Membrane Currents Carried by Ca, Sr, and Ba in Barnacle Muscle Fiber During Voltage Clamp

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ABSTRACT Membrane currents associated with voltage clamp of the giant muscle fibers of a barnacle, *Balanus nubilus*, were analyzed in terms of currents of the Ca and K channels. Although the activation of the K channel occurs more slowly than that of the Ca channel, both currents show a significant temporal overlap. The currents carried by Ca^{++}, Sr^{++}, or Ba^{++} through the Ca channel were compared under the conditions at which this overlap was the least. When only one divalent cation is present in the solution, Ba^{++} carries more current than Ca^{++} or Sr^{++} and the sequence of the current is Ba > Sr $\approx$ Ca. When the external solution contains a relatively high concentration of Co^{++}, which is a blocking agent for the Ca channel, inversion of the sequence occurs, to Ca > Sr > Ba. This is due to the fact that the blocking effect differs depending on which ion carries current through the Ca channel. The Ba current is most sensitive and the Ca current is least affected. Ba suppresses the current of the K channel, independently of its current-carrying function through the Ca channel.

INTRODUCTION

The action potential of various excitable tissues such as the squid giant axon can be described by two parallel conductance "channels" through the membrane, one which is primarily permeable to the Na ion and the other to the K ion (Hodgkin and Huxley, 1952 *b*). In certain other tissues the action potential is produced by an increase of the membrane permeability to Ca ions instead of Na ions (Fatt and Katz, 1953; Fatt and Ginsborg, 1958; Hagiwara and Naka, 1964; Hagiwara, 1973; Reuter, 1973). In a typical example of one of these systems, the barnacle muscle fiber, voltage clamp analysis of the membrane current (Hagiwara et al., 1969; Keynes et al., 1973) indicates that the Ca-dependent action potential can also be described in terms of two membrane conductance channels, i.e., the Ca and K channels. Besides their differential selectivities to Ca^{++} and K^{+}, these channels can be distinguished by their different responses to various blocking agents

and by different kinetics in response to changes in membrane potential. Various polyvalent cations, La^{+++}, Co^{++}, Mn^{++}, Ni^{++}, and Mg^{++}, block the Ca channel at concentrations at which no significant effect is seen on the current of the K channel (Hagiwara and Nakajima, 1966; Hagiwara and Takahashi, 1967; Hagiwara et al., 1969). Procaine and tetraethylammonium reduce the current of the K channel without affecting the current of the Ca channel (Hagiwara et al., 1964; Hagiwara et al., 1969; Keynes et al., 1973). With changes in membrane potential the activation of the Ca channel occurs earlier than that of the K channel. A similar difference is found between the Na and K channels of the Na-K system. In the previous work (Hagiwara et al., 1969, Fig. 3), it was suggested that the inactivation of the Ca current was relatively fast. Keynes et al. (1973) demonstrated that the apparent fast inactivation of the Ca current is due to the spatial inhomogeneity of the barnacle muscle fiber. A number of invaginations occur in the surface membrane and numerous transverse tubules originate from the invaginations as well as from the surface membrane. The invaginations and tubular lumens result in a distributed series resistance which leads to a spatial potential gradient and consequent current loops. Under such a condition, regenerative potential changes may occur in a membrane with a series resistance even when the overall membrane potential is maintained. Thus, the current of the Ca channel may decline not only because of the inactivation but also due to the change in the membrane potential in poorly controlled membrane areas. For the same reasons, the spatial inhomogeneity results in an oscillatory membrane current when both the inward and outward current mechanisms are active. Keynes et al. (1973) showed that the oscillation disappeared when the current of either the Ca or K channel was substantially reduced. When the K current is almost eliminated the Ca current shows little sign of fast inactivation. Therefore, fast inactivation cannot be considered an important factor in distinguishing the Ca channel from the K channel.

In Na-K systems, other ions besides Na may pass through the Na channels and are consequently capable of supporting action potentials (Hille, 1971, 1972). Similarly, in Ca systems it has been shown that when Ba^{++} and Sr^{++} replace Ca^{++} in the external bathing solution, action potentials are still present. This suggests that Ba or Sr ions can also carry current in the Ca channel. However, the selectivity of Ca^{++}, Sr^{++}, and Ba^{++} in the Ca channel of the membrane has not been studied. To answer these questions, the membrane current of the barnacle muscle fiber during voltage clamp was analyzed in Ca, Sr, and Ba media.

MATERIALS AND METHODS

Giant muscle fibers of a barnacle, *Balanus nubilus* Darwin, were used. Fibers were about 1–2 mm in diameter and about 4–5 cm in length. The preparation and the

general experimental arrangement were similar to those described elsewhere (Hagiwara et al., 1969). An isolated single muscle fiber was placed in a saline-filled lucite chamber which was divided into three compartments by partitions (see Fig. 1). After internal injection of the entire length of the fiber a pair of wire electrodes were introduced inside the fiber (see Fig. 1), one for passing currents and the other for recording potential changes. About 2 cm of the fiber from the tendon was in the saline chamber. The rest of the fiber (2–3 cm) was separated from the saline by a Vaseline (Cheseborough-Ponds, Inc., New York, N. Y.) block and was exposed to the air. The current electrode (E_1 in Fig. 1) was a platinum wire of 200-μm diameter. The wire was uninsulated for 2 cm from the tip and this portion was platinized. The uninsulated portion covered the entire length of the fiber in the saline chamber. The potential electrode (E_2 in Fig. 1) was a silver wire of 50-μm diameter with a portion of about 0.5 cm that was uninsulated and chlorided. This portion was situated at the center of the fiber in the middle compartment of the saline-filled chamber. Membrane potential changes were recorded as a potential difference between the internal wire electrode and the tip of a 3 M KCl-filled glass micropipette (E_3 in Fig. 1) in the external saline. The tip of the pipette was placed just outside the fiber membrane opposite to the exposed portion of the internal potential electrode. The absolute value of the resting potential was monitored by introducing a second 3 M KCl-filled glass micropipette (not shown in Fig. 1) into the fiber. Bundles of many fine chlorided silver wires (E_4, E_5, and E_6 in Fig. 1) were placed in each of three compartments. Those in the middle compartment were connected to a current-voltage converter to observe the membrane current of the fiber in the middle compartment and those in the other two compartments were connected to ground. The length of the middle compartment was 1 cm. The fiber in the chamber was superfused continuously with cooled saline solution (3–7°C). The general arrangement of the voltage clamp is shown in Fig. 1. The rise time constant for the voltage step was about 1 ms. The relatively large time constant was probably due to the large apparent membrane capac-

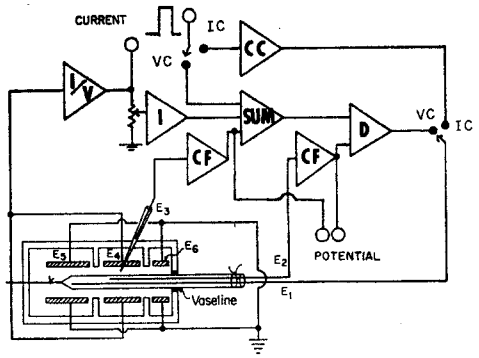


FIGURE 1. General experimental arrangement. CF, cathode follower. I, inverter for series resistance compensation I/V, current-voltage converter for current recording. SUM, summing circuit. CC, constant current generator. D differential amplifier for voltage clamp. VC, voltage clamp, and IC, current clamp. See text.

ity of the muscle fiber. This was not a major problem since the initial change of the membrane current was much slower than the time constant of the voltage step.

The compositions of major external solutions used are shown in Table I. A solution with the desired concentrations of Ca and Mg was made by mixing the Ca saline, Mg saline, and Ca-Mg-free saline in appropriate proportions. Ba or Sr solutions were made in a similar manner after replacing Ca in Ca saline with Ba or Sr. Tetraethylammonium chloride (TEA-Cl) solutions were made by replacing NaCl in the solution with an equimolar TEA-Cl. For the experiments employing Co^{++}, an appropriate amount of $CoCl_2$ was added to the solution. The hypertonicity of the solution due to this procedure did not seem to be significant since the concentration of $CoCl_2$ never exceeded 20 mM. The pH of the solution was buffered at 7.7 with 10 mM of Tris-HCl buffer. For experiments with $CoCl_2$, 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid)-NaOH buffer at pH 7.3 was used and no essential differences were found in the results obtained with the two buffer systems. The composition of the internal solution was KOH, 400 mM; EGTA, 100 mM;

TABLE I
COMPOSITIONS OF SOLUTIONS

	NaCl	KCl	$CaCl_2$	$MgCl_2$	Buffer
			mM		
Normal saline	461	8	20	12	10
Ca saline	59	8	300	—	10
Mg saline	9	8	—	333	10
Ca, Mg-free saline	509	8	—	—	10

Tris maleate, 20 mM; sucrose, 340 mM; methanesulfonic acid, 184 mM, and the pH was adjusted to 7.0 by adding methane-sulfonic acid.

The resting potentials of the fibers treated with the internal solution ranged from −55 to −65 mV in normal barnacle saline and were not much different from this value in test solutions examined in the present experiment. The voltage clamp was usually performed at a holding membrane potential between −62 and −65 mV. The conductance of the resting fiber membrane was determined with a small inward-going voltage pulse. The leakage currents were then calculated and subtracted from the recorded membrane currents. The average value of the specific membrane conductance in normal saline was 0.7 ± 0.3 mmho/cm². For this calculation invaginations of the surface membrane and the membrane of the transverse tubular system were ignored. According to Selverston (1967) this procedure underestimates the sarcolemal surface area at least by a factor of 10. The resting potential and the resting membrane conductance of intact muscle fibers measured with micropipettes in normal barnacle saline is −80 to −70 mV and 0.5 to 0.3 mmho/cm², respectively (Hagiwara and Naka, 1964). The smaller resting potential amplitude and membrane conductance of internally treated fibers is probably due to a shunt produced by the insertion of longitudinal electrodes.

RESULTS

Peak Amplitude of the Early Inward Current

The major aim of the present work is to compare the current of the Ca channel in Ca, Sr, and Ba solutions. As mentioned already the spatial inhomogeneity of the barnacle muscle fiber membrane makes it difficult to apply the quantitative current separation procedure originally used for the squid axon (Hodgkin and Huxley, 1952 *a*). Therefore, the peak amplitude of the early inward current during voltage clamp was used for the measure of the current of the Ca channel. The reliability of this procedure was examined in Ca media.

Fig. 2 A shows membrane currents associated with voltage clamp of a barnacle muscle fiber in a solution containing 40 mM Ca and 100 mM Mg. Two traces in each pair were obtained at the same membrane potential before and after the application of 20 mM Co^{++} which reduced the Ca current. The trace with a smaller inward current represents the current obtained with Co^{++}. The early inward current is followed by the late outward current. After reaching maximum amplitude the outward current declines during the maintained membrane depolarization (see traces listed 25 in Fig. 2 A). Like Keynes et al. (1973) we found that the membrane conductance is constant during this decline so the change seems to be a decrease of emf in the K channel as if K ions accumulate in the vicinity of the membrane. Records obtained without Co^{++} show oscillations. This is due to regenerative potential changes at a poorly controlled area found when the inward and outward current mechanisms are both active. Like Keynes et al. (1973) we found the current density along the length of the fiber to be uniform so the uncontrolled region is probably in internal clefts and tubules. The oscillation disappeared when the current of the Ca channel was reduced by Co^{++}. The peak amplitude of the early inward current and the maximum amplitude of the outward current obtained with and without Co^{++} are plotted against the membrane potential in Fig. 2 B. The peak inward current was reduced substantially by Co^{++}. In contrast, 20 mM Co^{++} did not alter the maximum outward current. This indicates that the relation between the maximum outward current and the membrane potential represents the current-voltage relation of the K channel alone. The K current first appears at membrane potentials substantially more positive than those giving appreciable transient inward current. The inward current increases rapidly with increase in membrane potential and reaches its maximum amplitude at -20 to -10 mV. At this membrane potential the amplitude of the K current is not yet significant. This suggests that the peak inward current may represent the maximum amplitude of the Ca current up to the membrane potential at

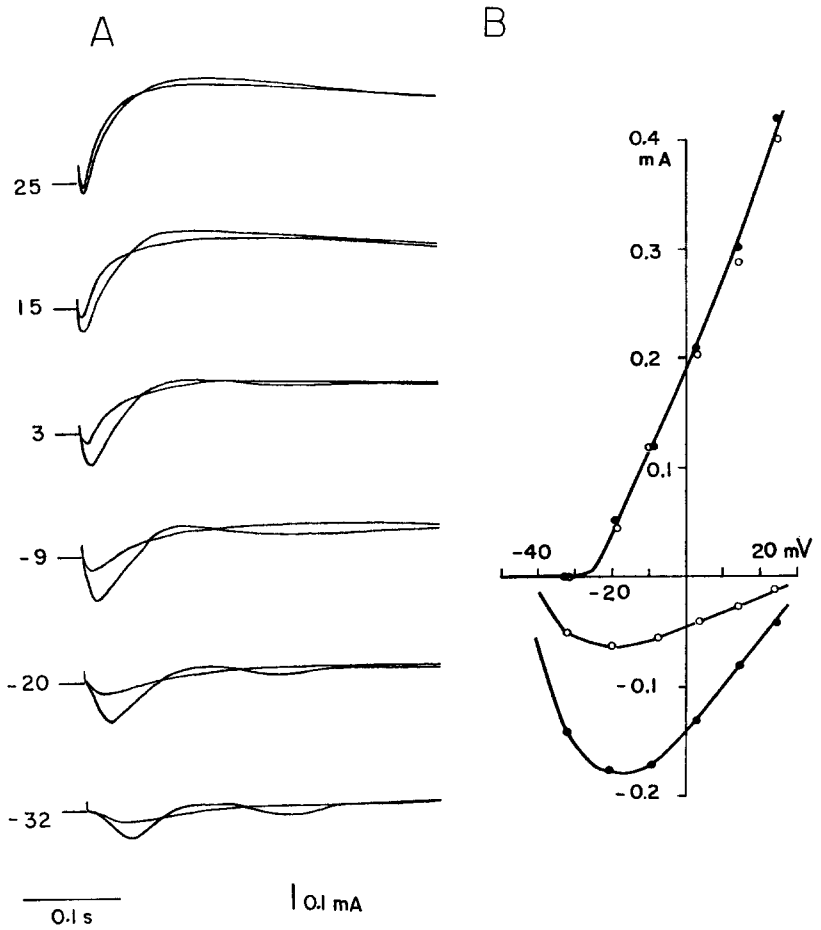


FIGURE 2. Membrane currents associated with voltage clamp of a barnacle muscle fiber. (A) The fiber was first in the solution containing 40 mM Ca and no Mg and then 20 mM $CoCl_2$ was added to the solution. The trace with a smaller peak inward current in each pair was obtained with Co^{++}. The number listed for each current record is the membrane potential during the voltage pulse. The holding membrane potential was −62 mV. Approximate surface area of the fiber was 0.9 cm^2. Leakage currents have been subtracted. (B) The peak amplitude of the inward current and the maximum amplitude of the outward current without (filled circles) and with (open circles) 20 mM Co^{++} are plotted against the membrane potential.

which the peak inward current becomes maximal. This identification can be further tested by using TEA to reduce K currents.

In the experiment shown by Fig. 3 A the fiber was first clamped in a solution containing 40 mM Ca, 100 mM Mg, and 50 mM TEA. The result is represented by the bottom trace of each pair. Membrane currents were predominantly inward since TEA reduced outward K current. The upper

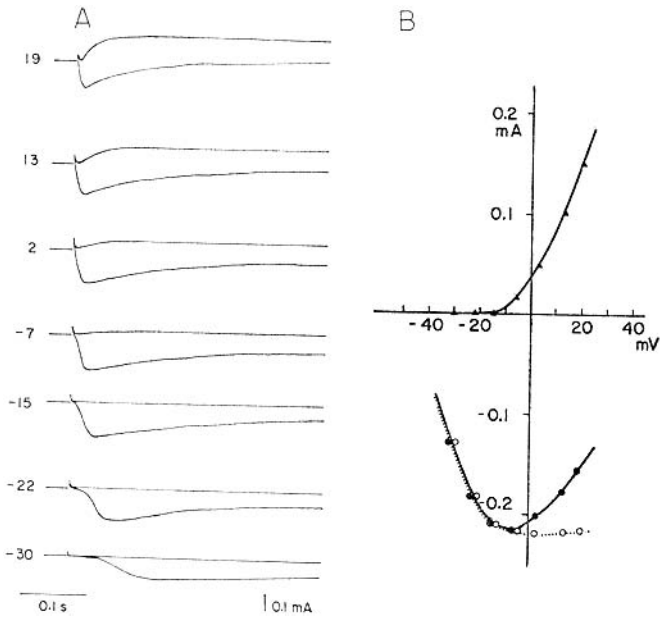


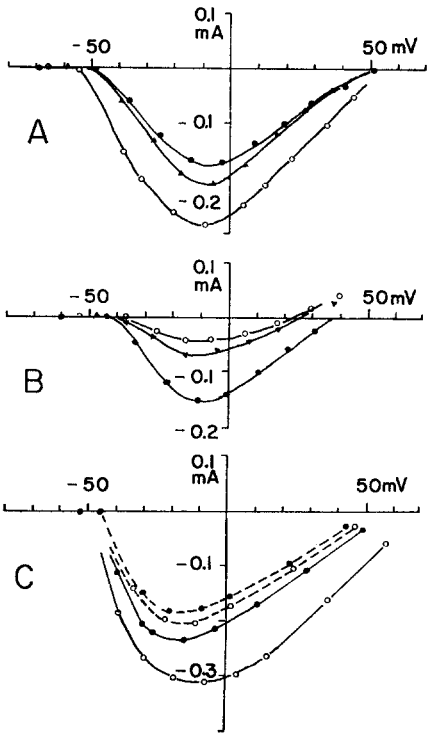
FIGURE 3. Membrane currents associated with voltage clamp of a barnacle muscle fiber. (A) The fiber was first in the solution containing 40 mM Ca, 100 mM Mg, and 50 mM TEA and then all of the $CaCl_2$ was removed from the solution by replacement with an isosmolar amount of NaCl. Each record consists of two traces obtained with (bottom trace) and without Ca^{++} (top trace). The number listed for each current record is the membrane potential during the voltage pulse. The holding membrane potential was −62 mV. Approximate surface area of fiber was 0.7 cm^2. Leakage currents have been subtracted. (B) The peak amplitude of the inward current in the bottom trace (filled circles), the maximum amplitude of the outward current in the top trace (filled triangles), and the maximum difference between currents of the paired traces (open circles) are plotted against the membrane potential.

trace of each pair was obtained with the same muscle fiber after the $CaCl_2$ had been replaced with isosmolar NaCl. The membrane current, after the removal of the external Ca^{++}, represents the residual K current in 50 mM TEA. The peak amplitude of the inward current obtained before the Ca removal and the maximum outward current obtained after Ca removal are plotted at different membrane potentials in Fig. 3 B. The maximum amplitude of the K current is negligible up to the membrane potential at which the peak inward current reaches its maximum amplitude. Furthermore, comparison of two traces in each pair in Fig. 3 A indicates that the K current develops with a time-course substantially slower than that of the Ca current. The membrane current obtained after Ca removal was subtracted from the current obtained at the same membrane potential before the removal and the maximum amplitude of the resulting current was plotted against the

membrane potential in Fig. 3 B. This coincided with the peak inward current up to the membrane potential at which the peak inward current reaches its maximum amplitude. For more positive membrane potentials they show a deviation, suggesting that there is a significant overlap between the outward K current and the inward Ca current even at the time when the Ca current reaches its peak amplitude. When the effect of TEA was examined in the Ca-free media, 50 mM TEA reduced the amplitude of the maximum outward current by a factor of 2–3. A TEA concentration of 150 mM reduced the outward current by 3–5 times. Thus, for the purpose of comparing the Ca^{++}, Sr^{++}, and Ba^{++} currents, the maximum amplitude of the peak inward current obtained in the presence of 150 mM TEA was used as a reliable measure of the inward current through the Ca channel.

Ca^{++}, Sr^{++}, and Ba^{++} Currents through the Ca Channel

In order to compare the currents carried by Ca^{++}, Sr^{++}, and Ba^{++} through the Ca channel, single muscle fibers were clamped in three solutions, each of which contained an equimolar quantity of Ca, Sr, or Ba in addition to 150 mM TEA. In the first set of experiments the solution contained no Mg^{++}, and Ca^{++}, Sr^{++}, or Ba^{++} was the only divalent cation in each solution. Fig. 4 A was obtained from such an experiment in which 20 mM Ca was first replaced with Sr and then with Ba. The amplitude of the peak inward current was measured for each voltage step and plotted against the membrane potential. Peak inward currents for Ca^{++}, Sr^{++}, and Ba^{+} became maximum at approximately the same membrane potential. Fig. 4 A shows that the maximum amplitude of the peak inward current has a sequence Ba > Sr > Ca. Similar experiments were performed with several other fibers and the results are summarized in Table II. The maximum amplitude of the peak inward current was normalized by taking the amplitude in Ca solution as unity. The maximum amplitude at 40 mM Ca corresponded to a current density of -0.3 ± -0.06 mA ($n = 10$) per cm^2 of the fiber surface. If the invagination of the surface membrane and the membrane of the transverse tubular system is taken into consideration the average current density may become more than 10 times smaller than this value. The concentration of Ca, Sr, or Ba was either 20 or 40 mM. No obvious difference of the ratios was detected at the two different concentrations. Therefore, average values were obtained with all fibers and they were Ca, 1.0, Sr, 1.05 ± 0.12 (SD), and Ba, 1.30 ± 0.16 (SD). Although the Sr current was slightly greater than the Ca current in the case shown in Fig. 4 A, the difference is not statistically significant. In contrast, the Ba current was invariably greater than the Ca or Sr current and the difference is significant. The sequence of the maximum inward current is, therefore, Ba > Sr $\approx$ Ca. As will be described in detail below, Ba^{++} suppresses the current of the K channel. Therefore, the greater inward



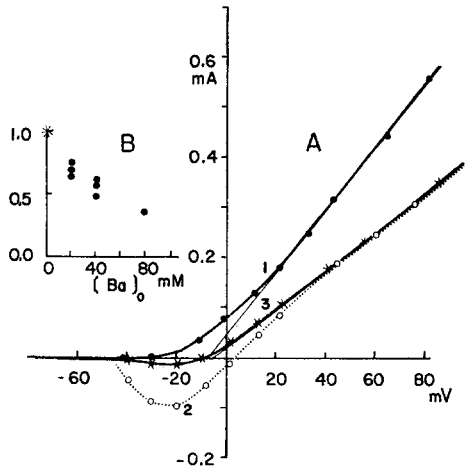


FIGURE 4

FIGURE 5

FIGURE 4. Relations between the amplitude of the peak inward current and the membrane potential. 150 mM TEA in the external solution. (A) Three curves obtained when the solution contained either 20 mM Ca (filled circles), Sr (filled triangles), or Ba (open circles) but no other divalent cations. The holding membrane potential, −65 mV. Temperature 4°C. Approximate area of fiber surface, 0.62 cm^2. (B) Three curves obtained when the solution contained 20 mM $CoCl_2$ in addition to 20 mM Ca, Sr, or Ba. The holding membrane potential, −65 mV. Temperature 4°C. Approximate fiber surface area, 0.55 cm^2. (C) Four curves obtained in 20 mM Ca (filled circles) or Ba (open circles) with (broken lines) or without 3 mM $CoCl_2$ (continuous lines). The solutions contained no Mg. The holding membrane potential, −62 mV. Temperature, 4°C. Approximate fiber surface area 0.75 cm^2. The leakage currents have been subtracted in all three cases.

FIGURE 5. (A) Relations between the amplitude of the maximum outward current and the membrane potential obtained during voltage clamp of a muscle fiber with a 0.4-s voltage pulse. Curve 1, in 40 mM Ca and no Mg; curve 2 in 40 mM Ba and no Mg; and curve 3 in 40 mM Ba and 200 mM Mg. For curves 2 and 3 the outward current did not reach a maximum within 0.4 s at membrane potentials smaller than +30 mV and, therefore, the amplitude of the current just before the end of the voltage pulse was plotted. The holding membrane potential, −65 mV. Temperature, 3°C. The leakage currents were subtracted. (B) The effect of Ba concentration upon the relative slope conductance. The slope conductance was calculated from the portion of the current-voltage relation for membrane potentials more positive than +30 mV and normalized by the slope conductance obtained with the same muscle fiber in the absence of Ba (in 20 or 40 mM Ca solution). For further explanation, see text.

TABLE II

RATIOS AMONG THE MAXIMUM VALUE OF PEAK INWARD CURRENT IN CA, SR, AND BA

	C	$[Co^{++}]_o$	Ca	Sr	Ba
A	20 mM	0	1.0	1.1	1.4
			1.0	1.2	1.6
			1.0	—	1.2
B	40 mM	0	1.0	1.1	1.1
			1.0	0.9	1.2
			1.0	0.9	1.4
			1.0	1.1	1.2
		Average	1.0	1.05	1.30
		SD		±0.12	±0.16
C	20	20	1.0	0.5	0.3
D	20	3	1.0	0.9	1.1
E	40	20	1.0	0.6	0.4
F	40	5	1.0	0.8	1.0

In each experiment $[Ca^{++}]_o = [Sr^{++}]_o = [Ba^{++}]_o = C$. 150 mM TEA in all solutions. The Mg concentration was 100 mM for E and F, and 0 for all other cases.

current in Ba solutions could be due partly to the reduction of the outward K current. The amplitude of the peak Ba current is greater than that of the Ca or Sr currents even in the range of membrane potential at which the K current is negligible. Therefore, in this experiment, Ba^{++} carries more current through the Ca channel, independent of any effect on the K^+ channel.

Co^{++} blocks the Ca channel (Hagiwara and Takahashi, 1967). A second set of experiments was performed in the presence of Co^{++}. The results shown in Fig. 4 B were obtained when the external saline contained 20 mM $CoCl_2$ and 150 mM TEA, with 20 mM Ca, Sr, or Ba. The sequence of the maximum amplitude of the peak inward currents was reversed and their ratios were Ca (1.0) > Sr (0.5) > Ba (0.3) (C in Table II). A similar inversion was also seen when the concentration of Ca, Sr, or Ba was 40 mM (E in Table II). When the concentration of Co^{++} was low the inversion of Ba > Ca to Ca > Ba did not occur but the ratio between the maximum Ba and Ca currents tended to approach unity (D and F in Table II). The external solutions for E and F of Table II contained 100 mM Mg^{++} in addition to Co^{++}. The blocking effect of Mg^{++} is small compared with that of Co^{++}. Their effect upon the maximum rate of rise shows that 1 mM of Co^{++} and 46 mM of Mg^{++} have almost the same blocking effect (Hagiwara and Takahashi, 1967).

The inversion of the sequence between the Ba and Ca currents is not due to the effect of Co^{++} upon the K current. Co^{++} neither alters the K current nor interferes with the suppressing effect of Ba upon the K current. The inversion is simply due to the differential effects of Co^{++} upon the Ca and Ba currents. In the experiment shown in Fig. 4 C a fiber was first clamped in

20 mM Ca or Ba in the absence of Co^{++}. The amplitude of the Ba current was significantly greater than that of the Ca current. Introduction of 3 mM Co^{++} into the solution reduced the maximum Ba current by a factor of 1.52 whereas the maximum Ca current was reduced by a factor of only 1.25. In other words, the blocking effect of Co^{++} differs, depending upon the ion species which carries the current through the Ca channel. Co^{++} of the same concentration reduces the Ba current significantly more than it does the Ca current. Thus, the amplitudes of the Ba and Ca currents in Fig. 4 C became almost equal after the application of 3 mM Co^{++}. If the Co^{++} concentration is increased the Ba current becomes smaller than the Ca current and consequently an inversion of the sequence occurs.

Effect of Ba upon the K Current

Curve 1 of Fig. 5 A shows the relation between the amplitude of the maximum outward current and the membrane potential when the fiber was clamped with a 0.4-s voltage pulse in a solution containing 40 mM Ca and no Mg. Neither the change in the Ca concentration to 20 or 80 mM nor the application of 20 mM Co^{++} or 200 mM Mg^{++} altered this relation significantly (see Fig. 2 B). Curve 2 was obtained after 40 mM Ca had been replaced with 40 mM Ba. For membrane potentials more positive than +30 mV the outward current reached its maximum amplitude before the end of a 0.4-s voltage pulse. For more negative membrane potentials, however, the current did not reach a final amplitude during the pulse and, therefore, the amplitude of the current just before the termination of the pulse was used to plot curve 2. For certain membrane potentials the currents were still inward.

The smaller amplitude of the outward current in Ba could be due to a long-lasting inward Ba current which counteracts the outward K current. If this is the case the amplitude of the outward current should be increased when the inward Ba current is reduced by blocking cations. Curve 3 shows the relation obtained after 200 mM $MgCl_2$ was introduced into the Ba solution by replacing with isosmolar NaCl. The result shows that the membrane current did shift in a more outward direction for membrane potentials smaller than +30 mV. However, the relation for membrane potentials greater than +30 mV remained unaltered, indicating that this portion of the current-voltage relation represents contribution of the K channel in the Ba solution. In other words, Ba does suppress the current of the K channel. The blocking cations do not interfere with the suppressing action of Ba. This shows that the suppressing effect of Ba upon the current of the K channel is independent of the current-carrying function of Ba in the Ca channel. The current-voltage relation for membrane potentials greater than +30 mV can be approximated by a straight line for curve 3 as well as for curve 1. The two straight lines intercept the membrane potential axis at approximately

the same membrane potential. Therefore, the change in the slope conductance of the linear portion of the current-voltage relation can be used to describe the reduction of the outward current in the Ba solution. In the case of Fig. 5 A, 40 mM Ba reduced the slope conductance from 5.8 mmho to 3.5 mmho. The ratio between the conductances with and without 40 mM Ba was, therefore, 0.6. Similar experiments were performed at different Ba concentrations, and the resulting conductance ratios are summarized in Fig. 5 B. The suppressing effect of Ba upon the K current increases with increasing Ba concentrations. The present results were obtained 20–30 min after application of Ba. A similar suppressing effect of Ba upon the K conductance has been found in vertebrate (Sperelakis et al., 1967) as well as other invertebrate muscle fibers (Werman et al., 1961; Werman and Grundfest, 1961).

DISCUSSION

By analyzing the maximum rate of rise of the Ca-dependent action potential in a barnacle muscle fiber, Hagiwara and Takahashi (1967) obtained evidence that the binding of Ca ion to a membrane site is an important step in the permeation of Ca ions through the membrane. The Ca channel has a site X and the site is either in free state X or the bound state XCa when Ca ions enter the channel. Since the internal Ca ion concentration is extremely small in EGTA injected fibers, the efflux of Ca ions can be neglected. Therefore, the steps involved with inward flow of Ca ions may be roughly represented by:

$$X + \mathrm{Ca}_o \rightleftarrows X\mathrm{Ca} \rightarrow X + \mathrm{Ca}_i.$$

The inward Ca current at a given membrane potential $I_{Ca}(V)$ is then given as a function of $[Ca^{++}]_o$ by

$$I_{Ca}(V) = \frac{I_{Ca\ max}(V)}{1 + \dfrac{K_{Ca}(V)}{[Ca^{++}]_o}}. \qquad (1)$$

$I_{Ca\ max}$ is I_{Ca} when all the sites are occupied by Ca and K_{Ca} is a dissociation constant of the site interacting with the external Ca ions. $I_{Ca\ max}$ and K_{Ca} are both membrane voltage dependent (see Hille, 1974). Hagiwara and Takahashi (1967) showed that Eq. 1 can describe satisfactorily the relation between $[Ca^{++}]_o$ and the maximum rate of rise of the Ca-dependent action potential.

Hagiwara and Nakajima (1966) and Hagiwara and Takahashi (1967) showed that various polyvalent cations block the Ca channel. The blocking cation M^{++} is not permeant through the Ca channel but may bind to the site X competitively, thereby blocking the Ca channel. In the presence of M^{++} the Ca current, $I_{Ca}(V)$ becomes,

$$I_{Ca}(V) = \frac{I_{Ca\ max}(V)}{1 + \frac{K_{Ca}(V)}{[Ca^{++}]_o} \cdot \left(1 + \frac{[M^{++}]_o}{K_M(V)}\right)}, \quad (2)$$

$K_M(V)$ being the dissociation constant of the site to the external M^{++}. Effects of La^{+++}, Co^{++}, Mn^{++}, Ni^{++}, and Mg^{++} upon the maximum rate of rise were examined and the experimental results were satisfactorily described by Eq. 2.

In the present experiment currents carried by Ca^{++}, Sr^{++}, or Ba^{++} were compared by observing the maximum amplitude of the peak inward current in equimolar solutions. Under these conditions the maximum peak inward current for Ca, Sr, or Ba occurs at the same membrane potential. Therefore, the ratios of the maximum Ca and Ba currents can be given by:

$$\frac{I_{Ba}(V)}{I_{Ca}(V)} = \frac{I_{Ba\ max}(V)}{I_{Ca\ max}(V)} \cdot \frac{C + a \cdot K_{Ca}(V)}{C + a \cdot K_{Ba}(V)}, \quad (3)$$

where

$$C = [Ca^{++}]_o = [Ba^{++}]_o \quad \text{and} \quad a = 1 + [Co^{++}]/K_{Co}(V).$$

The inversion of the Ca-Ba sequence by Co^{++} can be explained by referring to Eq. 3 if it is assumed that the ion species having a higher affinity for the membrane site has a lower mobility through the Ca channel, i.e., $I_{Ba\ max} > I_{Ca\ max}$ and $K_{Ba} > K_{Ca}$. The first and second factors of the product in the right-hand side of Eq. 3 can be called the mobility and affinity factors, respectively. The former is greater than unity and the latter is smaller than unity. If K_{Ca} and K_{Ba} are not significantly greater than C (20 and 40 mM), the ratio I_{Ba}/I_{Ca} in the absence of Co^{++} tends to be determined by the mobility factor and this results in the sequence: $I_{Ba} > I_{Ca}$. When a large amount of Co^{++} is added a becomes much greater than unity. Under this condition the affinity term becomes important in determining the sequence, and the sequence becomes $I_{Ca} > I_{Ba}$. Hence, the mechanism represented by Eq. 3 above explains the differential blocking effects of Co^{++} upon the Ca channel in Ca, Sr, and Ba solutions. This result indicates that the affinity sequence for the site X is Ca > Sr > Ba. The ratios of currents obtained at 20 mM Co^{++} suggest that K_{Ba}/K_{Ca} and K_{Sr}/K_{Ca} are at least 2–3 and 1.5–2.0, respectively. The foregoing results suggest that the Ca channel is a system capable of "saturation."

The overshoot of the action potential of a barnacle muscle fiber increases with increasing external Ca concentration with a maximum slope of 29 mV for a 10-fold change in the concentration. The slope for the Ba action potential is much greater and often reaches 60–85 mV/decade concentration change (Hagiwara and Naka, 1964). This behavior of the Ba action potential can be

explained by the result of the present work. A significant outward K current is likely to overlap the inward current of the Ca channel even at the time when the latter reaches its peak amplitude. Therefore, the overshoot of the action potential increases with an increasing inward Ca current as well as with decreasing K current. Ba ions suppress the K current and the degree of suppression increases with increasing Ba concentration. Therefore, the overshoot of the Ba action potential is increased with increasing Ba concentration not only because of an increase in the inward Ba current but also because of a decrease in the outward K current, whereas the overshoot of the Ca action potential increases only because of an increase in the inward Ca current.

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