Transport of Electrolytes in the Schwann Cell and Location of Sodium by Electron Microscopy

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Previous work from our laboratory (1–3), revealed that the Schwann cell of the tropical squid *Sepioteuthis sepioidea* maintains a potential difference of about −40 mv with respect to an external artificial sea water medium. This potential difference remains unchanged (within 1 mv) during the conduction of a single nerve impulse. It was also found that the sodium, potassium, and chloride ions are unequally distributed across the membrane of the Schwann cell. The most striking finding was the presence of a high sodium concentration in isolated sheaths of the giant axon, which was proposed to be located in the Schwann cell. The sodium of the sheath is about six times higher than that found in the axoplasm. It has been suggested that the Schwann cells may contribute to maintain the normal ionic composition of the axolemma-Schwann cell space, which is the actual extracellular environment of the axon (3).

The present work deals with the location of sodium in the nerve fiber sheath by means of electron microscopy and the analysis of the mechanism involved in the maintenance of the electrochemical potential differences across the membrane of the Schwann cell. The unpublished work on sodium location described in this paper represents the results of experiments carried out in collaboration with F. Rawlins.

ELECTROLYTE CONCENTRATIONS AND MEMBRANE POTENTIAL

Determinations of sodium, potassium, and chloride in isolated nerve fiber sheaths revealed that their concentrations in the Schwann cell, expressed in millimoles/liter of cell water, are 312 for sodium, 220 for potassium, and 167 for chloride (3). An estimate can be made of the equilibrium potentials for these ions from the values of their concentrations. The values obtained with this procedure (in millivolts) are +9 for sodium, −79 for potassium, and −32 for chloride, as referred to the sea water bath. The measured Schwann cell membrane potential is −40 mv. (1, 2). Thus, sodium and potassium would be distributed against their electrochemical potential differences, whereas chloride would be almost in equilibrium across the cell membrane. It is also possible that the Schwann cell membrane is impermeable to some of these ions or to all of them. A third alternative for potassium and chloride would be

that their activity coefficients are significatively lower in the cell than in the external fluid.

Sodium

The high sodium concentration found in the Schwann cell is the same when the nerve fiber sheaths are isolated in sea water or in isosmolal sucrose solution. Sodium location in the nerve fiber sheaths was determined by electron microscopy for investigation of the possible existence of sodium bound to extracellular material. The method used was similar to that described by Zadunaisky for muscle fibers (4). Sodium is precipitated as sodium pyroantimonate, when the tissue is put in contact with soluble potassium pyroantimonate. The pyroantimonate precipitate has a high electron opacity and a low water solubility.

In all experiments referred to in this paper, single nerve fibers dissected from *S. sepioidea* were used during the first 2 hr after decapitation of the animal. All experiments were carried out at room temperature (20–22°C).

Fig. 1 shows electron micrographs of cross-sections from two intact nerve fibers. Both fibers were soaked for 5 min in isosmolal sucrose solution and then fixed in glutaraldehyde and postfixed in osmium tetroxide. One fiber was fixed in the presence of potassium pyroantimonate and the other was used as control. In the fiber treated with the reagent, but not in the control, the presence of an electron-opaque, irregularly shaped material inside the Schwann cell cytoplasm can be observed. It is considered to be formed by precipitation of sodium pyroantimonate. The precipitate appears to be associated with dense patches within the cell cytoplasm. In the axon, the sectioned neurofibrils appear to mask the presence of sodium pyroantimonate. However, in longitudinal sections it was possible to distinguish the precipitate in the axoplasm. Few particles may be seen in the basement membrane, but none in the Schwann cell channels and endoneurium.

Fig. 2 shows electron micrographs of sections from two nerve fiber sheaths. The sheaths were isolated by slitting the axons while immersed in isosmolal sucrose solution. Total soaking time in the sucrose solution was 5 min. Fixation was similar to that used for fibers in Fig. 1. Cytoplasmic areas of increased density and clear cytoplasmic regions around invaginations of the basement membrane are observed both in the control and the treated nerve fiber sheaths. Again, the precipitate appears to be associated with dense patches within the cytoplasm of the Schwann cell. No appreciable amounts of the precipitate are found in the Schwann cell channels, the basement membrane and endoneurium. These results were very reproducible and appear to indicate that the sodium present in the nerve fiber sheaths is intracellular.

Fig. 3 shows the pyroantimonate method performed in an intact nerve fiber soaked for 60 min in isosmolal sucrose before fixation. It may be seen that despite the prolonged immersion in sucrose, the Schwann cell contains appreciable amounts of precipitate. As before, the precipitate appears to be associated with dense cytoplasmic patches. No precipitate is found in the basement membrane and Schwann cell channels. These results are compatible with the presence of sodium bound to some intracellular material in the Schwann cell.

It could be considered that the passive permeability of the Schwann cell membrane

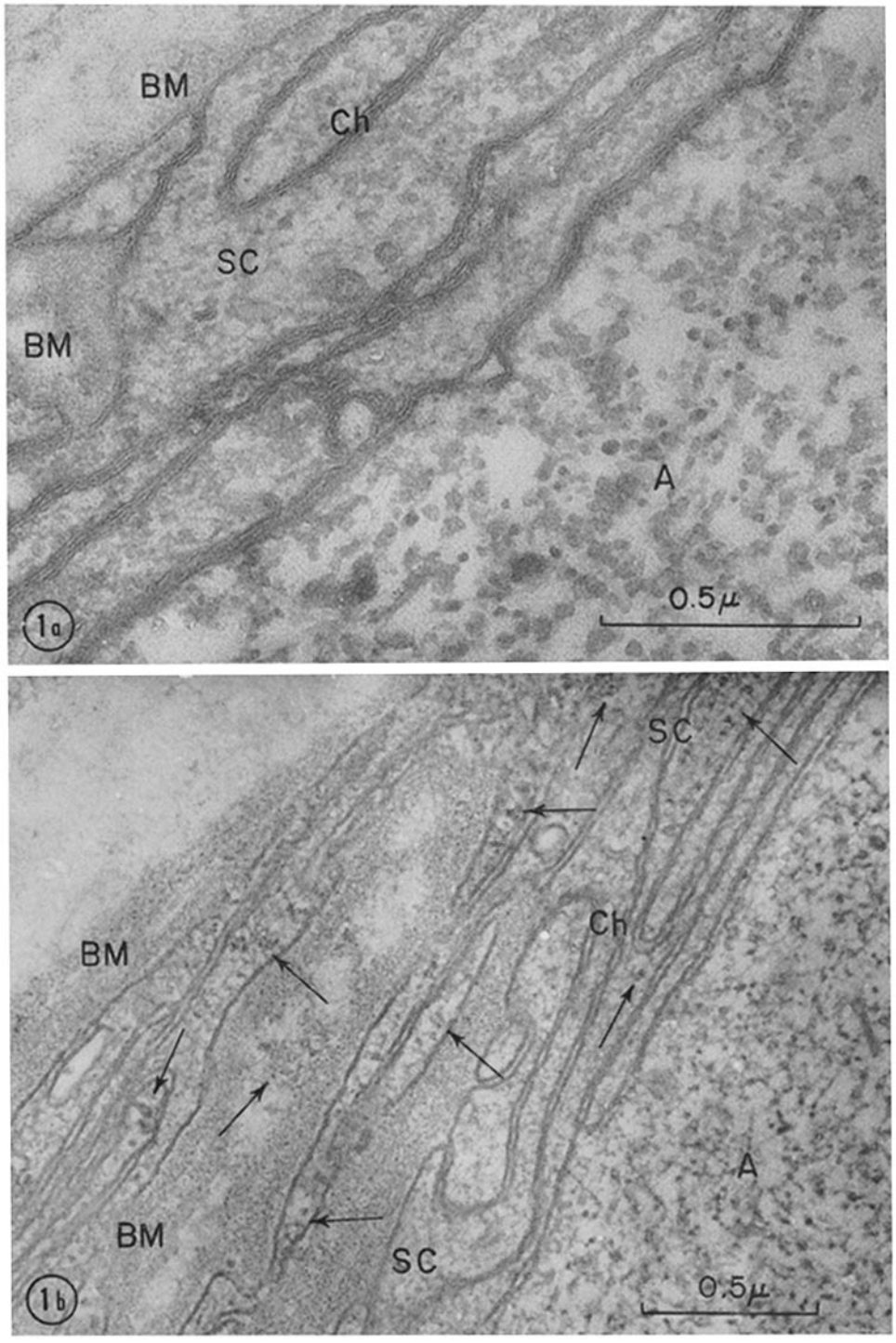

FIGURE 1. Electron micrographs of two *S. sepioidea* giant nerve fibers showing the location of sodium in the Schwann cell. Both fibers were soaked for 5 min in isosmolal sucrose solution before fixation. One fiber (Fig. 1 *a*) was used as control, and the other (Fig. 1 *b*) was fixed in the presence of potassium pyroantimonate. In the treated fiber, sodium pyroantimonate precipitate (arrows) is observed in high concentration in the Schwann cell cytoplasm (*SC*) and appears bound to some dense material. A few particles are also observed in the basement membrane (*BM*) whereas none are found in the Schwann cell channels (*Ch*). The presence of precipitate in the axoplasm (*A*) is masked by the sectioned neurofibrils. Glutaraldehyde-fixed, OsO_4-postfixed, Epon-embedded material. FIG. 1 *a*, × 73,400; FIG. 1 *b*, × 50,000.

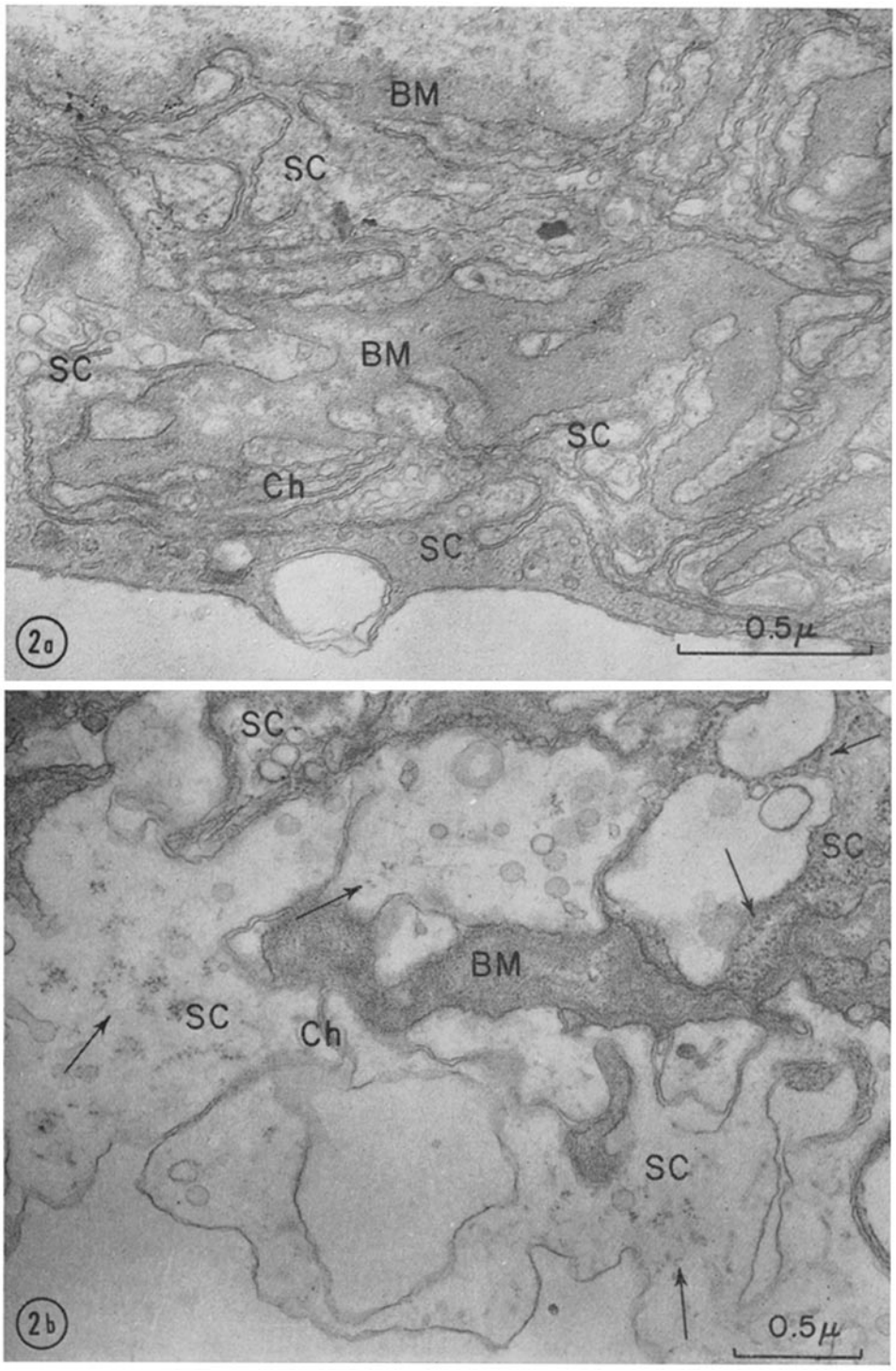

FIGURE 2. Electron micrographs of two *S. sepioidea* isolated nerve fiber sheaths showing the location of sodium in the Schwann cell. The sheaths were also soaked for 5 min in the sucrose solution and fixed as fibers in Fig. 1. Areas of increased density and clear cytoplasmic areas are seen in the Schwann cell (*SC*). In the treated sheath, the precipitate (arrows) is observed in the Schwann cell cytoplasm, but not in the basement membrane (*BM*) and Schwann cell channels (*Ch*). Again the precipitate appears bound to some cytoplasmic dense material. Glutaraldehyde-fixed, OsO_4-postfixed, Epon-embedded material. FIG. 2 *a*, × 53,600; FIG. 2 *b*, × 36,000.

to sodium is similar to that calculated for other cells. In agreement with this, large decreases of the external sodium concentration produce small variations of the Schwann cell electrical potential (5). Thus, in the steady state, sodium should be extruded from the Schwann cell against its electrochemical potential difference, as a balance to its passive entry.

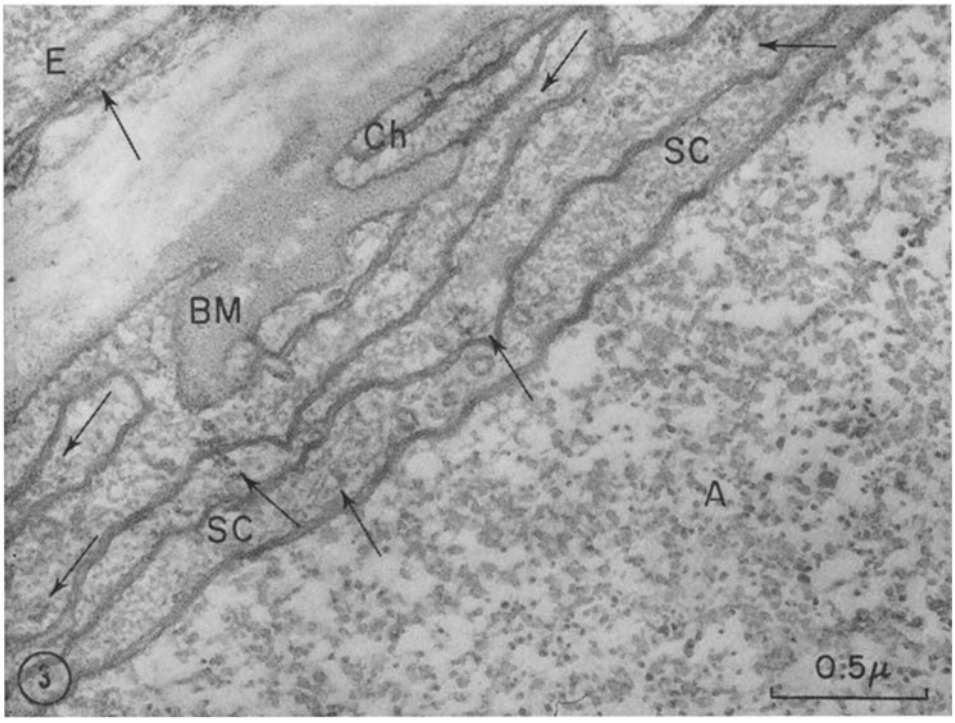

FIGURE 3. Electron micrograph of *S. sepioidea* giant nerve fiber showing the location of sodium in the Schwann cell. The fiber was soaked for 60 min in sucrose solution and then fixed as fibers in Fig. 1. Appreciable amounts of precipitate (arrows) are observed in the Schwann cell cytoplasm (*SC*) and endoneurium (*E*). It appears bound to some dense material. No precipitate is found in the basement membrane (*BM*) and Schwann cell channels (*Ch*). Again, the precipitate in the axoplasm (*A*) is masked by the sectioned neurofibrils. Glutaraldehyde-fixed, OSO_4-postfixed, Epon-embedded material. × 41,200.

Potassium

As mentioned above, the equilibrium potential for potassium estimated from the concentrations measured at both sides of the cell membrane is about −79 mv, whereas the measured membrane potential is only about −40 mv. Thus, it should be considered either that a large fraction of the Schwann cell potassium exists in a bound form or enclosed within cytoplasmic organelles, or that potassium is transported through the membrane towards the interior of the cell against its electrochemical potential difference.

Fig. 4 shows the relationship between the external potassium concentration and the Schwann cell membrane potential. It may be seen that at high external potassium concentrations the membrane potential approximates the values expected from the behavior of an ideal potassium electrode. The linear portion of the curve has a slope of 45 mv/10-fold change in concentration. These results seem to indicate that the Schwann cell membrane is more permeable to potassium than to other ions.

When the external potassium concentration equals the freely diffusible internal potassium concentration, the membrane potential is close to zero. Therefore, in plots of transmembrane potential differences as a function of $(K)_o$, $(K)_i$ can be estimated by extrapolating to zero membrane potential. The value obtained in this way is 210 mM, in agreement with 220 mM of total Schwann cell potassium concentration found by us from chemical analyses. Therefore, it is unlikely that an important fraction of the Schwann cell potassium exists in a nonfreely diffusible form. It seems more

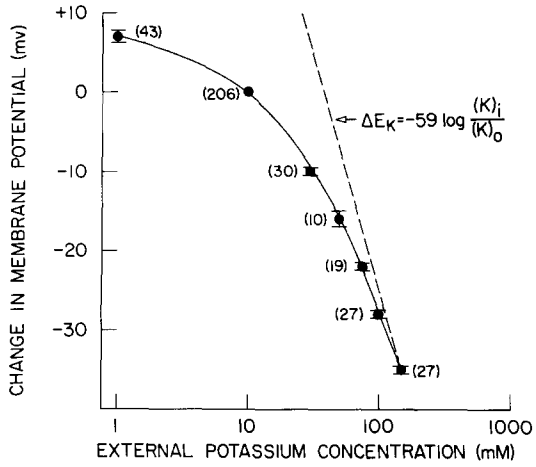

FIGURE 4. Relationship between Schwann cell electrical potential and external potassium concentration. Results obtained in 20 nerve fibers. The number of Schwann cells impaled in each solution is in parentheses. Values are mean ±1 SEM.

likely that the intracellular potassium in excess of the membrane potential is maintained constant by an inward movement of this ion through the cell membrane against its electrochemical potential difference. In agreement with this, prolonged immersion of the tissue in normal sea water containing a cardiac glycoside (K-strophanthoside) produces a significant decrease of the Schwann cell potassium (see below). The contribution of these and other ionic movements to the membrane potential was the subject of a previous paper (5).

Chloride

The chloride ion appears to be distributed almost in equilibrium across the Schwann cell membrane, since the equilibrium potential for chloride estimated above is −32 mv and the measured membrane potential is −40 mv. However, it is unlikely that the passive permeability of the membrane for chloride is as high as for potassium since large decreases in the external concentration of this ion produce only 1–3-mv variations in the Schwann cell membrane potential (5). Furthermore, the rate of

chloride loss from Schwann cells immersed in isosmolal sucrose solution is slower than that of sodium or potassium (see Fig. 1 in reference 3).

Calcium

At present, there are no direct measurements on the concentrations of divalent cations in the squid Schwann cell. Hodgkin and Keynes (6) found a relatively large residual amount of calcium in nerve fibers from which they had extruded most of the axoplasm. It is possible that a large fraction of their extra-axonal calcium was either bound to the membranes or within the cells in the Schwann layer and endoneurium.

The Schwann cell membrane potential in *S. sepioidea* is not modified by taking out the magnesium from the external medium or by large variations (9–44 mM) in the calcium concentration in magnesium-free sea water. However, increasing the concentration of calcium from 44 to 88 mM produces a 10 mv hyperpolarization of the Schwann cell (5). This would indicate that increasing $(Ca)_o$ either generates a potential at the external surface of the Schwann cell membrane, or decreases a net ionic

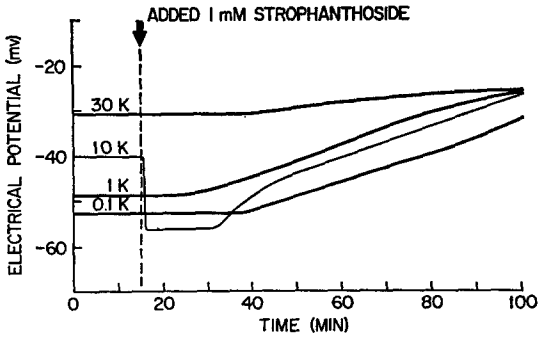

FIGURE 5. Effect of 10^{-3} M K-strophanthoside on the membrane potential of Schwann cells immersed in media with different potassium concentrations. $(K)_o$ is expressed in millimoles/liter of sea water. At the time indicated by the dashed line, the glycoside was added to the external solution.

movement through the membrane which had been lowering the membrane potential below the potassium equilibrium potential.

ION TRANSPORT AND MEMBRANE POTENTIAL

Since the sodium and potassium ions are distributed across the Schwann cell membrane against their electrochemical potential differences and since the cell membrane is mainly permeable to potassium and to a minor extent to sodium and chloride, an active uptake of potassium and an extrusion of sodium from the cell should balance passive movements of these ions through the membrane. Similar transport processes, which can be largely inhibited by cardiac glycosides, have been described in different tissues (7–10).

For an exploration of a possible relationship between ionic transport and membrane potential, the effects of cardiac glycosides on the Schwann cell potential and electrolyte concentrations were studied (5).

Fig. 5 is a schematic representation of the effect of K-strophanthoside on the electrical potential of Schwann cells immersed in media with different potassium concentrations. It shows that a normal $(K)_o$, which is 10 mM, the Schwann cell hyperpolarizes

within 1 min after the addition of K-strophanthoside. It stays hyperpolarized, and 20–30 min later it begins to gradually depolarize. At low $(K)_o$ only the late depolarization of the Schwann cell is observed, whereas at high $(K)_o$ the glycoside has no apparent effect on the potential within the experimental period. Thus, the glycoside requires an optimum potassium concentration to produce hyperpolarization of the cell.

The same glycoside concentration used above (10^{-3} M) produces further hyperpolarization of Schwann cells immersed in magnesium-free sea water with 88 mM of calcium, and a higher concentration of the glycoside (10^{-2} M) hyperpolarizes Schwann cells immersed in sea water with 30 mM of potassium (5). Thus, it seems that the hyperpolarization of the glycoside is independent, at least within the range studied by us, from the level of the Schwann cell membrane potential.

The biphasic effect of K-strophanthoside on the Schwann cell potential resembles

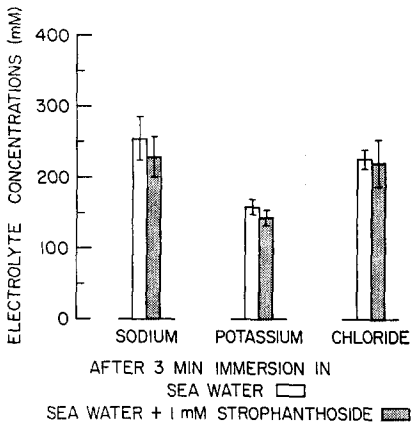

FIGURE 6. Effect of 10^{-3} M K-strophanthoside on the sodium, potassium, and chloride concentrations in the Schwann cell. Results obtained in 12 pairs of nerve fibers. One fiber of each pair was immersed for 3 min in artificial sea water or in sea water containing 1 mm K-strophanthoside. All fibers were slit, and then soaked for 3 min in isosmolal sucrose solution to wash out the extracellular electrolytes. The values are the mean ± 1 SEM.

that produced by low concentrations of ouabain on the electrical potential difference across the frog skin (11–12). However, lower concentrations of K-strophanthoside produce a smaller hyperpolarization of the Schwann cell. The hyperpolarization produced by a concentration of 10^{-5} M is about one-third of that shown in Fig. 5. The magnitude of the hyperpolarization is about the same whether K-strophanthoside or ouabain (G-strophanthin) are used. Thus, it seems that the initial hyperpolarization of the Schwann cell is due to neither an unspecific effect of a high concentration of the glycoside nor an initially low concentration of the drug in the vicinity of the cell.

The initial hyperpolarization of the Schwann cell can be accounted for by assuming either that the glycosides produce a rapid change in the intracellular electrolyte concentrations or that they alter a net ionic movement through the cell membrane. As an exploration of these alternatives, the electrolyte concentrations in the Schwann cell were determined at different times after addition of K-strophanthoside to the external medium.

Fig. 6 shows the sodium, potassium, and chloride concentrations found in the Schwann cell in 12 pairs of nerve fibers. One fiber of each pair was immersed for 3 min in artificial sea water or in artificial sea water containing K-strophanthoside. It

may be seen that the concentrations in both groups of nerve fibers are equal. These results seem to rule out the first alternative. Therefore, it appears necessary to consider that the initial hyperpolarization of the Schwann cell produced by the cardiac glycosides is related to a relative diminution of the entrance of a cation or the exit of an anion through the cell membrane.

Large variations in the sodium and chloride concentrations produce only a 1–3 mv change in the Schwann cell membrane potential. Thus, it is unlikely that a change in the passive permeabilities of the cell membrane to these ions can account for the initial hyperpolarization. It is also unlikely that the glycoside increases the permeability of the cell membrane to potassium, since at low external potassium concentrations K-strophanthoside has no hyperpolarizing effect. It is more likely that the initial hyperpolarization produced by the glycosides in the absence of changes in the intracellular electrolyte concentrations is due to an abrupt inhibition of an inward active potassium movement through the cell membrane.

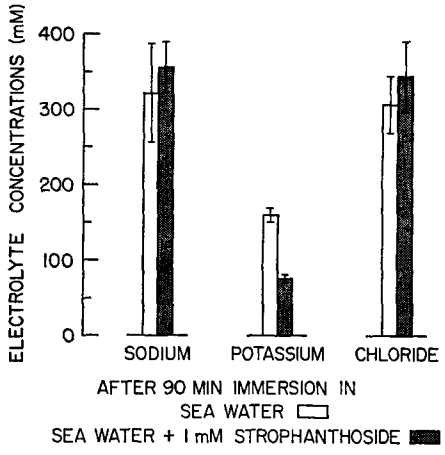

FIGURE 7. Effect of 10^{-3} M K-strophanthoside on the sodium, potassium and chloride concentrations in the Schwann cell. Results obtained in 11 pairs of nerve fibers. One fiber of each pair was immersed for 90 min in artificial sea water or in sea water containing 1 mM K-strophanthoside. All fibers were slit, and then soaked for 3 min in isosmolal sucrose solution to wash out the extracellular electrolytes. The values are the mean ± 1 SEM.

In agreement with the presence of an active potassium uptake mechanism in the Schwann cell, prolonged immersion of the nerve fiber in sea water containing K-strophanthoside produces a significant diminution of the Schwann cell potassium concentration. Fig. 7 shows the electrolyte concentrations in the Schwann cell of fibers immersed for 90 min in normal sea water or in sea water containing K-strophanthoside. It may be seen that the concentration of potassium in the Schwann cells treated with the glycoside is much lower than in the controls. If a large fraction of the Schwann cell sodium were bound, as previously suggested, it would mask changes in the free sodium concentration. Such changes would be also masked by the uncertainties in the values of the concentrations represented by their standard errors. The diminution of the Schwann cell potassium concentration could account for the late depolarization of the cell produced by K-strophanthoside.

Thus, the effects of K-strophanthoside and ouabain on the Schwann cell seem to put in evidence the presence of a cardiac glycoside-sensitive, active potassium transport towards the interior of the cell. This process seems to contribute directly to the mem-

brane potential and to the maintenance of the intracellular electrolyte concentrations in the Schwann cell.

CONCLUSIONS

The electron microscopic study herein described has revealed that the high sodium concentration determined in single sheaths of nerve fibers slit in isotonic sucrose solution or in sea water, and then soaked in isotonic sucrose solution, is located inside the Schwann cells. The results are compatible with the presence of nonfreely diffusible sodium in the Schwann cell cytoplasm. It also appears that in the living cell sodium should be actively extruded from the Schwann cell as a balance to its passive entry. The passive diffusion of sodium seems to contribute to the Schwann cell membrane potential.

Chloride appears to be passively distributed across the cell membrane, almost in equilibrium with the membrane potential. The diffusion of chloride through the membrane also seems to contribute to the membrane potential of the cell.

The potassium ion appears to be accumulated in the Schwann cell mainly as freely diffusible potassium. The passive permeability of the Schwann cell membrane is higher for potassium than for other ions. The effects of K-strophanthoside and ouabain, on the Schwann cell potential and electrolyte concentrations, indicate that the intracellular potassium in excess of the membrane potential is maintained constant by a cardiac glycoside-sensitive, active potassium transport towards the interior of the cell. This process, as the passive diffusion of sodium and chloride, seems to contribute directly to lower the membrane potential below the potassium equilibrium potential, whereas calcium appears to have an opposite effect.

The presence of a high internal concentration of sodium in the Schwann cell and the ability of the cell to concentrate potassium and extrude sodium are wholly compatible with the previously proposed hypothesis on the contribution of the Schwann cell to the maintenance of the normal ionic composition of the axolemma-Schwann cell space, which constitutes the immediate environment of the axon.

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BIBLIOGRAPHY

1. VILLEGAS, R., M. GIMENEZ, and L. VILLEGAS. 1962. The Schwann cell electrical potential in the squid nerve. *Biochim. Biophys. Acta* **62**:610.
2. VILLEGAS, R., L. VILLEGAS, M. GIMENEZ, and G. M. VILLEGAS. 1963. Schwann cell and axon electrical potential differences. *J. Gen. Physiol.* **46**:1047.
3. VILLEGAS, J., L. VILLEGAS, and R. VILLEGAS. 1965. Sodium, potassium, and chloride concentrations in the Schwann cell and axon of the squid nerve fiber. *J. Gen. Physiol.* **49**:1.
4. ZADUNAISKY, J. A. 1966. The location of sodium in the transverse tubules of skeletal muscle. *J. Cell Biol.* **31**:C11.

5. VILLEGAS, J., R. VILLEGAS, and M. GIMENEZ. 1968. Nature of the Schwann cell electrical potential: effects of the external ionic concentrations and a cardiac glycoside. *J. Gen. Physiol.* **51**:47.
6. HODGKIN, A. L., and R. D. KEYNES. 1957. Movements of labelled calcium in squid giant axons. *J. Physiol.* **138**:253.
7. SCHATZMANN, H. J. 1953. Herzglykoside als Hemmstoffe für den aktiven Kalium- und Natriumtransport durch die Erythrocytenmembran. *Helv. Physiol. Pharmacol. Acta* **11**:346.
8. GLYNN, I. M. 1957. The action of cardiac glycosides on sodium and potassium movements in human red cells. *J. Physiol.* **136**:148.
9. GLYNN, I. M. 1959. The sodium potassium exchange pump, in The method of isotopic tracers applied to the study of active ion transport. J. Coursaget, editor. Pergamon Press, Ltd., Oxford. 46.
10. CALDWELL, P. C., and R. D. KEYNES. 1959. The effect of ouabain on the efflux of sodium from a squid giant axon. *J. Physiol.* **148**:8P.
11. WILBRANDT, W., and E. M. WEISS. 1960. Antagonismus zwischen Herzglykosid und Corticosteroiden am Froschhaut potential. *Arzneimittel-Forsch.* **10**:409.
12. MACROBBIE, E. A. C., and H. H. USSING. 1961. Osmotic behaviour of the epithelial cells of frog skin. *Acta Physiol. Scand.* **53**:348.