Effects of Extracellular Calcium Ions, Verapamil, and Lanthanum on Active and Passive Properties of Canine Cardiac Purkinje Fibers

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SUMMARY. The effects of alteration of extracellular calcium ion concentration ([Ca++]o) were studied in isolated false tendons using microelectrode techniques. Several determinants of cellular excitability and conduction velocity were affected by extracellular calcium. Increasing [Ca++]o from 2 to 8 mM resulted in: (1) a progressive decrease in interelectrode conduction velocity (2) a 7-mV shift of the maximum upstroke velocity-membrane potential relation toward less negative potential, (3) an increase in rheobasic current, (4) a 14-mV shift of the voltage threshold for all-or-none depolarization to less negative potentials. (5) a 52% increase in internal longitudinal resistance per unit length, and (6) a 27% decrease in the capacitance filled by the foot of the action potential from 4.90 to 3.56 μF/cm2. Blockade of the slow inward current by Mn++ or verapamil did not alter the [Ca++]+-induced effects on the maximum upstroke velocity-membrane potential relation. Cable properties were determined during alteration of [Ca++]o in the presence of verapamil (3 × 10⁻⁶ and 1 × 10⁻⁵ M) or in the presence of La+++ (0.2 mM). Verapamil increased membrane resistance × unit length but did not affect internal longitudinal resistance per unit length. La+++ had no effects on either membrane resistance × unit length or internal longitudinal resistance per unit length. Verapamil did not block the increase in r, induced by elevation of [Ca++]. However, no change in r, occurred during an increase of [Ca++]o when La+++ was present. The results suggest that [Ca++]+-induced changes in internal longitudinal resistance may occur by the influx of calcium ions through the Na+/Ca++ exchange mechanism. (Circ Res 51: 637–651, 1982)
flects on internal longitudinal resistance and that these changes probably depend on Ca”entry into the cells through sites other than the slow channel.

Methods

Adult mongrel dogs (15–30 kg) were anesthetized with intraavenous sodium secobarbital (30 mg/kg) and the hearts rapidly excised. Long (8–17 mm; mean 12 mm) unbranched free-running false tendons (mean diameter, 450 μm) were selected from either ventricle, detached from the endocardium and mounted in a tissue bath. In some experiments, the false tendons were cut with fine scissors into short segments (1.9–2.5 mm). The preparations were superfused with warmed (37°C) oxygenated Tyrode’s solution of the following composition (mm): NaCl, 118; KCl 4; NaHCO3 22; MgCl2 0.5; CaCl2 1 to 8; NaH2PO4 0.9; and glucose 5.6. Changes in superfusate calcium concentration were made without compensation for changes in osmolarity. Solutions were aerated with 95% O2-5% CO2 to produce a pH of 7.3 to 7.4. Racemic verapamil (Knoll Pharmaceutical Co.) was added to the Tyrode’s solution from a stock solution of verapamil HCl in distilled water or by adding appropriate amounts of crystalline verapamil directly to the Tyrode’s solution (final concentrations 3 × 10−6 M, 1 × 10−5 M). Solutions containing manganese (3 mM) or lanthanum did not contain NaH2PO4. In the studies in which LaCl3 was used, the solution was buffered with either Tris 10 mM (+ HCl to pH 7.3) or Hapes 3–6 mM (N-2-hydroxyethyl-piperazine-N’-2-ethanesulfonic acid). Buffer substitution was necessary because La” is extremely insoluble in HCO3− or phosphate-containing solutions. Hapes was used as a substitute buffer in most of the experiments because it has been shown to be physiologically inert (Good et al., 1966), and solution pH is not temperature dependent. Care was required in the adjustment of solution pH to the physiological range because of formation of colloidal lanthanum (La(OH)3) at pH > 7.35 (G. Langer, personal communication). Tyrode’s solution containing Tris or Hapes was aerated with 100% O2 and pH stability was verified (± 0.02) by repeated determinations.

Free ionized-calcium concentration was measured in two experiments (Orion SS20 calcium ion-selective electrode system) to determine the extent to which soluble Ca” complexes [e.g., CaHCO3”, Ca(HCO3)2] lowered the total superfusate calcium concentration. In contrast to the results of Schaer (1974), but similar to the results of Pederson (1971), the ionized calcium concentration was 6–9% less than the total calcium concentration. Moreover, the percent decrease in free calcium concentration did not vary significantly with changes in total calcium from 1 to 8 mM. Thus, the relative change in free ionized calcium concentration (1.00:1.97:7.79) closely corresponded to the relative change in the total calcium concentration (1:2.8).

The preparations were stimulated at regular intervals (1.7–2 Hz) through bipolar wires isolated except at the tips. Twelve pacing stimuli were delivered prior to a test stimulus. Except where noted below, all test stimuli and measurements were performed during a pause in pacing with the coupling interval equal to the basic pacing interval. Glass microelectrodes with DC resistances of 5–12 MΩ were used to record transmembrane potentials and to inject intracellular current. Recording microelectrodes were filled with 3 mM KCl and current-injecting microelectrodes with either 3 mM KCl or 2.5 M K-citrato. A relay was used to share one microelectrode between recording and current injection modes. In the recording mode, the electrode was coupled to the input stage of a high impedance, capacitance-neutralizing amplifier. Electronic differentiation was used to obtain the first time derivative of the action potential upstroke (Vmax). A sawtooth was used to calibrate the differentiated signal in the 50–1000 V/sec range. In the current injection mode, the microelectrode was connected to a constant current source. The intensity of the current pulses was determined from the voltage drop across a precision 1 MΩ resistor interposed between the bath reference and ground. The signals were displayed on a memory oscilloscope (Tektronix D15), and measurements were taken from Polaroid pictures of the oscilloscopic traces. Fifteen to 20 minutes were allowed between changes of superfusate composition to allow changes (especially of cable properties) to reach steady state values. Intracellular impalements were maintained at the same sites for all measurements and repeated determinations were performed whenever possible. The order of alteration of extracellular calcium concentration was varied so that a consistent directional change of [Ca”]o would not bias the results. A stereomicroscope (Wild M5A) with a calibrated ocular grid (resolution ± 40 μm) was used for positioning and distance measurements. Transmembrane potential (Vm) measurements were made with a resolution of 1 mV (0.2 mV with additional 5- or 10-fold amplification for cable analysis) and current measurements with a resolution of 2 nA.

Two microelectrodes were used to measure conduction velocity (θ) during alteration of [Ca”]o. Bipolar stimulating electrodes were apposed to one end of the false tendon and pacing stimuli (2 msec duration) delivered at an intensity 1.5–2 times diastolic threshold. Action potentials were recorded from two widely spaced (4–9 mm) microelectrodes positioned along the same longitudinal axis with the proximal electrode 4–5 mm distant from the stimulating wires. The action potential upstroke and Vmax were displayed on the oscilloscope at a rapid sweep speed (1 msec/division), and interelectrode conduction time was measured as the time interval between the two upstrokes. We rejected experiments in which the two recorded action potentials did not arise from the same level of potential (±5 mV) or have comparable upstroke velocities (±15%). Interelectrode distance was measured with the stereomicroscope, and conduction velocity was calculated by dividing interelectrode distance by interelectrode conduction time.

Two methods were used to vary Vm in order to measure the Vmax−Vm relation of a propagated action potential with changes in [Ca”]o, from 1 to 8 mM. In the first method, keeping [Ca”]o constant, the extracellular K” concentration was varied from 4 to 10 mM over a 10-minute interval, and records of Vmax and Vm were taken during the transition. Measurements of changes in Vmax with Vm were made during both K”-depolarizing and K”-repolarizing limbs and the results averaged. While maintaining a stable impalement, the [Ca”]o was changed and the [K”] again varied. The results were plotted and a linear regression line fitted to the steep portion of the curve. Interpolation of the voltage at 50% inactivation of Vmax (V1/2) was done by calculation from the regression equation. The slope factor (k) of the Vmax−Vm relation was calculated using the data points and Vm in a semilogarithmic relation derived from the equation: h = (1 + exp [(Vm − V1/2)/k])−1; [where h = ratio of Vmax at test Vm to Vmax at the maximum Vm (Hodgkin and Huxley, 1952; Weidmann, 1955)].

In a second series of experiments, the Vmax−Vm relation was determined during changes in [Ca”]o, from 2 to 8 mM in the presence and absence of a slow channel-blocking agent (Mn” 2 mM or verapamil 3 × 10−6 M). A basic train...
of 10 stimuli was followed by a premature stimulus and the \( V_m \) and \( V_{mem} \) of the premature response measured. This pattern of stimuli and measurements was repeated with progressively more premature stimuli until the effective refractory period was reached. An automated process with on-line analysis of the action potential characteristics by a microcomputer system (Elharrar and Lovelace, 1979; Elharrar, 1980) facilitated rapid determinations. The resultant \( V_{mem} - V_m \) data were analyzed for \( V_h \) and \( k \) using the regression lines and equation described above.

The current threshold for all-or-none depolarization \( (I_{th}) \) was determined by injecting constant current pulses through a single intracellular microelectrode positioned near the midpoint of the false tendon. The response was visualized by returning to the recording mode after completion of current injection (switching time constant < 1 msec). The current threshold was defined as the minimum current intensity that resulted in an action potential. For each determination, multiple measurements were performed with several approaches to threshold using sub- and suprathreshold current intensities. The threshold current intensity was determined for pulses 0.2-100 msec in duration in long false tendons and 2-200 msec in shortened preparations.

The rheobasic current \( (I_{rh}) \) was determined with 100- and 200-msec current pulses in long and shortened preparations, respectively. The Lapicque equation \( (I_s = I_{rh}/(1 - \exp(-t/T_{gd})) \) (Lapicque, 1907)] was used to derive the time constant of the strength-duration curves \( (T_{gd}) \). \( T_{gd} \) was computed from least squares analysis of the relation between \( \log \) (1 - \( (I_s/I_{rh}) \)) and \( t \).

The apparent voltage threshold for all-or-none depolarization \( (V_{th}) \) was measured during changes in \( [Ca^{++}]_o \), by two different methods of intracellular current application. In the first method, two microelectrodes were inserted in close proximity (<100 \( \mu m \)) near the midpoint of the preparation. One microelectrode was used to apply 100-msec square pulses of intracellular current, and the second microelectrode was used to record transmembrane potentials near the site of current injection \( (x = 0) \). Both microelectrodes were positioned along the same longitudinal axis and maintained at the same sites during changes in \( [Ca^{++}]_o \). \( V_{th} \) was measured as the membrane potential at the end of a just-subthreshold current pulse (Fozzard and Schoenberg, 1972).

A possible complication in the \( V_{th} \) measured with a double microelectrode technique arises from three-dimensional spread of current from a point source. If calcium ions altered the magnitude of current spread from the point source, the true \( V_{th} \) could be constant and yet the measured \( V_{th} \) would change. Therefore, we also determined the change in \( V_{th} \) with \( [Ca^{++}]_o \), using a single sucrose gap technique for intracellular current injection. A false tendon was passed across a pair of latex membranes at the ends of a 1-mm partition separating side compartments of a three-chambered bath. The side compartments (1- to 1.5-ml volume) were superfused with warmed, oxygenated Tyrode’s solution and the middle compartment with isotonic sucrose solution (300 mm sucrose in distilled water) containing \( 10^{-5} \) \( m \) \( Ca^{++} \). Saturated vegetable oil (Crisco) was applied to the opening in the latex membranes to help ensure a tight seal around the preparation. The length of the false tendon in the test compartment was 3 to 4 mm.

Constant current pulses of varying duration (5-500 msec) were delivered to Ag-AgCl electrodes in the side compartments. Intracellular recordings of \( V_m \) were obtained approximately 100 \( \mu m \) from the site of exit of the false tendon from the latex membrane. An extracellular reference electrode was positioned close to the recording site to diminish voltage artifacts. As a control, current pulses were applied to the bath with the recording microelectrode in the extracellular space to ensure that no significant artifactual voltage deflection was present. The \( V_{th} \) was measured as the membrane potential at the end of a just-subthreshold current pulse.

Cable analysis during alteration of \( [Ca^{++}]_o \) was performed by means of a triple microelectrode technique. One microelectrode was inserted near the midpoint of the false tendon and used to inject 100- to 300-msec hyperpolarizing constant current pulses. The other microelectrodes were used to record transmembrane potential changes with distance. The proximal recording electrode was positioned greater than 1 Purkinje bundle diameter (mean placement 335 ± 19 \( \mu m \)) from the site of current injection to avoid problems with three-dimensional flow of current near the current electrode tip (Eisenberg and Johnson, 1970; Levin and Fozzard, 1981). The distal recording electrode was positioned a mean of 1221 ± 112 \( \mu m \) from the current site. Only hyperpolarizing current pulses were employed in the cable analysis. The current intensity (55 to 130 nA) was adjusted to result in a 5- to 8-mV deflection at the proximal recording site. Care was taken to position the microelectrodes along the same longitudinal axis. An experiment was considered successful only if recordings could be performed while maintaining the triple impalement sites during the change in \( [Ca^{++}]_o \). In each experiment, electrotonic potentials were recorded in duplicate or triplicate, and the measurements were averaged to minimize error due to variations with contraction.

The space constant \( (\lambda) \), input resistance \( (R_{in}) \), and membrane time constant \( (\tau_m) \) were calculated in long preparations (\( \geq 3A \) length from the site of current injection to termination) using the theory of an infinite coaxial cable (Hodgkin and Rushton, 1946; Weidmann, 1952; Jack et al., 1975). The space constant, defined as the distance corresponding to an e-fold change in voltage, was derived from a semilogarithmic plot of the steady state amplitude of the electrotonic potential \( (AV) \) vs. distance \( (x) \). The semilogarithmic plot of voltage vs. distance was extrapolated to the voltage \( (V_m) \) at the point of current input and divided by one-half the applied current to calculate the input resistance of a half length of fiber \( (R_{in} = V_m/[(1/2)] \), where \( 1 = \text{intensity of applied current} \). The time constant was derived from a plot of the half-time of rise of the electrotonic potential vs. distance (Hodgkin and Rushton, 1946; Jack et al., 1975). The slope of this relation equals \( \tau_m/2\lambda \). The time constant was calculated from the slope using the concurrently determined \( \lambda \). This relation was checked in several preliminary experiments in which the time to reach 84% of the total amplitude (current to recording electrode distance <100 \( \mu m \)) was compared to the above method for measuring \( \tau_m \). The results were comparable (<1 msec difference).

In preparations < 3A length from the site of current injection to termination, \( \lambda \), \( V_m \), and \( R_{in} \) were derived from measurements of \( AV \) and \( x \) using equations for a special case of cable theory (Weidmann, 1952; Delèze, 1970). In this case, the preparation was assumed to be terminated at a distance \( x = L \) (where \( L = \text{length of Purkinje bundle from the site of current input to its termination} \) by a short circuit. The terminal resistance was assumed to be short-circuited because, in most cases, a tiny piece of ventricular muscle remained attached to the false tendon. Using estimates of \( \lambda \) and \( V_m \) from infinite cable calculations (see above), a "plot" (using linear regression analysis) of \( \sinh^{-1} \left[ (AV/V_m) \sinh(L/\lambda) \right] \) vs. \( (L - x) \) yielded 1/\( \lambda \) as a slope. \( V_m \) was
recalculated using the new estimate of $\lambda$ and the following equation: $V_o = \{\Delta V \sinh(h/\lambda)/\sinh[(L-x)/\lambda]\}$. These estimates of $\lambda$ and $V_o$ were reinserted into the equations and the process repeated until the change in $\lambda$ and $V_o$ was less than 0.1% (5 to 10 iterations). $R_m$ and $\tau_m$ were calculated using the equations described above.

Dimensional analysis was performed in four experiments (conducted in parallel with the cable analysis measurements) to assess the structure of canine false tendons and provide estimates of specific cellular electrical properties. Canine false tendons of similar mean diameter to those undergoing cable analysis ($390 \pm 80 \mu m$) were quick frozen, sectioned perpendicular to the long axis and stained with hematoxylin and eosin. Purkinje cells were isolated by connective tissue into approximately circular and elliptical bundles composed of 5 to 24 (mean 12) cells juxtaposed in a bundle. Multiple circumscribed bundles (9 to 10) were present in cross-sections of false tendons near the junction with ventricular muscle but only one to three bundles in cross-sections of free-running segments (where microelectrodes were placed). The mean ($\pm$ SE) diameter of the Purkinje cells was $32 \pm 8 \mu m$ ($n = 45$); the mean cross-sectional area/cell was $780 \mu m^2$. The diameter of the bundles of Purkinje cells ranged from 70 to 220$\mu m$ with a mean ($\pm$ SE) of 132 $\pm 25 \mu m$.

If the bundles are assumed to be approximately circular in cross-section, the cells within a bundle closely coupled, and if each bundle is isolated electrically from the other bundles within the false tendon, then the geometry of a right circular cylinder with the mean diameter listed above can be used to calculate the specific electrical properties. In preparations where $L$ was $>3\lambda$ in length, the membrane resistance $\times$ unit length ($R_m$) and internal longitudinal resistance per unit length ($r_i$) were calculated from $\lambda$ and $R_m$ by the simultaneous solution of the following equations: $\lambda = \tau_m/r_i$, $R_m = \tau_m/r_i$. For preparations where the truncated cable equations were applied, $r_i$ and $r_m$ were calculated from the following equations: $n = R_m/\phi$, $R_m = \tau_m/r_i$, where $\phi = \lambda^2$. The specific membrane resistance ($R_m$) and specific internal longitudinal resistance ($R_i$) were calculated using the following equations: $R_i = \tau_i \lambda^2$, $R_m = \tau_m/r_i$, where $a = \lambda$. The calculations assumed that the radius of the conductive path was equal to the mean radius of the bundles listed above. Further discussion of the limitations of the geometrical assumptions is given below (see Limitations).

In a separate series of false tendons, the time constant of the foot of propagated action potentials ($TAP$) was measured during a change of $[Ca^{++}]_o$ from 1 to 8 mM. The $TAP$ was determined from phase-plane analysis (Jenerick, 1961, Sperelakis and Shumaker, 1968) of action potentials recorded at a large distance ($5$ to 10 mm) from the site of stimulation. The slope of the initial linear phase of the phase-plane [phase a (Sperelakis and Shumaker, 1968)] was used to measure $TAP$. In four of five experiments, the interelectrode conduction velocity ($\Theta$) was measured concurrently. The capacitance per unit length filled by the foot of the action potential ($C_{AP} \mu F/cm^2$) was calculated by the equation: $C_{AP} = \Theta TAP - 1/\Theta$, using the separately determined mean $\tau_i$ from the cable analysis at 2 and 8 mM $[Ca^{++}]_o$ (Tasaki and Fozzard, 1966). The specific capacitance of the foot ($C_{AP} \mu F/cm^2$) was calculated from the equation: $C_{AP} = \Theta TAP - 1/\Theta$, where $a = \lambda$.

Paired data analysis was performed using Student’s $t$-test (Snedecor and Cochran, 1967). Linear regression analysis was used to fit the data from which the various electrical properties were derived. Analysis of variance with repeated measures (Winer, 1971; Wallenstein et al., 1980) was performed to compare observations under three or more different experimental conditions. Where analysis of variance indicated a significant difference among the means ($P < 0.05$), a simultaneous multiple comparison procedure (Bonferroni method; Wallenstein et al., 1980) was used to determine the significance of differences among the means.

Limitations of the Method

The application of linear one-dimensional cable theory for cables of unlimited length requires some justification in a finite cardiac bundle of complex structure. Eisenberg and Johnson (1970) and Lieberman et al. (1975) have discussed in detail the limitations of the complex anatomy of cardiac tissue on linear cable analysis. To apply linear one-dimensional cable analysis, the Purkinje bundle is idealized as the simple geometry of a uniform right circular cylinder of infinite length with a core filled with material of uniform resistivity per unit length. The bundle is assumed to be immersed in an isopotential conductive solution of a volume much greater than the volume of the core so that the external longitudinal resistance is negligible compared to the internal longitudinal resistance. The membrane (sarclemma) enclosing the core is assumed to be represented by an ohmic resistor in parallel with a capacitor acting as a perfect dielectric. The electric field surrounding the current source is assumed to vary only along the longitudinal axis of the cylinder with the electric potential constant in any one cross-section. Voltage gradients about the current source are neglected. The cumulative effect of these assumptions is that unidimensional cable analysis represents only a first approximation of the electrical behavior of the cardiac Purkinje bundle to an applied point source of current.

The geometry of the Purkinje bundle is certainly more complex than a uniform circular cylinder. The bundle diameter varies along its length with the variation as great as $\pm 20\%$ (Weidmann, 1952). Furthermore, there is uncertainty introduced by the degree of folding of the membrane and by the geometry and dimensions of the clefts between Purkinje cells (Mobley and Page, 1972). The error introduced by neglecting these factors in calculating the membrane capacitance ($C_m$) from the membrane time constant is considerable. Therefore, we chose to determine an estimate of $C_m$ from the time constant of the action potential foot where the anatomic factors are less problematic.

Of more significance, error is introduced by neglecting the electric field in two-dimensions about the current source and by assuming a simple RC circuit as representative of the membrane electrical behavior. Two time constant characteristics of cardiac Purkinje fibers have been shown by Fozzard (1966) and Freygang and Trautwein (1970) and the time constant of a longitudinally oriented capacitance measured at 64 $\mu$sec (Freygang and Trautwein, 1970). Furthermore, the use of a point source of current is not ideal for the study of one-dimensional cable properties in a cylindrical structure. Radial voltage gradients near the point source cause the measured potential within the bundle to deviate seriously from the potential predicted by one-dimensional theory (Eisenberg and Johnson, 1970). The potential recorded by a microelectrode at a given distance from a nearby point source of current varies with the position of the electrode tip in the plane of the cross-section of the bundle (Eisenberg and Johnson, 1970). Therefore, to estimate roughly the degree of error introduced by the
simplifications of one-dimensional analysis, we measured the steady state voltage change over a range of distances from the point of current application. Figure 1 shows an experiment that illustrates the one- and three-dimensional decay of voltage with distance. Panel A shows the fitting of the data to a simple exponential. The fit between observation and the prediction of an infinite one-dimensional cable is close except for values near the current source (<200 μm). Panel B further emphasizes this close fit for distant recordings (x/a ≥ 2) and illustrates the complexity of the voltage changes near the current electrode tip. The expected behavior for voltage changes near the current electrode tip for the data in panel A is approximated by the smooth curve labeled λ/a = 15 (since λ = 1.83 mm and a = 110 μm, λ/a = 16.7). However, the observed points are more closely related to a much steeper voltage change with a ratio of λ/a = 2. This rapid decay suggests that the geometrical assumptions employed to derive the three-dimensional spread of voltage near a point source (namely, a right circular cylinder) are much too simple to represent the behavior of a canine Purkinje bundle. The very rapid decay of voltage near a point source has also been reported by Matsuda (1960) in terminal Purkinje fibers of dog ventricle and by Tanaka and Sasaki (1966) in mouse ventricle. Analytical studies of canine false tendons suggest that the transverse flow of current between neighboring cells in a bundle might account for the greater-than-expected voltage change near the current source (since the current would have to traverse membrane barriers in its spread through the cross-section of the bundle). Other explanations (e.g., branching and rejoining of the bundles) are also possible, but seem to be less likely in view of the distance involved (≤200 μm).

Because of the complex architecture of cardiac tissue, the question arises as to how one-dimensional cable analysis comes so close to approximating the steady state voltage recordings. Analysis of three-dimensional components of voltage decay (Eisenberg and Johnson, 1970; Levin and Fozzard, 1981; present Figure 1) shows that at distances greater than one bundle diameter from the current-injecting electrode, the value of the three-dimensional term is small and the one-dimensional term dominates. Nonlinearities of the cardiac membrane with respect to voltage and time are minimal in more negative ranges of membrane potential (−80 to −95 mV) and when long-lasting (100–200 msec) hyperpolarizing constant current pulses are applied (Hellam and Studt, 1974). Furthermore, according to Levin and Fozzard (1981), the charging of a Purkinje bundle deviates most from the Hodgkin-Rushton analysis (1946) at times comparable to the time constant for charging the internal membranes of the cleft, 1.7 msec (Schoenberg et al., 1975). In these experiments we have placed our recording electrodes within the region where one-dimensional analysis is a good approximation (Fig. 1) and maintained a constant position of the current-injecting and recording electrodes during experimental interventions. Since the three-dimensional component of voltage decay is a function of position and independent of membrane properties (Eisenberg and Johnson, 1970), the constant position of the impedances also tends to minimize the effects of neglecting three-dimensional voltage changes in interpreting the changes incurred during experimental interventions.

Another limitation concerns the amount of uncertainty introduced by using cable equations for infinite vs. finite lengths. The degree of error associated with the assumption...
of infinite length depends critically both on the ratio of the bundle length from the point of current injection (L) compared to λ and on the position of recording electrodes relative to the terminus of the bundle. We calculated the difference in λ and r, using equations for an infinite cable and those for a cable terminated by a short circuit (Weidmann, 1952). For a L/λ ratio of 4.0, λ for a finite cable was 0.2% higher and r 0.1% lower; for L/λ of 3.0, λ(finite) was 1.3% higher and r 0.6% lower; for L/λ of 2.5, λ(finite) was 3.8% higher and r 2.3% lower. The mean ± SEM for the cable experiments was 3.2 ± 0.4. Therefore, the approximate errors introduced by the simplifying assumption of infinite length were: 0.2 to 5% (λ) and 0.1 to 3% (r).

Results

Inter electrode Conduction Velocity and $V_{\text{max}} - V_{\text{m}}$ Relationship

The effects of varying $[\text{Ca}^{2+}]_o$ from 1 to 8 mM on the characteristics of the action potential upstroke and on inter electrode conduction velocity were measured in nine experiments. The results are shown in Table 1. With increasing $[\text{Ca}^{2+}]_o$, there was no change in $V_m$, an increase in overshoot, a 7-10% decrease in $V_{\text{max}}$, and a 20% decrease in inter electrode conduction velocity. When $[\text{Ca}^{2+}]_o$ was increased, conduction velocity consistently decreased but (in several experiments) variable changes in $V_{\text{max}}$ were observed with increasing $[\text{Ca}^{2+}]_o$. The significant changes in action potential characteristics and conduction velocity were noted when $[\text{Ca}^{2+}]_o$ was increased to 8 mM. No significant differences in overshoot, $V_{\text{max}}$, or conduction velocity were noted between $[\text{Ca}^{2+}]_o$ of 1 and 2 mM. Therefore, the remainder of the experiments were confined largely to studies of the effects of $[\text{Ca}^{2+}]_o$ in the 2 to 8 mM range.

The voltage-dependent effects of $[\text{Ca}^{2+}]_o$ on $V_{\text{max}}$ were investigated in five experiments in which alteration of $[K^+]_o$ was used to vary $V_m$. In one experiment (shown in Fig. 2), the $V_{\text{max}}$ - $V_m$ relationship was determined at $[\text{Ca}^{2+}]_o$ of 1, 2, and 8 mM. This experiment showed a progressive shift of the voltage at 50% inactivation of $V_{\text{max}}$ ($V_h$) by a mean of 7.4 mV toward less negative potentials, and increased $k$ from 4.9 to 6.4 mV. The increase in $k$ suggested a more gradual rate of change of $V_{\text{max}}$ with $V_m$ at a $[\text{Ca}^{2+}]_o$ of 8 mM (see Fig. 2). The equation $h = (1 + \text{exp} [(V_m - V_h)/k])^{-1}$ (where $h$ = ratio of $V_{\text{max}}$ at a test $V_m$ to $V_{\text{max}}$ at maximum diastolic potential) fitted the $V_{\text{max}} - V_m$ data with a mean correlation coefficient of 0.96 ± 0.01.

A premature stimulus method was also used in long preparations (six experiments) to study the $V_{\text{max}}$ - $V_m$ relationship during alteration of $[\text{Ca}^{2+}]_o$. The mean $V_h$ and $k$ parameters were similar to those shown in Table 2 ($V_h = -71.1$ mV ([Ca$^{2+}]_o$ 2 mM), $-64.7$ mV ([Ca$^{2+}]_o$ 8 mM) P < 0.001; $k = 4.3$ mV ([Ca$^{2+}]_o$ 2 mM), 6.0 mV ([Ca$^{2+}]_o$ 8 mM) P < 0.001) and the direction of the changes was unaltered. Mn$^{2+}$ 3 mM (four experiments) and verapamil 3 × 10$^{-6}$ M (two

Table 1

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Mean ± SEM shown; $V_m$ = membrane activation voltage; OVS = overshoot; $V_{\text{max}}$ = maximum upstroke velocity; $\theta$ = inter electrode conduction velocity; n = number of experiments.

* $P < 0.001$; † $P < 0.01$ compared to 2 mM $[\text{Ca}^{2+}]_o$.

FIGURE 2. Changes in $V_{\text{max}} - V_m$ relation with alteration of $[\text{Ca}^{2+}]_o$.

Ordinate: maximum upstroke velocity of propagated action potentials ($V_{\text{max}}$); abscissa: membrane activation voltage ($V_m$). Increasing $[\text{Ca}^{2+}]_o$ from 1 to 2 to 8 mM: (1) shifted the voltage at 50% inactivation of $V_{\text{max}}$ ($V_h$) to less negative potentials; (2) altered the contour of the $V_{\text{max}} - V_m$ curves to a progressively less rapid change of $V_{\text{max}}$ with $V_m$. Single impalement maintained for the duration of the experiment. $V_h$ values: −74.3 mV, −71.1 mV, and −61.6 mV at $[\text{Ca}^{2+}]_o$ of 1, 2, and 8 mM, respectively. Slope factor ($k$) values: 4.0 mV, 5.2 mV and 6.7 mV at $[\text{Ca}^{2+}]_o$ of 1, 2, and 8 mM, respectively. Changes in $[K^+]_o$ were used to vary $V_m$. 

<table>
<thead>
<tr>
<th>$[\text{Ca}^{2+}]_o$ (mM)</th>
<th>$V_m$ (mV)</th>
<th>$V_{\text{max}}$ (mV)</th>
<th>$\theta$ (mV/sec)</th>
<th>$\phi$ (mV/sec)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>−91</td>
<td>32</td>
<td>623</td>
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<td>0.10</td>
</tr>
<tr>
<td></td>
<td>±1</td>
<td>±1</td>
<td>±32</td>
<td>±0.13</td>
<td>±0.10</td>
</tr>
<tr>
<td>2</td>
<td>−90</td>
<td>34</td>
<td>642</td>
<td>2.04</td>
<td>1.68</td>
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<tr>
<td></td>
<td>±1</td>
<td>±1</td>
<td>±31</td>
<td>±0.12</td>
<td>±0.10</td>
</tr>
<tr>
<td>8</td>
<td>−91</td>
<td>39†</td>
<td>578†</td>
<td>1.68†</td>
<td>1.0†</td>
</tr>
</tbody>
</table>

Mean ± SEM shown; $V_m$ = membrane activation voltage; OVS = overshoot; $V_{\text{max}}$ = maximum upstroke velocity; $\theta$ = inter electrode conduction velocity; n = number of experiments.

* $P < 0.001$; † $P < 0.01$ compared to 2 mM $[\text{Ca}^{2+}]_o$.
**TABLE 2**

<table>
<thead>
<tr>
<th>[Ca++]o (mM)</th>
<th>Vm (mV)</th>
<th>OV5 (mV)</th>
<th>Vmax (V/sec)</th>
<th>Vb (mV)</th>
<th>k</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>-93</td>
<td>33</td>
<td>670</td>
<td>-70.3</td>
<td>4.9</td>
<td>5</td>
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<tr>
<td>±1</td>
<td>±1</td>
<td>±35</td>
<td>±1.3</td>
<td>±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-92</td>
<td>38†</td>
<td>620</td>
<td>62.9*</td>
<td>6.4*</td>
<td>5</td>
</tr>
<tr>
<td>±1</td>
<td>±1</td>
<td>±2.3</td>
<td>±0.9</td>
<td>±0.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vb = voltage at 50% inactivation of Vmax; k = slope factor of Vmax-Vb relationship.

* P < 0.001; † P < 0.01. For other abbreviations, see Table 1.

Experiments were added during these experiments to study the effect of slow channel blocking agents on the [Ca++]+-induced alterations in Vb and k. No significant changes in Vb, overshoot, Vmax, Vth, or k were observed when [Ca++]o was increased with additional concentrations of Mn++ or verapamil employed. Furthermore, when [Ca++]o was increased in the presence of Mn++ or verapamil, there was no significant change in the magnitude of the shift in Vth or shift in Vm observed with an increase in [Ca++]o alone. This finding occurred despite evidence of significant slow channel-blocking effects [i.e., shortening of the action potential duration at 50% of repolarization (APD50) and lengthening of APD90] from the addition of Mn++ or verapamil to the superfusate.

One additional experiment was performed to study the effects of alteration of [Ca++]o on Vmax and conduction velocity (θ). The Vm was depolarized to -65 mV by increasing [K+]o to 8 mM and measurements of Vmax and θ before and after an increase in [Ca++]o from 2 to 8 mM. At -65 mV, Vmax increased 23% with an increase in [Ca++]o (503 V/sec to 617 V/sec) but θ decreased by 18% (2.03 m/sec, [Ca++]o 2 mM to 1.66 m/sec, [Ca++]o 8 mM). This decrease in θ was similar in magnitude to the change in θ at more negative potentials (see Table 1). Thus, depolarization to -65 mV did not appear to affect the direction or magnitude of changes in θ with increasing [Ca++]o, but the changes in Vmax were in the opposite direction.

**Excitability as Measured by Intracellular Current Injection**

Strength-duration curves at [Ca++]o of 2 and 8 mM were performed in four shortened false tendons (mean length 2.28 mm) and in two long preparations (7.4 mm) to evaluate the effects of [Ca++]o on excitability. In an additional four long preparations, the change in rheobasic current with [Ca++]o was measured. There was no significant difference in maximum diastolic potential between experiments at 2 and 8 mM [Ca++]o. An example of one experiment in a shortened false tendon is shown in Figure 3. In every experiment, the non-normalized strength-duration curve shifted upward with increased [Ca++]o. This shift was due largely to a significant increase in the rheobasic current (Irh) at a [Ca++]o of 8 mM. There was no significant difference in the time constant of strength-duration curves (Tsd) at 2 and 8 mM [Ca++]o.

In the shortened false tendons, the mean (±SEM) Ih was 36 ± 8 nA at 2 mM [Ca++]o, and 62 ± 8 nA at 8 mM [Ca++]o (P < 0.02). In the long preparations, the mean Ih was 430 ± 72 nA at 2 mM [Ca++]o and 583 ± 79 nA at 8 mM [Ca++]o (P < 0.005). No consistent shift of the strength-duration curves was noted when the current and time axes were normalized by Ih and Tsd, respectively. However, due to deviations of the measurements from those predicted by the Lapicque equation (which was used to determine Tsd), uncertainty exists as to whether a real shift (albeit small) was masked by these deviations.

**Voltage Threshold for All-or-None Depolarization**

The change in the apparent voltage threshold (Vth) with [Ca++]o, was measured in five long false tendons using a double microelectrode technique. The mean maximum diastolic potential was -90 ± 2 mV at 2 mM [Ca++]o, and -87 ± 2 mV at 8 mM [Ca++]o (NS). There was a 14-mV shift of the Vth toward a less negative potential when [Ca++]o was increased. The mean Vth was -62 ± 2 mV at 2 mM [Ca++]o, and -48 ± 2 mV at 8 mM [Ca++]o (P < 0.01). The potential difference between Vm and Vth increased with increasing [Ca++]o: from 27 ± 1 mV at 2 mM [Ca++]o to 39 ± 2 mV at 8 mM [Ca++]o (P < 0.001).

Six additional experiments were performed using a single sucrose gap technique to apply intracellular current. Using this technique, the mean Vm was slightly less negative than in the previous experiments (-84 ± 3 mV, [Ca++]o 2 mM; -83 ± 2 mV, [Ca++]o 8 mM) but, again, there was no significant difference between the Vm at the two calcium concentrations. Increasing [Ca++]o resulted in a shift of Vth to less negative potentials: -62 ± 3 mV, [Ca++]o 2 mM; -48 ± 3 mV, [Ca++]o 8 mM (P < .0001).

Figure 4 shows one of two experiments in which the Vth was determined using the single sucrose gap technique and a variable current pulse duration (5 to
500 msec. The $V_h$ shifted to less negative potentials at both $[Ca^{++}]_0$ as the pulse duration increased (accommodation). However, the change in $V_h$ with pulse duration was much more pronounced at the higher $[Ca^{++}]_0$. The potential difference between $V_h$ at 2 and 8 mM $[Ca^{++}]_0$ was only 5 mV with a 5-msec current pulse but was 20 mV with a 200-msec current pulse. Thus, increasing $[Ca^{++}]_0$ not only shifted the $V_h$ to less negative potentials but increased the degree of accommodation of $V_h$ with the duration of current application.

### Cable Analysis

Cable analysis was performed in seven long false tendons at 2 mM and 8 mM Ca$^{++}$ to determine the effects of $[Ca^{++}]_0$ on the cable constants ($\lambda$, $R_{in}$, $\tau_m$) and the unit constants ($r_i$, $r_m$). The results are shown in Table 3. The mean length of the preparations was 11.4 ± 1.0 mm; the current microelectrode was a mean of 5.6 ± 0.9 mm from the termination of the false tendon (L); the mean intensity of the hyperpolarizing current pulses were 90 ± 12 nA. Six of the experiments were performed in a bicarbonate buffer and one (No. 3, Table 3) was performed in a Hepes buffer.

In all experiments, increasing $[Ca^{++}]_0$ from 2 to 8 mM resulted in a decrease in space constant, decrease in time constant, and increase in input resistance. There was a slight (3 mV) difference in $V_m$ between the preparations in 2 and 8 mM $[Ca^{++}]_0$ ($P < 0.05$). We believe that this small difference in $V_m$ did not influence substantially the cable analysis. Membrane resistance, which is the most membrane potential-sensitive property, is virtually independent of $V_m$ when measured with hyperpolarizing current pulses in the −80 to −100 mV range (Hellam and Studt, 1974; their Fig. 12).

From the individual measurements of $\lambda$ and $R_{in}$, the unit constants $r_i$ and $r_m$ were calculated. There was a 52% increase in longitudinal resistance per unit length when $[Ca^{++}]_0$ was increased from 2 to 8 mM: from 0.84 ± 0.13 m$\Omega$·cm, $[Ca^{++}]_0$ 2 mM to 1.28 ± 0.19 m$\Omega$·cm, $[Ca^{++}]_0$ 8 mM ($P < 0.005$). A small but not significant decrease in membrane resistance X unit length was observed: 42 ± 12 K$\Omega$·cm, $[Ca^{++}]_0$ 2 mM; 30 ± 6 K$\Omega$·cm, $[Ca^{++}]_0$ 8 mM. Using the mean bundle radius of 66 μm from the dimensional analysis, an estimate of the specific longitudinal resistance ($R_l$) and specific membrane resistance ($R_{im}$) was calculated. $R_l$ was 114 $\Omega$·cm at 2 mM $[Ca^{++}]_0$ and 174 $\Omega$·cm at 8 mM $[Ca^{++}]_0$. $R_{im}$ was 1736 $\Omega$·cm$^2$ at 2 mM $[Ca^{++}]_0$ and 1240 $\Omega$·cm$^2$ at 8 mM $[Ca^{++}]_0$.

To determine the effects of $[Ca^{++}]_0$ on the effective capacitance filled by the foot of the action potential, the time constant of the action potential foot ($\tau_{AP}$) was measured by phase-plane analysis in five experiments. A representative experiment with concurrently measured interelectrode conduction velocity is shown in Figure 5. As $[Ca^{++}]_0$ was varied from 1 to 2 to 8 mM, the mean $\tau_{AP}$ increased progressively from 0.115 ± 0.004 msec to 0.121 ± 0.004 msec to 0.160 ± 0.007 msec, respectively (n = 5; $P < 0.001$). The capacitance per unit length ($C_{AP}$) was 0.203 μF/cm at $[Ca^{++}]_0$ 2 mM and 0.147 μF/cm at $[Ca^{++}]_0$ 8 mM. The specific capacitance filled by the foot ($C_{AP}$) was 4.90 μF/cm² at

### Table 3

Effects of Calcium on Cable Properties

<table>
<thead>
<tr>
<th>$[Ca^{++}]_0$ 2 mM</th>
<th>$[Ca^{++}]_0$ 8 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>$V_m$ (mV)</td>
</tr>
<tr>
<td>-----</td>
<td>----------</td>
</tr>
<tr>
<td>1</td>
<td>-88</td>
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<tr>
<td>2</td>
<td>-82</td>
</tr>
<tr>
<td>3</td>
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</tr>
<tr>
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<td>-83</td>
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<td>-88</td>
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</tr>
<tr>
<td>Mean</td>
<td>-83</td>
</tr>
<tr>
<td>SEM</td>
<td>±0.3</td>
</tr>
</tbody>
</table>

No. = number of experiment; $V_m$ = diastolic membrane potential; $\lambda$ = space constant; $R_{in}$ = input resistance; $\tau_m$ = membrane time constant.

* $P < 0.01$; † $P < 0.025$. 
Verapamil significantly decreased the mean action potential duration (APD) at 50% repolarization and increased APD at 90 and 100% repolarization. No significant effects of verapamil at 3 × 10⁻⁶ M concentration were observed on maximum diastolic potential or overshoot. V_m depolarized by 5 and 23 mV at a verapamil concentration of 1 × 10⁻⁵ M. Table 4 shows results of the seven experiments with the values expressed as means ± sem. Verapamil significantly increased the membrane time constant from control. In addition, in all seven experiments, verapamil increased the space constant (P < 0.01). The effects of verapamil on the unit constants were evaluated by calculation of n and r_m from λ and R_m. Verapamil significantly increased r_m from control: 20 ± 4 KΩ·cm to 47 ± 18 KΩ·cm, P < 0.05. Verapamil had no significant effect on r_l.

When the [Ca++]_o was increased from 2 to 8 mM in the presence of verapamil, the space constant decreased (P < 0.02) and the input resistance increased (P < 0.01). The presence of verapamil did not significantly alter the magnitude of the [Ca++]_o-induced changes in λ and R_m from those changes previously observed during [Ca++]_o changes in the absence of verapamil. In the presence of verapamil, n increased from 0.67 ± 0.16 MΩ/cm at 2 mM [Ca++]_o to 1.31 ± 0.27 MΩ/cm at 8 mM [Ca++]_o (n = 7; P < 0.002); r_m remained unchanged during alteration of [Ca++]_o in the presence of verapamil. The lack of effect of verapamil on [Ca++]_o-induced increases in n was not concentration dependent because similar changes in n were observed at 1 × 10⁻⁵ M as in 3 × 10⁻⁵ M verapamil.

Six experiments were performed to test the effects of La+++ ions on [Ca++]_o-induced changes in cable properties. Alteration of the buffer from HCO₃⁻ to Hepes resulted in changes in both action potential characteristics and cable properties. Therefore, control measurements were performed with 2 mM [Ca++]_o in the Hepes buffer for the experiments in which La+++ was employed. In five experiments, the [La+++]_o was 0.2 mM and in one experiment, 1.0 mM. The 1.0 mM La+++ concentration resulted in irreversible depolarization to a resting potential of −40 to −50 mV. Irreversible depolarization to a resting potential of −40 mV also occurred in one of the five experiments at 0.2 mM La+++ in the other four experiments, La+++ had no significant effects on maximum diastolic potential, overshoot or APD at 50% repolarization. La+++ significantly increased APD at 100% repolarization from 349 ± 19 to 415 ± 31 msec (P < 0.05). An experiment showing the effects of La+++ and Ca++ is displayed in Figure 6. The results of cable analysis are shown in Table 5. No significant effects of La+++ were noted on mean λ, R_m, or τ_m. More importantly, there were no significant effects of [Ca++]_o on λ, R_m, or τ_m in the presence of La+++.

Therefore, n and r_m did not change in the presence of La+++ alone or with elevation of [Ca++]_o. The effects on n of changing [Ca++]_o in the absence and presence of verapamil and La+++ are summarized in Figure 7.

We performed additional experiments to determine whether the Hepes buffer alone could account for a lack of effect of increased [Ca++]_o on n. When La+++ was absent from the Hepes-buffered Tyrode’s solution (Table 3, experiment 3), increasing [Ca++]_o resulted in a 70% increase in n. In other experiments,
we precipitated La$$^{++}$$ by increasing the pH to 7.5. Under these conditions, increasing $$[\text{Ca}^{++}]_o$$ from 2 to 8 mM resulted in appropriate increases in $$r_l$$. Thus, the presence of La$$^{++}$$ ions and not the buffer, per se, was necessary to block the effect of increasing $$[\text{Ca}^{++}]_o$$ on $$r_l$$.

**Discussion**

The results of this study show that alteration of the extracellular calcium concentration affects several determinants of cellular excitability and conduction. Increasing $$[\text{Ca}^{++}]_o$$ from 2 to 8 mM resulted in: (1) a progressive decrease in conduction velocity, (2) a shift of $$V_{\text{max}}-V_m$$ relationship by 7 mV toward less negative potentials and an increase in slope factor (k), (3) an increase in rheobasic current, (4) a shift of the voltage threshold for all-or-none depolarization to less negative potentials, (5) a 52% increase in longitudinal resistance per unit length ($$n$$), and (6) a surprising 27% decrease in the capacitance filled by the foot of the action potential. Our data also demonstrated that blockade of the slow inward current by 3 mM Mn$$^{++}$$ or 3 $$\times$$ $$10^{-6}$$ M verapamil did not alter the effects of $$[\text{Ca}^{++}]_o$$ on the slope factor changes or voltage shifts of the $$V_{\text{max}}-V_m$$ relation. Furthermore, verapamil did not affect the $$[\text{Ca}^{++}]_o$$-induced changes in $$n$$, whereas 0.2 mM La$$^{+++}$$ completely blocked the effect of $$[\text{Ca}^{++}]_o$$ on $$r_l$$.

The changes in $$V_{\text{max}}$$, overshoot, $$I_{th}$$, and $$V_{th}$$ observed with varying $$[\text{Ca}^{++}]_o$$ probably are related to the effects of Ca$$^{++}$$ ions on the voltage- and time-dependent functions of the fast inward Na$$^+$$ current ($$i_{\text{Na}}$$) of Purkinje cells. Increasing $$[\text{Ca}^{++}]_o$$ resulted in voltage shifts of both the $$V_{\text{max}}-V_m$$ relation and $$I_{th}$$ toward less negative potentials. In the high Ca$$^{++}$$ medium, a greater degree of membrane depolarization was required to decrease $$V_{\text{max}}$$ as well as to reach threshold. On these attributes, there is a wide consensus in both nerve and muscle preparations regarding

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**TABLE 4**

**Effect of Verapamil and $$[\text{Ca}^{++}]_o$$ on Cable Properties**

<table>
<thead>
<tr>
<th>$$[\text{Ca}^{++}]_o$$</th>
<th>$$[\text{Ca}^{++}]_o$$</th>
<th>$$[\text{Ca}^{++}]_o$$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, 2 mM</td>
<td>Control, 2 mM + verapamil</td>
<td>Control, 2 mM + laurate</td>
</tr>
<tr>
<td>2 mM + verapamil</td>
<td>8 mM + verapamil</td>
<td>2 mM + laurate</td>
</tr>
<tr>
<td>Vm (mV)</td>
<td>Rin (msec)</td>
<td>n</td>
</tr>
<tr>
<td>3 $$\times$$ $$10^{-6}$$ M</td>
<td>84 ± 3</td>
<td>1.48 ± 0.26†</td>
</tr>
<tr>
<td>1 $$\times$$ $$10^{-5}$$ M</td>
<td>74 ± 4</td>
<td>1.84 ± 0.04</td>
</tr>
</tbody>
</table>

* P < 0.01 compared to $$[\text{Ca}^{++}]_o$$, 2 mM + verapamil; † P < 0.025 compared to $$[\text{Ca}^{++}]_o$$, 2 mM + verapamil; ‡ P < 0.05 compared to control.

For abbreviations see Table 3.

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**FIGURE 6.** Effects of $$[\text{Ca}^{++}]_o$$ on cable properties in the presence of La$$^{+++}$$. Each of the three columns show, in order from the top: the action potential, measurement of intracellular current intensity (I), voltage recorded from the proximal recording site ($$V_p$$), and voltage recorded from the distal recording site ($$V_d$$). Action potential duration increased with addition of La$$^{+++}$$ to the superfusate. No significant changes in internal longitudinal resistance per unit length ($$r_l$$) were observed. In this experiment, the membrane resistance $$\times$$ unit length ($$r_{m}$$) increased during La$$^{+++}$$ superfusion. $$r_l$$ values: 0.69 M$$\Omega$$-cm, 2 mM $$[\text{Ca}^{++}]_o$$; 0.67 M$$\Omega$$-cm, 2 mM $$[\text{Ca}^{++}]_o$$ + 0.2 mM La$$^{+++}$$; 0.64 M$$\Omega$$-cm, 8 mM $$[\text{Ca}^{++}]_o$$ + 0.2 mM La$$^{+++}$$; $$r_m$$ values: 17 k$$\Omega$$-cm, 20 k$$\Omega$$-cm, and 26 k$$\Omega$$-cm, respectively. The upstrokes of the action potentials were retouched for clarity; current records were retraced from original records.
The decrease in conduction velocity (Q) with increasing $[Ca^{++}]_0$ is the net result of changes in several parameters (such as the present data) affecting the shape of the $V_{\text{max}} - V_m$ relation (Walton and Fozzard, 1979). In propagated action potentials (such as the present data), the relationship between $V_{\text{max}}$ and sodium conductance is expected to be non-linear (Hunter et al., 1975; Cohen et al., 1981). Thus, there is too much uncertainty in the present work to draw conclusions regarding $[Ca^{++}]_0$-related changes in the kinetic parameters of $I_{Na}$. However, the $[Ca^{++}]_0$-related changes in the shape of the $V_{\text{max}} - V_m$ curves do suggest that the effects of $Ca^{++}$ on $I_{Na}$ may be more involved than simple voltage shifts of steady state activation and inactivation variables.
cellular factors: (1) changes in inward sodium current, (2) increased current threshold, (3) increased internal longitudinal resistance ($r_t$), and (4) changes in capacitance. Of these four factors, the change in $I_{Na}$ with varying [Ca++]o probably affected conduction velocity by only a minor amount. Evidence for this conclusion comes from indirect assessment of $I_{Na}$ (using measurements of $V_{max}$) during changes in [Ca++]o. Inasmuch as $V_{max}$ approximates the magnitude of $I_{Na}$, the amount of decrease in $V_{max}$ with a 4-fold increase in [Ca++]o was small (7-10%). Due to the increased rate of inactivation at lower [Ca++]o, without prior conditioning hyperpolarization, $I_{Na}$ may even increase when [Ca++]o is increased (Frankenhaeuser and Hodgkin, 1957; Frankenhaeuser, 1957). Furthermore, if [Ca++]o-induced changes in $I_{Na}$ were a major influence on changes in conduction velocity, the effect might change at depolarized potentials due to the voltage shifts in the $I_{Na} - V_m$ curves. With regard to this point, the experiment measuring [Ca++]o-induced changes in $V_{max}$ and $\Theta$ at a $V_m$ of $-65$ mV demonstrated that the direction and magnitude of [Ca++]o-induced changes in $\Theta$ was influenced little by depolarization. In fact, the changes in $V_{max}$ and $\Theta$ were in opposite directions (an observation also made by Peon et al., 1978). A more likely factor influencing conduction velocity (but one that is less clear theoretically) is the ratio between the current generated during the action potential upstroke and the current required to produce a threshold response. The strength-duration curves performed in shortened and long false tendons demonstrate that increased [Ca++]o increases the threshold intensity of current at all pulse durations. This increase in threshold current intensity makes the current generated during the upstroke a less effective depolarizing stimulus for adjacent cells at high [Ca++]o. In addition, the [Ca++]o-induced increase in internal longitudinal resistance provides a greater impedance to current flow between cells. The net effect of the [Ca++]o-induced increase in $I_{h}$ and $r_t$ is a decrease in conduction velocity. The decrease in capacitance with increasing [Ca++]o may partially offset the decrease in $\Theta$. The difficulty in assessing the influence of capacitance on $\Theta$ arises from the fact that it is a derived (not directly measured) quantity.

The 27% decrease in capacitance filled by the foot of the action potential ($C_{AP}$) particularly is surprising for several reasons. First, biomembrane capacitance is remarkably stable under physiological conditions and, hence, no changes might be expected to occur with increasing [Ca++]o. Second, if there is an effect of external calcium ions, the decrease in $\Theta$ and increase in $\tau_{AP}$ would allow more local current spread during propagation into membranes adjacent to the surface membrane (e.g., intercellular clefts), thereby increasing the area of membrane to be discharged during the foot. This would suggest that increasing [Ca++]o should increase (not decrease) the $C_{AP}$. Furthermore, the fact that $C_{AP}$ is calculated from several measured quantities ($\tau_{AP}$, $r_t$, and $\Theta$) compounds the chance for error and complicates the solution of the problem. However, several other pieces of information suggest that the decrease in $C_{AP}$ with increasing [Ca++]o may be real. Hodgkin and Nakajima (1972) have analyzed the capacitance of the foot of the action potential in frog skeletal muscle. Their analysis showed that $C_{AP}$ depends upon the product of an admittance term ($Y_f$) and the time constant of the foot ($\tau_{AP}$). Calculation of the change in $Y_f$ with increasing [Ca++]o (Equation 2, Hodgkin and Nakajima, 1972) shows that the decrease in $C_{AP}$ occurs because $Y_f$ decreases by a greater extent than $\tau_{AP}$ increases when [Ca++]o is elevated. The only problem with this calculation of $Y_f$ is that reliance is placed again upon the same measured quantities ($\tau_{AP}$, $r_t$, and $\Theta$) from which $C_{AP}$ was calculated. Thus, an assessment of capacitance independent of these quantities is desirable. Since the membrane time constant ($r_m$) is the product of $r_m$ and $c_m$ (membrane capacitance per unit length), an estimate of the percent change in $c_m$ with [Ca++]o can be obtained from the ratio of membrane time constants to the $r_m$ ratio (obtained from $\lambda$ and $R_{in}$). Using values of $r_m$ and $c_m$ from the cable analysis, $c_m$ decreased a mean of 20% for the increase in [Ca++]o from 2 to 8 mM. In addition, the equation proposed by Huxley ($\Theta \propto (V_{max}/[V_{p}c_{m}+(r_{t}+r_{o})])^{1/2}$, where $V_{p} =$ action potential amplitude, $r_{e} =$ extracellular resistance per unit length; cf. Fitzugh, 1969) also allows a percent change in $c_m$ to be calculated (assuming $r_{o}$ is negligible compared to $r_{t}$). Using the mean values of $V_{max}$, $V_{p}$, and $\Theta$ from Table 1 and the mean values of $r_{t}$ from cable analysis, $c_m$ decreased 16% for an increase from 2 to 8 mM [Ca++]o. The net result of these calculations is that capacitance decreases approximately 20% in raising [Ca++]o from 2 to 8 mM. This result becomes more understandable if the electrostatic definition of membrane capacitance is considered: $c_m =$ $|q|/V$, where $|q|$ = charge per unit area and $V =$ voltage applied across the membrane (for discussion, see Woo and Wei, 1973). A decrease in $c_m$ is the predicted result for an increase in [Ca++]o because increasing [Ca++]o is known to decrease membrane surface charge (McLaughlin et al., 1971; Brown and Noble, 1978) and yet not affect bulk phase membrane potential (i.e., the applied voltage). In a somewhat analogous situation, lowering pH to decrease surface charge density has been demonstrated to decrease $c_m$ of artificial lipid bilayers (Woo and Wei, 1973). Thus, the surprising finding that $C_{AP}$ decreases with increasing [Ca++]o in fact may be consistent with the predictions and findings of the effects of Ca++ ions on charged phospholipid membranes.

The effects of [Ca++]o on cardiac cable properties observed in this study contrast with the lack of significant effects reported by Reuter (1967) and De Mello (1975). We believe these differences relate partially to the fact that previous studies were performed on resting preparations, and superfusion times were relatively brief (2-10 minutes). The present experiments were performed during brief pauses in a constantly paced preparation. Changes in cable properties required 15-20 minutes of altered [Ca++]o superfusate.
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to reach steady state. Increasing [Ca\(^{++}\)]\(_o\) from 2 to 8 mM did not result in complete electrical uncoupling in any of the experiments. However, when [Ca\(^{++}\)]\(_o\) was increased 4-fold, a consistent and significant decrease in \(\lambda\) and increase in \(R_m\) were observed in all experiments. Application of cable theory allowed calculation of the changes in \(r_i\) and \(r_m\). The results show a mean increase of 52% in \(r_i\) and a 29% decrease in \(r_m\) with a 4-fold increase in [Ca\(^{++}\)]\(_o\). The change in \(r_i\) was highly significant, whereas the decrease in \(r_m\) was not significant. The effect of [Ca\(^{++}\)]\(_o\) on \(r_i\) is consistent with the postulated mechanism for the well-known action of Ca\(^{++}\) ions in the "healing-over" of injured cardiac cells (Délezé, 1970; De Mello and Dexter, 1970). In addition, the [Ca\(^{++}\)]\(_o\)-induced increase in \(r_i\) is consistent with the decoupling of cardiac cells produced by the intracellular injection of Ca\(^{++}\) ions (De Mello, 1975). The recent description of ultrastructural changes in gap junctional architecture with divalent cations (Peracchia and Peracchia, 1980; Dahl and Isenberg, 1980), together with the findings of this study, support the hypothesis that Ca\(^{++}\) ions control the normal cell-to-cell coupling in mammalian myocardium. Furthermore, the production of electrical uncoupling by metabolic inhibitors such as 2,4-dinitrophenol and reversal by intracellular injection of EDTA (De Mello, 1979a) provide support for the hypothesis that Ca\(^{++}\) ions may mediate the uncoupling of cells in diseased or damaged myocardium. Nevertheless, the lack of direct effects of verapamil on \(r_i\) and the persistence of [Ca\(^{++}\)]\(_o\)-induced changes in \(r_j\) during verapamil superfusion, and the observation that La\(^{+++}\) blocked completely the [Ca\(^{++}\)]\(_o\)-induced increase in \(r_i\) observed when raising [Ca\(^{++}\)]\(_o\) may best be explained by Ca\(^{++}\) entry into the cell via Na/Ca exchange.

The results show that 0.2 mM [La\(^{+++}\)]\(_o\) completely blocked the expected increase in \(r_i\) with [Ca\(^{++}\)]\(_o\). La\(^{+++}\) is known to block the slow inward current (Kass and Tsien, 1975), to displace \(^{45}\)Ca\(^{++}\) from superficial membrane binding sites (Sanborn and Langer, 1970; Chovan et al., 1979; Pang, 1980), and to inhibit the Na/Ca exchange mechanism (Langer and Frank, 1972; Reeves and Sutko, 1979). Verapamil is known to have no significant effect on the ability of cardiac microsomes or the sarcolemma to exchange or accumulate \(^{45}\)Ca\(^{++}\) (Nayler and Szeto, 1972; Langer et al., 1975). The effects of verapamil on superficial \(^{45}\)Ca\(^{++}\) binding are less certain because both significant displacement of bound Ca\(^{++}\) (Chovan et al., 1979; Pang, 1980) and no effect (Langer et al., 1975) have been reported. Nevertheless, the lack of direct effects of verapamil on \(r_i\) the persistence of [Ca\(^{++}\)]\(_o\)-induced changes in \(r_j\) during verapamil superfusion, and the observation that La\(^{+++}\) blocked completely the [Ca\(^{++}\)]\(_o\)-induced increase in \(r_i\) suggest that the increase in \(r_i\) observed when raising [Ca\(^{++}\)]\(_o\) may best be explained by Ca\(^{++}\) entry into the cell via Na/Ca exchange.

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**References**


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**FIGURE 8. Hypothesized pathways for the effects of extracellular calcium ions on internal longitudinal resistance (r).** A schematic diagram is shown of the sarcolemma, sarcoplasm, and gap junctional membrane of two adjacent cardiac cells. Internal longitudinal resistance is composed of a sarcoplasmic and junctional component in series. Extracellular calcium ions could affect \(r_i\) by any or all of the three pathways listed.
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