Nature of the Schwann Cell Electrical Potential

Effects of the external ionic concentrations and a cardiac glycoside

JORGE VILLEGAS, RAIMUNDO VILLEGAS, and MÁXIMO GIMÉNEZ

From Centro de Biofísica, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela

ABSTRACT The effects on the Schwann cell electrical potential of external ionic concentrations and of K-strophanthoside were investigated. Increasing $(K)_o$ depolarized the cell. The potential is related to the logarithm of $(K)_o$ in a quasi-linear fashion. The linear portion of the curve has a slope of 45 mv/ten-fold change in $(K)_o$. Diminutions of $(Na)_o$ and $(Cl)_o$ produced only small variations in the potential. Calcium and magnesium can be replaced by 44 mM calcium without altering the potential. Increase of $(Ca)_o$ to 88 mM produced about 10 mv hyperpolarization. The cell was hyperpolarized by 11 mv and 4 mv within 1 min after applying K-strophanthoside at concentrations of 10^{-3} and 10^{-5} M, respectively. No variations of cellular potassium, sodium, or chloride were observed 3 min after applying the glycoside. The hyperpolarization caused by 10^{-3} M K-strophanthoside was not observed when $(K)_o$ was diminished to 1 or 0.1 mM or was increased to 30 mM. At a $(K)_o$ of 30 mM, 10^{-2} M strophanthoside was required to produce the hyperpolarizing effect. In high calcium, the cell was further hyperpolarized by the glycoside. The initial hyperpolarization caused by the glycoside was followed by a gradual depolarization and a decrease of the cellular potassium concentration. The results indicate that the Schwann cell potential of about −40 mv is due to ionic diffusion, mainly of potassium, and to a cardiac glycoside-sensitive ion transport process.

Previous work (1, 2) carried out in single nerve fibers of the squid, *Sepioteuthis sepioidea*, revealed that across the plasma membrane of the Schwann cell (Del Rio Hortega's peripheral glial cell), there is an electrical potential difference of approximately −40 mv, the interior negative with respect to the outside solution. It was found also that the magnitude of the Schwann cell electrical potential, even the largest recorded, is always smaller than the resting potential of the neighboring axon. This electrical potential remains apparently unchanged, within 1 mv, during the conduction of a single nerve impulse.

The present investigation was undertaken to determine the nature of this electrical potential difference across the Schwann cell plasma membrane. The experiments deal with: (I) the effects on the Schwann cell and axon electrical potentials of different external potassium, sodium, chloride, and calcium concentrations, and (II) the effects on the electrical potential and the electrolyte concentrations of the Schwann cell of a cardiac glycoside, K-strophanthoside, added to normal seawater and to seawater with different potassium and calcium concentrations. The effect of the glycoside on the axon resting and action potentials was determined also in the same nerve fibers.

The results of the present work indicate that the Schwann cell electrical potential is due to the diffusion of ions, mainly of potassium, and also to a cardiac glycoside—sensitive ion transport process.

Preliminary results of this work have been reported (3, 4).

EXPERIMENTAL METHOD

General Procedure

Giant nerve fibers, 250 to 400 μ in diameter, from the hindmost stellar nerves of the tropical squid, *S. sepioidea*, were used in all experiments.

Immediately after decapitation of the living squid, the nerve fiber was isolated from the animal and placed in a Lucite holder containing artificial seawater where it was kept under slight tension by means of threads tied to each end. The fluid in the Lucite chamber holding the nerve fiber could be changed in less than 10 sec using a method described previously (5). All the experiments were performed at 20° to 22°C.

Experimental Solutions

(a) Artificial seawater (6), called normal seawater in the present work, was used as normal medium. The concentration of its components in millimols per liter was as follows: NaCl, 442; KCl, 10; $CaCl_2$, 11; $MgCl_2$, 53; $NaHCO_3$, 2.5. (b) Seawater in which part of its normal sodium chloride concentration was replaced, mole per mole, by choline chloride or, osmol per osmol, by sucrose. All other constituents of the normal seawater were kept at their normal concentrations. (c) Solutions containing potassium concentrations higher or lower than that of normal seawater. The media containing high potassium concentrations were prepared by replacing, mole per mole, sodium chloride by potassium chloride. Those containing low potassium concentrations were prepared by replacing potassium chloride by choline chloride. (d) Magnesium-free seawater was made by replacing all the magnesium chloride and calcium chloride in normal seawater by 44 mmoles of calcium chloride per liter (7). Isosmolarity was maintained with sucrose. The magnesium-free seawater was used as reference to prepare the high and low calcium solutions, by replacing sodium by calcium, or by substituting, osmol per osmol, calcium chloride by choline chloride plus sucrose. (e) Seawater solutions containing cardiac glycoside were prepared by adding the necessary amount of K-strophanthoside to the media. This glycoside is

soluble at room temperature in the different solutions used. K-strophanthoside was kindly supplied by Sandoz A. G. (Basel, Switzerland).

Measurement of the Electrical Potential Differences

Details of the experimental procedure utilized to measure the electrical potential differences across the Schwann cell membrane and across the axon membrane, were described and discussed at length in a previous work from our laboratory (2).

Glass micropipettes were filled with 3 M KCl solution by boiling under reduced pressure. The criteria for acceptable micropipettes were suitable shape, tip resistance between 8 and 20 megohms, and tip potential between 0 and -5 mv.

The exploring micropipette was provided with an Ag-AgCl electrode connected to an oscilloscope (Tektronix 502) via a neutralized input capacity compensated amplifier (Type NF1, Bioelectric Instruments, N.Y.; 10^{11} ohms input resistance, grid current less than 10^{-12} amp). An earthed reference electrode, Ag-AgCl in 3 M KCl solution, connected to the bath through a seawater agar-bridge, was used. The exploring micropipette resistance and tip potential were measured at frequent intervals during the experiment. The axon could be stimulated at will using two external fine platinum wires connected via a stimulus isolation unit (Grass SIU-4B) to a stimulator (Grass S4E).

Since the Schwann cells measure 60 to 100 μ in length and width and only up to 5 μ in thickness, the exploring micropipette was advanced into the nerve fiber along a line oblique to its surface. The exploring micropipette was mounted on a micromanipulator and advanced under a stereoscopic microscope. Schwann cells were impaled before entering the axon and, in some experiments, the exploring micropipette was made to cross the axon and the Schwann cells impaled at the side opposite to the penetration. Care was taken to avoid repeated impalement of a single Schwann cell.

Determination of the Electrolyte Concentrations

To determine the potassium, sodium, and chloride concentrations in the Schwann cell of giant nerve fibers immersed in normal seawater or in normal seawater containing K-strophanthoside, the following procedure was employed.

Prior to immersion in the solution, the small nerve fibers and the outermost portion of the endoneurium were removed, leaving at most a 6 μ thick endoneurium layer around the giant nerve fiber. The axon diameter was measured to within 3 μ. At the end of the immersion period in normal seawater or in the solution containing K-strophanthoside, the sheaths were slit longitudinally and their length measured to within 0.5 mm. Immediately after slitting, the axon sheath was soaked for 1 min in each one of a series of three baths of 5 ml sucrose solution, isosmolal with artificial seawater. Finally, the electrolytes in the sheath were analyzed. The total cellular volume in each slit sheath was calculated from the diameter and the length of the slit sheath, and the thickness of its cellular layers. Further details are given in previous work (8).

Sodium and potassium were extracted in 1 ml of 10% aqueous nitric acid solution for 12 hr at room temperature. The sodium and potassium concentrations in the

extract were determined by flame photometry. The chloride was extracted in 1 ml of 50% aqueous acetic acid solution for 12 hr at room temperature. The chloride in the extract was titrated potentiometrically (9).

RESULTS AND DISCUSSION

I. *Electrical Potential and External Ionic Concentrations*

The Schwann cell and axon electrical potentials were measured in each single nerve fiber, successively: (*a*) in normal seawater, for 10 min; (*b*) in one of the solutions with different ionic composition, for 20 min; and (*c*) after

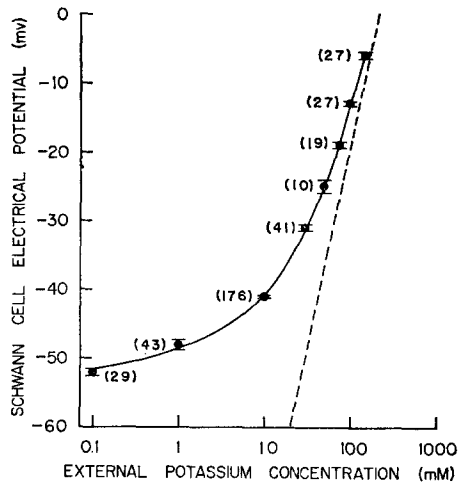

FIGURE 1. Relationship between Schwann cell electrical potential and external potassium concentration. Results obtained in 20 nerve fibers. The number of Schwann cells impaled in each solution is in parentheses. Values are mean ± 1 SEM. The dashed line has been drawn with a slope of 59 mv/tenfold change in concentration.

replacing the seawater solution used in *b*, by normal seawater, for 10 min. Measurements in solution b were considered acceptable only when the values obtained in solutions a and c agreed within 2 mv.

EFFECT OF THE EXTERNAL POTASSIUM CONCENTRATION

Fig. 1 shows the electrical potential difference across the Schwann cell membrane plotted as a function of $(K)_o$, the external potassium concentration. 20 nerve fibers were used in these experiments.

Fig. 1 shows that at high external potassium concentrations the Schwann cell electrical potential is related to the logarithm of $(K)_o$ in a quasi-linear fashion. The linear portion of the curve is characterized by a slope of 45 mv/tenfold change in $(K)_o$. This slope is smaller than that of the equivalent region of the curves obtained with the axons of the same nerve fibers and

axons of other squid species (6, 12, 13). The slope of the dashed line in Fig. 1, 59 mv/tenfold change in potassium concentration, represents ideal potassium electrode behavior.

Nicholls and Kuffler (10) and Kuffler, Nicholls, and Orkand (11) have found that glial cells of the central nervous system of the leech, *Hirudus medicinalis*, and the mudpuppy, *Necturus maculosus*, behave like perfect potassium electrodes over a wide range of $(K)_o$.

The slope of 45 mv/tenfold change in $(K)_o$ found in the squid Schwann cell reveals that at least under our experimental conditions, the behavior of the Schwann cell membrane seems to depart from that expected for a potassium electrode. The departure from ideal potassium electrode behavior probably cannot be ascribed to damage caused by the microelectrode for the following reasons. The Schwann cells measure 60–100 μ in length and width and up to 5 μ in thickness and the microelectrode was obliquely inserted into the cells. A stable potential could be registered during a single impalement of the cell for as long as 5 to 10 min. The potential remained steady rather than fluctuating or declining, indicating effective sealing around the electrode tip and little cellular damage. Intentional reinsertion of the microelectrode in a cell, to explore cellular damage, gave values within ± 3 mv of the initial value, thus indicating little damage due to the initial impalement.

Therefore it was necessary to explore the existence of other factors that in addition to the diffusion of potassium, could play a role in the genesis of its electrical potential. These factors might be the permeability of its membrane to other ions and the existence of an active ion transport process able to generate an electrical potential difference. This electrical potential would modify the potassium equilibrium potential. Deviation from ideal potassium electrode behavior is usually explained in other cells by assuming that the cell membrane is permeable to ions other than potassium (6, 14, 15), especially at low external potassium concentrations.

The variations of the axon resting and action potentials produced by the changes in $(K)_o$ were similar to those described for the axons of other squid species by Curtis and Cole (12), Hodgkin and Katz (6), and Hodgkin and Keynes (13). At external potassium concentrations above 30 mM, the linear portion of the curve which relates the axon resting potential to the logarithm of $(K)_o$ has a slope of 52 mv/tenfold change in concentration. The action potential declines rapidly when $(K)_o$ is increased above 30 mM.

EFFECT OF EXTERNAL SODIUM AND CHLORIDE CONCENTRATIONS

Fig. 2 shows the electrical potential differences across the Schwann cell membrane plotted as a function of the external sodium concentration. Sodium chloride was replaced by choline chloride or sucrose. 10 nerve fibers were used in these experiments.

The results indicate that the diminution of the external sodium and chloride concentrations produced only small variations (within 1 to 2 mv) of the Schwann cell electrical potential. Thus, it appears that the contribution of these ions to the potential is small.

The effects of the low sodium solutions on the resting and action potentials of the *S. sepioidea* axons were similar to those observed in *L. forbesi* by Hodgkin and Katz (12).

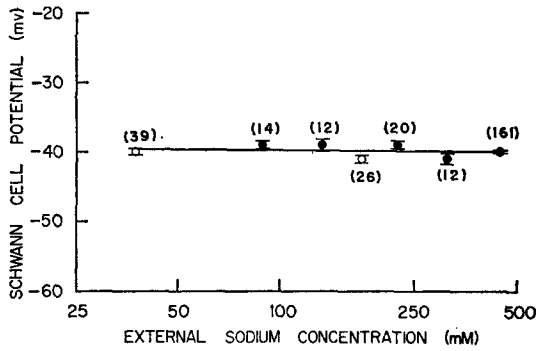

FIGURE 2. Relationship between Schwann cell electrical potential and external sodium concentration. The filled circles correspond to measurements made in low sodium solutions (sodium chloride replaced by choline chloride), and the open circles to measurements made in low sodium chloride solutions (sodium chloride replaced by sucrose). Results obtained in 10 nerve fibers. The number of Schwann cells impaled in each solution is in parentheses. Values are mean ± 1 SEM.

EFFECT OF THE EXTERNAL CALCIUM CONCENTRATION

The concentrations of calcium and magnesium in normal seawater are 11 and 53 mmoles per liter respectively.

The electrical potential of 118 Schwann cells from 13 nerve fibers, immersed in magnesium-free seawater containing 44 mmoles of calcium per liter, was 41 ± 0.2 mv (mean ± SEM). The corresponding value of 176 Schwann cells from 20 nerve fibers in normal seawater was also 41 ± 0.2 mv.

The axon electrical potentials of the *S. sepioidea* were not modified by replacing its normal seawater environment with magnesium-free seawater with 44 mmoles of calcium per liter. This is in agreement with observations of Frankenhaeuser, Hodgkin, and Keynes (cited in reference 7).

The effect of $(Ca)_o$, the external calcium concentration, was studied in 14 nerve fibers within a wide range of concentrations using as reference the magnesium-free seawater containing 44 mmoles of calcium per liter.

As shown in Fig. 3, a fivefold reduction of $(Ca)_o$ produced only a 1–2 mv hyperpolarization, whereas a twofold increment of the concentration pro-

duced hyperpolarization of the Schwann cell of about 10 mv. The hyperpolarizing effect of the high calcium concentration may be due to a decrease in the permeability of the cell membrane to ions other than potassium.

The variations of the electrical potentials of the axon produced by the changes in $(Ca)_o$ were similar to those described for the axons of other squid species by Huxley (16) and Adelman and Moore (17).

II. *Effect of a Cardiac Glycoside on the Electrical Potential*

To investigate whether, in addition to ionic diffusion, an active ionic transport process is directly involved in the genesis of the Schwann cell electrica

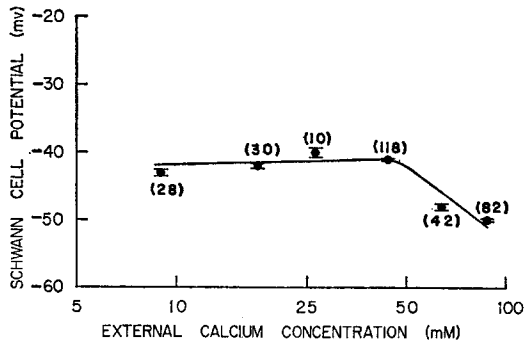

FIGURE 3. Relationship between Schwann cell electrical potential and externa calcium concentration. Magnesium-free seawater with 44 mM calcium was used as reference solution. Results obtained in 14 nerve fibers. The number of Schwann cells impaled in each solution is in parentheses. Values are mean ± 1 SEM.

potential, K-strophanthoside was used. Cardiac glycosides, when applied to the external surface of a variety of cells inhibit a large fraction of the active ion transport (18–20). Caldwell and Keynes (21) have shown that 10^{-5} M ouabain (strophanthin-G) markedly reduces sodium efflux from the axon of *L. forbesi* immediately after its addition to the external medium.

EFFECT OF DIFFERENT DOSES OF K-STROPHANTHOSIDE

The Schwann cell and axon electrical potentials were measured in nerve fibers immersed first in normal seawater and then in normal seawater containing K-strophanthoside at concentrations of 10^{-3} or 10^{-5} M.

Fig. 4 shows the results of one experiment on the effect of 10^{-3} M strophanthoside. The Schwann cell and the axon electrical potentials are plotted as a function of time. This figure shows that the Schwann cell becomes hyperpolarized by 10 to 15 mv within 1 min after addition of the glycoside and that it begins to depolarize gradually about 30 min later. In two groups of five nerve fibers each, the hyperpolarizations of the Schwann cells observed

immediately after applying the K-strophanthoside at concentrations of 10^{-3} and 10^{-5} M were 11 ± 1 and 4 ± 1 mv (mean ± SEM) respectively.

No changes in the resting and action potentials of the axon could be found within the first 2 hr of observation, as seen by Caldwell and Keynes (21) in *L. forbesi*.

EFFECT OF K-STROPHANTHOSIDE AT DIFFERENT EXTERNAL POTASSIUM CONCENTRATIONS

To explore a possible relationship between glycoside and potassium, the effect of K-strophanthoside on the Schwann cell and axon electrical potentials in media with different potassium concentrations was investigated.

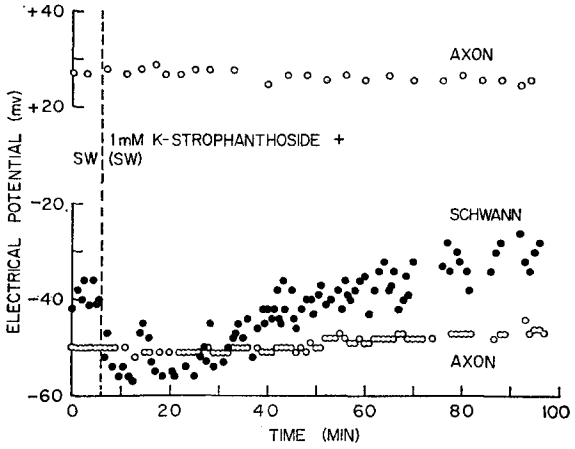

FIGURE 4. Effect of 1 mM K-strophanthoside on the Schwann cell and axon electrical potentials plotted as a function of time. The nerve fiber was first immersed in normal seawater (SW). At the time indicated by the vertical dashed line, this solution was replaced by a similar one containing 1 mM K-strophanthoside. The values plotted are the electrical potential differences measured in the Schwann cells (filled circles) and in the axon (open circles; resting potential, below; peak of the action potential, above).

Fig. 5 shows the results of one experiment on the effect of the glycoside in low potassium seawater. The Schwann cell and axon electrical potentials are plotted as a function of time. The electrical potentials were recorded with the nerve fiber in normal seawater ($(K)_o$ = 10 mM), and after replacing this medium, first by a solution with the potassium concentration reduced to 0.1 mM, and finally by a similar one containing 10^{-3} M K-strophanthoside. As described above, the reduction of $(K)_o$ produced hyperpolarization of the Schwann cell. Addition of the glycoside at this low $(K)_o$ did not further hyperpolarize the Schwann cell. The late depolarization described in normal seawater was observed. No further change in the resting potential of the axon, in addition to that produced by the change in $(K)_o$, was caused by the glycoside during 90 min of observation.

Figs. 6 and 7 show the results of experiments on the effect of two different concentrations of the glycoside in high potassium seawater. The electrical potentials were recorded with the nerve fiber in normal seawater, and after replacing this solution, first by seawater containing 30 mM potassium, and finally by a similar one with 10^{-3} M (Fig. 6) or 10^{-2} M (Fig. 7) K-strophanthoside. It may be seen that the increase of the external potassium concentration to 30 mM diminished the Schwann cell membrane potential as

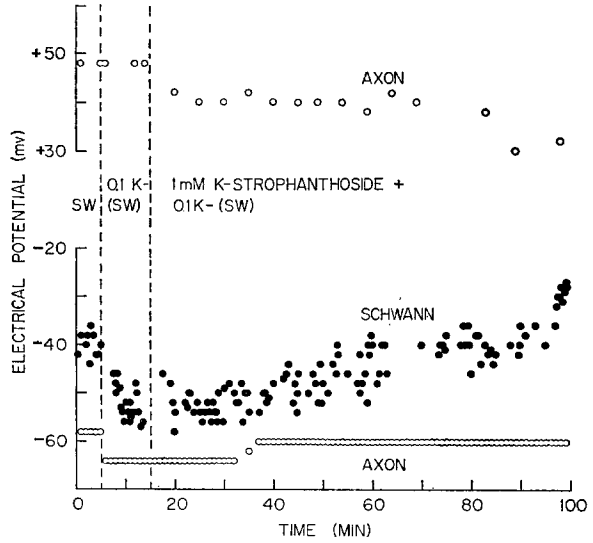

FIGURE 5. Effect of 1 mM K-strophanthoside in low potassium seawater on the Schwann cell and axon electrical potentials, plotted as a function of time. The nerve fiber was first immersed in normal seawater (SW). At the times indicated by the vertical dashed lines, this medium was replaced successively by seawater with the potassium concentration reduced to 0.1 mM (0.1 K-(SW)), and by a similar solution containing 1 mM K-strophanthoside. The values are the electrical potential differences measured in the Schwann cells (filled circles), and in the axon (open circles; resting potential, below; peak of the action potential, above).

already described in the present work. At this high $(K)_o$, the addition of 10^{-3} M K-strophanthoside did not produce any effect on the Schwann cell electrical potential during the experimental period. However, as may be seen in Fig. 7, at high $(K)_o$ a 10^{-2} M K-strophanthoside concentration was able to produce changes in the Schwann cell potential similar to those observed in normal seawater with only 10^{-3} M K-strophanthoside. It should be pointed out that at 10^{-2} M the glycoside also appears to have some early effect on the resting potential of these axons already diminished by immersion in the high potassium seawater.

Table I summarizes experiments in which the effect of K-strophanthoside

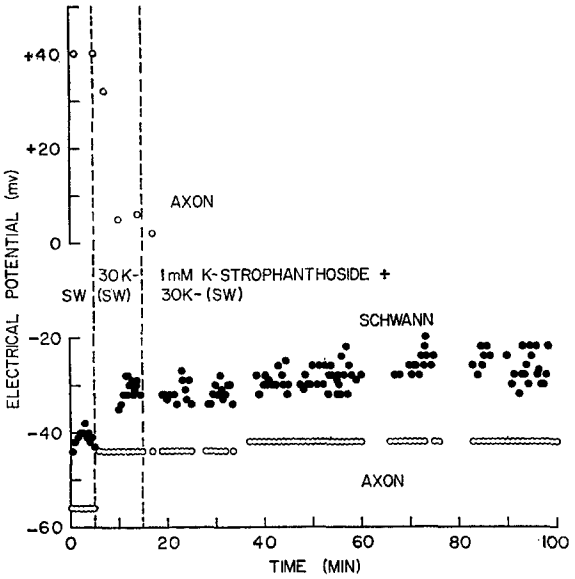

FIGURE 6. Effect of 1 mM K-strophanthoside in high potassium seawater on the Schwann cell and axon electrical potentials, plotted as a function of time. The nerve fiber was first immersed in normal seawater (SW). At the times indicated by the vertical dashed lines, this medium was replaced successively by seawater with the potassium concentration increased to 30 mM and by a similar one containing 1 mM K-strophanthoside. The values are the electrical potential differences measured in the Schwann cells (filled circles), and in the axon (open circles; resting potential, below; peak of the action potential, above).

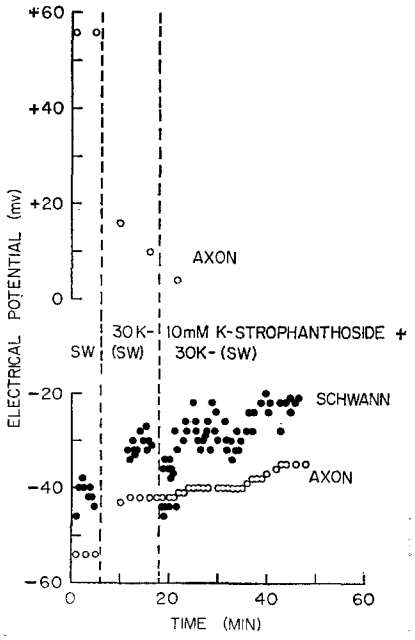

FIGURE 7. Effect of 10 mM K-strophanthoside in high potassium seawater on the Schwann cell and axon electrical potentials, plotted as a function of time. The nerve fiber was first immersed in normal seawater (SW). At the times indicated by the vertical dashed lines, this solution was replaced successively by seawater with the potassium concentration increased to 30 mM and by a similar solution containing 10 mM K-strophanthoside. The values are the electrical potential differences measured in the Schwann cells (filled circles), and in the axon (open circles; resting potential, below; peak of the action potential, above).

on the electrical potential of Schwann cells immersed in media with different $(K)_o$ was measured. Values are the mean ± SEM of the measurements made every 20 or 25 min, during intervals of 5 min.

The results, shown in Table I and in Figs. 5–7, suggest that the initial

TABLE I

EFFECT OF K-STROPHANTHOSIDE ON THE ELECTRICAL POTENTIAL OF SCHWANN CELLS IMMERSED IN SEAWATER WITH DIFFERENT POTASSIUM CONCENTRATIONS

Temperature 20–22°C

Nerve fiber No.	Electrical potential in mv* in each seawater solution (No. of cells in parentheses)					
			Change to strophanthoside-containing seawater with different $(K)_o$			
	Normal sea water $(K)_o$ in mM	Change to seawater with different $(K)_o$	Time interval after immersion, *min*			
			0–5	25–30	55–60	85–90
	$(K)_o$ = 10		1 mM strophanthoside; $(K)_o$ = 10 mM			
1	39 ± 1 (8)		54 ± 1 (5)	47 ± 1 (6)	36 ± 1 (5)	30 ± 1 (5)
2	39 ± 1 (8)		49 ± 1 (5)	49 ± 2 (4)	39 ± 1 (5)	33 ± 1 (5)
	$(K)_o$ = 10	$(K)_o$ = 0.1	1 mM strophanthoside; $(K)_o$ = 0.1 mM			
3	41 ± 1 (9)	52 ± 1 (11)	50 ± 1 (12)	50 ± 1 (6)	44 ± 2 (5)	35 ± 1 (13)
4	40 ± 1 (10)	52 ± 1 (19)	52 ± 2 (7)	48 ± 1 (8)	40 ± 1 (6)	38 ± 1 (3)
	$(K)_o$ = 10	$(K)_o$ = 1	1 mM strophanthoside; $(K)_o$ = 1 mM			
5	42 ± 1 (21)	49 ± 1 (35)	48 ± 1 (10)	46 ± 1 (13)	34 ± 1 (15)	
6	42 ± 1 (13)	50 ± 2 (11)	48 ± 2 (7)	51 ± 1 (6)	37 ± 1 (6)	35 ± 2 (9)
	$(K)_o$ = 10	$(K)_o$ = 30	1 mM strophanthoside; $(K)_o$ = 30 mM			
7	41 ± 1 (12)	31 ± 1 (16)	32 ± 0 (4)	29 ± 1 (11)	25 ± 1 (12)	27 ± 1 (10)
8	41 ± 1 (10)	32 ± 1 (16)	30 ± 1 (8)	30 ± 1 (7)	29 ± 1 (10)	29 ± 1 (5)
	$(K)_o$ = 10	$(K)_o$ = 30	10 mM strophanthoside; $(K)_o$ = 30 mM			
9	41 ± 1 (10)	30 ± 1 (16)	38 ± 1 (16)	23 ± 1 (7)		
10	42 ± 1 (10)	32 ± 1 (13)	37 ± 1 (17)	21 ± 1 (14)		

* Values are mean ± SEM.

hyperpolarization of the glycoside requires an optimum concentration of potassium in the external medium, above which a higher concentration of glycoside is needed to produce hyperpolarization. These results are similar to those found by Glynn (19) in red cells. This suggests that K-strophanthoside competes with potassium at the external surface of the Schwann cell.

EFFECT OF K-STROPHANTHOSIDE AT DIFFERENT EXTERNAL CALCIUM CONCENTRATIONS

As described above, increasing $(Ca)_o$ or adding K-strophanthoside produced hyperpolarization of the Schwann cell and not of the axon. To explore a possible relationship between glycoside and calcium, the effect of K-strophanthoside on the Schwann cell and axon electrical potentials at different $(Ca)_o$ was investigated. Magnesium-free seawater containing 44 mmoles of calcium per liter was used as reference.

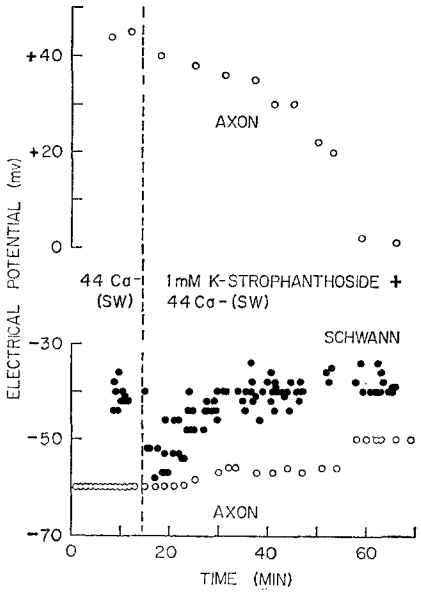

FIGURE 8. Effect of 1 mM K-strophanthoside in magnesium-free seawater with 44 mM calcium on the Schwann cell and axon potentials, plotted as a function of time. The nerve fiber was first immersed in magnesium-free seawater with 44 mM calcium (44 Ca-(SW)). At the time indicated by the vertical dashed line, this solution was replaced by a similar one to which 1 mM K-strophanthoside was added. The values are the electrical potential differences measured in the Schwann cells (filled circles), and in the axon (open circles; resting potential, below; peak of the action potential, above).

Fig. 8 shows the results of one experiment in which the electrical potentials were measured, first with the nerve fiber immersed in magnesium-free seawater with 44 mM calcium and after replacing this medium by a similar one with 10^{-3} M K-strophanthoside. The Schwann cell became hyperpolarized by about 15 mv immediately after addition of the glycoside, and it began to depolarize rapidly about 4 min later. It should be noticed that the glycoside caused a gradual depolarization of the axon and that the action potential decreased in magnitude faster than the resting potential. The rate of the depolarization of the axon caused by the glycoside in the magnesium-free seawater with 44 mM calcium is faster than that in normal seawater.

Fig. 9 shows the results of one experiment on the effect of K-strophanthoside in a high calcium medium. The nerve fiber was first immersed in magnesium-

free seawater with 44 mM calcium, then this medium was replaced by one with 88 mM calcium, and finally by a similar one with 10^{-3} M K-strophanthoside. The twofold increment of $(Ca)_o$ produced hyperpolarization of the Schwann cell and addition of the glycoside caused further hyperpolarization. This hyperpolarization is of the same magnitude as that produced by the same concentration of glycoside in normal seawater. However, the depolarization of the Schwann cell that follows the initial hyperpolarization was faster

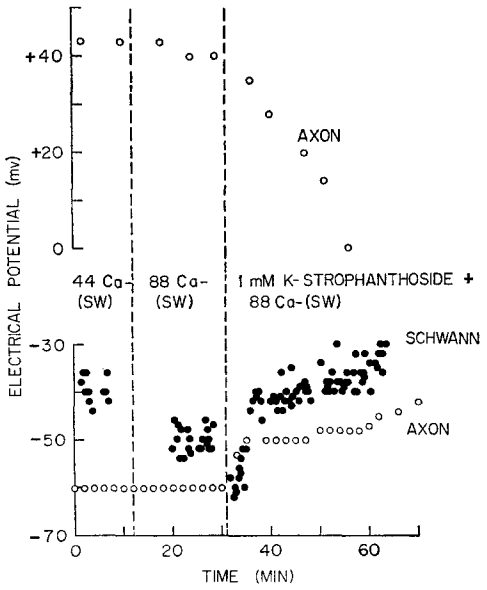

FIGURE 9. Effect of 1 mM K-strophanthoside in high calcium, magnesium-free seawater on the Schwann cell and axon electrical potentials, plotted as a function of time. The nerve fiber was first immersed in magnesium-free seawater with 44 mM calcium (44 Ca-(SW)). At the times indicated by the vertical dashed lines, this solution was replaced successively by seawater with the calcium concentration increased to 88 mM and by a similar one containing 1 mM K-strophanthoside. The values are the electrical potential differences measured in the Schwann cells (filled circles), and in the axon (open circles; resting potential, below; peak of the action potential, above).

than that observed at normal calcium concentration. The glycoside in the 88 mM calcium solution also caused axon depolarization. This depolarization was still faster than that observed in 44 mM calcium seawater.

Table II summarizes the results of five experiments on the effect of the K-strophanthoside at different $(Ca)_o$ on the Schwann cell electrical potential. Values are the mean ± 1 SEM These values were calculated from measurements made every 10 min, during intervals of 2 min.

The data in Table II suggest that the effects of calcium and the glycoside are additive.

EFFECT OF K-STROPHANTHOSIDE ON THE SODIUM, POTASSIUM, AND CHLORIDE CONCENTRATIONS

Values of the total potassium, sodium, and chloride concentrations of the *S. sepioidea* Schwann cell were measured in previous work (8). The values, expressed in millimoles per liter, are: potassium 220; sodium 312; and chloride 167. This sodium concentration is not different from that found in nerve

TABLE II

EFFECT OF K-STROPHANTHOSIDE ON THE ELECTRICAL POTENTIAL OF SCHWANN CELLS IMMERSED IN MAGNESIUM-FREE SEAWATER WITH DIFFERENT CALCIUM CONCENTRATIONS

Temperature 20–22°C

Nerve fiber No.	Electrical potential in mv* in each seawater solution (No. of cells in parentheses)					
			Change to Mg-free seawater containing strophanthoside, with different $(Ca)_o$			
	Mg-free seawater solution $(Ca)_o$ in mM	Change to Mg-free seawater with different $(Ca)_o$	Time interval after immersion, *min*			
			0–2	10–12	20–22	30–32
	$(Ca)_o$ = 44		1 mM strophanthoside; $(Ca)_o$ = 44 mM			
11	41 ± 1 (10)		50 ± 3 (7)	45 ± 1 (4)	40 ± 1 (9)	39 ± 1 (6)
12	41 ± 1 (11)		49 ± 1 (4)	42 ± 1 (6)	38 ± 1 (10)	
	$(Ca)_o$ = 44	$(Ca)_o$ = 88	1 mM strophanthoside; $(Ca)_o$ = 88 mM			
13	39 ± 1 (12)	50 ± 1 (20)	58 ± 5 (3)	31 ± 1 (8)		
14	40 ± 1 (12)	50 ± 1 (22)	60 ± 1 (7)	41 ± 1 (6)	37 ± 3 (3)	33 ± 1 (7)
15	40 ± 0 (14)	47 ± 1 (11)	55 ± 2 (9)	40 ± 1 (8)	24 ± 1 (6)	

* Values are mean ± SEM.

fibers slit in isosmolal sucrose solution. Recent experiments (Villegas, Rawlins, and Villegas, unpublished data) suggest that a fraction of the Schwann cell sodium may be bound.

The electrolyte concentrations in the Schwann cell were determined in pairs of nerve fiber sheaths; one sheath of each pair was immersed in normal seawater containing 10^{-3} M K-strophanthoside and the other was immersed in normal seawater without the glycoside. The immersion times were 3 min for one group of nerve fiber pairs and 90 min for another. At the end of the immersion period the extracellular electrolytes were washed out by soaking the sheaths in isosmolal sucrose solution for 3 min, and the concentrations in the cells were determined. Since 0.84 of the total cellular volume in the sheaths is composed of Schwann cells, the measurements are considered to be mainly those of the Schwann cell concentrations (8).

Table III shows concentrations in the cells of the nerve fiber sheaths immersed in normal seawater and of the paired sheaths immersed in normal seawater with 10^{-3} M K-strophanthoside.

The Schwann cell concentrations of the sheaths immersed for 3 min in normal, glycoside-free, seawater, are equal to those found in previous work (compare Fig. 1 of reference 8). On the other hand, the Schwann cells of the sheaths immersed for 90 min in normal, glycoside-free, seawater gain sodium and chloride and lose potassium with time.

TABLE III

EFFECT OF K-STROPHANTHOSIDE (10^{-3} M) ON THE SODIUM, POTASSIUM, AND CHLORIDE CONCENTRATIONS IN THE SCHWANN CELL OF THE SQUID NERVE FIBER*

Temperature 20–22°C

	Time of immersion 3 min			Time of immersion 90 min		
	No. of nerve fiber pairs	(*a*) Normal seawater	(*b*) Strophanthoside-containing seawater	No. of nerve fiber pairs	(*a*) Normal seawater	(*b*) Strophanthoside-containing seawater
		mM	mM		mM	mM
Sodium	6	254 ± 31	228 ± 29	5	322 ± 66	356 ± 34
Potassium	6	158 ± 11	143 ± 11	5	139 ± 9	77 ± 6
Chloride	6	225 ± 14	220 ± 33	6	306 ± 38	345 ± 47

* Values are mean ± SEM. Extracellular electrolytes were washed out by soaking the slit nerve fiber sheaths for 3 min in sucrose solutions, isosmolal with normal seawater. The Schwann cell concentrations in normal seawater, obtained by extrapolation to zero time of washing in isosmolal sucrose solution, are given in the text and in reference 8.

The sodium, potassium, and chloride concentrations in the Schwann cells were not influenced by the glycoside after an immersion time of 3 min.

The concentration of potassium in the Schwann cells treated with the glycoside for 90 min is lower than that in the Schwann cells of the sheaths immersed for the same period in glycoside-free, normal, seawater. If a large fraction of the Schwann cell sodium is bound, it would mask changes in the free sodium concentration.

III. *On the Nature of the Schwann Cell Electrical Potential*

The experimental results indicate that the electrical potential depends mainly upon the permeability of the Schwann cell membrane to potassium and the unequal distribution of this ion across the membrane. However, the electrical potential of the Schwann cell is smaller than the potassium equilibrium potential and the changes produced by variations of $(K)_o$ are also smaller than those expected for an ideal potassium electrode. Deviation from ideal potassium electrode behavior may be accounted for by assuming that the Schwann

cell membrane is permeable to ions other than potassium or that a nonelectroneutral ion transport process contributes to the potential.

It could be considered that the passive permeabilities of the membrane to sodium and chloride are similar to those calculated for other cells. In agreement with this, large decreases of $(Na)_o$ and $(Cl)_o$ produce only small variations of the Schwann cell electrical potential. However, the deviation of the slope of the linear portion of the curve relating the electrical potential to $(K)_o$ from the slope corresponding to an ideal potassium electrode, is larger than that expected from the changes in electrical potential caused by the variations in $(Na)_o$ and $(Cl)_o$. Thus, in addition to the role played by these ions an additional factor modifying the electrical potential should be considered.

As described above, addition of 10^{-3} or 10^{-5} M K-strophanthoside produces immediate hyperpolarization of the Schwann cell without significant changes in its ionic concentrations. The hyperpolarization caused by the glycoside could be explained either by an effect of the drug on the passive diffusion of ions through the cell membrane, or by an abrupt inhibition of a nonelectroneutral ion transport process. It is unlikely that a change in the passive permeabilities of the membrane to sodium and chloride could account for the observed hyperpolarization, since those permeabilities appear to be low and their increment would produce depolarization of the cell. It is also unlikely that the hyperpolarizing effect of the glycoside is due to an effect of the drug on the passive diffusion of potassium, since at low $(K)_o$ (either 1 or 0.1 mM external potassium concentrations), the K-strophanthoside has no hyperpolarizing effect. The glycoside requires an optimum $(K)_o$ to hyperpolarize the cell. Thus, we are left with the possibility that the initial hyperpolarizing effect of K-strophanthoside, in the absence of changes in the Schwann cell electrolyte concentrations, might be caused by an abrupt inhibition of a nonelectroneutral ion transport process.

As described above, increasing $(Ca)_o$ also produces hyperpolarization of the Schwann cell and not of the axon. Thus, it could be considered that calcium also inhibits the cardiac glycoside—sensitive ion transport process. If this were so one would expect that high $(Ca)_o$ would diminish the hyperpolarization induced by the cardiac glycoside. However, the effects of calcium and the glycoside on the potential appear to be additive, since at high $(Ca)_o$ addition of K-strophanthoside produces further hyperpolarization of the Schwann cell.

The hyperpolarization produced by the glycoside is followed by a gradual depolarization and a decrease in the Schwann cell potassium concentration. This late depolarization could be related to the late changes observed in the intracellular electrolyte concentrations.

This biphasic effect of K-strophanthoside on the electrical potential of the

Schwann cell resembles that produced by low concentrations of ouabain on the electrical potential difference across the frog skin (22, 23). This was explained as due to the low ouabain concentration employed in those experiments. A similar explanation for the biphasic effect of K-strophanthoside on the Schwann cell may be ruled out since the initial hyperpolarization of this cell is larger at 10^{-3} M than at 10^{-5} M.

It may be concluded (*a*) that the Schwann cell membrane potential is determined by the ionic concentration gradients and permeabilities, mainly of potassium, and to a minor extent of other ions, and (*b*) that also a net movement of ions, in all probability of potassium inward, contributes to the potential. K-strophanthoside blocks this latter contribution. It is tempting to suggest that a cardiac glycoside–sensitive nonelectroneutral pump is responsible for this net ionic movement.

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