containing 25 mM-K^+. Perfusion with a Tris substituted Na^+-free solution reversibly reduces the amplitude of, but does not block the inward current relaxations. *D1* and *2* show plots of the normalized inward relaxation amplitude, measured as the steady-state current (I_{ss}) flowing at the end of a 1·5 sec hyperpolarizing command to a given membrane potential (V_m) minus the instantaneous current (I_i) flowing at the start of the step command, normalized by dividing by ($I_{ss}-I_i$) recorded on stepping to -120 mV, plotted against the membrane potential during the step command. Holding potential indicated as V_h. Data points are the mean ± S.D. *D1* shows results obtained from nine neurones in medium containing 4·5 mM-K^+ in the presence and absence of Na^+. *D2* shows results obtained from ten neurones in medium containing 25 mM-K^+ in the presence and absence of Na^+. *D3* and *4* show examples of raw current voltage plots obtained in 4·5 (*D3*) and 25 (*D4*) mM-K^+ from which data was pooled to obtain the graphs shown in *D1* and *2*.

−120 and −60 mV showed a linear, i.e. ohmic, instantaneous (chord) relationship; the steady-state current–voltage relationship showed inward rectification, due to the activation of I_h. High concentrations of Cs^+ (1 and 10 mM) produced outward rectification in both the instantaneous and steady-state current–voltage relationships (Fig. 11). This voltage-dependent blocking action of Cs^+ is clearly seen in the records

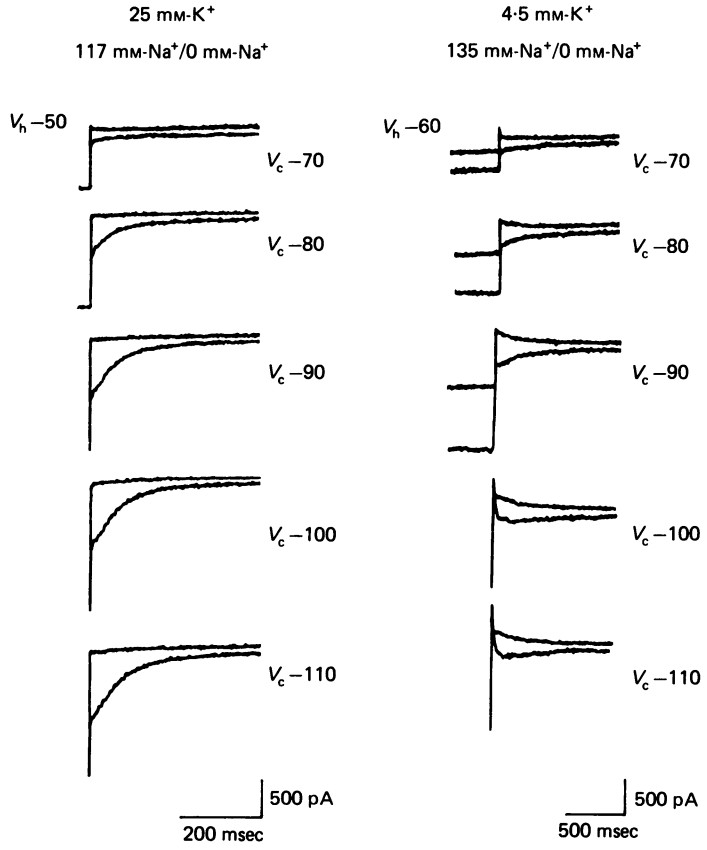


Fig. 9. Tail currents recorded in the presence and absence of Na^+. The series shown on the left were obtained from a neurone bathed in 25 mM-K^+ and voltage-clamped at a holding potential of −50 mV. The series shown on the right were obtained from another neurone bathed in 4·5 mM-K^+, and clamped at a holding potential of −60 mV. The membrane potential during 1·5 sec hyperpolarizing prepulses is indicated to the right of individual traces. Note that the tail currents are inward in the presence of Na^+, flat at −50 mV in Na^+-free 25 mM-K^+ medium and outward at −60 mV in Na^+-free 4·5 mM-K^+ medium.

shown in Fig. 10. In 200 μM-Cs^+ the steady-state current–voltage relationship again showed outward rectification, but the instantaneous relationship was less markedly influenced. A fast incompletely resolved outward relaxation was frequently recorded in Cs^+-containing solutions. It is possible this is due to a time- and voltage-dependent channel blocking action of Cs^+ (see Hagiwara *et al.* 1976).

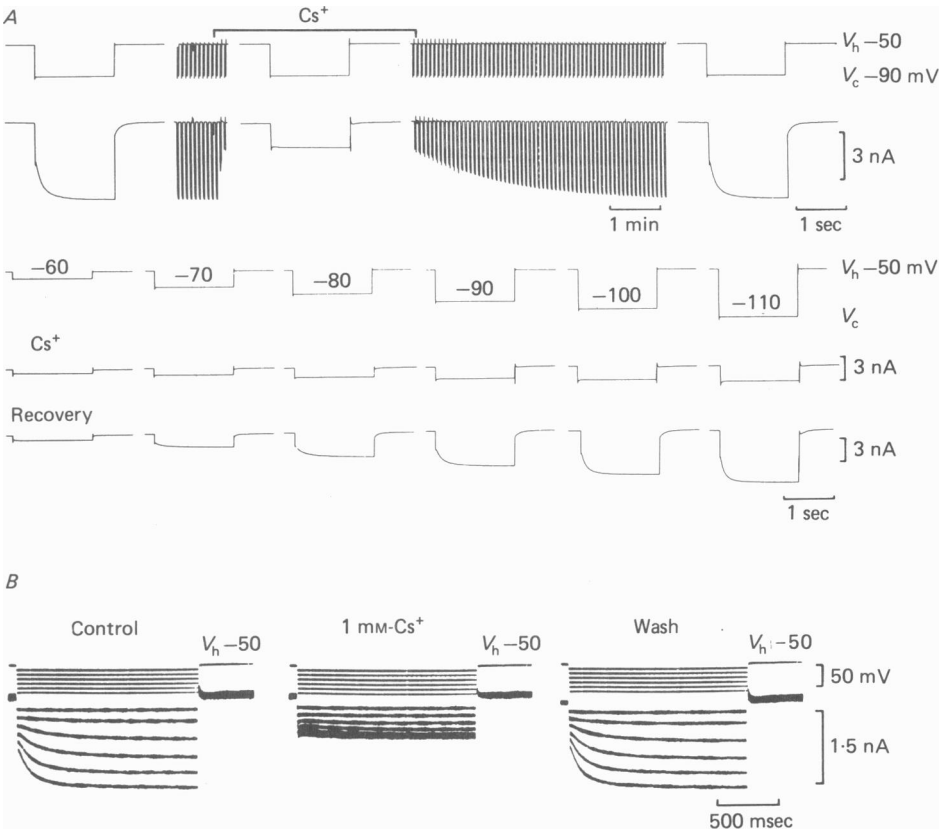


Fig. 10. Block of I_h by Cs^+. *A* shows (top row) the time course of the reversible block of inward relaxations evoked by 1·5 sec step changes of membrane potential from a holding potential (V_h) of −50 mV to a command potential (V_c) of −90 mV that is produced by diffusion of Cs^+ from a broken pipette containing 10 mM-CsCl. The lower rows show the relaxations produced by step commands to potentials between −60 to −110 mV during the presence of the CsCl diffusion pipette, and following recovery from the blocking action of Cs^+. The oscillographic records in *B* show the reversible block of I_h produced by application of 1 mM-CsCl from a perfusion pipette. The voltage-dependent blocking action of Cs^+ on the instantaneous leak current recorded on stepping from the holding potential of −50 mV is clearly visible.

Block of I_h by impermeable anion substitutes

The depolarized reversal potential of I_h is adequately explained by a mixed Na^+–K^+ conductance; indeed a similar mixed current system, labelled I_f, has been suggested to underlie the inward rectification shown by the sinoatrial node and by Purkinje fibres (DiFrancesco & Ojeda, 1980; DiFrancesco, 1981*a*, *b*). However, contribution of an anion conductance to the sinus node inward rectifier has been suggested by Seyama (1979) and by Yanagihara & Irasawa (1980).

In dorsal root ganglion neurones it seems likely that the Cl^- equilibrium potential (E_{Cl}) lies positive to the membrane potential range over which I_h activates. Thus an

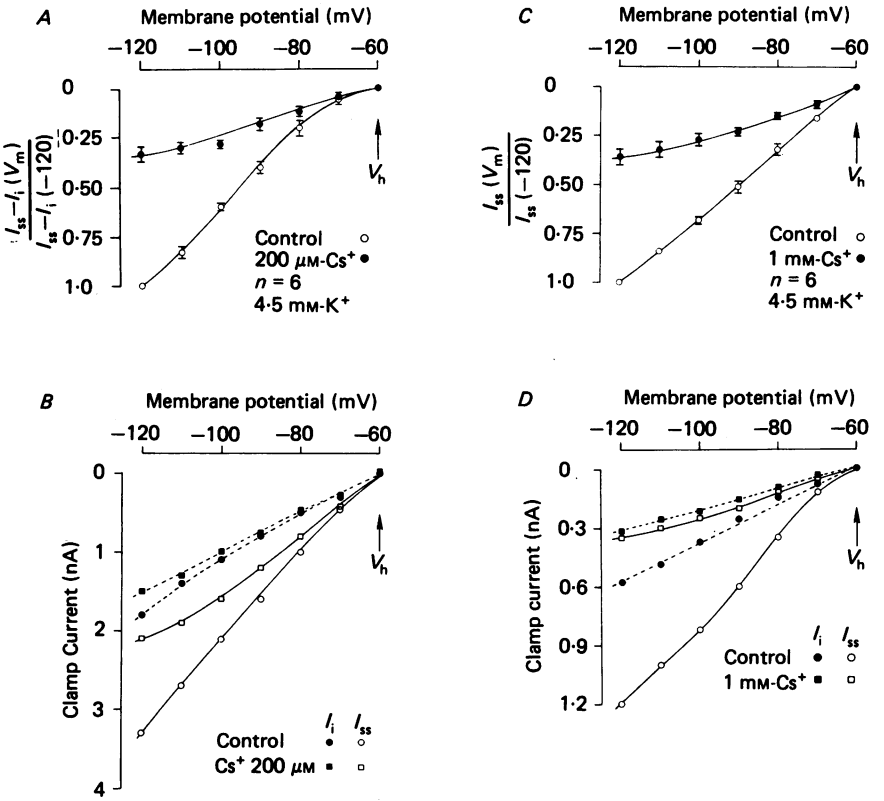


Fig. 11. *A* shows a plot of the inward relaxation amplitude measured as the difference between the steady-state (I_{ss}) and instantaneous (I_i) currents recorded on stepping to the membrane potential V_m, normalized for six neurones with respect to control values of $I_{ss}-I_i$ recorded on stepping to −120 mV from a holding potential (V_h) of −60 mV, plotted against the membrane potential during the step command, in the presence and absence of 200 μM-Cs^+. *B* shows an example of the relationship between I_i and I_{ss} plotted against V_m in the presence and absence of 200 μM-Cs^+ for a single neurone. *C* shows a plot of the steady-state current recorded at the command potential V_m normalized for six neurones with respect to the steady-state current recorded in control medium on stepping to −120 mV, in the presence and absence of 1 mM-CsCl, and plotted against the membrane potential during the step command. *D* shows an example from a single neurone of the relationship between I_i and I_{ss} plotted against V_m in the presence and absence of 1 mM-CsCl. Note the outward rectification present in both the instantaneous and steady-state current–voltage relationships. Data points in *A* and *C* are mean ± S.D.

inward Cl^- current might contribute to I_h. Although we have no information as to the value of E_{Cl} under our experimental conditions, experiments on dorsal root ganglion neurones of adult animals have revealed an inwardly directed Cl^- pump, which shifts E_{Cl} to around −23 mV (Gallagher, Higashi & Nishi, 1978). We therefore examined the effect on I_h of reducing the extracellular Cl^- concentration from 160 to 20 mM, using isethionate and methylsulphate as impermeant anions.

Under current clamp local perfusion of dorsal root ganglion neurones with this low-Cl^- solution produced a slowly developing membrane potential hyperpolarization associated with a membrane resistance *increase* (Fig. 12). Membrane potential

hyperpolarization by d.c. current injection alone produced a membrane resistance *decrease*. Recovery from the effects of the low-Cl^- solution was prolonged (Fig. 12).

Under voltage clamp, at a holding potential of -60 mV, perfusion with the low-Cl^- solution caused generation of a slowly developing outward holding current. The inward relaxations due to I_h were rapidly blocked by this solution (Fig. 12), as were

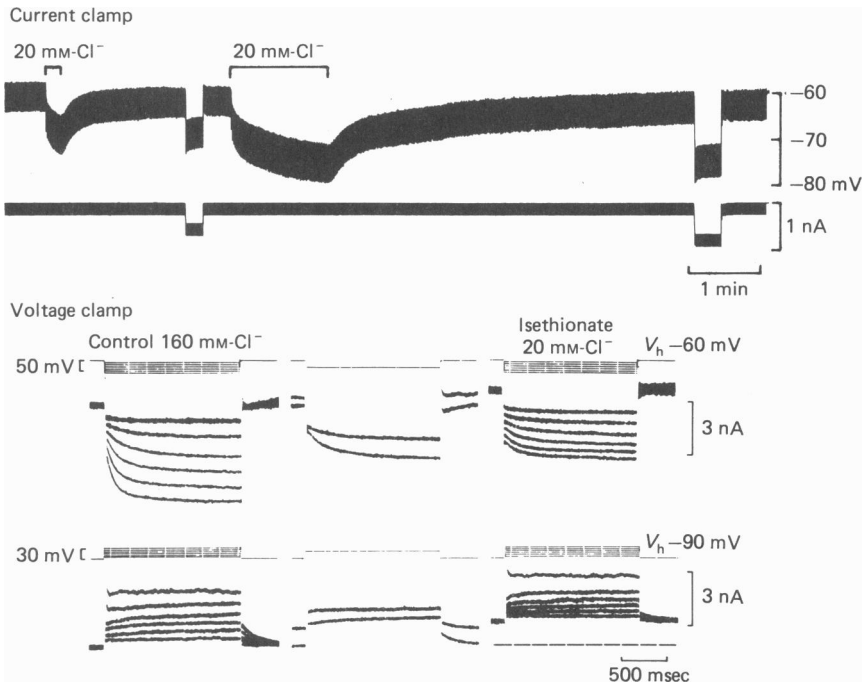


Fig. 12. Shows the action of low-Cl^- solution applied from a perfusion pipette on the membrane potential and input resistance (current clamp) of a dorsal root ganglion neurone impaled with separate current passing and voltage recording electrodes. D.c. current injection was used to assess the effect of membrane hyperpolarization *per se* on the input resistance. Under voltage-clamp perfusion with the same solution reduced the amplitude of the inward relaxations produced by step hyperpolarizing commands from a holding potential of -60 mV, and produced a slowly increasing outward shift of the holding current. At a holding potential of -90 mV a larger outward shift of the holding current was recorded, and the outward relaxations evoked by depolarizing commands were reduced in amplitude. The oscillographic records show families of current relaxations recorded before (left) and during (right) perfusion with the low-Cl^- solution at holding potentials (V_h) of -60 mV (top row) and -90 mV (lower row). The centre records show superimposed sweeps of the relaxations produced by a 30 mV hyperpolarizing command (top row) and a 20 mV depolarizing command (lower row) before and during perfusion with the low-Cl^- solution.

outward relaxations produced by depolarizing commands from a holding potential of -90 mV. We do not favour the most direct interpretation of this result, namely that an anion conductance contributes to I_h. Instead we believe that the isethionate–methylsulphate mixture used as an impermeant anion solution has a non-specific blocking action on the cation conductance G_h.

Examination of the instantaneous and steady-state current–voltage relationships recorded in low chloride solution revealed little change in the instantaneous relation-

ship, which remained linear, and a large reduction of the steady-state inward rectification produced by I_h (Fig. 13). Comparison between plots of the normalized inward relaxation amplitude *vs.* membrane potential obtained in normal, zero Na^+, Cs^+ and low-Cl^- media reveals interesting differences (compare Figs. 8, 11 and 13). In Cs^+ both the instantaneous and steady-state current–voltage relationships outward rectify, whereas in low-Cl^- solution the instantaneous relationship remains linear, but the steady-state curve no longer inward rectifies. In Na^+-free solution inward rectification remains, but is of reduced amplitude.

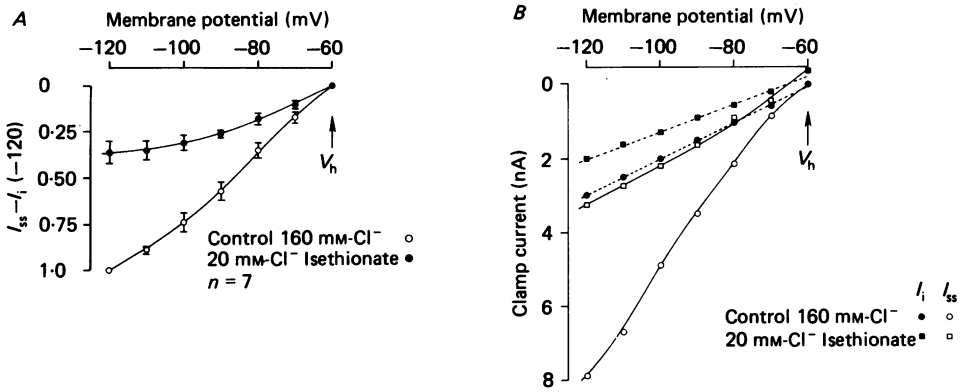


Fig. 13. Current–voltage relationships in low Cl^- medium. *A* shows the inward relaxation amplitude recorded on stepping to a membrane potential V_m plotted as the difference between the steady-state (I_{ss}) and instantaneous (I_i) currents recorded on stepping from the holding potential (V_h) to V_m, normalized by dividing by $I_{ss}-I_i$ recorded on stepping to −120 mV, plotted against the membrane potential during the step command (data from seven neurones; points are mean ± S.D.). *B* shows a plot of I_i and I_{ss} against V_m for a single neurone; note block of inward rectification during perfusion with low-Cl^- solution, and the approximately linear instantaneous current–voltage relationships.

DISCUSSION

Inward and anomalous rectification

Many biological membranes show inward rectification, that is the membrane conductance is greater for inward current than for outward current. The term anomalous rectification, as applied to slope-conductance measurements obtained under current clamp in neurones, has been used to describe both the apparent membrane resistance increase recorded on depolarization and the membrane resistance decrease recorded on hyperpolarization. Recent evidence from voltage-clamp experiments suggests that in some neurones depolarization may activate a voltage-sensitive conductance which carries a persistent inward Na^+ or Ca^{2+} current. This produces a region of negative slope conductance in the current–voltage relationship recorded depolarized to the resting potential (motoneurones: Schwindt & Crill, 1980; neocortical pyramidal cells *in vitro*: Stafstrom, Schwindt & Crill, 1982) and an *apparent* membrane resistance increase when voltage-recording under current clamp is used to construct current–voltage curves (Nelson & Frank, 1967; Nelson & Lux, 1970; Hotson *et al.* 1979). In contrast, in many neurones membrane hyperpolarization beyond about −70 mV activates a mechanism causing a time-dependent sag and

depolarizing overshoot in hyperpolarizing electrotonic potentials (Ito & Oshima, 1965; Nelson & Frank, 1967; Purpura *et al.* 1968; Gallego & Eyzaguirre, 1978; Gorke & Pierau, 1980; Mayer, Higashi, Gallagher & Shinnick-Gallagher, 1983). It seems unlikely that this response is generated by the same mechanism as that activated by membrane depolarization. Indeed, in spinal and visceral sensory ganglion neurones which show marked anomalous rectification during membrane hyperpolarization (Ito, 1957; Czéh *et al.* 1977; Gallego & Eyzaguirre, 1978, Görke & Pierau, 1980), depolarization beyond the resting potential activates only outward rectification (see. Fig. 1).

Recent experiments performed using a single micro-electrode switched voltage-clamp and brain-slice preparations *in vitro* have revealed conductances producing inward rectification in hippocampal and olfactory cortex neurones (Adams & Halliwell, 1982; Halliwell & Adams, 1982; Constanti & Galvan, 1983). The present results and those of Halliwell & Adams (1982) and Constanti & Galvan (1983) suggest that time-dependent anomalous rectification produced by membrane hyperpolarization is due to activation of voltage-sensitive conductances separate from those activated during membrane depolarization. The hyperpolarization-activated conductance produces a region of inward rectification in the steady-state current–voltage relationship. This latter type of anomalous rectification is closer in spirit to that originally described as such by Katz (1949), but the term inward rectification is more descriptive of the process (Noble, 1965; Adrian, 1969) and we refer to it here as such.

Hyperpolarizing electrotonic potentials recorded in sympathetic ganglion cells also show sags similar to those attributed to inward (anomalous) rectification in sensory ganglion neurones (Brown & Constanti, 1980; Constanti & Brown, 1981). However the responses recorded in sympathetic ganglion neurones are produced by a quite different mechanism: deactivation of a voltage-sensitive K^+ conductance carrying *outward* current at the resting potential (Brown & Adams, 1980; Constanti & Brown, 1981; Adams, Brown & Constanti, 1982). Under current clamp these two forms of *anomalous* rectification are easily distinguished. In sensory ganglion neurones the sag in the electrotonic potential increases with the amount of hyperpolarizing current injected, while in sympathetic neurones the sag disappears from strongly hyperpolarizing electrotonic potentials (Constanti & Brown, 1981; Adams *et al.* 1982).

Thus anomalous rectification as recorded under current clamp in neurones can be produced by at least three quite distinct mechanisms. It is only with the application of the voltage-clamp technique to vertebrate and mammalian neurones that these mechanisms are being identified.

Inward rectifiers in other membranes

It is also clear that inward rectification recorded in marine egg, heart, muscle, neuronal and photoreceptor membranes is not produced by a single common conductance mechanism. In all of these tissues the conductance is activated by hyperpolarization and is permeable to K^+. The inward rectifier of marine eggs, muscle and olfactory cortex neurones is a relatively K^+-specific conductance (Hagiwara & Takahashi, 1974; Standen & Stanfield, 1980; Constanti & Galvan, 1983) and is blocked by Ba^{2+} (Hagiwara *et al.* 1978; Standen & Stanfield, 1978*b*) and Cs^+ (Hagiwara *et al.* 1976; Gay & Stanfield, 1977; Constanti & Galvan, 1983). The

activation variable of this conductance shows $V-E_K$ dependence (Hagiwara *et al.* 1976; Leech & Stanfield, 1981; Hestrin, 1981; Constanti & Galvan, 1983); that is it appears as if the conductance depends on the difference between the membrane and K^+ equilibrium potentials, rather than on the membrane potential alone.

The conductance producing inward rectification in the cardiac sinoatrial node is not specific for K^+ and has a high permeability to Na^+ as well (DiFrancesco & Ojeda, 1980). A similar conductance present in Purkinje fibres is blocked by Cs^+ but not Ba^{2+} and does not appear to show $V-E_K$ dependence (DiFrancesco, 1981*a*, *b*). The inward rectifier in the photoreceptor membrane also shows poor Na^+–K^+ discrimination (Bader, Bertrand & Schwartz, 1982).

In neuronal membranes both types of inward rectifier may exist. In sensory ganglion neurones the properties of the inward rectifier conductance G_h include Na^+ dependence but not $V-E_K$ dependence, Cs^+ sensitivity and Ba^{2+} resistance, and resemble those of the sinoatrial node rectifier. Detailed information is not available on the ionic mechanism of the inward rectifier in hippocampal pyramidal neurones, but its Ba^{2+} resistance (Halliwell & Adams, 1982) suggests similarity to the sensory neurone and cardiac conductances, although the Na^+–K^+ permeability ratio may be lower in hippocampal neurones (Halliwell & Adams, 1982). In olfactory cortex neurones the inward rectifier shows Ba^{2+} sensitivity and $V-E_K$ dependence, and thus resembles the muscle and marine egg conductances (Constanti & Galvan, 1983).

Voltage-clamp studies on motoneurones *in vivo* (Barrett, Barrett & Crill, 1980) have also revealed a time-dependent conductance, activated on hyperpolarization from the resting potential, which produces a region of inward rectification in the steady-state current–voltage curve. The activation kinetics and ionic mechanism of this conductance were not investigated, but with a reversal potential close to -5 mV (Barrett *et al.* 1980) contribution of a Na^+ permeability change seems likely. Hyperpolarization activated *anomalous* rectification recorded under current clamp in vesical pelvic ganglion neurones is reduced in Na^+-free solution (Mayer *et al.* 1983), and the presence of a slowly activating inward rectifier under voltage clamp (M. L. Mayer, unpublished observations) suggests the presence in these neurones of a conductance similar to the inward rectifier present in sensory neurones and described here. Thus a hyperpolarization-activated conductance similar to G_h appears to be present in a wide variety of neurones.

Action of impermeable anion substituents

A detailed study on the ionic mechanism of the Purkinje fibre inward rectifier showed clearly that both Na^+ and K^+ ions carry current in this system (DiFrancesco, 1981*b*). No investigation of the role of anions was made, but Seyama (1979) and Yanagihara & Irisawa (1980) have reported that a variety of impermeable anion substitutes block inward rectification in this system. In sensory ganglion neurones isethionate–methylsulphate substitution also abolishes inward rectification. Such results suggest a major contribution of an anion conductance to the inward rectifier. It is possible however that impermeable anion substitutes block the inward rectifier by some pharmacological action. It is of interest that these anion substitutes reduce the K^+ permeability of ventricular muscle (Carmeliet & Verdonck, 1977), and block the transient outward current of Purkinje fibres independent of any action on Ca^{2+} activity (Siegelbaum & Tsien, 1980).

We suggest that in sensory ganglion neurones and perhaps also in the sinoatrial node (see Brown, 1982) elimination of inward rectification by impermeable anion substitutes does not indicate contribution of a large anion conductance to the rectifying process, but that these substitutes block the mixed Na^+–K^+ conductance by some unspecific process. This block is unlikely to occur in the channels carrying I_h, since the block might be expected to show some voltage dependence, i.e. relief as the electrical field across the membrane increases during hyperpolarization, since isethionate is an anion. Also little action of isethionate on the leak conductance was recorded. In contrast, the blocking action of Cs^+ on both the leak conductance and G_h is voltage-dependent.

Does I_h contribute to the resting membrane potential?

The current–voltage relationship of many neurones has a uniform slope around the resting potential and generally it has been assumed that electrotonic potentials recorded close to the resting potential reflect the *passive* properties of the membrane, which are usually modelled by a simple R–C network. However it has been known for some time (Araki, Ito & Oshima, 1961; Ito & Oshima, 1965) that active changes in membrane function can occur within 5 mV of the resting potential of healthy cells. It seems probable that for some neurones the resting potential is determined by the counterbalancing action of two voltage-sensitive conductances. One, an inward rectifier activated by membrane hyperpolarization, exerts a depolarizing influence. The other, a non-inactivating K^+ conductance activated by membrane depolarization, exerts a hyperpolarizing influence. One suitable candidate for the latter would be the M conductance (Adams *et al.* 1982; Halliwell & Adams, 1982).

The resting potential of sensory ganglion neurones, under our experimental conditions, is typically close to -60 mV in healthy cells. The inward rectifier conductance activates close to this potential and has a half-activation potential of approximately -80 mV. The inward rectifier in hippocampal neurones activates at approximately -80 mV and has a half-activation potential of approximately -95 mV (Halliwell & Adams, 1982). The resting potential of healthy hippocampal neurones is around -70 mV, i.e. hyperpolarized to that of sensory neurones. It is tempting to speculate that this difference in resting potentials results from the more positive threshold for activation of the inward rectifier in sensory neurones.

Functional significance of I_h

The role of an inward current activated by membrane hyperpolarization beyond the resting potential presents intriguing possibilities. In the sinoatrial node the current carried by the inward rectifier, I_f, has been identified as part of the pace-making apparatus (Brown & DiFrancesco, 1980; Brown, 1982). In our experiments sensory ganglion neurones were quiescent and showed rapid accommodation to a depolarizing current pulse, due to the presence of strong outward rectifiers which rapidly activated below the spike threshold (M. L. Mayer & G. L. Westbrook, unpublished observations). Thus any role of I_h in controlling spontaneous or repetitive activity will have to be studied in other neurones which lack such marked outward rectification. However in dorsal root ganglion neurones the activation of I_h during hyperpolarizing electrotonic potentials leads to a Cs^+-sensitive depolarizing overshoot, which is

capable of triggering an action potential. Thus I_h can contribute to the anode break excitation process.

In photoreceptors, which also contain a similar inward rectifier to that in sensory ganglion neurones (Bader *et al.* 1982), it has been suggested that the role of this conductance is to keep the membrane potential in a range suitable for the action and release of neurotransmitters (Fain & Lisman, 1981). The photoreceptor inward rectifier thus counterbalances the hyperpolarizing action of light during supression of the dark current (Fain, Quandt, Bastian & Gerschenfeld, 1978). It seems possible that the resting potential of sensory ganglion neurones is a similarly protected region, and that the inward rectifier is also present in nerve terminals where transmitter release occurs. Thus the role of I_h could be to counterbalance prolonged membrane hyperpolarization produced by Ca^{2+} entry during an action potential and subsequent outward current flow through a Ca^{2+}-activated K^+ conductance. It is of interest to note that *anomalous* rectification is considerably more pronounced in A- than C-fibre origin sensory ganglion neurones (Gallego & Eyzaguirre, 1978, Görke & Pierau, 1980), and that the frequency-following capability of the A-fibre neurones (and one assumes the temporal control of neurotransmitter release in the spinal cord) is the greater. The technical expertise needed for a voltage-clamp study of the conductance mechanisms present in nerve terminals seems unlikely to be achieved in the near future, but it is of interest to note that some form of anomalous rectification does occur in unmyelinated axons (Marsh, 1982), increasing the likelihood that this situation extends to the nerve terminal as well.

We thank Dr P. G. Nelson for his interest and the use of laboratory facilities, Dr H. Lecar for loan of the Leitz microscope, Dr B. Smith for constructing the voltage clamp, Dr J. Kimura for performing the activation variable curve-fitting analysis, Dr G. Guroff for the gift of nerve growth factor, and Sandy Fitzgerald for culturing the neurones. We are grateful to Drs A. B. MacDermott and T. G. Smith for their interest and advice during the course of this work. Drs C. R. Bader, A. Constanti and J. V. Halliwell kindly gave us copies of their papers in press. M.L.M. was a Harkness fellow of the Commonwealth Fund of New York.; G.L.W. is a P.R.A.T. Fellow of N.I.G.M.S., N.I.H.

REFERENCES

ADAMS, P. R., BROWN, D. A. & CONSTANTI, A. (1982). M-currents and other potassium currents in bullfrog sympathetic neurones. *J. Physiol.* **330**, 537–572.

ADAMS, P. R. & HALLIWELL, J. V. (1982). A hyperpolarization-induced inward current in hippocampal pyramidal cells. *J. Physiol.* **324**, 62–63.

ADRIAN, R. H. (1969). Rectification in muscle membrane. *Prog. Biophys. & molec. Biol.* **19**, 339–369.

ADRIAN, R. H. & FREYGANG, W. H. (1962*a*). The potassium and chloride conductance of frog muscle membrane. *J. Physiol.* **163**, 61–103.

ADRIAN, R. H. & FREYGANG, W. H. (1962*b*). Potassium conductance of frog muscle membrane under controlled voltage. *J. Physiol.* **163**, 104–114.

ALMERS, W. (1972). Potassium conductance changes in skeletal muscle and the potassium concentration in the transverse tubules. *J. Physiol.* **225**, 33–56.

ARAKI, T., ITO, M. & OSHIMA, T. (1961). Potential changes produced by application of current steps in motoneurones. *Nature, Lond.* **191**, 1104–1105.

BADER, C. R., BERTRAND, D. & SCHWARTZ, E. A. (1982). Voltage-activated and calcium-activated currents studied in solitary rod inner segments from the salamander retina. *J. Physiol.* **331**, 253–284.

BARRETT, E. F., BARRETT, J. N. & CRILL, W. E. (1980). Voltage-sensitive outward currents in cat motoneurones. *J. Physiol.* **304**, 251–276.

BROWN, D. A. & ADAMS, P. R. (1980). Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone. *Nature, Lond.* **283**, 673–676.

BROWN, D. A. & CONSTANTI, A. (1980). Intracellular observations on the effects of muscarinic agonists on rat sympathetic neurones. *Br. J. Pharmac.* **70**, 593–608.

BROWN, H. F. (1982). Electrophysiology of the sinoatrial node. *Physiol. Rev.* **62**, 505–530.

BROWN, H. & DIFRANCESCO, D. (1980). Voltage-clamp investigations of membrane currents underlying pace-maker activity in rabbit sino-atrial node. *J. Physiol.* **308**, 331–351.

BROWN, T. H., PERKEL, D. H., NORRIS, J. C. & PEACOCK, J. H. (1981). Electrotonic structure and specific membrane properties of mouse dorsal-root-ganglion neurons. *J. Neurophysiol.* **45**, 1–15.

CARMELIET, E. & VERDONCK, F. (1977). Reduction of potassium permeability by chloride substitution in cardiac cells. *J. Physiol.* **265**, 193–206.

CHOI, D. W. & FISCHBACH, G. D. (1981). GABA conductance of chick spinal cord and dorsal root ganglion neurons in cell culture. *J. Neurophysiol.* **45**, 605–620.

CONSTANTI, A. & BROWN, D. A. (1981). M-currents in voltage clamped mammalian sympathetic neurones. *Neurosci. Lett.* **24**, 298–294.

CONSTANTI, A. & GALVAN, M. (1983). Fast inward-rectifying current accounts for anomalous rectification in olfactory cortex neurones. *J. Physiol.* **335**, 153–178.

CZÉH, G., KUDO, N. & KUNO, M. (1977). Membrane properties and conduction velocity in sensory neurones following central or peripheral axotomy. *J. Physiol.* **270**, 165–180.

DIFRANCESCO, D. (1981*a*). A new interpretation of the pace-maker current in calf Purkinje fibres. *J. Physiol.* **314**, 351–376.

DIFRANCESCO, D. (1981*b*). A study of the ionic nature of the pace-maker current in calf Purkinje fibres. *J. Physiol.* **314**, 377–393.

DIFRANCESCO, D. (1982). Block and activation of the pace-maker channel in calf Purkinje fibres: effects of potassium, caesium and rubidium. *J. Physiol.* **329**, 485–507.

DIFRANCESCO, D. & OJEDA, C. (1980). Properties of the current I_f in the sino-atrial node of the rabbit composed with those of the current I_{K2} in Purkinje fibres. *J. Physiol.* **308**, 353–367.

DUNLAP, K. & FISCHBACH, G. D. (1981). Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. *J. Physiol.* **317**, 519–535.

EHRENSTEIN, G. & LECAR, H. (1977). Electrically gated ionic channels in lipid bilayers. *Q. Rev. Biophys.* **10**, 1–34.

FAIN, G. L. & LISMAN, J. E. (1981). Membrane conductances of photoreceptors. *Prog. Biophys. & molec. Biol.* **37**, 91–147.

FAIN, G. L., QUANDT, F. N., BASTIAN, B. L. & GERSCHENFELD, H. M. (1978). Contribution of a caesium-sensitive conductance increase to the rod photoresponse. *Nature, Lond.* **272**, 467–469.

FUKUSHIMA, Y. (1982). Blocking kinetics of the anomalous potassium rectifier of tunicate eggs studied by single channel recording. *J. Physiol.* **331**, 311–331.

GALLAGHER, J. P., HIGASHI, H. & NISHI, S. (1978). Characterization and ionic basis of GABA-induced depolarizations recorded *in vitro* from cat primary afferent neurones. *J. Physiol.* **275**, 263–282.

GALLEGO, R. & EYZAGUIRE, C. (1978). Membrane and action potential characteristics of A and C nodose ganglion cells studied in whole ganglia and in tissue slices. *J. Neurophysiol.* **41**, 1217–1232.

GAY, L. A. & STANFIELD, P. R. (1977). Cs^+ causes a voltage-dependent blockade of inward K currents in resting skeletal muscle fibres. *Nature, Lond.* **267**, 169–170.

GÖRKE, K. & PIERAU, F. K. (1980). Spike potentials and membrane properties of dorsal root ganglion cells in pigeons. *Pflügers Arch.* **386**, 21–28.

HAGIWARA, S., MIYAZAKI, S., MOODY, W. & PATLAK, J. (1978). Blocking effects of barium and hydrogen ions on the potassium current during anomalous rectification in the starfish egg. *J. Physiol.* **279**, 167–185.

HAGIWARA, S., MIYAZAKI, S. & ROSENTHAL, N. P. (1976). Potassium current and the effect of cesium on this current during anomalous rectification of the egg cell membrane of a starfish. *J. gen. Physiol.* **67**, 621–638.

HAGIWARA, S. & TAKAHASHI, K. (1974). The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *J. Membrane Biol.* **18**, 61–80.

HALLIWELL, J. V. & ADAMS, P. R. (1982). Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Res.* **250**, 71–92.

HESTRIN, S. (1981). The interaction of potassium with the activation of anomalous rectification in frog muscle membrane. *J. Physiol.* **317**, 497–508.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.* **148**, 127–160.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544.

HOTSON, J. R., PRINCE, D. A. & SCHWARTZKROIN, P. A. (1979). Anomalous inward rectification in hippocampal neurons. *J. Neurophysiol.* **42**, 889–895.

ITO, M. (1957). The electrical activity of spinal ganglion cells investigated with intracellular microelectrodes. *Jap. J. Physiol.* **7**, 297–323.

ITO, M. & OSHIMA, T. (1965). Electrical behaviour of the motoneurone membrane during intracellularly applied current steps. *J. Physiol.* **180**, 607–635.

KANDEL, E. R. & TAUC, L. (1966). Anomalous rectification in the metacerebral giant cells and its consequences for synaptic transmission. *J. Physiol.* **183**, 287–304.

KATZ, B. (1949). Les constantes électriques de la membrane du muscle. *Archs Sci. physiol.* **3**, 285–299.

KOSTYUK, P. G., VESELOVSKY, N. S., FEDULOVA, S. A. & TSYNDRENKO, A. Y. (1981). Ionic currents in the somatic membrane of rat dorsal root ganglion neurones-III. Potassium currents. *Neuroscience* **6**, 2439–2444.

LEECH, C. A. & STANFIELD, P. R. (1981). Inward rectification in frog skeletal muscle fibres and its dependence on membrane potential and external potassium. *J. Physiol.* **319**, 295–309.

MARCHAIS, D. & MARTY, A. (1979). Interaction of permeant ions with channels activated by acetycholine in *Aplysia* neurones. *J. Physiol.* **297**, 9–45.

MARSH, S. (1982). Conductance measurements in mammalian unmyelinated nerves. *J. Physiol.* **325**, 5–6*P*.

MAYER, M. L., HIGASHI, H., GALLAGHER, J. & SHINNICK-GALLAGHER, P. (1983). On the mechanism of action of GABA in pelvic vesical ganglia: biphasic responses evoked by two opposing actions on membrane conductance. *Brain Res.* **260**, 233–248.

MAYER, M. L. & WESTBROOK, G. L. (1982). 'Anomalous' rectification in mouse spinal sensory neurones generated by a time- and voltage-dependent inward current. *J. Physiol.* **332**, 24–25*P*.

MAYER, M. L. & WESTBROOK, G. L. (1983). Ionic mechanism of I_h in mouse spinal sensory ganglion neurones. *J. Physiol.* **334**, 116–117*P*.

MIYAZAKI, S., TAKAHASHI, K., TSUDA, K. & YOSHII, M. (1974). Analysis of non-linearity observed in the current–voltage relation of the tunicate embryo. *J. Physiol.* **238**, 55–77.

NAKAJIMA, S., IWASAKI, S. & OBATA, K. (1962). Delayed rectification and anomalous rectification in frog's skeletal muscle membrane. *J. gen. Physiol.* **46**, 97–115.

NELSON, P. G. & FRANK, K. (1967). Anomalous rectification in cat spinal motoneurons and effect of polarizing currents on excitatory postsynaptic potentials. *J. Neurophysiol.* **30**, 1097–1113.

NELSON, P. G. & LUX, H. D. (1970). Some electrical measurements of motoneurone parameters. *Biophys. J.* **10**, 55–73.

NOBLE, D. (1965). Electrical properties of cardiac muscle attributable to inward going (anomalous) rectification. *J. cell Physiol.* **66**, Suppl. 2, 127–135.

PURPURA, D. P., PRELEVIC, S. & SANTINI, M. (1968). Hyperpolarizing increase in membrane conductance in hippocampal neurons. *Brain Res.* **7**, 310–312.

RANSOM, B. R. & HOLZ, R. W. (1977). Ionic determinants of excitability in cultured mouse dorsal root ganglion and spinal cord cells. *Brain Res.* **136**, 445–453.

RANSOM, B. R., NEALE, E., HENKART, M., BULLOCK, P. N. & NELSON, P. G. (1977). Mouse spinal cord in cell culture. I. Morphology and intrinsic neuronal electrophysiological properties. *J. Neurophysiol.* **40**, 1132–1150.

SCHWINDT, P. C. & CRILL, W. E. (1980). Properties of a persistent inward current in normal and TEA-injected motoneurones. *J. Neurophysiol.* **43**, 827–846.

SEYAMA, I. (1979). Characteristics of the anion channel in the sino-atrial node cell of the rabbit. *J. Physiol.* **294**, 447–460.

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

STAFSTROM, C. E., SCHWINDT, P. C. & CRILL, W. E. (1982). Negative slope conductance due to a persistant subthreshold sodium current in cat neocortical neurones *in vitro*. *Brain Res.* **236**, 221–226.

STANDEN, N. B. & STANFIELD, P. R. (1978*a*). A mechanism for the fall in resting potassium conductance of frog skeletal muscle fibres occurring under extreme hyperpolarization. *J. Physiol.* **282**, 18–19*P*.

STANDEN, N. B. & STANFIELD, P. R. (1978*b*). A potential- and time-dependent blockade of inward rectification in frog skeletal muscle fibres by barium and strontium ions. *J. Physiol.* **280**, 169–191.

STANDEN, N. B. & STANFIELD, P. R. (1978*c*). Inward rectification in frog skeletal muscle: a blocking particle model. *Pflügers Arch.* **378**, 173–176.

STANDEN, N. B. & STANFIELD, P. R. (1979). Potassium depletion and sodium block of potassium currents under hyperpolarization in frog sartorius muscle. *J. Physiol.* **294**, 497–520.

STANDEN, N. B. & STANFIELD, P. R. (1980). Rubidium block and rubidium permeability of the inward rectifier of frog skeletal muscle fibres. *J. Physiol.* **304**, 415–435.

STANFIELD, P. R., ASHCROFT, F. M. & PLANT, T. D. (1981). Gating of a muscle K^+ channel and its dependence on the permeating ion species. *Nature, Lond.* **289**, 509–511.

YANAGIHARA, K. & IRISAWA, H. (1980). Inward current activated during hyperpolarization in the rabbit sino atrial node cell. *Pflügers Arch.* **385**, 11–19.