Low-Threshold, Persistent Sodium Current in Rat Large Dorsal Root Ganglion Neurons in Culture

MARK D. BAKER AND HUGH BOSTOCK

Sobell Department of Neurophysiology, Institute of Neurology, London WC1N 3BG, United Kingdom

Baker, Mark D. and Hugh Bostock. Low-threshold, persistent sodium current in rat large dorsal root ganglion neurons in culture. J. Neurophysiol. 77: 1503–1513, 1997. Dorsal root ganglion neurons from adult rats (≥200 g) were maintained in culture for between 1 and 3 days. Membrane currents generated by large neurons (50–75 μm apparent diameter) were recorded with the whole cell patch-clamp technique. Large neurons generated transient Na+ currents and at least two types of inward current that persisted throughout 200-ms voltage-clamp steps to +20 mV. One persistent current activated close to −35 mV (high threshold), whereas in about half of the cells another persistent current began to activate negative to −70 mV (low threshold). The high-threshold persistent current was identified as a Ca2+ current, as previously described in these neurons. The low-threshold current was reversibly suppressed either by replacing external Na+ with tetramethylammonium ions or by reducing external Na+ concentration ([Na+]i) and simultaneously raising external [Ca2+]. It was blocked by tetrodotoxin (TTX) with an apparent equilibrium dissociation constant in the single nanomolar range. We conclude that the low-threshold current is a TTX-sensitive, persistent Na+ current. The persistent TTX-sensitive current contributed to steady-state membrane current from at least −70 mV to 0 mV, a wider potential range than predicted by activation-inactivation gating overlap for transient Na+ current. Because of its low threshold and fast activation kinetics, the persistent Na+ current is expected to play an important role in determining membrane excitability.

INTRODUCTION

Recent evidence from studies of the tetrodotoxin (TTX) sensitivity of the demarcation potential in rat optic nerve (Stys et al. 1993), and from the characteristics of “late addition” in human sensory nerve (Bostock and Rothwell 1995, 1997), has implied the presence of functionally significant, steady-state inward currents in mammalian axons, operating at or near the resting potential. The current present in human sensory nerve must be generated at the nodes of Ranvier in large axons to contribute to changes in excitability brought about by very brief transcutaneously applied currents. The membrane current must also be voltage dependent and have fast deactivation kinetics, properties that were inferred from the response to brief hyperpolarizations. Bostock and Rothwell (1995, 1997) were unable to draw any conclusion regarding the ionic nature of the membrane current involved, because their data were necessarily obtained by indirect means. However, experiments on rat optic nerve (Stys et al. 1993) suggest that a steady-state inward current, operating near the resting potential, is a Na+ current because it is sensitive to 2 μM TTX. No such persistent inward currents have been described for isolated voltage clamped nodes of Ranvier from mammalian nerve. However, Dubois and Bergman (1975) reported the presence of a small, TTX-sensitive, “late” Na+ current at frog sensory nodes of Ranvier that was sustained for over 140 ms, even when the membrane was held at positive potentials. This current appeared to activate at more negative potentials than the transient current, but its kinetic properties were not described.

Large dorsal root ganglion (DRG) neurons would be expected to support the largest-diameter somatosensory axons, analogous to those studied by Bostock and Rothwell in humans. Such neurons generate transient Na+ currents (Caffrey et al. 1992; Fedulova et al. 1991), similar to Na+ currents recorded from voltage-clamped nodes of Ranvier (e.g., Brismar 1980; Schwarz and Eikhof 1987). We now present evidence that in addition to transient Na+ currents, some adult rat large DRG neurons also generate a persistent Na+ current, which is TTX sensitive and apparently similar to persistent Na+ currents already recorded from other cells, including hippocampal neurons, trigeminal motoneurons, and cardiac myocytes (Chandler et al. 1994; French et al. 1990; Saint et al. 1992). If present in axons, this current could account for the characteristics of latent addition described for human sensory axons and could also lead to TTX sensitivity of the resting potential. A part of the evidence for the persistent Na+ current in DRG neurons has already appeared in abstract form (Baker and Bostock 1996).

METHODS

Cell culture

Primary cultures of neurons were prepared from the lumbar DRGs of male Wistar rats (200–300 g). The rats were anesthetized with ketamine/mixetomidine (75 and 0.5 mg/kg, respectively) administered by intraperitoneal injection, and, once the ganglia were removed, the animals were overdosed with anesthetic and exsanguinated. After a laminectomy was performed, −6–8 ganglia (mainly L1–L4) were dissected free and pooled together in a Ca2+- and Mg2+-free, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-buffered dissociation solution containing (in mM) 155 NaCl, 4.8 Na-HEPES, 5.6 HEPES, 1.5 K2HPO4, and 10 glucose. The solution also contained protease (3 mg/ml Dispase, Sigma) and collagenase (0.6 mg/ml Type XI, Sigma). After the ganglia were incubated in the dissociation solution for 1 h at 37°C, they were gently triturated and washed in enzyme-free solution, and then the dissociated cells were plated onto poly-L-lysine-coated glass coverslips in the culture wells of 12-well plates (Falcon). The culture medium was a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12, to which were added t-glutamine (2 mM), penicillin/streptomycin (100 U/ml and 0.1 mg/ml, respectively) and 10% heat-inactivated foetal bovine serum. All media and additives were obtained from Sigma. The cells were kept in a 37°C incubator with a 5% CO2 atmosphere.
Electrophysiology

Coverslips with adherent neurons were mounted in a 35-mm plastic petri dish recording chamber. To isolate the inward membrane current, K⁺ currents and any outward Cl⁻ currents were suppressed with the use of a combination of ion replacement and pharmacology. Almost all external Cl⁻ was replaced with gluconate, and the external solutions incorporated tetraethylammonium (TEA⁺) ions, 4-aminopyridine (4-AP), and Cs⁺ ions to block outward and inward rectification. The normal extracellular solution contained (in mM) 135.6 sodium gluconate, 4.54 HEPES (Na⁺), 5.46 HEPES, 1.1 calcium gluconate, 1.2 magnesium gluconate, 5.4-AP, 10 cesium gluconate, and 10 TEA Cl, pH 7.2–7.3. Tetramethylammonium (TMA) gluconate replaced sodium gluconate in some experiments. A stock solution of TMA gluconate was obtained by neutralizing TMA OH with gluconic acid (Sigma). The normal internal solution contained (in mM) 143 CsCl, 3 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (Na), 6.04 HEPES (Na), 3.96 HEPES, 1.21 CaCl₂, 1.21 MgCl₂, and 10 TEA Cl, pH 7.2–7.3. Recordings were made with the use of an Axopatch 200 amplifier (Axon Instruments) with CV 202 headstage, and were filtered with the use of the four-pole Bessel filter on the amplifier (~3 dB at 2 kHz). Control of command pulse protocols and data collection was carried out by an IBM PC running pClamp version 5 or 6 (Axon Instruments). All electrodes used were made from thin-wall glass, coated with Sylgard, and fire polished. Their initial resistances, when filled with recording solution, were usually between 1 and 2 MΩ. For experiments in which external solutions containing sodium gluconate were used, the gluconate-chloride junction potential at the tip of the electrode was eliminated by applying a ~10-mV offset to the holding potential (see Baker and Ritchie 1994). TTX was observed to be virtual.

During whole cell recordings, the capacity current transient, generated on a hyperpolarizing step change in command potential, was routinely cancelled with the use of the simple resistance-capacitance circuit within the amplifier. Estimates of series resistance (Rₛ) and membrane capacity (Cₘ) could be read from the front panel potentiometers for each cell. Sometimes cancellation appeared complete, indicative of an excellent space clamp. The Rₛ/Cₘ prediction facility of the Axopatch amplifier was always used, allowing fast charging of the cell Cₘ during imposed step changes in membrane potential. In this way, the effective time constant for charging the membrane in response to an imposed step was reduced by up to 90%. Feedback Rₛ compensation was also always used, and was set to ≥70%. Imperfect Rₛ compensation commonly gave rise to a substantial voltage error across the uncompensated fraction of Rₛ (IR drop) on activation of a large, transient Na⁺ current, resulting in loss of transmembrane voltage control. Because of this, the membrane potential briefly diverged from the command potential by up to +25 mV. However, voltage control was quickly regained when the transient current inactivated.

Voltage-clamp protocols

Families of whole cell currents were evoked by a series of voltage-clamp steps from a negative prepulse potential. The membrane potential was held at ~90 mV, stepped to a prepulse potential of ~110 mV for 100 ms, and then stepped to a range of more positive potentials in either 5-, 10-, or 20-mV increments. Quasi-steady-state currents were also recorded in the whole cell configuration, where the membrane was held at a prepulse potential of ~20 mV for between 50 and 200 ms, to fully inactivate transient Na⁺ current. The potential was then stepped to a series of values between ~90 and ~20 mV, in increments of 10 mV, for 100 ms. Leak and transient currents were eliminated in some data sets by adding appropriately scaled currents evoked in response to slow, reversed-polarity clamp steps, recorded over a range of negative potentials.

In some particularly stable recordings, difference currents could be derived by subtracting a family of currents recorded in the presence of TTX, TMA, or low Na⁺ plus raised Ca²⁺, from control currents.

Evoked currents were measured as the mean current over 5 or 10 ms, at the end of a voltage-clamp test step, or as a peak current value. For both transient and sustained Na⁺ currents, a value of current (I) was converted to a permeability (P) with the use of the Goldman-Hodkin-Katz constant-field current equation for a monovalent cation

\[ P = I/(\langle E_m F^2/RT \rangle) \frac{1}{(1 - \exp(-FE_m/RT))} \]

where \( E_m \) is the value of membrane potential; \( F \) is Faraday's constant; \( R \) is the gas constant; \( T \) is the absolute temperature; and \( c_{na} \) and \( c_{out} \) are the concentrations of Na⁺ in the internal and external solutions, respectively. Plots of Na⁺ permeability versus membrane potential (i.e., P-E plots) were fitted by Boltzmann relations of the form

\[ P = P_{max}/[1 + \exp(E_{1/2} - E_{m} / \sigma_{E})] \]

where \( P_{max} \) is the limiting maximum permeability, \( E_{1/2} \) is the membrane potential at 0.5 \( P_{max} \), and \( \sigma_{E} \) is the steepness parameter (equal to \( RT/\sigma_{E} \), where \( z \) is an effective gating charge).

To estimate the apparent equilibrium dissociation constant (\( K_D \)) for block of the persistent Na⁺ current by TTX in the presence of residual outward rectification, the fractional block (\( B \)) was fitted by a rectangular hyperbola, on the assumption of 1:1 binding and block

\[ B = D/(D + K_D) = (I_0 - I_f)/(I_0 - I_e) \]

where \( I_0 \) is the amplitude of the steady-state whole cell current in the absence of TTX, \( I_f \) the current amplitude in the presence of a single concentration (\( D \)) of TTX, and \( I_e \) is the residual current with full block. For each cell, \( I_0 \) and \( K_D \) were estimated from measured values of \( I_0 \) and \( I_f \) by least-squares procedure.

All experiments were performed at 20–22°C.

RESULTS

A high proportion of dissociated DRG neurons appears almost round in culture, and it is therefore quite easy to estimate an apparent cell diameter. The diameters of the neurons studied varied between 50 and 75 μm. These were the largest cells in the cultures, selected to correspond to the cell bodies of the largest myelinated afferent fibers, with the fastest conduction velocities (Harper and Lawson 1985). Although the estimation of a diameter provides a simple method of categorizing neurons, we found that cells could vary by 20 or 25 μm in diameter and yet apparently have very similar somatic cell Cₘs.

In some cells, the whole cell quasi-steady-state current–membrane potential (I-E) relation was dominated by a low-threshold component (Fig. 1A, ○). In others, the low-threshold component was either absent or negligible, and in these cases the I-E relation was dominated by a high-threshold component (○). A low-threshold, persistent current was certainly not present in all neurons studied, whereas a high-threshold current was probably present in all cells in which calcium currents were not blocked (see below). A small high-threshold current contributes to the I-E relation dominated by the low-threshold current in Fig. 1A. An inward current operating over the low-threshold potential range was...
observed in 29 of 54 cells, and varied in maximum amplitude from <100 pA to >1 nA. We conclude that a low-threshold persistent current was present in about half of the large neurons. The mean I-E relation for 16 randomly selected neurons, from which recordings were made with the use of appropriate voltage-clamp protocols, is shown in Fig. 1B.

Nature of the high-threshold, persistent current

The high-threshold, persistent current was selectively blocked by superfusing Cd^{2+} (20–25 μM), a blocker of N- and L-type Ca^{2+} currents (Fox et al. 1987) (Fig. 2A). Computed difference currents (inset), describe an I-E relation appropriate for the high-threshold current alone. The high-threshold current had an apparent threshold close to −35 mV and gave rise to a peak inward current around −10 to 0 mV. Raising external Ca^{2+} initially caused a dramatic increase in the amplitude of the high-threshold current. However, it also gave rise to a clear phase of inactivation, and subsequently led to the gradual disappearance of the current, over several minutes (Fig. 2B). These characteristics are well-described properties of Ca^{2+} channels in neonatal rat DRG neurons (Fedulova et al. 1985; Kostyuk et al. 1981). Introducing 5 mM Ba^{2+} into the superfusate increased the current amplitude by >3 times (Fig. 2C), indicating that Ba^{2+} ions could act as a charge carrier for the current. In this experiment the transient Na^{+} current and the low-threshold persistent current were suppressed by partially replacing external sodium gluconate with TMA gluconate. The derived I-E relation for the Ba^{2+} current is similar to the quasi-steady-state I-E relation for the high-threshold current, for example that given in A. We conclude that the high-threshold, persistent current is a Ca^{2+} current.

Ionic basis of low-threshold, persistent current

Replacing extracellular sodium gluconate by superfusing a solution of TMA gluconate reversibly suppressed both the transient Na^{+} current and the low-threshold, persistent current. As expected, the apparent voltage dependence of the remaining inward currents behaved as though the 10-mV offset allowed for the Cl^{-} / gluconate^{-} junction potential (see METHODS) was abolished by the solution change. Computing the difference in quasi-steady-state current amplitudes before and after replacing Na^{+} gave a family of “difference currents.” Plotting the magnitude of the difference currents against membrane potential gives the I-E relation for the membrane current that was suppressed by reducing Na^{+} (Fig. 3A). Similar Na^{+} replacement observations were made on two other cells with a conspicuous, low-threshold persistent current, and these data strongly suggest that the low-threshold persistent current recorded in adult neurons is a Na^{+} current. The current apparently activates close to −75 mV and becomes maximal near −40 mV. In Fig. 3B, the current amplitudes have been converted to permeabilities (according to Eq. 1), assuming that the low-threshold channels are Na^{+} selective, and the resulting values are plotted against membrane potential. A Boltzmann relation (Eq. 2), given by best-fit parameter values, is superimposed on the data points. The I-E relation for the difference currents was similar to that for the low-threshold current in another cell in which residual outward rectification was small (but not completely eliminated) and the high-threshold current was blocked by 25 μM Cd^{2+} (Fig. 3C).

Effects of Ca^{2+} ions on low-threshold, persistent current

Fedulova et al. (1985) reported that the kinetically fast, low-threshold Ca^{2+} current in neonatal rat DRG neurons did not fully inactivate at potentials more negative than −40 mV. Similarly, Fox et al. (1987) found that a sustained portion of T-type Ca^{2+} current can operate in chick sensory neurons over the same potential range as the low-threshold, persistent current reported here. Furthermore, at least some Ca^{2+} channels are known to lose their ionic selectivity and

![Fig. 1](http://jn.physiology.org/)

**FIG. 1.** Steady-state current–membrane potential (I-E) relations reveal low- and high-threshold persistent inward currents. A: example quasi-steady-state I-E relations for 2 different cells. The membrane potential was held at +20 mV for 200 ms and then stepped to between −90 and +20 mV for 100 ms. In 1 cell, the relation was dominated by a low-threshold inward current, activating close to −70 mV (●). In the other cell, the low-threshold current was absent, and the I-E relation was dominated by a high-threshold inward current, activating near −35 mV (○). B: mean I-E relation derived from recordings made in 16 neurons with the use of voltage-clamp protocols similar to that used in A. Continuous fine lines: mean ± SE. A low-threshold inward current did not appear to be present in 7 cells. Note that some residual outward rectification contributed to the I-E relations in all cells, particularly at positive potentials, so that the apparent reversal potentials in this figure are misleading. Linear leakage currents have been subtracted from the measured current values in A and B.
M. D. BAKER AND H. BOSTOCK

FIG. 2. High-threshold persistent inward current is a Ca$^{2+}$ current. A: superfusion of 20 μM Cd$^{2+}$ selectively blocked the high-threshold persistent current. The whole cell quasi-steady-state I-E relation is given before (●) and after (○) the introduction of Cd$^{2+}$ into the superfuse. The I-E relation includes an inward current, operating over a more negative potential range, which is unaffected by the presence of extracellular Ca$^{2+}$. Insert: computed difference currents. B: peak high-threshold inward current is increased by raising extracellular Ca$^{2+}$ from 1.1 to 24 mM. Under these conditions, the current evoked by a step change in potential (from −110 to −10 mV) clearly inactivates (heavy trace). After 3 min, the current was smaller (light trace), as the evoked peak current declined over several min of recording. C: family of inward currents evoked by voltage-clamp steps to between 0 and −20 mV, from a prepulse potential of −110 mV, with 5 mM extracellular Ba$^{2+}$. Superfusion of 5 mM Ba$^{2+}$ enhanced the high-threshold inward current (see text). Records in B and C are leak and residual capacity transient subtracted. D: mean current values from final 10 ms of the traces in C, plotted against membrane potential; ordinate scale same as in C.

become permeable to Na$^+$ ions when the extracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) is low, i.e., <100 μM (e.g., Almers and McCleskey 1984). It was therefore important to rule out the possibility that the low-threshold current may represent Na$^+$ ion permeation through Ca$^{2+}$ channels. In the present series of experiments the extracellular [Ca$^{2+}$] was always ≥1.1 mM, and for this reason Na$^+$ ion permeation through Ca$^{2+}$ channels was considered unlikely. To provide a more definitive answer, we tested the effect of changing external [Ca$^{2+}$] on the low-threshold, persistent current and found that it was blocked by Ca$^{2+}$ over the millimolar concentration range (Fig. 3A). A positive shift in the voltage dependence of gating occurred when Ca$^{2+}$ was raised from 1.1 to 20 mM. The magnitude of this shift appeared to be close to 10 mV (as measured from the shift in activation threshold for the low-threshold component), and a 10-mV shift was allowed for in subsequent analysis. Converting the currents to permeabilities, the block of the low-threshold component by 20 mM Ca$^{2+}$ was 39% at −40 mV. We found that the degree of block attained by Ca$^{2+}$ ions depended inversely and steeply on the [Na$^+$] in the extracellular solution; in half normal [Na$^+$] (73 mM), 24 mM Ca$^{2+}$ produced complete block of the low-threshold component (Fig. 4B). The initial, control I-E relation is dominated by a low-threshold component (solid circles). The combination of raised Ca$^{2+}$ and low Na$^+$ blocked the low-threshold component, but enhanced the high-threshold component (open circles, open squares). In 105 mM Na$^+$, 20 mM Ca$^{2+}$ caused 80–90% block at the same potential (Fig. 4C, n ≈ 2 cells, all conditions). These results indicate that the channels underly-
FIG. 3. Low-threshold persistent inward current is a Na\(^+\) current. Difference currents, computed from quasi-steady-state current values before and after Na\(^+\) replacement by tetramethylammonium (TMA), are plotted against membrane potential (I-E plot) in A. Converting the difference current values to permeabilities and plotting against membrane potential gives a Na\(^+\) permeability–membrane potential (P-E) plot, B. The smooth line is predicted by a Boltzmann relation, calculated with the use of best-fit parameter values;
\[
P_{\text{max}} = 1.84 \times 10^{-11} \text{ cm}^2 \text{s}^{-1},
\]
\[
E_{1/2} = -50.3 \text{ mV},
\]
\[
g = 5.8 \text{ mV}.
\]
C: example of a quasi-steady-state I-E relation, for another cell, dominated by a large low-threshold component. These inward currents clearly resemble the difference currents in A. The high-threshold current in this cell was blocked by the continuous presence of 25 \(\mu\)M Cd\(^{2+}\). Probable small, residual outward rectification gives rise to an apparent reversal potential more negative than theoretical sodium reversal potential. Plotted current values are linear leak subtracted.

**Action of TTX on the low-threshold, persistent current**

Superfusion of TTX reversibly blocked both the transient Na\(^+\) current and the low-threshold, persistent current. Fig. 5A shows a family of membrane currents evoked by clamp steps from \(-110\) mV to \(-90\), \(-70\), and \(-50\) mV. At \(-50\) mV, a transient Na\(^+\) current was evoked, followed by a persistent inward current. Both the transient and persistent currents appeared to be fully blocked by 100 nM TTX. (Note that in this patch-clamp experiment, voltage control was lost during the large transient current because of an \(IR\) drop generated across the residual \(\text{R}_c\). This results in the inactivation rate for the transient current appearing to be inappropriately high for the command potential). Measurements of the degree of block brought about by superfusion of TTX over the concentration range of 2.5–100 nM (6 cells) imply that the maximum possible value of \(K_D\) is close to 10 nM (assuming that the concentration dependence of block may be described as a Langmuir adsorption isotherm). For two neurons, the \(K_D\)s estimated were 7.1 and 11.5 nM. The percentage block of quasi-steady-state inward current is plotted against the concentration of superfused TTX for the same two neurons in Fig. 5B. The smooth curve is a rectangular hyperbola (Eq. 3) where \(K_D = 8 \text{ nM}\).

The activation of the transient and persistent currents in this cell can be seen in more detail in Fig. 6A, which shows TTX-sensitive difference currents evoked by voltage steps from \(-110\) mV to between \(-90\) and \(-50\) mV in 5-mV increments. The lowest-threshold TTX-sensitive current persisted throughout the test step. At the most negative potentials giving rise to a perceptible inward current, the current was almost rectangular in waveform. This is consistent with non-inactivation over at least the initial part of the potential range of activation. The transient current dominated the responses to some of the more positive pulses. We suggest that the simplest interpretation of the currents presented is that transient Na\(^+\) current was recruited at around \(-55\) mV, and superimposed on a persistent inward current that activated at more negative potentials. The voltage dependence of the TTX-sensitive persistent current can be seen clearly in Fig. 6B. The persistent current activated close to \(-70\) mV, i.e., ~15 mV more negative than transient current (recorded both in this neuron and in others not generating the persistent current).

An example of a quasi-steady-state I-E relation in which TTX-sensitive difference currents are plotted is shown in Fig. 6C. The data were obtained from a different neuron than that in A and B. A comparison of the relation obtained for the TTX-sensitive current with that for the current suppressed by Na\(^+\) replacement (Fig. 3) suggests that in both
FIG. 4. Low-threshold persistent current is blocked by Ca\textsuperscript{2+} ions. A: quasi-steady-state I-E relations for a neuron in 1.1, 10, and 20 mM external Ca\textsuperscript{2+}. The control relation (1.1 mM) is dominated by a low-threshold component (●). Raising external Ca\textsuperscript{2+} to 10 and 20 mM (○, ○, respectively) blocked the low-threshold component progressively, whereas the high-threshold component was enhanced. Plotted current values are linear leak subtracted. B: block by Ca\textsuperscript{2+} is steeply Na\textsuperscript{+} dependent. Raising external Ca\textsuperscript{2+} from 1.1 to 24 mM blocks the low-threshold component completely when the external Na\textsuperscript{+} concentration (Na\textsuperscript{+}) is simultaneously lowered to 73 mM. Solid circles: initial control I-E relation. Open circles: raised Ca\textsuperscript{2+} and low Na\textsuperscript{+}. Solid squares: return to control conditions reverses block. Progressive block of outward rectification was evident during this experiment, so that the 2 sets of control data do not superimpose. Open squares: return to raised Ca\textsuperscript{2+} and low Na\textsuperscript{+}. Plotted current values are linear leak subtracted. C: plot of % block of persistent Na\textsuperscript{+} current by 20 mM (in 1 case 24 mM) Ca\textsuperscript{2+} vs. external Na\textsuperscript{+}. Smooth curve fitted by eye and of no theoretical significance.

types of experiment the same current was being affected. The mean current amplitudes are converted to permeabilities, assuming that the channels generating the persistent current were Na\textsuperscript{+} selective (Eq. 1), and the derived values are plotted in a P-E plot (Fig. 6D). A Boltzmann relation (Eq. 2), given by best-fit parameter values, is superimposed on the data points.

Whole cell quasi-steady-state current reversed polarity at membrane potentials more negative than the sodium or calcium reversal potentials, probably because residual outward rectification contributed to the current, particularly at positive potentials (e.g., Fig. 1). Block of the low-threshold persistent current by TTX invariably led to a shift in the reversal potential for whole cell steady-state current to less positive potentials, indicating that the reversal potential for the blocked current was more positive than that for the total current.

Voltage dependence of the persistent Na\textsuperscript{+} current

To quantify the voltage dependence of activation for the low-threshold, persistent current, differences in the steady membrane current (measured \(\approx 35\) ms after the clamp step) brought about by replacing extracellular Na\textsuperscript{+} with TMA, lowering extracellular [Na\textsuperscript{+}] while simultaneously raising extracellular [Ca\textsuperscript{2+}], and superfusing TTX were measured over a range of membrane potentials and converted to values of permeability (Eq. 1). In Fig. 7, normalized permeability values obtained from difference currents recorded in four experiments are pooled and presented as means ± SE. Two of the original data sets included in this analysis have already been presented in Figs. 3 and 6. Values of the best estimates for the midpoint and slope factor defining the fitted Boltzmann were: \(E_{1/2} = -49.0\) mV and \(a_g = 7.2\) mV. These values are not different from those presented by French et al. (1990) for a voltage-dependent persistent Na\textsuperscript{+} current in mammalian hippocampal neurons (\(E_{1/2} \approx -50\) mV, and \(a_g = 5\)–9 mV). French et al. (1990) utilized voltage-clamp protocols of similar durations to those used in the present experiments, but they treated the current as though it were generated by a conductance, rather than by a permeability. Differences in apparent voltage dependence arising through these two assumptions should be minimal over a negative voltage range but become more marked on approaching the sodium reversal potential.
Persistent Na\(^+\) current activation kinetics

Quantifying the activation kinetics of the persistent Na\(^+\) current is problematic, and especially so in large cells. Imperfect subtraction of residual capacity transients obscures the activation of the persistent Na\(^+\) current close to threshold (see below). Membrane charging on step depolarization is limited by the clamp time constant. This time constant also acts on the recorded currents as a low-pass filter, and in this way limits the apparent rate of gating, even where the \(R_C\) prediction facility, provided by the amplifier, can dramatically reduce the time taken to charge the membrane in response to a command step. In these neurons \(C_m\) is large and thus the compensated clamp time constant can be as high as 100 \(\mu\)s. Another problem affecting the analysis of current activation is that at most potentials the transient Na\(^+\) current superimposes on the persistent current and dominates the recordings. Because of these limitations, we have not attempted to fit the activation of the persistent Na\(^+\) current.

Difference currents already presented (Fig. 6A) indicate that activation kinetics for the persistent Na\(^+\) current, at potentials more negative than −55 mV, are fast. The shortest latency to maximum inward current in three cells, with good voltage control (residual somatic charging time constant 22–25 \(\mu\)s) was always <2.5 ms, where the membrane potential was stepped from −110 mV to between −70 and −50 mV in 5-mV increments. The presence of residual capacity transients prevented a more precise measure of the time taken for the current to activate. However, these data confirm that the persistent Na\(^+\) current activates quickly, with kinetics comparable with that of the transient Na\(^+\) current.

Persistent Na\(^+\) current sustained over minutes

The voltage-clamp protocols described for this study so far have been limited to 300 ms in duration. It is clear from the data already presented that at potentials more positive than the apparent activation threshold, the persistent Na\(^+\) current was sustained throughout such a protocol. A different type of experiment was undertaken to determine whether the persistent Na\(^+\) current was sustained over many minutes, or whether it underwent complete inactivation. In Fig. 8A are records of holding current for one cell in which the membrane was clamped at a constant potential over minutes. At the times indicated by the solid circles, the normal superfuse was switched to one containing 20 nM TTX. The membrane potential was held constant, at one of a range of potentials between −70 and 0 mV, for ≥1.5 min before superfusion of TTX began. TTX caused a repeatable and reversible reduction in the holding current that was consistent with the block of a sustained inward current. No hysteresis was found on returning to negative holding potentials. Similar effects were seen in three cells. Plotting the maximum fall in holding current against membrane potential gives the \(I-E\) relation shown in Fig. 8B.

These data indicate that a sustained TTX-sensitive current is active in this cell over a wider membrane potential range than would be expected for the “window current” generated...
FIG. 6. TTX-sensitive persistent current activates at more negative potentials than transient Na\(^+\) current. A: family of difference currents computed from recordings made before and after the superfusion of 100 nM TTX, same neuron as in Fig. 5: clamp steps from a prepulse potential of \(-110\) mV, to between \(-90\) and \(-50\) mV, in 5-mV increments. B: I-E plot for currents presented in A, where mean current values were calculated for the final 5 ms of the clamp step. C: TTX-sensitive difference currents from quasi-steady-state measurements of membrane current, in a different neuron, presented as an I-E plot. Concentration of TTX in superfusate was 2 µM. D: I-P plot for same neuron as in C, where the values of current have been converted to permeabilities, assuming the channels generating the current are Na\(^+\) selective. Smooth line is a Boltzmann relation given by best-fit parameter values; \(P_{\text{max}} = 2.46 \times 10^{-11}\) cm\(^3\) s\(^{-1}\), \(E_{1/2} = -57.0\) mV, \(a_g = 5.2\) mV.

DISCUSSION

We have provided evidence that many large DRG neurons from adult rats generate a persistent Na\(^+\) current that activates \(-15\) mV more negative than the transient Na\(^+\) current in the same cells. It is not known why the current is generated in only a fraction of the total population of large neurons. Although we have not recognized any morphological characteristics systematically related to the presence or absence of the current (or to its magnitude), it seems unlikely that the population could be functionally homogeneous, and differences in ion channel expression between cells might therefore be expected.

The TTX concentration dependence of block of the low-threshold persistent current implies a \(K_D\) within the single nanomolar range. This is within 1 order of magnitude of values estimated for the block of fast transient Na\(^+\) current in guinea pig spinal root ganglion neurons (Fukuda and Kameyama 1980) and for block of Na\(^+\) channels in rabbit C fibers (Colquhoun and Ritchie 1972), which are 3 nM and between 1 and 5 nM, respectively. The high sensitivity of the persistent current to TTX suggests that it is a Na\(^+\) current, rather than (for example) a Ca\(^{2+}\) current. In this respect, the persistent current differs from other nonclassical, kinetically slow Na\(^+\) currents generated by small DRG neurons. These latter currents are TTX insensitive. The persistent Na\(^+\) current is also blocked by external Ca\(^{2+}\) ions, a characteristic shared with transient Na\(^+\) currents (e.g., Woodhull 1973; Yamamoto et al. 1984). The apparent \(K_D\) for Ca\(^{2+}\) ion block of toxin-modified transient Na\(^+\) channels is \(~20–30\) mM at 0 mV (Worley et al. 1986; Yamamoto et al. 1984). Because the block of transient channels is voltage dependent, the binding site is interpreted to be \(~0.25\) times the width of the membrane electric field removed from the
outer membrane surface. Thus, at −40 mV, half-block could theoretically be attained by 9–14 mM. The propensity of Ca\(^{2+}\) ions to block the persistent current may not be different from this. We have found that the persistent current is blocked by Ca\(^{2+}\) ions in a Na\(^{+}\)-dependent manner. Reducing the [Na\(^{+}\)] from 140 to 73 mM dramatically enhances the block: 20 mM Ca\(^{2+}\) gives rise to 39% and 100% block in the two solutions, respectively. This steep dependence on [Na\(^{+}\)] may involve the selective screening of negative surface charge by Na\(^{+}\) ions, where unscreened charges act to increase the [Ca\(^{2+}\)] in the vicinity of the channels.

Channel type underlying the persistent Na\(^{+}\) current

Schwindt and Crill (1977) recorded a persistent inward current in 13 of 35 cat lumbar motoneurons in vivo, and the analogous current in trigeminal motoneurons was later demonstrated to be sensitive to TTX (Chandler et al. 1994), suggesting that it was a Na\(^{+}\) current. Persistent Na\(^{+}\) currents are generated by other central neurons (reviewed by Crill 1990), and also by ventricular myocytes (Saint et al. 1992). French et al. (1990) reasoned that the persistent Na\(^{+}\) current in hippocampal neurons could not be explained in terms of mh overlap for transient Na\(^{+}\) channels, because the known voltage dependence of the gating parameters for the transient current could not give rise to the observed persistent I-E relation. They also concluded that because the relative amounts of transient and persistent Na\(^{+}\) current were highly variable from cell to cell, the persistent current probably did not result from a minor fraction of the transient channels normally operating in a gating mode without inactivation. Thus they favored the possibility that the current was generated by another type of Na\(^{+}\) channel, distinct from the transient Na\(^{+}\) channel. In ventricular myocytes, the transient Na\(^{+}\) current is TTX insensitive, but the persistent Na\(^{+}\) current is TTX sensitive (Saint et al. 1992). This difference in sensitivity to block by TTX strongly implies that the amino acid sequence of the transient and persistent Na\(^{+}\) channels differ, at least at the TTX binding site, and that therefore the two channels must be different molecules.

Alternatively, Alzheimer et al. (1993) and Brown et al. (1994) suggest that at least some of the Na\(^{+}\) channels in cortical neurons must have fast, voltage-independent inactivation, described most fully for channels in neuroblastoma cells (Aldrich and Stevens 1987), and that such Na\(^{+}\) channels can, on occasion, normally operate in a gating mode without inactivation. These noninactivating channels would give rise to the persistent Na\(^{+}\) current. Such a view implies that transient Na\(^{+}\) currents and persistent Na\(^{+}\) currents are generated by the same channels.

Like French et al. (1990), we also discount the possibility that an mh overlap current can account for the persistent Na\(^{+}\) current in DRG neurons, because the predicted I-E relation clearly differs from the one observed in two respects. First, at potentials progressively more positive than that of apparent threshold, mh overlap is predicted to reach a peak and then decline. The decline is caused by the diminishing value of h. However, persistent Na\(^{+}\) current operates at membrane potentials at which inactivation of transient Na\(^{+}\) current is virtually complete (at −0 mV), and thus has a

**Fig. 8.** TTX-sensitive holding current over a wide range of constant membrane potentials. A: brief superfusion of 20 nM TTX caused reversible decreases in holding current over a wide range of membrane potentials. Membrane potential was held constant within the range of −70–0 mV for ±1.5 min before TTX was introduced into the superfusate. Solid circles: could not give rise to the observed persistent I-E plot. Some holding potentials have been corrected for residual series resistance (R\(_s\)) errors, where leakage currents were large.
different potential dependence from that predicted for \( mh \) overlap. Second, the current operates at membrane potentials more negative than the apparent activation threshold for transient \( \text{Na}^+ \) current [i.e., where the degree of steady-state activation (\( m^+ \)) is close to 0)], and can give rise to a significant current at \(-70 \text{ mV}\). We have also found a wide variation in the amplitude of the persistent current in DRG neurons. It is certain that many neurons generate transient \( \text{Na}^+ \) current but do not generate persistent \( \text{Na}^+ \) current. If the \( \text{Na}^+ \) channels in DRG neurons were of the neuroblastoma type, the kinetic characteristics of a current generated by channels operating in a nonactivating mode would be very different from those we have recorded. One would predict that the activation kinetics of the persistent \( \text{Na}^+ \) current at membrane potentials more negative than the activation threshold for the transient current would be very slow (time to peak \( >40 \text{ ms} \); Gonoi and Hille 1987), but in fact they appear to be as fast as transient currents. The results of other experiments on peripheral nerve \( \text{Na}^+ \) channels do not conform to those expected for the neuroblastoma type. Removal of inactivation by a variety of methods has not been reported to increase the peak amplitude of the \( \text{Na}^+ \) current at nodes of Ranvier (Neumcke et al. 1987). A similar situation is found for the \( \text{Na}^+ \) channels of skeletal muscle (e.g., Nonner et al. 1980). A dramatic increase in current amplitude on removal of inactivation is necessarily predicted for channels with fast, voltage-independent inactivation, and this discrepancy seems to imply that the mechanism of inactivation for the peripheral nerve \( \text{Na}^+ \) channel differs from that of the channel in neuroblastoma cells.

Our data are therefore most consistent with the presence of a \( \text{Na}^+ \) channel in about half of the large DRG neurons, different from the transient channel in terms of its activation voltage dependence and characteristics of inactivation. Whether these differences result from a primary amino acid sequence difference, or from some other mechanism, such as chemical modification of transient channels (e.g., oxidation) is unknown.

**Persistent \( \text{Na}^+ \) current in axons**

Dubois and Bergman (1975) reported a late, TTX-sensitive current in voltage clamped frog nodes of Ranvier that persisted for \( >140 \text{ ms} \), activated at more negative potentials than the transient current, and was larger in sensory than motor fibers. The low-threshold persistent \( \text{Na}^+ \) current we have recorded in rat DRG neurons corresponds well with the limited information available on this axonal late current. So far as we are aware, however, studies of sodium currents at mammalian nodes have not identified a comparable \( \text{Na}^+ \) current. Late openings of single \( \text{Na}^+ \) channels have been recorded from axon-attached patches of demyelinated rat spinal root axons by Mitrovic et al. (1993). They recorded characteristic motor/sensory differences that they related to the presence, in motor patches only, of a second, slower component of transient \( \text{Na}^+ \) current inactivation, which was removed by glutathione. The relationship of the late openings of Mitrovic et al. to the late TTX-sensitive current of Dubois and Bergman and our persistent \( \text{Na}^+ \) current is unclear, because the late openings would produce very little average current in sensory fibers, and their voltage dependence was not described.

Evidence for a low-threshold persistent current or “threshold current” in human peripheral nerve has come from an analysis of the different excitability properties of motor and sensory axons, as shown by their “strength-duration” and “latent addition” behavior (Bostock and Rotbwell 1995, 1997). Excitability at sensory and motor nodes of Ranvier, following brief (60 \text{ \mu s}) hyperpolarizing current pulses, returned to normal with different time courses. A slow, voltage-dependent component of recovery in sensory fibers implied that a kinetically fast, regenerative membrane current was operating at rest. Bostock and Rothwell (1997) were able to model their latent addition data by replacing a small fraction of transient \( \text{Na}^+ \) channels (more at sensory than at motor nodes) with persistent \( \text{Na}^+ \) channels, activating at 20 \text{ mV} more negative than the transient channels and at half the rate. (These characteristics were taken from a model of the persistent \( \text{Na}^+ \) current in cerebellar Purkinje cells, according to De Schutter and Bower 1994). The persistent \( \text{Na}^+ \) current we have found in DRG neurons corresponds closely to this inferred threshold current at nodes of Ranvier. Differences in the amount of persistent \( \text{Na}^+ \) current generated by sensory and motor axons could explain the marked differences in stimulus strength-duration characteristics between anatomically similar sensory and motor axons (e.g., Panizza et al. 1992). The persistent \( \text{Na}^+ \) current would also account for the lower rheobase estimated for sensory relative to motor axons with similar conduction velocities (Mogyoros et al. 1996). The strategic activation range of the persistent current means that any depolarizing influence (e.g., a generator current from a sensory nerve ending, or current applied from an external electrode), must activate the current, which then amplifies and prolongs the resulting depolarization.

This work was supported by a grant from the Wellcome Trust. Address for reprint requests: M. D. Baker, Sobell Dept. of Neurophysiology, Institute of Neurology, Queen Square, London WC1N 3BG, UK.

Received 30 September 1996; accepted in final form 2 December 1996.

**REFERENCES**


Brown, A. M., Schwindt, P. C., and Crill, W. E. Different voltage dependence of transient and persistent \( \text{Na}^+ \) currents is compatible with modal-


