Microscopic heterogeneity in unitary N-type calcium currents in rat sympathetic neurons

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1. Single N-type calcium (Ca^{2+}) channels in rat superior cervical ganglion neurons display complex patterns of activity in both inactivating and non-inactivating gating modes. Unitary currents were elicited by holding the patch at −90 mV and stepping to +30 mV for 740 ms. Barium (110 mM) was used as the charge carrier. The dihydropyridine agonist (+)-202-791 was included in the bath to ensure that single channel recordings showed no L-type Ca^{2+} channel mode 2 activity. Using this protocol, we characterized three additional patterns of N-type Ca^{2+} channel activity named: (1) LLP for large unitary current amplitude ($i = -0{\cdot}92$ pA) and low open probability ($P_o = 0{\cdot}26$); (2) SLP for small unitary current amplitude ($i = -0{\cdot}77$ pA) and low open probability ($P_o = 0{\cdot}25$); and (3) SHP for its small unitary current ($i = -0{\cdot}77$ pA) and higher open probability ($P_o = 0{\cdot}39$).

2. Transitions among these patterns of activity occur more slowly than transitions between closed and open states, resulting in significant clustering of like sweeps. Thus, the complicated gating of single N-type Ca^{2+} channels can be dissected into multiple, independent modes, each with the same reproducible pattern of activity.

3. This heterogeneous activity is not unique to sympathetic neurons, for inactivating (4), non-inactivating (4), SLP (4) and SHP (3 patches) gating modes were also observed in cell-attached patch recordings ($n = 4$) of single N-type Ca^{2+} channels in differentiated phaeochromocytoma (PC12) cells.

4. The 1568 sweeps from four single N-type Ca^{2+} channel recordings that used the same voltage protocol were categorized by mode to determine the frequency of occurrence of each. Of the 54 % of sweeps that showed activity, 42 % were inactivating and 58 % were non-inactivating. The contribution by each mode to the sustained current was estimated using the equation: $I = NP_o i$, where N is the frequency of occurrence of each mode and P_o and i are the mean values of open probability and unitary current amplitude respectively. The LLP mode contributed 18 %, the SLP mode 16 %, and the SHP mode 66 % of the sustained whole cell N-type Ba^{2+} current.

5. The variability in the incidence among these modes in other cell types may resolve some of the controversy surrounding the characterization of N- and L-type whole cell Ca^{2+} current components in peripheral neurons. In addition, the number of different modes provides a source of plasticity that may be a target of modulation by neurotransmitters and cellular signals.

A number of voltage-activated ion channels display modal gating (Moczydlowski & Latorre, 1983; Hess, Lansman & Tsien, 1984; Patlak & Ortiz, 1989). One of the best described examples is the single L-type calcium (Ca^{2+}) channel which displays three distinct modes of gating, characterized by clusters of sweeps with the same pattern of activity. Mode 0 is characterized by sweeps with no openings, mode 1 by sweeps with infrequent, short openings and mode 2 by sweeps with long openings of 10–20 ms (Hess *et al.* 1984; Kokubun & Reuter, 1984; Ochi, Hino & Niimi, 1984). Transitions between these modes occur abruptly and apparently randomly but are much slower than the transitions between open and closed states. The rates of transition among the modes of the L-type channel can be influenced by voltage

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(Pietrobon & Hess, 1990; Artalejo, Rossie, Perlman & Fox, 1992), drugs (Hess *et al.* 1984; Kokubun & Reuter, 1984; Ochi *et al.* 1984), neurotransmitters and second messengers (Yue, Herzig & Maraban, 1990). In comparison, less is known about the details of N-type Ca^{2+} channel gating. Unitary N-type Ca^{2+} currents, like L-type Ca^{2+} currents, have been dissected into two modes of activity (Plummer & Hess, 1991) based on changes in inactivation. Non-inactivating gating displays clusters of sweeps with bursts of activity that show no indication of inactivation even with a test pulse duration of 1·5 s. In contrast, the burst of activity that occurs in clusters of sweeps exhibiting the inactivating mode inactivates with $\tau = 40$ ms (Plummer & Hess, 1991). Here we report that single N-type Ca^{2+} channels in rat sympathetic neurons exhibit three additional modes of activity based on changes in unitary conductance and mean open time. The frequency of incidence of these five (six including nulls) modes of activity can account for the whole cell N-type current kinetics and provides a source of plasticity that may be a target of modulation by neurotransmitters and cellular signals.

METHODS

Superior cervical ganglia (SCG) from rats 1–3 days old were removed from the animals by anaesthetizing with CO_2 before decapitation. Both ganglia were dissected from the neck area and enzymatically dissociated using standard methods (Hawrot & Patterson, 1979). The outer connective tissue surrounding each ganglion was teased away. Ganglia were then cut in half and incubated at 37 °C in a 1 mg collagenase B (ml dispase)$^{-1}$ (Boerhinger Mannheim Corp., Indianapolis, IN, USA) solution for 1–2 h. The dissociated cells were pelleted, resuspended in Dulbecco's modified Eagle's medium (DMEM), plated on collagen-coated coverslips (murine Type IV, Collaborative Research, Bedford, MA, USA) and cultured in DMEM containing 7·5 % calf serum (Sigma, St Louis, MO, USA), 7·5 % fetal bovine serum (HyClone, Logan, UT, USA), 4 mM glutamine (Sigma), 1 % penicillin–streptomycin (Gibco, Grand Island, NY, USA), and 2 μg ml^{-1} nerve growth factor isolated from mouse salivary glands. Recordings were made from cells cultured from 2–9 days. PC12 cells were differentiated using the same culture conditions described above and were used after 10 days in culture. Single cell-attached patch recordings were made with standard techniques (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). The pipette solution contained 110 mM $BaCl_2$ and 10 mM Hepes–TEA (pH 7·5). The membrane potential of the cell outside the patch was zeroed by using a bath solution of 140 mM potassium aspartate, 10 mM Hepes and 5 mM EGTA (pH 7·5). In addition, 500 nM of the dihydropyridine agonist (+)-202-791 (a gift from Dr Hof, Sandoz, Basel, Switzerland) was included in the bath solution to ensure that single channel recordings showed no L-type Ca^{2+} channel mode 2 activity (Hess *et al.* 1984), and were therefore N-type currents. The currents were recorded at room temperature with a Dagan patch clamp amplifier (Minneapolis, MN, USA), sampled every 200 μs, filtered at 1 kHz (−3 dB, 8-pole Bessel filter), and digitized at 5 times the filter cut-off frequency. The recordings were saved and analysed on a PDP-11/73 computer (DEC, Sunnyvale, CA, USA). For kinetic analysis, channel openings and closings were classified by a mid-line crossing detector and the histograms were fitted to single decaying exponential curves by a non-linear χ^2 minimization routine (Bevington, 1969). Amplitude histograms were plotted directly from the data with the bin size equal to 204·8 points per picoamp. Data were analysed statistically using a two-tailed Student's t test for two means.

RESULTS

Unitary N-type Ca^{2+} currents display modal activity

Single N-type Ca^{2+} channels in rat superior cervical ganglion (SCG) neurons display complex patterns of activity that have not been described previously. Unitary currents were elicited by holding the patch at −90 mV and then stepping to +30 mV for 740 ms every 5 s. Recordings were made in the presence of the dihydropyridine (+)-202-791 (500 nM), a specific L-type Ca^{2+} channel agonist that enhances mode 2 activity (Kokubun, Prod'hom, Beckor, Porzig & Reuter, 1986). If the channel showed no dihydropyridine-induced long openings, it was identified as N-type. Using this protocol, three distinct patterns of activity can be observed in single channel cell-attached patch recordings based on changes in unitary conductance and mean open time in addition to the changes in inactivation described by Plummer & Hess (1991).

Although present in both inactivating and non-inactivating sweeps, the characteristics of the three gating patterns are most easily analysed in the non-inactivating sweeps where there are more channel openings. Figure 1 shows examples of these three patterns of activity in consecutive non-inactivating sweeps recorded from a single N-type channel. The first pattern of activity shown in the left-hand panel is characterized by a unitary current of −0·92 pA and a low open probability ($P_o = 0{\cdot}24$). We have named this activity LLP for large unitary current, low P_o. This pattern of activity can clearly be discriminated from the second mode of activity shown in the middle panel. While the second pattern of activity has a similar low P_o ($P_o = 0{\cdot}25$), the unitary current is clearly smaller ($i = 0{\cdot}74$ pA). We have called this activity SLP for small unitary current, low P_o. In contrast, the third pattern of activity shares the same unitary current amplitude as the second pattern, but has a significantly ($P < 0{\cdot}01$) higher P_o

Table 1. Summary of non-inactivating modal gating of single N-type Ca^{2+} channels

Cells	i (pA)	P_o
SCG		
LLP	0·924 ± 0·055	0·258 ± 0·017
SLP	0·768 ± 0·035	0·246 ± 0·015
SHP	0·766 ± 0·030	0·394 ± 0·033
PC12		
SLP	0·694 ± 0·027	0·289 ± 0·036
SHP	0·709 ± 0·016	0·389 ± 0·043

Mean unitary current (i) and open probability (P_o) were measured at a test pulse to +30 mV for non-inactivating sweeps of the LLP, SLP and SHP modes in SCG and PC12 cells. Values are means ± S.E.M., $n = 4$ single channel patches for both SCG neurons and PC12 cells.

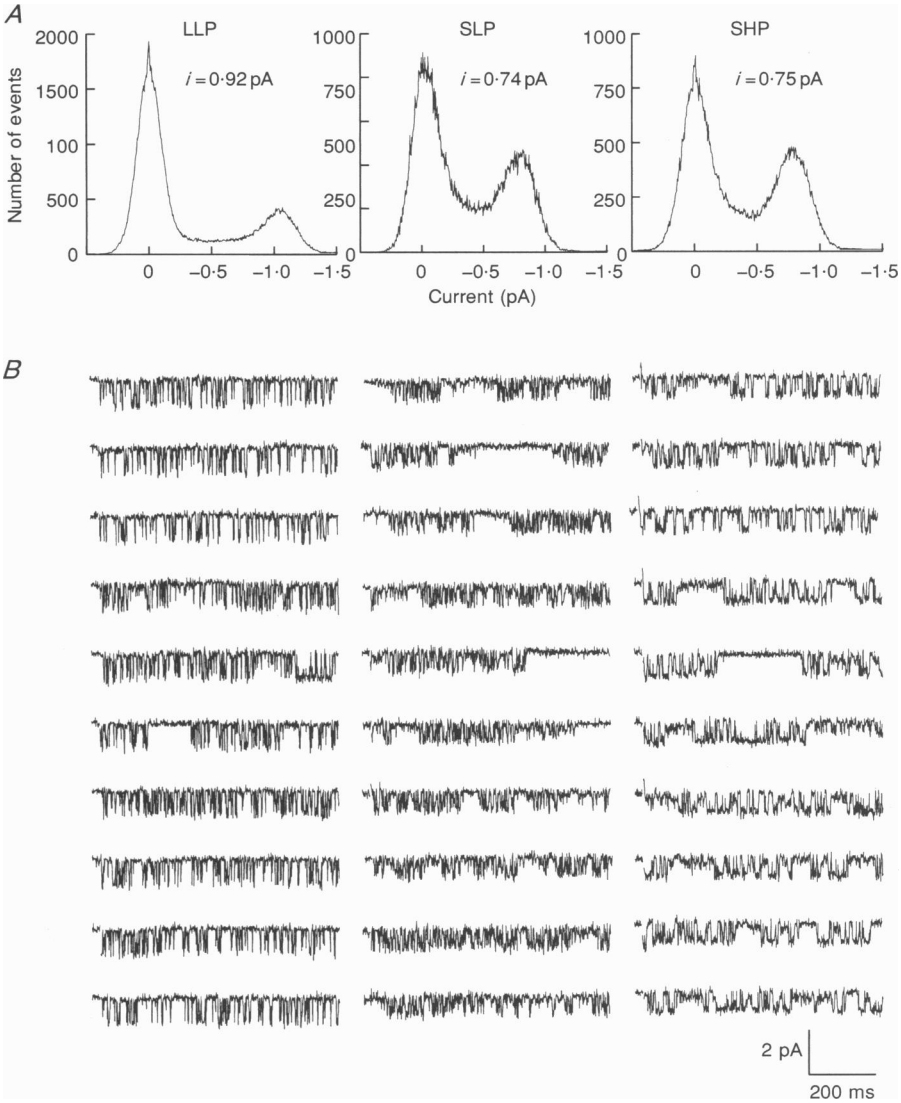

Figure 1. Sweeps illustrating the three non-inactivating patterns of N-type channel activity
Single cell-attached patch recordings of N-type Ca^{2+} channels were made from cultured neonatal rat SCG neurons by holding the patch at −90 mV and stepping to +30 mV for 740 ms. Taken from the same single channel recording, each panel shows the amplitude histogram (*A*) and activity from ten consecutive, non-inactivating sweeps (*B*). The amplitude histograms for each pattern of activity demonstrate that there is a single channel present in the patch and that this channel exhibits various patterns of activity. LLP for large conductance, low P_o; SLP for small conductance, low P_o; SHP for small conductance, higher P_o.

($P_o = 0{\cdot}39$; Table 1). We have called this activity SHP for small unitary current, higher P_o.

From diary plots of recordings from four single channel patches (Figs 2*A* and 3) it is clear that these patterns of activity are superimposed on the inactivating and non-inactivating modes of gating described previously by Plummer & Hess (1991). In the diary plots shown in Figs 2*A* and 3*A*, the frequency of occurrence of the LLP, SLP and SHP modes in inactivating and non-inactivating sweeps is recorded over time. Clustering of sweeps occurs for each combination. For example, the change to the LLP mode from the SHP mode that occurs in the diary plot in Fig. 2*A* at B, is expressed in both inactivating and non-inactivating sweeps which are shown in Fig. 2*B*. Likewise, clustering of the SHP mode at C and the SLP mode at D are also apparent in sweeps exhibiting the inactivating mode (Fig. 2*C* and *D*) in addition to sweeps displaying the non-inactivating mode (Figs 1 and 4*A*). There is a significant clustering of like sweeps in the diary plot in Fig. 2*A*, when testing for a statistical correlation among the LLP, SLP and SHP modes ($P < 0{\cdot}001$) or when comparing inactivating and non-inactivating sweeps within each of the LLP, SLP or SHP modes ($P < 0{\cdot}001$) using Fisher's exact test (See Plummer & Hess, 1991 for details of the analysis). The diary plot in Fig. 3*A* is

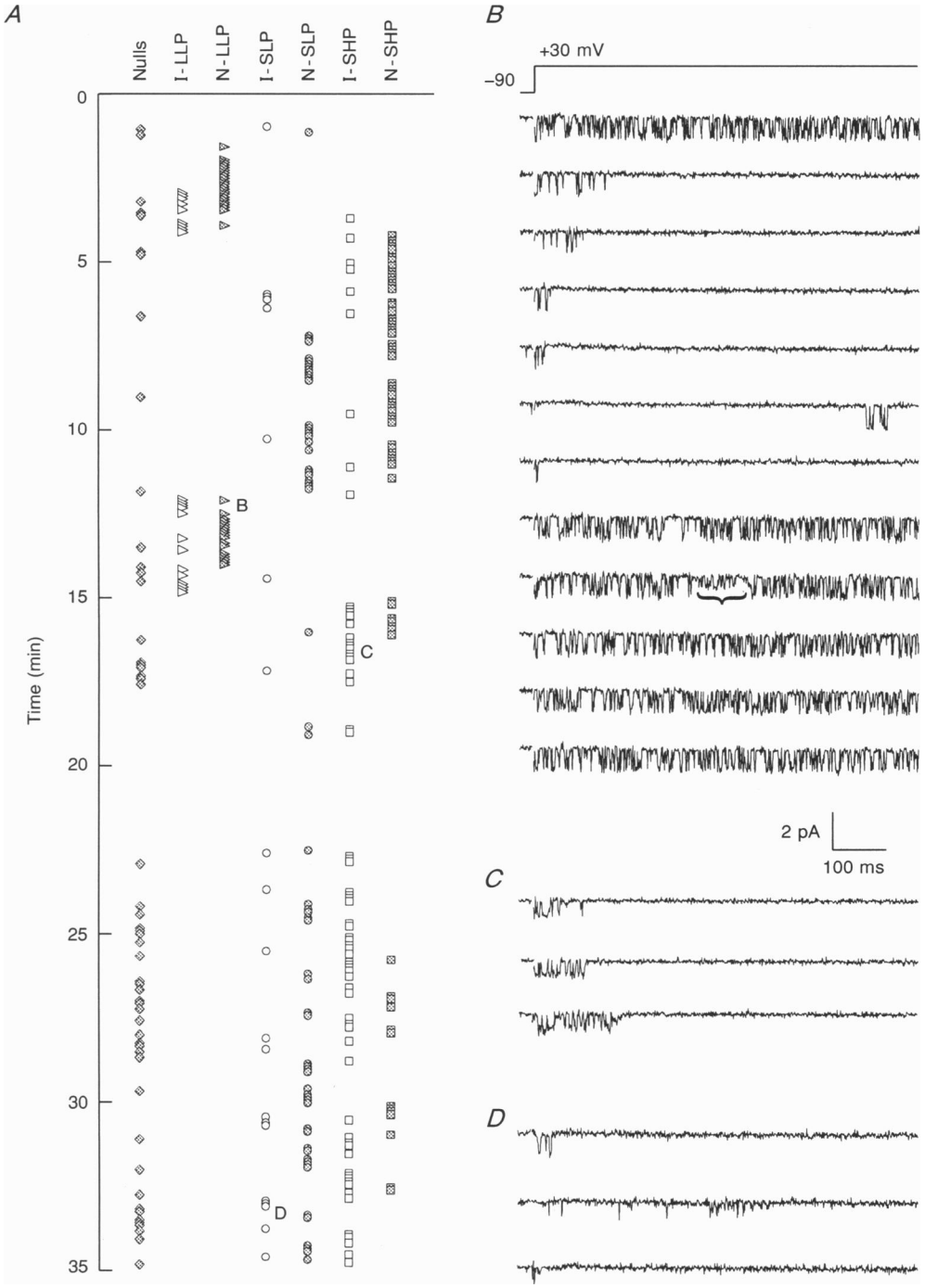

Figure 2. LLP, SLP and SHP activities superimposed on the inactivating (I-) and non-inactivating (N-) modes of a single N-type Ca^{2+} channel

A, diary plot of the occurrence of each type of pattern of activity. From approximately minutes 19–23 different test potentials were used to construct a current–voltage relationship, and therefore these sweeps were not included in the diary plot. *B*, non-inactivating and inactivating sweeps exhibiting LLP activity. These sweeps were selected from the diary plot at *B*. As has been reported previously for rat sympathetic N-type Ca^{2+} channels (Plummer *et al.* 1989) additional complexity is conferred by a subconductance state that occurs within modes as shown here at the rotated parentheses. *C*, inactivating sweeps from the diary plot at *C* that display the SHP pattern of activity. *D*, inactivating sweeps from the diary plot at *D* that display the SLP pattern of activity.

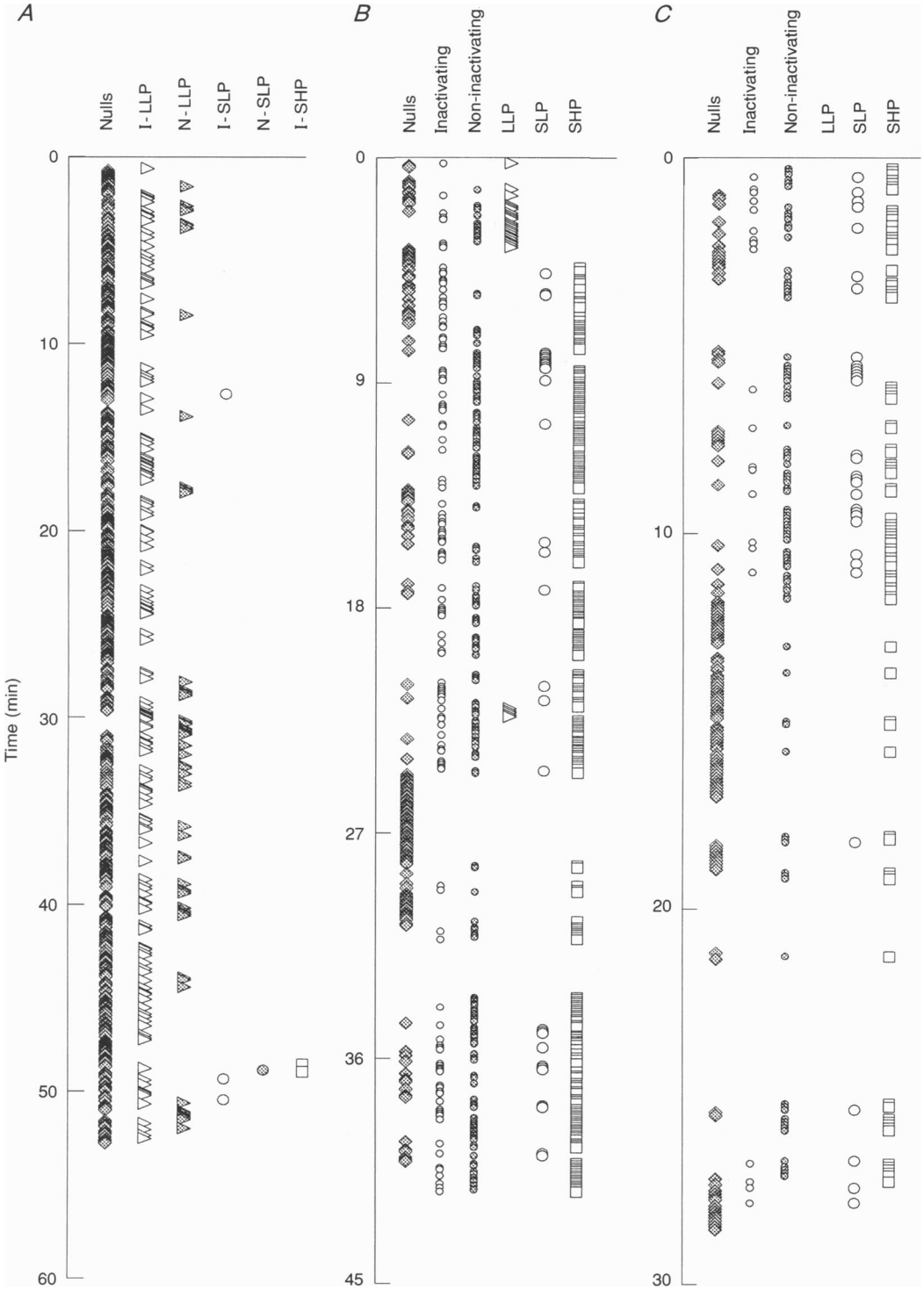

Figure 3. Additional diary plots of single N-type channel activity
A, this plot, similar to the one in Fig. 2*A*, exhibits mainly LLP activity and no SHP activity. The LLP mode was present in both the inactivating and non-inactivating sweeps. Diary plot *A* has no N-SHP gating. *B* and *C*, each sweep was scored as null, inactivating or non-inactivating. Non-null sweeps were further scored as exhibiting the LLP, SLP or SHP mode. Diary plot *C* has no LLP modal gating.

plotted as Fig. 2*A* and illustrates the variety of distribution of each pattern of activity in each recording. The majority of the sweeps in the diary plot in Fig. 3*A* exhibit the LLP but none of the SHP pattern of activity. Again, there is significant clustering ($P < 0{\cdot}001$) of the inactivating LLP activity compared to the non-inactivating LLP activity. To emphasize the simultaneous expression of the inactivating and non-inactivating modes with the LLP, SLP and SHP patterns of activity, two additional diary plots were expressed in a slightly different way (Fig. 3*B* and *C*). Each sweep was scored as a null, inactivating or non-inactivating sweep. Non-nulls were further categorized as LLP, SLP or SHP. In both plots, when enough sweeps of a particular pattern of activity are displayed, there is again significant clustering of sweeps with like patterns of activity ($P < 0{\cdot}02$), whether categorized by inactivation, current amplitude or open probability.

As with the inactivating and non-inactivating gating, these three additional types of activity appear to be modal for the following reasons. First, the transitions between the patterns of activity are much slower than the transitions between the closed and open states, resulting in clusters of sweeps of each type of mode as described above (Figs 2, 3 and 4). In the rare case when a transition from the SLP to the LLP is caught within the sweep, it occurs abruptly. An example of this is shown in Fig. 4*B* where the unitary current amplitude abruptly increases. Once shifted, the current remains in the LLP mode for multiple sweeps. In this particular case, transitions between the SLP and LLP modes are not due to a gradual shift in the holding potential because further along in the recording there is an abrupt switch back to the identical, smaller amplitude unitary current. Second, as can be seen in the diary plots (Figs 2 and 3), there appears to be no pattern to the frequency or sequence of occurrence of the modes suggesting that each mode occurs independently of the others. For instance, in the diary plot of the cell recording shown in Fig. 2*A*, the LLP mode occurs only at the beginning of the recording while in the diary plot in Fig. 3*A*, the LLP mode is dominant throughout the recording. Third, all three patterns of activity can be observed in single N-type channel patches (Figs 1, 2 and 4), that never showed overlapping openings, indicating that these modes of activity are not from different types of channels. With these characteristics, it seems reasonable to regard these patterns of activity as distinct modes, each with its own reproducible pattern of openings, closings and channel size.

Square root–log plots of open time and closed time distributions were calculated from the non-inactivating sweeps from the recordings shown in Figs 2*A* and 3 ($n = 4$) to quantitatively describe the striking kinetic differences among the three additional modes of activity (Fig. 5).

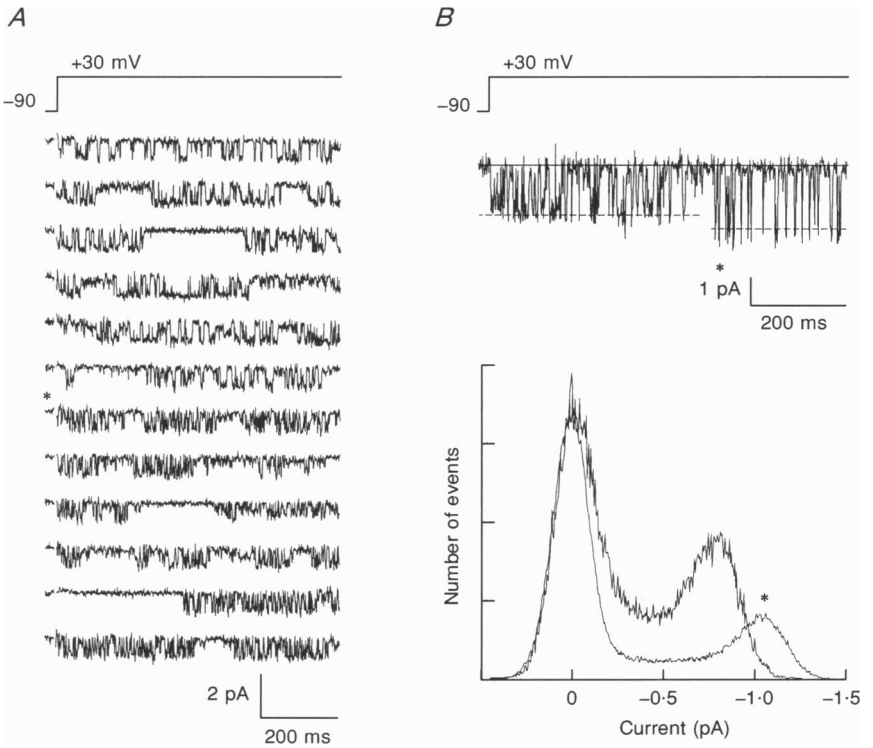

Figure 4. Transitions between the patterns of activity are abrupt
A, transition between sweeps from the SHP to the SLP activity at the asterisk. *B*, a rare occurrence of a transition between the SLP and LLP activities during a sweep occurred at the asterisk . Once shifted to the larger unitary current, the channel displayed this LLP pattern of activity for more than twenty sweeps before abruptly shifting back to the SLP pattern of activity. The two dashed lines define the average unitary current amplitude for each mode measured from their respective amplitude histograms shown superimposed (*C*).

Although these three patterns are present in the inactivating sweeps, we have not analysed their kinetics quantitatively because of insufficient channel openings per sweep. While the LLP and SLP modes are clearly distinguishable by their conductances, their open time distributions are similar. In contrast, the SHP mode which has the same conductance as the SLP mode is clearly distinguishable by its kinetics. The open time distributions of the SLP and LLP modes have a component with a time constant around 1 ms, but the SHP mode has a long-lasting component with a mean open time of approximately 3 ms, 3 times as long as in the LLP and SLP modes. In addition to sharing the same unitary current amplitude, the SLP and SHP modes both have a similar short open time component ($\tau = 0{\cdot}42$ ms). All three of these modes share similar closed time distributions in that the closed times are usually best described by three exponentials. The LLP mode differs, however, in that its longest closed time is less than one-half as long as in the SLP and SHP modes.

Comparison of high P_o activity of N-type and L-type Ca^{2+} channels

The higher P_o activity that characterizes the SHP mode is somewhat reminiscent of mode 2 activity (Hess *et al.* 1984) induced by dihydropyridine agonists in L-type Ca^{2+} channels. Figure 6 compares ten consecutive non-inactivating sweeps of the SHP mode from a single N-type channel recording with ten consecutive sweeps from a single L-type channel. Both recordings were made in the presence of the dihydropyridine agonist (+)-202-791 (500 nM). The differences between the SHP mode of the N-type channel and mode 2 of the L-type channel in SCG neurons are clear even in the raw traces. The amplitude histograms (Fig. 6*A*) and the mean currents (Fig. 6*C*) show that the L-type unitary current and mean current amplitudes are larger than those of the N-type channel. All single N-type current recordings ($n = 6$; $g = 20 \pm 3$ pS) had conductances (Fig. 7*A*) less than the L-type channels ($n = 3$; $g = 25 \pm 0$ pS; see also Plummer, Logothetis & Hess, 1989). From the sweeps (Fig. 6*B*) it is quite obvious that the dihydropyridine-induced long openings of the L-type channel are much longer than the long openings of the SHP mode of the N-type channel. The open time distribution of the SHP mode is best fitted by the sum of two exponentials with a longer open time component having a $\tau = 3$–4 ms as described earlier, whereas the L-type channel mode 2 activity is best fitted by the sum of three exponentials with the longest open time component having a $\tau \approx 10$–20 ms (Fig. 7*B*). In addition, the closings of the L-type channel are better resolved in the sweeps, suggesting that it closes more slowly than the N-type channel. While both of these modes have closed time distributions that are fitted by the sum of three to four exponentials, the mean closed time for the N-type channel is much greater than that of the L-type channel (Fig. 7*C*).

One concern is whether the presence of the dihydropyridine agonist in the bath has caused the heterogeneous modal activity of the N-type Ca^{2+} channel. To test this

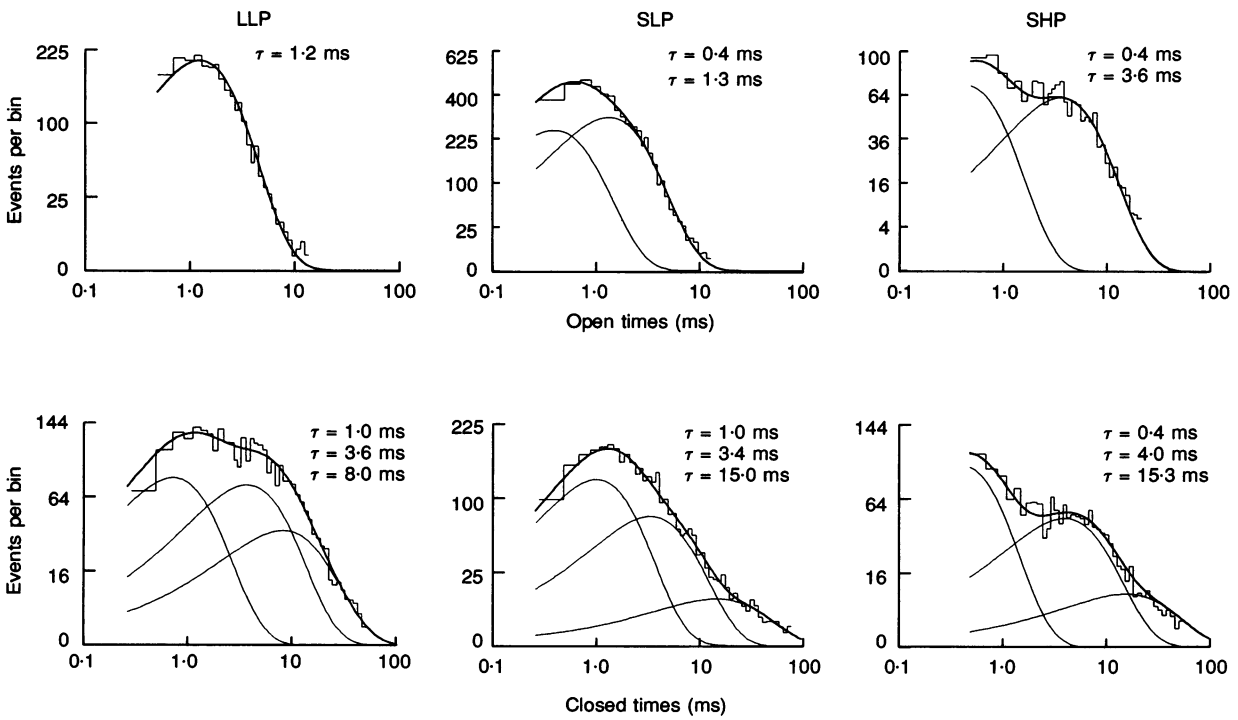

Figure 5. Kinetic analysis of the mean open and closed times for each mode of activity
Open time and closed time distributions for the LLP, SLP and SHP modes were plotted as square root–log plots from the single channel, cell-attached patch recording shown in Figs 1 and 2. The open and closed times of each of the modes is fitted by the sum of exponential curves (thick line).

possibility we made recordings from patches containing one or two N-type channels in the absence of the dihydropyridine agonist ($n = 5$). While it is much more difficult under these conditions to be certain that the unitary currents are from an N-type channel, we identified a channel as N-type by observing non-inactivating and inactivating modal patterns and by never observing mode 2 long openings. Under these conditions, modal activity was still observed (Fig. 8), indicating that the existence of modal activity is independent of the presence of the dihydropyridine agonist. We have identified inactivating (5), non-inactivating (4), LLP (1), SLP (4) and SHP (5 patches) modal activity in these recordings. As in the presence of (+)-202-791, the LLP, SLP and SHP modes appear to occur independently of the inactivating and non-inactivating modes. Thus, the SLP and SHP modes were observed in both inactivating and non-inactivating sweeps.

These modes of activity are not unique to N-type channels in SCG neurons, but were also identified in differentiated PC12 cells. We have observed the inactivating (4) and non-inactivating (4 patches) modes as well as the SLP (4) and SHP (3 patches) modes in cell-attached patch recordings of single N-type Ca^{2+} channels in differentiated PC12 cells. Patches were held at −90 mV and stepped to +20 or +30 mV for 740 ms to elicit modal activity. As with SCG neurons, strong depolarizing test potentials were required in order to observe the non-inactivating and SHP modes. The kinetic and conductance characteristics are

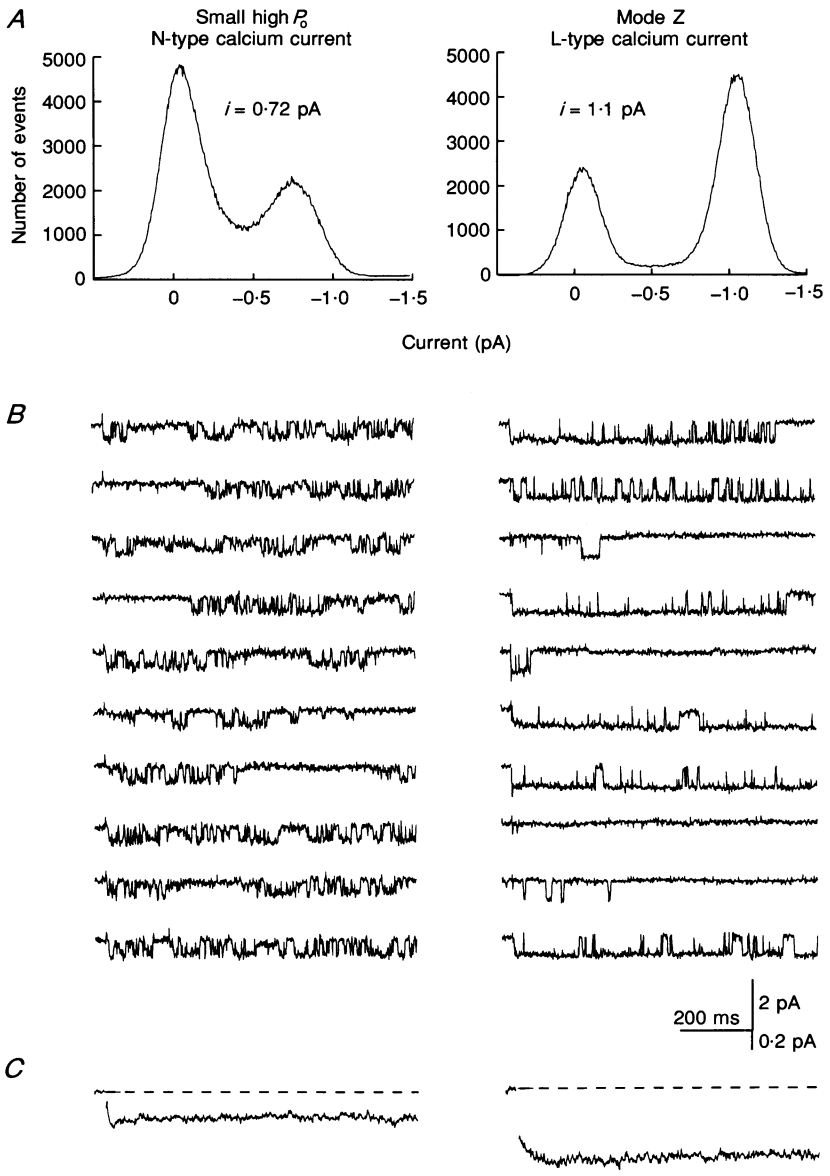

Figure 6. SHP modal gating of single N-type Ca^{2+} currents is different from mode 2 activity of single L-type Ca^{2+} currents

The amplitude histograms (*A*), consecutive sweeps (*B*) and mean currents (*C*) illustrate some of the differences between these two channels. The diary plot of this N-type channel is shown in Fig. 3*B*.

Table 2. Summary of the frequency of occurrence of the different modes of N-type Ca^{2+} channel activity

	Sweeps (n)	Percentage total	Percentage non-nulls	Percentage non-inactivating	Percentage whole cell current
Total	1568	100	—	—	—
Null	721	46	—	—	—
Non-null	847	54	100	—	—
Inactivating	358	—	42	—	—
Non-inactivating	489	—	58	100	100
LLP	97	—	—	20	18
SLP	109	—	—	22	16
SHP	283	—	—	58	66

$n = 4$ patches

The percentage of total activity and the number of sweeps are shown for each type of activity. Percentage of sustained whole cell current was calculated for the LLP, SLP and SHP modes from: (percentage of total sweeps) × (probability of being open) × (unitary current amplitude).

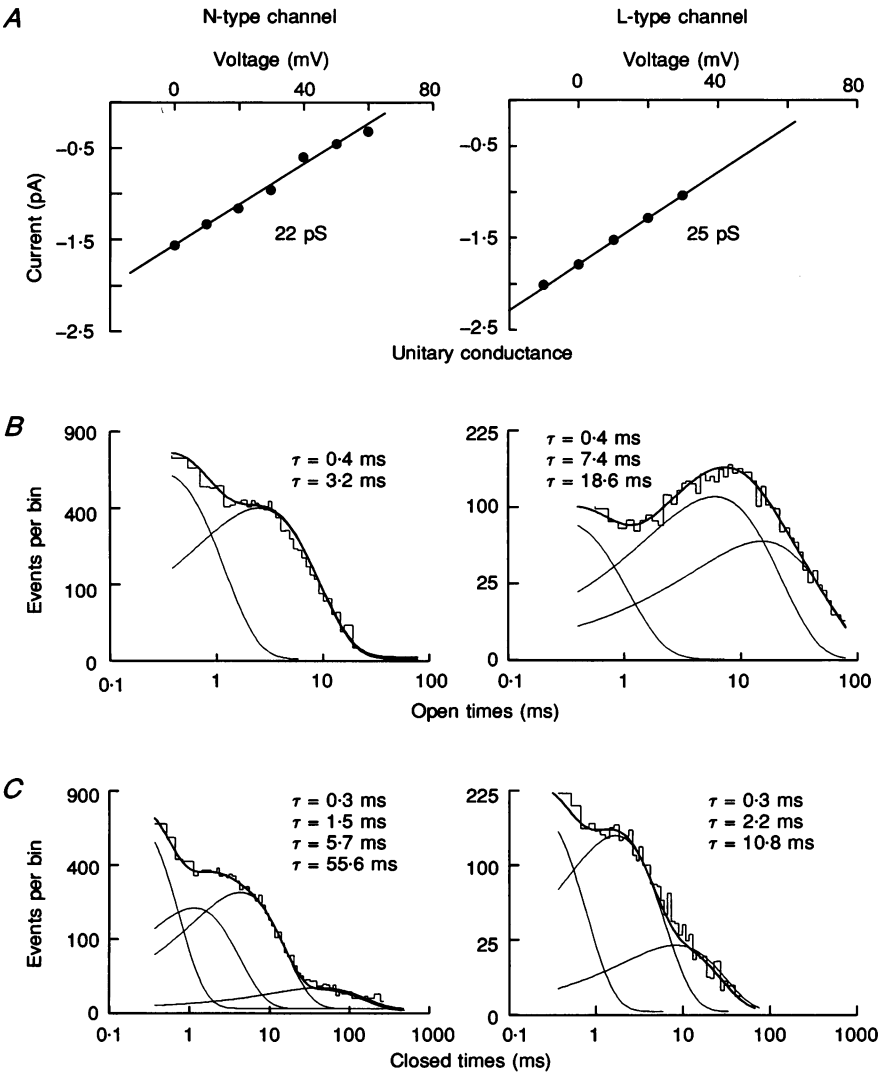

Figure 7. Kinetic differences between SHP currents of N-type channels and mode 2 currents of L-type Ca^{2+} channels

Current–voltage relationships (*A*) and square root–log plots of open time (*B*) and closed time (*C*) distributions of the channel activity shown in Fig. 5.

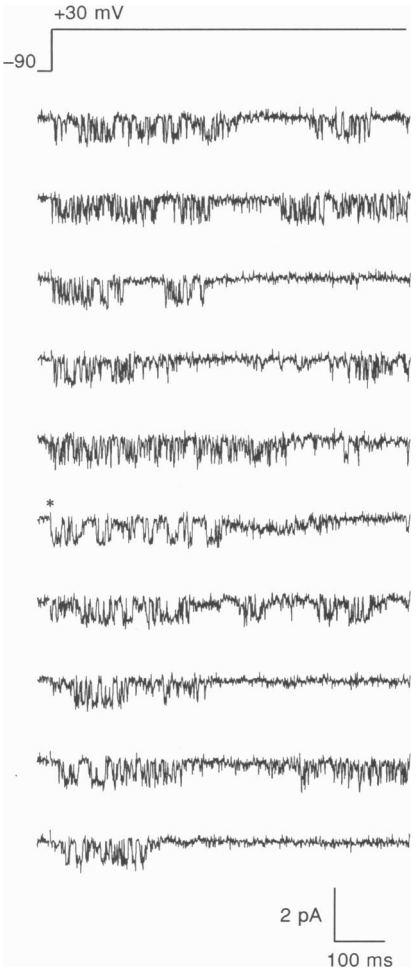

Figure 8. High P_o activity is observed in single N-type Ca^{2+} channels when the dihydropyridine agonist is absent from the bath
These ten consecutive sweeps demonstrate that clusters of the SLP and SHP modes of activity still can be observed in single channel patches when (+)-202-791 is not present. Asterisk indicates the transition from the SLP to the SHP mode.

similar to those seen in the corresponding modes of single N-type channels in SCG neurons (Table 1).

Contribution of different modes of N-type channel activity to the whole cell current

We have analysed the modal activity from six single N-type Ca^{2+} channels in the presence of the dihydropyridine agonist (+)-202-791. All patches exhibited the inactivating, SLP and SHP modes. Five of these patches also showed the non-inactivating and LLP modes. From four of these single channel recordings that used the same voltage protocol, 1568 sweeps were categorized by the mode displayed (Table 2), 46 % of the sweeps were nulls, while 54 % displayed activity. Of the sweeps showing activity, 42 % were inactivating and 58 % non-inactivating, indicating that at the whole cell level, there should be a fairly large sustained component of whole cell N-type current. If these four cells are representative of the normal frequency of the different modes, then 20 % of the sustained current is comprised of sweeps that exhibit the LLP mode, 22 % display the SLP mode and 58 % exhibit the SHP mode of activity. To estimate the contribution of the current mediated by each mode to the sustained current, we used the equation:

$$I = NP_o i,$$

where N is the frequency of occurrence of each mode and P_o and i are the mean values of open probability and unitary current amplitude respectively, shown in Table 2. We estimate that the LLP mode contributes 18 %, the SLP mode 16 % and the SHP mode 66 % of the sustained whole cell N-type Ba^{2+} current.

DISCUSSION

Modes of activity underlie the heterogeneous activity of the N-type Ca^{2+} current

A unique aspect of the modal activity of the N-type Ca^{2+} current is that different modes of activity appear to occur simultaneously and relatively independently of one another, resulting in complex patterns of activity. For example, the long 3–4 ms openings of the SHP mode can occur in both the inactivating and non-inactivating sweeps. The one combination that we rarely observed was the higher P_o activity with the larger unitary conductance. The switch to the large unitary conductance appears to stabilize the channel in the low P_o mode, which then appears to inhibit transitions to the higher P_o activity. We consider this heterogeneous gating of single N-type Ca^{2+} channels modal in that the transitions among at least six discrete patterns of activity are much slower than the transitions between open and closed states. Modal activity of N-type Ca^{2+} channels was observed in the presence or absence of low concentrations

(500 nM) of the dihydropyridine agonist (+)-202-791 and therefore could not be caused by non-specific effects of dihydropyridines that can occur at concentrations higher than 3 μM (Aosaki & Kasai, 1989; Jones & Jacobs, 1990; Zernig, 1990).

The number of modes of activity may help explain some of the differences between the whole cell and single channel kinetics as well as the variability observed in the kinetics of the whole cell N-type Ca^{2+} currents among different types of neurons. In SCG neurons, approximately 30–50 % of the whole cell N-type current is transient and inactivates within 100 ms (Plummer & Hess, 1991). We have found from our recordings of single N-type Ca^{2+} channels that 42 % of the sweeps inactivate, consistent with a transient component of the whole cell current in rat (Plummer *et al.* 1989) and frog (Jones & Marks, 1989) sympathetic neurons. Both differentiated PC12 cells (shown here) and frog sympathetic neurons (Delcour & Tsien, 1993) display similar modal activity at the single channel level. Delcour & Tsien (1993) have also observed clustering of nulls, low and high P_o sweeps that appear very similar to the modes described here. Like this report, they have observed changes in conductance associated with low P_o activity. In addition, they have been able to resolve the existence of a medium P_o mode. Whether N-type Ca^{2+} channels in other cell types, such as dorsal root ganglion (DRG) cells and central neurons, display similar modal activity has not yet been addressed. In DRG neurons that have been shown pharmacologically to have primarily N-type current, a transient and sustained component of the whole cell current (Scroggs & Fox, 1992) is present, indicating that modal activity also occurs in these neurons. However, it is not clear whether some of the differences in inactivation kinetics between N-type current in DRG and SCG neurons are due to variations in the rate constants among the modes or to other differences such as changes in the sequence of the proteins that form the channel complex (Williams, Velicelebi, Ellis & Harpold, 1992). Furthermore, a voltage dependence has been correlated with the different modes. While we have not systematically studied the voltage dependence of each mode, it is clear that non-inactivating sweeps were never seen at test potentials less than +10 mV, in agreement with the three-state cyclic model of the N-type Ca^{2+} channel (Jones & Marks, 1989) which predicts that inactivation occurs more rapidly with more negative test potentials. Inactivation may not be the only mode sensitive to voltage for the SHP mode observed here as well as the high P_o mode in frog sympathetic neurons (Delcour & Tsien, 1993) appear to require strong depolarizing test pulses.

The realization that N-type Ca^{2+} channels have openings that last 3–4 ms may explain why previously it seemed so much easier to find and record from single L-type than N-type Ca^{2+} channels. This seemed contradictory to the observation that approximately 10–20 % of the whole cell current in SCG neurons is L-type current with the remainder being N-type current (Plummer *et al.* 1989). It may be that the fairly frequently occurring long openings of the N-type Ca^{2+} channel, observed in the SHP mode, have been mistaken for dihydropyridine agonist-induced mode 2 activity of the L-type channel. In single and multichannel patches, we find that the ratio of L- to N-type channels is approximately one to five, consistent with the relative contributions of N- and L-type currents to whole cell Ba^{2+} currents in SCG neurons.

Physiological implications of modal activity

Modal activity is not unique for N-type currents in sympathetic neurons. A variety of ion channels display distinct patterns of activity that appear modal when examined at the single channel level, including L-type Ca^{2+} channels (Hess *et al.* 1984), sodium channels (Kirsch & Brown, 1989; Patlak & Ortiz, 1989; French, Sah, Buckett & Cage, 1990; Cannon, Brown & Corey, 1991; Zhou, Potts, Trimmer, Agnew & Sigworth, 1991), Ca^{2+}-activated K^+ channels (Moczydlowski & Latorre, 1983; McManus & Magleby, 1988) and delayed rectifier K^+ channels (Perozo, Vandenberg, Jong & Bezanilla, 1991; Ruppersberg, Stocker, Pongs, Heinemann, Frank & Koeren, 1991). Thus, modal activity may be a fundamental property of many types of voltage-activated ion channels. If modal activity is common, we may expect to see clustering of sweeps with distinct patterns of gating in other high threshold-activated Ca^{2+} channels such as the P-type Ca^{2+} channel.

At present, it is not clear what causes the variations in inactivation, open time and unitary conductance that define the modal gating of the N-type Ca^{2+} channel. Plummer & Hess (1991) have hypothesized that the slow transition rates between the inactivating and non-inactivating modes suggest a reversible, covalent modification of the N-type Ca^{2+} channel protein. The different modal activity observed here may be the result of random 'noise' in cell signalling pathways that can act on the N-type channels. If this is so, stimulation of one of these pathways may stabilize a particular mode, increasing its incidence. An example of this at the whole cell level is that okadaic acid results in a large increase in the amplitude of the transient component with no change in the sustained component of whole cell Ba^{2+} currents of frog sympathetic neurons (Werz, Elmslie & Jones, 1992). These data indicate that phosphorylation enhances the inactivating mode. Modifications of other types of ion channels also result in modal changes in the kinetics of the currents. For example, positive voltage (Lee, 1987; Pietrobon & Hess, 1990; Artalejo *et al.* 1992), dihydropyridine agonists (Hess *et al.* 1984), β-adrenergic agonists (Reuter, Stevens, Tsien & Yellen, 1982) and phosphorylating conditions (Brum, Osterrieder & Trautwein, 1984; Yue *et al.* 1990) each increase the incidence of long openings (mode 2 activity) of single L-type Ca^{2+} channels. In addition, increases in the L-type current conductance has been observed in the presence of a dihydropyridine agonist, which may be caused simply by a slowed closing of the channel (Kokubun & Reuter, 1984). Three non-inactivating K^+ currents expressed in oocytes and recorded in cell-attached patches become inactivating when the patch is ripped off (Ruppersberg *et al.* 1991). If the reducing agent glutathione is added to the bath, the currents revert to their

non-inactivating character. They hypothesize that the reduction of a disulphide bond that normally restrains the inactivation ball may account for the appearance of inactivating currents. In contrast, increases in channel open times and reopenings occur with inside-out patches of sodium channels compared to cell-attached channel activity (Cannon *et al.* 1991). While some of these experiments show opposite effects, they nevertheless demonstrate the importance of a variety of factors, e.g. voltage, phosphorylation and redox potential of a cell, that chronically modulate the kinetic characteristics of ion currents. Whether these changes result in completely new rate constants between closed, open and inactivated states as has been suggested previously (Hess *et al.* 1984) or in changes in rate constants between particular states is unclear.

Changes in the occurrence of ion channel modes can have profound physiological and pathological effects. For example, an increase in the occurrence of mode 2 activity accounts for the enhancement of cardiac L-type currents by β-adrenergic stimulation (Yue *et al.* 1990). Genetic defects in a skeletal muscle Na^+ channel increase the occurrence of a non-inactivating mode of gating such that transient paralysis in humans can be triggered merely by a small rise in extracellular K^+ (Cannon *et al.* 1991). A number of neurotransmitters inhibit whole cell N-type current in SCG nerve cells by decreasing the rate of current activation (Plummer, Rittenhouse, Kanevesky & Hess, 1991). We have not yet examined the effects of neurotransmitters on either the frequency of occurrence of each of these modes of activity or on changes in the gating within each of these modes. However, with single N-type Ca^{2+} channels, the variety of modal activities may be prime targets for modulation by neurotransmitters. Furthermore, this new understanding of the modal gating of N-type Ca^{2+} channels opens up exciting possibilities for designing new pharmaceutical agents that act to stabilize N-type channels in modes that result in a lower Ca^{2+} influx. This may help minimize cytotoxicity due to over stimulation or acute ischaemia and reperfusion that can occur during epilepsy, stroke or other trauma to the brain.

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