the steroids of maternal origin, did not modify receptor distribution in the endometrium of pregnant rats. We cannot completely exclude the possibility that the blastocyst secretes an unknown factor that enhances receptor translocation from cytosol to nucleus. Nevertheless, our observations are in agreement with the hypothesis that assigns an active role to the blastocyst in implantation.

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7. After ovariectomy on the morning of day 5, normal implantation occurs on the morning of day 6. In the absence of progesterone, however, implantation is not maintained thereafter (P. Sartor, in preparation).
8. Cytosolic receptors for estradiol were present in concentrations of 5.49 ± 0.38 and 2.42 ± 0.81 pmole per milligram of DNA (four determinations) at the implantation sites and in the intermediary regions, respectively. The values for cytosolic receptors for progesterone were 4.17 ± 0.09 and 2.23 ± 0.26, respectively, in the same regions. Thus ovariectomy caused an important increase in the total cellular concentration of progesterone receptors at the implantation sites.
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Calcium Transients During Excitation-Contraction Coupling in Mammalian Heart: Aequorin Signals of Canine Purkinje Fibers

Abstract. *Aequorin signals in mammalian heart muscle cells reveal the existence of two temporally distinct processes that increase cytoplasmic calcium ions after membrane excitation. The differential dependence of these processes on the pattern of stimulation suggests that the first process is, or is closely related to, calcium entry through the surface membrane and that the second is calcium release from intracellular storage sites.*

The ability to directly observe intracellular Ca^{2+} transients is central to the solution of problems in the investigation of excitation-contraction coupling (*1*). In this report I show that through the use of the Ca^{2+}-sensitive photoprotein aequorin, such direct observations of intracellular Ca^{2+} transients have now been made in mammalian heart muscle. These observations are relevant to several concepts about excitation-contraction coupling in mammalian heart cells: First, Ca^{2+} enters the cell during the plateau of the action potential (*2*). As part of this process the ion channels inactivate but later recover in a simple exponential fashion (*3*) during the repolarized interval between action potentials. Second, during the action potential Ca^{2+} is released into the cytoplasm from intracellular storage sites (stores) (*4*). The stores are replenished by the Ca^{2+} entering during the action potential, and by active sequestration of the previously released Ca^{2+}. The Ca^{2+} taken up by the storage mechanism is not again available for release until after a delay. As a consequence (of replenishment and delayed reavailability), the amount of Ca^{2+} released during an action potential has been thought to depend in a rather complex way on the pattern of stimulation (*4*). The aequorin signals in canine Purkinje fibers have features consistent with these concepts.

Strands of Purkinje tissue were excised from either ventricle of canine hearts and mounted in a small (1 ml), temperature controlled (35° ± 0.5°C), recording chamber. The superfusing solution flowed continuously (4 ml/min) and was equilibrated with 95 percent O_2 and 5 percent CO_2 and contained 123 m*M* NaCl, 5.4 m*M* KCl, 2.7 m*M* $CaCl_2$, 0.42 m*M* NaH_2PO_4, 23.8 m*M* $NaHCO_3$, 1.0 m*M* $MgCl_2$, and 5.5 m*M* glucose. The strand was electrically stimulated by 0.5-msec pulses of current flowing between a punctate cathode near one end of the strand and a distant anode. The force of contraction was measured with an AME semiconductor force transducer. Membrane potential was measured with the micropipettes used to pressure-inject aequorin. The extraction and purification of aequorin was as described in (*5*). Light from the aequorin-injected strand was detected with an EMI 9635 B photomultiplier tube (PMT) and recorded as anode current. Signals (light, force, membrane potential) were stored on magnetic tape for later analysis (signal averaging). In each muscle 10 to 20 intracellular microinjections of aequorin were made along a 3-mm length of the strand.

Figure 1A shows examples of the three kinds of signals routinely recorded in these experiments. Light and isometric force development were normally recorded simultaneously throughout the experiment. Membrane potential was recorded before and after microinjection, but not during light recording.

Light begins to rise to levels detectably above the photomultiplier tube noise about 10 msec after the upstroke of the action potential. It then continues to rise rapidly for approximately 18 msec. In a distinctly separate phase, the light rises more slowly over a period of about 55 msec and reaches a peak roughly 80 msec after the onset of excitation. In this signal light declined exponentially (τ = 31 msec) over a period of 80 msec beginning about 10 msec after peak light intensity.

The configuration of aequorin signals is highly dependent on the inotropic state of the muscle (*6*, *7*). In the present experiments variations in inotropic state and the aequorin signal were produced by varying the pattern of stimulation. Figure 1B illustrates the simplest situation—that of stimulation at regular intervals. In this protocol stimulation was begun at the longest interval and progressed through successively shorter intervals as a steady-state response was achieved at each interval. The resulting relation between stimulus interval and steady-state maximum isometric force qualitatively resembled that observed in mammalian atrium (*8*) and sheep Purkinje fibers (*3*) in that the contractions at long intervals were large. In Fig. 1B, for intervals of 5 seconds and less, the aequorin signal consists of the initial rapid rise followed either by a plateau or an additional inflection on the declining phase. The light

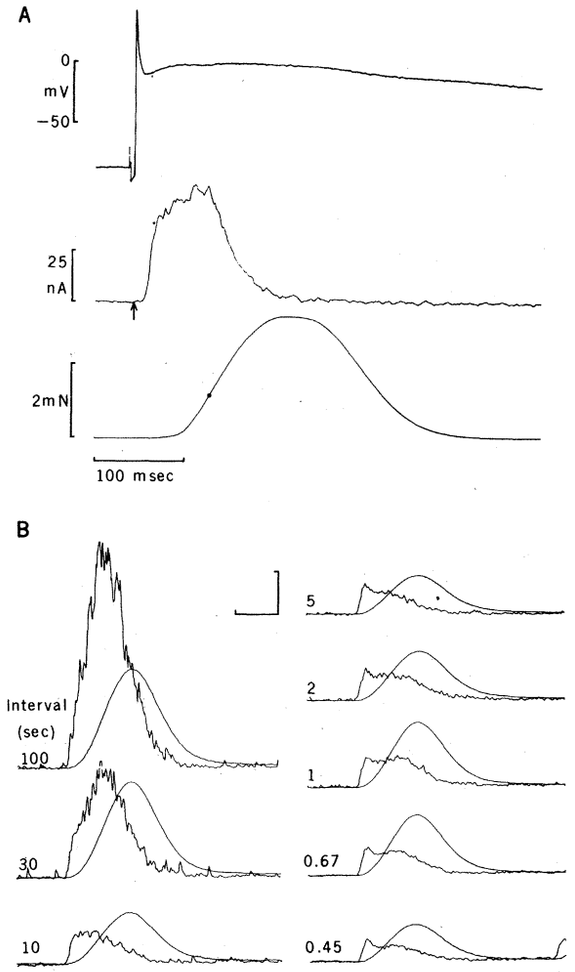

Fig. 1. (A) Recordings of membrane potential (top trace), light (middle trace), and force of isometric contraction (bottom trace). The arrow marks the time of action potential upstroke as seen in upper trace. The point in the force tracing marks the time at which the rate of force development was greatest. A total of 128 light and force signals were averaged. Paired stimulation: 5-second interval alternating with 1-second interval. The responses shown were preceded by a 5-second interval. Action potential duration: 700 msec. (B) Influence of the interval between stimuli on the aequorin signal and force of contraction. The stimulus interval (in seconds) is indicated at the beginning of each set of superimposed light and force tracings. Vertical calibration bar: 5 nA or 0.5 mN. Horizontal calibration bar: 100 msec.

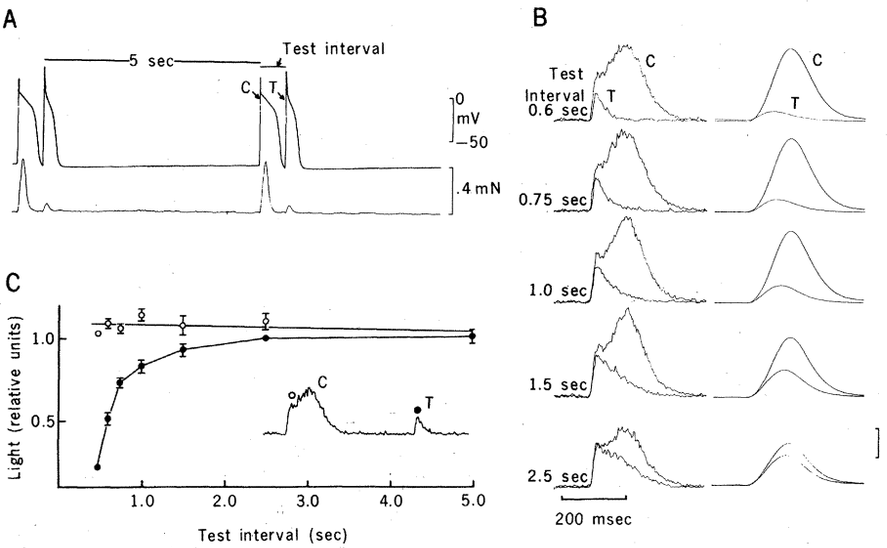

Fig. 2. (A) Illustration of stimulus protocol; records of membrane potential and contractions. Conditioning responses (*C*) were always preceded by a 5-second stimulus interval. Test responses (*T*) were generated after a variable test interval. (B) Light and force records from an experiment in which the protocol was as shown in (A). The conditioning and test responses at each test interval (indicated) are shown superimposed for the light signals (on the left) and force (on the right). The conditioning response is always the larger one of each pair. The vertical calibration bar represents 2.5 nA or 0.4 mN; 128 signals were averaged. (C) Graphical representation of the result of four experiments as shown in (A) and (B). Points are means ± standard error except for the single value point at the 0.45-second interval. Symbols: ○, amplitude of initial phase of conditioning aequorin signals; ●, amplitude of initial phase of test aequorin signals. Amplitudes have been normalized in each experiment to the amplitude of the test signal in that experiment at a test interval of 2.5 seconds. Lines were drawn by eye.

measured 100 msec after the stimulus varies in the same direction as does the peak force. There is a clear progressive flattening and diminution of this part of the aequorin signal at intervals on either side of 1 second and this is paralleled by changes in force development. The amplitude of the initial rapidly rising phase does not have the same dependence on the stimulus interval, being reduced at intervals of 0.45 and 0.67 second but constant for intervals up to and including 10 seconds. In the signal at 30-second intervals an initial rapidly rising phase is still discernible, occurring before a larger, more slowly rising phase. At 100-second intervals the signal consists of a rapid increase to a peak followed by an almost equally rapid decline. In a minority of muscles the contractions and aequorin signals at long intervals were smaller than at intermediate intervals.

The peak amplitude of the initial rapid phase is reduced at short intervals but seems to be relatively constant at longer intervals (up until it becomes indistinguishable at very long intervals). It is as if the underlying process recovers during the diastolic interval, with most of the recovery occurring in about 2 seconds. To examine this idea directly, experiments were done according to the protocol shown in Fig. 2A. Pairs of stimuli were given to generate first a conditioning response (C) and then, after a variable test interval, a test response (T). The interval preceding each conditioning response (5 seconds) was long enough so that the conditioning action potential configuration was relatively constant. The results of this kind of experiment are shown in Fig. 2, B and C. The peak amplitude of the rapid phase of the test aequorin signal recovers rapidly as the test interval is lengthened, until recovery is complete at 2.5 seconds. This result is shown graphically in Fig. 2C in which the data from four such experiments have been plotted. An additional striking effect of this protocol is that in the conditioning aequorin signals, particularly at the short test intervals, light intensity after the initial rapid phase is markedly increased over what it was during stimulation at regular 5-second intervals compare Fig. 1B; Figs. 1B and 2B illustrate data from the same preparation). The test aequorin signals at the shorter test intervals also differ distinctly in shape from signals occurring during stimulation at regular intervals; after the reduced initial rapid phase the light declines monophasically.

Some of the factors involved in the quantitative interpretation of aequorin

signals have been recognized and discussed elsewhere (*5, 7, 9*). For the interpretations in this study, two of the most important of these factors are: (i) during the aequorin signal spatial gradients of calcium concentration [Ca^{2+}] may exist within the cell (*10*), and (ii) the relation between [Ca^{2+}] and aequorin luminescence is nonlinear (*11*). Thus, transient aequorin signals may not represent even an "average" cellular [Ca^{2+}], as would a linear Ca^{2+} indicator. For spatially uniform [Ca^{2+}] the limit of detection in these experiments has been estimated, by the method of Allen and Blinks (*7*), to be $10^{-7}M$ (*12*).

The best available evidence (*13*) indicates that at 35°C aequorin luminescence rises with a half-time ($T_{1/2}$) of 3 msec after a rapid increase in [Ca^{2+}]. Only the initial rise of the signal is rapid enough ($T_{1/2}$ = 9 msec) to have been even slightly affected by aequorin kinetics.

Potential problems arise in the present study because of the use of a multicellular, multiply injected preparation. If one assumes a conduction velocity of 2 m/sec, the temporal relation between membrane excitation and the aequorin signal (Fig. 1A) is probably not in error by more than ± 1.5 msec, since the electrically recorded cell and the other injected cells were never more than 3 mm apart. Similarly, the aequorin signal is probably not temporally dispersed by more than 3 msec.

The observed interval dependence of the initial rapid phase strongly suggests that an increase in Ca^{2+} during this part of the signal is related to the entry of Ca^{2+} through the surface membrane via the slow inward current (I_{si}). Voltage clamp experiments show that recovery of I_{si} in Purkinje fibers is a relatively rapid exponential process at resting membrane voltage (*3*) (τ = 0.67 second, 95 percent complete within 2 seconds; compare Fig. 2C). Although the interval dependence of peak I_{si} and peak initial light are believed similar, the temporal occurrence of the two signals may be less so; for example, there is evidence that Ca^{2+} entry promotes rapid repolarization (*14*), an event that is nearly over before luminescence is detectable. An alternative hypothesis, that the initial phase represents predominantly release of Ca^{2+} from stores, cannot at present be excluded, but this would require that such release has a simple interval dependence similar to that of I_{si} rather than the more complex interval dependence usually attributed to release of Ca^{2+} from internal stores (*4*).

The decline in light that may immediately follow the initial rapid phase cannot be interpreted unequivocally. It certainly represents a decrease in [Ca^{2+}] but this decrease could be due to redistribution by diffusion, as well as active uptake into an aequorin-free compartment, or passive binding.

After the initial rapid rise, the time course and peak amplitude of the aequorin signal are not related in a simple way to the interval between stimuli. The signal may decline monophasically, it may have a plateau, or continue to rise to a peak before declining. The first case is usually evident only in depressed inotropic states (extrasystole, Fig. 2B), the second may occur during steady stimulation at regular intervals, and the last also occurs during steady stimulation at long intervals, but occurs particularly in potentiated inotropic states (Fig. 2B). Thus, this part of the aequorin signal is increased in potentiated inotropic states and decreased in depressed inotropic states. However, a direct relation between peak light intensity and peak force does not exist. The dependence of this part of the aequorin signal on the stimulus interval and the pattern of stimulation is not similar to any described interval dependence of I_{si}. This raises the possibility that an underlying process is release of Ca^{2+} from intracellular storage sites. This interpretation would be consistent with numerous studies that have implicated increased release of Ca^{2+} from storage sites as the major cause of inotropic effects produced by certain changes in the pattern of stimulation, for example, paired stimulation (*4*) (compare Fig. 2B).

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12. For an experiment in which a single cell was injected with aequorin and 700 responses were averaged, the minimum detectable L/L_{max} (L is light intensity during the response, and L_{max} is peak light intensity in saturating [Ca^{2+}] calculated from total light emitted during lysis of the cell) was 1.3×10^{-6}. This would correspond to $10^{-7}M$ Ca^{2+} for aequorin at 37°C, in the presence of 2 mM Mg^{2+} and 150 mM KCl. This is believed to be comparable to the more typical experimental situation in which ten cells were injected and 64 to 128 responses were averaged.
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Parathyroid Hormone: A Coronary Artery Vasodilator

Abstract. *Injection of synthetic bovine parathyroid hormone (the amino terminal peptide containing the first 34 amino acids) to the coronary circulation of the dog resulted in a marked coronary vasodilation. The vasodilatory response was dose-dependent and amounted to a 161 percent increase over the resting flow rate at a concentration of 1.0 unit per kilogram.*

A synthetic preparation of bovine parathyroid hormone containing the first 34 amino acids [bPTH(1-34)] was tested for possible vasoactive effects on the coronary circulation and other cardiovascular responses in the dog.

Five mongrel dogs of either sex and weighing 19 to 38 kg were used. The dogs were anesthetized with sodium pentobarbital (30 mg/kg), tracheostomized, and ventilated with room air by means of a positive pressure respirator (Harvard Apparatus). A left thoracotomy was performed through the fifth intercostal space. The pericardium was incised and sutured to the chest wall to form a cradle for the heart. A length (2 cm) of the left circumflex coronary artery was freed of surrounding connective tissue and a 7- to 9-mm electromagnetic flow probe was placed on the artery. The flow rate in the left circumflex artery was determined by means of a Carolina model 501D electromagnetic flowmeter (Carolina Medical Electronics). A 23-gauge thin-wall vein needle tip was inserted into the artery distal to the flow probe and allowed to remain indwelling during the course of the experiment for bPTH(1-34) or isotonic saline (control) injections.

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