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SOME FURTHER OBSERVATIONS ON THE ELECTROGENIC SODIUM PUMP IN NON-MYELINATED NERVE FIBRES

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SUMMARY

1. A study has been made of the hyperpolarization that follows a period of electrical activity (post-tetanic hyperpolarization) and of the hyperpolarization which develops when potassium is readmitted after bathing the desheathed vagus nerve of the rabbit in potassium-free Locke solution during 15 min (potassium-activated response).

2. Reduction of the external chloride concentration increases the membrane resistance and the potassium-activated response without changing the time constant of the response. A linear relation between the amplitude of the potassium-activated response and the membrane resistance was found. When chloride was replaced completely by isethionate (sodium salt) and by sulphate (other salts) the potassium-activated response increased by a factor of 5.

3. The membrane resistance is decreased during the post-tetanic hyperpolarization elicited in isethionate Locke solution: the decrease is more pronounced after a longer period of electrical stimulation of the nerve.

4. A small increase of the membrane resistance was found during the potassium-activated response. The changed membrane potential during the response can account for the alteration of the membrane resistance observed.

5. The amplitude of the potassium-activated response is increased during hyperpolarization and reduced during external depolarization of the nerve, whereas the time constant is not affected. The potassium-activated response appears to be independent of polarization of the membrane after correction for the changed membrane resistance.

6. The maximum amplitude of the activated response and the external potassium concentration are related following Michaelis–Menten kinetics; the time constant of the response is inversely related to the external potassium concentration.

7. The area of the electrogenic response activated by high potassium concentrations (5·6–20 mM) is almost constant, but is reduced at lower potassium concentrations. The amplitude and area of the thallium-activated response are increased (about 1·5 times) compared with the potassium-activated response.

8. It was concluded that the electrogenic response, reflected by post-tetanic hyperpolarization, is not directly related to activity of the electrogenic pump, which is probably due to accumulation of potassium in the periaxonal space; that the potassium activated response is produced entirely by activity of the electrogenic sodium pump; and that the current produced by activity of the electrogenic sodium pump is independent of the electrochemical gradient and membrane resistance.

INTRODUCTION

The metabolic processes occurring in nerve during recovery from activity are accompanied by rather slow changes in the membrane potential (Kerkut & York, 1971; Thomas, 1972). Thus, myelinated and non-myelinated nerve fibres hyperpolarize after a period of stimulation. This post-tetanic hyperpolarization is attributed to the increased rate of sodium extrusion that follows a period of activity (Ritchie & Straub, 1957; Connelly, 1962; Rang & Ritchie, 1968*b*; Holmes, 1962; Den Hertog, Greengard & Ritchie, 1969; Den Hertog & Ritchie, 1969).

The size of the potential generated by an electrogenic operating pump should be related to the membrane resistance. Rang & Ritchie (1968*b*) tried to examine the effect of changing the membrane resistance by changing the external chloride, but they could not decide from their observations whether the increase in the post-tetanic hyperpolarization in the absence of chloride was related to an increase in membrane resistance or enhancement of the rate of electrogenic extrusion of sodium.

In many tissues the sodium pump is activated by potassium and other cations such as thallium, rubidium and caesium (Whittam & Ager, 1964; Baker & Connelly, 1966; Rang & Ritchie, 1968*b*). The discrepancy between the activated electrogenic response and the oxygen consumption in C-fibres in the presence of potassium and thallium respectively (Rang & Ritchie, 1968*b*) remained inconclusive until now.

Acceleration of the sodium pump produced by a lowered electrochemical gradient as suggested for striated muscle (Conway, Kernan & Zadunaisky, 1961; Mullins & Frumento, 1963) cannot be important in C-fibres, for both the electrochemical gradient and the oxygen consumption were increased after addition of potassium (Rang & Ritchie, 1968*a*, *b*). However, when the electrochemical gradient was changed by polarizing current, the post-tetanic hyperpolarization was affected (Rang & Ritchie, 1968*b*).

The present experiments examine the relation between the electrochemical gradient, the membrane resistance and the coupling ratio, respectively, and the electrogenic response.

METHODS

The cervical vagus of the rabbit, killed by the injection of air into an ear vein, was desheathed under a dissecting microscope and mounted in a double-sucrose gap apparatus for measuring changes in membrane potential and of the electrotonic potential (Berger, 1963; Bülbring & Tomita, 1969). The desheathed vagus nerve was inserted in a channel (25 mm long; 1·0 mm wide) and fixed on both sides. The 'indifferent' outerside of the nerve was perfused with isotonic potassium chloride solution. The central part of the nerve (length: 0·8 mm or less) was perfused with the test solution (flow: 1·0 ml./min) and the two adjoining parts were perfused with isotonic sucrose solutions (flow: 0·1 ml./min; Fig. 1).

Polarizing currents (10^{-8}–10^{-6} A) delivered by a battery could be applied via a series resistor of 100 MΩ to one of the sucrose bridges by means of the two indwelling platinum rings (Fig. 1: electrodes 3 and 4) at a distance of 5 mm from each other. This circuit could be connected or disconnected by an electrotonic switch, operated by a stimulator, to deliver pulses with a duration of 1·0 sec and 5·0 sec. The central part of the nerve perfused with the test solution could be stimulated by pulses (duration: 0·3 msec) delivered by a stimulator via an isolation unit by means of the platinum electrodes (2 and 3) at a distance of 0·8 mm from each other.

The space constant of the non-myelinated and myelinated fibres appears to be 0·14–0·25 mm and 2·7–7·2 mm, respectively, using the data presented by Evans & Murray (1954), Keynes & Ritchie (1965) and Huxley & Stämpfli (1949, 1951). From this it is likely that changes in the electrotonic potential are due almost completely to changes in the membrane properties of the non-myelinated fibres. Changes in the amplitude of the electrotonic potential are assumed to be linked with the membrane resistance of the non-myelinated fibres in accordance with the cable theory (Hodgkin & Rushton, 1946; Ohashi, 1971).

To test the contribution of the myelinated fibres to the electrotonic potential, produced by applying current to all the nerve fibres, the central part of the nerve was perfused with isotonic potassium chloride during 20 min. The amplitude of the electrotonic potential (duration: 2–5 sec) was reduced during exposure to isotonic potassium solution to 11·4 % (S.E. of mean: 1·2 %; $n = 3$) of its original value in 5·6 M potassium chloride-free Locke solution. From the observations made by Hodgkin & Huxley (1952*a*) and Rang & Ritchie (1968*b*) it is likely that the membrane resistance of non-myelinated nerve fibres found in chloride-free Locke solution is reduced to 3 % in the presence of isothonic potassium chloride. Thus, it seems fair to assume that the myelinated fibres contribute for 8 % to the membrane resistance in chloride-free Locke solution. The electrotonic potentials measured in the present experiments are corrected for the contribution of the myelinated fibres of the vagus nerve.

The Locke solution contained (mM): NaCl, 154; K_2SO_4, 2·8; $CaSO_4$, 5·0; tris (hydroxymethyl) amino-methane (Tris) brought to pH 7·2 with sulphuric acid, 4·0; D-glucose, 5·0. In chloride-free Locke solution sodium chloride was replaced by sodium isethionate, a presumably impermeant anion (Rang & Ritchie, 1968*b*). In the experiments in which the external potassium (or thallium) concentration was varied, sodium was added as isethionate (134 mM) and sulphate. Potassium (or thallium) sulphate was substituted for sodium sulphate (the total concentration of

potassium- and sodium sulphate was 22·8 mM). The isotonic pressure of these solutions was maintained by the addition of sucrose.

The potentials were recorded via a cathode follower by means of calomel electrodes making contact with the solution on both sides of the sucrose gap and registrated on a servo driven potentiometric pen recorder. The experiments were carried out at room temperature (20–22° C). All data given are means ± S.E. of the mean; the S.E. is not shown if this value is too small to be represented in the graphs. The regression lines were calculated using the least-squares method; the area of the responses were measured with a planimeter.

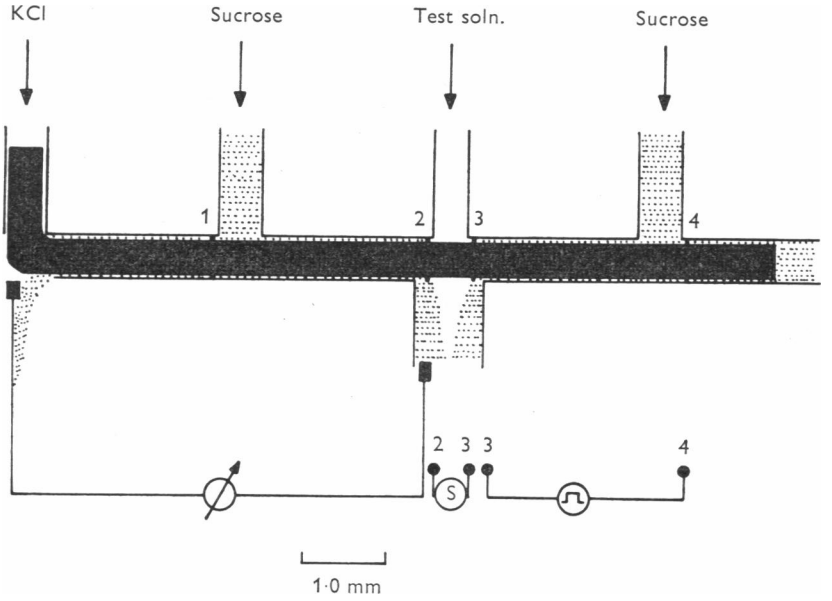

Fig. 1. Schematic diagram of stimulating and recording arrangements. A piece of cervical vagus of the rabbit was mounted in a channel and fixed on both ends. The tissue was bathed in isotonic potassium chloride, isotonic sucrose and test solution. Only 0·8 mm or less was exposed to the test solution between the two sucrose channels. Current pulses were applied through a 100 MΩ resistor via electrodes 3 and 4 to one side of the nerve bathed in sucrose. The changes in membrane potential were recorded across the left sucrose gap. The nerve could be stimulated by applying pulses (duration: 0·3 msec) via the platinum electrodes 2 and 3.

RESULTS

The membrane resistance during the post-tetanic response

The membrane resistance represented by the amplitude of the electrotonic potential was measured during the post-tetanic hyperpolarization (Fig. 2). The amplitude of the electrotonic potential fell at the peak of the post-tetanic hyperpolarization (to 84·2 ± 4·0 % of the value before the response; $n = 3$) and recovered during the decline of the post-tetanic

response. The effect of polarization on the membrane resistance was examined by measuring the electrotonic potential during external hyperpolarization of the nerve to the same extent as the maximum amplitude of the post-tetanic hyperpolarization. The electrotonic potential appears to increase during hyperpolarization ($117{\cdot}0 \pm 4{\cdot}6\,\%$ of the value found without polarization; $n = 3$) rather than to decrease as during the post-tetanic response. This difference might be due to the short-circuiting of

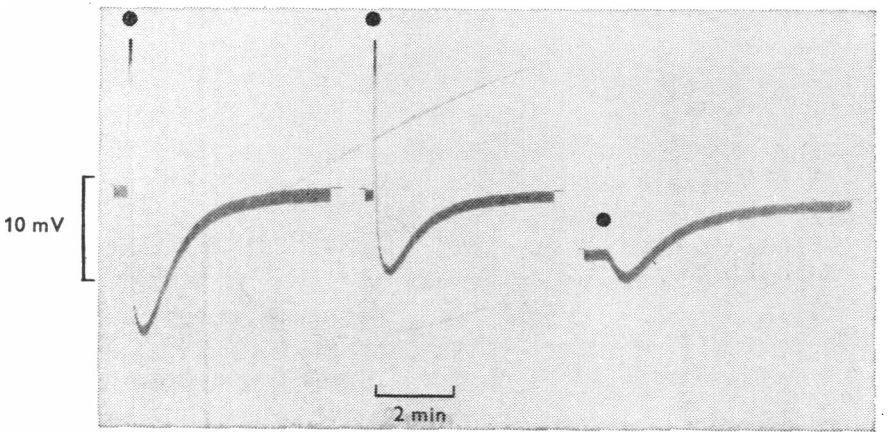

Fig. 2. The electrotonic potential evoked during the post-tetanic hyperpolarization and potassium-activated response in chloride-free isethionate Locke solution of the non-myelinated fibres of a rabbit desheathed vagus nerve. The electrotonic potential is generated by constant current pulses (intensity: 2×10^{-8} A; duration: 1 sec). The post-tetanic hyperpolarization elicited after stimulation of the nerve during 5 sec and 3 sec at a rate of 30/sec (indicated by a dot) are shown in the left-hand curve and the middle response, respectively. The potassium-activated response (right-hand response) was evoked by readmission of potassium (5·6 mM; indicated by a dot) after leaving the nerve in potassium free solution for 15 min.

the post-tetanic hyperpolarization by potassium accumulating in the priaxonal space during repetitive stimulation of the nerve. The short-circuiting factor, which depends on the potassium concentration just outside the nerve fibres, would therefore depend on the period of stimulation. In agreement with this the size of the electrotonic potential was reduced to 96·5, 82·5 and 74·5 % (mean value; $n = 2$) of the amplitude measured just before the post-tetanic hyperpolarization after stimulation of the nerve for 3, 5 and 10 sec respectively.

The membrane resistance during the potassium-activated response

Readmission of potassium after bathing the preparation in potassium-free Locke solution for 15 min reactivates the sodium pump and excess sodium is pumped out. The sodium extrusion is accomplished by hyperpolarization of the membrane. This hyperpolarization called the potassium-activated response, is the same as that studied by Rang & Ritchie who used a somewhat different procedure loading the fibres with sodium.

To examine the membrane resistance during the potassium-activated response, electrotonic potentials were generated simultaneously (Fig. 2). The amplitude of the electrotonic potential increases during the potassium-activated response (to $105{\cdot}0 \pm 0{\cdot}7\,\%$ at the peak of the response; $n = 3$). External hyperpolarization of the membrane to the same extent as observed at the peak of the potassium-activated response also caused an increase of the electrotonic potential (to $106{\cdot}0 \pm 1{\cdot}0\,\%$; $n = 3$). From these results it is likely that the changed membrane resistance during the potassium-activated response is due only to the non-linearity of the voltage–current relation.

The effect of external chloride on the potassium-activated response

Since chloride ions are responsible for the bulk of the membrane resistance in most excitable tissues, the resistance might also be altered in non-myelinated nerve fibres of the rabbit by changing the external chloride concentration. The potassium-activated response and the electrotonic potential were measured to determine the relation between both phenomena. The potassium-activated response was elicited after leaving the nerve in potassium-free Locke solution for 15 min of which the last 8 min were in the presence of 0, 10, 20, 40, 80 or 150 mM chloride. Electrotonic potentials were generated simultaneously with the response (Fig. 3). The time constant of decay of the potassium-activated response remains constant in different external chloride concentrations (Fig. 4), which suggest that chloride does not interfere with the activity of the sodium pump. The relation between the maximum amplitude of the response (ΔV) and the total membrane resistance (R_t), represented by the electrotonic potential, appears to be linear (Fig. 4). This linear relationship shows that the electrogenic sodium current (I_{Na}) is independent of the membrane resistance and can be described by

$$\Delta V = I_{Na} R_t = I_{Na} (R_o + R_{Cl}), \qquad (1)$$

where R_{Cl} is the membrane resistance due to the chloride ions and R_o is the membrane resistance due to all the other ions. The electrotonic potential is reduced to about 23 % when isethionate is replaced by chloride

(150 mM; Fig. 4). The contribution of chloride to the total membrane resistance, therefore, is about 77 %, which is consistent with the value found by Rang & Ritchie (1968*b*). Unfortunately, correction for changes in the permeability of the ions in this system except chloride (R_o) caused by the changed membrane potential after alteration of the external chloride concentration could not be made in view of the different junction potentials (Armett & Ritchie, 1960). However, the linear relationship

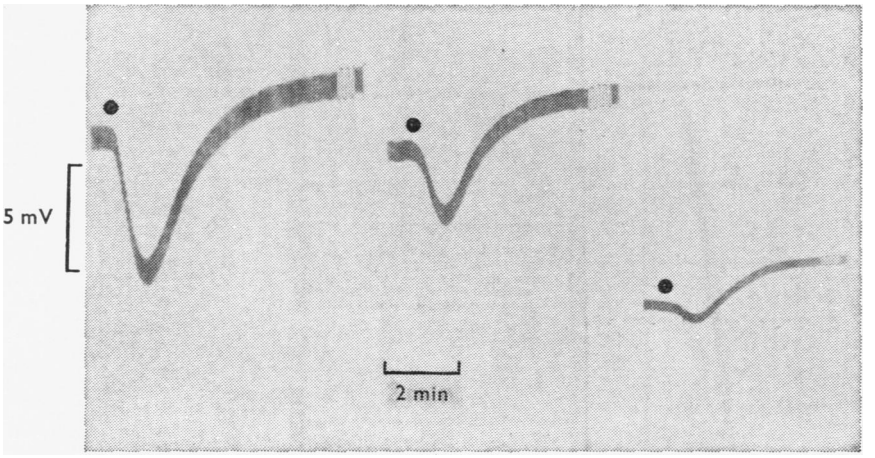

Fig. 3. The effect of external chloride on the potassium-activated response and the electrotonic potential. The potassium-activated response was evoked by readmission of potassium (5·6 mM; indicated by a dot) after leaving the nerve in potassium-free solution during 15 min of which the last 8 min were in the presence of respectively 0 mM (left-hand response), 40 mM (middle curve) and 150 mM (right-hand response) chloride. The electrotonic potential was generated during the responses by constant current pulses (intensity: $2{\cdot}0 \times 10^{-8}$ A; duration: 1 sec; at the end of each response electrotonic potentials with a duration of 5·0 sec were generated during about 20 sec).

between the amplitude of the potassium-activated response and the total membrane resistance, which was varied by changing the chloride dependent membrane resistance, suggest that the contribution of the other ions to the total membrane resistance is negligible.

A marked non-linearity of the relation between the maximum amplitude of the potassium-activated response and the external chloride concentration was found (Fig. 4). To correct for limitation of the chloride ions mobility at higher concentrations, the chloride activity (a_{Cl}) has been chosen as a parameter instead of the concentration. If the membrane resistance is proportional to the chloride activity (proportionality constant: K)

of the external medium and independent of all the other ions (R_o) then relation (1) would become:

$$\Delta V.a_{Cl} = \frac{I_{Na}}{K} - \frac{\Delta V}{K.R_o}. \quad (2)$$

The plotted relation between the product of the maximum amplitude of the potassium-activated response ΔV and the chloride activity of the external solution (a_{Cl}), respectively, and the amplitude of the response fits well with the expected linearity between both parameters (Fig. 4; eqn. (2)).

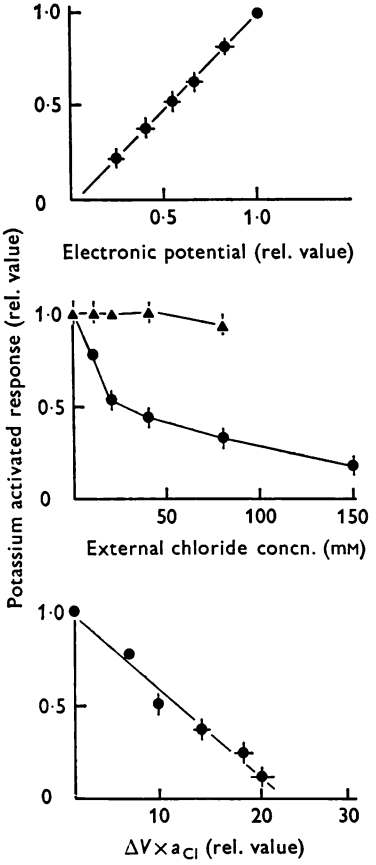

Fig. 4. The relation between the potassium-activated response and respectively the electrotonic potential and external chloride. The values of the potassium-activated response and the electrotonic potential found in chloride-free Locke solution were taken as unity (amplitude of the potassium-activated response (●): 1·0 = 5·3 ± 0·9 mV; time constant (▲): 1·0 = 2·04 ± 0·10 min; electrotonic potential: 1·0 = 1·4 ± 0·1 mV; n = 4). The straight lines represent the regression lines. In the lower graph the product of the amplitude of the potassium-activated response and the chloride activity is plotted on the abscissa (see eqn. (2)).

The effect of polarizing current on the potassium-activated response

To investigate if the electrogenic component of the sodium pump is capable of operating against a potential difference, the electrogenic response was measured during polarization of the membrane. After leaving the nerve for 12 min in potassium-free Locke solution the membrane was polarized by constant current. The membrane potential appeared to be constant within 3 min after onset of the polarization. After 15 min perfusion with potassium-free solution the polarized nerve was exposed to

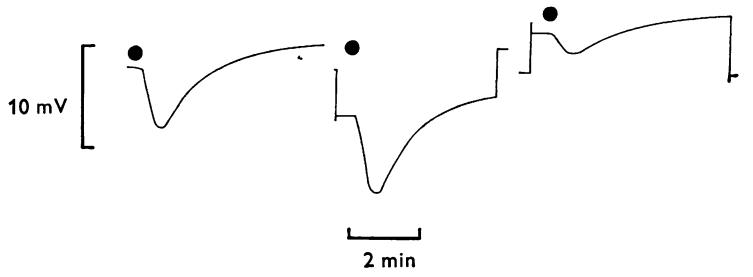

Fig. 5. The effect of polarization on the potassium-activated response elicited by readmission of potassium (5·6 mM; indicated by a dot) after leaving the nerve in potassium-free Locke solution during 15 min. Chloride was replaced by sulphate and isethionate. The membrane was polarized by a conditioning current 3 min before the potassium-activated response. The left hand response was recorded without polarization of the membrane followed by the potassium-activated response during external hyperpolarization of the membrane (current: 4×10^{-8} A). The right hand response was recorded during depolarization of the membrane (current: 8×10^{-8} A).

potassium containing Locke solution, to elicit the potassium-activated response. Modification of the sodium load of the fibres during perfusion with potassium-free solution by external polarization is not likely; polarization for 3 and 12 min, respectively, before addition of potassium did not alter the potassium-activated response (polarizing current: 4×10^{-8} A; two expts.). The potassium-activated response elicited without and during external polarization of the nerve are shown in Fig. 5. The size of the potassium-activated response is increased during hyperpolarization and reduced during depolarization of the nerve, while the time constant is not altered (Fig. 6). The unchanged time constant suggests that the rate at which sodium is extruded is not influenced by polarization.

A changed membrane resistance or a changed coupling ratio (which is that fraction of outwardly pumped sodium that is extruded in exchange for inwardly pumped potassium), might account for the changed amplitude of the potassium-activated response. To distinguish between both

possibilities the membrane resistance during external polarization was calculated from the slope of the voltage–current relation (Fig. 6). The amplitude of the potassium-activated response observed during polarization is represented by a horizontal bar in the graph. The maximum elec-

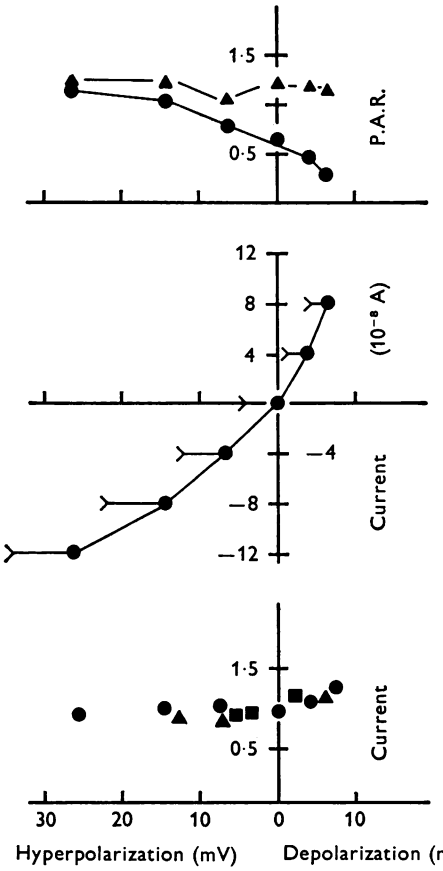

Fig. 6. The potassium-activated response during external polarization. The relation between the maximum amplitude of the potassium-activated response and the time constant, respectively, and membrane polarization is shown in the upper graph. The amplitude of the response (●) was 4·0 mV, while the time constant (▲) was 1·68 min without polarization of the membrane. The middle graph shows the voltage–current relation; the dots represent the membrane polarization evoked by different external current intensities. The horizontal bars represent the amplitude of the response during polarization as given on the abscissa. The lower graph shows the calculated current, generated by the electrogenic sodium pump, at different membrane polarization. The electrogenic current in three different experiments (different symbols) found without polarization of the membrane were 1·9, 2·0 and $2{\cdot}5 \times 10^{-8}$ A respectively, which are taken as unity in the graph.

trogenic current can be calculated from the amplitude at the peak of the response and the membrane resistance at a given external polarization. It appears that the electrogenic sodium current (I_{Na}) is not affected by external polarization of the nerve, although the sodium current tends to increase during depolarization of the membrane (Fig. 6). Thus, the effect on the size of the potassium-activated response during polarization can be explained entirely by the changed membrane resistance.

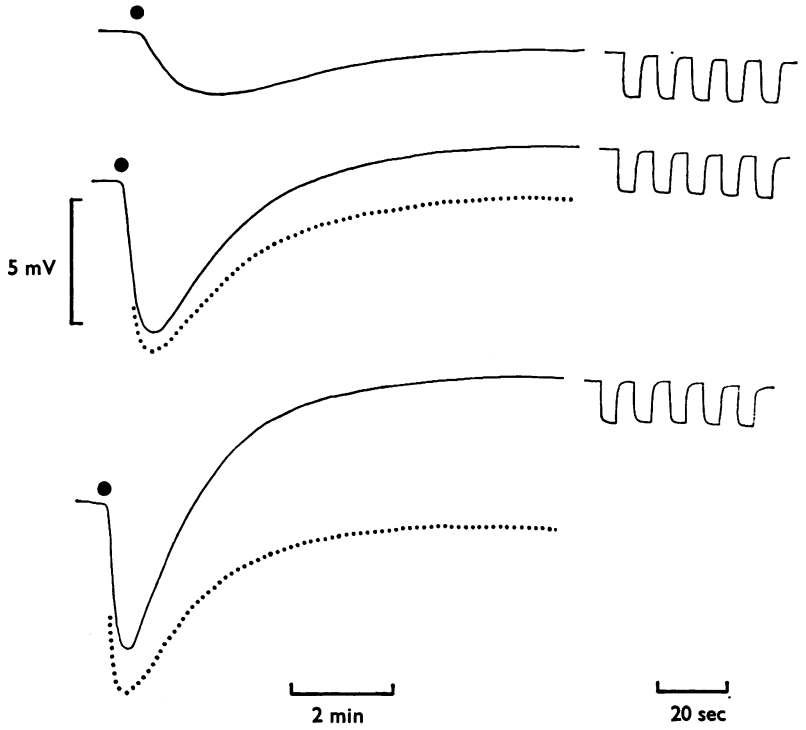

Fig. 7. The recorded and corrected (dotted curve) potassium-activated response and the electrotonic potential in the presence of different external potassium concentrations. The activated response was elicited by readmission (indicated by a dot) of 1 mM (upper trace), 5·6 and 12 mM (lower trace) potassium respectively after leaving the nerve in potassium-free solution during 15 min. The electrotonic potential (generated by constant current pulses; intensity: $2{\cdot}4 \times 10^{-8}$ A; duration: 5·0 sec) in different potassium concentrations are shown at the right-hand side of the Figure.

The potassium- and thallium activated response

To know whether the coupling factor or the membrane resistance is responsible for the reduction of the area of the potassium-activated response at higher potassium concentrations as observed by Rang & Ritchie (1968*b*), the electrotonic potential was measured. Responses were elicited

by addition of different potassium concentrations after leaving the nerve for 15 min in potassium-free solution. The potassium-activated response and the electrotonic potential recorded during exposure to different external potassium concentrations are shown in Fig. 7. The electrotonic potential was not altered when the potassium concentration was changed from 0·5 up to 12 mM, but was decreased at 20 mM potassium (to 53·0 ± 6·1 %) after 5 min exposure (n = 4). The activated response evoked after addition of 5·6 mM potassium or more is composed of a hyperpolarizing and a depolarizing phase; the changed electrochemical gradient can account

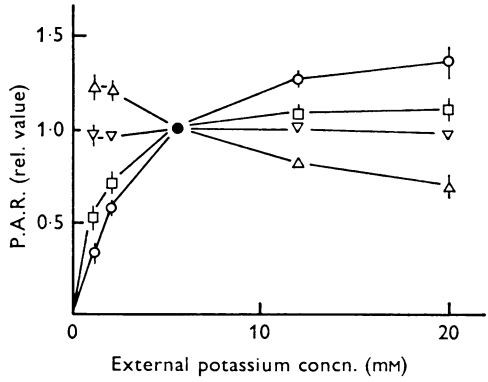

Fig. 8. The relation between the potassium-activated response and the electrotonic potential, respectively, and the external potassium concentration. The mean values of the maximum amplitude (○), the time constant (△), the area (□) of the potassium-activated response and the electrotonic potential (▽), respectively, are taken as unity at an external potassium concentration of 5·6 mM (a dot; amplitude: 1·0 = 4·9 ± 0·4 mV; time constant: 1·0 = 2·02 ± 0·12 min; area: 1·0 = 10·04 ± 0·30 mV min; electrotonic potential: 1·0 = 2·2 ± 0·2 mV; n = 5).

for the depolarization. The response was corrected for the depolarization to yield the real electrogenic component of the sodium pump (Fig. 7). This correction factor was found by changing the external potassium concentration from 1 mM up to 5·6, 12 or 20 mM potassium. As reference 1 mM potassium was taken to prevent inhibition of the sodium pump; the difference in membrane potential recorded between 1 mM and potassium-free Locke solution was negligible. The potassium-activated response was also corrected for the reduction of the membrane resistance found at 20 mM potassium (using the same procedure as given in previous section). The relation between the corrected maximum amplitude of the potassium-activated response and the external potassium concentration is shown in Fig. 8. Activation of the sodium pump with potassium follows Michaelis–Menten kinetics, which is shown by the linear relationship

between the reciprocal maximum amplitude of the electrogenic response and the reciprocal potassium concentration (Lineweaver–Burk plot; Fig. 9). The area of the electrogenic response (corrected for changes in the membrane resistance at 20 mM potassium) increased about 10 % when the external potassium was raised from 5·6 up to 20 mM and was reduced at lower potassium concentrations (Fig. 8).

Activation of the sodium pump with thallium is also reflected by hyperpolarization of the membrane (the thallium-activated response). There is a marked discrepancy between the potency of potassium and thallium in producing the activated response on membrane potential and oxygen consumption (Rang & Ritchie, 1968*b*). To know if there is a difference in the

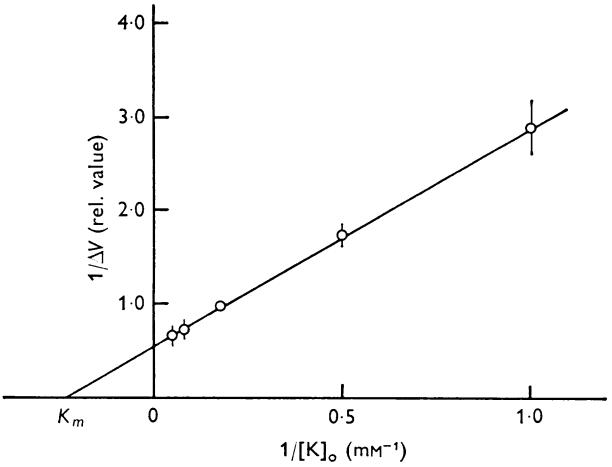

Fig. 9. The relation between the reciprocal value of the maximum amplitude of the potassium-activated response and the reciprocal potassium concentration (Lineweaver–Burk plot). The amplitude was taken as unity at an external potassium concentration of 5·6 mM (1·0 = 4·9 ± 0·4 mV; n = 5).

short-circuiting factor using potassium or thallium, the membrane resistance was determined simultaneously with the electrogenic response. The activated responses were corrected for the depolarizing phase caused by readmission of potassium or thallium using the same procedure as described before. It was found that the electrotonic potential is the same in the presence of potassium and thallium (5·6 and 11·2 mM). The maximum amplitude and the area of the thallium-activated response are higher than the corresponding response elicited with potassium, while the time constant of decay is not altered. The amplitude of the 5·6 mM thallium response was increased with about 35 % (1·42 and 1·29 times the potassium response (two expts.); the area of the response was altered in the same manner as the amplitude (1·45 and 1·23 times the potassium response).

The amplitude of the response activated with 11·2 mM thallium was 1·50 and 1·89 times the response evoked with 5·6 mM potassium (two expts.) and the area was increased to 1·57 and 1·58 times the area of the potassium response.

DISCUSSION

The membrane resistance during the electrogenic response

The present data and those of Rang & Ritchie (1968*b*), leave little doubt that the post-tetanic hyperpolarization and the potassium-activated response reflect activity of the sodium pump. However, in addition the membrane resistance appears to be reduced during the post-tetanic hyperpolarization. This decrease at the peak of the post-tetanic hyperpolarization is about the same as that obtained by perfusing of the nerve with isethionate-Locke solution containing 15 mM potassium instead of the normal 5·6 mM. This suggests that potassium accumulation in the periaxonal space, caused by the potassium efflux during the action potential, raising the periaxonal concentration to 15 mM, might account for the decrease of the membrane resistance following repetitive stimulation. The reduction of the membrane resistance during the electrogenic response is transient because of re-uptake of periaxonal potassium and diffusion to the bulk of the external solution. On the other hand a passive increase in potassium permeability after stimulation (Hodgkin & Huxley, 1952*b*; Frankenhaeuser & Hodgkin, 1956) might also contribute to the reduction of the membrane resistance. Thus, the post-tetanic potassium concentration in the periaxonal space may be less than 15 mM, but the present experiments do not allow one to distinguish between these possibilities.

Reduction of the membrane resistance by either of these mechanisms cannot be expected during the potassium-activated response. Indeed a small increase in membrane resistance was observed during the potassium-activated response, caused by the non-linearity of the voltage–current relation.

Chloride and the electrogenic response

The amplitude of the potassium-activated response is about 5 times higher in isethionate Locke solution than in chloride Locke, which can be explained by a different membrane permeability of these anions. From the present observations it appears that the total membrane resistance is reduced to about 20 % if chloride is substituted for isethionate (Fig. 4). The linear relationship between the amplitude of the potassium-activated response and membrane resistance (Fig. 4) indicates that the electrogenic sodium current, which produces the response, is independent of the membrane resistance. In agreement with this, the time constant of decay

of the electrogenic response appears to be independent of the external chloride concentration.

The relation between the amplitude of the potassium-activated response and external chloride can be described by eqn. (2); the electrogenic sodium current was shown to be constant. Rang & Ritchie (1968*b*) observed that chloride produced a disproportional large decrease in membrane resistance at lower chloride concentrations. This was explained by mobility of chloride ions in association with a saturable carrier. In the present experiments the limited chloride ion mobility at high concentrations was taken into account. With this modification the relation between the amplitude of the potassium-activated response and the product of the amplitude of the electrogenic response and the chloride activity appears to be linear (Fig. 4). From these results it is likely that the mobility of chloride across the membrane is dependent only on the electrochemical gradient.

Membrane polarization and the electrogenic response

Conway *et al.* (1961) have suggested that the sodium pump is accelerated by lowering the electrochemical gradient across the nerve fibre membrane. It was assumed by Rang & Ritchie (1968*b*) that such an activation of the sodium pump was not pronounced in C-fibres for they found only a small reduction of the post-tetanic hyperpolarization during external hyperpolarization of the nerve. In contrast with this observation an increase in the size of the electrogenic response (potassium-activated response) during external hyperpolarization of the membrane was found in the present experiments. The leakage of sodium inwardly in the absence of external potassium is independent on polarization. Thus, it is unlikely that the potassium-activated response is influenced by polarization due to alteration of the sodium load as suggested for the post-tetanic hyperpolarization, which might explain the different observations made by Rang & Ritchie and in the present experiments. The marked change in amplitude of the potassium-activated response during external polarization can be accounted for entirely by the changed membrane resistance. This indicates that the electrogenic current is not dependent on the electrochemical gradient; this is also suggested by the unchanged time constant of the potassium-activated response during polarization (Fig. 6). Limitation of the sodium pump activity by a critical energy barrier, therefore, as suggested by Conway and co-workers (1961), is not likely in non-myelinated nerve fibres.

The activated response

The sodium pump is activated by a number of cations (Baker & Connelly, 1966; Rang & Ritchie, 1968*b*) such as potassium and thallium. From the relation between the amplitude of the potassium-activated response and potassium concentration it is clear that a carrier system is involved in the uphill transport of sodium and potassium (Fig. 9). The amplitude of the electrogenic response appears to increase when the potassium concentration ranges from 1 up to 20 mM conform the observations made by Rang & Ritchie (1968*b*). However, reduction of the area of the response as observed by these authors was not seen in the present experiments. This discrepancy might be due to the biphasic nature of the response observed with potassium concentrations of 5·6 mM or more. The potassium-activated response, therefore, was corrected for the depolarizing phase following addition of potassium in the present experiments. The area of the response was found to be almost constant above 5·6 mM potassium but is reduced at lower concentrations, although the membrane resistance does not change at this concentration range (Fig. 8). This means that the coupling factor might be inversely related to the potassium concentration.

The discrepancy between the potencies of potassium and thallium in changing oxygen consumption and membrane potential (Rang & Ritchie, 1968*b*) cannot be explained by a changed membrane resistance (see Results). Furthermore, the area of the response activated with thallium is greater than the electrogenic response found after readmission of potassium. The present observation that the membrane resistance does not depend on the cation used for activation and the results presented by Rang & Ritchie (1968*a*) that potassium and thallium were equally effective in increasing the oxygen consumption suggest that the coupling ratio is changed in the presence of thallium.

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