

# Physiological Role and Selectivity of the In Situ Potassium Channel of the Sarcoplasmic Reticulum in Skinned Frog Skeletal Muscle Fibers

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**ABSTRACT** The role of  $K^+$  as a counterion during  $Ca^{2+}$  release from the sarcoplasmic reticulum (SR) has been investigated. An optical technique using the  $Ca^{2+}$ -sensitive dye antipyrylazo III monitored  $Ca^{2+}$  release from skinned (sarcolemma removed) muscle fibers of the frog. Skinned fibers were used since the removal of the sarcolemma allows direct access to the SR membrane. Releases were stimulated by caffeine, which activates  $Ca^{2+}$  release directly by binding to a receptor on the SR. Two different methods were used to decrease the SR  $K^+$  conductance so that its effect on  $Ca^{2+}$  release could be assessed: (a) the SR  $K^+$  channel blocker, 1,10-bis-quanidino-*n*-decane (bisG10) was used to eliminate current pathways and (b) substitution of the impermeant ion choline for  $K^+$  was used to decrease charge carriers. Both bisG10 and choline substitution caused a concentration-dependent decrease in the  $Ca^{2+}$  release rate. Therefore we conclude that  $K^+$  is an important counterion for  $Ca^{2+}$  during its release from the SR. The selectivity of the in situ SR  $K^+$  channel to several monovalent cations was determined by substituting them for  $K^+$  and comparing their effect on  $Ca^{2+}$  release. The substituted ions were expected to affect  $Ca^{2+}$  release in proportion to their ability to support a counterion flux, which is, in turn, a function of their relative conductance through the SR  $K^+$  channel. The selectivity sequence determined by these experiments was  $K^+ = Rb^+ = Na^+ > Cs^+ > Li^+ > choline$ .

## INTRODUCTION

Skeletal muscle activation is triggered by the release of  $Ca^{2+}$  from the sarcoplasmic reticulum (SR), an intracellular organelle, into the myoplasm. The permeability of the SR membrane to biological ions has been well studied and is reviewed by Meissner (1983). Information has been obtained from isolated SR membrane vesicles that exhibit a high permeability to small monovalent cations and anions. Using various experimental techniques, three separate pathways have been identified; a  $K^+$ ,  $Na^+$  channel, an anion channel, and a  $H^+$  channel (Meissner and McKinley,

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1976, 1982; Kometani and Kasai, 1978; McKinley and Meissner, 1978; Miller, 1978; Kasai et al., 1979; Meissner and Young, 1980). The K<sup>+</sup>-selective channel was found in ~65% of the SR vesicle population and demonstrated a K<sup>+</sup> efflux too rapid to resolve (McKinley and Meissner, 1978). To obtain more information on its electrical behavior Miller and co-workers have inserted the SR K<sup>+</sup> channel into lipid bilayers (reviewed by Miller et al., 1984). The channel has two states, open and closed, with the open state favored as the voltage outside the SR is made increasingly positive (Miller, 1978; Labarca et al., 1980). The conductive properties of this channel have been studied with specific blockers and ion-binding techniques and is characterized by a single-channel K<sup>+</sup> conductance of 214 pS, which is a saturable function of ion activity (Coronado et al., 1980). The resting K<sup>+</sup> permeability of in situ SR membrane has been determined with an isotopic tracer flux technique (Best and Abramcheck, 1985a). The resultant value of  $1.7 \times 10^{-7}$  cm/s while less than values determined in isolated membrane preparations, clearly demonstrates that significant K<sup>+</sup> movement is possible.

It is known that except for Ca<sup>2+</sup> the distribution of intracellular ions between the myoplasm and the SR is homogenous (Somlyo et al., 1977; Caputo, 1984) and therefore a steady state SR membrane potential different from zero is unlikely. However, rapid efflux of Ca<sup>2+</sup> during activation might polarize the SR membrane causing a transient change in voltage. The extent of the electrical potential change that actually occurs is unknown but has been a topic of debate since the control of Ca<sup>2+</sup> release is important in regulating contraction (Nakajima and Endo, 1973; Detliker, 1982; Meissner, 1983; Baylor et al., 1984; Miller et al., 1984). If Ca<sup>2+</sup> were the only ion to cross the membrane during release then the SR membrane potential would rapidly approach the Ca<sup>2+</sup> equilibrium potential ( $E_{Ca}$ ), reducing the driving force on the ion to zero and, therefore, limiting the extent of Ca<sup>2+</sup> release. Movement of other ions during Ca<sup>2+</sup> release would contribute, either positively or negatively, to the change in voltage and would also have a significant effect on the Ca<sup>2+</sup> release. Thus, counterion movement is important physiologically since it should help determine the magnitude of the Ca<sup>2+</sup> efflux from the SR. As the K<sup>+</sup> ion is present in the myoplasm at a high concentration and since a highly conductive K<sup>+</sup>-selective channel exists in the SR membrane, K<sup>+</sup> is likely to act as a counterion to Ca<sup>2+</sup> efflux across the SR.

We have used an optical technique to monitor Ca<sup>2+</sup> release from the SR of single skinned skeletal muscle fibers. One objective of this study was to determine directly whether counterion movement through the SR K<sup>+</sup> channel is physiologically important in modulating Ca<sup>2+</sup> movement. Results with 1,10-bis-quanidino-*n*-decane (bisG10) a SR K<sup>+</sup> channel blocker (Garcia and Miller, 1984a), and replacement of K<sup>+</sup> with the impermeant ion choline demonstrate a dose-dependent decrease in the Ca<sup>2+</sup> release rate. These results strongly suggest a physiological role of the SR K<sup>+</sup> channel conductance. We have also investigated the selectivity of the in situ SR K<sup>+</sup> channel by substituting K<sup>+</sup> with other monovalent cations. Their effect on Ca<sup>2+</sup> release rate is presumably related to their conductance through the SR K<sup>+</sup> channel. The more permeable ions will be able to support a larger Ca<sup>2+</sup> release. The selectivity sequence we have determined for the in situ SR K<sup>+</sup> channel is in good agreement with that reported previously for the isolated SR K<sup>+</sup> channel in lipid bilayers. Pre-

liminary reports of some of the data reported here have appeared previously (Abramcheck and Best, 1986, 1987).

## METHODS

### *Spectrophotometric Studies of Dye Solutions*

**Solutions.** The basic solution used in these experiments was calculated to contain 110 mM monovalent cations ( $K^+$  and  $Na^+$ ), 2 mM MgATP, 0.1 mM Mg, 15 mM creatine phosphate, a pCa of 8, and ~35 mM MOPS buffer (pH = 7.0 at 22°C), ionic strength = 0.15. A computer program was used to solve the system of equilibrium reactions needed to describe a solution containing multiple chelating species (Donaldson and Kerrick, 1975). The stability constants used in the program were taken from Godt and Lindley (1982) and were appropriate for 22°C. The  $pK_a$  of MOPS was taken to be 7.20 at this temperature. Solutions were checked for  $Na^+$  and  $K^+$  by flame photometry, for chloride with a chloridometer, and for osmolarity. Stock solutions containing 50 mM caffeine, 1 mM bisG10, and 0.8 mM antipyrilazo III (AP III) (Sigma Chemical Co., St. Louis, MO) were prepared by adding dry chemical to the basic solution. These solutions were then serially diluted and mixed as appropriate ensuring that all paired solutions contained the same concentration of AP III, ~0.4 mM. AP III is a  $Ca^{2+}$ -sensitive metallochromic indicator dye which has been used to monitor  $Ca^{2+}$  transients in other tissues and predominately forms a 1:2  $Ca^{2+}$ /dye complex at the  $Ca^{2+}$  and dye concentrations used in our studies (Hollingsworth et al., 1987).

**BisG10 synthesis.** BisG10 was synthesized according to Garcia and Miller (1984a). Equal volumes of 1,10-diaminodecane, 100 mM in 50% methanol, and 300 mM S-methyl-isothioureia hemisulfate were mixed together. The reaction was stirred for 2 d at room temperature under a fume hood and the pH was periodically adjusted to 9.5 by titration with a 1-M NaOH solution. The main product is the bis-guanidinated compound, which precipitates out at these conditions. The primary contaminant is the monoguanidinated compound, which should remain soluble. The precipitate was extensively washed with cold water, ethanol, and ether, and then dried overnight in a desiccator under a vacuum. The purity of the compound was tested with H-NMR using a 99% pure bisG10 sample kindly supplied by Dr. C. Miller as a fingerprint. The results showed our bisG10 sample to be as pure as that supplied by Miller and free of contaminants since no additional peaks were observed.

**Determination of the apparent dissociation constant of AP III.** AP III is known to interact with several divalent ions and compounds resulting in wavelength-dependent shifts in its absorption spectrum (Scarpa, 1979; Palade and Vergara, 1982; Baylor et al., 1986). Of the constituents in our basic solution it is known that  $Mg^{2+}$  forms a 1:1 complex with AP III (Rios and Schneider, 1981). Arsenazo III, another metallochromic  $Ca^{2+}$  indicator dye, has been shown to interact with caffeine with a 1:1 stoichiometry (Best and Abramcheck, 1985b). Therefore, we investigated the interaction between AP III and caffeine. The drug bisG10 was also checked for any effect on AP III. AP III was titrated with each compound (caffeine, bisG10) separately to assess the extent of its binding. Difference spectra were recorded from 350 to 750 nm using a scanning spectrophotometer (Lambda 3A; Perkin-Elmer Corp., Norwalk, CT) balanced with the basic solution. The presence of maxima or minima in the difference spectra indicated that significant binding had occurred. Double reciprocal plots of the concentration of the compound and the absorption change at the maximum of a difference peak were then done to determine the  $K_D$  of the compound and AP III interaction.

The  $K_D$ 's of the interactions between AP III and caffeine or bisG10 are needed to calculate the apparent dissociation constant of AP III and  $Ca^{2+}$ . Rios and Schneider (1981) have shown that  $Mg^{2+}$  binds to AP III with a  $K_D$  of 6.7 mM, and they have derived equations for the

equilibrium of the dye in the presence of both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . These equations give rise to an apparent dissociation constant of AP III and  $\text{Ca}^{2+}$ . In our studies both  $\text{Mg}^{2+}$  and caffeine were present in the dye solutions. An apparent dissociation constant of AP III and  $\text{Ca}^{2+}$  under these conditions can be calculated following the method of Rios and Schneider. In the presence of  $\text{Ca}^{2+}$  the following equilibrium condition holds:

$$K = \text{Ca}(\text{D}_T - 2\text{CaD}_2)/\text{CaD}_2, \quad (1)$$

where  $\text{D}_T$  is total dye and  $\text{CaD}_2$  is the calcium-dye complex. When  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and caffeine (Cf) are present the appropriate equation is:

$$K\{(\text{Cf}/K_{\text{Cf}}) + [(\text{Mg} + K_{\text{Mg}})/K_{\text{Mg}}]\}^2 = [\text{Ca}(\text{D}_T - 2\text{CaD}_2)^2]/\text{CaD}_2. \quad (2)$$

Eq. 2 is analogous to Eq. 1 and, as pointed out by Rios and Schneider, for  $\text{Mg}^{2+}$  alone it demonstrates that the same equilibrium equation of 1:2 stoichiometry for  $\text{Ca}^{2+}$  and AP III

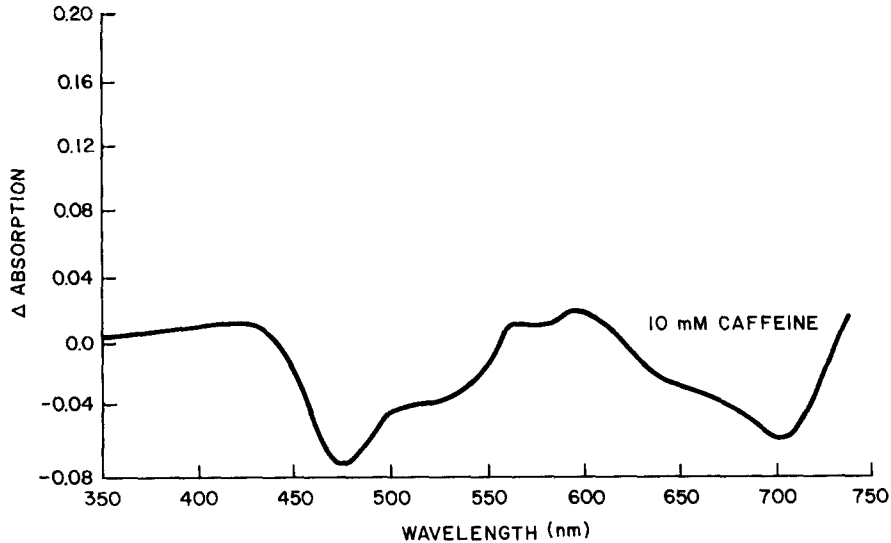


FIGURE 1. Difference spectrum of AP III and caffeine. The spectrum shows the effect of 10 mM caffeine on the absorbance of a 400  $\mu\text{M}$  AP III solution. The broad negative peaks are at 475 and 700 nm.

holds when both  $\text{Mg}^{2+}$  and caffeine are present. The apparent second-order dissociation constant  $K'$  is related to the absolute constant  $K$  by

$$K' = K\{(\text{Cf}/K_{\text{Cf}}) + [(\text{Mg} + K_{\text{Mg}})/K_{\text{Mg}}]\}^2. \quad (3)$$

In the studies using bisG10 we need to account for the binding of  $\text{Mg}^{2+}$ , caffeine, and bisG10 to AP III. The calculations follow the same logic with the added equations for bisG10 binding. The apparent second-order dissociation constant  $K'$  is now related to the absolute constant  $K$  by

$$K' = K\{(\text{Cf}/K_{\text{Cf}}) + [(\text{Mg} + K_{\text{Mg}})/K_{\text{Mg}}] + (\text{bis}/K_{\text{bis}})\}^2. \quad (4)$$

The value of  $K'$  used was 8,000  $\mu\text{M}^2$  (W. M. Kwok, X.-P. Xu, and P. M. Best, manuscript in preparation).

*Spectrophotometric studies of dye solutions.* It was determined that the stock solutions of caffeine and bisG10 without AP III had no significant absorbance ( $\leq 0.007$ ). Therefore, no

direct correction of the absorption transients from the optical studies for an inherent absorption of these compounds was necessary. These compounds were then tested for interactions with AP III and any subsequent effect on the AP III spectrum.

Titration of AP III with caffeine from 2.5 to 20 mM caused a shift in the AP III spectra. The difference spectra of AP III and caffeine demonstrates this concentration dependent shift due to formation of a caffeine-dye complex (Fig. 1). A significant effect of caffeine binding to arsenazo III, another metallochromic  $\text{Ca}^{2+}$  indicator dye, has also been demonstrated (Best and Abramcheck, 1985b). The primary characteristics of the difference spectra of AP III and caffeine are broad negative peaks at 475 and 700 nm. The double reciprocal plot for the caffeine experiment at 630 nm (Fig. 2), the isosbestic point of AP III and  $\text{Ca}^{2+}$ , shows a linear function (correlation coefficient, 0.994) and gives a  $K_D$  of 3.6 mM. In the optical studies caffeine was used in the release solutions and its effect on  $\text{Ca}^{2+}$  binding to AP III is corrected for in Eq. 4. The effect of 5 mM caffeine and 0.1 mM  $\text{Mg}^{2+}$  is to increase the apparent dissociation constant of AP III for  $\text{Ca}^{2+}$  by a factor of 5.78 compared with a factor of 1.01 for  $\text{Mg}^{2+}$  alone.

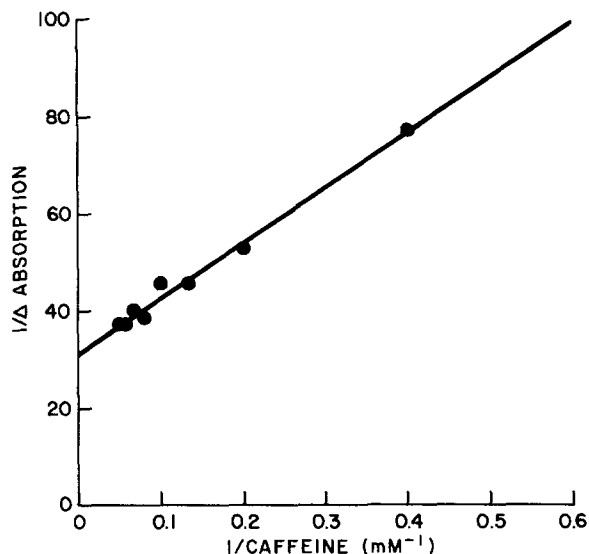


FIGURE 2. Interaction of AP III and caffeine. Graph is a double-reciprocal plot of the caffeine titration of AP III. Absorbance values are taken at 630 nm. The line is a linear least-squares fit to the data points (correlation coefficient = 0.994). The slope is 31.27 and the  $y$ -axis intercept is 113.24. The calculated  $K_D$  is 3.6 mM.

Titration of AP III with bisG10 ranging from 0.01 to 0.5 mM caused wavelength-dependent shifts in the AP III spectra. The difference spectra of AP III and bisG10 demonstrate the shift in the AP III spectra due to bisG10 binding (Fig. 3). The bisG10 concentration-dependent shift is positive above 580 nm with a negative peak developing at  $\sim 540$  nm. The double reciprocal plot using absorbance values at 720 nm is a linear function with a correlation coefficient of 0.997 (Fig. 4). The calculated  $K_D$  was 2.4 mM, which represents significant binding between bisG10 and AP III. Their binding is accounted for in the calculation of the apparent dissociation constant of AP III and  $\text{Ca}^{2+}$  as discussed (see Eq. 4). For example, at the highest bisG10 concentration used (0.5 mM) the apparent  $K_D$  of the dye for  $\text{Ca}^{2+}$  is 6.82 times the absolute  $K_D$ .

#### Optical Studies

**Solutions.** The dissected frog muscle was kept in a Ringer solution titrated to a pH of 7.0, which contained 115.5 mM NaCl, 2.5 mM KCl, 1.8 mM  $\text{CaCl}_2$ , 1.55 mM  $\text{NaH}_2\text{PO}_4$ , and 1.55 mM  $\text{Na}_2\text{HPO}_4$ .

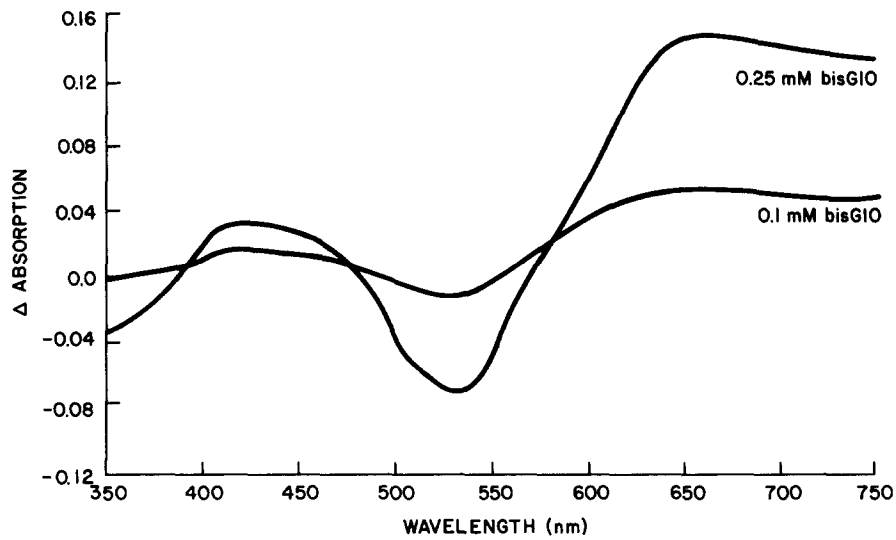


FIGURE 3. Difference spectra of AP III and bisG10. The spectra show the effect of 0.1 and 0.25 mM bisG10 on the absorbance of a 400- $\mu$ M AP III solution. The negative peak maximum is at 540 nm.

In experiments using skinned muscle fibers, solutions were designed to mimic intracellular conditions. Five different solutions were used in these experiments: relax. depletion, load, prerelease, and release. The solution compositions were calculated with the computer program described earlier with stability constants appropriate for 10°C. The  $pK_a$  of MOPS was taken to be 7.28 at this temperature. All solutions were calculated to contain 2 mM MgATP, 0.1 mM free  $Mg^{2+}$ , 110 mM monovalent cations ( $Na^+$  and  $K^+$ ), 15 mM creatine phosphate, and ionic strength = 0.15 (pH 7.0 at 10°C). Monovalent anions were limited by the use of

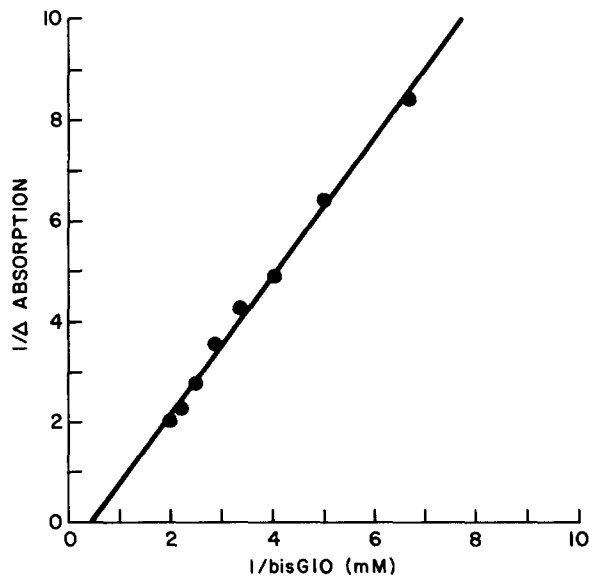


FIGURE 4. Interaction of AP III and bisG10. Graph is a double-reciprocal plot of the bisG10 titration of AP III. Absorbance values are taken at 720 nm. The line is a linear least-squares fit to the data points (correlation coefficient = 0.997). The slope is 1.37 and the y-axis intercept is  $-0.584$ . The calculated  $K_D$  is 2.3 mM.

propionate or methyl sulfate salts (7 mM Cl). The pCa of the solutions was calculated to be 8.0, except for the load solution in which it was 5.0. The actual salts and their concentrations are shown in Table I. The prerelease and release solutions were made by splitting a volume of solution containing 0.5 mM AP III added as the dry powder, and bisG10 where applicable, and adding 5 mM caffeine as the dry powder to half the volume to create the release solution. This assured equal dye and drug concentrations in both solutions. In the monovalent cation substitution experiments the prerelease and release solutions had a percentage of the K<sup>+</sup> propionate concentration replaced by an equivalent amount of the propionate salt of the substituted ion. In the case of choline the impermeable anion methyl sulfate was used. The depletion solution was made by adding 10 mM caffeine as the dry powder to a volume of relax solution. In the 100% K<sup>+</sup> substitution experiments the prerelease and release solutions lacked creatine phosphate. It was eliminated in these experiments since it brought 30 mM Na<sup>+</sup> to the solutions which could not be replaced by the substituting ion.

*Fiber preparation and release protocol.* The semitendinosus muscle was removed from *Rana pipiens berlandieri*, placed in Ringer solution, and kept on ice. A small bundle of fibers was removed, blotted, and placed in light mineral oil. Using a dissecting microscope a single

TABLE I  
*Composition of Solutions for Ca<sup>2+</sup> Release Experiments*

Solution type	KOH	MOPS	Na <sub>2</sub> ATP	Na <sub>2</sub> creatine phosphate	MgCl <sub>2</sub>	CaCl <sub>2</sub>	K <sub>2</sub> EGTA	K propionate
Relax, depletion	17.15	43.98	2.18	15.60	3.65	0.047	2	53.26
Load	17.20	31.11	2.19	15.60	3.60	1.94	2	53.28
Prerelease, release	15.08	28.40	3.74	15.05	2.16	0	0	57.96

Concentrations are expressed in millimolar.

fiber was teased out of the group and skinned by physical removal of the sarcolemma. Aluminum foil clips were then attached to both ends of the fiber. The fiber was mounted in the center section of a glass chamber housed in a water-cooled brass plate (~10°C) via stationary and mobile hooks. The fiber was then stretched ~20% of its resting length. A coverslip was secured over this central chamber and held in place with a spring clip. Fiber diameter was recorded by changing the plane of focus through the fiber and recording the distance from the fine focus knob. Sarcomere lengths were determined with a filar eyepiece. The solution surrounding the fiber could be rapidly changed via vacuum-driven syringe pumps that were controlled by a series of solenoid valves. The fiber chamber volume was ~60 μl, and ~300 μl of one of six precooled solutions could be rinsed through in ~100 ms. The timing of solution changes was controlled by a computer. A standardized protocol consisting of a test release bracketed by control releases was used. The fiber was depleted of its endogenous Ca<sup>2+</sup> store by alternating exposure to the high caffeine depletion and the relax solutions, which both contained EGTA. Once depleted, the fiber was exposed to the load solution for 1 min and then returned to the relax solution for 15 s. The fiber was then incubated in the prerelease solution for a set time interval; 1 min for bisG10 experiments due to its apparent binding kinetics, and 5 min for ion substitution experiments to allow for stabilization of possible osmotic effects. This also allowed for equilibration of the fiber and dye before initiating Ca<sup>2+</sup> release. Exposure to the release solution triggers Ca<sup>2+</sup> release which is then terminated by returning the fiber to the high EGTA relax solution. The fiber was allowed to rest in relax solution a few minutes before the next solution cycle was started.

*Optical recording.* A computer-interfaced optical system that allows measurements of optical absorption in single fibers during Ca<sup>2+</sup> release was used. The system has been

described in detail (Best and Fill, 1986). The absorption-measuring system allowed dual wavelength measurements to be made over relatively long time intervals (seconds).

Two different wavelengths were monitored to determine the absorption change due to  $\text{Ca}^{2+}$  release. AP III has a  $\text{Ca}^{2+}$ -sensitive wavelength at 720 nm where absorption changes indicate the formation of the AP III- $\text{Ca}^{2+}$  complex. The second wavelength used was 790 nm to account for absorption changes unrelated to changes in  $\text{Ca}^{2+}$  concentration (i.e., fiber movement). The difference spectra of 720–790 nm was calculated and the early slope of the absorption transient was used to determine the initial  $\text{Ca}^{2+}$  release rate. Caffeine was used to stimulate  $\text{Ca}^{2+}$  release from the SR. Changes in dye absorption (720–790 nm) indicating the presence of  $\text{Ca}^{2+}$  in the myofilament space were monitored and analyzed by computer. An individual trace consists of several parts, as shown in Fig. 5. The initial section of the trace is

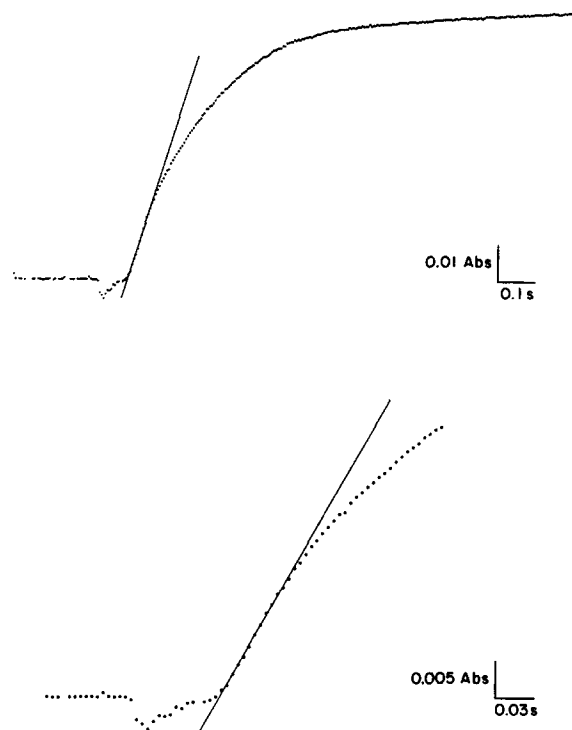


FIGURE 5. Individual absorption trace from a control release. Both traces are from the same release. The time scale of the second is one-third that of the first in order to show more detail in the region of initial absorption increase. The lines are from a linear regression on the first nine points indicating the steepest change in absorption. The slope is 0.343.

constant at zero absorption and is followed by a brief solution change artifact that lasts no longer than 100 ms. This is followed by an increase in absorption, which indicates the formation of the dye- $\text{Ca}^{2+}$  complex. The rate of absorption change decreases and then reaches a plateau indicating dye saturation. Titration of AP III with  $\text{Ca}^{2+}$  allows one to relate the absorption values at any point in the release trace to a specific concentration of myoplasmic  $\text{Ca}^{2+}$  (data not shown). For example, the change in dye absorption for a pCa of 5 is  $\sim 0.05$  at 720 nm ( $l = 0.1$  cm). Allowing for a roughly tenfold difference in path length, the fiber absorbance value correlating to this pCa is 0.005, which is reached before the plateau of the release trace. Dye saturation at the end of the release occurs at a pCa of  $\sim 3$ . The relationship between dye absorption and  $\text{Ca}^{2+}$  concentration is linear up to a pCa of  $\sim 5.5$ , which corresponds to about the first 100 ms of our release trace. Consequently, we expect the change in absorption to be linear with respect to  $\text{Ca}^{2+}$  and the dye to be far from saturation during this time. Therefore, our measurements of  $dA/dt$  were made as early as possible after the end of



the solution change artifact. A linear regression was performed on ~8–12 points that corresponded to the steepest change in absorption. This value was then used to calculate the early  $\text{Ca}^{2+}$  release rate as described below.

The release protocol used was that of a test release bracketed by releases under control conditions. Its reproducibility was tested in control experiments consisting of three successive releases. The average of three control releases was considered as 100%, and then each release was expressed as a percentage of this value. For the bisG10 experimental protocol the mean  $\pm$  SE was  $100.1 \pm 0.1$  ( $n = 10$ ), which indicates essentially no variability in release rate for the three consecutive releases. For the ion substitution experimental protocol the mean  $\pm$  SE was  $100.1 \pm 0.1$  ( $n = 5$ ).

Using Beer's law, dye stoichiometry and the fiber diameter, the value for  $dA/dt$ , can be converted to the rate of change of the  $\text{Ca}^{2+}$ -dye complex ( $d\text{CaD}_2/dt$ ). A correction factor  $p$  can be derived that relates  $d\text{CaD}_2/dt$  to the  $\text{Ca}^{2+}$  release rate  $R$ , assuming a system of rapidly equilibrating  $\text{Ca}^{2+}$  buffers (Rakowski et al., 1985). The derivation also assumes that the buffer systems are far from saturation, which holds during the initial stages of  $\text{Ca}^{2+}$  release. The release rate  $R$  is:

$$R = p * d\text{CaD}_2/dt (\mu\text{M}/\text{ms}) \quad (5)$$

with the correction factor  $p$  given by:

$$p = 1 + \frac{[\text{T}] * K' + K' * K_T}{[\text{D}]^2 * K_T} \quad (6)$$

where  $[\text{T}]$  is the troponin concentration (240 mM),  $K_T$  is the dissociation constant for troponin and calcium (2  $\mu\text{M}$ ),  $K'$  is the apparent constant for AP III and Ca, and D is dye concentration.

Release rates were calculated for all releases using Eq. 5. The test release rate was then expressed as a percent of the average release rate of the bracketing controls. The average control release rate from all the fibers used in this study was  $52.7 \pm 6.2 \mu\text{M}/\text{ms}$  ( $\bar{X} \pm \text{SE}$ ,  $n = 236$ ), which is somewhat greater than what was reported previously by this lab (Best and Fill, 1986). The lower Mg concentration used probably accounts for the higher release rate obtained in this study. Other laboratories have reported similar rates of release in intact cells (Baylor et al., 1983; Rakowski et al., 1985).

## RESULTS

### *The Effect of Decreased $\text{K}^+$ Conductance on $\text{Ca}^{2+}$ Release*

During release, if  $\text{Ca}^{2+}$  is the only ion to cross the SR membrane its efflux should be self-limiting. Both the duration and extent of the  $\text{Ca}^{2+}$  release would be increased if there were an ion flux opposing the development of a SR membrane potential equal to  $E_{\text{Ca}}$ . An influx of  $\text{K}^+$  would act in such a supportive role. Hence studies were performed to assess the effect of a decreased SR  $\text{K}^+$  conductance on the  $\text{Ca}^{2+}$  release rate. The first approach used the  $\text{K}^+$  channel blocker bisG10 to decrease the  $\text{K}^+$  conductance by removing available current pathways. Tension studies on single skinned muscle fibers demonstrated a concentration-dependent decrease in peak height and rate of rise of caffeine-stimulated releases after bisG10 exposure (data not shown). These studies also indicated that short times (<1 min) were needed for bisG10 to become effective and to be washed off. The optical studies using bisG10 were done to obtain a more quantitative measure of the bisG10 blocking effect on

$\text{Ca}^{2+}$  release rate. An example of individual traces from an experiment with a 60-s exposure to 0.3 mM bisG10 is shown in Fig. 6. The lines are the result of a least-squares fit to  $\sim 12$  points during the initial portion of the trace, and the test release clearly shows a decrease in slope from that of the bracketing controls. Note that the second control release indicates full recovery from bisG10 exposure, which indicates that its binding is reversible. All values ( $dA/dt$ ) were converted to  $\text{Ca}^{2+}$  release rates using the equations described earlier. Test release rates were then expressed as a percent of controls. The control release correction factor  $p$  was calculated with the apparent dissociation constant for AP III binding corrected for caffeine and  $\text{Mg}^{2+}$  binding ( $p = 23.4$ ). The test release correction factor  $p$  was calculated for each bisG10 concentration with the  $K'$  corrected for caffeine,  $\text{Mg}^{2+}$ , and bisG10 binding

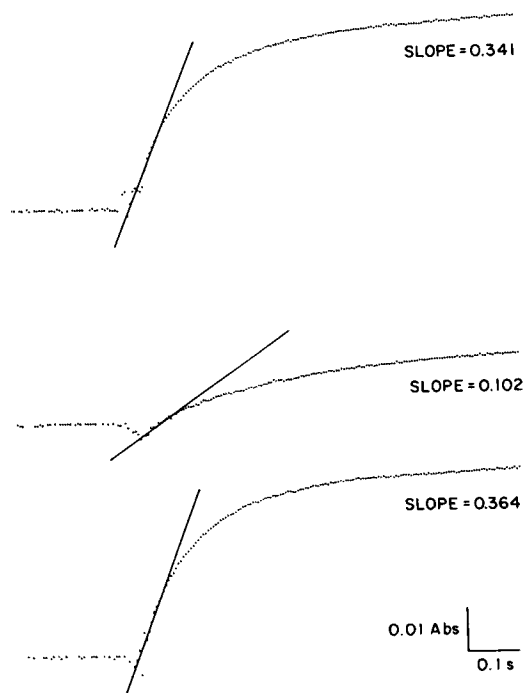


FIGURE 6. Effect of 0.3 mM bisG10 on early  $\text{Ca}^{2+}$  release rate. Control releases (top, bottom) bracket a test release in 0.3 mM bisG10. The lines are from a linear regression of the initial points indicating the steepest change in absorbance and the slopes are indicated on each trace. The test slope is 28.9% of the averaged controls.

to AP III. The  $p$  values ranged from 23.7 to 27.4, indicating a relatively small additional effect of bisG10 binding to AP III. The relative test release rates were plotted as a function of the bisG10 concentration (Fig. 7) and demonstrate a dose-dependent decrease in the early  $\text{Ca}^{2+}$  release rate. There is a clear and consistent inhibitory effect of bisG10 on the early  $\text{Ca}^{2+}$  release rate. This is exemplified by the drastic reduction seen with 0.5 mM bisG10.

An alternate explanation for the bisG10 results discussed above is that the drug competes for the SR caffeine-binding site or directly affects the SR  $\text{Ca}^{2+}$  channel or  $\text{Ca}^{2+}$  pump. If any of these possibilities were true then the observed effect of bisG10 would not necessarily be caused solely by the blockade of the  $\text{K}^+$  conductance pathway. Therefore, experiments to test the effect of an alternate method of decreasing

the  $K^+$  conductance that did not involve pharmacological intervention were performed.

Substitution of the impermeant cation choline for  $K^+$  was used as another method to decrease  $K^+$  conductance. This substitution effectively reduces the available charge carriers. An example of individual traces from an experiment with a 5-min incubation in a prerelease solution in which 34% of the total permeant monovalent cations ( $K^+$  and  $Na^+$ ) were substituted by choline methyl sulfate is seen in Fig. 8. The lines are derived from a linear regression and the slope of the test release is clearly less than the control release slopes. The second control demonstrates a good release supporting the belief that there is no residual choline or effects from its presence. Changes in absorption were converted to  $Ca^{2+}$  release rates using the correction factor  $p$  for all releases. This correction accounts only for the  $Mg^{2+}$  and caffeine AP III interactions since any choline methyl sulfate interaction was

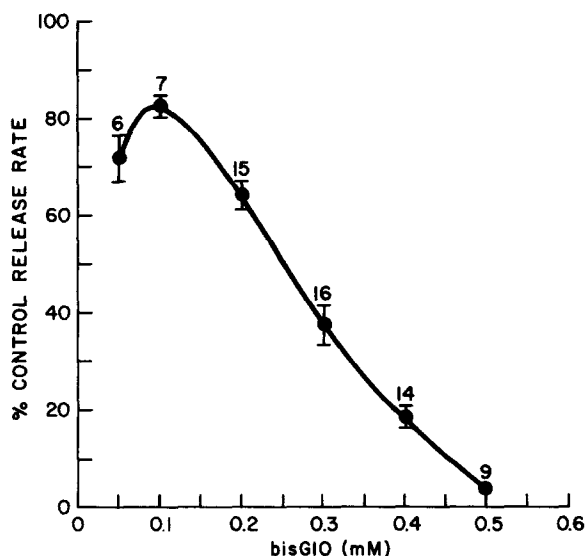


FIGURE 7. Response of  $Ca^{2+}$  release rate to bisG10 exposure. Points represent the mean  $\pm$  SE of the data at each bisG10 concentration. The line is a smooth curve drawn through the data points.

assumed to be equivalent to that of  $K^+$  propionate. The test release rate is then expressed as a percentage of control rates, hence the value of  $p$  is not critical to our results. Therefore, the conclusion drawn from these experiments is not dependent on a valid correction factor since it cancels when calculating percents. The relative rates of the test releases are compared with the concentration of choline in the test prerelease and release solutions (Fig. 9). All substitutions caused a decrease in  $Ca^{2+}$  release rate with the effect of 7 mM permeant monovalent cation giving ~25% of control. Clearly this method of lowering  $K^+$  conductance causes a decrease in the  $Ca^{2+}$  release rate, which strongly indicates a physiological role for the SR  $K^+$  channel.

#### *Determination of Ion Selectivity*

The experiments discussed above show that counterion flux is necessary to support  $Ca^{2+}$  release. The smaller the ionic conductance through the SR  $K^+$  channel, the

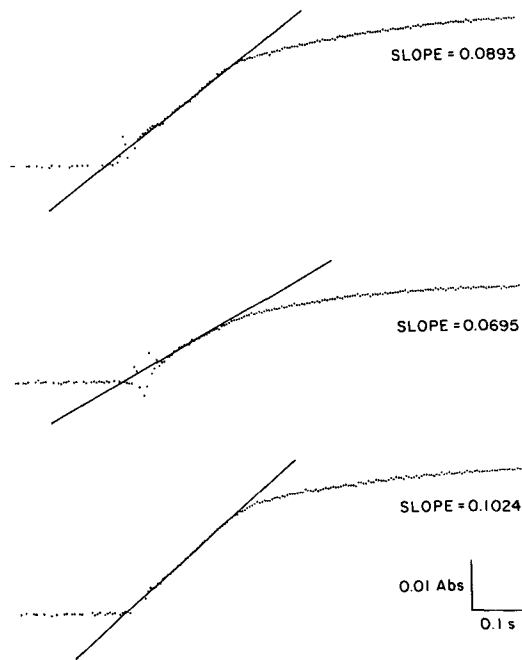


FIGURE 8. Effect of 37 mM choline on early  $\text{Ca}^{2+}$  release rate. Control traces (top, bottom) bracket a test release in 37 mM choline. The lines are the result of a linear regression on the initial points indicating the fastest change in absorption. The slopes are indicated on each trace. The test slope (middle trace) is 72.5% of averaged bracketing controls.

slower  $\text{Ca}^{2+}$  leaves the SR. The interrelationship between monovalent cation conductance and  $\text{Ca}^{2+}$  release rate can be used to determine the ion selectivity of the in situ SR  $\text{K}^+$  channel. The relative ability of any particular monovalent cation, compared with  $\text{K}^+$ , to support  $\text{Ca}^{2+}$  release should be related to its conductance through the SR  $\text{K}^+$  channel.

The following monovalent cations were chosen for the  $\text{K}^+$  substitution experiments:  $\text{Na}^+$ ,  $\text{Rb}^+$ ,  $\text{Li}^+$ , and  $\text{Cs}^+$ . Both partial and complete (leaving 7 mM  $\text{Na}^+$  pres-

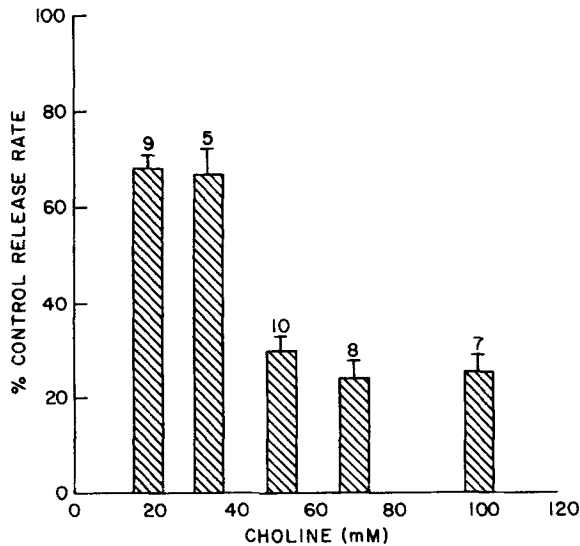


FIGURE 9. Response of  $\text{Ca}^{2+}$  release rate to choline substitution. The bars represent the mean  $\pm$  SE of the data at each choline concentration with the number of determinations on top of each bar. The effect of choline substitution is concentration dependent and appears to peak at  $\sim 60$  mM choline. Actual choline concentrations were (from left to right) 22, 37, 55, 73, and 104 mM.

ent) substitutions were done.  $\text{Na}^+$  was chosen to see if it is as permeable as  $\text{K}^+$  in an intact SR membrane preparation. In isolated systems  $\text{Rb}^+$  has been shown to be about one half as conductive as  $\text{K}^+$ , and  $\text{Li}^+$  was determined to be poorly conductive, so they should show a clear effect on  $\text{Ca}^{2+}$  release (Coronado et al., 1980).  $\text{Cs}^+$  has been shown to have a low conductance and a blocking action in isolated SR  $\text{K}^+$  channels (Coronado and Miller, 1979) and is of interest since in cut fiber preparations replacement of  $\text{K}^+$  with  $\text{Cs}^+$  appears to have no effect on  $\text{Ca}^{2+}$  release (Palade and Vergara, 1982).

Shown as representative of the behavior of all monovalent cation substitution experiments are the individual traces from a 100% substitution of  $\text{Li}^+$  for  $\text{K}^+$  (Fig. 10). The lines are from linear regression and the slope of the release in the presence

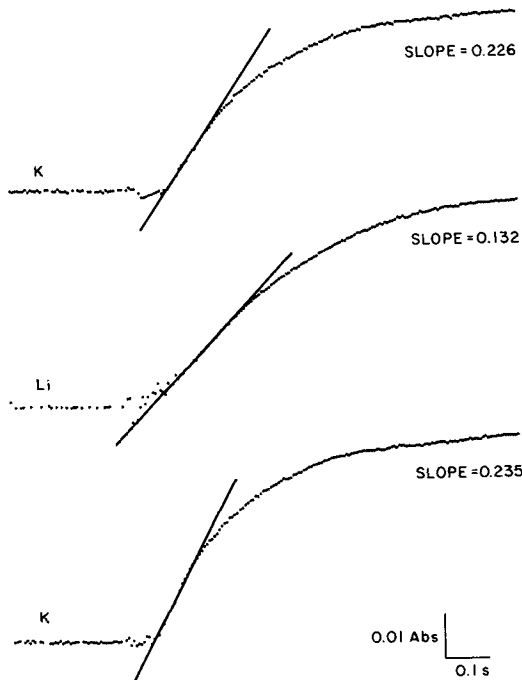


FIGURE 10. Effect of  $\text{Li}^+$  substitution on early  $\text{Ca}^{2+}$  release rate. Individual absorption traces are from an experiment with a 5-min exposure to 104 mM  $\text{Li}^+$ . The lines are from a linear regression on the initial points indicating the steepest change in absorption. The slopes are indicated on each trace. The test (middle) release is 57.3% of the averaged control.

of  $\text{Li}^+$  shows a clear decrease in release rate to 55% of control. The second control release demonstrates that there is no residual effect from  $\text{Li}^+$  substitution. The slopes of the absorption traces were converted to  $\text{Ca}^{2+}$  release rates using a correction factor  $p$  of 96.95 for all ion substitution experiments. Again ratios of release rates are used so the correction factor has minimal impact on the results.

The effect of replacing all or part of the  $\text{K}^+$  by various monovalent cations is summarized in Fig. 11. Neither  $\text{Rb}^+$  nor  $\text{Na}^+$  showed a significantly different effect from  $\text{K}^+$  on  $\text{Ca}^{2+}$  release rate at partial or complete substitution. These results indicate that  $\text{Rb}^+$ ,  $\text{Na}^+$ , and  $\text{K}^+$  are equivalently conductive. In contrast, the  $\text{Li}^+$  substitution gives a concentration-dependent decrease in  $\text{Ca}^{2+}$  release rate; the more  $\text{Li}^+$  present the lower the  $\text{Ca}^{2+}$  release rate. This result indicates that  $\text{Li}^+$  has a significantly lower conductance than  $\text{K}^+$  and that it does not appear to block the channel.

The two Cs<sup>+</sup> substitution results are identical, giving ~85% of the control release rate, which suggests a weak blocking action of Cs<sup>+</sup>. Blocking of the channel was assumed when there was no further reduction in the Ca<sup>2+</sup> release rate when the concentration of the substituted ion was increased. Also, the block was assumed to be equally effective at both ion concentrations. Partial and complete substitution of K<sup>+</sup> by choline gave ~25% of the control release rate. These results can also be explained by a blocking action of choline, though it was much stronger than that of Cs<sup>+</sup>. These combined results show that the relative selectivity of the in situ SR K<sup>+</sup> channel is K<sup>+</sup> = Rb<sup>+</sup> = Na<sup>+</sup> > Cs<sup>+</sup> > Li<sup>+</sup> > choline.

## DISCUSSION

### *Spectrophotometric Studies of Dye Solutions*

AP III, a metallochromic indicator dye, has been widely used to monitor changes in cytoplasmic Ca<sup>2+</sup> concentrations in frog skeletal muscle fibers (Rios and Schneider,

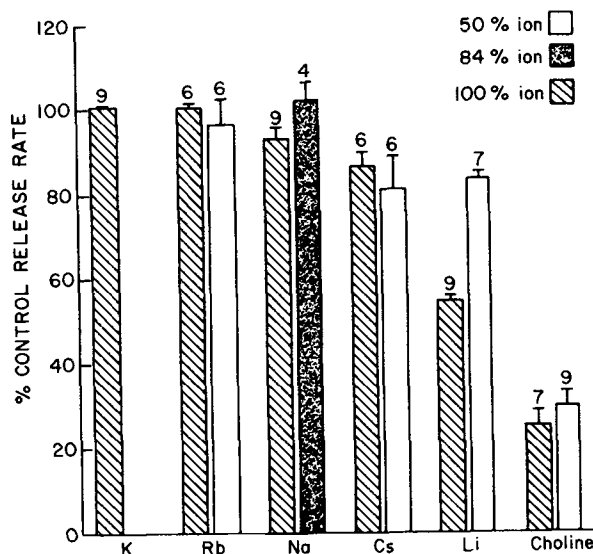


FIGURE 11. Response of early Ca<sup>2+</sup> release rate to substitution of K<sup>+</sup> by various monovalent cations. The values are the mean ± SE. The hashed bars represent 100% ion substitution, the solid bar represents 84% ion substitution, and the open bars represent 50% ion substitution.

1981; Baylor et al., 1982; Kovacs et al., 1983). Characterization of the interactions between AP III and compounds or ions used in experiments is of interest since it can lead to a better understanding of the dye chemistry. Baylor and co-workers have extensively studied AP III and its reaction with Ca<sup>2+</sup>. AP III was found to form metal-free dimers and a large fraction (75–89%) of the dye appears bound within a fiber since it is not freely diffusible (Baylor et al., 1986). The extent of AP III binding within the myoplasm sheds doubt on the accuracy of  $K_D$ 's and extinction coefficients determined in vitro when applied to intracellular conditions. AP III also forms more than one complex with Ca<sup>2+</sup> depending on their relative concentrations (Hollingsworth et al., 1987), but under the conditions found in our experiments the 1:2 Ca<sup>2+</sup>-dye stoichiometry predominates. AP III is a reactive chemical and when doing a quantitative analysis of absorption signals in cells, shifts in the absorption

spectra or changes in absorption caused by ions or compounds other than  $\text{Ca}^{2+}$  need to be considered. Significant binding of caffeine and bisG10 to AP III was demonstrated in our studies. However, their binding did not directly affect the  $\text{Ca}^{2+}$ -dependent absorption change used in our calculations since the compounds have no inherent absorption and were kept at constant concentrations. An equilibrium between AP III and  $\text{Mg}^{2+}$ , caffeine, and, when present, bisG10, had been established before the  $\text{Ca}^{2+}$  release is initiated. Hence the dissociation constant for  $\text{Ca}^{2+}$ -AP III binding will be increased. Therefore, an apparent dissociation constant  $K'$  derived from the equilibrium condition of all dye-binding species is used. These spectrophotometric clearly demonstrate the need to check for binding between AP III and other compounds found in experimental solutions to determine the correct  $K_D$ . This is especially important if their concentration changes.

#### *Optical Studies of $\text{Ca}^{2+}$ Release in Single Muscle Cells*

*The role of  $\text{K}^+$  as a counterion during  $\text{Ca}^{2+}$  release.* The function of the intact SR  $\text{K}^+$  channel has been a topic of speculation for years. McKinley and Meissner suggested that the purpose of the  $\text{K}^+$ ,  $\text{Na}^+$  channel found in SR vesicles was to minimize charge and osmotic effects during  $\text{Ca}^{2+}$  release and reuptake (1977, 1978). Similarly, from studies on the isolated SR  $\text{K}^+$  channel, Miller and co-workers suggested they function as that of a "leak" to allow  $\text{Ca}^{2+}$  to rapidly cross the SR membrane without establishing a potential (Garcia and Miller, 1984a; Miller et al., 1984). The experiments described in this study were designed to directly investigate the influence of charge movement through the SR  $\text{K}^+$  channel on  $\text{Ca}^{2+}$  release from intact SR. The decrease in  $\text{Ca}^{2+}$  release seen with a decreased  $\text{K}^+$  conductance supports the hypothesis of an important physiological role of the SR  $\text{K}^+$  channel.

If  $\text{K}^+$  is acting as a counterion and its movement is inhibited, the SR membrane potential should approach  $E_{\text{Ca}}$  at the beginning of release thus limiting the extent of  $\text{Ca}^{2+}$  movement. The optical technique used in these experiments allowed an estimate of the early  $\text{Ca}^{2+}$  release rate, which from the argument above should be sensitive to changes in SR membrane potential. Caffeine was used to trigger  $\text{Ca}^{2+}$  release since it is known to stimulate SR  $\text{Ca}^{2+}$  channels directly, bypassing the normal excitation-contraction coupling mechanism. Therefore, any effect seen on the  $\text{Ca}^{2+}$  release will be due to factors that regulate the  $\text{Ca}^{2+}$  efflux once it has been initiated. The optical studies were performed to determine the physiological effect of a decreased SR membrane  $\text{K}^+$  conductance. Two different methods were used to lower the  $\text{K}^+$  conductance: blockade of the  $\text{K}^+$  channel by bisG10 and substitution of the impermeant ion choline for  $\text{K}^+$ .

The compound bisG10 has been shown to be a very effective SR  $\text{K}^+$  channel blocker. The half-inhibition of  $\text{K}^+$  conductance by bisG10 in bilayers is  $50 \mu\text{M}$  (Garcia and Miller, 1984a). In studies in which  $\text{Tl}^+$  (which is thought to pass through the SR  $\text{K}^+$  channel) entry into isolated SR vesicles was investigated, the concentration of bisG10 causing half-inhibition of conductance was  $75 \mu\text{M}$  (Garcia and Miller, 1984b). In the optical studies described here bisG10 exposure clearly caused a dose-dependent decrease in early  $\text{Ca}^{2+}$  release rate and nearly abolished it at a concentration of  $0.5 \text{ mM}$ . From these data it is seen that to obtain a relative release rate of 50%  $\sim 270 \mu\text{M}$  bisG10 would be needed. This is a somewhat larger value than that

obtained in reconstituted channels as described above. The skinned fiber preparation used contains intact SR membrane and hence the affinity of bisG10 for the  $K^+$  channel could be different than that in isolated channel preparations. Presumably the environment of the  $K^+$  channel is different in isolated vs. intact SR so direct comparisons are not possible. Also, the effective concentration of bisG10 is lowered by the formation of a complex with AP III. For example, at the highest bisG10 concentration of 0.5 mM, 22% is bound to AP III. We found no effect of bisG10 on submaximal or maximal tension generation in skinned fibers (X.-P. Xu, personal communication), which suggests that the drug does not bind to the contractile filaments.

Choline has been established as being impermeant to the SR membrane (Kometani and Kasai, 1978; McKinley and Meissner, 1978). Using a stopped-flow fluorescence quenching technique on SR vesicles, no conductance path was found for choline (Garcia and Miller, 1984a). In studies with the SR  $K^+$  channel inserted into planar bilayers surrounded by 400-mM salt solutions the choline conductance was found to be  $\leq 1$  pS (Coronado and Miller, 1982). In the optical studies reported here, replacing choline for  $K^+$  caused a concentration-dependent reduction in  $Ca^{2+}$  release rate. This is consistent with the notion that a lower  $K^+$  concentration would decrease the counterion flow causing a less extensive  $Ca^{2+}$  efflux. This result is also consistent with the idea that the bisG10 effect is due to the drug binding to the  $K^+$  channel. The combined results of the bisG10 and choline substitution experiments clearly demonstrate an important physiological role of the SR  $K^+$  conductance.

That a significant  $Ca^{2+}$  release (25% of control) was seen even in the presence of 104 mM choline (total substitution) indicates that  $K^+$  is not the sole counterion in our system. Release solutions contain 7 mM  $Na^+$  and  $Cl^-$ , which could partially support  $Ca^{2+}$  release via charge redistribution through the SR  $K^+$  and anion channel, respectively. Another possible pathway for counterion flow in our preparation is the SR  $H^+$  channel. It is a major determinate of SR membrane permeability with its density estimated to be at least  $50/\mu m^2$  and its permeability coefficient at least  $10^{-3}$  cm/s (Meissner and Young, 1980). Diffusion potentials from pH gradients have been studied in SR vesicles with a voltage-sensitive dye. The results demonstrated that transient or steady state SR membrane potentials can be reduced by the movement of  $H^+$  (Meissner and Young, 1980). It has also been shown that during  $Ca^{2+}$  release there is a proton release from the SR in excess of that explained by ATP splitting during  $Ca^{2+}$  reuptake (Chiesi and Inesi, 1980). The hypothesis that  $H^+$  also acts as a counterion to  $Ca^{2+}$  during release has been suggested by Somlyo and co-workers (Somlyo et al., 1981). In electron probe studies on tetanized muscle fibers  $Ca^{2+}$  release is shown to be accompanied by the uptake of  $K^+$  into the terminal cisternae of the SR. But their results showed that the amount of  $K^+$  moved is approximately half the amount of charge carried from the SR by  $Ca^{2+}$  movement. Baylor and co-workers (1987) have used the pH indicator dye phenol red in intact muscle fibers to follow the change in myoplasmic pH after  $Ca^{2+}$  release. The apparent pH change can be accounted for by a proton flux from the myoplasm into the SR, the magnitude of which could account for roughly a third to half the charge imbalance obtained during  $Ca^{2+}$  release. Hence, it appears clear that the  $H^+$  conductance might also play a role in charge neutralization of the  $Ca^{2+}$  current during



release. Our results do suggest, however, that  $K^+$  plays a major role physiologically in maintaining charge balance during  $Ca^{2+}$  release since release was severely curtailed by the elimination of  $K^+$  from the solutions, even in the presence of 7 mM  $Cl^-$  and hydrogen ion.

Given a potential role of  $H^+$  and  $Cl^-$  as charge carriers in our system, it is surprising that bisG10 blocked  $Ca^{2+}$  release almost completely in our experiments. One possible explanation is that bisG10 blocks the SR  $H^+$  channels as well as the  $K^+$  channel. The drug is related chemically to the quaternary amines, some of which (e.g., decamethonium) are potent channel blockers. BisG10 has been studied in whole muscle preparations and causes relaxation of muscle without any stimulatory effect (Ozawa et al., 1962). If it did block both the SR  $K^+$  and  $H^+$  channels, and with nominal permeant anions (7 mM  $Cl^-$ ) present in our experimental conditions, there would be few pathways or charge carriers to support a counterion flux resulting in a very small  $Ca^{2+}$  release.

Previously, we measured the  $K^+$  permeability of resting SR using passive  $^{42}K$  fluxes from frog skinned fibers (Best and Abramcheck, 1985a, b) and obtained a value of  $1.7 \times 10^{-7}$  cm/s. To determine whether the  $K^+$  permeability changes during  $Ca^{2+}$  release, we have estimated the upper limit of the  $K^+$  permeability for the SR during  $Ca^{2+}$  release in the following manner. We assume the simplest equilibrium condition in which the resting potential difference across the SR is zero and  $K^+$  concentrations are identical within the SR and in the myoplasm (Somlyo et al., 1977). Using an average  $Ca^{2+}$  release rate of 10  $\mu M/ms$  (appropriate for 1 mM  $Mg^{2+}$ ; Best and Fill, 1986) we can calculate an average  $Ca^{2+}$  efflux of  $2.5 \times 10^{-4}$  mol/cm<sup>2</sup> s for a 100- $\mu M$  fiber. Assuming a  $K^+$  influx twice that of  $Ca^{2+}$  ( $K^+$  acts as a perfect counterion) we can use the following equations describing unidirectional flux to calculate the  $K^+$  permeability:

$$g_K = \frac{(ZF)^2}{RT} J^{oi} \quad (7)$$

$$P_K = g_K \left/ \frac{(ZF)^2}{RT} C_K^o \right., \quad (8)$$

where  $C_K$  is the K concentration,  $J^{oi}$  is the flux from outside to inside the SR, and  $Z$ ,  $F$ ,  $R$ , and  $T$  have their customary meanings. The value we obtain,  $2.3 \times 10^{-7}$  cm/s, is not appreciably different from that which we obtained for the resting SR, which suggests that the SR  $K^+$  permeability does not change during  $Ca^{2+}$  release. We interpret this to mean that the resting  $K^+$  permeability is sufficiently high to allow enough  $K^+$  to move during  $Ca^{2+}$  release to maintain charge balance across the SR membrane.

*Ion selectivity of the in situ SR  $K^+$  channel.* Since  $K^+$  carries a significant counterion flux during  $Ca^{2+}$  release, changes in the  $Ca^{2+}$  release rate can be used as an indirect measure of the changes in SR  $K^+$  channel conductance. Substitution of other monovalent cations for  $K^+$  was performed to determine the relative selectivity of the in situ SR  $K^+$  channel. Its characteristics can then be compared with those of the isolated channel. The efflux of radioactive ions from SR vesicles demonstrates a permeability pathway that appears equally selective for  $K^+$  and  $Na^+$ . This "cation

channel" has a cut-off diameter for cations with a cross section  $>0.4 \times 0.6$  nm (McKinley and Meissner, 1978). This estimate is in good agreement with the  $K^+$ -selective channel described by Miller exhibiting a channel cut-off diameter of 0.4–0.5 nm determined in lipid bilayers (Coronado and Miller, 1982). Hence, for the choline and bisG10 experiments we assumed  $Na^+$  to be as permeable as  $K^+$ . This assumption was confirmed from our  $Na^+$  substitution experiments, which indicated that the high permeability to  $Na^+$  is a characteristic of the in situ SR  $K^+$  channel and not an artifact of the isolation procedure.

The relatively high permeability of  $Cs^+$  was not expected since it has been shown to block surface membrane  $K^+$ -conducting channels in many excitable membranes (Hille, 1973; Armstrong, 1975). The blocking action is believed to be due to the  $Cs^+$  entering the channel and sterically preventing  $K^+$  ions from crossing the membrane.  $Cs^+$  is frequently used in perfusion solutions of cut muscle fiber preparations. These fibers show "normal"  $Ca^{2+}$  release with all internal  $K^+$  replaced by  $Cs^+$ , which would seem to indicate that the ion is permeable in the SR  $K^+$  channel (Palade and Vergara, 1982). However, Coronado and Miller (1979) found that  $Cs^+$  blocks the  $K^+$  conductance of isolated rabbit SR  $K^+$  channels in lipid bilayers. The normal  $K^+$  conductance of 130 pS with 0.1 M  $K^+$  was undetectably low ( $<3$  pS) in 0.1 M  $Cs^+$ .  $Cs^+$  binds to the open channel with a  $K_D$  of 18 mM at a  $V_m$  of +50 mV. Further bilayer studies with 1-M  $Cs^+$  salt solutions found no fluctuation in single-channel conductance, and also that the  $Cs^+$  block is competitive with  $K^+$  (Coronado et al., 1980). However more recent bilayer studies on the SR  $K^+$  channel show measurable single-channel currents in the presence of only  $Cs^+$  salts (Cukierman et al., 1985). Currents were not seen before due to the high  $Cs^+$  concentrations. Since  $Cs^+$  binds tightly inside the  $K^+$  channel most channels were occupied and hence remained open. It was concluded that  $Cs^+$  is almost as permeant as  $K^+$  but 20-fold less conductive. Our  $Cs^+$  substitution experiments show support of ~85% of control  $Ca^{2+}$  release rate, which indicates a weak blocking action of the ion. The blocking action of  $Cs^+$  was suggested by the result that no further decrease in  $Ca^{2+}$  release rate was seen when going from 50 to 100%  $Cs^+$  substitution. These results along with observations in cut fiber experiments indicate that  $Cs^+$  can permeate the in situ SR  $K^+$  channel.

The ion selectivity of the SR  $K^+$ -selective channel from frog leg muscle was studied in lipid bilayers by Labarca and Miller (1981). The selectivity sequence (conductance values in pS) follows:  $K^+$  (97)  $>$   $NH_4^+$  (57)  $>$   $Rb^+$  (48)  $>$   $Na^+$  (23)  $>$   $Li^+$  (7.6)  $>$   $Cs^+$  ( $<1$ ). This is the same selectivity sequence found for the extensively studied rabbit SR membrane  $K^+$  channel although the conductance values for the frog are lower (Coronado et al., 1980). The more recent bilayer studies showing  $Cs^+$  as very permeable (Cukierman et al., 1985) would place the ion before  $Li^+$  in the selectivity sequence. From our ion substitution studies on the in situ SR  $K^+$  channel we obtained the following selectivity sequence:  $K^+ = Rb^+ = Na^+ > Cs^+ > Li^+ >$  choline. Comparison of the two sequences demonstrates very good agreement which strongly suggests that they describe the same channel.

This study was supported by grants from the National Institutes of Health (AR-32062) and Muscular Dystrophy Association to P. M. Best. C. W. Abramcheck was supported by a predoctoral training grant from the Public Health Service (GM07143-10).

*Original version received 9 April 1988 and accepted version received 5 July 1988.*

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