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ON THE PERMEABILITY OF MAMMALIAN NON-MYELINATED FIBRES TO SODIUM AND TO LITHIUM IONS

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Electrically excitable cells at rest are characteristically highly permeable to potassium ions and relatively impermeable to sodium ions. Thus, the permeability of the membrane of frog muscle fibres to sodium ions is only 0·01 times that to potassium ions (Hodgkin & Horowicz, 1959); for the squid giant axon the corresponding value is 0·04 (Hodgkin & Katz, 1949). It seems from the present experiments that the relative permeability of the membrane of some mammalian non-myelinated (C) fibres to sodium ions is a good deal higher than these values, and this may partially account for the different kinds of after-potential characteristic of different types of mammalian C fibres.

METHODS

Cervical vagus nerves were rapidly dissected from rabbits killed by the injection of air into an ear vein, and hypogastric nerves were dissected from cats anaesthetized with pentobarbital sodium (30 mg/kg). Both types of nerve consist mainly of non-myelinated fibres, and it has been assumed that the changes in potential of the whole nerve trunk, which occur when the ionic composition of the external bathing medium is changed, could be attributed mainly to these fibres. Each nerve was desheathed under a microscope ($\times 40$) and mounted in a sucrose-gap apparatus for measuring changes in resting potential (Stämpfli, 1954; Straub, 1956, 1957; Armett & Ritchie, 1960). The part of the nerve on the 'indifferent' side of the sucrose gap was perfused with a Locke's solution whose composition remained constant throughout the experiment. The part on the 'recording' side of the sucrose gap was bathed with a variety of modified Locke's solutions, the change in resting potential when switching from one solution to another being recorded with little or no artifact. Preparations which did not almost completely fill the lumen of the sucrose-containing tube on the 'recording' side were rejected, because a large area of contact between the sucrose solution and the perfusing solution (which would necessarily follow from a loose fit) would have been the seat of a confusing diffusion potential in parallel with the nerve potential. During these experiments measurements were made of the potential changes that resulted from switching to bathing solutions containing more potassium or more sodium than a reference solution. The depolarizations so produced were always rapid and largely complete within a few minutes. Readings of the new potentials were usually made after the preparation had soaked for about 5–10 min in the new solution, at a time when the potential was again steady.

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Locke's solution normally contains (mM): NaCl 154; KCl 5·6; $CaCl_2$ 2·2; buffer 1–2; glucose 5·0. This chloride-Locke's solution was used in experiments where the action potentials of the rabbit's vagus and the cat's hypogastric nerves were examined; a few such experiments were done on the rabbit's saphenous nerve, which also contains large numbers of sensory C fibres, there being about four times as many non-myelinated as myelinated fibres (Ranson & Davenport, 1931). However in most of the experiments, when changes in resting potential were being studied, sulphate was used in preference to chloride as the anion in the solutions bathing the nerve, it being assumed that mammalian nerve fibres like other nerve and muscle cells are impermeable to sulphate. This was done to prevent the fibres from swelling when the external potassium concentration was increased; had chloride or some other permeable anion been used, swelling would have been inevitable (Boyle & Conway, 1941; Adrian, 1956). The solutions bathing the nerve were prepared from isotonic solutions of sodium sulphate (113 mM), lithium sulphate (113 mM), potassium sulphate (121 mM) and sucrose (270 mM), to each of which was added tris(hydroxymethyl)aminomethane (Tris) 1 mM as buffer, brought to pH 7·4 with sulphuric acid. Usually enough calcium sulphate was added to each of the bathing solutions to produce a concentration of ionized calcium of about 1 mM (see Hodgkin & Horowicz, 1959). The compositions of the individual bathing solutions, which were all equilibrated with oxygen are described in the text. Unless otherwise stated, experiments were carried out at 25° C (room temperature).

RESULTS

Experiments on the rabbit's vagus

Sodium permeability. In the first type of experiment the nerve was equilibrated for at least 30 min before the experiment began in a potassium-free solution containing 53·3 mM sodium sulphate, 1 mM Tris sulphate and 142·6 mM sucrose; the solution was saturated with solid calcium sulphate. This solution is isotonic with Locke's solution, has the same ionic strength, has a *total* concentration of calcium of about 10 mM and an *ionized* calcium concentration of about 1 mM. Figure 1 (open circles) shows the changes in resting potential produced by replacing part of the sodium with equivalent amounts of potassium. It can be seen that even at high potassium concentrations the slope of the line relating the resting potential to the logarithm of the potassium concentration is considerably smaller than the 58 mV for a tenfold increase in external potassium concentration, which would be expected if the membrane behaved as a simple potassium electrode. Furthermore, at potassium concentrations below about 5 mM the resting potential is relatively insensitive to changes in potassium concentration. Hodgkin & Horowicz (1959) have shown that the similar but much smaller deviation which occurs in frog muscle is of the kind expected from a very slight permeability to the sodium ion. Thus, the membrane potential (E) in the absence of any contribution by chloride seems to be determined by the equation,

$$E = -\frac{RT}{F} \ln \frac{[K]_i + \alpha[Na]_i}{[K]_o + \alpha[Na]_o},$$

where $[\ ]_i$ and $[\ ]_o$ represent the concentrations inside and outside the fibre, R is the gas constant, T the absolute temperature and F is Faraday's constant. The constant α is equal to the ratio of the permeabilities of the muscle membrane to sodium and to potassium (i.e. P_{Na}/P_K) and has a value of approximately 0·01. The straight line drawn through the solid circles in Fig. 1 shows that this equation also applies to mammalian non-myelinated fibres and adequately describes the relationship between the

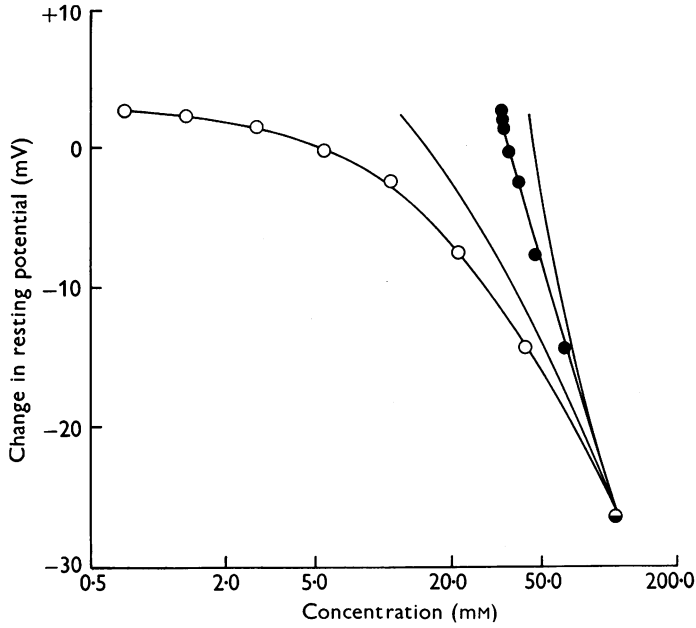

Fig. 1. The relationship between the external potassium concentration and the membrane potential of a desheathed rabbit's vagus nerve. The potential (○) is expressed relative to the resting potential obtaining at the potassium concentration of Locke's solution (5·6 mM). Abscissae: Potassium concentration, open circles; potassium concentration +0·3 (sodium concentration), solid circles. The concentration is plotted on a logarithmic scale. For explanation of the rest of the figure, see text. Temperature, 25° C.

membrane potential and the external concentration of sodium and potassium ions if the value of P_{Na}/P_K is taken to be about 0·3. The assumption of this extraordinarily high permeability of the membrane to sodium ions not only gives a linear relationship, but the slope of the line becomes equal to 58 mV for a tenfold change in $[K]_o + (P_{Na}/P_K)[Na]_o$. The remaining two lines in Fig.1 were calculated for two other values of P_{Na}/P_K: when P_{Na}/P_K is taken as 0·1 the relation is concave towards the origin, when it is taken as 0·4 it becomes convex.

Similar experiments were done in bathing solutions containing no

sucrose. The total ionic strength and the amount of all ions, except calcium, were thus higher. The amount of ionized calcium present in these solutions was necessarily smaller than in the experiments just described, because the increased concentration of sulphate ions restricts the ionization of calcium sulphate; this decrease in the amount of ionized calcium cannot be remedied by increasing the concentration of calcium sulphate, because

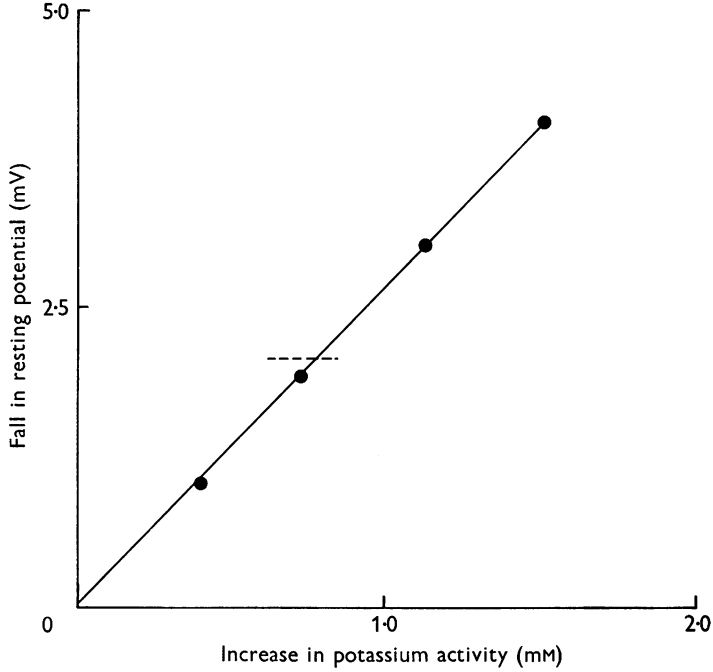

Fig. 2. The relationship showing the decrease in resting potential produced by increasing the activity of potassium in the solution bathing a desheathed rabbit's vagus nerve (●). The interrupted line indicates the decrease the membrane potential produced by adding 4·5 mM sodium (as the sulphate) to the reference Locke's solution. Temperature, 25° C.

the solubility of this compound is limited. The permeability of the membrane to sodium ions determined in these experiments was again about 1/4 of that to potassium ions. In spite of the abnormal composition of the sulphate bathing solutions used above, conduction of the action potential was maintained for many hours with no sign of deterioration when the solutions contained some calcium ions and the usual amount of potassium (5·6 mM).

These experiments, although they consistently indicated that the permeability of the nerve membrane to sodium ions was high, were somewhat unsatisfactory for accurate determinations of P_{Na}/P_K, in that the same

experimental points could be fitted to straight lines, as in Fig. 1, with quite a large range of values. A more sensitive method for determining the relative sodium permeability is illustrated in Fig. 2. Nerve preparations were equilibrated for some time in a standard chloride-free sucrose solution containing 2·8 mM potassium sulphate, 1 mM Tris sulphate and 1 mM ionized calcium. Conduction of the action potential was of course absent, but was restored almost immediately if enough sodium ions, more than 9 mM, were introduced. The calcium was added to the solutions as solid calcium sulphate and the amount of ionized calcium was calculated from the dissociation constant of calcium sulphate, $5{\cdot}3 \times 10^{-3}$ mole/l. (Brink, 1954). In the first part of these experiments the potassium concentration in the bathing solution was slightly increased by replacing some of the sucrose by equiosmotic quantities of potassium sulphate, and the consequent changes in the resting potential were noted. This procedure changed the ionic strength of the solution and consequently changed also the activity coefficient of the potassium ion. In Fig. 2, therefore, the changes in membrane potential are plotted against the changes in potassium *activity* rather than the changes in potassium *concentration*, the activity of the potassium in the standard sucrose solution being 4·7 mM. The activity of the potassium ion in the solutions of different ionic strengths was determined from graphs constructed from tables of the mean ionic activity coefficient of potassium sulphate given by Taylor (1931). It should be noted that the relationship between the depolarization and the increase in potassium activity in Fig. 2 is linear. This linearity presumably occurs because the change $[\Delta K]_o$ in potassium activity is small, so that the expected potential fall $(RT/F)\ln([K]_o + [\Delta K]_o)/[K]_o$ then becomes $(RT/F) \times [\Delta K]_o/[K]_o$. More generally, if there is any degree of short-circuiting in the sucrose gap, the potential change would be $f(RT/F)[\Delta K]_o/[K]_o$, where f is the short-circuiting factor whose value lies between 0 and 1. In the second part of this experiment sodium sulphate was added, instead of extra potassium sulphate, to the original standard solution and the resulting depolarization noted. The permeability to sodium was then calculated as follows. In the experiment of Fig. 2 increasing the potassium *activity* by 0·8 mM caused a depolarization of 2·1 mV. The same depolarization was produced by increasing the sodium *concentration* by 4·5 mM. In the latter solution the total sodium activity was calculated to be 3·7 mM; although in the solution with the added sodium ions the potassium *concentration* was not changed, its *activity* decreased from the value of 4·7 mM obtaining in the standard sucrose solution to 4·6 mM because of the increased ionic strength. The relative sodium permeability is thus given by the relation

$$-0{\cdot}1 + 3{\cdot}7(P_{Na}/P_K) = 0{\cdot}8$$

i.e. P_{Na}/P_K is 0·24. Thirteen such experiments gave a value for P_{Na}/P_K of $0{\cdot}25 \pm 0{\cdot}02$ (S.E.). In most of these experiments the *total* calcium concentration was kept constant, so that a slight decrease in the concentration of *ionized* calcium must have occurred when the extra sodium or potassium sulphate was added. However, the maximum change in the amount of ionized calcium was calculated to be less than 20 %. That this change was probably too slight to alter the interpretation of the experiments seems to be borne out by the results of three experiments where the total calcium sulphate concentration was altered whenever the composition of the solution was altered, so as to maintain a constant concentration of ionized calcium (1 mM); the values obtained for the relative sodium permeability in these experiments, 0·29, 0·27 and 0·23, clearly did not differ appreciably from the over-all mean of 0·25.

Lithium permeability. Keynes & Swan (1959) have shown that although the mechanism responsible for generating the action potential does not discriminate between sodium and lithium ions, the active transport mechanism is capable of extruding lithium from the interior of muscle fibres at only about a tenth or less of the rate that it extrudes sodium ions. The passive permeability of the resting membrane to this ion, which was not specifically determined in their experiments, could be estimated with the present methods. Eight experiments similar to that illustrated in Fig. 2 showed that the permeability of vagal C fibres to lithium is about $0{\cdot}19 \pm 0{\cdot}01$ (S.E.) that to potassium, and $0{\cdot}71 \pm 0{\cdot}03$ (S.E.) that to sodium.

Experiments on the cat's hypogastric nerve

Gasser (1950, 1955, 1956, 1958) has emphasized the fact that there are differences in the electrical responses of different kinds of mammalian C fibres. The rabbit's vagal C fibres just described have a pronounced positive after-potential soon after the spike (Fig. 3*b*), and are like the C fibres of the saphenous nerve which Gasser (1950) classified as 'dr C fibres'. It seemed worth while to examine the other type of C fibres described by Gasser (1950), those in the sympathetic nervous system. These sympathetic fibres characteristically have a pronounced negative after-potential (Fig. 3*a*). This negative after-potential may be preceded by a slight tendency to develop an early positivity (P_1 in Fig. 3*a*); it may also be succeeded by a very small positive after-potential (P_2 in Fig. 3*a*) which appears in Fig. 3*a* as a thickening of the base line a few hundred milliseconds after the spike. Four experiments similar to those described in Fig. 2 were carried out on the cat's hypogastric nerve. They showed that the permeability of the membrane of these fibres to sodium and to lithium was much less than the corresponding values for the rabbit's vagus nerve, being $0{\cdot}10 \pm 0{\cdot}02$ (S.E.) and $0{\cdot}07 \pm 0{\cdot}02$ (S.E.) of that to potassium respectively; the per-

meability of the membrane to lithium was $0{\cdot}73 \pm 0{\cdot}04$ (S.E.) of that to sodium.

Because the permeability to sodium in these nerves was lower than in the vagus, larger amounts of sodium sulphate (up to 22·5 mM) were required to produce depolarizations to match those produced by potassium sulphate. The changes in ionized calcium concentration which would have been the result of the large increases in the sulphate concentration would

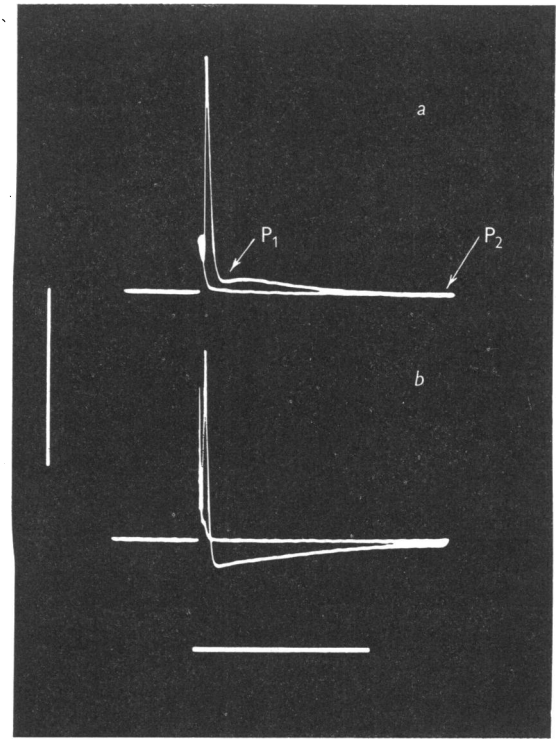

Fig. 3. Records of the compound action potential of the cat's hypogastric (*a*) and the rabbit's vagus (*b*) nerves. Each record consists of two superimposed traces. The first, that of the A or B potentials of the myelinated fibres, acts as a base line for the second trace, whose main deflexion is the C potential of the non-myelinated fibres. P_1 and P_2 indicate the positions of the two positive after-potentials which may be observed in the hypogastric nerve. The horizontal line represents 250 msec and the vertical bar 10 mV. Temperature, 25° C.

thus have been large. They were prevented, as in an earlier experiment, by adding enough solid calcium sulphate to maintain a constant concentration of ionized calcium (1 mM). Measurements of P_{Na}/P_K in these C fibre preparations, however, seem relatively insensitive to changes in the calcium-ion concentration. This is illustrated by the results of experi-

ments on four other cat's hypogastric nerves, where the total calcium concentration was kept constant, so that, according to calculation, the concentration of ionized calcium fell from 1 mM in the standard solution to about 0·3 mM in the solution with the added sodium sulphate. These experiments gave a value of P_{Na}/P_K of $0{\cdot}12 \pm 0{\cdot}01$ (S.E.).

DISCUSSION

The results of these experiments may help to explain one of the major differences between the action potential of mammalian *sensory* C fibres, where there is a pronounced positive after-potential, and those of mammalian sympathetic *motor* C fibres where there is a pronounced negative after-potential (Fig. 3). Hodgkin & Keynes (1955) and Frankenhaeuser & Hodgkin (1956) have shown for squid fibres—and it has subsequently been confirmed for mammalian C fibres (Greengard & Straub, 1958; Ritchie, 1961)—that the positive after-potential immediately following the spike is due to the fact that the membrane potential is normally below the potassium equilibrium potential. After the action potential, however, the potassium permeability which is increased during the spike does not return at once to its original level. Therefore for a short time the membrane behaves more closely as a potassium electrode and the membrane potential approaches the potassium equilibrium potential, i.e. there is a brief phase of hyperpolarization. The positive after-potential is thus a natural consequence of the fact that the nerve membrane does not behave like a potassium electrode. If the nerve membrane did behave as a potassium electrode, it is clear from the sort of argument put forward by Greengard & Straub (1958) that the main after-potential should not be positive, but rather negative, owing to the fact that potassium ions have been released during the spike and have accumulated in the small space of about 150 Å between the Schwann cell and the nerve membrane; because of the very small size, and thus the large surface:volume ratio, of mammalian non-myelinated fibres, this negative after-potential would be particularly pronounced. These considerations suggest how the present experimental results may at least partially account for the differences in the after-potential of different types of C fibre. Because the rabbit's vagal fibres have a much higher permeability to sodium ions than the cat's sympathetic C fibres, the resting membrane potential is likely to deviate further from the potassium equilibrium potential than the membrane potential of the sympathetic fibres. On the one hand, therefore, one would expect the vagal C fibres to conform more to the scheme proposed by Hodgkin & Keynes (1955) and Frankenhaeuser & Hodgkin (1956), where a positive after-potential is expected; on the other hand, sympathetic C fibres would

be expected to conform more to the scheme proposed by Greengard & Straub (1958), which predicts a negative after-potential.

The fact that the resting permeability of C fibres to lithium is about 70 % of that to sodium accounts for the increase in resting potential noted when lithium is substituted for sodium in Locke's solution (Ritchie & Straub, 1957; Greengard & Straub, 1958), for the membrane potential would then deviate less from the potential expected of a simple potassium electrode. An interesting consequence of the membrane then behaving more nearly as a potassium electrode is that the positive after-potential might be expected to be decreased. This has in fact been found to be the case by Greengard & Straub (1958) for the rather small positive after-potential (indicated by P_2 in Fig. 3*a*) which occasionally succeeds the main negative after-potential in sympathetic C fibres, although their interpretation of the disappearance of this positive after-potential involved the mechanism for the active extrusion of sodium ions. During the present experiments with vagal C fibres or with saphenous-nerve C fibres it was found that when the sodium chloride of ordinary Locke's solution was replaced by lithium chloride the large positive after-potential was often markedly reduced, or was even abolished. Greengard & Straub's explanation, although not excluded, would seem less satisfactory in explaining the results with these sensory C fibres because the marked positive after-potential in these fibres seems to be accounted for by an increased permeability to potassium outlasting the spike (Ritchie, 1961), and thus could hardly be affected by an action on the sodium pump. The two different factors which have been suggested to account for the disappearance in lithium solutions of the positive after-potential are fundamentally quite different, but it seems difficult with the available evidence to decide how far each factor is involved or whether even other factors must be invoked to explain the effect of lithium on the after-potential.

The above conclusions strictly apply only under the present experimental conditions. It is clearly possible that the nerves were damaged by the unphysiological bathing media used or by the temperature, which was well below body temperature. However, this is unlikely for in all the bathing solutions used the nerves were fully capable of conducting: if they were not already conducting (because of the absence of sodium ions) the action potential could be restored immediately by adding sufficient sodium (about 9 mM) to the medium. Nor did the temperature of 25° C seem to affect conduction adversely; indeed, at an even lower temperature (about 8° C) conduction was maintained for several days. The difference in the kind of after-potential—positive for the vagal and saphenous-nerve C fibres and negative for the sympathetic C fibres—is present both when the temperature of the nerve is about 25° C and when it is at body

temperature (see Ritchie, 1961, Fig. 8). Therefore it seems probable that the difference in the permeability characteristics of the membrane observed in the present experiments also exists in the nerve in the body.

If the high value of P_{Na}/P_K were due to the *absolute* permeability of the membrane to sodium being very high, the metabolic cost to the fibre of maintaining a low intracellular sodium and the high intracellular concentration of potassium necessary for conduction would be large—perhaps too large to be reasonable under physiological conditions. However, the high value of P_{Na}/P_K may well result from a rather low resting permeability to potassium ions; in this case no extraordinary metabolic load would be put on the C fibres.

SUMMARY

1. Experiments were done to determine the relative permeability of the membranes of sensory and motor mammalian C fibres both to sodium and to lithium.

2. The value of P_{Na}/P_K for rabbit's vagal C fibres is $0{\cdot}25 \pm 0{\cdot}02$ (S.E.) and the value for the cat's hypogastric nerve is $0{\cdot}10 \pm 0{\cdot}02$ (S.E.).

3. The permeability of the membranes of both types of C fibres to lithium ions is about 70% of that to sodium ions.

4. Mammalian C fibres thus seem to be much more permeable to sodium ions than are other excitable membranes.

5. These differences in the permeability of the membranes of the two main types of mammalian non-myelinated nerve fibres to sodium ions could account for the characteristic differences in their after-potential.

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