Long Duration Responses in Squid Giant Axons Injected with ^{134}Cesium Sulfate Solutions

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ABSTRACT Giant axons from the squid were injected with 1.5 M cesium sulfate solutions containing the radioactive isotopes ^{42}K and ^{134}Cs. These axons, when stimulated, gave characteristic long duration action potentials lasting between 5 and 45 msec. The effluxes of ^{42}K and ^{134}Cs were measured both under resting conditions and during periods of repetitive stimulation. During the lengthened responses there were considerable increases in potassium efflux but only small increases in cesium efflux. The selectivity of the delayed rectification process was about 9 times greater for potassium ions than for cesium ions. The data suggest that internal cesium ions inhibit the outward potassium movement occurring during an action potential. The extra potassium effluxes taking place during excitation appear to be reduced in the presence of cesium ions to values between 7 and 22% of those expected in the absence of cesium inhibition.

If the series of alkali cations K^+, Rb^+, Cs^+ is considered, it seems evident that, in the squid axon, K^+ and Rb^+ tend to behave in a similar manner while the Cs^+ ion gives rise to marked differences in behavior. Baker, Hodgkin, and Shaw (1962) perfused squid giant axons internally with Rb_2SO_4 solutions and also with Cs_2SO_4 solutions. The rubidium medium prolonged the action potential and appeared to affect the declining phase, but excitability was maintained. The cesium perfusion medium, however, first prolonged the action potential and then led to membrane depolarization and eventual loss of excitability. Pickard et al. (1964) observed little if any depolarizing action when squid axons were bathed in a Cs^+-rich sea water ($[Cs^+]$ = 423 mM). Also, axons undergoing voltage clamp (sucrose-gap technique) in the high Cs^+ medium showed negligible early current but a normally appearing delayed outward current. Cesium ions, when present externally, do not seem to carry significant amounts of membrane current. Chandler and Meves (1965) observed that in voltage-clamped axons internally perfused with a medium having half the K^+ replaced with Cs^+, the delayed current was reduced to 2 to 5% of the control value observed in axons perfused with a normal high K^+ medium.

The work cited shows that the squid axon membrane is relatively impermeable to cesium ions and that when present internally, cesium ions interfere with the outward movement of potassium ions. The purpose of this paper is to report the results obtained in cases in which both ^{42}K and ^{134}Cs efflux are measured at rest and when axons are giving long duration responses due to the presence of a high internal cesium concentration.

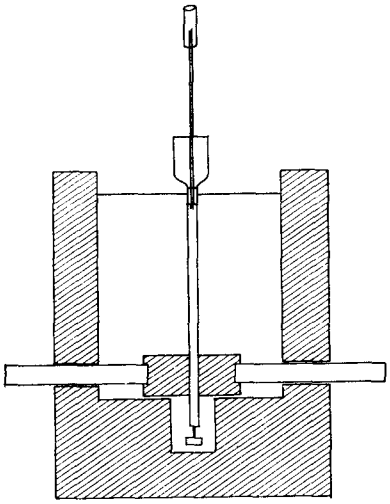

FIGURE 1. The chamber used for the microinjection of radioactive solutions into axons is diagramed schematically. The axon to be injected was tied to a cannula shown at the top end of the axon. A weight was tied to the bottom end and the lower part of the vertically suspended axon was held in the clamp illustrated. A microcapillary was attached to the microliter syringe (not shown) and this was led downward into the axon via the cannula.

METHODS

Giant axons from the squid, *Loligo pealii*, were carefully dissected from the hindmost stellar nerve and cleaned of adhering smaller fibers over a 5 cm length. Axons were then mounted vertically in the chamber depicted in Fig. 1 where they were microinjected with the desired solution. The general method of microinjection was similar to that employed by Hodgkin and Keynes (1956) and to the method described in detail by Brinley and Mullins (1965). A Hamilton microsyringe of 1 μl capacity was filled with the radioactive solution (mixtures of ^{42}K and ^{134}Cs in 1.5 M Cs_2SO_4 carrier solution) and a microcapillary attached to the syringe was introduced into the axon through a cannula at the top end. Approximately 7 mm of axon length was injected with the dyed (phenol red) radioactive mixture and the axon was removed from the apparatus and mounted in the cell depicted in Fig. 2. The injected portion could thus be situated in the center pool region of the efflux measurement chamber, the length of this region being 0.5 cm. Potassium- and cesium-free sea water of the following composition was perfused externally over the portion of axon situated in the center

pool: $[Na^+]$ = 433 mM, $[Ca^{++}]$ = 9.3 mM, $[Mg^{++}]$ = 48.5 mM, $[Cl^-]$ = 497 mM, $[SO_4^=]$ = 25.5 mM, $[HCO_3^-]$ = 2.2 mM, pH = 7.9. The normal sea water used to bathe the axon while in preparation differed from this solution only in containing K^+ ions at a concentration of 10 mM and in having a sodium concentration of 423 mM. Flowing isosmotic sucrose solutions provided electrical insulation of the axon section in the center pool from the portions of axon in the two end pools which contained isosmotic KCl. A suction device was employed to collect the sea water that passed over the axon in the center region. An approximately 1 cc sample was collected at

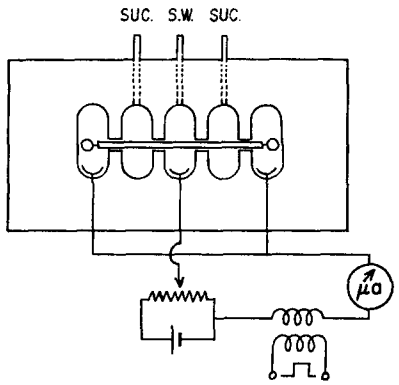

FIGURE 2. The chamber used for the measurement of the effluxes of the radioactive tracers employed is shown diagramatically. Axons were mounted in the center groove and vaseline was used to seal off the portion of axon situated in each pool of the chamber. Cooled K- and Cs-free sea water were passed into the center compartment and subsequently collected for the assay of ^{42}K and ^{134}Cs radioactivity. Current for hyperpolarization was passed through the membrane using the electrodes in the center and end compartments and the voltage divider circuit illustrated. Isosmotic sucrose solutions were passed into the pools labeled "Suc." to provide electrical insulation of the portions of axon situated in these pools. The membrane in the center compartment was excited in each experiment by square wave pulses of 0.1 msec duration introduced into the membrane current circuit.

1 min intervals during each experiment. Membrane and action potentials of the axon in the center pool were measured using the usual 3 M KCl-filled microelectrode, a high impedance preamplifier, and a Tektronix oscilloscope. The membrane potential of the axon in the region of measurement was hyperpolarized by means of the current-passing circuit illustrated. Axoplasm samples from the region of axon situated in the center pool were taken at the termination of each experiment. Each sample was weighed and ashed in a platinum crucible at 550°C for 10 hr. The ash was dissolved in a drop of 1 N HNO_3 and diluted to 5 cc with deionized water. An aliquot, usually 0.1 cc, was taken for assay of radioactivity. The axoplasm aliquot and all efflux samples collected were dried on planchets and counted by means of a low level counting system. The first counting gave the sum of ^{42}K and ^{134}Cs counts. The samples were allowed to decay for 6 days after which another counting was made to obtain the ^{134}Cs counts. This period of radioactive decay was sufficient to reduce the ^{42}K counts to a

level below background. The remainder of the axoplasm solution was used for flame photometer analyses of K and Cs. Axoplasmic specific activities of K and Cs were computed from the data and these can be regarded as constant since the collection solution did not contain K^+ or Cs^+ ions. Effluxes were measured in the manner described by Sjodin and Mullins (1967).

RESULTS

The following results were obtained on axons having an internal cesium concentration in the range from 152 to 180 mM depending on the size of the axon and the amount of cesium sulfate microinjected. When such axons were mounted in the cell depicted and penetrated with a microelectrode, the initial membrane potential recorded varied within the range from −45 mv to −54 mv. The average value obtained for 6 axons was −51 mv. These potential values were obtained while the axons were perfused externally with K-free, Cs-free sea water. Normally, under these conditions, the recorded membrane potentials would lie within the range from −60 mv to −67 mv. The high internal cesium concentration therefore exerts a depolarizing action on the axon membrane. This result was to be expected in view of the work of Baker, Hodgkin, and Shaw (1962).

Most of the axons studied gave membrane action potentials of reduced magnitude at the resting potential with a high internal cesium concentration. When the segment of membrane in the center pool control region was polarized to a potential of at least −60 mv by passage of an inward current, action potentials were obtained, upon stimulation, which had durations of up to 45 msec compared with a normal duration at the same temperature of around 2 msec. Such action potentials often had characteristic plateau regions as shown in Fig. 3 and were similar in appearance to the longer duration responses observed by Adelman, Dyro, and Senft (1965). To a first approximation at least, the rising phase of the long duration "cesium action potentials" is normal and the sodium activation process is unaffected by internal cesium ions. The processes which restore the membrane to the initial state following excitation, however, are strongly affected by the high internal cesium concentration.

The longest responses had durations around 45 msec. Such a response is shown in Fig. 3A. The record shows an oscillatory plateau lasting about 35 msec. The oscillation was not due to noise derived from electrical power lines. The instability in the "plateau oscillation" is apparent in the record from another axon shown in Fig. 3B. The record was obtained during stimulation at a repetition rate of 1 per sec. The shorter duration response was the most frequent one observed but about every third pulse gave a response like the longer one superposed on the record. The process responsible for maintaining the plateau thus exhibits thresholdlike characteristics similar to those

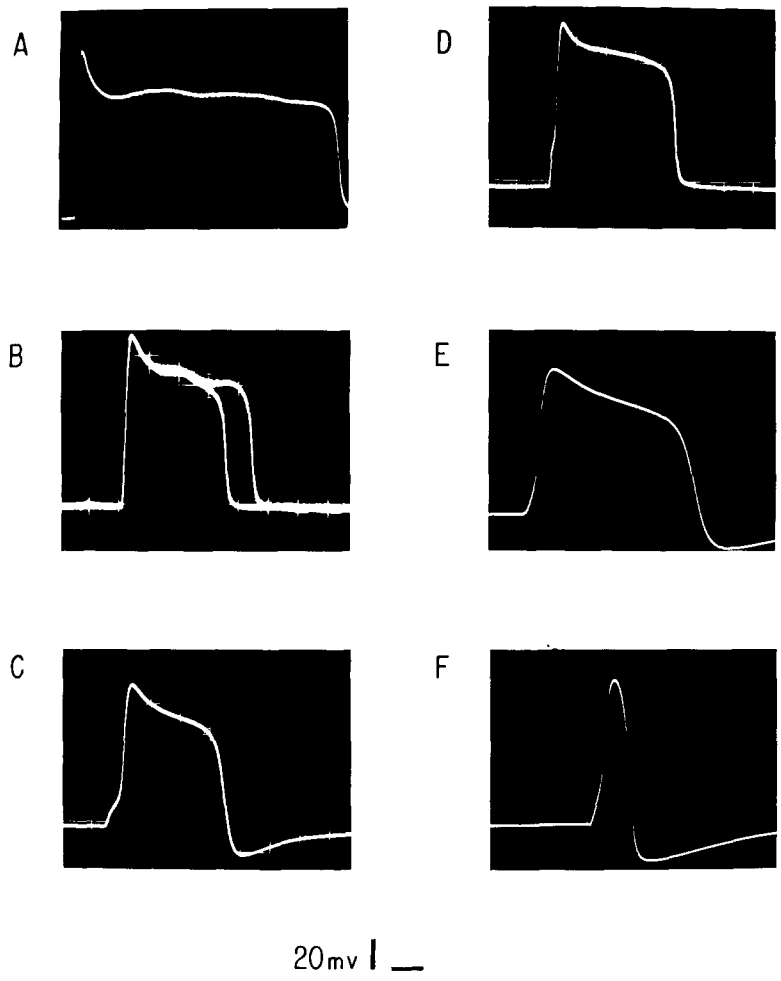

FIGURE 3. Typical responses given by axons injected with cesium sulfate are illustrated. The vertical calibration bar represents 20 mv and this applies to all action potentials shown. The horizontal bar represents 5 msec for part A, 5 msec for B, 2 msec for C, 5 msec for D, 1 msec for E, and 1 msec for F. The action potential photographs are as follows: A, action potential with oscillatory membrane potential plateau typical of the longest duration responses observed. B, action potential from a high cesium axon stimulated at a rate of one per sec. Every third or fourth response was a longer duration response superposed on the shorter one. C, the duration of the action potential shown is about 6 msec. This response is typical of those responses given by the axons stimulated at a rate of 100 per sec in Table I. D, the action potential shown was given by axon 84A in Table I as it was stimulated for the determination of extra ^{42}K and ^{134}Cs efflux. E, the action potential given by axon 713A (Table I) is illustrated. F, a normal action potential given by an axon containing no cesium ions is shown for comparison. In all cases the temperatures were close to 12°C. The exact temperatures for some of the action potentials are stated in Table I.

shown by oscillatory action potentials observed at the normal resting potential (Sjodin and Mullins, 1958). In general, the shapes and durations of the cesium action potentials varied and in some preparations the plateau region showed a steady decline to a membrane potential value where a rapid recovery process occurred. A series of observed types of responses is illustrated in Fig. 3. The magnitudes of the plateau membrane potentials varied from preparation to preparation but plateau potentials that persisted were in the range of +20 to +30 mv.

The data on K and Cs efflux at rest and during stimulation are presented in Table I. Representative action potentials given by the axons yielding the efflux data in Table I are shown in Fig. 3. Axons having action potentials of

TABLE I

POTASSIUM AND CESIUM EFFLUXES FROM GIANT AXONS WITH HIGH INTERNAL CESIUM CONCENTRATIONS

Axon No.	Temperature	Axoplasmic concentrations		Resting effluxes		Repetition rate	Action potential Duration	Extra efflux per impulse		Fraction of outward $K^+ + Cs^+$ Current carried by K ions
		[K]	[Cs]	ϕ_K	ϕ_{Cs}			K	Cs	
	°C	mM		pmoles/cm² sec		sec^{-1}	msec	pmoles/cm²		
630A	12.5	217	180	30.1	12.4	100	5	3.24	0.46	0.88
713A	12.9	163	152	55.8	18.5	100	6	1.71	0.28	0.86
83A	11.2	232	174	40.9	20.6	100	6	5.38	0.0*	1.0
84A	11.8	185	172	69.1	15.5	10	25	42.7	2.6	0.94

* Less than 0.05.

around 5 to 6 msec duration were stimulated at a rate of 100 per sec. Axon 84A gave an action potential of 25 msec duration and a stimulation rate of 10 per sec was used to obtain the extra effluxes occurring during the action potential. The appearance of the action potential given by axon 84A as observed during the stimulation and tracer collection interval is shown in Fig. 3D.

The data in Table I indicate that the resting Cs efflux was always lower than the corresponding K efflux even though the respective internal concentrations were comparable. The data also show that significant extra effluxes of K^+ ions are associated with the long duration responses. The extra effluxes of Cs^+ ions, however, were small and varied from zero to 16% of the extra K^+ efflux. The selectivity of the delayed rectification process for K^+ ions relative to that for Cs^+ ions is high, as evidenced by the fraction of the total $K^+ + Cs^+$ current carried by K^+ ions during an action potential (last column of Table I).

DISCUSSION

These experiments indicate that the squid giant axon membrane discriminates markedly between potassium and cesium ions during an action potential. This differs from the conclusions of Tasaki and Spyropoulos (1961) who reported that the axon membrane shows no great difference in selectivity between K^+ and Cs^+ ions. The two investigations are not directly comparable, however, as the internal cesium and potassium concentrations were approximately equal to each other in the present work. The discrimination between K^+ and Cs^+ ions shown by the resting membrane is less marked as seen in Table I. The reason for this may be that a fraction of the resting efflux occurs through nonspecific leakage regions of the membrane (Mullins, 1966). It is not likely that this is the sole explanation, however.

It is interesting to examine the flux data in the light of voltage clamp experiments under similar conditions. Chandler and Meves (1965) observed the delayed current to be reduced to very small values in a depolarizing voltage clamp when half of the internal potassium was replaced with cesium during internal perfusion. Adelman and Senft (1966) have, in similar perfusions, observed an inhibitory effect of cesium ions on the nonsodium steady-state current. It can be concluded that the delayed rectification pathways have a very low permeability to cesium ions and that Cs^+ ions have a blocking effect on the passage of K^+ ions through the same pathways. The low permeability of delayed rectification channels to cesium ions has been verified by the efflux data obtained in this investigation. The average selectivity of the membrane during an action potential deduced from K^+ and Cs^+ flux data is about 9 to 1 in favor of K^+. This estimate is made on the basis that the extra K^+ and Cs^+ losses during an action potential follow a similar time course. If part of the Cs^+ loss occurs during a process temporally separated from the delayed rectification process, the selectivity ratio of K^+ to Cs^+ during delayed rectification would be higher. Chandler and Meves (1965), however, found the membrane permeability to cesium ions during the early current in a voltage clamp to be about 1/60th of the permeability to sodium ions. This information applied to the present data indicates that less than 10% of the observed cesium loss during an action potential can be attributed to leakage through sodium channels.

Though the data of Adelman and Senft (1966) do not permit the assignment of relative K^+ and Cs^+ permeabilities to the delayed current channels, the degree of cesium interference with potassium movement can be estimated. When the internal potassium and cesium ion concentrations were of comparable magnitude, a marked reduction in the nonsodium steady-state current was apparent in the cases in which depolarizations were large. The degree of current reduction was potential-dependent and became less severe with diminishing depolarization. Little or no current reduction was apparent very

near the resting potential. The resting potassium ion effluxes measured in this work are consistent with the latter observation as their values lie within the range of normally observed values. There is no way to directly determine the degree of cesium interference in the present flux experiments. The reason is that there is no way of knowing what the potassium effluxes would have been for the same action potential durations under the same conditions in the absence of cesium ions. An estimate can be made, however. The normal K^+ loss per impulse near the temperatures employed varies between 3.7 and 15 pmoles/cm² (Brinley and Mullins, 1965; Sjodin (unpublished observations); Caldwell and Keynes, 1960). The average value for ten axons taken from these sources is 8.5 pmoles/cm². The first three axons reported in Table I had action potentials of about 3 times the normal duration. A rough estimate of the predicted average K^+ loss during such action potentials with no flux interference would be about 25 pmoles/cm² per impulse. On this basis, the per cent of normal potassium current occurring in the presence of internal cesium ions is estimated as follows: axon 630A: 13%, axon 713A: 7%, axon 83A: 22%, or about 14% on the average.

The data obtained on axon 84A can be readily analyzed because of the long duration of the action potential and its approximation in shape to a square wave of 90 mv amplitude and 25 msec duration (Fig. 3D). The resting potential measured for this axon was −54 mv and the membrane was hyperpolarized to −60 mv during the experiment. The peak-to-peak height of the action potential was 110 mv and a prolonged potential plateau occurs around +30 mv. The action potential can be compared with a depolarizing voltage clamp of 90 mv amplitude and 25 msec duration. The steady-state potassium conductance increase during such a voltage clamp is about 80-fold (Hodgkin and Huxley, 1952) and the maximum value is achieved in about 2 msec at the temperature employed in the present experiments. There is no direct measurement of the membrane conductance during the potential plateaus observed but an estimate can be made of the extent of flux inhibition caused by internal cesium ions. One can calculate the potassium flux increase observed during the long duration action potential and compare this with the flux increase expected in the stated voltage clamp in the absence of cesium ions. Assuming that the extra potassium loss during the long duration response occurs uniformly throughout the potential plateau, the extra potassium efflux of 42.7 pmoles/cm² per impulse observed is equivalent to a 25-fold increase in potassium efflux during the action potential. To estimate the flux increase expected during the voltage clamp, use must be made of the relation $I_K = F\phi_K = g_K(E - E_K)$ where I_K refers to potassium current, ϕ to flux, g_K to conductance, E to membrane potential, and E_K to the equilibrium potential for potassium ions. A difficulty in applying this relation to the present results is that the experiments were performed in K-free sea water when E_K is for-

mally infinite. The "underswing" of the action potential obtained in K-free sea water (normal axon) indicates, however, that E_K actually reaches a limiting value of around -90 mv as the external potassium concentration is reduced to zero. This value is used to calculate that the driving force term $(E - E_K)$ increases fourfold upon altering the membrane potential from -60 mv to $+30$ mv. The conductance increase is 80-fold and the expected flux increase is thus 320-fold. The 25-fold flux increase observed indicates that cesium ions have lowered the activity of the potassium current–carrying system to 25/320 or 8% of the normal activity.

At a membrane potential corresponding to the plateau value of $+30$ mv for axon 84A, the results of Adelman and Senft (1966) predict a reduction of the nonsodium steady-state current to about 12% of the normal value. There appears to be agreement between the tracer flux and voltage clamp experiments both at rest and during the long duration action potentials.

Though the present results do not provide information on the movements of ions other than K^+ and Cs^+, it is clear that significant movements of at least one other ion must occur during the long responses. The ions most likely to be involved are Na^+ and Cl^-. The work of Adelman and Senft (1966) strongly suggests that additional current is carried mainly by sodium ions.

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