PROPERTIES OF POTASSIUM AND SODIUM CHANNELS IN FROG INTERNODE

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SUMMARY

1. Voltage-clamp experiments were performed on single frog internodes after acute demyelination with lysolecithin. The action of lysolecithin was stopped by washing out the lysolecithin with normal Ringer solution containing bovine albumin when the first delayed current was observed. After washing, the temperature was lowered from 25 to 15 °C. These procedures greatly prolonged the survival of the demyelinated internode up to 1 h.

2. External tetraethylammonium chloride (TEA⁺, 110 mM) reduced the K⁺ current in the internode only to 11 % of the control value. 110 mM-TEA⁺ increased the time constant τ_n of K⁺ activation by a factor of two in the node and by a factor of four in the internode. 120 mM-CsCl at the cut ends of the fibre also reduced the delayed outward current recorded at 60 mV in the internode to 11% of the control value, hardly changing the time constant τ_n .

3. After a depolarization, the K⁺ tail current decayed in two phases, suggesting that the K⁺ conductance of the internodal membrane may be composed of at least two components, a slow one (g_{Ks}) and a fast one (g_{Kf}) . As in the node, the fast K⁺ conductance of the internode can be further decomposed into two components $(g_{Kf1}$ and g_{Kf2}) with different activation potential ranges. The fast phase of the tail current was blocked by external application of 1 mM-4-aminopyridine (4-AP). The slow phase was almost unaltered by 1 mM-4-AP. The extrapolated slow tail current was 33 % of the total tail current in the internode and 15 % at the node, i.e. the proportion of slow K⁺ channels is larger in the internode than in the node.

4. Tetrodotoxin (TTX)-sensitive transient inward currents could be measured in the demyelinated internode, provided the large K^+ currents were blocked by internal Cs⁺. The time course, TTX sensitivity, reversal potential and steady-state inactivation of the transient early inward current indicate that this current is caused mainly by Na⁺ passing through Na⁺ channels.

5. The density of K^+ and Na^+ channels in the demyelinated internode is estimated from the size of the K^+ and Na^+ current, respectively, and the capacity of the demyelinated segment. The K^+ channel density of the internode seems to be about 20 times smaller than in the node, whereas the Na^+ channel density in the internode appears to be about 500 times smaller than in the node.

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INTRODUCTION

In frog myelinated nerve fibres, K^+ channels are not restricted to the node but also occur in the internode (Chiu & Ritchie, 1982). During acute demyelination of a single frog internode, the internodal segment shows a steady increase in capacity and leakage current, after which a delayed outward current is revealed. The sensitivity to tetraethylammonium chloride (TEA⁺) and a K⁺-dependent reversal potential led Chiu & Ritchie (1982) to suggest that this delayed current is a K⁺ current similar to that normally present in the frog node and in squid nerve.

The purpose of my experiments was to investigate whether the three components of K^+ current that have been found in the node of Ranvier (Dubois, 1981*a*; see also Plant, 1986) are also present in the internode. In the node, K^+ tail currents decrease in two phases: a fast and a slow one. From measurements of the activation potential range and the inactivation time constants of the fast K^+ conductance, Dubois (1981*a*) suggested that the fast K^+ conductance can be decomposed into two components. This means that the K^+ conductance of the nodal membrane is composed of three components which may correspond to three different types of K^+ channels. My experiments show that these three types of K^+ channels are also present in the internode.

After blocking the internodal K^+ currents with internal Cs^+ , I was able to record small internodal Na⁺ inward currents. This contrasts with the results of Chiu & Ritchie (1982), who did not observe any Na⁺ inward current after demyelination. Likewise, in binding experiments (Ritchie & Rogart, 1977) and cytochemical studies (Quick & Waxman, 1977) no evidence for the presence of Na⁺ channels in the internode was obtained. In freeze-fracture investigations (Kristol, Sandri & Akert, 1978), however, particles that seem to be the voltage-sensitive Na⁺ channels because they are concentrated at nodal regions, appear also in the internode with a density about 100 times smaller than in the node.

Some of the results have been reported in a preliminary communication (Grissmer, 1986).

METHODS

The experiments were carried out on single myelinated nerve fibres of the frog Rana esculenta. Only sensory fibres were investigated, because in sensory fibres the two fast components of K⁺ conductance (g_{K}) are more easily distinguished, at least in the node (Dubois, 1981a). They were isolated from the sensory branch (ramus cutaneus cruris posterior) innervating the skin above the m. gastrocnemius (Stämpfli & Hille, 1976). Membrane currents were recorded under voltage-clamp conditions (Nonner, 1969). The pool A, which would normally contain a node in conventional nodal voltage-clamp studies, now contained a small segment of internode. The two nodes of Ranvier to the left and right of this segment lay in the two end-pools (C and E). The fibres were cut on both sides of the investigated internode at a distance of about 0.75 mm; thereby, the neighbouring nodes were removed. Normally, the end-pools contained 120 mm-KCl. The normal Ringer solution was composed of (mM): NaCl, 110; KCl, 2.5; CaCl₂, 1.8; Tris HCl buffer, 5; pH 7.1-7.3. In KCl Ringer solution, NaCl was replaced by KCl. For acute demyelination, the segment in pool A was exposed at 25 °C to a Ringer solution containing 0.2% (w/v) lysolecithin (Sigma) prepared immediately before use. To stop demyelination, the lysolecithin was washed out with normal Ringer solution with bovine albumin (2% (w/v)). After 10 min wash-out, the solution was changed to normal Ringer solution and the temperature was lowered from 25 to 15 °C. This procedure prolonged the survival of the demyelinated fibres up to 1 h.

The absolute values of the membrane potential in the internode were determined in a few experiments as the voltage displacement in pool A when the naked internodal axolemma was destroyed by a strong hyperpolarizing pulse at the end of the experiment. The average absolute membrane potential determined in this way was $-69 \pm 2 \text{ mV}$ (mean $\pm \text{s.e.}$ of mean; n = 6). In most experiments the membrane potential was assumed to be -70 mV. The internode was held at a 20 mV more negative value, i.e. at an assumed holding potential of -90 mV.

In control experiments with a node in pool A, the holding potential was adjusted so that the peak Na⁺ current was 70% of the maximum Na⁺ current measured after a -40 mV pre-pulse of 50 ms duration (the steady-state value, h_{∞} , of the inactivation variable, h (Hodgkin & Huxley, 1952 a, b) is 0.7). For $h_{\infty} = 0.7$ the membrane potential can be assumed to equal the normal resting potential of -70 mV (see Frankenhaeuser, 1959). Subsequently, the holding potential was shifted to -90 mV. It remained at -90 mV during the entire experiment. In experiments on the node, 310 nm-tetrodotoxin (TTX) was added to all solutions to block Na⁺ currents totally.

The command voltage pulses were generated by a microcomputer (DEC LSI 11/23), which was also used for sampling the data at 10 and 100 μ s intervals and for off-line analysis. All current records were filtered with a 10 kHz low-pass filter and stored on floppy disks. The pulse duration was 5 or 50 ms. If not otherwise mentioned, currents and tail currents were corrected for capacitive and leakage currents. For this purpose, the current associated with a -100 mV pulse of 5, 50 or 100 ms duration was measured before each run. The leakage current was the current at the end of this hyperpolarizing pulse. The capacitive current was the current remaining after subtraction of the leakage current. Leakage current and capacitive current were multiplied by -1, suitably scaled and subtracted from the total current.

The capacitive current recorded from the demyelinated internode consisted of a fast and a slow component (see Fig. 3C of Chiu & Ritchie, 1982). A double-exponential fit of the integrated capacitive current gave the charges Q_t and Q_s associated with the fast and slow component, respectively. The time constants of the two components ranged from 40 to 70 μ s and from 0.5 to 1.0 ms. Dividing Q_t by the amplitude of the hyperpolarizing pulse gave the capacity C_t . The value of C_t determined in this way should be independent of the filter frequency.

Absolute values for the membrane currents were obtained by assuming a longitudinal axoplasmic resistance of 10 M Ω , corresponding to a value of 140 M Ω /cm for the resistance per unit length of the axis cylinder of a frog nerve fibre with a diameter of 14 μ m (Stämpfli & Hille, 1976).

RESULTS

K^+ currents

General observations. Fig. 1 A shows the total current across an intact internodal segment in pool A in response to a 50 ms pulse to 10 mV and a 50 ms pulse to -190 mV. These membrane currents reflect the leakage properties of the intact myelin. The internodal segment was then demyelinated by application of a Ringer solution containing 0.2% lysolecithin (Chiu & Ritchie, 1982). The current records in Fig. 1B were taken 30 min after the beginning of lysolecithin treatment in response to the same pulses as in Fig. 1A. In comparison to Fig. 1A the leakage current had increased about 10-fold, but no specific current could be seen. After 42 min demyelination the first K⁺ currents could be detected. In order to prevent destruction of the axon by the lysolecithin, the lysolecithin was washed away with a normal Ringer solution containing 2% bovine albumin for 10 min. After the wash, the solution was changed to KCl Ringer solution and the temperature was lowered from 25 to 15 °C. After 10 min in KCl Ringer solution the currents shown in Fig. 1C were measured in response to the same pulses as in Fig. 1A and B. A clear outward current can be seen during the depolarizing pulse and a large inward tail current after the pulse. The current associated with the hyperpolarizing pulse showed no sign of inward rectifier K^+ currents as proposed by Bostock & Grafe (1985). The outward current

and the leakage current shown in Fig. 1*C* did not change appreciably during the experiment as can be seen from Fig. 1*D* representing the currents after 40 min treatment with KCl Ringer solution. It seems that washing away the lysolecithin with bovine albumin and cooling the Ringer solution or test solution stabilizes the delayed K^+ currents in the internode. This conclusion is confirmed by Fig. 2*A* and *B*.



Fig. 1. Membrane currents $I_{\rm M}$ in the internode before (A), during (B) and after (C and D) demyelination with 0.2% lysolecithin Ringer solution. A was obtained before treatment, B after 30 min demyelination and C and D after 42 min demyelination, 10 min washing with 2% bovine albumin Ringer solution and 10 min (C) or 40 min (D) washing with KCl Ringer solution. $I_{\rm M}$ associated with pulses of 50 ms duration to 10 mV and -190 mV. Membrane currents were not corrected for capacitive and leakage current. The absolute holding potential was assumed to be -90 mV (see Methods). Temperature = 25 °C for A and B. Temperature = 15 °C for C and D. Fibre 283.



Fig. 2. A, K⁺ inward current $I_{\rm K}$ in the internode after 37 min demyelination with 0.2% lysolecithin Ringer solution, 10 min wash with 2% bovine albumin Ringer solution, 30 min with normal Ringer solution and 10 min or 30 min with KCl Ringer solution. $I_{\rm K}$ associated with a 50 ms depolarizing pulse to -50 mV. B, current–voltage relations measured at the same times as in A. Maximum K⁺ currents $I_{\rm K}$ obtained with 50 ms depolarizing pulses of varying amplitude are plotted against the absolute membrane potential E in mV. Δ , 10 min KCl; \Box , 30 min KCl. The absolute holding potential was assumed to be -90 mV (see Methods). Temperature = 15 °C. Fibre 282.

In Fig. 2A the K⁺ inward current $I_{\rm K}$ is shown 40 and 60 min after washing away the lysolecithin with bovine albumin for 10 min. There is hardly any change in the inward current associated with a 50 ms depolarizing pulse to -50 mV during 20 min washing with KCl Ringer solution. In Fig. 2B the current-voltage relations measured at the same times as in Fig. 2A are shown. The current was flat at the reversal potential, confirming Fig. 8C of Chiu & Ritchie (1982). The reversal potential in KCl Ringer solution (-8 mV) roughly agrees with the 0 mV expected for an absolute holding potential of -90 mV on the basis of a Nernst potential for K⁺. The points \triangle and \square almost coincide, indicating that the internodal K⁺ currents are stable over a period of 20 min. In those experiments in which the absolute membrane potential was determined, the measured reversal potential of the K⁺ current in KCl Ringer solution was 5 ± 3 mV (mean \pm s.D.; n = 5) more negative than the theoretical value.



Fig. 3. K⁺ outward current $I_{\rm K}$ in the node and in the internode in normal Ringer solution (control) and 10 min after application of TEA⁺ Ringer solution. TEA⁺ Ringer solution was prepared by replacing the NaCl by 110 mm-TEA⁺. K⁺ outward current associated with a 50 ms pulse to 60 mV. The absolute holding potential in the internode was assumed to be -90 mV (see Methods). Temperature = 15 °C. Fibres 280 (node) and 282 (internode). Same internode as in Fig. 2.

Effect of external TEA⁺ and internal Cs⁺. Fig. 3 compares outward $I_{\rm K}$ in normal Ringer solution in the node with that in the internode. The shape of the current is similar but the tail current decays more slowly in the internode; it is not clear whether the very slowly decaying component of the internodal tail current is real or due to external K⁺ accumulation during the depolarizing pulse. A detailed investigation of the tail currents is presented below. Fig. 3 also illustrates the effect of 110 mm-TEA⁺ on currents in the node and in the internode. In the node 110 mm-TEA⁺ reduced $I_{\rm K}$ (measured at the end of a 50 ms pulse to 60 mV) to 2% of the control value. This is in approximate agreement with Hille (1967) whose dose-response curve shows that at a TEA⁺ concentration of 110 mm there is little K⁺ current left. In the internode, 110 mm-TEA⁺ was only able to reduce $I_{\rm K}$ to 11% of the control value. In three experiments the reduction was to $11\pm 3\%$ (mean \pm s.D.). Thus there is more TEA⁺-insensitive K⁺ current in the internode than in the node. The slow and voltage-dependent turning on of this current in 110 mm-TEA⁺ (see Fig. 5) suggests that this current is a K^+ current and not a non-linear, voltage-dependent leakage current.

The effect of 20 mM-TEA⁺ on the K⁺ inward current $I_{\rm K}$ (recorded in KCl Ringer solution) in the node and in the internode is shown in Fig. 4A. $I_{\rm K}$ was measured with a 50 ms pulse to -50 mV. In the node 20 mM-TEA⁺ reduced $I_{\rm K}$ to 5% of the control values. The control values were measured before and after TEA⁺ treatment. In the internode 20 mM-TEA⁺ was only able to reduce $I_{\rm K}$ to 10% of the control values. Thus, the effect of 20 mM-TEA⁺ on the K⁺ inward current in the internode is half of that in the node. This different effectiveness of TEA⁺ is further illustrated in Fig. 4B which shows current-voltage relations in the node and in the internode measured at the same times as in Fig. 4A. For absolute membrane potential E = 110 mV, the current remaining in 20 mM-TEA⁺ is 7% of the control value in the node, but 19% in the internode. In both node and internode, the *I*-*E* curves in 20 mM-TEA⁺ have the same reversal potential as the control *I*-*E* curves in KCl Ringer solution, namely -10 mV (node) and -8 mV (internode).



Fig. 4. K⁺ current $I_{\rm K}$ in the node and in the internode in KCl Ringer solution before, during and after application of 20 mm-TEA⁺. A, K⁺ inward current $I_{\rm K}$ associated with a 50 ms pulse to -50 mV. B, current-voltage relations in the node and in the internode in KCl Ringer solution before (\Box), during (\triangle) and after (*) application of 20 mm-TEA⁺. Maximum K⁺currents obtained with 50 ms depolarizing pulses are plotted against the absolute membrane potential E in mV. The absolute holding potential in the internode was assumed to be -90 mV (see Methods). Temperature = 15 °C. Fibres 280 (node) and 282 (internode). Same fibres as in Fig. 3.

I compared τ_n , the time constant of K⁺ activation in normal Ringer solution, with τ_n in 110 mm-TEA⁺ Ringer solution. Fig. 5 shows such a comparison in the node and in the internode. Because the currents in 110 mm-TEA⁺ Ringer solution are very small, very high amplification had to be used; even then τ_n could be obtained only for large depolarizations (node: $E \ge 20$ mV; internode: $E \ge -10$ mV). The control



Fig. 5. Time constants τ_n of K⁺ currents in the node and in the internode in normal Ringer solution (control) and 110 mm-TEA⁺ Ringer solution, respectively. The time constants were obtained by fitting the currents associated with 50 ms pulses to different potentials with the equation $I_{\rm K} = I_{\rm K,ss} n^4 k$, (1)

where

$$n = n_{\infty} - (n_{\infty} - n_0) \exp\left(-t/\tau_n\right),$$

with $n_0 = 0$, $n_{\infty} = 1$ and $k = k_{\infty} - k_{\infty} - k_0$) exp $(-t/\tau_k)$ similar to the term *h* of the Na permeability (Hodgkin & Huxley, 1952*a*, *b*; Frankenhaeuser, 1963; Schwarz & Vogel, 1971). $I_{k,ss}$ is the steady-state K⁺ current. The absolute holding potential in the internode was assumed to be -90 mV (see Methods). Temperature = 15 °C. Fibres 280 (node) and 282 (internode). Same fibres as in Fig. 3. The curves through the points were fitted with the equation $\tau_n = 1/(\alpha_n + \beta_n)$ with $\alpha_n = A(V-B)/(1 - \exp((B-V)/C))$ and $\beta_n = A(B-V)/(1 - \exp((V-B)/C))$ (see Frankenhaeuser, 1963; Frankenhaeuser & Huxley, 1964). The computer was allowed to vary A, B and C for α_n while A, B and C for β_n were fixed to the values given by Frankenhaeuser (1963) for long cathodal steps. Values for A, B and C are:

	Node			Internode		
	A (mV/ms)	B (mV)	С (mV)	A (mV/ms)	B (mV)	C (mV)
α_n normal Ringer solution	0.013	64	46	0.009	124	66
$\alpha_n \text{TEA}^+ \text{Ringer}$ solution	0.007	25	12	0.002	55	27
β_n (Frankenhaeuser, 1963)	0.02	12	10	0.02	12	10

 τ_n values in the node and in the internode were hardly different. For example, in the node, in normal Ringer solution, τ_n for depolarizations from the holding potential (-90 mV) to E = 60 mV and 110 mV was $0.64 \pm 0.08 \text{ ms}$ and $0.48 \pm 0.06 \text{ ms}$, respectively (mean \pm s.E. of mean; n = 5). These values are in good agreement with the values given in Table 1 of Koppenhöfer (1967), whereas Hille (1968) reported somewhat larger τ_n values. In the internode, in normal Ringer solution, τ_n for depolarizations from the holding potential (-90 mV) to E = 60 mV and 110 mV was $0.65 \pm 0.06 \text{ ms}$ (n = 14) and $0.52 \pm 0.04 \text{ ms}$ (n = 13), respectively (mean \pm s.E. of mean). In the node in 110 mM-TEA⁺, τ_n was about 2 times larger than in normal

Ringer solution. In the internode 110 mm-TEA⁺ increased τ_n for $E \ge -10$ mV by a factor of four (see Fig. 5). Two other experiments confirmed this factor of four.

It seemed possible that the effect of TEA⁺ on τ_n is complicated by a series resistance artifact. To see whether the activation time constant τ_n is affected by the voltage drop across a resistance in series with the internodal membrane (see Taylor, Moore & Cole, 1960) experiments were carried out with internal Cs^+ to reduce the K^+ outward current. 120 mM internal CsCl reduced the current amplitude measured at 5 ms depolarization to 60 mV to about 11% of the current amplitude recorded with 120 mM internal KCl, hardly altering the activation time constant τ_n . The simplest explanation is that the series resistance artifact does not affect the activation time constant τ_n . Records taken 20 or 40 min after changing the solution in the end-pools were nearly identical with records taken 60 min after the solution change, suggesting that already after 20 min the internal Cs⁺ concentration had reached a steady state. Presumably, 60 min after changing the solution in the end-pools to 120 mm-CsCl there is very little or no K^+ inside the fibre. This means that the delayed outward current recorded 60 min after the solution change is mainly carried by Cs⁺, which are able to pass through the K^+ channel at strong depolarizations (Hille, 1979; Clay & Shlesinger, 1984).

Fast and slow component of $g_{\rm K}$. Fig. 4A shows that the tail current at the pulse end is much slower in the internode than in the node (see also Fig. 3). This phenomenon is further illustrated by Fig. 6A and B. The tail currents are recorded in KCl Ringer solution in order to avoid complications due to K⁺ accumulation (Dubois & Bergman, 1975). The tail currents in the node could be fitted with a double-exponential function with a fast $(\tau_{\rm f})$ and a slow $(\tau_{\rm s})$ time constant $(\tau_{\rm f} = 2.9 \text{ ms}, \tau_{\rm s} = 21.5 \text{ ms}$ for a conditioning depolarization to -40 mV; $\tau_f = 2.6 \text{ ms}$, $\tau_s = 23.3 \text{ ms}$ for a conditioning depolarization to 30 mV), showing that the K⁺ conductance, activated by conditioning depolarizations, deactivates in two phases: a fast and a slow one (Dubois, 1981 a). The time constants of the fast K^+ conductance are in reasonable agreement with the values 3-6 ms given by Dubois (1981*a*), whereas the time constants of the slow K⁺ conductance are somewhat smaller than the time constants $\tau_s = 40-60$ ms of Dubois (1981b) measured at -90 mV after a 50 ms conditioning depolarization. Following Dubois (1981a), the instantaneous slow current was obtained by extrapolating the later part of the tail current to time zero of repolarization. This extrapolated slow current was 15 % of the instantaneous value of the total tail current for both conditioning depolarizations. The fraction of 15% for the slow K⁺ conductance in the node is in reasonable agreement with the fraction of $19\pm5\%$ $(\text{mean}\pm s.E. \text{ of mean})$ for sensory fibres in Table 1 of Dubois (1981a). A direct comparison, however, is complicated by the fact that the amplitude of the slow component depends on the duration of the depolarization (Dubois, 1981a) which in Dubois' experiments was 100 ms as against 50 ms in my experiments.

In the internode, too, the tail currents could be fitted with a double-exponential function ($\tau_{\rm f} = 3.6$ ms, $\tau_{\rm s} = 27.7$ ms for a conditioning depolarization to -40 mV; $\tau_{\rm f} = 5.0$ ms, $\tau_{\rm s} = 40.1$ ms for a conditioning depolarization to 30 mV). In seven measurements with conditioning depolarizations to -40 mV or 30 mV the time constants $\tau_{\rm f}$ ranged from 3.4 to 5.0 ms and from 2.4 to 5.0 ms, respectively, the time constants $\tau_{\rm s}$ ranged from 22 to 80 ms and from 22 to 65 ms, respectively. This means



Fig. 6. K⁺ tail currents $I_{K,tail}$ at E = -90 mV and temperature = 15 °C in KCl Ringer solution. A, from a node (fibre 280); 50 ms conditioning depolarization to -40 and 30 mV. B, from an internode (fibre 282), same internode as in Fig. 2; conditioning depolarization as in A. C, from another internode (fibre 283) before and after application of KCl Ringer solution with 1 mM-4-aminopyridine (4-AP), same internode as in Fig. 1 but 20 min later than Fig. 1 D; 50 ms conditioning pulse to 110 mV.

that the time constants for the fast and the slow K^+ conductance measured in the internode are comparable with those in the node. However, the fraction of the slow K^+ conductance is much larger in the internode than in the node. In Fig. 6*B*, the extrapolated slow current in the internode amounted to 30% of the instantaneous value of the total tail current for both conditioning depolarizations. For larger conditioning depolarizations (to 70–110 mV) this fraction was 33%. In fourteen measurements on the internode the fraction of the slow K^+ conductance for conditioning depolarizations to 70–110 mV was $33 \pm 2\%$ (mean \pm s.E. of mean) of the total K^+ conductance (for the node: 15%, see above). Thus, the proportion of slow and fast K^+ channels in the internode differs from that in the node.

In the node, Dubois (1981*a*, *b*) described a complete block of the fast K⁺ conductance (g_{Kf}) by 1 mm-4-aminopyridine (4-AP) while the slow K⁺ conductance (g_{Ks}) was unaffected. With the same concentration (1 mm), Pappone & Cahalan (1984) found a 95% block of g_{Kf} and, in addition, a 48% block of g_{Ks} . Fig. 6*C* shows that in the internode 4-AP is able to block the fast channels, hardly altering the slow phase of the K⁺ conductance. The extrapolated slow phase of the tail current in KCl Ringer

solution amounted to -6.8 nA. The tail current in KCl Ringer solution + 1 mM-4-AP could be fitted with a single exponential function with exactly the same time constant as the slow phase of the tail current in KCl Ringer solution ($\tau_s = 16.1$ ms) and an initial value of -6.2 nA.



Fig. 7. The fast K⁺ conductance (g_{kl}) was obtained in KCl Ringer solution from the total tail current minus the extrapolated slow tail current recorded at -90 mV after activation of the conductance by conditioning depolarizations of 50 ms duration to different potentials (abscissa). The absolute holding potential in the internode was -90 mV. Temperature = 15 °C. Fibres 286 (node) and 288 (internode).

The fast K⁺ conductance in the node can be decomposed into two components, one (g_{Kf1}) activating between -80 and -30 mV, the other (g_{Kf2}) activating between -40 and +30 mV (Dubois, 1981*a*). Fig. 7 compares the fast K⁺ conductance in the node and in the internode. The fast K⁺ conductance was calculated from the fast component of the tail current after subtraction of the slow current from the total tail current. The fast conductance-voltage curves in the node and in the internode are similar: both have a bend near E = -40 mV. Dubois (1981*a*) explained the form of the $g_{Kf}-E$ curve as the sum of two superimposed conductance-voltage curves reflecting two fast K⁺ currents which activate over different voltage ranges.

Na⁺ currents

Fig. 8 demonstrates that TTX-sensitive Na⁺ currents can be recorded from the central portion of the internode after demyelination. The Figure shows membrane currents in the internode with cut ends of fibre in 120 mm-CsCl. During demyelination with lysolecithin the end-pools contained 120 mm-KCl and during the wash with bovine albumin the solution at the cut ends of fibre was changed to 120 mm-CsCl. Changing the solution in the end-pools resulted in a marked reduction of the delayed outward current. In the upper row of Fig. 8 membrane currents associated with pulses to -10 and -210 mV can be seen. To reduce the noise ten records were averaged. After subtraction of the current during negative pulses from the current was reversibly blocked by Ringer solution + 310 nm-TTX. A TTX-sensitive Na⁺ inward current in the central portion of the internode after demyelination was observed in a total of four experiments with 120 mm-CsCl or 115 mm-CsCl+5 mm-NaCl as the

Internal CsCl

Before





Fig. 8. Membrane currents in the internode in normal Ringer solution with cut ends of fibre in 120 mM-CsCl. The solution in the end-pools was changed from 120 mM-KCl during demyelination with lysolecithin (52 min) to 120 mM-CsCl during wash with bovine albumin. Upper row: Membrane currents associated with pulses to -10 and -210 mV, not corrected for capacitive and leakage currents. Ten records averaged. Lower row: subtraction of current during negative pulse from current during positive pulse yields transient inward current that is reversibly blocked by Ringer solution + 310 nM-TTX. Holding potential assumed to be -110 mV. Temperature = 15 °C. Fibre 298.

internal solution. In eight other experiments, the TTX sensitivity of the early inward current was not investigated, but the time course of this current indicated that it was a Na⁺ current. In five of these experiments the early inward current was so large that it could be observed without blocking K⁺ currents; the three remaining experiments were carried out with 20 mm-external TEA⁺ to reduce the K⁺ current. The range of the Na⁺ current amplitude in different experiments is illustrated in Fig. 10.

In order to characterize these TTX-sensitive currents, current-voltage relations were measured as shown in Fig. 9A. For this purpose 115 mm-CsCl+5 mm-NaCl was used as internal solution. Peak Na⁺ currents were obtained with 5 ms depolarizing pulses. The current-voltage curve in the internode is similar to that measured in the node. The measured reversal potential (69 mV) roughly agrees with the theoretical value of 70–76 mV using a permeability of Cs⁺ relative to Na⁺ between 0.013 (Hille, 1972) and zero.

The effect of steady-state membrane potential on the peak Na⁺ current associated with a test potential step is shown in Fig. 9*B*. The relation between peak Na⁺ current and membrane potential was S-shaped as described for the node of *Rana esculenta* (Dodge & Frankenhaeuser, 1958) and the node of *Xenopus laevis* (Frankenhaeuser,

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Fig. 9. Current-voltage curve and steady-state inactivation of the internodal Na⁺ current. A, current-voltage relation in normal Ringer solution. Peak Na⁺ currents (I_{Na}) obtained with 5 ms depolarizing pulses plotted against the absolute membrane potential E during test pulses. The absolute holding potential was assumed to be -90 mV (see Methods). Under this assumption, the reversal potential was $E_{Na} = 69 \text{ mV}$ and the potential at which the Na⁺ current had reached half its maximum was $E_{1/2} = -38$ mV. Solution at the cut ends of fibre was changed from 120 mm-KCl during demyelination to 115 mm-CsCl+5 mm-NaCl during wash with bovine albumin. Temperature = 15 °C. Fibre 296. B, steady-state inactivation. Normalized peak Na⁺ currents associated with a 5 ms depolarizing pulse to E = 10 mV plotted against the absolute membrane potential during 50 ms pre-pulses. h_{∞} is the Na⁺ current that is rapidly available to the test pulse as a fraction of that available after strong hyperpolarizations. For $h_{\infty} = 1.0$ the Na⁺ current is 0.62 nA. The continuous line was calculated from $h_{\infty} = 1/(1 + \exp(((E_{\rm h} - E)/k_{\rm h})))$ with $E_{\rm h} = -66 \text{ mV}$ and $k_{\rm h} = 8.56 \text{ mV}$. The absolute holding potential was assumed to be -70 mV (see Methods). Solution at the cut ends of fibre was changed from 120 mm-KCl during demyelination to 115 mm-CsCl+5 mm-NaCl during wash with bovine albumin. Temperature = $15 \,^{\circ}$ C. Fibre 297.

1959). An empirical equation of the same form as that used for the node was therefore fitted to the experimental values (continuous line in Fig. 9*B*). The experimental values for $E_{\rm h}$ and $k_{\rm h}$ ($E_{\rm h}$ is the potential at which the peak sodium inward current reached half its maximum corresponding to $h_{\infty} = 0.5$; $k_{\rm h}$ is a measure of the steepness of the curve) roughly agree with the mean values in Table 1 of Frankenhaeuser (1959) ($V_{\rm h} = 9.15 \text{ mV} \doteq E_{\rm h} = -60.85 \text{ mV}$ and $k_{\rm h} = 8.17 \text{ mV}$). That means that there is hardly any difference in steady-state inactivation of Na⁺ channels in node and internode.

Chiu & Ritchie (1982) reported a correlation between the fast membrane capacity $C_{\rm f}$ and the delayed outward current $I_{\rm K}$. My experiments confirm this correlation; for example, $I_{\rm K} = 60$ nA measured at the end of a 50 ms depolarizing pulse to 60 mV was associated with $C_{\rm f} = 44$ pF, whereas $I_{\rm K} = 30$ nA measured in another experiment with the same depolarizing pulse was associated with $C_{\rm f} = 20.5$ pF. To demonstrate a correlation between $C_{\rm f}$ and Na⁺ inward current $(I_{\rm Na})$, $C_{\rm f}$ was plotted against $I_{\rm Na}$ as shown in Fig. 10. The points could be fitted by a straight line through the origin (slope 0.059 nA/pF), indicating that $I_{\rm Na}$ is linearly proportional to $C_{\rm f}$. The values for



Fig. 10. Plot of fast membrane capacity C_t versus Na⁺ inward current $I_{\rm Na}$ in demyelinated internodes of seven different experiments. The values for C_t were calculated as described under Methods. The values for $I_{\rm Na}$ were the maximum peak currents attainable in each experiment. The line through the points was fitted by a least-squares method with a slope of 0.059 nA/pF. The absolute holding potential was assumed to be -70 or -90 mV (see Methods). Temperature = 15 °C.

 $C_{\rm f}$ in Fig. 10 were obtained by fitting the integrated capacitive current $I_{\rm C}$ with a double exponential (see Methods). A correlation between $C_{\rm f}$ and $I_{\rm Na}$ could also be demonstrated when $C_{\rm f}$ was defined to be the portion of the capacity associated with the initial 90 μ s of $\int I_{\rm C} dt$ (see Chiu & Ritchie, 1982); in this case the slope of the straight line through the points was somewhat steeper (0.08 nA/pF).

DISCUSSION

The present experiments confirm the finding of Chiu & Ritchie (1982) that K^+ currents can be recorded from an internode acutely demyelinated with lysolecithin. By washing out the lysolecithin with bovine albumin it becomes possible to keep the demyelinated internode in a stable condition for about an hour. The internodal K^+ conductance, like the nodal, consists of two components, a fast and a slow, but the fraction of the slow component is twice as large as in the node. In agreement with the observations of Dubois (1981*a*,*b*) on the node, only the fast component is blocked by 4-AP. It was possible to decompose the fast K^+ conductance into two components as has been done in the node by Dubois (1981*a*).

An interesting observation is that 11 % of the internodal K⁺ current is insensitive to TEA⁺ even in the relatively high concentration of 110 mm. In mammalian nerve fibres Chiu & Ritchie (1980, 1981) found that internal TEA⁺ (80 mM) could block the K⁺ current in the paranodal region of acutely demyelinated nerve fibres only to 30 %. External application of TEA⁺ (20–60 mM) was even less effective than internal. A TEA⁺-insensitive K⁺ current exists also in frog muscle fibres (Stanfield, 1970; Almers & Palade, 1981) and in mollusc neurones (Neher & Lux, 1972; Thompson, 1977) (for detail see review of Stanfield, 1983).

TEA⁺ in the relatively high concentration of 110 mm increased the time constant τ_n in the node and internode by a factor of two or four, respectively. An increase of

 τ_n by small concentrations of TEA⁺ in the node was reported by Koppenhöfer (1967) and confirmed by Ilyin, Katina, Lonskii, Makovsky & Polishchuk (1980). The latter authors suggested that the increase of τ_n could be accounted for by a smaller contribution of the fast component to the over-all I_K in the presence of TEA⁺. Van den Berg, Siebenga & De Bruin (1977) and Dubois (1981*a*), too, found that the slow K⁺ conductance is less sensitive to TEA⁺ than the fast K⁺ conductance.

A surprising finding was the existence of an internodal Na⁺ inward current. Chiu & Ritchie (1982) could not detect any Na⁺ inward current after demyelination. Several possibilities could account for the discrepancy. The first is that their internodal membrane area was smaller than mine because their internodal segment in pool A was shorter (40–60 μ m) than the 100–200 μ m in my experiments; the second is that Na⁺ currents could have escaped their detection because of the low gain used to record the large K⁺ currents; a further possibility is that Na⁺ channels are indeed absent in the internodal region of nerve fibres from the bull-frog. The first possibility seems unlikely, because the capacity of their internodal segment was not different from mine. This leaves the two other possibilities, namely the insensitivity of their measuring system or a species difference between *Rana esculenta* and *Rana catesbeiana*. Likewise, the failure to find internodal Na⁺ channels in binding experiments and cytochemical investigations (see Introduction) is probably either due to the insensitivity of the insensitivity of the methods employed or to species differences.

The size of Na⁺ or K⁺ current in the internode, compared to that in a normal node, is a good measure of the number of ion channels in the internode. The capacity of a normal node with an assumed nodal area of 50 μ m² (Stämpfli & Uhrík, 1980) is about 1 pF (Stämpfli & Hille, 1976). The size of the K⁺ current in a normal node measured at depolarizations to E = 60 mV is about 30 nA and the maximum size of the Na⁺ current is about 30 nA. In the internode $I_{\rm K} = 30$ nA at E = 60 mV is associated with $C_{\rm f} = 20.5$ pF (see p. 130) and $I_{\rm Na}$ is 0.059 nA/pF (see legend of Fig. 10). Hence, the density of K⁺ channels in the internode is about 20 times smaller than in the node, whereas the Na⁺ channel density is about 500 times smaller than in the node. This calculation can only be an approximate estimation, with the assumption that node and internode have a homogeneous capacity distribution.

A lower limit for the Na⁺ channel density in the internode can be estimated from the geometry of the investigated internodal segment in pool A. With the length of the internodal segment $L \sim 100-200 \ \mu m$ and a diameter of the demyelinated fibre $d \sim 8-12 \ \mu m$, the maximum internodal membrane area which can be exposed, is $F = d \ \pi L \sim 2500-7500 \ \mu m^2$, i.e. 50-150 times larger than the nodal area. The mean value of the maximum internodal Na⁺ currents measured in seven experiments is 4 ± 1 nA (mean \pm s.E. of mean) (see Fig. 10) as opposed to 30 nA in a normal node. Hence, the density of Na⁺ channels in the internode is 400-1000 times smaller than in the node. If the internodal membrane in pool A is for example only partly exposed, the Na⁺ channel density in the internode would be larger.

From current fluctuations (Conti, Hille, Neumcke, Nonner & Stämpfli, 1976) and gating current measurements (Nonner, Rojas & Stämpfli, 1975) the Na⁺ channel density for the frog node of Ranvier was found to be 2000 and $5000/\mu m^2$, respectively. Hence, the internodal Na⁺ channel density is about $4-10/\mu m^2$. This is not in contrast to the binding studies of Ritchie & Rogart (1977). Finding no difference in the binding capacity of tritium-labelled saxitoxin to intact and homogenized rabbit nerves, they concluded that the internodal membrane has not more than about 25 Na⁺ channels/ μ m². A channel density of 4–10/ μ m² is in about the same range as the channel density determined by TTX-binding studies in rabbit vagus nerve, garfish olfactory nerve or lobster leg nerve (see Table 6 of Colquhoun, Rang & Ritchie, 1974). The nerve fibres of these nerves, however, have a smaller diameter than frog nerve fibres. Therefore they require a smaller Na⁺ channel density to propagate a nervous impulse. However, the Na⁺ channels in the internode may contribute to the continuous conduction as described for demyelinated nerve fibres by Bostock & Sears (1978).

In conclusion, the internodal $g_{\rm K}$ like the nodal $g_{\rm K}$ consists of three components $g_{\rm Kf1}$, $g_{\rm Kf2}$, $g_{\rm Ks}$. The relative size of the slow component in the internode is twice as large as in the node. The TEA⁺-insensitive fraction of $g_{\rm K}$ (which is negligible in the node) amounts to 11% in the internode. The time course, TTX sensitivity, reversal potential and steady-state inactivation of the transient early inward current measured in the internode after blockage of the large K⁺ currents constitute strong evidence that this current is caused by Na⁺ passing through Na⁺ channels.

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