J. Membrane Biol. 132, 13–25 (1993)

The Journal of
Membrane Biology

Potassium Channels in Squid Neuron Cell Bodies: Comparison to Axonal Channels

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Summary. The squid giant axon is formed from the fusion of many axons whose cell bodies are located in the giant fiber lobe (GFL) of the stellate ganglion. We measured macroscopic potassium channel currents in both squid giant axons and in the cell bodies. These currents appear similar in many ways, but were differently affected by the amino group modifying reagent, trinitrobenzene sulfonic acid (TNBS). TNBS increased the steady-state amplitude of the axonal currents but decreased the somal currents. We also studied single K channel currents in the giant fiber lobe cells, for comparison with single K channel currents measured previously in the axons. We found two common classes of K channels, distinguished by their conductances (11 and 25 pS) and kinetic properties. The two channel types resemble the 10 and 20 pS channels that have been described in squid giant axons. These results suggest that the cell bodies and axons express fundamentally similar K channel types. The different effects of amino group modification on macroscopic K currents in the two regions may arise from small (perhaps post-translational) modifications of the core proteins.

Key Words K channels · patch clamp · axons · soma · single channels · amino groups

Introduction

Different parts of the neuron serve different functions. The cell body integrates input from presynaptic sources before generating an action potential. The axon serves to propagate the impulse to the terminal where depolarization may lead to transmitter release. Such spatial segregation of function may depend upon spatially distinct populations of ion channels. This segregation may be investigated using squid giant axons and their cell bodies in the giant fiber lobe (GFL) of the stellate ganglion. Using this preparation, Gilly, Lucero, and Horrigan (1990) found evidence that glycosylation of sodium channels is involved in controlling their spatial localization.

Macroscopic potassium currents in squid giant axons and GFL cell bodies are similar in their activation time course, voltage dependence, and pharmacological sensitivity (Llano & Bookman, 1986) and in their sensitivity to divalent cations (Gilly & Armstrong, 1982; Spires & Begenisich, 1992*b*). These currents do, however, differ in their inactivation kinetics, with time constants near 100 msec for GFL cells (Llano & Bookman, 1986) and over 1 sec for axons (Ehrenstein & Gilbert, 1966; Chabala, 1984).

Llano and Bookman (1986) also investigated single channel potassium currents in GFL cells and concluded that there is a single class of K channels with a conductance near 20 pS. Subsequent studies of unitary currents in the axon revealed three classes of K channels (Llano, Webb & Bezanilla, 1988). These are distinguishable by their conductances of about 10, 20, and 40 pS, and by their kinetic properties.

Since the GFL somata possess the protein synthetic machinery for the axons (Gainer et al., 1984; but *see also* Capano et al., 1987), the absence of the 10 and 40 pS channels in the somata would require a mechanism for the differential expression of channels in the two regions. As a first step in investigating this mechanism, we examined the unitary K channel currents in GFL cell bodies. In contrast to the conclusion of Llano and Bookman (1986), we found that the GFL cell bodies expressed two commonly occurring K channels with conductances near 10 and 20 pS. The distribution of open times of the somal 20 pS channel had two components, as does the axonal 20 pS channel (Llano et al., 1988; Perozo, Jong & Bezanilla, 1991*a*; Perozo et al., 1991*b*). We also observed some single channel activity in the GFL cells consistent with the 40 pS axonal channel.

Treatment of squid axons with amino group

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modifying reagents slows macroscopic K currents and increases steady-state currents at large depolarizations (Spires & Begenisich, 1992*a*). We examined the actions of the amino specific reagent, trinitrobenzene sulfonic acid (TNBS), on both macroscopic and single K channels in GFL cell bodies. We found that TNBS slowed the macroscopic GFL cell currents consistent with the effects on axonal K currents. In contrast to the enhancement of axonal K current, TNBS reduced the macroscopic GFL cell currents. TNBS had no effect on the amplitude of currents through the somal 10 or 20 pS channel or on the open times of the 20 pS channel. We found evidence for a TNBS-induced increase in latency for the 20 pS channel, an effect which may underlie the slowing of macroscopic currents produced by this reagent.

Some of the results have been reported in abstract form (Spires et al., 1988).

Materials and Methods

Biological Preparations

The data in this report were obtained with giant axons and cells from the giant fiber lobe in the stellate ganglia of the squid, *Loligo pealei,* available at the Marine Biological Laboratory, Woods Hole, MA. The GFL cells are the cell bodies whose axons fuse to form the third-order giant axons (Young, 1939). The cells were dissociated using techniques similar to those of Llano and Bookman (1986) and Brismar and Gilly (1987). The dissociated cells were plated on glass coverslips coated with CellTak (Collaborative Research, Bedford, MA) and cultured for several hours to five days before use.

Voltage Clamps

Giant axons were internally perfused and voltage clamped with axial wire techniques that have been described previously (Begenisich & Lynch, 1974; Busath & Begenisich, 1982). Internal (axoplasmic) potentials were measured with a glass pipette filled with 0.56 M KCl. External voltages were measured with an agar-filled, 3 M KCl electrode. Membrane voltages have been corrected for the measured liquid junction potentials.

Macroscopic K channel currents from the GFL cell bodies were obtained with the whole-cell configuration of the patch-clamp technique. We used a circuit of our own design with a wide bandwidth amplifier (model 3554, Burr Brown, Tucson, AZ) and a 10 MΩ feedback resistance. This circuit allowed compensation for 92–95% of the measured series resistance. This high level of compensation was possible because of the quality of the cancellation of stray (fast) capacitance (Sigworth, 1983). When filled with internal solution, the electrodes had resistances below 1 MΩ and often below 0.5 MΩ.

Uncompensated series resistance can, especially with large currents, introduce significant voltage clamp errors (*see* Armstrong and Gilly, 1992, for a recent discussion). While the K channel currents in these cells can be as large as 100 nA (*see* Fig. 1), typical values are 20–40 nA (Spires & Begenisich, 1992*b*). The low pipette resistances and high series resistance compensation of our clamp circuit, resulted in uncompensated series resistances of less than 150 kΩ. Consequently, series resistance errors can be estimated at less than 6 mV for 40 nA currents. Furthermore, we have found the kinetics of the K channel currents to be quite reproducible in spite of large variability in current magnitude (e.g., Spires & Begenisich, 1992*b*)

Cancellation of cell (slow) capacitance was not used in experiments with macroscopic K channel currents. The complete duration of the cell capacity current with series resistance compensation was always less than 50 μsec and often near 35 μsec. Membrane voltages have been corrected for the appropriate liquid junction potentials.

Single channel currents were measured from inside/out patches and, occasionally, from cell-attached patches. These measurements used conventional patch clamp electronics with a 10 GΩ headstage. Pipette resistances were 2–10 MΩ. Most of the cell capacitance was subtracted as described by Sigworth (1983). Residual capacity current was subtracted from single channel records with a template obtained under identical voltage clamp conditions. This template was made either from the average of many records with no channel openings or by fitting an exponential function to a single record with no openings.

Membrane currents were acquired with a 12-bit analog/digital converter controlled by a laboratory personal computer. The voltage-clamp pulses were generated by a 12-bit digital/analog converter controlled by the computer system. For macroscopic current measurements, linear capacitive and leakage currents were subtracted using a -P/4 procedure (Bezanilla & Armstrong, 1977). Currents were filtered at 10 or 20 kHz (macroscopic currents) or at 2 or 3 kHz (unitary currents) with a four-pole Bessel filter.

Solutions

Our standard artificial sea water (ASW) consisted of (in mM): 440 NaCl, 50 $MgCl_2$, 10 $CaCl_2$, 5 to 15 HEPES, pH 7.4. Macroscopic K channel currents from both axons and cell bodies were measured in 50 K ASW: 390 NaCl, 50 KCl, 50 $MgCl_2$, 10 $CaCl_2$, 5 or 10 HEPES, pH 7.4. Axons were internally perfused with a solution containing 350 mM K: 270 K-glutamate, 50 KF, 15 K_2HPO_4, 390 glycine, pH 7.4. The internal (pipette) solution for whole-cell measurements on the cell bodies differed slightly from this solution but contained the same K concentration: 250 K-glutamate, 30 KF, 20 KCl, 20 KOH, 15 K_2HPO_4, 10 EGTA, 350 glycine, pH 7.4. All measurements of macroscopic currents were done at 15°C.

The bath solution for inside/out and cell-attached patches was an elevated K solution that consisted of (in mM): 440 KCl, 5 $MgCl_2$, 175 glycine, 20 glucose, 10 HEPES, pH 7.4. In a few experiments 100 μM $CaCl_2$ was added. Na channel currents, when present, were eliminated with 300 nM or 1 μM tetrodotoxin. The pipette solution for these experiments was the standard ASW described above. Single channel measurements were done at 20–22°C.

As described by Spires and Begenisich (1992*a*), treatment of giant axons with amino group reagents significantly modifies macroscopic K channel currents. In the experiments reported here, amino group modification was produced by treatment with 2.5 or 5 mM trinitrobenzene sulfonic acid (TNBS) added to ASW or 50 K ASW. Because TNBS reacts with the neutral form of amino groups, treatment was done in solutions of elevated pH

(usually pH 8.2 or 9.0 buffered with 15 mM CHES). Control experiments showed that treatment with the elevated pH solutions in the absence of TNBS had no irreversible effects. More details on amino group modification and TNBS action on squid axons can be found in Spires and Begenisich (1992*a*).

Data Analysis

The quantitative analysis of our data included the fitting of exponential time functions to macroscopic current records. The final approach to steady-state K channel current can be fit by a single exponential time function for both axons (White & Bezanilla, 1985; Spires & Begenisich, 1989) and cell bodies (Spires & Begenisich, 1992*b*). Therefore, we obtained an estimate of the K channel ionic current activation time constant by fitting an exponential function between 50–65% and 90–95% of the current maximum. Ionic tail currents were also fit with a single exponential time function from 90–95% of maximum to about 20% or less. Time constants obtained in this way are called deactivation time constants. All fits of theoretical functions used the "simplex" algorithm (Caceci & Cacheris, 1984).

The major problem in measuring the kinetic properties of single K channels in the cell bodies was the high channel density. Most patches contained more than three channels and none contained only a single channel. The same problem exists in the axon and Llano et al. (1988) used a depolarized holding potential to reduce (via the slow inactivation process) the number of active channels. We also used this approach for many patches.

Analysis of single channel current data included current amplitude, open time, and first latency histograms. Two types of amplitude histograms were obtained. In the first method, all data points at the test potential were used. In the second, only selected sections of the data were included. These methods are discussed further in Results. The data in both types of histograms were fit by the sum of n Gaussian functions of the form:

$$N = A_1 e^{-\frac{1}{2}\left(\frac{1}{\sigma_1}\right)^2} + \sum_{k=2}^{n} A_k e^{-\frac{1}{2}\left(\frac{i<i_k>}{\sigma_k}\right)^2}$$

where i is the current amplitude, N is the number of events at that amplitude. A_k, $<i_k>$, and σ_k characterize the amplitude, mean, and dispersion of the k^{th} Gaussian component. Note that the first Gaussian component is defined to have a mean value of 0. Since all the amplitude histograms have a component at 0 current, this is an appropriate simplification and has the advantage of eliminating one free parameter from the fit.

Determining the distributions of channel open times was complicated by the presence of two or more channels in our patches. There were often two or more overlapping channel openings in the data records; when two channels overlapped, the open durations were randomly assigned (Aldrich, Corey & Stevens, 1983; Hoshi & Aldrich, 1988). Records with more than two overlapping channels were discarded. Open times were determined from idealized records with a threshold of 50% of mean channel current amplitude (Colquhoun & Sigworth, 1983). Open time histograms were fit with a function consisting of one or two exponential components.

Time to first opening (first latency) histograms were also determined from idealized records. As with open time determinations, the presence of several channels in a patch complicates the interpretation of first latency data since it is impossible to tell which of several channels was the first to open. Corrections can be applied if the number of channels is known (Patlak & Horn, 1982; Aldrich et al., 1983). For n channels in the patch, the correction involves taking the n^{th} root of the multi-channel cumulative distribution. Even for only three channels, this correction is large and requires a large number of observations to produce a reasonably smooth distribution. Uncorrected first latency distributions, however, can be used to compare patches containing the same number of channels. This is the method we used.

Results

Amino Group Modification of Axonal and Cell Body K Currents

Although macroscopic K currents of the giant axons and GFL cells appear similar in many respects, they differ in their responses to amino group modification induced by TNBS treatment. This difference is illustrated in Fig. 1. The currents in the upper and lower panels were recorded from an axon and a cell body, respectively, under the same conditions. The currents on the left side of each panel were recorded before treatment with TNBS and the currents on the right were recorded after treatment. The upper panel shows that amino group modification slowed both activation and deactivation of axonal K channel current as previously described (Spires & Begenisich, 1992*a*). The kinetics of the currents in GFL cells were similarly modified (lower panel).

The kinetic effects are analyzed quantitatively in Fig. 2, where the voltage dependence of K current time constants is shown before (open symbols) and after (filled symbols) TNBS treatment, for the axon (circles) and the soma (squares) of Fig. 1. Not only were the control time constants similar for the axon and cell body but TNBS slowed the channel kinetics by nearly identical amounts.

In contrast to the similarity of the kinetic effects induced by amino group modification, the steady-state current levels in the axon and cell body responded differently to TNBS treatment (Fig. 1). Axonal currents in response to steps to about 60 mV are increased by 20–65% after TNBS treatment, and the increase is independent of holding potential (Spires & Begenisich, 1992*a*). In 18 similar experiments on GFL cell bodies, only one cell showed an increase in current and this was only 7%. This cell was treated with 2.5 mM TNBS at pH 8.2 for 20 min. In the other 17 cells, TNBS reduced the current by an amount that depended somewhat on the treatment conditions. Treatment with 2.5 mM TNBS at pH 8.2 for 13–15 min reduced the current by 14 ± 2% (SEM, n = 3). Treatment for 3–4 min with 2.5 mM TNBS at pH 9 produced a 14 ± 1% (4) reduction. One cell treated at pH 8.6 with 2.5 mM TNBS for 9

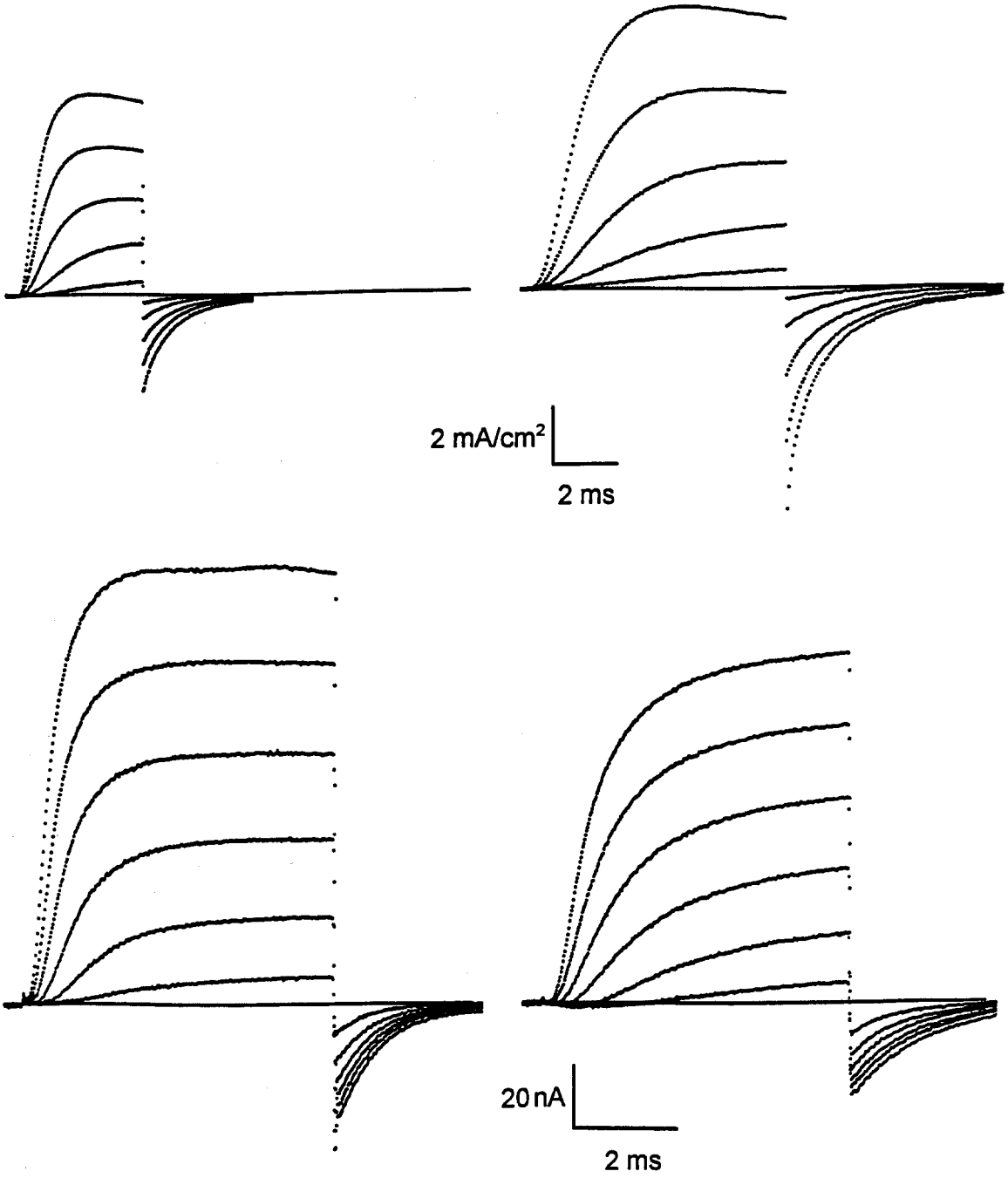

Fig. 1. TNBS modification of K channel currents. (Upper) Axonal K channel currents before (left) and after (right) a 6-min treatment with 2.5 mM TNBS at pH 9. The currents (in pH 7.4, 50 K ASW) shown were produced by voltage steps to −23, −3, 17, 37, and 57 mV from a holding potential of −63 mV. Calibration bars: 2 mA/cm², 2 msec. Axon SQDXP. (Lower) Cell body K channel currents before (left) and after (right) a 5.5-min treatment with 5 mM TNBS at pH 9. The currents (in pH 7.4, 50 K ASW) shown were produced by voltage steps to −23, −8, 7, 22, 37, and 52 mV from a holding potential of −68 mV. Calibration bars: 20 nA, 2 msec. Cell body CELDZ.

min showed a 48% decrease. Currents were reduced by 20 and 30% in two cells treated for 5.5 and 6 min with 5 mM TNBS at pH 9. The most severe treatment (5 mM at pH 9 for 9–10 min) reduced the currents by 43 ± 4% (7).

Single K Channels in Cell Bodies

In order to learn more about the mechanism of TNBS action, we measured some of the properties of single K channels in the GFL cell bodies before

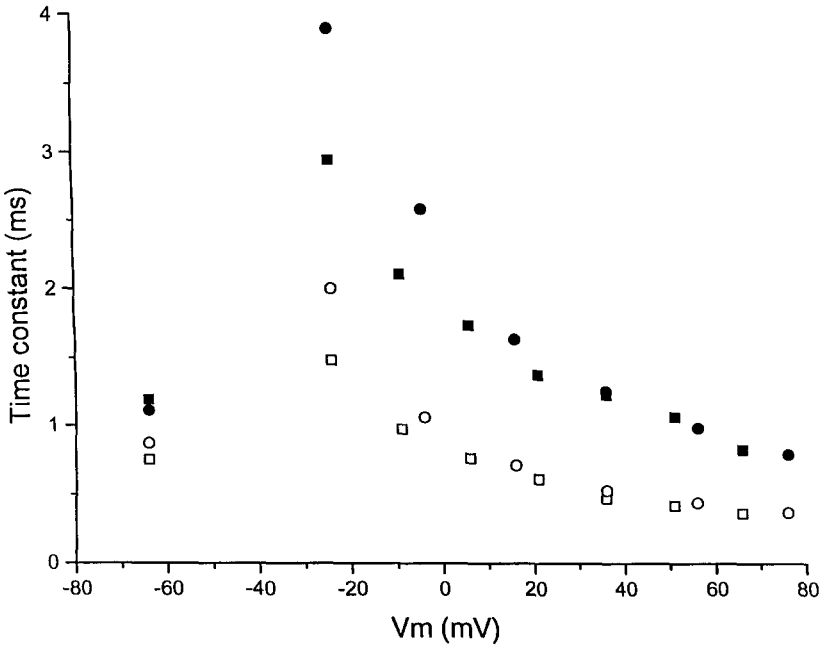

Fig. 2. K channel time constants and TNBS treatment. K channel time constants before (open symbols) and after (filled symbols) TNBS treatment of an axon (circles) and a cell body (squares). The values at −68 mV are deactivation time constants; the rest are activation time constants.

and after TNBS treatment. The measurements in untreated cells also allow comparison to previous descriptions of axonal channels.

The inset of Fig. 3 shows current records at +4 mV from an inside/out patch of a GFL cell. This patch contained two or more channels, as indicated by the double openings in some of the current records. Inspection of the records suggests that the channels in the patch had the same current level. In order to quantitatively determine the unitary current levels, we constructed an all-points histogram of current amplitude as described in Materials and Methods. This histogram constitutes the main part of Fig. 3. There are three peaks in this histogram and the data are reasonably described by a sum of three Gaussian components. The two nonzero peaks are at 2.0 and 3.9 pA, consistent with the activity of two channels with identical current levels of about 2 pA.

Currents from another, similar experiment, also at +4 mV, are illustrated in the inset of Fig. 4. Again, a single unitary current level is apparent and the main part of the figure shows the all-points histogram. The nonzero components in the histogram were 1.47 and 3.1 pA, consistent with the activity of two identical channels with an open channel current level of about 1.5 pA. This level is rather different than the value of 2 pA of Fig. 3 even though the recording conditions were identical. This difference suggests that the cell bodies express two different types of K channels, distinguished by their single channel current levels. This suggestion is strengthened by the data illustrated in Fig. 5.

The inset of Fig. 5 contains records from an inside/out patch at 24 mV. Unlike the data in the insets of Figs. 3 and 4, there appear to be two different unitary current levels: one of about 3 pA (top two records of the inset) and a smaller one (middle trace). Both levels are apparent in the bottom two records. The associated all-points histogram is more complex than those in Figs. 3 and 4 and seems consistent with the two current levels seen in the inset. In order to better resolve the underlying unitary currents, we determined amplitude histograms by including only the small or the large channel level. An example of this analysis for the data of Fig. 5 is illustrated in Fig. 6.

The upper panel shows the amplitude histogram of only small amplitude openings. These data are fit by the sum of two Gaussian components (solid line), with a nonzero level of 1.38 pA. The lower panel is the histogram with isolated large channel openings. These data are fit by the sum of three Gaussian components with nonzero levels of 2.7 and 5.3 pA. While 1.38 and 2.7 are close to a factor of two apart, the probability that all the large channel events are two simultaneous openings and closings of the smaller level is extremely low. Indeed, the data in Fig. 7 show that the two channel levels found were not, in general, a factor of two different.

In Fig. 7, unitary current levels from inside/out patches of both the large (○) and small (△) current events are shown at various membrane potentials. These two levels are well separated at large depolarizations but are not far apart at potentials below 0 mV. Linear fits to the data are shown as solid lines.

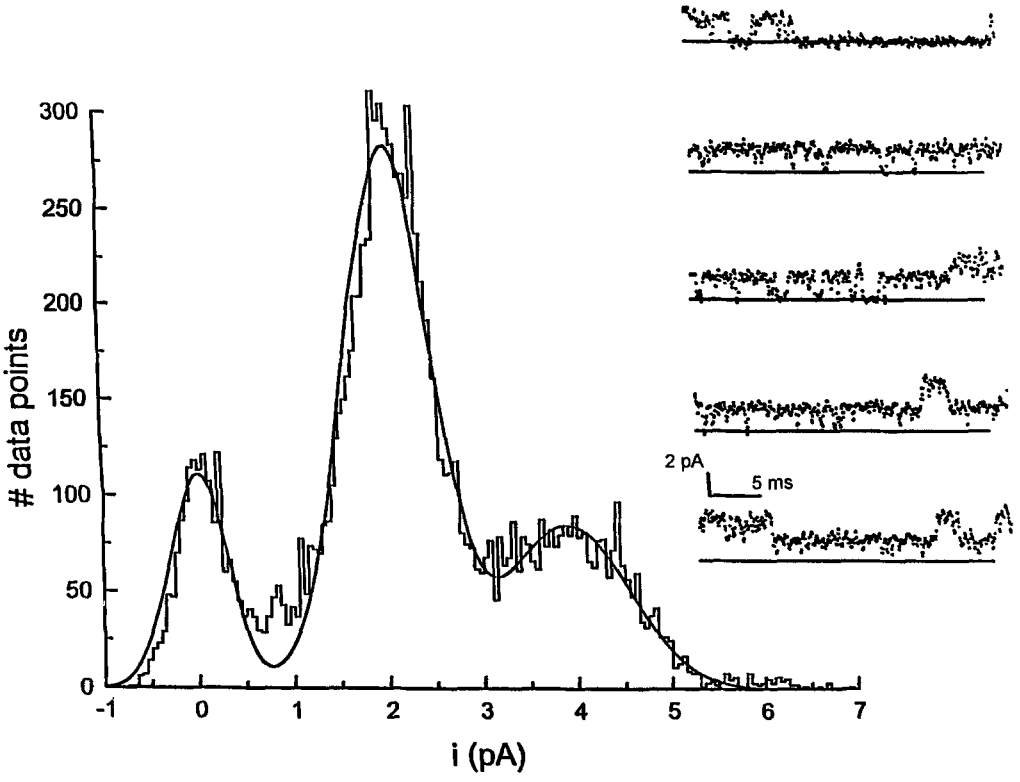

Fig. 3. Cell body single K channel currents—25 pS channel. (Inset) Selected records recorded at a maintained potential of +4 mV. Calibration Bars: 2 pA, 5 msec. (Main) All-points histogram from approximately 60 records similar to those in the inset. The solid line is the fit of a function consisting of three Gaussian components. The current levels at the Gaussian peaks from the fits are 0.0, 2.0, and 3.9 pA.

The slopes of these lines establish single channel, slope conductances of 11 and 25 pS for the two channel types (referred to as the 10 and 25 pS channels).

It is difficult to quantify the relative occurrence of the two channel types. Most patches had too many channels to allow analysis. A few patches had only one channel type; some had both. The appearance of the two channel levels was not a result of patch excision as on-cell patches also exhibited 25 pS (□) and 10 pS levels (▽).

Also included in Fig. 7 are unitary currents obtained from cells that had been treated with TNBS. These included both 10 (▲) and 25 pS (●) levels from inside/out patches and a 10 pS channel from a cell-attached patch (▼). There does not appear to be any clear effect of TNBS treatment on the single channel current amplitudes.

Almost all of the channel openings in hundreds of records from dozens of patches could be identified with either the 10 or the 25 pS channels. However, in six records from four patches, very large current levels were found. These are shown in Fig. 8. The top three records were obtained from three different patches at +64 mV. In the top record, two levels are seen: an approximately 2 pA opening, consistent with the 10 pS channel type, and a much larger level of about 6 pA. In the third record, there appears to be an approximately 6.5 pA opening and, later in time, an additional opening of about 3.5 pA. This smaller level is about that expected from the 25 pS channel at this voltage. The lower two records (at +4 mV) contain openings of 3.5 to 4 pA. These very large current levels at +4 and +64 mV represent a slope conductance of 40–50 pS.

In patches with three or fewer channels, as described in Materials and Methods, we were able to obtain histograms of the open times for the 25 pS channel. The upper panel of Fig. 9 shows one such histogram obtained from data recorded at 4 mV. The number of observations with openings in the first bin is underestimated because of the effect of filtering on event detection (Colquhoun & Sigworth, 1983). The lines in the figure are the results of fitting a one- (dashed line) or two-exponential (solid line) function to the data (not including the first bin). The two-exponential function appears to be a better representation of the data and has time constants of 0.16 and 1.4 msec.

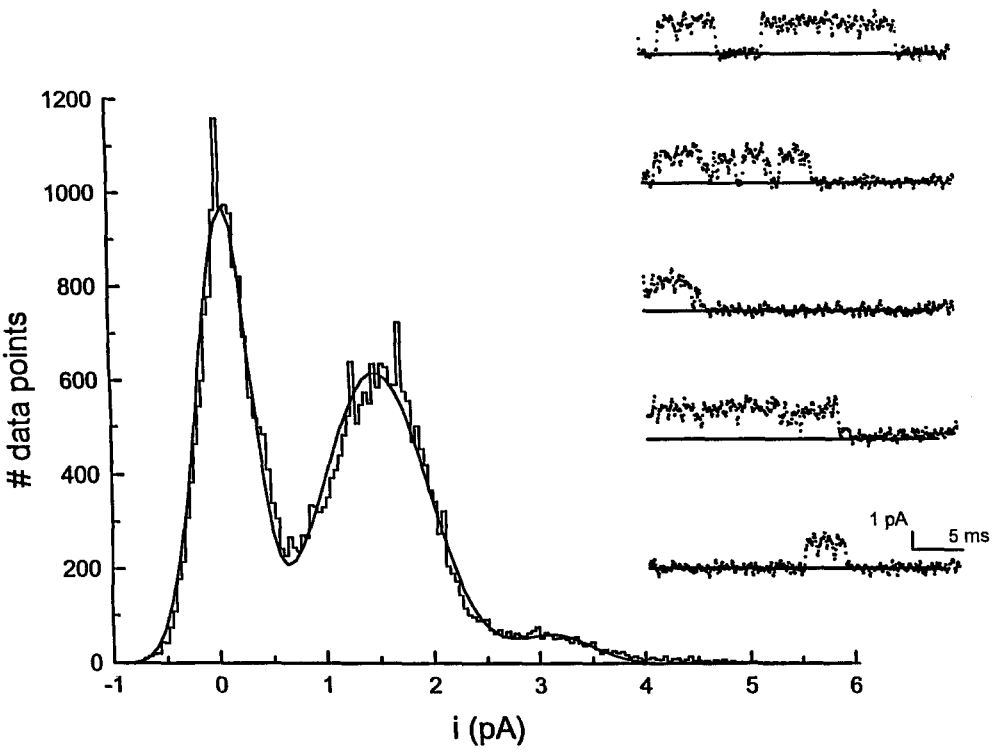

Fig. 4. Cell body single K channel currents—10 pS channel. (Inset) Selected records recorded at a maintained potential of 4 mV. Calibration Bars: 1 pA, 5 msec. (Main) All-points histogram from approximately 100 records similar to those in the inset. The solid line is the fit of a function consisting of three Gaussian components. The current levels at the Gaussian peaks from the fits are 0.0, 1.47, and 3.1 pA.

The fast time constant from the two-exponential fit in the upper part of this figure is not much slower than the approximately 0.11 msec rise time of the Bessel filter (at 3 kHz) used in data collection (Colquhoun & Sigworth, 1983). Thus, this parameter cannot be determined with suitably high reliability. We used only the slower time constant for further analysis.

The lower panel of Fig. 9 shows the slow time constant at several membrane voltages. These data are from five inside/out patches and one on-cell patch that include untreated (open symbols) and TNBS treated cells (filled symbols). There appears to be, at most, only a slight voltage dependence of the slow component and no significant effect of TNBS treatment.

One hallmark of delayed rectifier macroscopic current is the sigmoid time course of channel activation in response to membrane depolarization. This delay is due to transitions through many closed channel conformations (White & Bezanilla, 1985; Spires & Begenisich, 1989). At the single channel level, these processes control the time to first channel opening. To investigate whether TNBS exerted its effect on K current kinetics (Fig. 1) by slowing the time to first opening, we compared first latency distributions for untreated and TNBS-treated cells. Such a comparison for two patches, each containing three channels, is illustrated in Fig. 10. Time-to-first-latency data from an untreated cell are plotted with dashed lines and data from a TNBS-treated cell as solid lines. In the untreated patch, all channel openings occurred within 1.2 msec. In the TNBS-treated patch, in contrast, one-third of the openings occurred after 1.2 msec with several openings more than an order of magnitude later. Any openings later than 30 msec (the duration of the voltage pulse) would not have been observed.

Discussion

Similarities between K Channel Types in Axons and Cell Bodies

Llano et al. (1988) measured single K channels from the giant axon by patching the internal surface of the cut-open axon preparation. They reported three single channel levels of approximately 10, 20, and 40 pS conductance. In GFL cell bodies, Llano and Bookman (1986) reported only a single class of K channels with a conductance near 20 pS.

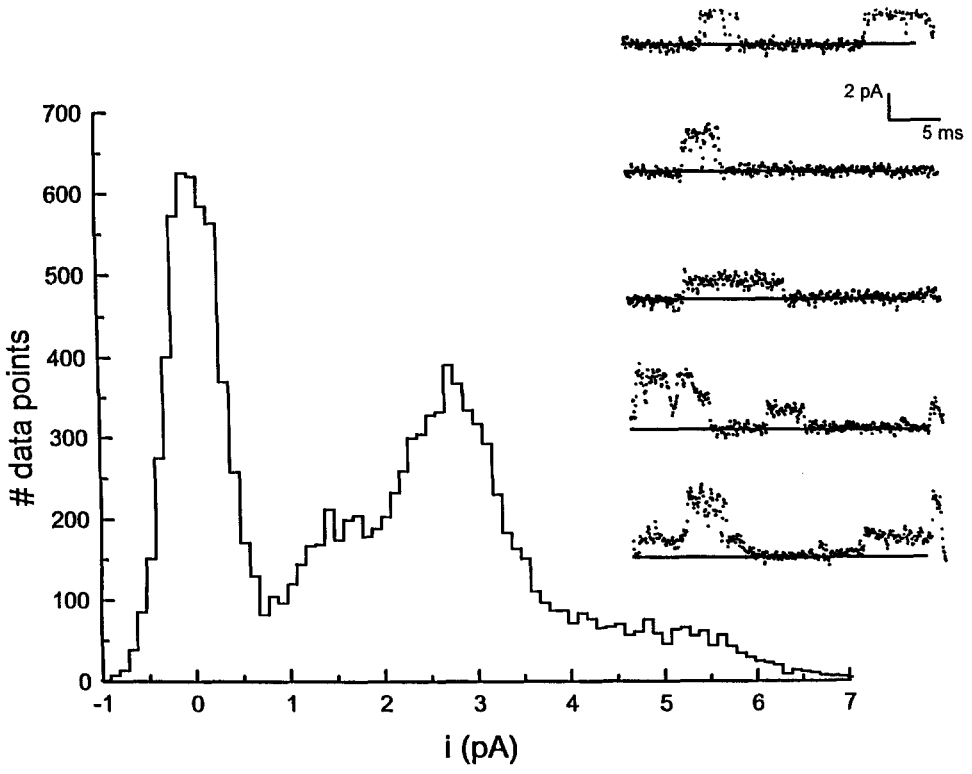

Fig. 5. Cell body single K channel currents—both 10 and 25 pS channels. (Inset) Selected records recorded in response to potential steps to 24 mV from a holding potential of 4 mV. Calibration bars: 2 pA, 5 msec. (Main) All-points histogram from approximately 80 records similar to those in the inset.

20 pS Channel

The 20 pS channel identified in axon patches has all the appropriate properties to produce the macroscopic delayed rectifier K current. It activates within a few msec (Llano et al., 1988) and has a large, voltage-dependent open probability (Perozo et al., 1991*b*). These channels in axon patches do not undergo inactivation on the time scale of a few tens of msec but are subject to slow inactivation (Llano et al., 1988; Perozo et al., 1991*b*).

The single channel currents measured by Llano and Bookman (1986) in GFL cells were from outside/out patches at 10–12°C with external and internal K concentrations of 5 and 530 mM, respectively. Although the lower K and higher temperature in our experiments complicate comparison, these two differences would tend to have opposite effects on channel conductance. Therefore, it seems likely that the 25 pS channel that we observed and the 20 pS channel reported by Llano and Bookman are the same.

The distribution of open times of the axonal 20 pS channel has two components (Llano et al., 1988; Perozo et al., 1991*b*) We found that the distribution of open times of the 25 pS channel in GFL cells also had two components, consistent with a report on these cells in abstract form (Llano, Armstrong & Bookman, 1986). In Fig. 9 (lower panel), we compare the slow component of our open time data with the slow components from the axonal (+, Llano et al., 1988) and GFL 20 pS channel data (x, Llano et al., 1986). These earlier studies were done at 13 and 10°C and our data were obtained at 20°C. We assumed a thermal Q_{10} of 3 to convert these earlier data to values comparable to our data. With this correction, there is little apparent difference in the open times of the axonal 20 pS channel and the GFL 25 pS channel.

10 pS Channel

The smallest channel (10 pS) reported in axons has a longer latency than the 20 pS channel and exhibits less flicker (Llano et al., 1988). There are no reports of open or closed times of this channel type. While Llano and Bookman (1986) reported only 20 pS channels in GFL cells, we observed a channel with a slope conductance of 11 pS, near the axonal value of 10 pS. The 11 pS cell body channel exhibited latency and flicker properties consistent with the

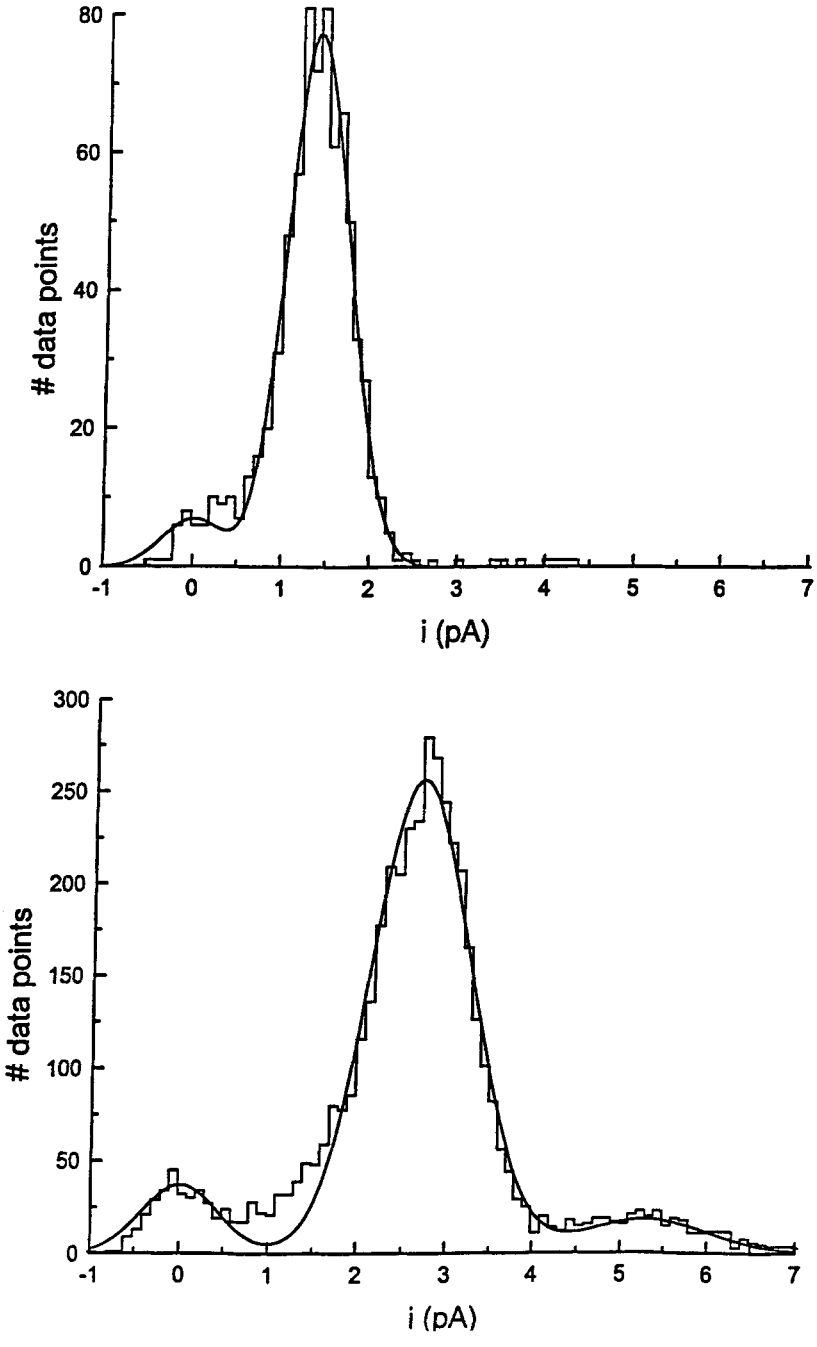

Fig. 6. Separation of single channel current levels. (Upper) Histograms of data from the 10 pS channel obtained as described in Materials and Methods. Solid line is a fit of the sum of two Gaussian components with the nonzero peak at 1.38 pA. (Lower) Histogram of data from 25 pS channel. Solid line is a fit of the sum of three Gaussian components with nonzero peaks at 2.7 and 5.3 pA.

axonal 10 pS channel, but a quantitative comparison is hampered by the absence of published data on these kinetic properties.

Llano et al. (1988) observed the 10 pS channel in axons only after exposing the axoplasmic surface to a 0 K solution which reduced the activity of the 20 pS channel, presumably inactivating it by depolarization. They suggested that the 10 pS channel might by induced by the 0 K solution or might even be a converted form of the 20 pS channel. Our results show that the 10 pS channel is likely to be a separate channel type, at least in cell bodies. It occurred often in our excised patch experiments (with K^+ ions present on the axoplasmic side), occasionally as the only channel type in a patch. Furthermore, it was found in cell-attached patches with the cell and its membrane intact.

40 pS Channel

Llano et al. (1988) found the activity of the very large (40 pS) axonal channel to be quite variable. The

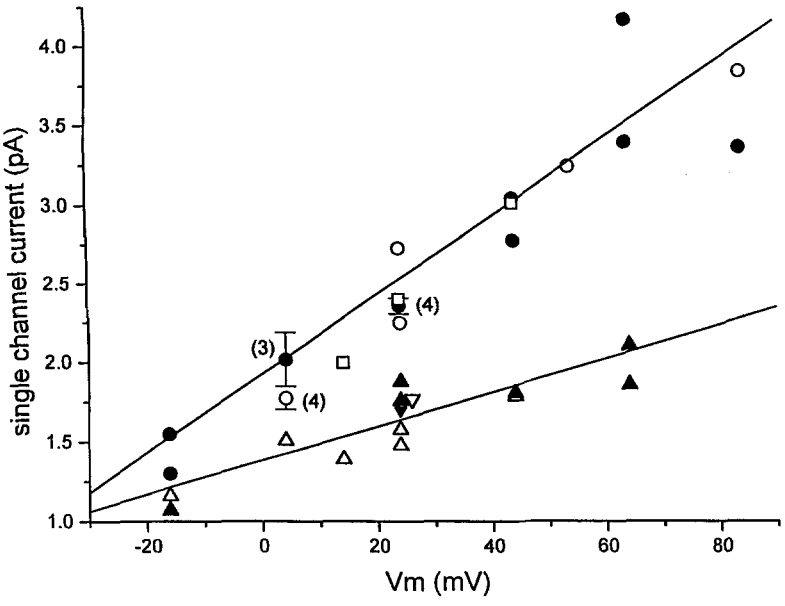

Fig. 7. Single channel current-voltage relations. Currents from both untreated (open symbols) and TNBS treated cells (filled symbols). Data include 25 pS (○, ●) and 10 pS (△, ▲) conductance channels from inside/out patches. Symbols represent single measurements except where mean and SEM limits are shown with number of measurements in parentheses. The lines are least-squares fits to the data. The slopes of these lines represent 11 and 25 pS conductances. Measurements from four on-cell patches are included for untreated, 25 pS (□) and 10 pS channels (▽, displaced by 2 mV for clarity) and for TNBS-treated, 10 pS (▼) channels. The holding potentials of five of the nine inside/out patches and three of the four on-cell patches were −40 to −60 mV; the rest of the patches were maintained at a depolarized (usually 0 mV) potential.

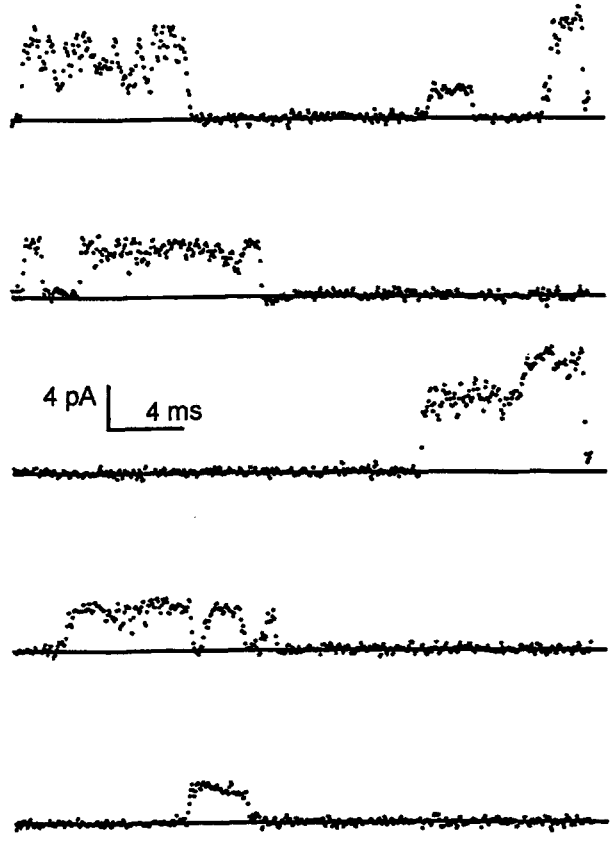

Fig. 8. Very large conductance openings. Data from five patches at two potentials: +64 mV (top three records) and +4 mV (bottom two records). All but the bottom record were from TNBS treated cells. Calibration bars: 4 pA; 4 msec.

open probability increases in conditions designed to promote protein phosphorylation but to a maximum level of only 0.14 (Perozo et al., 1991*a*). This channel also activates very slowly (seconds) with depolarization (Perozo et al., 1991*a*). For these reasons, this channel is unlikely to underlie the delayed rectifier K current in axons. We observed large unitary currents (Fig. 8) in a few records out of many hundreds. While these large openings appear consistent with the axonal 40 pS channel, their rare occurrence in the cell bodies precludes a definitive comparison.

In summary, we have observed in GFL cell bodies two commonly occurring channels with conductances (11 and 25 pS) similar to those of the two main axonal K channels described by Llano et al. (1988). The open times of the 20 and 25 pS channels are also similar. We also observed a few very large unitary currents consistent with the third (40 pS) channel type seen in axons. These observations suggest that all three channel types in the axon are also expressed in the cell bodies. On the other hand, there do appear to be some differences between the channels in axons and cell bodies, as discussed in the next section.

Differences between Axonal and Cell Body K Channels

The macroscopic currents in giant axons and GFL cell bodies responded differently to TNBS modification: both types of currents were slowed by modification but axonal steady-state currents increased and cell body currents decreased. This was true even

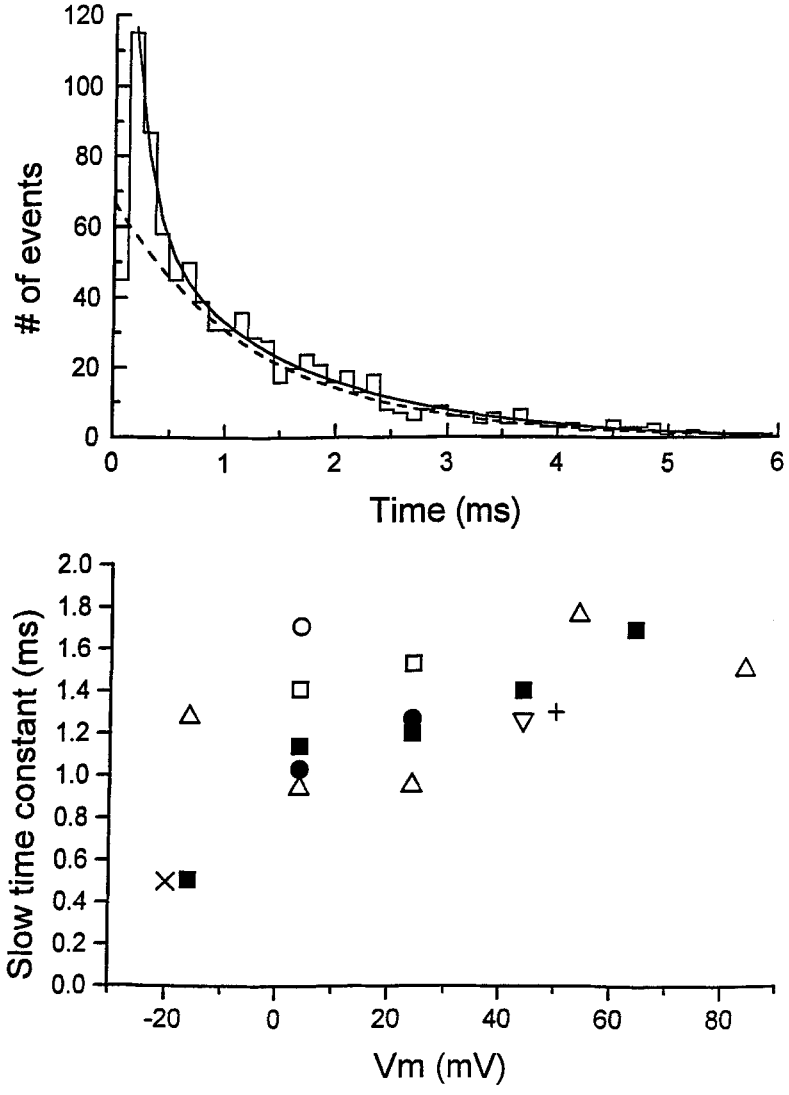

Fig. 9. Open times for the 25 pS channel. (Upper) Distribution of open times at +4 mV. Dashed line is a single exponential time function fit to the data with a time constant of 1.3 msec. The solid line represents a two-exponential fit with time constants of 0.16 msec and 1.4 msec. (Lower) Slow time constant for the 25 pS channel. Ordinate values obtained from the slower component of the fit, as shown in the upper part of the figure. Each symbol represents a different patch and the data include untreated (open symbols) and TNBS-treated cells (filled symbols). All data from inside out patches except for (▽) which was an on-cell patch; (×) and (+) are computed (as described in text) from outside-out patch data from a GFL cell and a giant axon, respectively.

for treatment conditions milder than those used on axons (Spires & Begenisich, 1992*a*).

In addition, there may be a difference, in the inactivation properties of the channels in the giant axons and GFL cell bodies. Llano and Bookman (1986) added together their single channel records from the GFL cell bodies to form a representation of the ensemble behavior. The ensemble current exhibited substantial inactivation (within 100 msec at 0 mV and 10–12°C) not seen with ensemble averages of axonal single channel currents (Llano et al., 1988; Hille, 1992, p. 69). These results are consistent with the inactivation properties of the macroscopic currents from the two cell regions. Macroscopic K channel current in the GFL cell bodies exhibits inactivation with time constants on the order of 100 msec (Llano & Bookman, 1986). Inactivation of axonal K channel currents studied with the axial wire voltage-clamp method has a much slower time course, on the order of seconds (Ehrenstein & Gilbert, 1966; Chabala, 1984), but has not been examined at positive potentials due to the complications induced by K^+ accumulation in the periaxonal Schwann cell space surrounding the axon (Adelman, Palti & Senft, 1973).

Mechanism of Action of TNBS

Amino group modification with TNBS both slowed and decreased the macroscopic K currents in GFL cells (Fig. 1). TNBS modification had no detectable effect on the current level of either the 10 pS or 25 pS channel type (Fig. 7). Thus, the decrease in steady-state macroscopic K currents is not due to reduced single-channel current levels. Consequently, the current decrease must be due to either

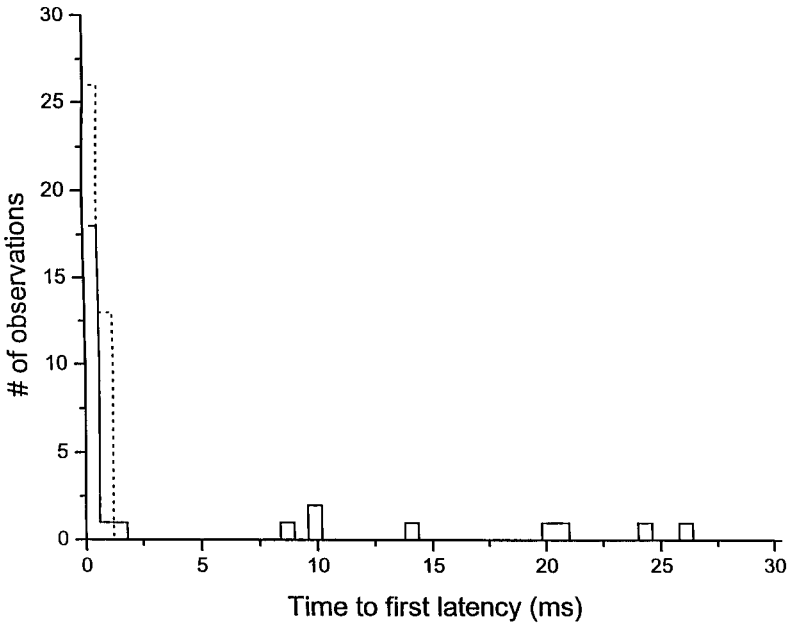

Fig. 10. Time to first opening histogram. Latency data from steps of the membrane potential to +44 mV. The histogram illustrated by the dashed line is from a patch with three 25 pS channels. The histogram in solid lines is a three-channel patch from a cell body treated with TNBS. The first latencies were determined as described in Materials and Methods.

a reduction of the number of channels or the probability of channel opening (or both). We do not have the data to distinguish between these two mechanisms.

The kinetic effect of TNBS on GFL currents did not result from any change in the slow component of the open time distribution for the 25 pS channel (Fig. 9). While the large number of channels in our patches made analysis difficult, the data of Fig. 10 suggest that TNBS had a significant effect on the opening latency of the 25 pS channel. Thus, it may be TNBS slows channel kinetics by reducing rate constant(s) between closed states.

Conclusion

Our findings suggest that the three types of K channels present in the axon are also expressed in the cell bodies. Thus, it is not necessary to postulate a special mechanism for the differential expression of these channels in the two regions of the neuron. On the other hand, the differences in inactivation and the differential effects of TNBS on K currents in these two cell regions, suggest that these protein channels are not identical. It may be that, as for Na channels in this same preparation (Gilly et al., 1990), differences between the properties of channels in the axon and cell body arise from differences in post-translational modifications.

We are grateful to the Director and staff of the Marine Biological Laboratory for their assistance. We thank Drs. F. Bezanilla and W. F. Gilly for reading the manuscript. We also thank Gerry Harris and Martin Gira for construction of the recording apparatus and voltage-clamp electronics, and John Young of Scientific Associates, Rochester, NY, for help with the data acquisition system.

This work was supported by U.S. Public Health Service grant NS-14138.

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Received 25 June, 1992; revised 2 October 1992