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Calcium currents in squid giant axon

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Voltage-clamp experiments were carried out on intracellularly perfused squid giant axons in a Na-free solution of 100 mM $CaCl_2$ + sucrose. The internal solution was 25 mM CsF + sucrose or 100 mM RbF + 50 mM tetraethylammonium chloride + sucrose. Depolarizing voltage clamp steps produced small inward currents; at large depolarizations the inward current reversed into an outward current. Tetrodotoxin completely blocked the inward current and part of the outward current. No inward current was seen with 100 mM $MgCl_2$ + sucrose as external solution. It is concluded that the inward current is carried by Ca ions moving through the sodium channel. The reversal potential of the tetrodotoxin-sensitive current was +54 mV with 25 mM CsF + sucrose inside and +10 mV with 100 mM RbF + 50 mM tetraethylammonium chloride + sucrose inside. From the reversal potentials measured with varying external and internal solutions the relative permeabilities of the sodium channel for Ca, Cs and Na were calculated by means of the constant field equations. The results of the voltage-clamp experiments are compared with measurements of the Ca entry in intact axons.

Introduction

Calcium ions enter the squid axon during an action potential or during a depolarizing voltage-clamp pulse. This has been shown with radioactive Ca (Hodgkin & Keynes 1957; Tasaki, Watanabe & Lerman 1967) and with aequorin as Ca indicator (Baker, Hodgkin & Ridgway 1971; Baker, Meves & Ridgway 1973*a*, *b*). The aequorin experiments indicated two phases of Ca entry: an early phase that is blocked by tetrodotoxin and seems to reflect Ca entering through the sodium channels and a late phase that is insensitive to tetrodotoxin and tetraethylammonium, but blocked by Mn and the organic Ca antagonist D-600.

Further evidence for Ca entry comes from the work of Tasaki, Watanabe & Singer (1966) and Tasaki *et al.* (1967). They showed that fibres internally perfused with 25 mM CsF + glycerol and immersed in a Na-free solution of 100 or 200 mM $CaCl_2$ + glycerol are able to produce action potentials, arising from an influx of Ca ions, for several hours.

The amount of Ca entering the fibre is very small. With 112 mM Ca in the external solution the Ca entry measured with the tracer technique is in the order of 0.08 pmol/cm² per action potential (Hodgkin & Keynes 1957), corresponding to an electric charge of less than 0.02 μC/cm². Tracer measurements on perfused fibres with 25 mM CsF + glycerol inside and 100 mM $CaCl_2$ + glycerol outside also indicate a very small Ca entry (Takenaka & Yumoto 1969).

It was the purpose of the voltage-clamp experiments described below to measure the small electric currents which are carried by Ca ions moving across the membrane. In an intact axon with normal seawater outside the small calcium inward current will be totally obscured by the large Na inward and K outward currents. The experiments were therefore done on fibres immersed in a Na-free solution containing a high concentration of Ca and internally perfused with a K-free salt solution; the delayed K channel was blocked by internal Cs or tetraethylammonium. In the first experiments the same solutions as in the experiments of

Tasaki *et al.* (1966, 1967) were used. In a later stage the composition of the solutions was varied, e.g. by using 100 mM RbF + 50 mM tetraethylammonium chloride instead of 25 mM CsF as the internal electrolyte.

The voltage-clamp experiments with 25 mM CsF as perfusion fluid have recently been reported elsewhere (Meves & Vogel 1973). The main findings are summarized below. The experiments with 100 mM RbF + 50 mM tetraethylammonium chloride as internal solution will be described in detail.

Methods

The experiments were done on giant axons of *Loligo forbesi* and occasionally *Loligo vulgaris*. The uncleaned axon was extruded and perfused by the method of Baker, Hodgkin & Shaw (1962). Special care had to be taken to remove the external Na completely, especially from the space between the small nerve fibres which surround the giant axon. The fibre was washed for three consecutive periods of 8–10 min in Na-free tris seawater. It was then bathed in a Na-free solution of 100 mM $CaCl_2$ + sucrose for at least another 30 min. The voltage-clamp method has been described in previous publications (Chandler & Meves 1965; Meves & Vogel 1973). The membrane currents were measured with a double C electrode. They were usually recorded with an oscilloscope. In some experiments the membrane currents were averaged with a Biomac 1000 signal analyser and recorded with a pen recorder. The resting potential measurements were corrected for junction potentials which were determined at the beginning and end of each experiment. The external and internal solutions were made isotonic with seawater by adding appropriate amounts of sucrose. The pH was 7.3–7.8 for the external solutions and 7.2–7.5 for the internal solutions and was adjusted with tris-HCl buffer (final concentration 0.1–1.0 mM). The Ca activity in the external 100 mM $CaCl_2$ + sucrose solution was determined with a calcium electrode as 35.5 mmol/kg H_2O. The Cs activity in 25 mM CsF + sucrose and the Rb activity in 100 mM RbF + 50 mM tetraethylammonium chloride + sucrose were assumed to be the same as the K activities in corresponding solutions with KF; the latter were measured with a K-sensitive electrode; from these measurements [Cs] and [Rb] were obtained as 26 and 99 mmol/kg H_2O, respectively.

Results

Experiments with 25 mM-CsF *as internal electrolyte*

Figure 1 shows the voltage clamp currents in a fibre with 25 mM CsF + sucrose as internal solution and 100 mM $CaCl_2$ + sucrose as external solution. Between clamp pulses the membrane was held at the resting potential (−37.5 mV). A hyperpolarizing pulse to −65.5 mV produced a short capacity transient followed by a small leakage inward current; from the latter the leakage resistance was estimated as 80 kΩ cm². A depolarizing pulse to +9.5 mV led to a small sustained inward current. The inward current reached a maximum of 5–6 μA/cm² at a potential of +29.5 mV and decreased again for larger depolarizations. At +52.5 mV the current was outward during the whole pulse. With stronger pulses the outward current grew rapidly in size. The inward and outward currents were maintained during the whole 60 ms clamp pulses. There was, however, a clear decay of the inward currents and a lesser decay of the outward currents. Experiments with longer pulses indicated that even after 300 ms there was still a large fraction of the inward current left.

The next step was to find out whether the membrane currents were affected by tetrodotoxin, the selective inhibitor of the sodium channel. Figure 2 shows records without tetrodotoxin (*a*) and with tetrodotoxin (*b*). The currents in (*a*) are similar to those in figure 1; the maximum inward current occurred at a potential of +32.5 mV. The records in (*b*) were taken 18 min after adding 2 μM tetrodotoxin to the external fluid. The potential during the clamp pulses was approximately the same as in the records in (*a*). The inward current has completely disappeared, the outward current is much smaller.

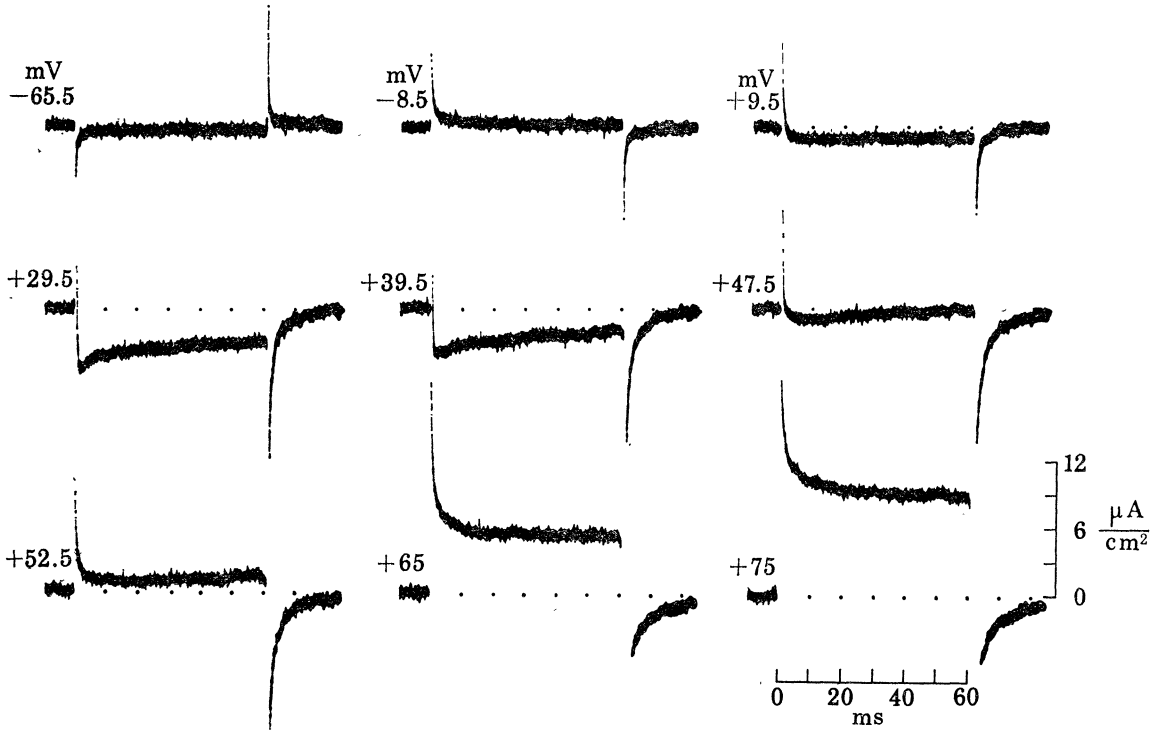

FIGURE 1. Membrane currents associated with step changes in membrane potential. External solution: 100 mM-$CaCl_2$ + sucrose. Internal solution: 25 mM CsF + sucrose. Resting potential = holding potential = −37.5 mV. The internal potential during the potential step is given above each trace. Outward current upward. The current tails at the end of the two largest pulses (+65 and +75 mV) were partly off screen. Records were taken after bathing the fibre in Na-free solution for 72 min. Axon diameter 840 μm. Temperature 16.9 °C. (From Meves & Vogel 1973.)

The quantitative analysis of a tetrodotoxin experiment is illustrated in figure 3. The open circles give the membrane currents recorded in the presence of 1.5 μM tetrodotoxin and measured 20 ms after the beginning of the clamp pulse; measurements at 10 or 60 ms gave similar values. The measuring points follow a straight line (slope 33.1 kΩ cm²) over a wide potential range, but deviate clearly for internal potentials larger than 25 mV. The deviation seems not to be due to delayed rectification because a similar deviation was seen with 100 mM RbF + 50 mM tetraethylammonium chloride as internal electrolyte (see figure 5); it probably represents leakage rectification. The filled symbols in figure 3 show the tetrodotoxin-sensitive current which is the difference between the current measured before application of tetrodotoxin and the current recorded at the same potential in the presence of tetrodotoxin. The currents were measured at a fixed time (20 ms) after the beginning of the clamp pulse; this was done because the peak values of the currents (which are normally plotted in a current–voltage relation)

could not be measured reliably. As illustrated in figure 3, the tetrodotoxin-sensitive inward current started to turn on between −10 and 0 mV, reached its peak (4.5 μA/cm²) at about +30 mV and decreased for more positive potentials. The tetrodotoxin-sensitive current reversed its sign at +54.5 mV and became outward for larger potentials. In a total of ten experiments the average value of the reversal potential was +54.1 mV (s.e. ± 2.1 mV).

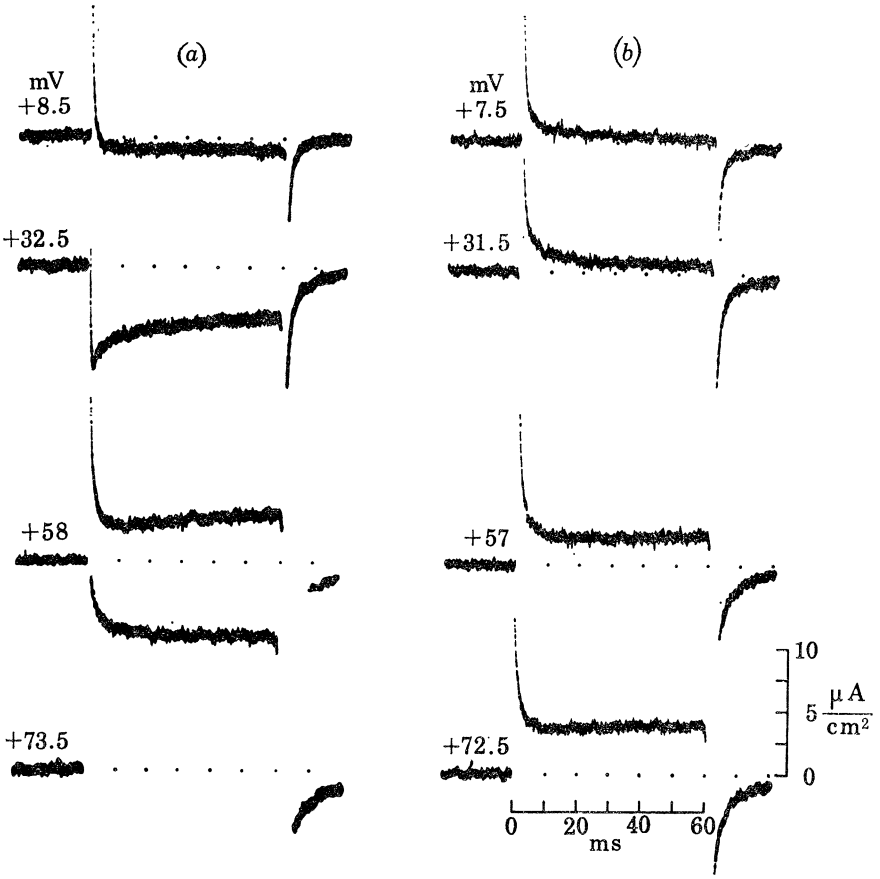

FIGURE 2. Effect of tetrodotoxin on membrane currents. (*a*) 100 mM $CaCl_2$ + sucrose (without tetrodotoxin) as external solution; (*b*) 18 min after adding tetrodotoxin, final concentration 2 μM. Internal solution: 25 mM CsF + sucrose. (*a*) Resting potential = holding potential = −44.5 mV; (*b*) resting potential = −35.5 mV, holding potential −45.5 mV. Records in *a* were taken after bathing the fibre in Na-free solution for 65 min. Axon diameter 960 μm. Temperature 17.0 °C. (From Meves & Vogel 1973.)

The experiment in figure 3 indicates that there is a tetrodotoxin-sensitive inward current (presumably carried by external Ca ions) and a tetrodotoxin-sensitive outward current (presumably carried by internal Cs ions) which are equal and opposite at a potential of about +54 mV. It was essential to rule out other ions (external Cl, residual external Na, internal F) as current carriers. Substituting Ca acetate for $CaCl_2$ in the external solution did not abolish the tetrodotoxin-sensitive outward current, indicating that this current is not carried by external Cl. As mentioned above, the fibres were bathed in Na-free solution for at least an hour before starting the experiment; this should be sufficient to remove the external Na completely. Nevertheless, it was important to demonstrate directly that the small inward currents are not carried by residual external Na ions. This was done by using 100 mM $MgCl_2$ instead of 100 mM $CaCl_2$ as external electrolyte. No inward currents were seen with $MgCl_2$ whereas the

same fibre gave clear inward currents with $CaCl_2$. The absence of inward currents in $MgCl_2$ cannot be attributed to inactivation of the sodium conductance because large inward currents were obtained when 20 mM NaCl was added to the 100 mM $MgCl_2$ + sucrose solution. This experiment makes it unlikely that the inward currents seen with 100 mM $CaCl_2$ + sucrose as external solution are carried by residual external Na. It also argues against the somewhat remote possibility that internal F is the current carrier. Increasing or decreasing the external Ca concentration increased or decreased the reversal potential of the tetrodotoxin-sensitive current. This gives further support to the idea that the tetrodotoxin-sensitive inward current is carried by external Ca ions.

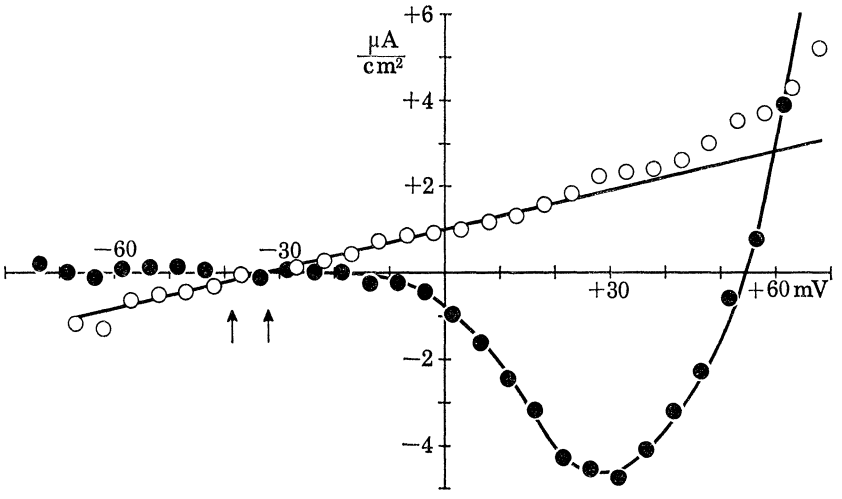

FIGURE 3. Current–voltage relation for a fibre with 100 mM $CaCl_2$ + sucrose outside and 25 mM CsF + sucrose inside. ●, Tetrodotoxin-sensitive current = (current before application of tetrodotoxin) − (current after application of tetrodotoxin). ○, Tetrodotoxin-insensitive current = current after application of tetrodotoxin. The currents before application of tetrodotoxin were recorded with a holding potential of −38.5 mV (= resting potential). The currents after application of tetrotodoxin were recorded 16 min after adding tetrodotoxin (final concentration 1.5 μM), resting potential = holding potential = −32 mV. Holding potentials indicated by arrows. All currents were measured 20 ms after beginning of clamp pulse. Abscissa: internal potential during pulse. Axon diameter 990 μm. Temperature 16.5 °C. (From Meves & Vogel 1973.)

From the average reversal potential of the tetrodotoxin-sensitive current the relative permeability of the sodium channel for Ca and Cs was calculated. The calculation was based on the constant field equations (Goldman 1943; Hodgkin & Katz 1949) which were modified by assuming a large fixed negative charge potential at the inside of the membrane. This modification which was first introduced by Frankenhaeuser (1960) was suggested by Sir Alan Hodgkin. For the calculation the ion activities as measured with ion-selective electrodes were used. The average permeability ratio P_{Ca}/P_{Cs} was obtained as 1/0.62 or 1.61/1, i.e. the permeability of the sodium channel for Ca is about 1.6 times larger than for Cs under our experimental conditions.

It seemed of particular interest to obtain information about the relative permeability of the sodium channel for Ca and Na. Two series of experiments were done to determine P_{Ca}/P_{Na}. In the first series the internal 25 mM CsF was totally or partially replaced by NaF or Na phosphate; this led to a shift of the reversal potential towards less positive internal potentials. In the second series small amounts of NaCl (5–50 mM) were added to the external 100 mM

$CaCl_2$ + sucrose solution; this produced a shift of the reversal potential towards more positive internal potentials. From the changes in reversal potential observed in these two series of experiments the ratio P_{Ca}/P_{Na} was calculated, using again the modified constant field equations and the measured ion activities. P_{Ca}/P_{Na} was found to be 1/10–1/7, indicating that the sodium channel is 7–10 times more permeable to Na than to Ca under our experimental conditions; these numerical values depend on the use of the constant field equations for the interpretation of the experimental results.

The main properties of the Ca inward currents recorded in fibres with 25 mM CsF as internal electrolyte can be summarized as follows:

(1) The inward currents are very small (maximum size 4–8 $\mu A/cm^2$). This explains the small rate of rise (2 V/s at 16–17 °C) of the action potentials recorded under these conditions (Tasaki *et al.* 1966, 1967; Meves & Vogel 1973).

(2) The Ca inward currents are completely abolished by tetrodotoxin. Tetrodotoxin-insensitive Ca inward currents, corresponding to the late Ca entry in aequorin experiments on intact axons, have not been observed. A possible explanation would be that the late Ca entry seen with aequorin represents an electro-neutral exchange, but other explanations are not ruled out.

(3) The Ca inward currents inactivate very slowly. This is quite different from the transient time course of the tetrodotoxin-sensitive Ca entry in aequorin-injected intact axons. The slow inactivation is probably due to the perfusion with a CsF solution of low ionic strength. Internal CsF is known to produce slow and incomplete sodium inactivation (Chandler & Meves 1970); there is also evidence for a delayed sodium inactivation in fibres perfused with a solution of low salt concentration.

(4) The permeability ratio P_{Ca}/P_{Na} of the sodium channel is estimated as 1/10–1/7. This is more than 10 times larger than the ratio 1/100 which Baker *et al.* (1971) deduced from aequorin experiments on intact axons, indicating a loss of selectivity under our experimental conditions. The two figures are difficult to compare because they were obtained in completely different ways. However, a substantial loss of selectivity is also indicated by measurements of the permeability ratio P_{Cs}/P_{Na}; this ratio was 1/22–1/9 as opposed to 1/61 in previous experiments with perfusion fluids of normal ionic strength (Chandler & Meves 1965). The loss of selectivity is probably due to the low ionic strength of the internal solution.

(5) The time course of the Ca inward current is different from the time course of the Na inward current, the latter showing a pronounced initial peak (see Figs 14 and 15 of Meves & Vogel 1973).

The general conclusion is that the experimental results obtained on axons intracellularly perfused with 25 mM CsF + sucrose are certainly not directly applicable to intact axons. This raises the question whether we can make the experimental conditions more 'normal' and still get Ca inward currents. Experiments designed to answer this question are described in the following section.

Experiments with 100 mM RbF + 50 mM *tetraethylammonium chloride as internal electrolyte*

A solution of 100 mM RbF + sucrose was chosen as a 'more normal' perfusion fluid, i.e. the Cs ions were replaced by the more potassium-like Rb and the internal salt concentration was increased by a factor of 4. Fifty mM tetraethylammonium chloride were added to the internal

solution in order to block the current through the delayed channel; this was necessary because the delayed channel which is almost completely blocked by internal Cs (Chandler & Meves 1965; Adelman & Senft 1966) has an appreciable permeability for Rb. The external solution was again 100 mM $CaCl_2$ + sucrose. The inward currents obtained with 100 mM RbF + 50 mM tetraethylammonium chloride as internal electrolyte were smaller than those with 25 mM CsF inside. This was to be expected because the sodium channel is more permeable for Rb than for Cs (Chandler & Meves 1965) and is also to some extent permeable for tetraethylammonium ions (Binstock & Lecar 1969). The internal concentration of Rb and tetraethylammonium is relatively high; consequently, a large part of the Ca inward current will

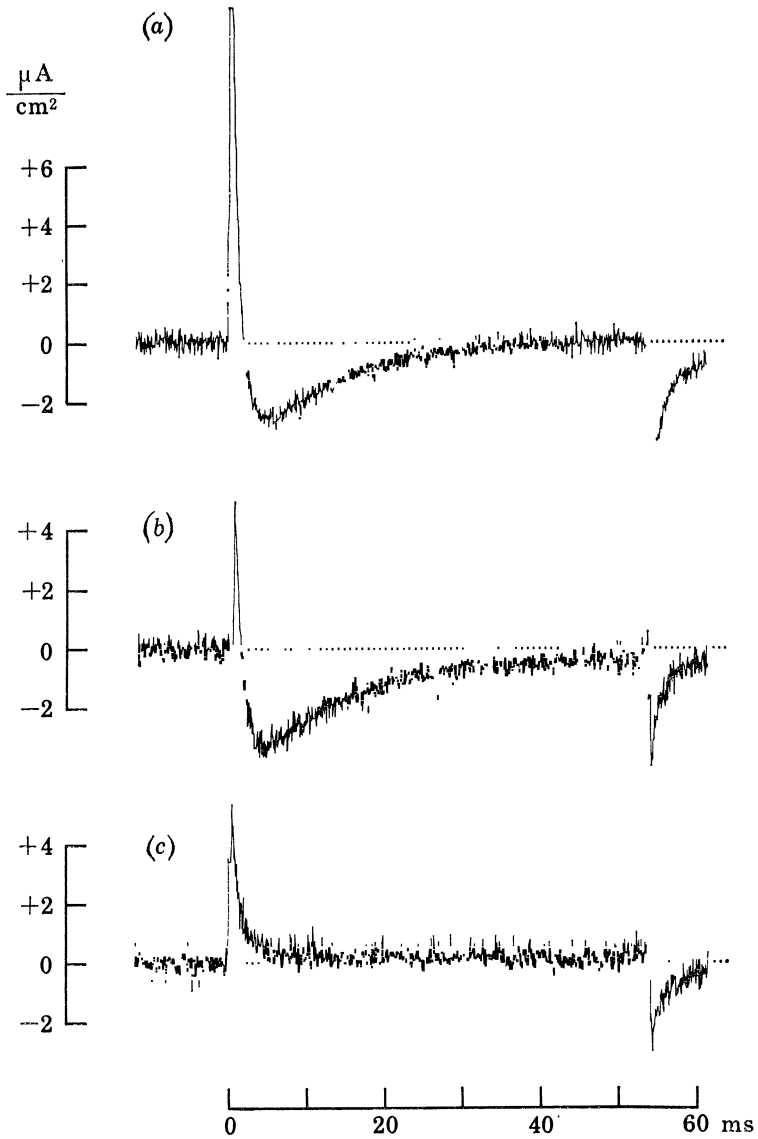

FIGURE 4. Averaged membrane currents recorded with a pen recorder. (*a*) Eight depolarizing pulses of 60 mV amplitude; (*b*, *c*) eight depolarizing and eight hyperpolarizing pulses of 60 mV amplitude. Pulse frequency 1.5/s. External solution: 100 mM $CaCl_2$ + sucrose in *a* and *b*; same with 2 μM tetrodotoxin in *c*. Internal solution: 100 mM RbF + 50 mM tetraethylammonium chloride + sucrose. Resting potential: −43.5 mV in *a*, −44.5 mV in *b* and *c*. Holding potential: −63.5 mV in *a*, −64.5 mV in *b* and *c*. The capacitative current at the end of the pulses in *a* was only partly recorded. Calibration applies to a single current record. Axon diameter 910 μm. Temperature: 1 °C.

be cancelled by the simultaneous outward movement of Rb and tetraethylammonium ions. To improve the signal to noise ratio eight current records were averaged with a Biomac 1000 signal analyser. The temperature was lowered to 1 °C to improve the time resolution.

Figure 4 shows averaged membrane currents from a fibre perfused with 100 mM RbF + 50 mM tetraethylammonium chloride + sucrose. The experiment was started after washing the fibre for 80 min in Na-free solution. The membrane was held at a potential of −63.5 to −64.5 mV between the clamp pulses. Record *a* was obtained by averaging the membrane currents associated with eight depolarizing pulses of 60 mV amplitude. Following the capacitative current there was a transient inward current which reached a peak value of 2.5 μA/cm² and declined to zero during the 54 ms clamp pulse. The capacitative current at the end was only partly recorded. The inward current in record *a* is partly cancelled by the simultaneous leakage outward current. To subtract the leakage outward current the membrane currents associated with eight depolarizing and eight hyperpolarizing pulses, both of exactly 60 mV height, were averaged: record *b*. In this way the symmetrical part of the leakage current and the symmetrical part of the capacitative current are automatically removed; only their asymmetrical parts remain. Record *b* reveals a very short outward current peak at the beginning and a corresponding inward current peak at the end. The inward current is somewhat larger than in *a* and its beginning is less rounded. It decays to a small sustained component of inward current which had not been visible in record *a* and lasts until the end of the 54 ms pulse; the sustained component suggests incomplete inactivation of the sodium channel, similar to that observed on NaF and CsF perfused fibres (Chandler & Meves 1970). Record *c* was taken with the same technique as record *b* (again averaging eight depolarizing and eight hyperpolarizing pulses), but 20 min after adding 2 μM tetrodotoxin to the external fluid. The inward current has completely vanished. There remained a transient outward current at the beginning of the clamp pulses, decaying to a very small sustained outward current, and a transient inward current at the end of the clamp pulses. These current transients represent the asymmetrical part of the capacitative current which has been interpreted as sodium gating current by Armstrong & Bezanilla (1973) and Keynes & Rojas (1973) (see also Meves, Shaw & Vogel 1974).

The current–voltage relation for the same fibre is shown in figure 5. The measuring points were taken from high gain oscilloscope records of individual membrane currents because averaged records for a sufficiently large number of different pulse amplitudes were not available. The meaning of the symbols is the same as in figure 3. The filled circles give the tetrodotoxin-sensitive current (= current before application of tetrodotoxin − current after application of tetrodotoxin) at 10 ms after the beginning of the clamp pulse. The time 10 ms was chosen since reliable measurements at shorter times were difficult because of the long-lasting tail of the capacitative current. The tetrodotoxin-sensitive current was inward for small depolarizations, had a maximum of 3.5 μA/cm² at about −10 mV, reversed its sign at an internal potential of +10 mV and became outward for larger depolarizations. The empty circles represent the tetrodotoxin-insensitive current which was measured 20 ms after the beginning of the clamp pulse; at this time the slow tail of the capacitative current had completely disappeared. Nearly identical values were obtained at 54 ms. The measuring points follow a straight line (slope 68.5 kΩ cm²) for internal potentials between −100 and 0 mV, but deviate clearly for positive internal potentials (leakage rectification).

The reversal potential +10 mV in the experiment of figure 5 is significantly smaller than the average value of +54.1 mV (s.e. ± 2.1 mV) found for fibres with 25 mM CsF as internal

electrolyte. It is in satisfactory agreement with the reversal potential predicted from equation (6) of Meves & Vogel (1973)

$$V_e = 54.4 \lg \frac{4P_{Ca}[Ca]_o}{P_{Rb}[Rb]_i},$$

which is here written for 1 °C and Rb as the internal cation; the contribution of tetraethylammonium ions is neglected. $[Ca]_o$ and $[Rb]_i$ are the measured activities of Ca and Rb (35.5 and 99 mmol/kg H_2O, respectively, see Methods). With $P_{Ca}/P_{Cs} = 1/0.62$ (see p. 381 and $P_{Rb}/P_{Cs} = 61/40$ (see Chandler & Meves 1965) one obtains $P_{Ca}/P_{Rb} = 1/0.95$ and $V_e = +9.8$ mV.

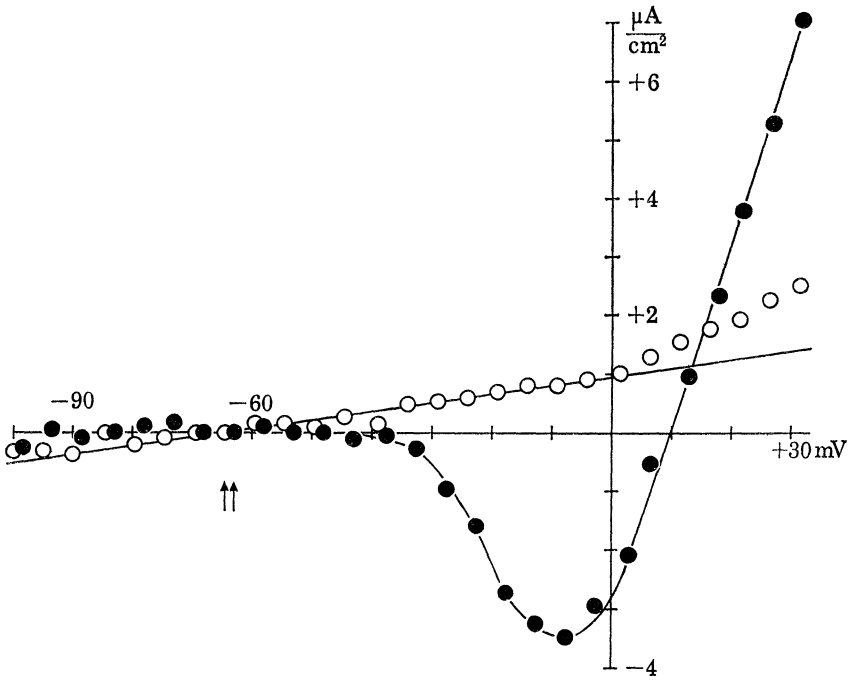

FIGURE 5. Current–voltage relation for a fibre with 100 mM $CaCl_2$ + sucrose outside and 100 mM RbF + 50 mM tetraethylammonium chloride + sucrose inside. Same experiment as in figure 4. The currents were measured from individual oscilloscope records. ●, Tetrodotoxin-sensitive current = (current before application of tetrodotoxin) − (current after application of tetrodotoxin), both measured 10 ms after beginning of clamp pulse. ○, Tetrodotoxin-insensitive current = current after application of tetrodotoxin, measured 20 ms after beginning of clamp pulse. The currents before application of tetrodotoxin were recorded with a holding potential of −63 mV (resting potential −43 mV). The currents after application of tetrodotoxin were recorded 17 min after adding tetrodotoxin (final concentration 2 μM) at a holding potential of −64.5 mV (resting potential −44.5 mV). Holding potentials indicated by arrows. Abscissa: internal potential during pulse.

The time course of the Ca inward current in figure 4 is clearly faster than the time course of the inward current in fibres perfused with 25 mM CsF + sucrose (see figures 1 and 2). At the end of the 54 ms pulse in figure 4*b* the current had decayed to a very small value whereas in figures 1 and 2 at this time an appreciable fraction of the current was still left, in spite of the 16 °C higher temperature. The perfusion with 100 mM RbF + 50 mM tetraethylammonium chloride + sucrose did not lead to the appearance of a tetrodotoxin-insensitive Ca inward current.

The experiment illustrated in figures 4 and 5 was repeated on five other fibres, also perfused with 100 mM RbF + 50 mM tetraethylammonium chloride + sucrose; the external solution

contained again 100 mM $CaCl_2$, using either sucrose or tris to maintain isotonicity. All five fibres showed tetrodotoxin-sensitive inward currents, similar in time course to those in figure 4. The inward currents were, however, smaller in size and, in most cases, did not exceed the leakage outward current; this was partly due to a lower leakage resistance which was 12.8–59.7 kΩ cm² as compared with 68.5 kΩ cm² for the fibre in figure 4. No inward currents were found with Na-free tris seawater containing 11 mM $CaCl_2$ and 55 mM $MgCl_2$.

Discussion

The main conclusion which can be drawn from the experiments is that the sodium channel of the squid axon membrane is clearly to some extent permeable to Ca ions. This is consistent with the observation that part of the Ca entry in aequorin-injected intact axons is blocked by tetrodotoxin. It seems likely that the relative Ca permeability of the sodium channel is higher under our experimental conditions than in intact axons.

An interesting question is whether other divalent cations are also able to permeate through the sodium channel. As mentioned on page 380, no inward currents were seen when the external Ca was replaced by Mg. Similarly, Tasaki *et al.* (1966, 1967) obtained no action potentials with Mg instead of Ca in the external solution. They were, however, able to record tetrodotoxin-sensitive action potentials with $SrCl_2$ or $BaCl_2$ as the external electrolyte (see also Watanabe, Tasaki, Singer & Lerman 1967). Quite interesting is the recent observation of Mn action potentials which were obtained with 20–50 mM $MnCl_2$ outside and 25 mM CsF inside (Yamagishi 1973). In conclusion, it seems that the sodium channel, at least under certain experimental conditions, is to some extent permeable for Sr, Ba, Mn, but not for Mg.

Finally, the question arises whether the Ca ions that enter the nerve fibre through the sodium channel (and through the tetrodotoxin-insensitive late Ca channel) have any noticeable effect on the membrane itself. The amount of Ca entry during a single action potential is small, but maintained repetitive activity may cause a significant increase of the calcium concentration at the inner side of the membrane. In molluscan neurons Meech (1972) found that small amounts of calcium salts injected into the nerve cell body increase the potassium permeability. Dr R. W. Meech and I are presently studying the effect of small intracellular calcium concentrations on the sodium and potassium permeability of squid giant axons.

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