British Journal of Pharmacology (2000) 129, 1617–1626
www.nature.com/bjp

Selective block of late Na^+ current by local anaesthetics in rat large sensory neurones

*,[1]Mark D. Baker

[1]Sobell department of Neurophysiology, Institute of Neurology, Queen Square, London WC1N 3BG

1 The actions of lignocaine and benzocaine on transient and late Na^+ current generated by large diameter ($\geqslant 50$ μm) adult rat dorsal root ganglion neurones, were studied using patch-clamp techniques.

2 Both drugs blocked whole-cell late Na^+ current in a concentration-dependent manner. At 200 ms following the onset of a clamp step from -110 to -40 mV, the apparent K for block of late Na^+ current by lignocaine was 57.8 ± 15 μM (mean $\pm$ s.e.mean, $n=4$). The value for benzocaine was 24.9 ± 3.3 μM, (mean $\pm$ s.e.mean, $n=3$).

3 The effect of lignocaine on transient current, in randomly selected neurones, appeared variable ($n=8$, half-block from ~ 50 to 400 μM). Half-block by benzocaine was not attained, but both whole-cell ($n=11$) and patch data suggested a high apparent K, >250 μM. Transient current always remained after late current was blocked.

4 The voltage-dependence of residual late current steady-state inactivation was not shifted by 20 μM benzocaine ($n=3$), whereas 200 μM benzocaine shifted the voltage-dependence of transient current steady-state inactivation by -18.7 ± 5.9 mV (mean $\pm$ s.e.mean, $n=4$).

5 In current-clamp, benzocaine (250 μM) could block subthreshold, voltage-dependent inward current, increasing the threshold for eliciting action potentials, without preventing their generation ($n=2$).

6 Block of late Na^+ current by systemic local anaesthetic may play a part in preventing ectopic impulse generation in sensory neurones.

British Journal of Pharmacology (2000) **129,** 1617–1626

Keywords: Patch-clamp; Na^+ channel; local anaesthetic; sensory neurone

Abbreviations: DRG, dorsal root ganglion; TTX, tetrodotoxin

Introduction

Many large diameter (>50 μM) dorsal root ganglion (DRG) neurones generate both transient and late Na^+ current. A portion of the late current fails to inactivate and gives rise to a steady-state or 'persistent' current (Baker & Bostock, 1997; 1998a). The late current is generated by Na^+ channels operating in at least three gating modes (*c.f.* Patlak & Ortiz, 1986; Alzheimer *et al*., 1993), characterized by two forms of burst opening and ultra-brief, sporadic openings (Baker & Bostock, 1998a). Late channel gating mode is probably pH sensitive, acidification promoting behaviours leading to inactivation and reducing the number of late openings (Baker & Bostock, 1999a). Late current activates about 20 mV more negative than transient current, and can operate at, or close to, the resting membrane potential. Because the current activates rapidly, and is regenerative, it has been proposed to play a key role in controlling membrane excitability. Whether the transient and late components of Na^+ current are generated by one sub-type of Na^+ channel with a wide repertoire of gating modes, or by more than one sub-type is not known. There is good evidence that late Na^+ current in cerebellar Purkinje neurones is generated by a different molecular sub-type from that which generates the transient Na^+ current (Raman *et al*., 1997; de Miera *et al*., 1997). The late and transient current are hypothesised to be generated by homologues of *Scn8a* (NaCh6) and rat brain type I, respectively (de Miera *et al*., 1997). Large sensory neurones also manufacture the mRNA for both of these channels (Black *et al*., 1996) and by analogy this could allow a similar delegation of function.

One potentially useful approach to characterizing late current and investigating its function, would be to attempt to find a selective blocker. For this reason the effects of local anaesthetics were investigated, as they are known to bring about their useful effects by blocking voltage-gated Na^+ channels. The mechanism of channel block in axonal preparations has been studied in detail; a charged local anaesthetic molecule requires an open channel to access its binding site, and once bound, stabilizes or promotes channel inactivation (Strichartz, 1973; Hille, 1977; reviewed by Hille, 1992; Scheuer, 1999). There is a wide range of sensitivity to local anaesthetics among Na^+ currents. Some sub-types of Na^+ channel are particularly resistant to block by local anaesthetics. Pharmacological characterization of the tetrodotoxin (TTX) -resistant channel, SNS, by Akopian *et al*. (1996) revealed that 1 mM lignocaine reduced the peak current by less than 50%. In contrast, the TTX-sensitive transient currents recorded for some dorsal root ganglion (DRG) neurones are half blocked by 40–50 μM lignocaine during similar protocols (Roy & Narahashi, 1992; Scholz *et al*., 1998). Intriguingly, these currents are much more sensitive to the drug than reported for currents recorded at voltage-clamped nodes of Ranvier (reviewed by Hille, 1992) or CA1 pyramidal neurones (Kaneda *et al*., 1989), and this difference seems surprising given the functional similarity of the currents. One obvious possibility is that molecular differences between channel sub-types account for at least a part of the difference in sensitivity.

*Author for correspondence; E-mail: mbaker@ion.ucl.ac.uk

A second possibility is that because drug binding depends upon the channel state, different channel gating behaviours might alter the propensity with which channels are blocked, by changing the availability of a preferred state or states. For example, block could depend upon channel open-time (Ju *et al*., 1992).

Given that differences in molecular structure or gating behaviour might be expected to affect drug-channel interaction, the present experiments were conducted to ascertain whether local anaesthetics can discriminate between transient and late Na^+ currents, already known to be similarly sensitive to TTX (Baker & Bostock, 1997), and in so doing help to define the physiological role of the late Na^+ current.

Methods

Cell culture

Primary cultures of neurones were prepared from lumbar dorsal root ganglia of male Wistar rats (100–300 g), using a standard technique described elsewhere (Baker & Bostock, 1997). Dissociated neurones were plated upon poly-L-lysine (Sigma, Poole, Dorset, U.K.) coated coverslips, previously seeded with Schwann cells and fibroblasts obtained from dissociated spinal roots, in the culture wells of 12-well plates (Falcon, Becton Dickson & Co., NJ, U.S.A.). The neurones were kept in a 37°C incubator with a 5% CO_2 atmosphere for up to 3 days.

Electrophysiology

Coverslips with adherent neurones were placed within a 35 mm plastic Petri dish, which formed the recording chamber, and mounted on the stage of an inverted microscope. Experiments were carried out on neurones $>50\ \mu$m in apparent diameter. For voltage-clamp, the normal solutions contained the following (in mM): extracellular, Na-gluconate (135.6), K-gluconate (1), Na-HEPES (4.54), HEPES (5.46), Ca-gluconate$_2$ (1.1), Mg-gluconate$_2$ (1.2), 4-aminopyridine (4-AP) (5), CsCl (10), tetraethylammonium (TEA) Cl (10). 4-AP was neutralized by the addition of gluconic acid; intracellular, CsCl (143), (Na) EGTA (3), Na-HEPES (6.04), HEPES (3.96), $CaCl_2$ (1.21), $MgCl_2$ (1.21). In some experiments internal CsCl was replaced by CsF, and when external Na^+ was reduced to 20 mM in order to control the large transient currents, external Na-gluconate was equimolarly replaced by Tetramethylammonium (TMA)-gluconate. For current clamp, the normal solutions contained the following (in mM):- extracellular, NaCl (145.6), Na-HEPES (4.54), HEPES (5.46), $CaCl_2$ (1.1), $MgCl_2$ (1.2), KCl (2.5); intracellular, KCl (143), (Na) EGTA (3), Na-HEPES (6.04), HEPES (3.96), $CaCl_2$ (1.21), and $MgCl_2$ (1.21). The pH of solutions was 7.2–7.3. Although the voltage-clamp solutions were designed to block K^+ currents, maximal block usually took many minutes to occur in whole-cell recordings. No attempt was made to block high-threshold voltage activated Ca^{2+} currents normally generated by the neurones. These currents did not contribute to those recorded at -40 mV or more negative, and late Na^+ currents at more positive potentials (recorded during h_∞ protocols) were recorded as TTX-sensitive difference currents. Superfusion of solutions was gravity driven, and all experiments were carried out at room temperature (20–22°C). Lignocaine (lidocaine) and benzocaine were obtained from Sigma (Poole, Dorset, U.K.). Benzocaine was made up as a 200 mM stock solution in ethanol, and the vehicle was usually not more than 0.1% of the experimental solution volume, commonly much less. Higher concentrations of ethanol alone had small effects on transient Na^+ current amplitude.

Recordings were made using an Axopatch 200 amplifier (Axon Instruments, Foster City, CA, U.S.A.) with a CV202 headstage, and were filtered using the 4-pole Bessel filter on the amplifier; -3 dB at 2 KHz (whole-cell recordings) or 5 KHz (membrane patch recordings). All electrodes were fabricated using thin wall glass capillaries (GC150TF-15, Clark Electromedical, Pangbourne, U.K.), coated with Sylgard (Dow Corning) and fire-polished. Their initial resistance once filled with recording solution was usually between 2 and 3 MΩ. The whole-cell capacity current transient generated on an imposed step in potential was routinely cancelled using the simple resistance-capacitance (*RC*) circuit within the amplifier. The charging-time-constant prediction facility of the Axopatch amplifier was always used, allowing rapid charging of the cell membrane capacity. In this way, the effective time constant for charging the membrane in response to an imposed step was reduced by up to 90%. Estimated R_s values at the start of whole-cell recording sessions fell within the range 1.8–5.8 MΩ ($n=34$). Feedback series resistance (R_s) compensation was also always used, and was set to at least 70%. When using external gluconate solutions, the junction potential between the bath and the KCl bridge was allowed for by offsetting the holding potential by -10 mV (e.g. Baker & Bostock, 1997). Control of command pulse protocols and data collection was carried out by a Dell PC running pClamp version 6 (Axon Instruments).

The voltage-dependent activation of Na^+ channels in outside-out patches occurred at a more negative potential than that in the whole-cell, as previously reported (Baker & Bostock, 1998a). One possible reason for an apparent potential shift is a Donnan equilibrium potential that exists upon attaining the whole-cell recording configuration (e.g. Marty & Neher, 1983). This potential is largest upon initially attaining the whole-cell configuration (perhaps amounting to 10 mV). However, in the present experiments, the intracellular contents of the neurones equilibrated with the pipette solution over several 10s of minutes before recording began (to allow outward rectification to be blocked maximally), and it therefore seems unlikely that this effect could account for anything but a small fraction of the shift in the potential-dependence of channel gating. Activation of both late and transient current occurred at potentials 20 mV more negative than in whole-cell (in the presence of the same solutions), and data on steady-state transient current inactivation in patches indicates that the voltage-dependence was shifted even more substantially in the negative direction, by up to 40 mV. The mechanism was not investigated, but it was important to take the effect into account because unless the holding potential was sufficiently negative (to remove inactivation), then the transient current in patches appeared more sensitive to local anaesthetic than it did in the whole-cell, and the discrimination between transient and late current was less marked.

Voltage-clamp protocols

Whole-cell late currents were commonly recorded using voltage-clamp steps from a negative pre-pulse potential (-110 mV) to a range of membrane potentials in 5 or 10 mV increments. Families of membrane currents were elicited by long duration clamp steps (e.g. 200 ms). Whole-cell leakage and residual capacity currents were usually removed by subtracting appropriately scaled currents evoked by reversed polarity clamp steps. During particularly stable experiments, it was possible to record sufficient data to subsequently compute

'difference currents' from families of currents recorded before and after the superfusion of TTX, benzocaine or lignocaine. In membrane patches, leakage current and residual capacity transients generated in response to imposed potential steps were subtracted either by constructing records from traces without channel activity, or by scaling responses to small, negative going clamp steps. Voltage-clamp protocol repeat frequencies did not usually exceed 1 Hz, and no significant frequency-dependent block using lignocaine was evident. In one experiment using benzocaine, the protocol repeat frequency was 2 Hz.

Data analysis

Plots of the value of steady-state current availability (h_∞) for late Na^+ current (measured after 2 s duration pre-pulses) *versus* membrane potential (E_m) were fitted with a Boltzmann relation of the form:

$$h_\infty = 1 - (A/\{1 + \exp[(E_{1/2} - E_m)/a_h]\}) \quad (1)$$

using a least-squares procedure (SigmaPlot, Jandel); where A is the maximum degree of inactivation, $E_{1/2}$ is the membrane potential at which inactivation is half maximal, a_h is the steepness parameter.

Late current amplitudes were measured as the mean current over 10 ms, at the end of a long duration voltage-clamp step, and the value of current (I) was converted to a permeability (P) according to the Goldman-Hodgkin-Katz constant-field current equation for a monovalent cation:

$$P = I/((E_m F^2/RT)\{[c_{in} - c_{out}\exp(-FE_m/RT)]/[1 - \exp(-FE_m/RT)]\}) \quad (2)$$

where F is Faraday's constant; R is the gas constant; T is the absolute temperature; and c_{in} and c_{out} are the concentrations of Na^+ in the internal and external solutions, respectively. E_m is as previously defined. Plots of Na^+ permeability versus membrane potential were fitted by Boltzmann relations of the form:

$$P = P_{max}/\{1 + \exp[(E_{1/2} - E_m)/a_p]\} \quad (3)$$

where P_{max} is the limiting maximum permeability, $E_{1/2}$ is the membrane potential at 0.5 P_{max}, and a_p is the steepness parameter.

Values of apparent equilibrium dissociation constant, K, were determined by fitting single rectangular hyperbolae to current amplitude values, measured in the presence of a range of drug concentrations. For late current amplitude values the fit asymptote was not constrained to zero, which allowed for the presence of residual outward rectification.

Wherever possible, experimentally determined values are quoted as mean ± s.e.mean.

Results

The actions of lignocaine

Transient and late inward currents were evoked by voltage-clamp steps from a negative pre-pulse potential (−110 mV). Superfusion of lignocaine (500 μM) reversibly abolished the late current while the transient current, although slightly reduced in amplitude, was clearly still present (Figure 1a and inset). The data are shown without leakage subtraction, in order to demonstrate clearly that the changes in late current cannot be attributed to changes in input resistance. The block of late current by lignocaine can exhibit time-dependence (Baker & Bostock, 1998b). When subject to a sufficiently large depolarizing step, late current normally declines, because it is subject to a voltage-dependent, slow inactivation (Baker & Bostock, 1998a). Superfusion of lignocaine increased the rate of decline, and this effect was clearest at potentials close to the threshold for activating the current, where the degree of native inactivation is small (Figure 1b). The amplitude of the current blocked at a latency of 190–200 ms, in the same neurone, exhibited a voltage-dependence similar to that previously reported for late Na^+ current (Figure 1c). The sensitivity of the transient current was variable. Attempts were made to control the current in randomly selected cells by reducing extracellular Na^+ (see Methods) or recording in a nonsaturating concentration of TTX (15 nM). The results indicated that half-block could occur over a wide range of concentrations (50–400 μM; $n=8$), and that transient current can still be present on exposure to 900 μM lignocaine (data not shown). In other experiments, where block of late current was studied, the transient current sensitivity implied half-block at the upper end of this range, as reported for transient Na^+ current in hippocampal pyramidal neurones (Kaneda *et al*., 1989). The wide range of sensitivities reported here might partly be explained by variable rates of transient channel activation, which depends upon the quality of the voltage-clamp. Because of the difficulties involved in making good recordings of large transient Na^+ currents, in cells with a considerable capacity (>100 pF), other possible sources of variability were not investigated. Determining the late Na^+ current amplitude at −40 mV as a fraction of the total current subsequently abolished by the superfusion of 100 nM TTX, or making allowance for the presence of residual outward rectification in the analysis, allowed the estimate of K for block of late Na^+ current as 57.8 ± 15 μM, mean ± s.e.mean, $n=4$ (e.g. Figure 1d).

The actions of benzocaine

Benzocaine appeared even better at discriminating between transient and late Na^+ current than lignocaine (Figure 2a). Benzocaine was more effective than lignocaine at blocking late current ($K = 24.9 \pm 3.3$ μM at −40 mV, mean ± s.e.mean, $n=3$), and less effective at blocking transient current. A high concentration of the drug (250 μM) seemed to be equally good at blocking late current over its entire activation range (Figure 2b,c). The properties of the Boltzmann curve (Figure 2c), drawn according to best-fit parameters through derived permeability values, are similar to those previously published for activation of late Na^+ current isolated by other means (Baker & Bostock, 1997) and this suggests that block does not exhibit substantial voltage-dependence. The apparent K for block of late current, shown in Figure 2d, was not different at −60 mV (19.2 and 23.8 μM at −40 and −60 mV, respectively), a potential nearer the activation threshold for late current, and below the normal activation threshold for transient current in these cells. Data from whole-cell experiments ($n=11$) suggested a consistently high apparent K for block of transient current (>250 μM). Excised patch data indicates that with a pre-pulse potential sufficient to remove inactivation, the value of K may actually exceed 700 μM (see below).

Local anaesthetics have been proposed to stabilize or favour fast inactivation of transient Na^+ currents (e.g. Hille, 1977, Scheuer, 1999), although the drug may actually associate with a state on the activation pathway rather than the inactivated

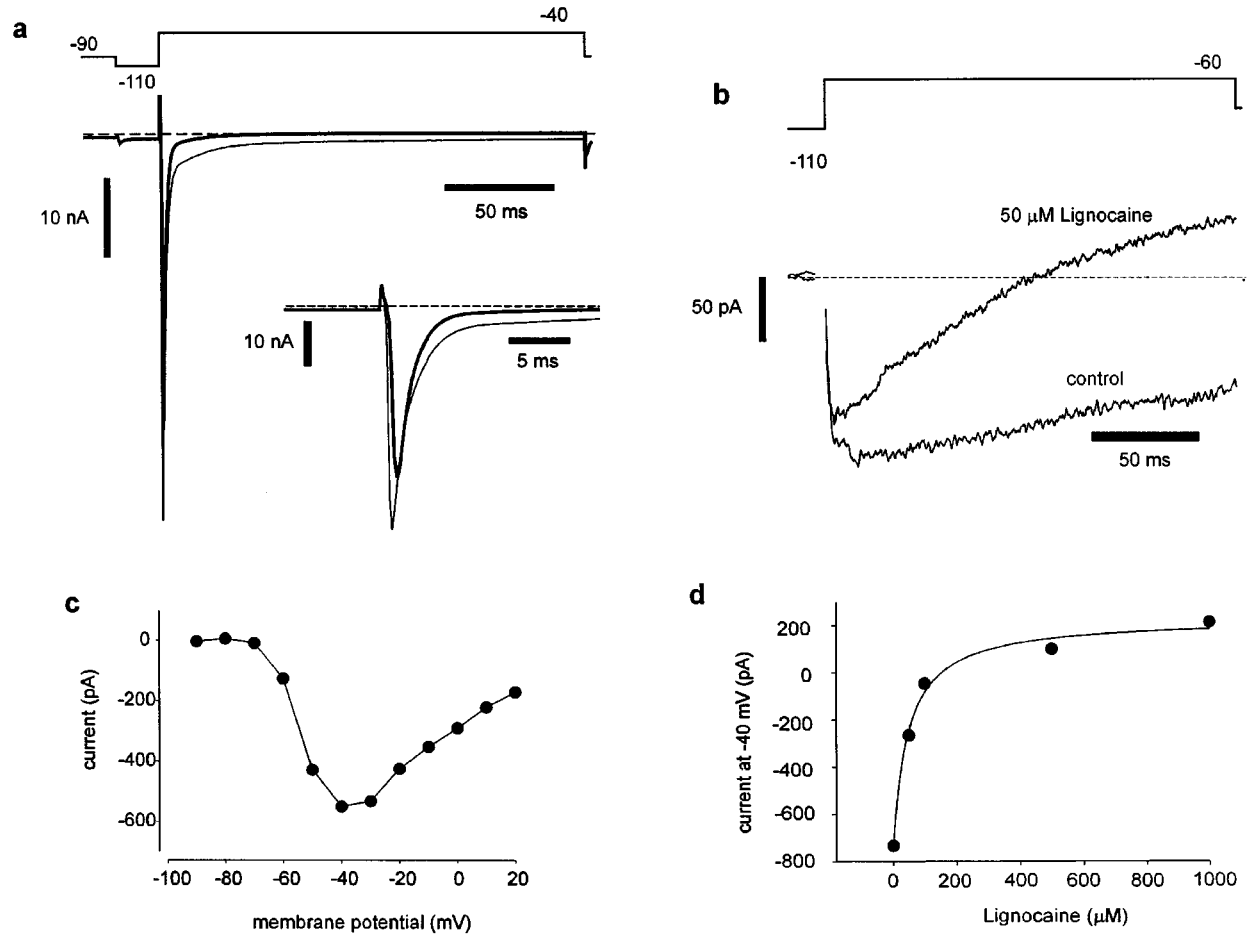

Figure 1 Selective block of late Na^+ current by lignocaine. (a) Transient and late Na^+ currents evoked in response to 200 ms duration clamp steps to −40 mV in the absence (light trace) and presence (heavy trace) of 500 μM lignocaine. No residual capacity or leakage current subtraction. Zero current is represented by a broken line. Inset: (same records) most transient current remains in the presence of drug. (b) Lignocaine (50 μM) can cause a decay in the late current, at a potential more negative than that at which transient Na^+ current activates. In the presence of drug, current becomes outward during the clamp step due to residual outward-rectification. (c) Current-membrane potential plot for currents blocked by the superfusion of 50 μM lignocaine, at equilibrium. Lignocaine sensitive current operates over same potential range as late Na^+ current. Same cell as in (b). (d) Concentration-dependence of block in another neurone at −40 mV and at 200 ms is well described by a single rectangular hyperbola (fit allows for residual outward rectification). Apparent value of K is 47.8 μM.

state (Vedantham & Cannon, 1999). However, exposure to a near 50% blocking concentration of benzocaine (20 μM) did not shift the residual late current h_∞ relation in the hyperpolarizing direction, which would be predicted for favoured inactivation. Rather, it reduced the slope of the relation, suggesting that drug binding does not promote inactivation of late current, and may slightly retard it (Figure 2e). The data presented were derived from TTX-sensitive records, and are compared with a Boltzmann relation previously presented as a best-fit to control channel availability data (Baker & Bostock, 1998a).

Benzocaine (300 μM) blocked late sodium currents in an outside-out patch exhibiting both transient and late openings, while transient current amplitude was only slightly reduced (Figure 3a). It is difficult to be certain from whole-cell recordings whether late current is blocked before it activates, or whether there is rapid block upon activation, during the residual capacity current. However, block of late current in a second membrane patch was present from very early in the clamp-step (i.e. 1–2 ms), consistent with block of closed channels (Figure 3b and inset). Benzocaine (25 μM) block of macroscopic late current at near threshold potentials, was also present from early in the clamp-step (data not shown). The ensemble-mean current blocked by benzocaine exhibits the slow, partial inactivation previously reported for late current (Baker & Bostock, 1998a). The effect of benzocaine upon individual traces suggests that the drug may block late current by reducing the number of late openings. In a third patch, exhibiting channels giving rise to brief, sporadic openings (and therefore more nearly conforming to an ideal where openings are homogeneous and do not overlap), it was possible to count the number of openings occurring over 100 ms (Figure 3c), according to a simple (5pS) threshold-crossing rule. Benzocaine (200 μM) dramatically and reversibly reduced the number of openings, suggesting that an important element of its action is an interaction with late channels in non-conducting states, which could include closed-states.

Benzocaine shifts the voltage-dependence of transient Na^+ channel inactivation

In patches, the maximal transient current evoked by a sequence of incrementing clamp-steps was blocked by ~20% by 200 μM benzocaine ($n=2$). In these experiments, the pre-

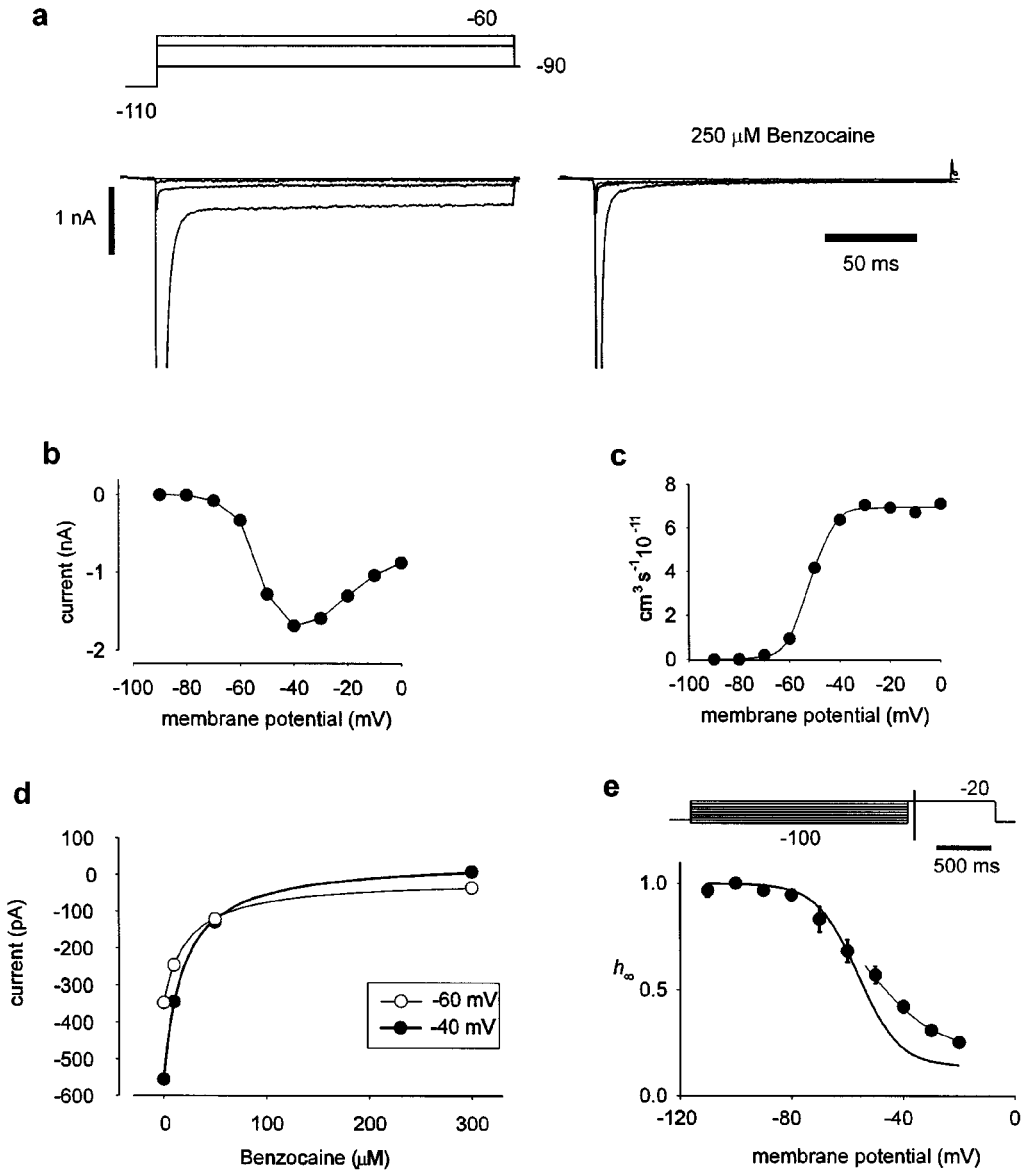

Figure 2 Characteristics of current blocked by low concentrations of benzocaine. (a) Late and transient Na^+ currents evoked by clamp steps up to -60 mV (left hand traces). Late current abolished after superfusion of 250 μM benzocaine (currents subsequently recorded in the presence of 200 nM TTX subtracted from original records). (b) Current-membrane potential plot for benzocaine (250 μM) sensitive component, measured at the end of 200 ms duration voltage-clamp steps, for the same neurone as in (a). Permeability-membrane potential plot (c), derived from the values of current in (b). Smooth curve is a Boltzmann relation, where, $P_{max}=6.96\ 10^{-11}$ cm^3 s^{-1}, $E_{1/2}=-51.8$ mV and $a_p=4.6$ mV. (d) Similar values of K found for block of late current at -40 mV (where the current is maximal), and at -60 mV, at the end of 200 ms duration voltage-clamp steps: 19.2 and 23.8 μM respectively. (e) Effect of 20 μM benzocaine upon steady-state current availability (h_∞) after 2 s pre-pulses. TTX (200 nM)-sensitive currents measured in three neurones between 60 and 70 ms after a clamp step to -20 mV (as indicated). Main intracellular anion was Cl^- rather than F^-. Heavy curve is the Boltzmann relation with best-fit parameters for mean control data. Data obtained in the presence of the drug diverge from control values at potentials more positive than -60 mV. Parameters for fit to control data are: $E_{1/2}=-56.2$ mV, $a_h=7.4$ mV and $A=0.86$. Corresponding values for data in the presence of drug are, -53.1 mV, 12.5 mV and 0.79, respectively, but only a part of the curve is shown.

pulse potential was held sufficiently negative (-140 mV) to remove control steady-state inactivation. Thus the transient current, when activated from a negative holding potential, may require more than 700 μM benzocaine to produce 50% block. However, the effectiveness of benzocaine as a blocker of transient Na^+ current in both whole-cell or membrane patch recordings was clearly affected by the holding or pre-pulse potential. For this reason, the effect of 200 μM benzocaine upon the voltage-dependence of steady-state fast inactivation was investigated in other patches using a range of pre-pulse potentials (Figure 4a). Block of the maximal transient current when preceded by the most negative pre-pulse (to -150 mV) was by $18.5\pm1.9\%$ (mean$\pm$s.e.mean, $n=4$); data obtained from one neurone is shown in Figure 4a,b. The drug shifted the voltage-dependence of inactivation by -18.7 ± 5.9 mV (mean$\pm$s.e.mean, $n=4$), Figure 4c.

Functional consequences of selective block of late Na^+ current

In a neurone with a sufficiently negative resting membrane potential, benzocaine spares the transient current (and hence the action potential), while blocking the late current operating at sub-threshold potentials (Figure 5a) (Baker & Bostock, 1999b). This discrimination probably occurs for the following reasons. With a membrane potential near -70 mV there

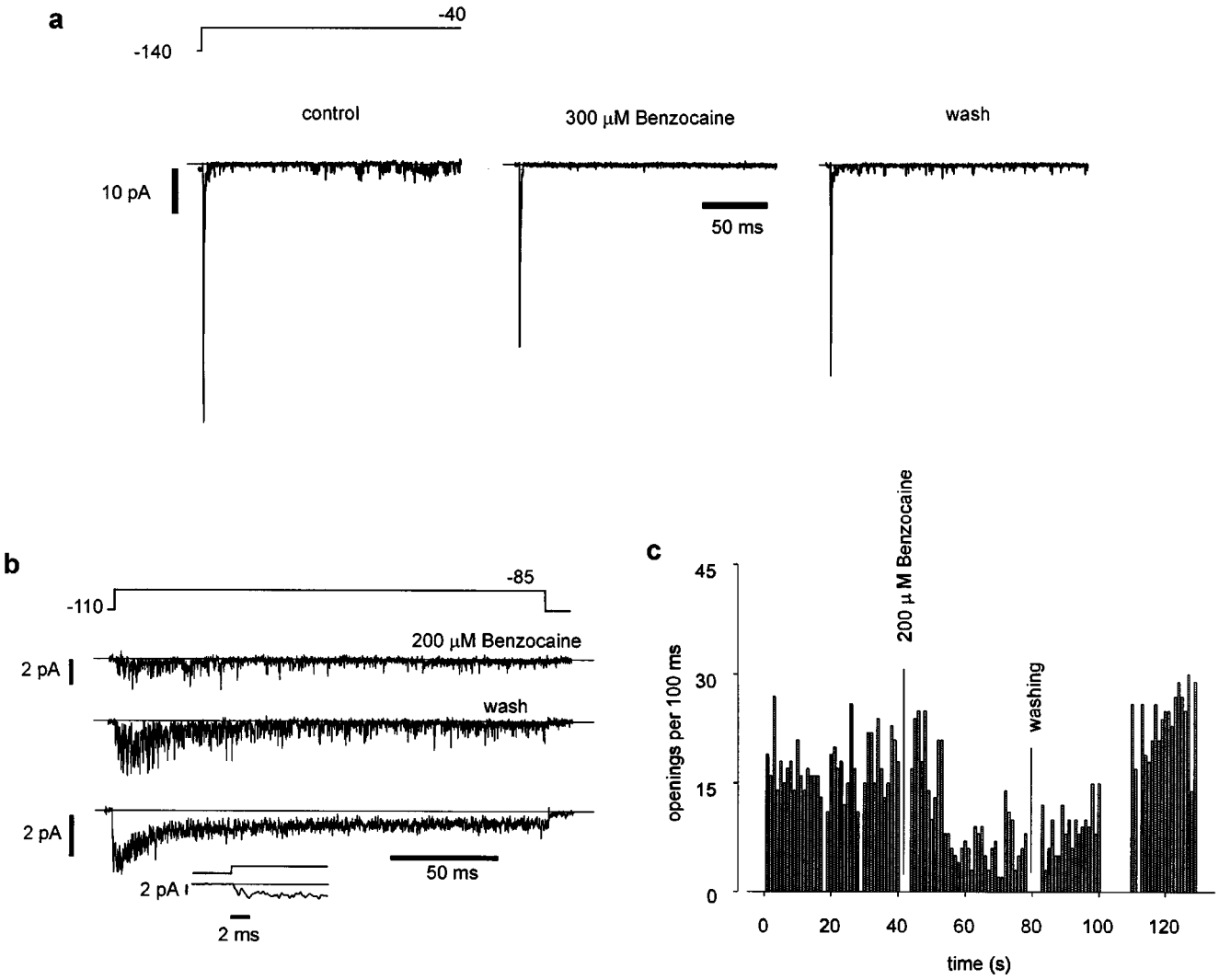

Figure 3 Selective block of late current in outside-out patches by benzocaine. (a) Benzocaine (300 μM) reversibly eliminated late channel openings, while transient current amplitude was slightly reduced in the same patch. (b) Benzocaine (200 μM) reversibly blocked late channel currents in another patch, throughout 200 ms duration clamp steps at potentials more negative than threshold for transient current (upper two single example traces). Lower trace and inset, ensemble-mean difference current between recordings in the absence and presence of drug (51 traces with drug, 31 traces during wash). Substantial current block apparent at 1–2 ms following the onset of the clamp step (inset). (c) Benzocaine (200 μM) reversibly reduced the number of late openings in a third patch. Channel openings (sufficient to cross a 5 pS threshold) recorded during clamp steps to −40 mV, counted between 60 and 160 ms following the clamp step onset. The channel openings appeared homogeneous and of the brief, sporadic type. Gaps appear in the histogram either where noise prevented analysis, or between recording sessions.

should be ~70% transient channel availability. The availability is high because in the whole-cell configuration, the voltage-dependence of control transient current inactivation appears to be up to 40 mV more positive than that found in patches (see Methods, data not shown). As a result, more than 250 μM benzocaine is required to half-block the transient Na^+ current. Action potential latency is short, and transient channel inactivation kinetics near the observed resting potential are relatively slow, so that inactivation will not increase very much before the transient channels activate. In comparison, superfusion of 100 nM TTX, a concentration of the toxin that would cause a similar degree of block of late current as 250 μM benzocaine, prevents action potential generation and anaesthetizes the neurone ($n=2$).

Because late Na^+ current activates at more negative potentials than transient current, it is hypothesized to contribute to sub-threshold depolarizing events, amplifying and prolonging them. The present experiments provide evidence that in large sensory neurones late Na^+ current can indeed amplify sub-threshold depolarizations (Figure 5b), but because this action is curtailed by stronger activation of fast delayed rectification, the extra depolarization is not prolonged. Thus, in the presence of fast K^+ channels, late Na^+ current would be expected to be most effective at amplifying depolarizations with a rapid rate of rise. The reversible effects of 250 μM benzocaine and the effect of 100 nM TTX on sub-threshold membrane potential changes were compared in the same neurone as that in Figure 5b, and found to be similar. Unfortunately, because benzocaine is not perfectly selective, it is not possible to assign all the increase in threshold to block of late current alone. However, a reasonable expectation is that lower concentrations of benzocaine (e.g. 25–50 μM) would lower excitability in large sensory neurones almost exclusively through an action on late current.

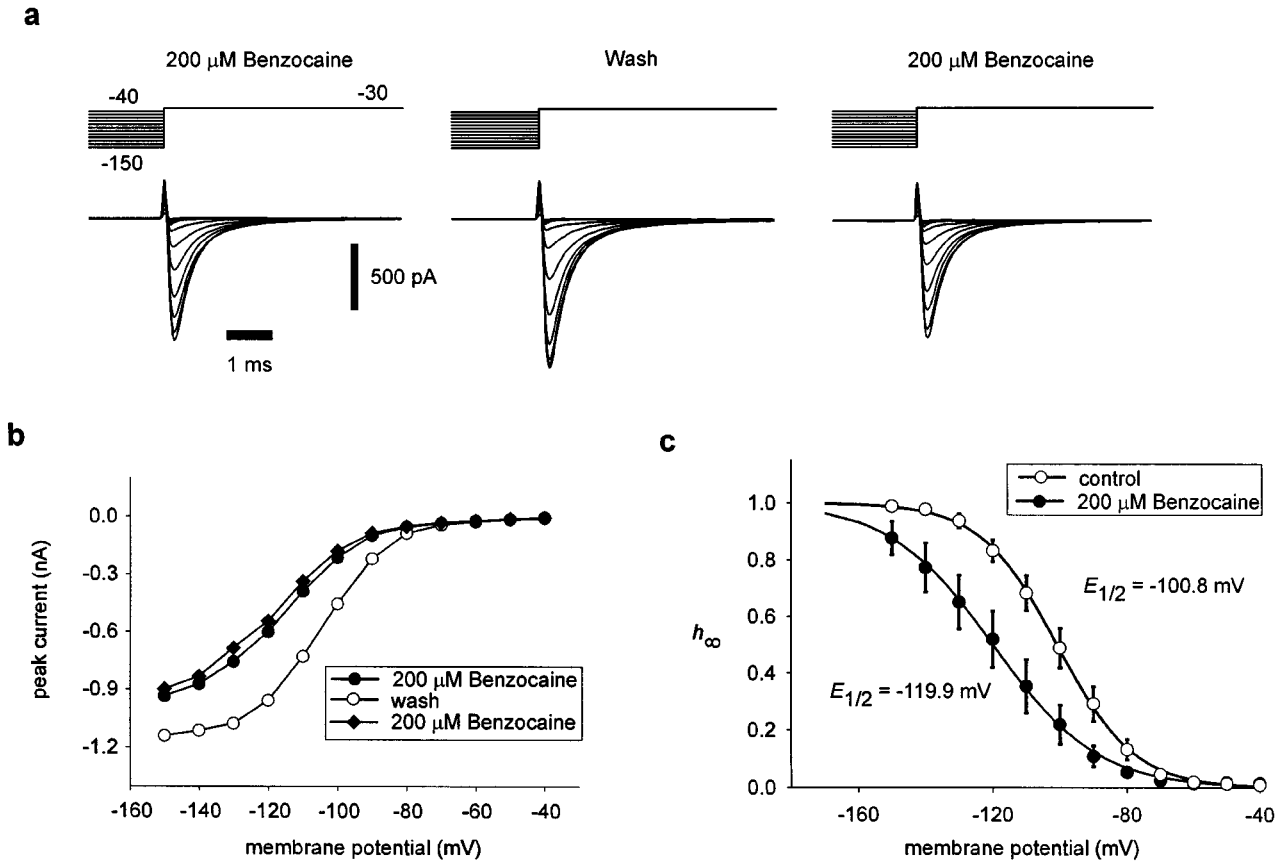

Figure 4 Benzocaine block of transient Na^+ current in membrane patches is achieved by promoting inactivation (a) Sequentially recorded families of transient Na^+ currents evoked by a clamp-step to -30 mV, following a 50 ms pre-pulse to a range of membrane potentials. Currents were recorded in the presence of 200 μM benzocaine (left hand panel), during wash (centre panel), and after re-exposure to the drug (right hand panel). (b) Potential-dependence of steady-state Na^+ current availability (h_∞), measured from the peak amplitudes of the currents shown in (a). Benzocaine reduces the maximal transient current (extrapolated values from Boltzmann fits) by 14–16%. From raw data, where pre-pulse is -150 mV, block was $<21\%$. (c) Mean derived h_∞ relations from data obtained in four patches including that in (b). Benzocaine (200 μM) shifts $E_{1/2}$ for steady-state inactivation by -19 mV. Smooth curves are Boltzmann relations, drawn according to best-fit parameters. $E_{1/2}$ and $a_h = -100.8$ and 11.4 mV, and -119.9 and 15.3 mV, for control and drug values, respectively.

Discussion

I have presented evidence showing that low concentrations of lignocaine and benzocaine can act selectively upon late Na^+ current in large dorsal root ganglion neurones. Both agents exhibited a similar preference for late current, although benzocaine appeared to be more selective, at least in voltage-clamp. Lignocaine block of late current could exhibit time-dependence, which was not observed for block by benzocaine. These findings, though novel for peripheral neurones, are consistent with other recently published findings for central neurones. Pharmacological studies of late Na^+ currents in pyramidal neurones indicate that they can also be targeted preferentially by local anaesthetics and anti-convulsants. (Hammerström & Gage, 1998; Lampl *et al*., 1998). A subtle though potentially important aspect of drug action on Na^+ channels is revealed by these experiments, and they provide further evidence for the non-homogeneous nature of Na^+ current in mammalian neurones.

Possible mechanisms of selective block

Characterisation of Na^+ currents in large sensory neurones has revealed that the contributing channels, although all sensitive to TTX, are not functionally uniform. Most, if not all of the late and steady-state current recorded, appears to be generated by a minor fraction of the total population of Na^+ channels, and these channels undergo reopening (Baker & Bostock, 1997). One simple explanation for the accumulation of block by a low concentration of lignocaine, is that there is an open channel requirement for access to the binding site, and block proceeds slowly because channel open-probability is low and channel openings are brief. Time dependent block of inactivation-deficient mutant and chemically modified Na^+ channels has been reported (e.g. Balser *et al*., 1996; Vedantham & Cannon, 1999), which appears similar to the action of lignocaine reported here. The current decay induced by exposure to lignocaine could actually be due to an allosteric effect, accelerating the slow inactivation (*c.f.* Balser *et al*., 1996), rather than giving rise to an open-channel block. However, in large sensory neurones, favoured inactivation of the late current seems improbable, as strong enhancement of late current inactivation (with a shift in the voltage-dependence of the h_∞ relation) does not occur on exposure to benzocaine.

Closed transient channels are resistant to block by local anaesthetic, so that significant block of transient channels can only take place when channels have been activated by depolarization. This is a result in agreement with descriptions of the action of local anaesthetics on voltage-clamped nodes of Ranvier, and in cardiac muscle (Hille, 1977; Bean *et al*., 1983).

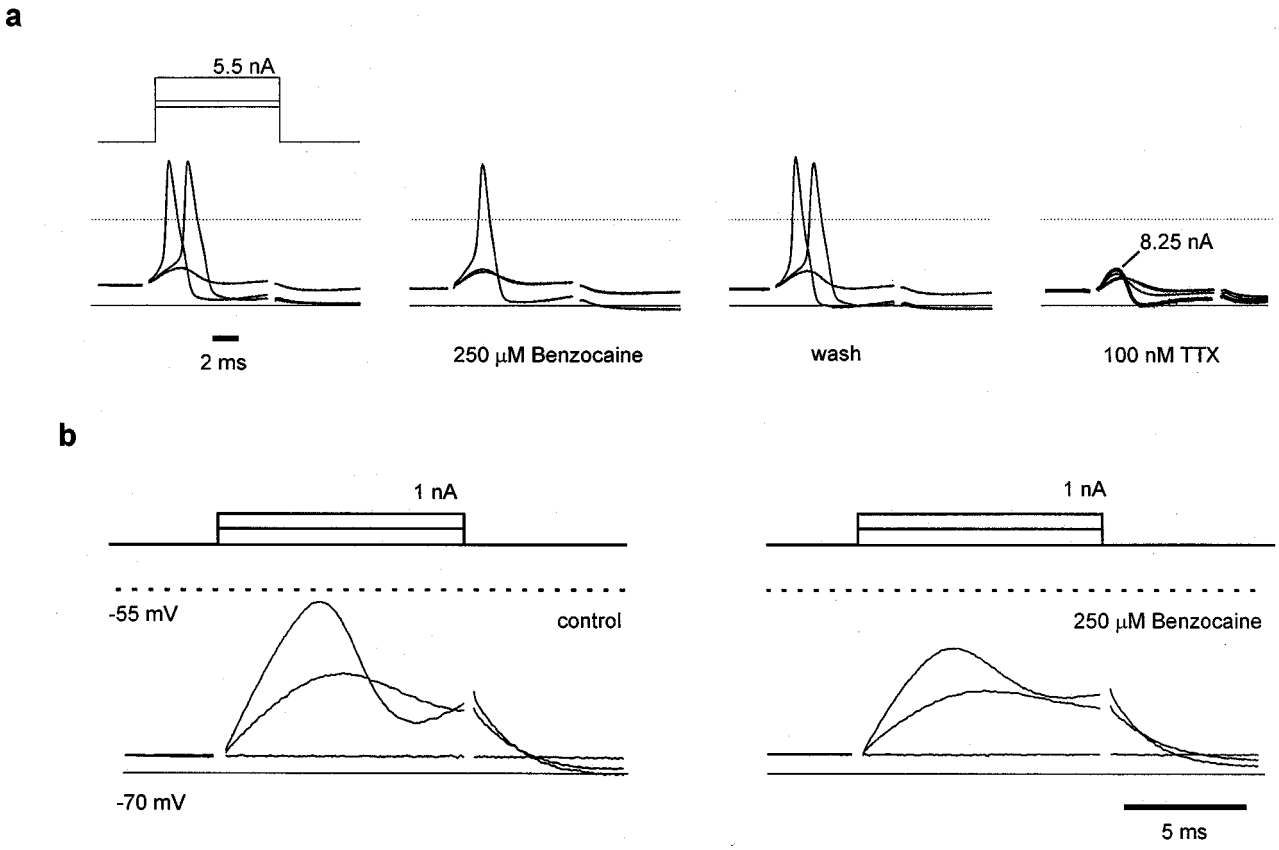

Figure 5 Benzocaine can block sub-threshold Na^+ current while sparing action potentials, whereas a matched concentration of TTX renders neurones inexcitable. (a) Rectangular current pulses applied to a neurone in current-clamp elicits action potentials. Voltage responses to three depolarizing current steps (upper insert) promoting sub-, just supra-threshold, and clearly supra-threshold depolarizations under control conditions (left hand panel). Benzocaine (250 μM) reversibly raises the current threshold (>40%) but fails to prevent action potential generation (middle panels). A matched concentration of TTX, expected to block late current by >90% anaesthetizes the neurone (right hand panel). In the presence of TTX, the neurone remains inexcitable, even when applied current is increased to 8.25 nA. Dotted lines and solid lines indicate 0 and −80 mV, respectively. (b) Sub-threshold potential changes in absence (control) and presence of 250 μM benzocaine (different neurone than in (a)). Benzocaine blocks amplification of passive depolarizing, electrotonic response (increasing threshold >60%). All recordings corrected for series resistance errors, and residual artefacts have been blanked.

In contrast, the evidence presented for benzocaine block of the late current suggests that if channel-state dependent binding is a factor, then it cannot be substantial, and it seems probable that block of closed channels must occur. Block is associated with a fall in the number of brief, sporadic channel openings, suggesting an interaction with non-conducting channels. At potentials near the activation threshold (where channel open-probability is low), block of late current is present immediately upon depolarization (e.g. Figure 3b), suggesting that the channels do not have to open before they are blocked. Block of late current at 200 ms does not appear to be substantially voltage-dependent, and finally, there is no shift in the voltage-dependence of the late current h_∞ relation on exposure to the drug.

While some aspects of the differential block of transient and late currents may be ascribed to differences in channel gating, selection on a molecular basis may also play a part and cannot be ruled-out. The corresponding class I antiarrhythmic action of lignocaine upon the heart may be attributable to a selective action on persistent Na^+ current (Ju *et al.*, 1992). Block of steady-state Na^+ current is achieved at low, therapeutic concentrations (Colatsky, 1982; Ju *et al.*, 1992; Rosen, 1996), that minimally affect transient current. Ventricular muscle expresses a TTX-resistant transient current, and a TTX-sensitive late current (Saint *et al.*, 1992). As the binding of TTX is well established to depend critically upon the channel amino-acid sequence, it is probable that the channels generating these two portions of the total Na^+ current represent at least two different Na^+ channel sub-types.

Late current and spontaneous impulse generation in nerve

Following nerve transection, sensory nerve fibres of all diameters can become spontaneously active, impulses arising at sites of nerve regeneration (sprouting) and from axotomized DRG neurones (Wall & Gutnick, 1974; Wall & Devor, 1983; Burchiel, 1984; reviewed by Devor, 1995). A minor subset of normal DRG neurones can also generate spontaneous activity (e.g. Burchiel, 1984). Devor *et al.* (1992) reported that after injury the spontaneous, ectopic discharges in large diameter fibres, arising at both sites, are ultra-sensitive to systemic lignocaine. The discharges are suppressed by doses too low to obviously affect axonal excitability to applied currents or attributes of normal impulse transmission. These authors report that a cumulative dose of approximately 1 mg kg^{-1} was sufficient to reduce activity arising within axotomized neurones by 50%. Assuming the distribution and fate of the drug in rat is comparable with those in man, the resulting plasma concentration may have been less than 10 μM (*c.f.* Rosen,

1996). In contrast, Jaffe & Rowe (1996) reported that 50% conduction failure in rat dorsal root axons *in vitro*, when stimulated at low frequency, occurs after exposure to 230 μM lignocaine. The present findings shed light upon these effects and suggest a special role for the late current in the generation of ectopic impulses in diseased sensory nerve, especially within the soma of DRG neurones. Although Devor *et al*. (1992) reported a differential effect of lignocaine on cell bodies and sprouting afferents (the soma being more sensitive to the drug), this pharmacological discrimination does not necessarily imply fundamentally different mechanisms of electrogenesis because the local relative densities of Na^+ and K^+ channels may differ. For example, accumulation of Na^+ channels may occur at the end of a regenerating nerve, and this has been suggested to account for hyperexcitable nerve sprouts and neuromas (e.g. Devor *et al*., 1989; England *et al*., 1994; 1996), although whether K^+ channels also accumulate is not known.

In demyelinated spinal root axons, spontaneous, ectopic activity is driven by a pacemaker potential at the active site, consistent with the operation of an inward current at subthreshold potentials (Baker & Bostock, 1992). The spontaneous firing (5–20 Hz) can be highly regular, the rate being controlled by kinetically slow K^+ channels. Exposure to tetraethylammonium (TEA) ions, that block the slow K^+ channels, increase the rate of discharge. In demyelinated dorsal column and sciatic nerve axons, spontaneous activity of a similar type has also been reported (Kapoor *et al*., 1997). A sinusoidal, slow membrane potential oscillation at near threshold potentials appears to be responsible for the regular excitation, and the oscillation can also be recorded in the absence of action potential generation. Importantly, Kapoor *et al*. (1997) discovered that the membrane potential oscillation could be abolished by exposure to TTX, implicating a Na^+ current in its generation. Thus, the inward current initiating membrane potential instability and ectopic activity in demyelinated nerve may be synonymous with late Na^+ current. Consistent with this hypothesis, unitary currents similar to those underlying at least a part of the late current in DRG neurones (brief, sporadic activity; Baker & Bostock, 1997), have also been found in axonal membrane patches (Mitrović *et al*., 1993).

It is therefore probable that the effectiveness of systemic local anaesthetics in the treatment of spontaneous, ectopic impulse generation in diseased and damaged nerve is due, at least in part, to a selective action on late Na^+ current.

I would like to thank Hugh Bostock for reading and commenting upon a version of this manuscript, and I am grateful to the reviewers for their constructive criticism. This study was supported by a grant from the Medical Research Council.

References

AKOPIAN, A.N., SIVILOTTI, L. & WOOD, J.N. (1996). A tetrodotoxin-resistant, voltage-gated sodium channel expressed by sensory neurons. *Nature,* **379,** 257–262.

ALZHEIMER, C., SCHWINDT, P.C. & CRILL, W.E. (1993). Modal gating of Na^+ channels as a mechanism of persistent Na^+ current in pyramidal neurones from rat and cat sensorimotor cortex. *J. Neurosci.,* **13,** 660–673.

BAKER, M. & BOSTOCK, H. (1992). Ectopic activity in demyelinated spinal root axons of the rat. *J. Physiol.,* **451,** 539–552.

BAKER, M.D. & BOSTOCK, H. (1997). Low-threshold, persistent sodium current in rat large dorsal root ganglion neurons in culture. *J. Neurophysiol.,* **77,** 1503–1513.

BAKER, M.D. & BOSTOCK, H. (1998a). Inactivation of macroscopic late Na^+ current and characteristics of unitary late Na^+ currents in sensory neurons. *J. Neurophysiol.,* **80,** 2538–2549.

BAKER, M.D. & BOSTOCK, H. (1998b). Selective block of persistent Na^+ current by lidocaine in cultured rat sensory neurones. *J. Physiol.,* **511P,** 123*P*.

BAKER, M.D. & BOSTOCK, H. (1999a). The pH dependence of late sodium current in large sensory neurons. *Neurosci.,* **92,** 1119–1130.

BAKER, M.D. & BOSTOCK, H. (1999b). Benzocaine discriminates between transient and late Na^+ current in rat cultured large sensory neurones. *J. Physiol.,* **518P,** 151*P*.

BALSER, J.R., NUSS, H.B., ORIAS, D.W., JOHNS, D.C., MARBAN, E. TOMASELLI, G.F. & LAWRENCE, J.H. (1996). Local anesthetics as effectors of allosteric gating. Lidocaine effects on inactivation-deficient rat skeletal muscle Na channels. *J. Clin. Invest.,* **98,** 2874–2886.

BEAN, B.P., COHEN, C.J. & TSIEN, R.W. (1983). Lidocaine block of cardiac sodium channels. *J. Gen. Physiol.,* **81,** 613–642.

BLACK, J.A., DID-HAJJ, S., McNABOLA, K., JESTE, S., RIZZO, M.A., KOCSIS, J.D. & WAXMAN, S.G. (1996). Spinal sensory neurons express multiple sodium channel a-subunit mRNAs. *Mol. Brain Res.,* **43,** 117–131.

BURCHIEL, K.J. (1984). Spontaneous impulse generation in normal and denervated dorsal root ganglia: sensitivity to alpha-adrenergic stimulation and hypoxia. *Exp. Neurol.,* **85,** 257–272.

COLATSKY, T.J. (1982). Mechanisms of action of lidocaine and quinidine on action potential duration in rabbit cardiac Purkinje-fibers – an effect on steady-state sodium currents. *Circulation Res.,* **50,** 17–27.

DE MIERA, E.V.S., RUDY, B., SUGIMORI, M. & LLINAS, R. (1997). Molecular characterization of the sodium channel subunits expressed in mammalian cerebellar Purkinje cells. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 7059–7064.

DEVOR, M. (1995). Abnormal excitability in injured axons. In: *The Axon***.** Waxman, S.G., Kocsis, J.D. & Stys, P.K. (eds). Ch. 27, pp. 530–552, OUP: New York.

DEVOR, M., KELLER, C.H., DEERINCK, T.J. & ELLISMAN, M.H. (1989). Na^+ channel accumulation on axolemma of afferent endings in nerve end neuromas in *Apteronotus*. *Neurosci. Lett.,* **102,** 149–154.

DEVOR, M., WALL, P.D. & CATALAN, N. (1992). Systemic lidocaine silences ectopic neuroma and DRG discharge without blocking nerve conduction. *Pain,* **48,** 261–268.

ENGLAND, J.D., GAMBONI, F., FERGUSON, M.A. & LEVINSON, S.R. (1994). Sodium channels accumulate at the tips of injured axons. *Muscle Nerve,* **17,** 593–598.

ENGLAND, J.D., HAPPEL, L.T., KLINE, D.G., GAMBONI, F., THOURON, C.L., LIU, Z.P. & LEVINSON, S.R. (1996). Sodium channel accumulation in humans with painful neuromas. *Neurology,* **47,** 272–276.

HAMMARSTRÖM, A.K.M. & GAGE, P.W. (1998). Inhibition of oxidative metabolism increases persistent sodium current in rat CA1 hippocampal neurons. *J. Physiol.,* **510,** 735–741.

HILLE, B. (1977). Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen Physiol.,* **69,** 475–496.

HILLE, B. (1992). *Ionic Channels of Excitable Membranes*. Ch. 15, pp. 390–422, Sunderland, M.A.: Sinauer Associates Inc.

JAFFE, R.A. & ROWE, M.A. (1996). Differential nerve block – direct measurements on individual myelinated and unmyelinated dorsal root axons. *Anesthesiology,* **84,** 1455–1464.

JU, Y.K., SAINT, D.A. & GAGE, P.W. (1992). Effects of lignocaine and quinidine on the persistent sodium current in rat ventricular myocytes. *Br. J. Pharmacol.,* **107,** 311–316.

KANEDA, M., OYAMA, Y., IKEMOTO, Y. & AKAIKE, N. (1989). Blockade of the voltage-dependent sodium current in isolated rat hippocampal neurons by tetrodotoxin and lidocaine. *Brain Res.,* **484,** 348–351.

KAPOOR, R., LI, Y.G. & SMITH, K.J. (1997). Slow sodium-dependent potential oscillations contribute to ectopic firing in mammalian demyelinated axons. *Brain,* **120,** 647–652.

LAMPL, I., SCHWINDT, P. & CRILL, W. (1998). Reduction of cortical pyramidal neuron excitability by the action of Phenytoin on persistent Na^+ current. *J. Pharmacol. Exp. Ther.,* **284,** 228–237.

MARTY, A. & NEHER, E. (1983). Tight-seal whole-cell recording. In: *Single-channel recording*. Sakmann, B. & Neher, E. (eds). Ch. 7, pp. 107–121, Plenum: New York.

MITROVIĆ, N., QUASTHOFF, S. & GRAFE, P. (1993). Sodium channel inactivation kinetics of rat sensory and motor nerve fibres and their modulation by glutathione. *Pflügers Arch.,* **425,** 453–461.

PATLAK, J.B. & ORTIZ, M. (1986). Two modes of gating during late Na^+ channel currents in frog sartorius muscle. *J. Gen. Physiol.,* **87,** 305–326.

RAMAN, I.M., SPRUNGER, L.K., MEISLER, M.H. & BEAN, B.P. (1997). Altered subthreshold sodium currents and disrupted firing patterns in Purkinje neurons of *Scn8a* mutant mice. *Neuron,* **19,** 881–891.

ROSEN, D.M. (1996). Antiarrhythmic drugs. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. Hardman, J.G., Gilman, A.G. & Limbird, L.E. (eds.). Ch. 35, pp. 839–874, McGraw-Hill: New York.

ROY, M.L. & NARAHASHI, T. (1992). Differential properties of tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels in rat dorsal root ganglion neurons. *J. Neurosci.,* **12,** 2104–2111.

SAINT, D.A., JU, Y.-K. & GAGE, P.W. (1992). A persistent sodium current in rat ventricular myocytes. *J. Physiol.,* **453,** 219–231.

SCHEUER, T. (1999). A revised view of local anaesthetic action: what channel state is really stabilized? *J. Gen. Physiol.,* **113,** 3–6.

SCHOLZ, A., KUBOYAMA, N., HEMPELMANN, G. & VOGEL, W. (1998). Complex blockade of TTX-resistant Na^+ currents by lidocaine and bupivacaine reduce firing frequency in DRG neurons. *J. Neurophysiol.,* **79,** 1746–1754.

STRICHARTZ, G.R. (1973). The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J. Gen. Physiol.,* **62,** 37–57.

VEDANTHAM, V. & CANNON, S.C. (1999). The position of the fast-inactivation gate during lidocaine block of voltage-gated Na^+ channels. *J. Gen. Physiol.,* **113,** 7–16.

WALL, P.D. & DEVOR, M. (1983). Sensory afferent impulses originate from dorsal root ganglia as well as from the periphery in normal and nerve-injured rats. *Pain,* **17,** 321–339.

WALL, P. & GUTNICK, M. (1974). Properties of afferent nerve impulses originating from a neuroma. *Nature,* **248,** 740–743.

(Received September 20, 1999
Revised January 24, 2000
Accepted January 27, 2000)