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THE EFFECTS OF RUBIDIUM IONS ON COMPONENTS OF THE POTASSIUM CONDUCTANCE IN THE FROG NODE OF RANVIER

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SUMMARY

1. The effects of replacement of external and internal K^+ ions by Rb^+ ions on the two fast components (g_{f1} and g_{f2}) and slow component (g_s) of the K^+ conductance (g_K) in frog nodes of Ranvier were investigated under voltage- and current-clamp conditions.

2. Fast and slow components of g_K were separated by double exponential fits to tail currents following long depolarizing pre-pulses, or by the use of short pre-pulses which activate little g_s. g_s was also isolated by 1 mM-4-aminopyridine (4-AP). g_{f1} and g_{f2} were distinguished in the fast conductance–voltage curve by their different voltage dependences, g_{f1} activating at more negative potentials.

3. Reversal potential measurements indicated that Rb^+ is less permeant than K^+, and measurements in 4-AP indicated that the slow component has a lower Rb^+ permeability than the fast. In a 50% K^+, 50% Rb^+ mixture P_{Rb}/P_K was less than that in 100% Rb^+ suggesting that P_{Rb}/P_K is mole-fraction dependent.

4. With external Rb^+ the current–voltage relation was shifted by *ca.* −10 mV compared to that in K^+, an effect on g_f ($= g_{f1}+g_{f2}$). The slow conductance (g_s) and, under similar conditions, the Na^+ current–voltage relation were not shifted.

5. g_f, calculated from inward tail currents, was reduced with external Rb^+ at potentials where g_{f2} was activated. Instantaneous current–voltage relations following pre-pulses which activate different components of g_f confirmed these observations. In K^+ the instantaneous current–voltage relation showed some inward rectification which was largely abolished with Rb^+. Comparison of g_f calculated from outward (g_o) and inward (g_i) currents confirmed this, and showed that inward g_{f2} was reduced with Rb^+ such that $g_o = g_i$. Outward currents were little affected by external Rb^+.

6. External Rb^+ slowed the fast inward tail current following all pre-pulses which activate g_f, but had no effect on the time course of the slow component of the tail current.

7. Regenerative responses, which occur in high [K^+] (+300 nM-tetrodotoxin) solutions in current clamp did not repolarize in Rb^+. Voltage-clamp experiments showed that inactivation of inward currents is slowed when Rb^+ is the charge carrier.

8. Replacement of internal K^+, by application of Rb^+ to the cut ends of the fibre, shifted the reversal potential to more positive potentials but had no effect on the conductance or kinetics.

9. External Rb^+ has a large number of effects on inward currents, but little effect on outward currents. Internal Rb^+ had little effect on outward or inward currents. These results suggest that the properties of the channel depend on the direction and nature of the current.

INTRODUCTION

Potassium (K^+) channels in nerve fibre membranes are highly selective and only a few ion species are permeant. Reversal potential measurements indicated that, of the alkali metal cations, only Rb^+ other than K^+ itself was readily permeant (Moore, Anderson, Blaustein, Takata, Lettvin, Pickard, Bernstein & Pooler, 1966; Hille, 1973). In contrast to the reversal potential measurements, current measurements with Rb^+, externally in the node of Ranvier (Hille, 1973; Århem, 1980) and both externally and internally in the squid giant axon (Chandler & Meves, 1965; Bezanilla & Armstrong, 1972; Clay & Shlesinger, 1983) show deviations from the independence principle (Goldman, 1943; Hodgkin & Katz, 1949). Currents in Rb^+ are smaller than predicted from the permeability ratio estimated from the change in reversal potential. In addition, in the presence of Rb^+, closing of K^+ channels is slowed (Århem, 1980; Swenson & Armstrong, 1981; Beam & Donaldson, 1983; Cahalan & Pappone, 1983; Cahalan, Chandy, DeCoursey & Gupta, 1985) while activation is little affected or shifted to more negative potentials (Cahalan & Pappone, 1983).

Recently, it has been suggested that the total K^+ conductance (g_K) in the frog node of Ranvier is the sum of a number of components with different kinetic and pharmacological properties, which may be separate populations of channels or a single population of channels with multiple states (Schwarz & Vogel, 1971; Ilyin, Katina, Lonskii, Makovsky & Polishchuk, 1980; Dubois, 1981*b*, 1982*a*, 1983; Hu & Rubly, 1983; Pappone & Cahalan, 1984; Conti, Hille & Nonner, 1984).

The experiments described in this paper investigated the effects of Rb^+, both externally and internally (applied to the cut ends), on the components of g_K in the frog node of Ranvier. A preliminary report of some of these results has been presented (Plant, 1984).

METHODS

Single motor or sensory myelinated nerve fibres were dissected from the tibial nerve of the frog *Rana esculenta* (Stämpfli & Hille, 1976), and current or voltage clamped at 15 °C as described by Nonner (1969). The internodes were cut, at a distance of ~ 0·75 mm on both sides of the node under investigation, in 117 mM-KCl or -RbCl. At the beginning of the experiment, the node was superfused with Ringer solution which contained (mM): NaCl, 110; KCl, 2·5; $CaCl_2$, 1·8; Tris HCl, 5; pH 7·2 and was allowed to stabilize for 20–30 min. The potential at which the Na^+ current was 70% of its maximum value, i.e. $h_\infty = 0{\cdot}7$ (measured with a test pulse (V) of 50–60 mV with and without a 50 ms pre-pulse of $V = -40$ mV) was assumed to be the resting potential ($E = -70$ mV).

The fibre type, motor or sensory, was further determined from its electrophysiological properties; in current clamp from the ability of sensory fibres to fire repetitively in response to long depolarizing stimuli, and in voltage clamp from the ratio of the steady-state K^+ current at $E = 50$ mV to the peak Na^+ current at $E = -10$ mV which is 0·43 in motor and 0·69 in sensory fibres (see Neumcke (1981) for a review of differences between motor and sensory fibres). For further measurements, the holding potential was adjusted to -90 or -100 mV, to remove steady-state K^+ current inactivation (Schwarz & Vogel, 1971) and to reduce the number of K^+ channels open at the holding potential. All measurements of K^+ channel current were performed in external solutions with high

concentrations (117 mM) of the permeant ions (K^+ or Rb^+), and a solution of the same total permeant ion concentration bathing the cut ends. External solutions contained (mM): KCl or RbCl, 117; $CaCl_2$, 1·8; Tris HCl, 5; pH 7·2. These solutions reduce the problems of accumulation of permeant ions in, or depletion of permeant ions from the perinodal space or axoplasm near the nodal membrane (Dubois & Bergman, 1975*b*; Dubois, 1981*a*). Some accumulation, though relatively small compared to that in Ringer solution, was observed following large outward currents, particularly in some sensory fibres (see also Palti, Moran & Stämpfli, 1980). External accumulation results in a shift of the reversal potential, E_r (zero current potential) measured by graphical interpolation of the instantaneous current–voltage relation, to more positive potentials (though this was < 10 mV). This results in a failure of the conductance g_K, calculated from inward tail currents using the value of E_r measured following a pre-pulse to 0 mV (during which little current flowed), to saturate at positive potentials. This effect of accumulation was small and not systematically corrected. In most experiments solutions also contained 300 nM-tetrodotoxin (TTX) to block current through Na^+ channels. Tetraethylammonium chloride (TEA Cl; 10 mM), and 4-aminopyridine (4-AP; 1 mM), were added in some experiments. The effects of internally applied ions were investigated by exchanging the KCl solution bathing the cut ends for one containing the test ion and allowing the test ion to enter the fibre by diffusion (Koppenhöfer & Vogel, 1969).

Command pulses were generated, and the corresponding membrane currents, filtered at 5–10 kHz, sampled by computer (DEC LSI 11/23). Currents were sampled at intervals of 10 or 100 μs, or for inactivation experiments 10 and 100 ms, by a twelve-bit A/D converter. In 4-AP the signal-to-noise ratio was improved by averaging four current records. Correction for linear leakage and capacitive currents was achieved by analogue subtraction or by the addition or subtraction of an appropriately scaled negative pulse. Absolute currents were calculated assuming a longitudinal axoplasmic resistance of 10 MΩ, which corresponds to the value of 140 MΩ/cm for the resistance per unit length of axis cylinder of a 14 μm diameter frog nerve fibre (Table 1, Stämpfli & Hille, 1976). Where appropriate, results are given as mean ±standard error of the mean.

RESULTS

Effects of external Rb^+ on K^+ channel currents

Replacement of external K^+ (117 mM) by Rb^+ had a number of reversible effects on the currents flowing through K^+ channels (Fig. 1), confirming previous results (see Introduction). In Rb^+ the negative resistance branch of the current–voltage relation and reversal potential were shifted to more negative potentials (Fig. 1*C*), and the inward tail currents observed on repolarization to the holding potential were slowed considerably (Fig. 1*A* and *B*). The mean difference between the reversal potential in Rb^+ and that in K^+ (ΔE_r) was $-7{\cdot}0 \pm 0{\cdot}5$ mV ($n = 9$). From $P_{Rb}/P_K = e^{\Delta E_r F/RT}$, this corresponds to a permeability ratio (P_{Rb}/P_K) of $0{\cdot}76 \pm 0{\cdot}01$ ($n = 9$). In mixtures of 50% Rb^+ + 50% K^+ ($[Rb^+] + [K^+] = 117$ mM), P_{Rb}/P_K was $0{\cdot}58 \pm 0{\cdot}01$ ($n = 4$). Similar shifts of reversal potential were also measured from instantaneous and from isochronal current–voltage relations obtained following pre-pulses of different durations and amplitude. The half-maximum inward current, measured from current–voltage curves normalized to the peak inward current value, was shifted by $-9{\cdot}8 \pm 0{\cdot}5$ mV ($n = 7$) on changing from K^+ to Rb^+.

Shift with external Rb^+ is specific for K^+ channels

It has been shown in squid axon that the Na^+ conductance and kinetics are dependent on $[K^+]_o$ (Adelman & Palti, 1969*a*, *b*; Gillespie & Meves, 1981). The possibility that the Na^+ permeability is also shifted when external K^+ is replaced by Rb^+ was investigated in hypertonic solutions containing 117 mM-K^+ or -Rb^+ plus 50 mM-NaCl. The initial transient current following the depolarizing step was

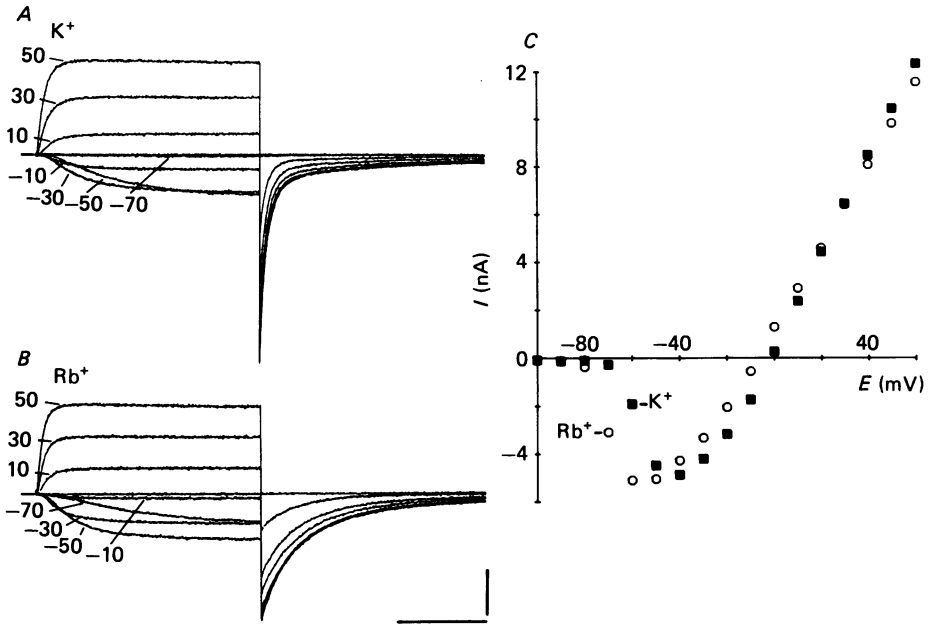

Fig. 1. The effect of replacement of external K^+ by Rb^+ on currents through K^+ channels. *A*, currents recorded during and after 50 ms pulses to potentials between −70 and +50 mV from a holding potential of −90 mV in 117 mM-K^+ and *B*, in 117 mM-Rb^+. Calibration bars; vertical 5 nA, horizontal 20 ms. *C*, peak current–voltage relations from *A* and *B* for K^+ (■) and Rb^+ (○). 117 mM-KCl on cut ends. Motor fibre.

assumed to be current through Na^+ channels and current at the end of the 55 ms pulse K^+ current (Fig. 2*C*). The validity of these assumptions and absence of temporal overlap was confirmed by the addition of TTX (300 nM) in which the pulse protocol was repeated and TTX-sensitive and TTX-insensitive currents determined. When Rb^+ replaced external K^+ the K^+ current–voltage relation was shifted to more negative potentials (Fig. 2*B*), as described above. No such shift of the Na^+ current–voltage relation was observed, only a reduction of approximately 20% in the maximum Na^+ current and a shift of the reversal potential to more negative potentials (Fig. 2*A*).

These effects of the removal of external K^+ on the Na^+ current probably occurred because K^+ is measurably permeant through Na^+ channels ($P_K/P_{Na} = 0{\cdot}086$: Hille, 1972) while Rb^+ is much less permeant ($P_{Rb}/P_{Na} \leqslant 0{\cdot}012$: Hille, 1972). Using these values of the permeability ratios the difference in current at a particular potential which is predicted assuming independence can be calculated from the equation:

$$\frac{I'}{I} = \frac{[Na^+]'_o + \alpha[Rb^+]'_o + \beta[K^+]'_o - ([Na^+]'_i + \alpha[Rb^+]'_i + \beta[K^+]'_i)\exp(FE/RT)}{[Na^+]_o + \alpha[Rb^+]_o + \beta[K^+]_o - ([Na^+]_i + \alpha[Rb^+]_i + \beta[K^+]_i)\exp(FE/RT)} \quad (1)$$

(Binstock & Lecar, 1969), where I' is the current when Rb^+ replaced external K^+, I is the current in K^+, $\alpha = P_{Rb}/P_{Na}$, $\beta = P_K/P_{Na}$, and F, E, R and T have their normal meanings. Assuming that $[Na^+]_i$ and $[Rb^+]_i$ are 0, eqn. (1) predicts reductions of I_{Na} of 14 and 16·2% at 0 and −10 mV respectively. These compare with mean

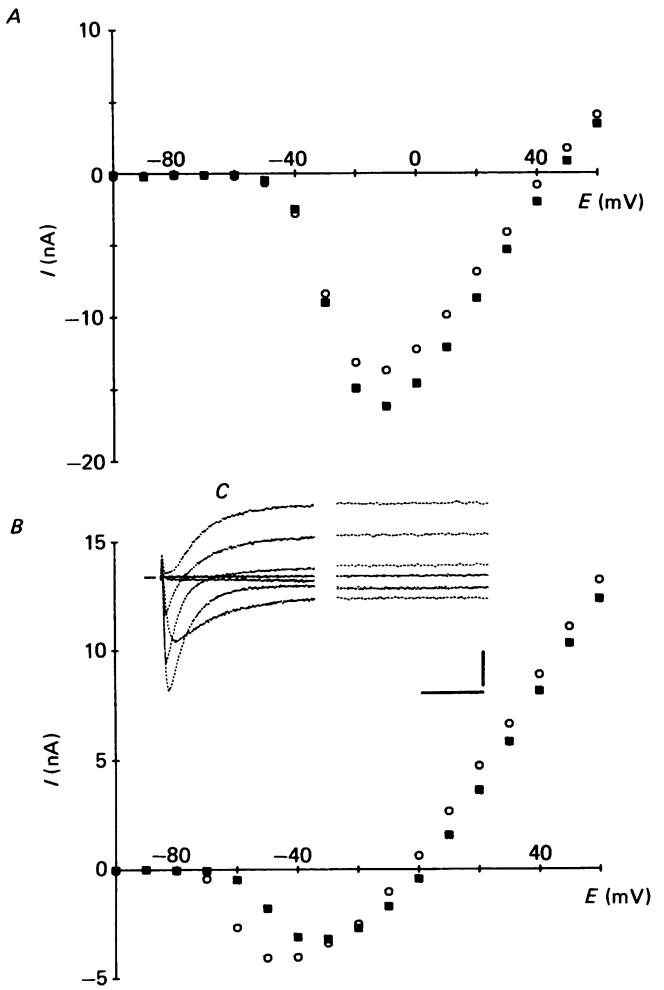

Fig. 2. Comparison of the effects of Rb^+ on Na^+ and K^+ currents. *A*, current–voltage relation for the peak initial transient current during, and *B*, steady-state current at the end, of 55 ms pulses in hypertonic solutions containing 117 mM-K^+ (■) and 117 mM-Rb^+ (○) plus 50 mM-Na^+. *C*, current records at the beginning, sampled at 100 kHz (left), and end, sampled at 10 kHz (right), of a 55 ms pulse in 117 mM-K^+, 50 mM-Na^+. Calibration bars; vertical 5 nA, horizontal 2 ms. Potentials from −90 to 50 mV in 20 mV steps. Motor fibre.

experimental values of 15·1 % ($n = 3$) at 0 mV and 14·7 % ($n = 3$) at −10 mV. This suggests that the reduction in I_{Na} observed occurs as a result of replacement of K^+, which is slightly permeant, by the relatively impermeant Rb^+. Similarly, the shift of E_r (−4 mV in Fig. 2*A*, mean = − 4·4 mV ($n = 3$)) is also consistent with that predicted from independence (−3·9 mV) using the values of permeability ratios from Hille (1972).

External Rb^+ also produces a shift in 20 mM-Ca^{2+}

The effect of Rb^+ in shifting the conductance and current–voltage curves may be

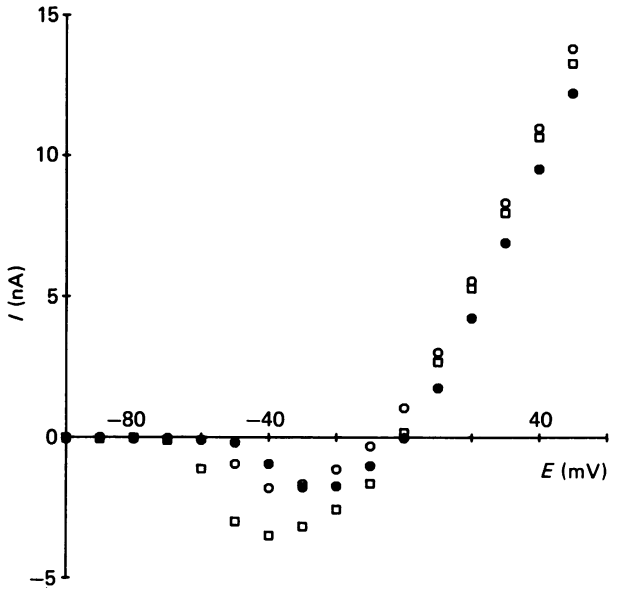

Fig. 3. Effect of raising $[Ca^{2+}]_o$ on the shift of the current–voltage relation induced by Rb^+. Currents were measured at the end of a 55 ms pulse in 117 mM-K^+, 1·8 mM-Ca^{2+} (□), 117 mM-K^+, 20 mM-Ca^{2+} (●) and 117 mM-Rb^+, 20 mM-Ca^{2+} (○). Sensory fibre.

by competing more strongly than K^+ with Ca^{2+} for negative sites, thus affecting the membrane voltage field. To test this possibility, external K^+ was replaced by Rb^+ in solutions with high $[Ca^{2+}]$ (20 mM) in two fibres. Changing from 1·8 to 20 mM-Ca^{2+} shifted the current–voltage curve by 16·2 and 18·1 mV to more positive potentials (Fig. 3). Similar shifts (−9·5 and −10·5 mV) of current–voltage relations to those in 1·8 mM-$CaCl_2$ (−9·8±0·5 mV, see p. 83) were observed when Rb^+ replaced K^+ externally.

Separation of g into components

Instantaneous current–voltage relations in the node of Ranvier are approximately linear at all external K^+ concentrations. Therefore the K^+ permeability system can be described in terms of the conductance (g) (Dodge, 1963; Dubois & Bergman, 1977; Attwell, Dubois & Ojeda, 1980).

The conductance was calculated from the instantaneous current on repolarization to the holding potential, or more negative potentials ($E \leqslant -90$ mV), following conditioning pulses to various potentials. Following short (< 10 ms) pulses the deactivation of I_K can be approximated by a single exponential function, but following longer pulses a slow component of deactivation becomes apparent and the tail currents are better described by the sum of two exponential functions (see also Dubois, 1981*b*). It has been suggested that the total K^+ conductance is composed of a number of components (see Introduction). The fast component (g_f) can be isolated from the slow (g_s) by the use of short conditioning pulses or by subtraction of the fit to the slow component from the total tail current.

The amplitude of the slow component of the tail current at the time of repolarization ($t(0)$) was estimated by extrapolation of the fit to the slow component to $t(0)$, and

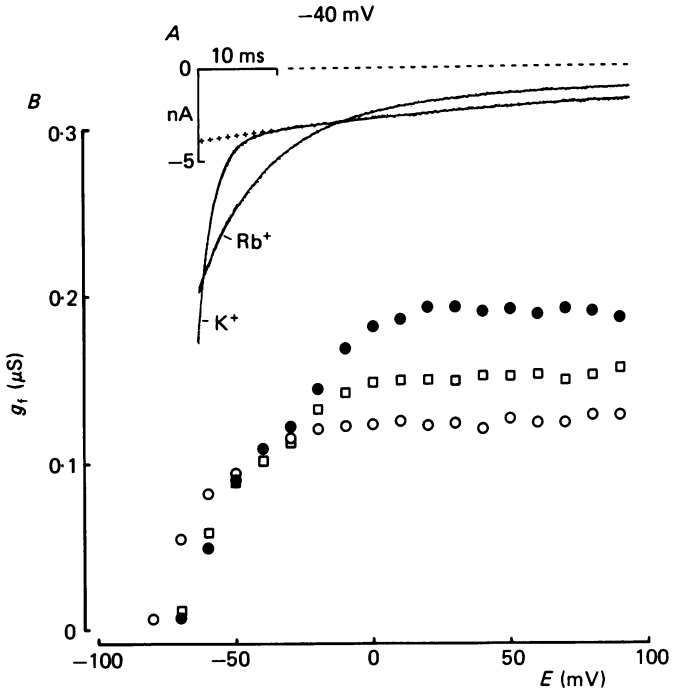

Fig. 4. Tail currents and fast conductance, calculated from tail currents, in K^+ and Rb^+. *A*, tail currents recorded at −90 mV following a 200 ms pre-pulse to −40 mV fitted to the sum of two exponential functions (by a least-squares method) with, in K^+, 65·0 % of the current decaying fast; τ_f = 1·8 ms, and 35·0 % decaying slowly; τ_s = 57·9 ms, and in Rb^+, 72·8 % fast; τ_f = 7·9 ms and 27·2 % slow; τ_s = 58·2 ms. + + + +, extrapolated slow component in K^+. *B*, fast conductance in K^+ (●), 50 % K^+ + 50 % Rb^+ (□) and Rb^+ (○), calculated as described in the text. Motor fibre.

the amplitude of the fast component by subtraction of $I_{s,t(0)}$ from the total instantaneous current (Fig. 4*A*). Currents were fitted using a least-squares method. Separation of the fast and slow tail currents in Rb^+ was complicated by a change in the kinetics of deactivation due to a slowing of the fast component (see below).

With external K^+, the fast conductance curve (g_f) has two components with an inflexion at $E = -20$ to -40 mV (Fig. 4*B*), suggesting the presence of two components of g_f; g_{f1} activated at $E > -70$ mV, and g_{f2} activated at $E > -40$ mV (Dubois, 1981*b*). Similar differences between motor ($g_{f1} > g_{f2}$) and sensory ($g_{f1} < g_{f2}$) fibres to those described by Dubois (1981*b*) were observed.

Following replacement of K^+ by Rb^+, the slow component of the tail current is reduced (Fig. 4*A*) at all potentials. The fast conductance at negative potentials was shifted by approximately −10 mV, reached a similar amplitude to that in K^+ at the inflexion point, but increased little above this with more positive conditioning pre-pulses (Fig. 4*B*). Rb^+ reduces g_{f2} while g_{f1} is shifted but not reduced. A mixture of 50 % Rb^+ + 50 % K^+ had an intermediate effect (Fig. 4*B*).

Instantaneous current–voltage curves for the fast component, measured using short (< 10 ms) conditioning pulses which activate little g_s, confirmed the effects of Rb^+ seen on the fast conductance–voltage relation. At negative potentials, where the

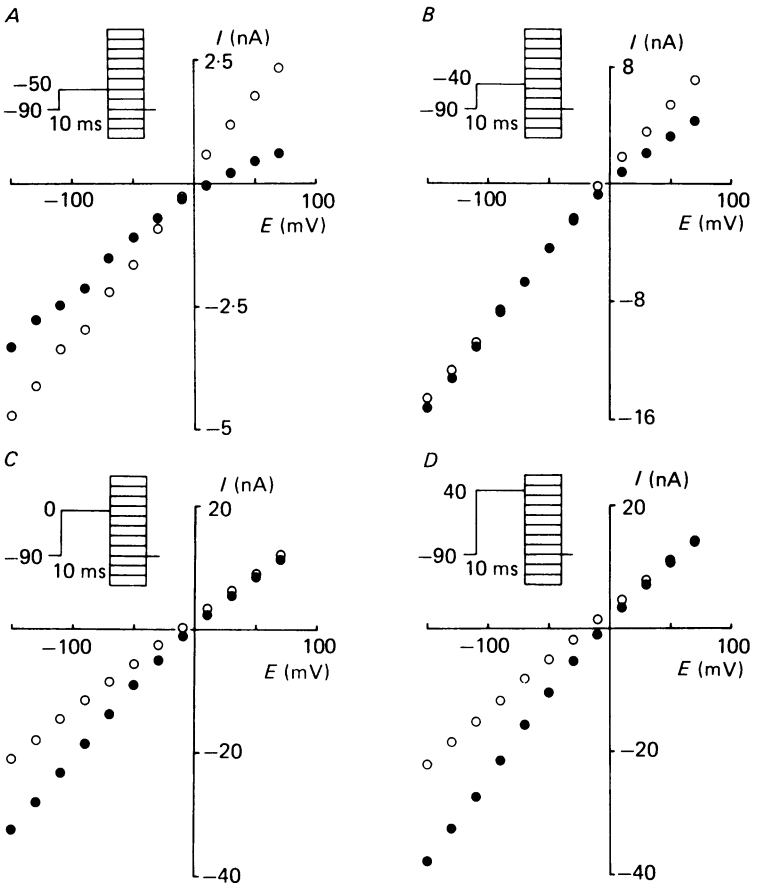

Fig. 5. Instantaneous current–voltage relations for currents recorded 150 μs following pre-pulses of different amplitude which activate different proportions of the components of K^+ conductance in K^+ (●) and Rb^+ (○). Pulse protocols are illustrated in the insets. Note that *A* is from a different fibre to *B*, *C* and *D*, and that the ordinates have different scales.

shift is apparent in Rb^+, the slope of the current–voltage relation is increased in Rb^+ (Fig. 5*A*), reflecting the increase in g. Around the point of inflexion, where the conductances are similar, the current–voltage relations correspond for inward currents (Fig. 5*B*), whilst at more positive pre-pulse potentials the inward currents are reduced in Rb^+ (Fig. 5*C* and *D*). The extent of the reduction in inward current following more positive pre-pulse potentials showed very little or no voltage dependence, suggesting that the effect of Rb^+ is not a strong voltage-dependent block of inward current.

Instantaneous current–voltage relations in K^+ show some inward rectification which is largely removed in Rb^+ (Fig. 5). The measurements in Fig. 5 underestimate the extent of rectification, particularly at more negative potentials where the current decays very rapidly (Fig. 6*A*) and resolution is limited by uncompensated capacitive current. Estimates of the initial current by extrapolation of the fit to the tail current to $t(0)$ show that the current may be underestimated by $\sim 30\%$ at -150 mV. The

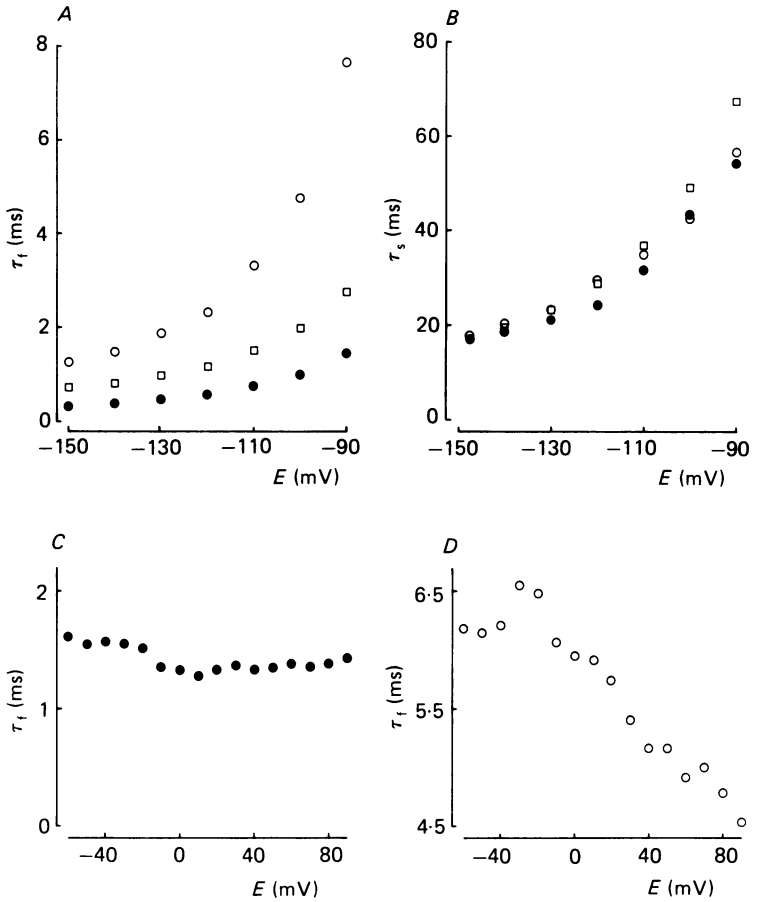

Fig. 6. Potential dependence of tail current time constants. Time constants for the fast (*A*) and slow (*B*) components of the tail currents in solutions containing K^+ (●), 50% K^++50%Rb^+ (□) and Rb^+ (○) on stepping to potentials between −150 and −90 mV following a 200 ms pre-pulse to 0 mV. Same fibre as Fig. 4 measured at a different time. *C* and *D*, dependence of fast tail current time constant, on repolarization to −90 mV, on the potential during the 200 ms pre-pulse in K^+ (*C*) and Rb^+ (*D*). Note that the ordinate in *D* has a minimum of 4·5 and not 0 as in *C*. *C* and *D* from a different fibre to *A* and *B*.

error decreases rapidly with more positive test pulse potentials. Rb^+ tail currents are slower at all potentials and less prone to underestimation. The extent to which inward current is reduced in Rb^+ compared to K^+ increases with increasing pre-pulse potential, the inward currents in Rb^+ being of a similar amplitude following pre-pulses to 0 and +40 mV (Fig. 5*C* and *D*).

Effect of external Rb^+ on deactivation kinetics

In the presence of external Rb^+ deactivation of K^+ channels is slowed (see Introduction). This effect of Rb^+ was also clear in the experiments described here (Fig. 1*B* and 4*A*). The time constants obtained from the fits of tail currents to two exponential functions for the fibre in Fig. 4 are shown in Fig. 6 for K^+, 50%

$K^+ + 50\%$ Rb^+, and Rb^+. In this experiment, tail currents were measured on repolarization to potentials between -150 and -90 mV following a 200 ms pre-pulse to 0 mV. Deactivation of the fast component is slowed by external Rb^+, the extent of slowing being dependent on $[Rb^+]_o$ and on the membrane potential (Fig. 6*A*; see also Cahalan & Pappone, 1983; Beam & Donaldson, 1983). However, the time constant of the slow component (τ_s) was little affected by Rb^+ (Fig. 6*B*). Estimates of τ_s are more difficult in Rb^+ because the amplitude of I_s is reduced and τ_f is increased, resulting in more temporal overlap of the two components. The use of brief (< 10 ms) pre-pulses, which activate little of the slow component, showed that the slowing of deactivation in Rb^+ was an effect on the fast component, and not an altered relaxation of g_s.

With external Rb^+ and internal K^+, τ_f was dependent on the amplitude of the conditioning pre-pulse, decreasing with increasing pre-pulse potential at potentials where the current during the pre-pulse was outward (Fig. 6*D*). This results in a cross-over of Rb^+ tail currents in some fibres, in contrast to the normal parallel time course of K^+ tails. A possible explanation for this effect is a small increase in $[K^+]$ at the outer membrane surface, during pre-pulses positive to the reversal potential, causing an increase in the closing rate of the channels. The results in Fig. 6*A* suggest that the effect of K^+ may dominate, since in a 50% $K^+ + 50\%$ Rb^+ mixture the slowing effect of Rb^+ was not 50% of that in 100% Rb^+.

τ_f in K^+ and Rb^+ was not influenced by the length of the conditioning depolarization, but τ_s became larger with increasing pre-pulse duration, confirming the observations of Dubois (1981*c*). As reported by Cahalan & Pappone (1983), no effect of Rb^+ on activation kinetics was detected when the times at which currents reached the half-maximum value were determined.

Slow conductance (g_s) in 4-AP

To investigate the effects of Rb^+ on the slow conductance with reduced contamination by the fast conductance, currents were recorded in the presence of 1 mM-4-AP. Dubois (1981*b*, 1982*b*) described a complete block of g_f by 1 mM-4-AP, without the frequency- and voltage-dependent removal of block which occurs with low $[4\text{-AP}]_o$ (Ulbricht & Wagner, 1976), while g_s was unaffected. With the same concentration (1 mM), Pappone & Cahalan (1984) reported a 95% block of g_f and, in addition, a 48% block of g_s after 270 s.

In 4-AP the fast component of the tail current was irreversibly abolished at low depolarizations, but following large positive pulses a small, fast component of the tail was observed, suggesting that removal of a small part of the 4-AP block occurs during the depolarization. The slow tails in 4-AP were fitted at times > 7 ms to reduce the possibility of contamination (by the remaining fast tail current and by uncompensated capacitive currents) and because of the presence, following larger depolarizations, of a delay before the decline of the tail current. A similar delay before the decline of the slow tail current was described in some fibres by Ilyin *et al.* (1980). The reversal potential, measured from the instantaneous current–voltage relation following a 200 ms pre-pulse to 0 mV, was unaffected by the addition of 4-AP. Run-down of the slow component was a considerable problem in all measurements (including those in the absence of 4-AP), making quantitative estimates difficult.

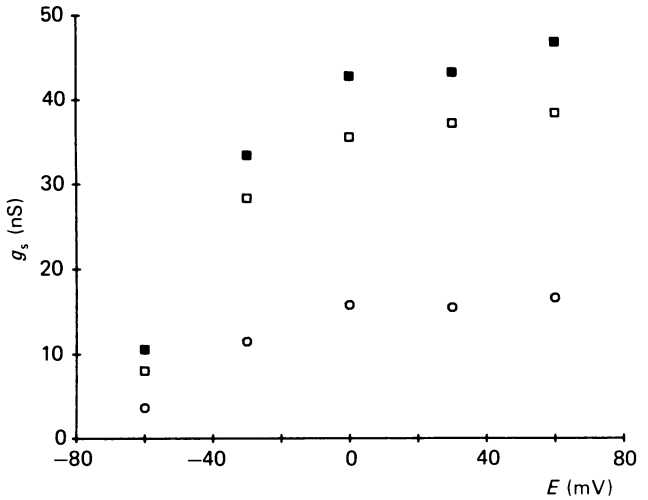

Fig. 7. Effects of 4-AP and Rb^+ on the slow component of the conductance. Slow conductance was calculated from the fit to the slow current component extrapolated to $t(0)$ in 117 mM-K^+ (■), to the current remaining after the addition of 1 mM-4-AP (□), and the current in 117 mM-Rb^+ + 1 mM-4-AP (○). Experiment used a limited number of voltage steps to reduce the problems of run-down. Motor fibre.

In seven out of eight fibres, addition of 1 mM-4-AP reduced the amplitude of g_s at +60 mV by between 5 and 51 % (see Fig. 7); in one fibre there was a small (6·5 %) increase (mean reduction 19·0 ± 6·6 % ($n = 8$)). This supports the observation of Pappone & Cahalan (1984) that 4-AP also reduces g_s, though to a lesser extent than g_f.

Replacement of external K^+ by Rb^+ further reduced the amplitude of g_s (Fig. 7), an effect which was completely or partially reversible in five out of eight fibres. The lack of reversibility in some fibres was probably due to run-down since measurements in fibres where fewer potential steps were used indicated a reversible effect. In no fibre in Rb^+ was a shift of the slow conductance–voltage curve to more negative potentials observed, in contrast to the effect on g_f. The reduction in g_s at +60 mV on changing from external K^+ to Rb^+ was 41 ± 5 % ($n = 8$). E_r in Rb^+ was also shifted to more negative potentials. The mean shift of E_r was −9·3 ± 1·3 mV ($n = 8$), corresponding to a P_{Rb}/P_K of 0·70 ± 0·04 ($n = 8$). This value is different from the value of 0·76 ± 0·01 ($n = 9$) estimated for the fast component ($t = 1·56$).

Effects of internal Rb^+

In internally perfused squid axons, replacement of internal K^+ by Rb^+ prolongs the action potential (Baker, Hodgkin & Shaw, 1962) and results in a considerable reduction in the delayed outward current (Chandler & Meves, 1965; Bezanilla & Armstrong, 1972). It was of interest to investigate the ability of internal Rb^+ to carry current through K^+ channels and to determine whether internal Rb^+ could produce similar effects to those described above for the action of external Rb^+.

Currents were first recorded with 117 mM-KCl bathing the cut ends and then 20 min following replacement of KCl in the end pools by 117 mM-RbCl. Unfortunately

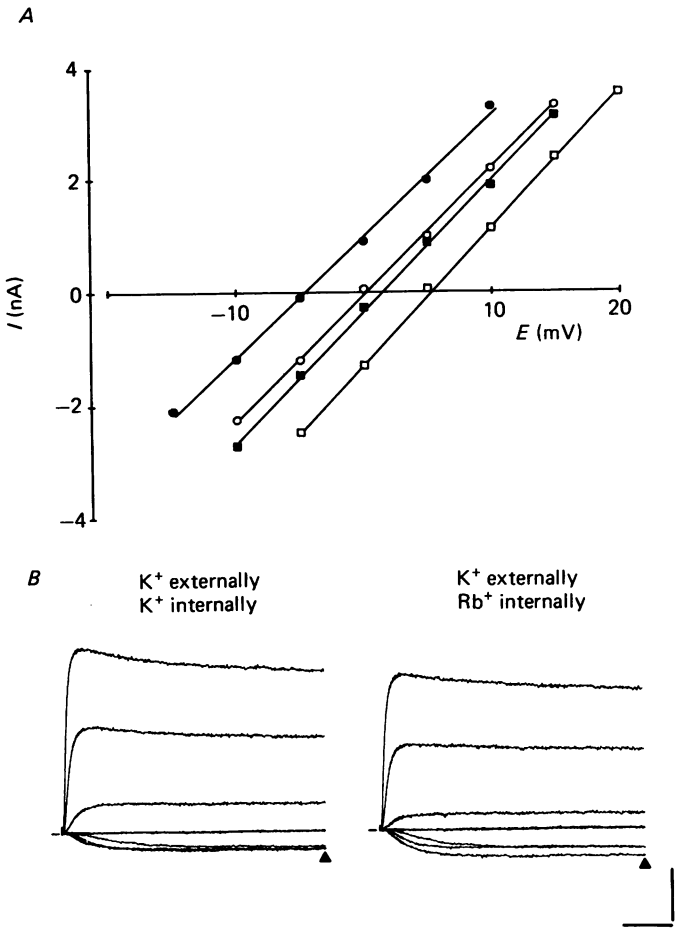

Fig. 8. *A*, Instantaneous current–voltage relations measured 150 μs following a 200 ms conditioning pulse to 0 mV with different external and internal solutions; K^+ externally and internally (■); Rb^+ externally, K^+ internally (●); Rb^+ externally and internally (○); K^+ externally, Rb^+ internally (□). *B*, current records from the same fibre as in *A*, with K^+ externally and K^+ internally (left) and Rb^+ internally (right) for potentials between −90 mV and 50 mV in 20 mV steps. The first inward current was observed at −50 mV and the further depolarizations can be identified by the increase in activation rate. For outward currents the activation half-times were little affected, e.g. at 30 mV: 1·60 ms K^+, 1·67 ms Rb^+ at 50 mV: 1·11 ms K^+ and 1·15 ms Rb^+. ▲ indicates the current at −10 mV. Calibration bars; vertical 5 nA, horizontal 10 ms. Sensory fibre.

with this technique it is difficult to estimate the extent of exchange of K^+ for Rb^+. Fig. 8*A* shows the effect of internal and external Rb^+ on the reversal potential in one fibre. The reversal potential with K^+ externally and internally was +1·6 mV. Following replacement of external K^+ by Rb^+, E_r was shifted by −6·2 mV to −4·6 mV. KCl bathing the cut ends was exchanged for RbCl resulting in a shift of E_r back to a value (0 mV) close to that with K^+ both externally and internally. When K^+ was then applied externally E_r was +5·1 mV. Internal Rb^+ therefore produced shifts of E_r in the direction which would be predicted for a less permeant ion ($P_{Rb} < P_K$).

With external K^+, replacement of internal K^+ by Rb^+ produced shifts of E_r to more positive potentials. The mean shift was $5{\cdot}0 \pm 0{\cdot}6$ mV ($n = 3$). When, with internal Rb^+, external K^+ was replaced by Rb^+, E_r was close to that with K^+ both externally and internally, suggesting that there was a good exchange of Rb^+ for K^+. In the presence of external K^+, the shift of E_r on application of internal Rb^+ was never as large as the shift when Rb^+ replaced external K^+, even when later comparison of E_r with Rb^+ externally and internally with E_r with K^+ externally and internally indicated that there was a good exchange of internal solution. Oxford & Adams (1981) showed that the sequence of relative permeabilities for internal cations was similar to that for external cations. These authors have also shown that the permeability ratio estimated when internal K^+ was replaced by Rb^+ was larger than when external K^+ was replaced by Rb^+ (D. J. Adams & G. S. Oxford, personal communication). This is consistent with the shifts of E_r described above.

Apart from the effect on E_r, no other effect of internal Rb^+ was observed. Outward currents were little affected in amplitude or kinetics by the change in permeant cation (Fig. 8*B*). The times at which the outward currents reached their half-maximum value are given in the legend to Fig. 8. Note that the inward current at -10 mV was larger than at -30 mV with internal Rb^+ in this sensory fibre (see below).

The conductance was calculated from instantaneous currents on stepping to a positive potential ($+50$ mV) where currents were outward, and negative potentials (-100 mV) where currents were inward, following short conditioning depolarizations. These conditioning potentials were 8 or 10 ms in duration so as to activate little of the slow component which, unlike that present during inward tail currents, could not be separated from the fast component at positive potentials. Fig. 9 shows conductance–voltage curves obtained from such an experiment, in the different external and internal solutions. Reversal potentials were estimated from instantaneous current–voltage relations following an 8 ms pre-pulse to 0 mV. Both 'inward' (g_i) and 'outward' (g_o) conductance–voltage curves have a similar shape, showing an inflexion at similar potentials. The conductance with external K^+ calculated from outward currents at $+50$ mV was always smaller than that from inward currents at -100 mV ($g_o \approx 0{\cdot}7\ g_i$), consistent with the rectification of the instantaneous current–voltage relation (Fig. 5). Replacement of internal K^+ by Rb^+ had no effect on either curve. When external K^+ was replaced by Rb^+ both curves were shifted to more negative potentials, and at more positive potentials ($E > -10$ mV) g_i was reduced considerably but g_o relatively little affected. The slight reduction of g_o in Fig. 9 was not observed in all fibres. g_i was reduced such that the rectification of g at positive pre-pulse potentials was abolished. With external K^+, g_o/g_i at $+60$ mV was $0{\cdot}73 \pm 0{\cdot}02$ ($n = 4$), and with external Rb^+, $1{\cdot}03 \pm 0{\cdot}03$ ($n = 4$). Similar results were obtained with internal K^+ and internal Rb^+ suggesting that the internal cation is not important.

Presence of multiple components in the current–voltage relation

To date, the presence of more than one fast component of g_K has only been clearly revealed from conductance–voltage relations and not from current–voltage relations. This is probably because, under the experimental conditions normally used, the jump in g is close to the reversal potential where the driving force ($E - E_K$) is low. E_r can

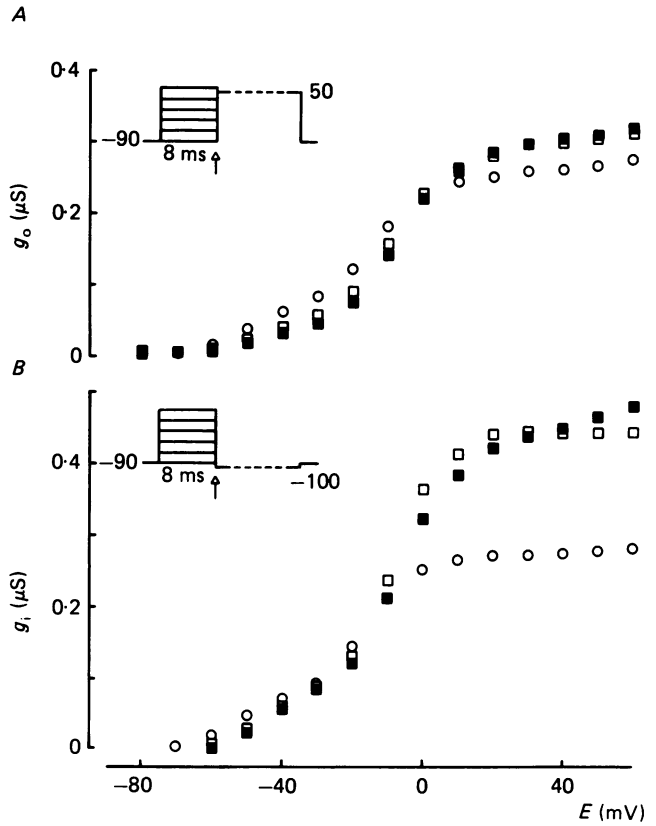

Fig. 9. Comparison of the effects of internal and external Rb^+ on the fast conductance calculated from instantaneous outward (*A*) or inward (*B*) currents. Conductance was first measured with K^+ externally and internally (■) then after application of Rb^+ to the cut ends of the fibre (□) and finally following replacement of external K^+ by Rb^+ (○). The pulse protocol is shown in the insets. Currents were measured at the point indicated by the arrows. Sensory fibre.

be shifted to more positive potentials by replacement of internal K^+ by relatively impermeant ions such as tetramethylammonium (Hille, 1973) or caesium (Dubois & Bergman, 1975*a*).

Currents were recorded from fibres with 117 mM-CsCl applied to the cut ends. E_r was shifted by 20–40 mV from E_r with K^+ both externally and internally and outward currents were reduced considerably (Fig. 10*B*). In sensory fibres, where g_{f2} is much larger than g_{f1} (Dubois, 1981*b*), the current–voltage relation shows two components (Fig. 10*A*), the break occurring at the same potential as the inflexion in the conductance–voltage curve measured from tail currents for the same fibre (Fig. 10*C*). No such break in the current–voltage relation was observed for motor fibres where the difference in the relative amplitudes of g_{f1} and g_{f2} is not as great. This also explains the effect of internal Rb^+ on the inward currents in Fig. 8*B*, where in the sensory fibre illustrated, g_{f2} was very much larger than g_{f1} and the small shift in E_r produced by internal Rb^+ was sufficient to reveal the jump.

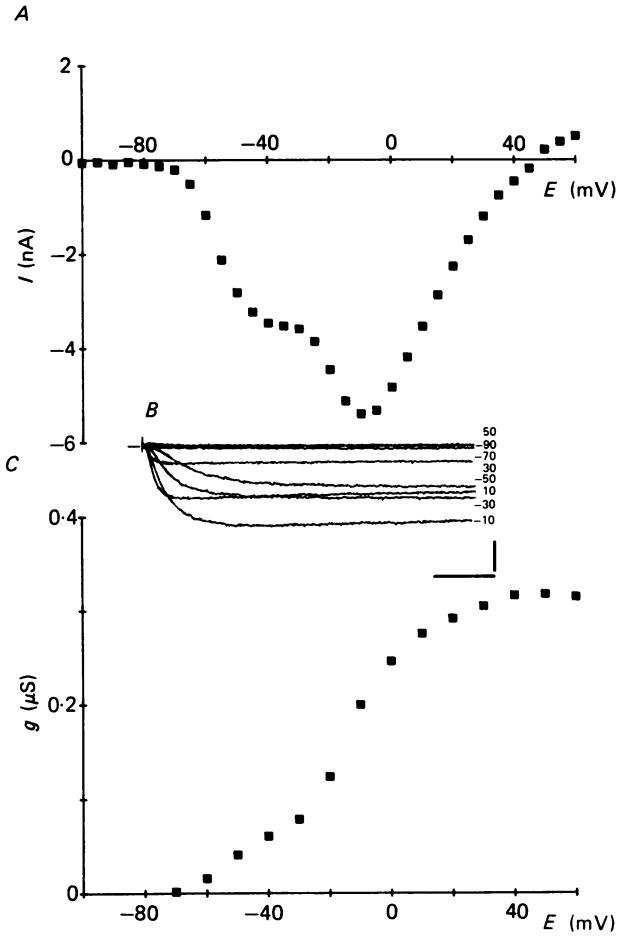

Fig. 10. Replacement of internal K^+ by an impermeant cation (Cs^+) reveals the presence of two components in the current–voltage relation in sensory fibres. *A*, current–voltage relation for currents measured at the end of a 55 ms pulse, from the current records in *B*. *B*, current records for the indicated potentials (mV). Calibration bars; vertical 2 nA, horizontal 10 ms. *C*, total conductance calculated from the fits of tail currents, extrapolated to the time of repolarization, following 55 ms conditioning potentials. External solution 117 mM-KCl, internal solution 117 mM-CsCl. Sensory fibre.

Rb^+ prolongs K^+ channel action potentials

In solutions containing high [K^+], long duration action potentials can be elicited when the membrane, repolarized by anodal current, is depolarized (Müller, 1958). Lüttgau (1961) showed that Rb^+ could replace K^+ as charge carrier but that inactivation of the K^+ system was slowed.

Fig. 11*A* shows tracings of pen-recorder records of K^+ action potentials recorded in 117 mM-K^+, 300 nM-TTX Ringer solution. These action potentials were sensitive to TEA^+ (10 mM), the effects of which were reversible, and to 4-AP (1 mM), which was not completely reversible. In K^+ the duration of the action potential was approximately 30 s. Following application of Rb^+, the membrane hyperpolarized by around

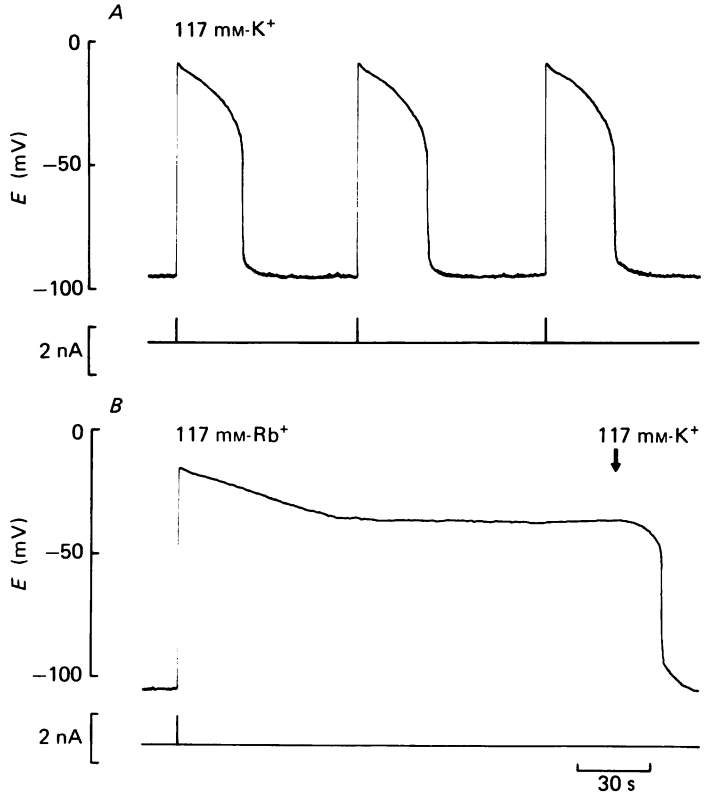

Fig. 11. Effect of Rb^+ on regenerative responses in current clamp. *A*, K^+ regenerative responses induced by 5 ms current pulses. *B*, response to a similar current pulse in Rb^+; repolarization was induced by changing the solution to K^+ at the arrow. Upper trace, membrane potential; lower trace, current. Solution 117 mM-K^+ or 117 mM-Rb^+ (+300 nM-TTX).

10 mV and action potentials on depolarization reached a peak, declined slowly to a plateau level but did not repolarize unless external Rb^+ was replaced by K^+ (Fig. 11*B*) or large anodal currents were applied. The effects of Rb^+ on the action potentials were completely reversible.

Inactivation of the K^+ system is probably responsible for the slow phase of repolarization of K^+ action potentials and the data are consistent with a slowing of this process by Rb^+.

Inactivation in K^+ and Rb^+

Inactivation of I_K can be more directly studied in voltage clamp and is slow, compared to activation, and incomplete (Schwarz & Vogel, 1971; Dubois, 1981*b*).

Currents were measured during 45 s voltage-clamp pulses in 117 mM-KCl or -RbCl (+300 nM-TTX). Owing to the longer pulse duration in these experiments, accumulation and depletion of the permeant ion may be more significant.

Inward currents in K^+ decline slowly to a steady-state level (Fig. 12) and can be fitted by the sum of a single exponential function plus constant. Inactivation of outward currents is more complicated (Fig. 12) requiring the sum of two exponential

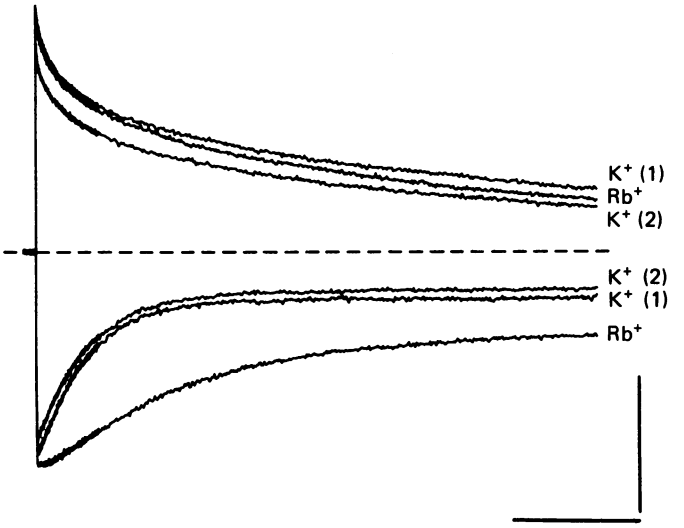

Fig. 12. Inactivation of inward and outward currents with external K^+ and with external Rb^+ (117 mM). The records show currents recorded with the fibre superfused by K^+ before ($K^+(1)$) and after ($K^+(2)$) superfusion of the fibre with Rb^+. Inward currents were recorded at −50 mV and outward currents at 30 mV for 45 s. The dashed line is the holding current. Calibration bars; vertical 5 nA, horizontal 10 s.

functions, because of the presence of a rapid component, plus a constant term. Outward current inactivation was very similar to that observed by Schwarz & Vogel (1971) in *Xenopus* fibres in Ringer solution. These authors also found that at low depolarizations the fast phase of inactivation was 'nearly missing'. The time constant of the slowly inactivating component was voltage dependent, increasing with depolarization in a similar way to τ_2 of Schwarz & Vogel (1971) while the fast time constant τ_1 showed little potential dependence.

When Rb^+ replaced K^+ in the external solution inactivation of inward current was slowed considerably (τ for the inward current in Fig. 12 was increased from 3·9 s in K^+ to 11·9 s in Rb^+, neglecting the initial 1 s of the Rb^+ current), while inactivation of outward currents was little affected. Such slowing of inactivation could account for the prolongation of Rb^+ action potentials.

DISCUSSION

The experiments confirm and further investigate the previously described effects of Rb^+ on the K^+ permeability of the node of Ranvier and, in addition, show that the action of Rb^+ is dependent on the side of the membrane to which it is applied and the current direction, and that Rb^+ does not act on all components of the K^+ conductance in the same manner.

Rb^+ permeability and conductance

Rb^+ permeability measured from E_r. Reversal potential measurements in the node of Ranvier where all external K^+ was replaced by Rb^+ have indicated that Rb^+ is less permeant than K^+ through K^+ channels with a permeability ratio P_{Rb}/P_K in the

range 0·76–0·91 (see above; Hille, 1973; Cahalan & Pappone, 1983). The value 0·76 obtained in these experiments is the lowest in this preparation but is close to values described for voltage-dependent delayed rectifier K^+ channels in other tissues, e.g. 0·74 in snail neurones (Reuter & Stevens, 1980) and 0·77 in human T lymphocytes (Cahalan *et al.* 1985). When the external solution was 50 % K^+ + 50 % Rb^+, P_{Rb}/P_K was 0·58, suggesting that the permeability ratio is mole-fraction dependent. In 4-AP, when g_f is blocked, P_{Rb}/P_K was 0·7 and different from that for the total conductance. However, the measurements were not made on the same fibres. Under similar conditions a P_{Rb}/P_K of 0·6 has been reported (Fig. 3*b* of Dubois, 1981*c*).

Rb^+ and the components of K^+ conductance. Hille (1973) found that, following large depolarizing pulses, inward tail currents in Rb^+ were smaller than expected from the permeability ratio, assuming independence between K^+ and Rb^+ fluxes. This result is confirmed in these experiments where, at pre-pulse potentials more positive than −30 mV, the fast conductance (g_f) calculated from inward tail currents is reduced in Rb^+, while at more negative potentials the conductance is similar, or increased owing to a shift.

The presence of two fast components is apparent as an inflexion in the fast conductance–voltage curve at around $E = -30$ mV. Previously this inflexion has only been observed in conductance–voltage curves (Palti *et al.* 1980; Dubois, 1981*b*; Cahalan & Pappone, 1983) but it may also be seen in the current–voltage relations from sensory fibres when E_r is shifted to more positive potentials by the application of an impermeant cation, such as Cs^+, internally (Fig. 10).

With external K^+, the conductance calculated from inward currents is approximately 30 % larger than that calculated from outward currents (see Fig. 9). The maximum fast conductance ($g_f = g_{f1} + g_{f2}$) measured at −100 mV is larger than that at +50 mV, an observation consistent with the inward rectification of the instantaneous current–voltage relation measured after short pre-pulses. Such rectification is also apparent in Fig. 3*A* of Attwell *et al.* (1980). This rectification was largely abolished with external Rb^+, resulting in a near linear instantaneous current–voltage relation, and g_f from inward currents was reduced such that g_f calculated from inward and outward currents was approximately equal.

Recently, Cahalan *et al.* (1985) have shown that the instantaneous current–voltage relation in human T lymphocytes shows inward rectification in high $[K^+]_o$ and that this is a property of the open single channel. Similarly, single Ca^{2+}-activated K^+ channels in human erythrocytes exhibit inward rectification and Rb^+ reduces the single channel conductance by ~ 50 %, the reduction showing a slight voltage dependence (Grygorczyk & Schwarz, 1985).

The reduction in inward current seen in these experiments is as would be expected for an ion less permeant than K^+ (though greater than that which would be predicted from P_{Rb}/P_K calculated from E_r) or for an ion which blocks the channel to some extent. Application of Rb^+ internally, however, has little effect on outward currents and Rb^+ behaves like K^+ as charge carrier.

The effect of internal Rb^+ is different from that in squid axon where complete replacement of internal K^+ by Rb^+ results in a reduction of the delayed outward current (Chandler & Meves, 1965; Bezanilla & Armstrong, 1972). One possible

explanation for the difference is that the squid experiments were performed in K^+-free or low $[K^+]_o$ solutions, whereas these experiments all used high $[K^+]_o$ solutions. Interestingly, Chandler & Meves (1965), using K^+-free external solutions, observed a larger effect of internal Rb^+ than Bezanilla & Armstrong (1972) with low-K^+ (10 mM) solutions. It is possible that $[K^+]_o$ reduces the blocking effect of internal Rb^+, which it does for the blocks by internal Na^+, Cs^+ and Ba^{2+} (Bezanilla & Armstrong, 1972; Armstrong & Taylor, 1980). External K^+ also speeds the recovery from block by internal quaternary ammonium ions (Armstrong, 1975). It is thought that K^+ in the channel displaces the blocking ion from a binding site within the pore.

A number of the observations described here are clearly deviations from independence. Such deviations have been described for membrane K^+ channels (Hille & Schwarz, 1978) and also for Na^+ channels (Hille, 1975; Begenisich & Cahalan, 1980*a*, *b*). Many of the properties of K^+ channels can be reproduced by assuming that they are multi-ion single-file pores with an energy profile which is a linear sequence of at least four barriers and three wells, the wells representing ion binding sites (Hille & Schwarz, 1978). Rectification of the instantaneous current–voltage relation can also be predicted by such models (Woodbury, 1971; Begenisich & Cahalan, 1980*b*). The effect of Rb^+ on rectification and the sidedness of the Rb^+ effect seen in these experiments could possibly be explained by differences in the energy profile in Rb^+, where, with external Rb^+, the heights of energy barriers and/or depths of energy wells are changed, but this has not been tested. In squid axon, the permeability ratios for the Na^+ channel are dependent on the internal concentration of K^+, Cs^+ or ammonium ions, but independent of the external concentration of K^+ or ammonium (Chandler & Meves, 1965; Begenisich & Cahalan, 1980*a*). Begenisich & Cahalan (1980*a*, *b*) showed that a three-barrier, two-site model, with equally spaced barriers and wells, but asymmetric with respect to barrier height and well depth, could account qualitatively for their observations. The energy profile of K^+ channels could be modified by the presence of Rb^+ at binding sites in the channel or alternatively by modulatory binding sites at the outer membrane surface. The existence of K^+ binding sites within the channel has been proposed to explain the dependence of g_K and activation on external K^+ in the node of Ranvier (Dubois & Bergman, 1977; Dubois, 1981*a*, *b*). Such a site or sites could modulate the permeability properties, and also kinetics, of the ion channel. The existence of modulatory sites outside the permeation pathway has been used to model the effects of ions on the conductance and kinetics of inward rectifier channels in starfish egg cells (Ciani, Krasne, Miyazaki & Hagiwara, 1978) and cation-selective channels isolated from the sarcoplasmic reticulum (Fox & Ciani, 1985).

Recently, Läuger (1985) has shown that many of the properties of ion channels, including rectification, can be explained by the existence of conformational substates of the channel with voltage-dependent transitions between the substates. Such a model can also predict the effect of permeating ions on channel kinetics (see below) when interaction between an ion and its binding site in the channel influences the rate constants of the conformational transitions.

Effects of Rb^+ on kinetics

Deactivation. In squid axon the decrease in slope of the instantaneous current–voltage relation by external Rb^+ has been interpreted as a voltage-dependent block by Rb^+ at a site within the pore (Clay & Shlesinger, 1983) or a lower mobility of Rb^+ in the channel (Swenson & Armstrong, 1981). In the latter, the action of external Rb^+ on the instantaneous current–voltage relation was linked with a slowing of channel closing on repolarization which is dependent on the occupancy of the pore by the permeant ion. A slowing effect of high $[K^+]_o$ on channel closing has been observed (Stühmer & Conti, 1979; Swenson & Armstrong, 1981; Cahalan & Pappone, 1983; Cahalan *et al.* 1985) but this effect is more marked in the presence of external Rb^+ (Århem, 1980; Swenson & Armstrong, 1981; Cahalan & Pappone, 1983; Beam & Donaldson, 1983; Spruce, Standen, Stanfield & Wilson, 1984; Cahalan *et al.* 1985). Rb^+ increases the open time of single voltage-dependent K^+ channels in vesicles of frog sarcolemma (Spruce *et al.* 1984).

The results presented here show similar effects of external Rb^+ on the deactivation of g_f in the node of Ranvier to those of Cahalan & Pappone (1983) who used short conditioning pulses. Deactivation of g_f was slowed over the whole potential range over which g_f was activated and not limited to potentials where the slope of the instantaneous current–voltage relation was reduced or g_{f2} reduced. This suggests that the effect of external Rb^+ on deactivation and the slope of the instantaneous current–voltage relation may not be related (cf. Swenson & Armstrong, 1981). There was no evidence for two populations of fast K^+ channels in the external Rb^+ effect on deactivation, all fast channels were slowed. External Rb^+ had little effect on the deactivation of g_s. The kinetics of outward currents were unaffected by the replacement of internal K^+ by Rb^+ as charge carrier.

Cahalan & Pappone (1983) proposed an alternative model for the slowing action of external Rb^+ and trinitrobenzene sulphonic acid (TNBS) on deactivation using a single energy barrier model for the gating process. The effect of external Rb^+ was to reduce the energy of the open state relative to the energy of transition to the closed state.

Inactivation. Rb^+ inhibited the repolarization of regenerative responses in current-clamped fibres; repolarization only occurred when K^+ was readmitted or the membrane hyperpolarized (see Fig. 11). Lüttgau (1961) also showed that inactivation, measured in current clamp, was slowed by external Rb^+. This was confirmed in voltage clamp in these experiments, though the decreased rate of inactivation was only observed when the current was carried inwards by Rb^+ (see Fig. 12). Outward K^+ current kinetics were unaffected by external Rb^+. The effect of internal Rb^+ on inactivation of outward currents was not investigated using long pulses, but the turn-off (inactivation) of outward currents during short pulses did not appear to be greatly affected (Fig. 8*B*). Inward K^+ current inactivation was fitted by a single exponential, while in Rb^+, currents of similar or larger amplitude decayed exponentially (after 1 s) but much more slowly. This is evidence against depletion of K^+ from the perinodal space or accumulation at the inner membrane surface as mechanisms of slow current decline in K^+.

From these experiments it was not possible to determine whether the monoexponential decline of inward I_K compared to the biexponential decay of outward I_K was a property of the current direction or a potential-dependent process. Schwarz & Vogel (1971) showed that, in Ringer solution, at low potentials (< -30 mV) decay of I_K was monoexponential but at more positive potentials biexponential. Dubois (1981*b*) showed that g_{f2}, which is activated at more positive potentials, inactivates more rapidly than g_{f1}. It is probable that two phases of inactivation were not seen for inward currents in these experiments because the potential at which the fast phase of inactivation is first observed is close to E_r where K^+ currents are small and difficult to measure. One possibility to investigate this problem would be to shift E_r using an impermeant ion internally, as in Fig. 10.

The action of external Rb^+ in slowing inactivation is similar to its effect on deactivation, though on a very different time scale, and appears to be an action on g_{f1} which is activated in the relevant potential range. Effects of permeant ions on inactivation kinetics have been described for the inward rectifier channels in frog skeletal muscle (Stanfield, Ashcroft & Plant, 1981) and Na^+ channels in frog node of Ranvier (Spires & Shrager, 1984).

Interestingly, the actions of Rb^+, albeit on different components of the K^+ conductance and processes with very different time courses, are mainly restricted to conditions where Rb^+ carries inward current, outward Rb^+ currents being similar to those in K^+. The conductance for Rb^+ inward movement is reduced compared to K^+, in a particular potential range, and both deactivation and inactivation of inward currents, where Rb^+ is the charge carrier, are slower than in K^+. It is, however, difficult to connect these effects because of different time courses and voltage dependences.

Shift of K^+ conductance by external Rb^+

The model of Cahalan & Pappone (1983) which predicted the effects of external Rb^+ and TNBS on deactivation, also predicted the shift in the steady-state conductance–voltage curve which they observed and was also seen for both conductance–voltage and current–voltage curves in these experiments. This shift is specific for the K^+ conductance (Na^+ currents were unaffected) and also limited to g_f; g_s was not shifted (see Figs. 2, 4, 7 and 9). From g_f measured from outward currents with external Rb^+ it appeared that both g_{f1} and g_{f2} were shifted. An effect of Rb^+ on surface potential is a possibility which must also be considered. A shift of K^+ conductance to more negative potentials when $[K^+]_o$ is raised has been reported in squid axon, and it was supposed that K^+ impedes the binding of Ca^{2+} to negative surface charges (Ehrenstein & Gilbert, 1968). Similarly in squid, Na^+ conductance parameters are shifted to more negative potentials by small increases in the concentration of K^+, Rb^+ and Cs^+, observations compatible with an inhibition of Ca^{2+} binding to fixed negative charges (Adelman & Palti, 1969*a*, *b*; Gillespie & Meves, 1981). It is possible that external Rb^+ inhibits the binding of Ca^{2+} to fixed negative charges more effectively than K^+, thereby causing a shift. However, these charges must be close to or associated with g_f since g_s and I_{Na} were not shifted. Differential effects of Ca^{2+} and pH, which also influence the surface potential, on Na^+ and K^+ conductance parameters have been reported (Shrager, 1974; Begenisich, 1975; Schauf

TABLE 1. Effects of various agents on components of the K^+ conductance in frog node of Ranvier

Substance or agent	Effect	Components	References
TEA^+	Block	$g_{f1} = g_{f2} > g_s$	2, 5
4-AP	Block	$g_{f1} = g_{f2} \gg g_s$	2, 3, 6, 7
Capsaicin	Block (time dependent)	$g_{f2} > g_{f1}$	2, 8
Morphine Naloxone	Block	g_{f2}, g_s	4
Rb^+ (external)	Reduction of inward currents	g_{f2}, g_s	7
Rb^+ (external)	Slows inward tail currents	g_{f1}, g_{f2}	1, 7
Rb^+ (external)	Slows inward current inact.	g_{f1}	7
Rb^+ (external)	Negative shift	g_{f1}, g_{f2}	1, 7
TNBS	Conversion	$g_f \rightarrow g_s$	1, 6
High pH (11·5)	Conversion	$g_f \rightarrow g_s$	1, 6
High pH (11·5)	Negative shift	g_{f1}	1

= equal effect, > greater effect, → conversion.

References: 1, Cahalan & Pappone, 1983; 2, Dubois, 1981*b*; 3, Dubois, 1982; 4, Hu & Rubly, 1983; 5, Ilyin *et al.* 1980; 6, Pappone & Cahalan, 1984; 7, this paper; 8, T. D. Plant, unpublished.

& Davis, 1976). The shifting effect of Rb^+ was also seen when $[Ca^{2+}]_o$ was raised from 1·8 to 20 mM (see Fig. 3).

Are there different populations of K^+ channels?

Multiexponential deactivation of I_K does not necessarily indicate the presence of different populations of K^+ channels and can also be explained by a more complicated gating process (Cahalan & Pappone, 1983). However, there is increasing pharmacological and kinetic evidence which suggests that there are different populations or conducting states of K^+ channels in the node of Ranvier. Effects of various substances on the different components are summarized in Table 1.

The evidence does not clearly distinguish whether there are separate populations of channels or forms of the same channel with pharmacological and kinetic differences. External Rb^+ has different actions on the two fast components and slow component. g_{f2} and g_s measured from inward tails are both reduced by external Rb^+ but activate over different potential ranges. g_{f1} and g_{f2} are shifted to more negative potentials by external Rb^+, while g_s is not shifted. The fast tail current ($g_{f1}+g_{f2}$), is slowed considerably by external Rb^+, the slow tail component being rather little affected. While g_f is slowed, it is not slowed to an extent where it could be considered that g_f was converted to g_s, which appears to be the action of high pH and TNBS (Pappone & Cahalan, 1984). The slow current induced by TNBS and high pH is also resistant to 4-AP, as is g_s, actions which suggested that the fast and slow components are interconvertible and may be different forms of the same channel. This was not the case for the current slowed by Rb^+ which was blocked by 4-AP.

Inactivation kinetics also indicate the presence of different components of g_K (Schwarz & Vogel, 1971; Dubois, 1981*b*). Non-stationary current fluctuation measurements show that there are multiple channel states or populations with different single channel conductances and that some of these may interconvert slowly (Conti *et al.* 1984). As yet, no clear physiological role for the different components has been discovered.

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