

FLUCTUATIONS IN MEMBRANE CURRENT DRIVEN BY INTRACELLULAR CALCIUM IN CARDIAC PURKINJE FIBERS

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ABSTRACT Spontaneous oscillatory fluctuations in membrane potential are often observed in heart cells, but their basis remains controversial. Such activity is enhanced in cardiac Purkinje fibers by exposure to digitalis or K-free solutions. Under these conditions, we find that voltage noise is generated by current fluctuations that persist when membrane potential is voltage-clamped. Power spectra of current signals are not made up of single time-constant components, as expected from gating of independent channels, but are dominated by resonant characteristics between 0.5 and 2 Hz. Our evidence suggests that the periodicity arises from oscillatory variations in intracellular free Ca that control ion movements across the surface membrane. The current fluctuations are strongly cross-correlated with oscillatory fluctuations in contractile force, and are inhibited by removing extracellular Ca or exposure to D600. Chelating intracellular Ca with injected EGTA also abolishes the current fluctuations. The oscillatory mechanism may involve cycles of Ca (or Sr) movement between sarcoplasmic reticulum and myoplasm, as previously suggested for skinned cardiac preparations. Our experiments in intact cells indicate that changes in surface membrane potential can modulate cytoplasmic Ca oscillations in frequency and perhaps amplitude as well. A two-way interaction between surface membrane potential and intracellular Ca stores may be a common feature of heart, neuron, and other cell types.

INTRODUCTION

In a wide variety of excitable cells, the ionic current across the surface membrane undergoes fluctuations when measured under voltage clamp. These fluctuations are interesting because they often reflect the regulation of ion permeation at the molecular level. Axon or end-plate membranes, the most closely studied systems, show current fluctuations arising from the spontaneous opening and closing of individual ionic pores (for reviews, see Verveen and DeFelice, 1974; Conti and Wanke, 1975; Neher and Stevens, 1977). The probability of opening or closing can be influenced by membrane potential, transmitter concentration, or other physiological variables. But when these factors are held constant, the membrane current fluctuations have power spectra with single time-constant components, consistent with independent channels opening and closing at random (Stevens, 1972).

In addition to the noise expected from conventional channel gating, heart cells can display a fundamentally different kind of membrane current fluctuation whose characteristics are oscillatory rather than random. Such oscillatory fluctuations have been recorded under voltage

clamp in Purkinje fibers (Lederer, 1976; Lederer and Tsien, 1976; Kass et al., 1976; Aronson and Gelles, 1977; Eisner and Lederer, 1979), as well as single Purkinje cells (Mehdi and Sachs, 1978) and sinoatrial node (Irisawa and Noma, 1977). These fluctuations have several distinguishing properties. Their power spectra are not made up of single time-constant (Lorentzian) components, but show peaks at oscillatory frequencies near 1 Hz (Kass et al., 1976). The current fluctuations are associated with spontaneous contractile fluctuations. Both electrical and mechanical fluctuations are enhanced when intracellular free calcium (Ca_i) is elevated by bathing solutions containing high Ca_o , low Na_o , low K_o , or toxic concentrations of digitalis. Under these conditions, the oscillatory current fluctuations are much larger than the fluctuations expected from random opening and closing of channels in the resting membrane. The electromechanical fluctuations seem related to damped oscillations in membrane current or force that are triggered by a hyperpolarizing voltage change (see Tsien et al., 1979, for earlier references; see also Eisner and Lederer, 1979; Vassalle and Mugelli, 1981).

In this paper, we provide the first description of oscilla-

tory membrane current fluctuations using techniques of spectral analysis. Our experiments were carried out with a number of questions in mind. What is the relationship between the fluctuations in membrane current and contractile force? How do the fluctuations respond when intracellular calcium is buffered by EGTA or replaced by strontium? What influence does membrane potential have on the magnitude and frequency of the fluctuations? What is the relationship between spontaneous fluctuations and oscillations evoked by a hyperpolarizing potential change?

Some preliminary aspects of this work were reported to the Biophysical Society (Kass et al., 1976).

METHODS

Membrane potential, membrane current, and contractile force were measured in short calf Purkinje fiber bundles as described previously (Kass et al., 1978a). During the initial mounting and impalement procedures, the preparations were superfused with a modified Tyrode solution with the following composition (in mM): 150 Na, 4 K, 5.4 Ca, 0.5 Mg, 155.8 Cl, 10 Tris-maleate (pH 7.2–7.4). The solutions also contained 5 mM glucose and were pregassed with 100% O₂. To enhance oscillatory phenomena, including spontaneous current fluctuations, a state of “Ca-overload” was produced by repetitive stimulation in the presence of 1–2 μ M strophanthidin (Sigma Chemical Co., St. Louis, MO), or by exposure to 0-K Tyrode solution (see Eisner and Lederer, 1979).

In EGTA-injection experiments, the preparation was impaled with a bevelled micropipette containing 0.5 M K-EGTA (neutralized to pH 7 with KOH). This micropipette was used to pass current during voltage-clamp runs, and for iontophoretic injection of EGTA with negative current pulses during other periods. The amount of EGTA injected is expressed roughly as cumulative negative iontophoretic charge (see Siegelbaum and Tsien, 1980).

For noise analysis, membrane current or force signals were filtered with a 10-Hz cut-off frequency, digitized at 40 Hz, and analyzed for spectral density (Bendat and Piersol, 1971). Most of the experiments were performed at the University of Rochester, using a PDP-11 computer (Digital Equipment Corp., Maynard, MA) and our own programs to calculate power density spectra. Figs. 7, 9, and 10 are results from an earlier series of experiments at Yale University, where analysis of power spectra or cross-correlograms was carried out on a PDP-8 computer using PAFFT or DAQUAN programs provided by the Digital Equipment Corporation. The spectra in Figs. 9 and 10 are smoother because of spectral averaging and digital filtering associated with the PAFFT program. In all the power spectra, power density and frequency are plotted on linear scales, and not on the logarithmic scales used for channel noise analysis. Spectra are shown without subtraction of noise due to nonperiodic sources because these are negligible over the frequency range of interest. This point is illustrated in Figs. 3 and 4, where the periodic component was experimentally suppressed. It is also borne out by comparison of current noise before and after strophanthidin intoxication (see Lederer and Tsien, 1976, Fig. 2). When we examined this point in an experiment with spectral analysis, we found that the total noise power between 0 and 5 Hz was increased 17-fold above control by strophanthidin treatment.

RESULTS

Voltage and Current Fluctuations

Membrane electrical fluctuations are usually studied in one of two ways: measurement of voltage fluctuations with either zero or constant applied current (for example, Verveen and Derksen, 1965; Katz and Miledi, 1972) or

measurement of current fluctuations with membrane potential under voltage clamp (e.g., Poussart, 1969; Anderson and Stevens, 1973). In principle either approach should lead to the same conclusions. However, as applied to oscillatory phenomena in cardiac preparations, the two approaches have led to widely different interpretations.

Fig. 1 compares voltage and current fluctuations from the same preparation (cf. Conti and Wanke, 1975). A short Purkinje fiber was impaled with current-passing and voltage-measuring microelectrodes, then exposed to 0-K Tyrode solution to evoke oscillatory fluctuations. *A* shows fluctuations in membrane potential under conditions of constant current. The voltage fluctuations have a peak-to-peak amplitude of ~ 2 mV, and a sinusoidal character that is evident from the signal itself, or from its power spectrum, shown below. Almost all the power is concentrated in the sharp peak near 0.52 Hz.

Voltage fluctuations of comparable magnitude and periodicity have been recorded by many workers, starting with Bozler (1943) and continuing up to the present. At least

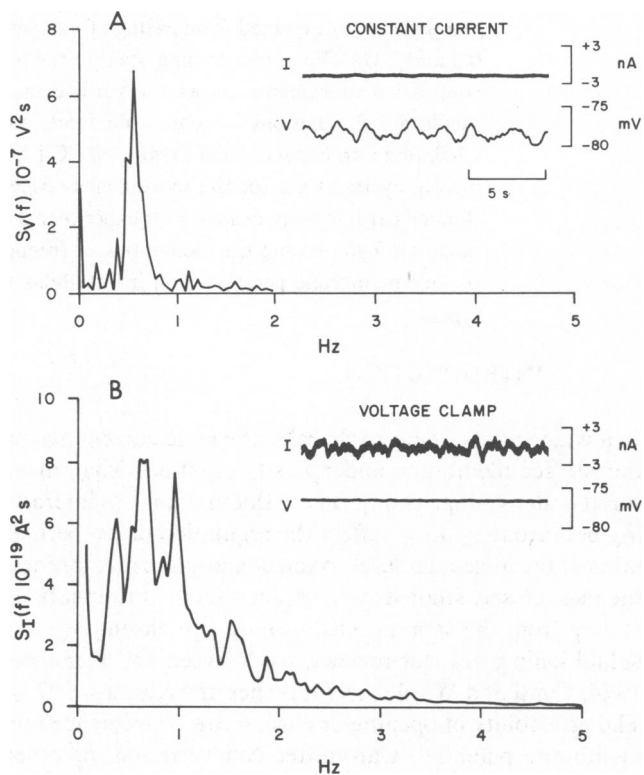
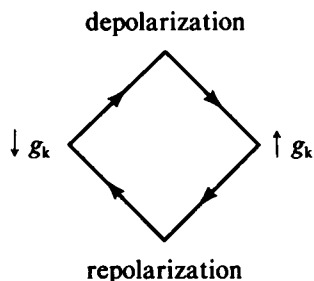


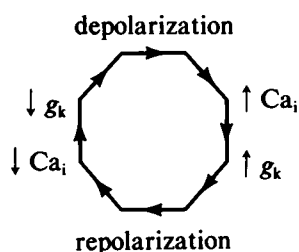
FIGURE 1 Comparison of voltage fluctuations and current fluctuations. Recordings from a calf Purkinje fiber bundle in the presence of 0-K, 5.4-mM Ca Tyrode. *A* shows voltage fluctuations with constant applied current, taken just before imposing voltage clamp. *B* shows current fluctuations under voltage clamp, 30 s later. Graphs show corresponding plots of power spectral density. *A*, voltage noise spectrum taken from analysis of a single 25.6 s voltage record. *B*, Current noise spectrum. Six individual spectra were obtained from consecutive 25.6 s records, averaged, and Hanning filtered. Preparation RK124-01, apparent cylindrical surface area, 0.008 cm².

four different mechanisms have been proposed:

(a) Membrane potential oscillations involving a Hodgkin-Huxley type channel. For example, assuming a time- and voltage-dependent K channel, the fluctuations could arise from the following oscillatory cycle:



(b) Membrane potential oscillations involving a Ca-activated K channel:



This explanation was proposed by Akselrod et al. (1979) for voltage fluctuations in carp atrial muscle fibers.

(c) Random membrane current fluctuations, arising from independent opening and closing of ionic channels, filtered by the resonant impedance of the resting membrane. The resonance arises from time- and voltage-dependent conductance changes that behave like an inductance in parallel with the membrane capacitance. This explanation was put forward by Clay et al. (1979) to account for voltage fluctuations in embryonic heart cell aggregates.

(d) Oscillatory membrane current fluctuations, filtered across the membrane impedance (Lederer, 1976; Kass et al., 1976).

Which mechanism dominates under the present experimental conditions? Fig. 1 *B* shows the critical test, the measurement of membrane current under voltage clamp. The traces were taken a few seconds after switching from constant current to constant voltage. Fluctuations now appear on the current trace. This result argues against explanations such as (a) and (b), in which variations in membrane potential are crucial to the oscillatory cycle. In their simplest form, these hypotheses would predict a flat current signal at fixed membrane potential. The remaining two mechanisms can be distinguished by considering the nature of the current noise. If random opening and closing of ion channels were the major noise source, as in hypothesis (c), one would expect a spectrum comprised of one or

more single time-constant components, each with maximum power at zero frequency, and each falling off at f^{-2} beyond its own corner frequency. Thus, noise power would decrease monotonically with increasing frequency. In fact, the measured power spectrum rises to a maximum between 0.5 and 1 Hz, and the periodicity is also apparent in the current signal itself. From experiments of this type, we conclude that oscillatory fluctuations in membrane current are predominant under our experimental conditions. Voltage-clamp experiments in the preparations used by Akselrod et al. (1979) and Clay et al. (1979) would be useful to see if the same explanation applies to their systems.

Although voltage-dependent conductance changes are not crucial to the generation of the basic oscillation, they are no doubt important in the expression of current oscillations as fluctuations in membrane potential. The voltage fluctuations in Fig. 1 *A* show a much narrower spectral distribution than the current fluctuations in Fig. 1 *B*. This is not very surprising. According to theory (see Stevens, 1972, or Wanke et al., 1974), the voltage power spectrum $S_V(f)$ and the current power spectrum $S_I(f)$ should be related through the membrane complex impedance $Z(f)$ as follows:

$$S_V(f) = S_I(f) |Z(f)|^2. \quad (1)$$

Thus, the spectra $S_V(f)$ and $S_I(f)$ can be quite different if $|Z(f)|^2$, the modulus of the membrane impedance, varies strongly near frequencies of interest, for example, as a result of channel gating. In fact, calculations of membrane impedance by Clapham (1978) and Clapham and DeFelice (1979), based on an empirical description of Purkinje fiber electrical properties (McAllister et al., 1975), predict

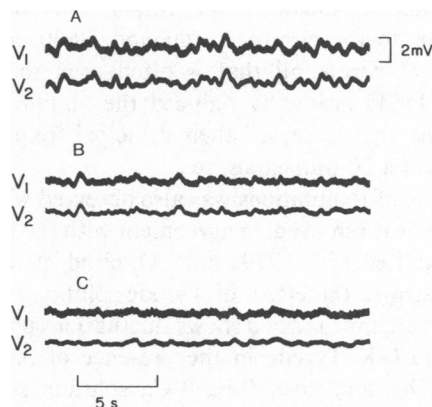


FIGURE 2 Synchronous voltage fluctuations recorded at two impalement sites separated by 275 μm along the axis of a 2 mm-long Purkinje fiber bundle. *A*, 4-mM K, 5.4-mM Ca Tyrode, no drug present. *B*, *C*, after exposure to 5×10^{-6} g/ml D600 for 12 and 24 min, respectively. The membrane potential remained within a few millivolts of -34 mV throughout. In this range of potentials, Ca channels may carry a steady inward current that can be blocked by D600 (Kass, et al. 1976a). The depolarization was produced by the second microelectrode impalement; a steady -2 -nA hyperpolarizing current was sufficient to restore normal excitability. Preparation S05-1.

that $|Z(f)|$ should reach a peak near 0.5 Hz and then fall off steeply at higher frequencies, in reasonable agreement with the results shown in Fig. 1.

Most of the analysis in this paper will involve current fluctuation measurements, because these measurements bypass filtering by the membrane impedance. Although voltage fluctuation measurements are generally less useful, they provide a convenient method for assessing electrical coupling between cells within a multicellular preparation (see, for example, DeFelice and DeHaan, 1977). Good coupling and restricted voltage non-uniformity are important in the interpretation of current records from voltage clamp experiments. Fig. 2 shows microelectrode recordings of membrane potential from two impalement sites spaced a number of cell lengths apart along the Purkinje fiber cell column. The upper trace is thickened by high frequency noise due to a higher microelectrode resistance, but in other respects the two voltage signals are practically identical. Synchronous voltage fluctuations of the same amplitude appear at both points along the cable. Evidently, cells can remain well coupled during spontaneous oscillations. This conclusion fits with earlier results of Lederer and Tsien (1976), who compared voltage signals from separate cells during current oscillations evoked by hyperpolarizing steps that are termed "transient inward current" (TI) (see Fig. 5 *A*). Because spontaneous fluctuations are usually much smaller than evoked current oscillations in current density, they are even less likely to produce serious longitudinal non-uniformity.

Evidence for Involvement of Intracellular Ca

Fig. 2 *B* and *C* illustrate the response of the voltage fluctuations to the organic compound D600, a methoxy derivative of verapamil that is often used to block Ca channels. D600 gradually reduced the amplitude of the fluctuations and decreased their principal frequency over the course of a 20-min exposure.

Inhibition of fluctuations was also observed when extracellular Ca was removed, in agreement with the findings of Eisner and Lederer (1979) and Akselrod et al. (1979). Fig. 3 illustrates the effect of Tyrode solution with nominally zero calcium. Trace *a* shows fluctuations produced by exposure to 0-K Tyrode in the presence of 5.4-mM Ca solution. Changing to a 0-K, 0-Ca solution sharply decreased the current fluctuations (*b*). The corresponding power spectra (*A*, *B*) show a marked reduction of spectral density at all frequencies. The response of the current fluctuations to Ca removal was consistent and reversible. When Ca was restored following a period in 0 Ca, the fluctuations recovered quickly and often showed a transient overshoot in amplitude beyond the original control value that subsided after several minutes.

Current fluctuations resemble evoked current oscillations (TI) in their response to Ca removal or Ca channel

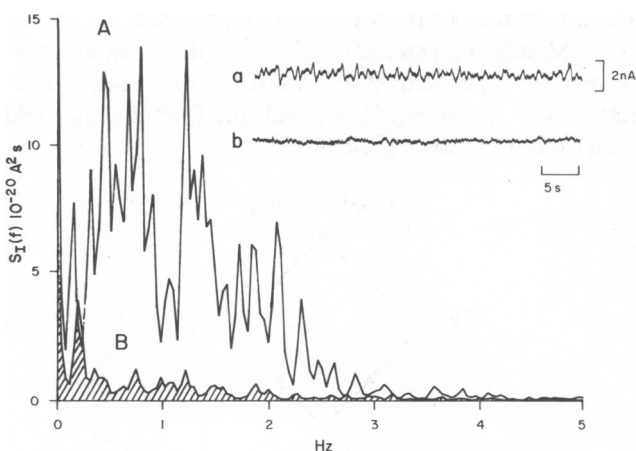


FIGURE 3 Effect of removing extracellular Ca on current fluctuations (inset, *a* and *b*) and their spectra. Records were taken after 5 min exposure to 0-K, 5.4-mM Ca Tyrode (*a*, *A*) and 10 min after switching to 0-K, 0-Ca Tyrode (*b*, *B*). Holding potential was -40 mV throughout. Preparation RK89-02, apparent cylindrical surface area, 0.0046 cm².

blockers (see Tsien et al., 1979, for references). These results can be interpreted in terms of an intracellular Ca hypothesis (Kass et al., 1978*a*). According to the hypothesis, spontaneous current fluctuations and evoked transient currents are reflections of a subcellular mechanism involving oscillatory movements of Ca between the myoplasm and an intracellular store such as the sarcoplasmic reticulum. Oscillatory movements of this kind have been documented in skinned cardiac cells by Fabiato and Fabiato (1975) and others (see below). In intact cells, inhibition of oscillations by removal of Ca_o or blockers of Ca channels is expected because these interventions reduce Ca entry and thereby lower intracellular Ca below the level needed to sustain the oscillatory movements between myoplasm and intracellular store.

If the hypothesis of a subcellular Ca oscillator is correct, one would expect that evoked or spontaneous current oscillations would also be inhibited by buffering the intracellular Ca at a low value. We tested this prediction in experiments where the Ca chelator EGTA was introduced by intracellular iontophoresis. Fig. 4 shows the effect of intracellular EGTA injections on spontaneous current fluctuations induced by 0-K Tyrode.

Trace *a* and its companion spectrum *A* were obtained during a 0-K challenge, before significant EGTA injection had taken place. The bathing solution was then changed to 4-mM K Tyrode, and EGTA was injected by passing negative current pulses (see Methods). After a cumulative charge transfer of 25 μ C, a second 0-K challenge was imposed, and record *b* was taken. The amplitude of oscillations was considerably reduced. Further inhibition of the fluctuations was evident during a third 0-K challenge that followed an additional period of iontophoretic injection. This experiment is representative of a total of five preparations where EGTA injection reduced spontaneous current fluctuations. Similar effects of EGTA injection are

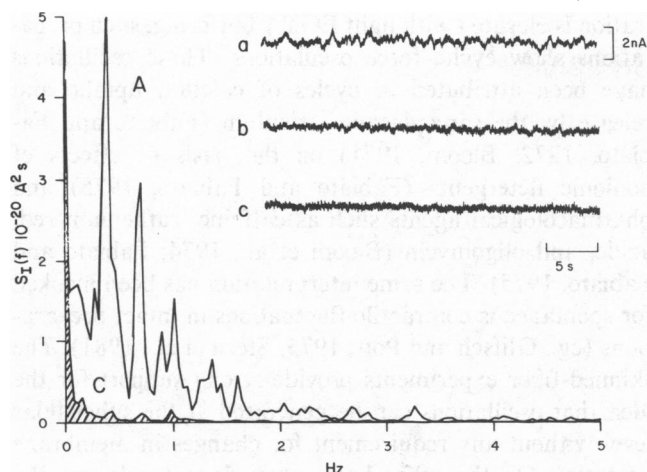


FIGURE 4 Effect of intracellular iontophoretic injection of EGTA on current fluctuations (*inset*) and current spectral density. Records were taken during three separate exposures to 0-K, 5.4-mM Ca Tyrode. *a*, at $t = 0$, just before hyperpolarizing current pulses from the EGTA-containing micropipette. *b*, at $t = 16$ min, after passing hyperpolarizing pulses giving a cumulative charge transfer of $-25 \mu\text{C}$. *c*, at $t = 22$ min, after an additional charge transfer of $-11 \mu\text{C}$. In the lower graph, spectra *A* and *C* correspond to records *a* and *c* (intermediate spectra omitted for clarity). Preparation RK87-03, apparent cylindrical surface area, 0.011 cm^2 .

mentioned in a preliminary report on isolated Purkinje cells by Mehdi and Sachs (1978).

According to our hypothesis, intracellular EGTA should also prevent evoked oscillations. Fig. 5 shows the effect of EGTA injection on the electro-mechanical oscillations evoked by a hyperpolarizing voltage step. All the records in this figure were obtained in 0-K solution. *A* shows the slow inward current (I_{si}) and twitch following the "make" of a depolarizing pulse and the evoked TI and aftercontraction (AC) following the "break." After EGTA was injected over a period of 38 min in 4-mM K Tyrode, the bathing

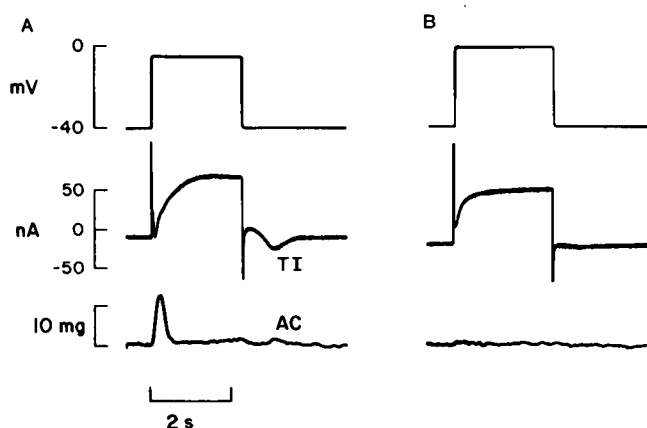


FIGURE 5 Effect of intracellular EGTA on TI and AC. Records of membrane potential, membrane current, and force, recorded during exposures to 0-K, 5.4-mM Ca Tyrode. *A*, before EGTA injection. *B*, at $t = 38$ min, after passing hyperpolarizing pulses from an EGTA-containing micropipette for a cumulative charge transfer of $-47.5 \mu\text{C}$. Preparation RK87-02, apparent cylindrical surface area, 0.013 cm^2 .

solution was changed back to 0-K Tyrode. As *B* shows, the twitch is blocked, as expected if EGTA is acting as an effective intracellular Ca buffer. The TI and aftercontraction are almost completely abolished, as predicted by the hypothesis.

Correlation between Fluctuations in Current and Contractile Force

Starting with the experiments of Bozler (1943), many investigators have observed contractile fluctuations in heart cells, especially under conditions of Ca overload. The spontaneous mechanical activity provides another clue that intracellular Ca is a factor in the oscillatory mechanism. Because calcium ions are known to activate contraction, the contractile activity can be taken as an expression of intracellular Ca fluctuations. If membrane current fluctuations reflect the same Ca fluctuations, as our hypothesis proposes, the electrical and mechanical signals ought to be closely related.

Fig. 6 shows records of force and membrane current from a voltage-clamped Purkinje fiber that had been intoxicated with strophanthidin. In the presence of 5.4-mM Ca Tyrode (*A*), both signals fluctuate in a rather sinusoidal manner. Spectral analysis (not shown) confirms this impression: the force and current spectra peak at the same resonant frequency, 0.84 Hz. Closer examination of the records in *A* indicates that the signals have a definite phase relationship: peaks in the force signal seem roughly synchronous with troughs in the membrane current.

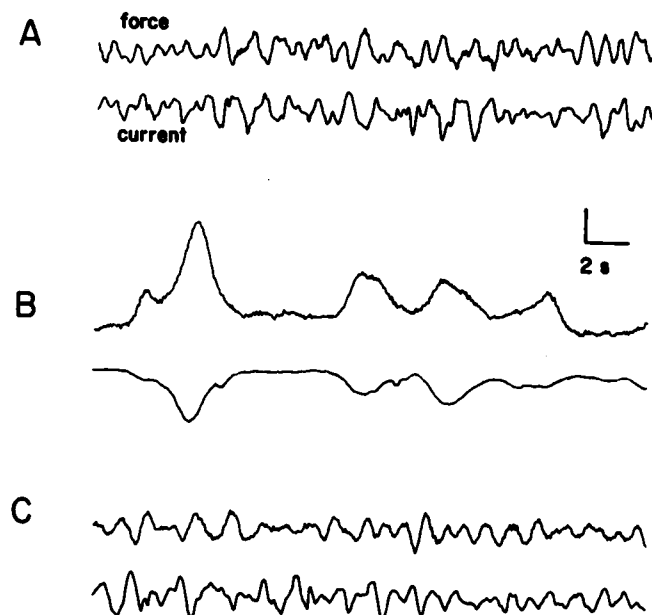


FIGURE 6 Simultaneous recordings of fluctuations in membrane current and contractile force from a Purkinje fiber bundle during continuous exposure to $1 \mu\text{M}$ strophanthidin. *A*, 5.4-mM Ca Tyrode. *B*, 12 min after bathing solution was changed to 5.4-mM Sr Tyrode. *C*, 5 min after returning to 5.4-mM Ca Tyrode. Preparation R10-1. Membrane potential held at -43 mV throughout. Horizontal bar indicates 2 s for all panels. Vertical calibration, 0.16-mg force, 100-nA current.

Fig. 6 B shows records from the same preparation after the bathing calcium had been completely replaced by strontium. The fluctuations became slower and more dramatic in Sr, and the correspondence between upward peaks in force and inward peaks in membrane current becomes very obvious. When the original Ca Tyrode was restored (Fig. 6 C), the fluctuations reverted to their original form.

Cross-correlational analysis provides a convenient method for examining the temporal relationship between current and force signals in Ca Tyrode. Fig. 7 shows the cross correlogram between inward current ($-I$) and force (F), which is defined as $R_{-IF}(\tau) = \int -I(t)F(t+\tau) dt$. The correlogram is shown on two time scales. On a slow time base, the correlogram has a damped sinusoidal appearance that reflects the common periodic character of the current and force signals. If peaks of force and inward current were exactly synchronous, $R(\tau)$ would show a maximum at $\tau = 0$. In fact, as the expanded time base plot indicates, $R(\tau)$ reaches its maximum value at $\tau = 70$ ms. In other words, peak force lags behind peak inward current by 70 ms. This result agrees rather well with previous analysis of the temporal relationship between transient inward currents and aftercontractions. Kass et al. (1978a) found lags ranging between 50–140 ms, and Eisner and Lederer (1979) report a delay of the order of 100 ms. Evidently, inward current across the surface membrane responds more quickly to a rise in free intracellular Ca than does externally measured force.

Fluctuations in contractile force are a common feature of intact cardiac cells under Ca overload and skinned cardiac preparations where the surface membrane is mechanically stripped away (Fabiato and Fabiato, 1972; 1977) or rendered permeable to small molecules by treatment with EDTA (Müller, 1976) or saponin (Endo and Kitzawa, 1978). When the myoplasmic calcium concen-

tration is elevated with light EGTA buffering, such preparations show cyclic force oscillations. These oscillations have been attributed to cycles of calcium uptake and release by the sarcoplasmic reticulum (Fabiato and Fabiato, 1972; Bloom, 1971) on the basis of effects of nonionic detergents (Fabiato and Fabiato, 1975) and pharmacological agents such as caffeine, ruthenium red, azide, and oligomycin (Bloom et al., 1974; Fabiato and Fabiato, 1975). The same interpretation has been invoked for spontaneous contractile fluctuations in intact preparations (eg., Glitsch and Pott, 1975; Stern et al., 1981). The skinned-fiber experiments provide strong support for the idea that oscillations can be generated at the subcellular level without any requirement for changes in membrane potential. On the other hand, they do not rule out the possibility that in intact cells, membrane potential may exert a modulatory influence on the magnitude, frequency, or coherence of the subcellular oscillations.

Voltage-Dependence of Fluctuation Magnitude

The most obvious type of voltage-dependence involves changes in driving force on the ions carrying the current. If the ionic pathway(s) for the current have a reversal potential (E_{rev}) where net charge transfer is zero, one would expect the noise variance to be minimal at this potential (cf. Dionne and Ruff, 1977). According to previous analysis, evoked current oscillations show a reversal potential ranging from -5 mV in 150-mM Na Tyrode, to more negative values in sodium-deficient solutions.

Fig. 8 shows the results of an experiment in the presence of 37-mM Na solution. This concentration was chosen because it puts the expected minimum in noise variance amidst the range of potentials that can easily be maintained for many seconds without large outward holding currents. Spontaneous current fluctuations were induced by a 15-min exposure to 2- μ M strophanthidin and measured at a series of membrane potentials ranging from 0 to -80 mV. The variance of the current signal was determined for each measurement and plotted against membrane potential. As Fig. 8 shows, the variance approaches a minimum near -40 mV. This value falls within the range of reversal potentials (-22 to -42 mV), determined for evoked oscillations under similar ionic conditions (Kass et al., 1978b, Fig. 4).

The minimum in noise variance does not correspond to the Nernst potential for any individual ion. It presumably represents a point of balance between opposite ionic fluxes that are jointly regulated by the local free Ca near the inside surface of the sarcolemmal membrane. In an earlier paper (Kass et al., 1978b), we proposed that intracellular Ca acts through activation of a nonselective cation channel, with roughly equal permeability to Na^+ and K^+ ions, but impermeable to Cl^- , somewhat analogous to acetylcholine-activated channels at the motor end plate. Recently, this hypothesis has received direct support from record-

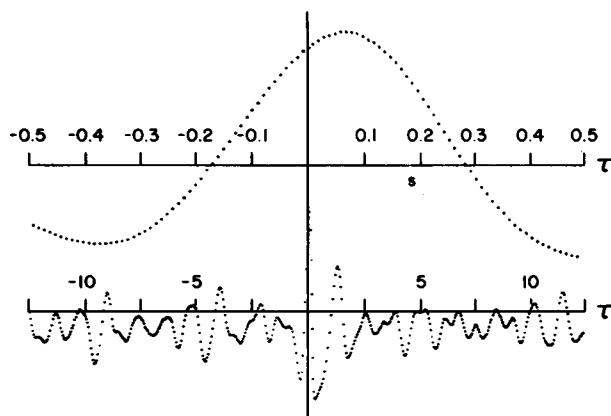


FIGURE 7 Cross-correlogram of contractile force and inward membrane current signals illustrated in Fig. 6A. *Top*, correlogram taken at relatively fine time resolution, 10 ms between adjacent points. Rightward displacement from origin corresponds to increasing time lag of force following inward current. *Bottom*, correlogram taken at coarser time resolution to show behavior over longer times; 50 ms between adjacent points. Analysis using LAB 8/e correlation program (Digital Equipment Corp.).

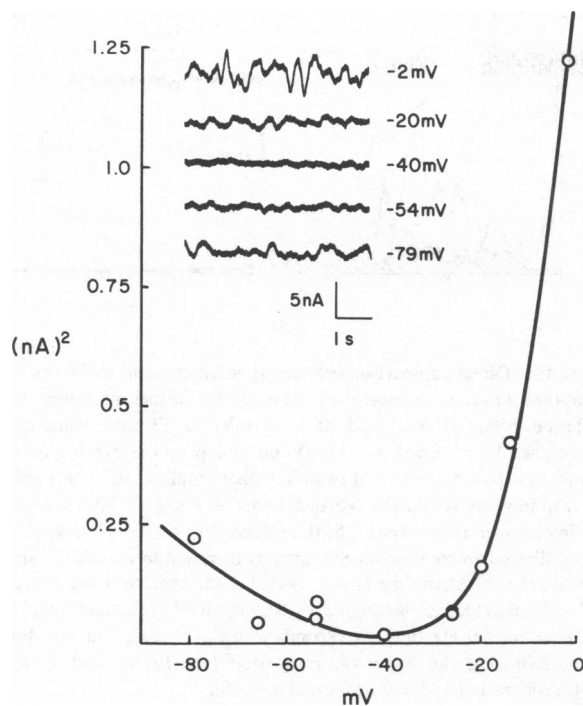


FIGURE 8 Voltage dependence of noise variance. *Inset*, representative traces showing current fluctuations at various holding potentials. Graph, noise variance calculated from these records and others from the same experiments. Smooth curve drawn by eye. Preparation RK76-1, 0-K, 5.4-mM Ca, 37-mM Na Tyrode, apparent cylindrical surface area, 0.01 cm².

ings of single channel currents in cultured heart cells (Collquhoun et al., 1981).

Effect of Membrane Potential on the Oscillatory Frequency

Experiments of this type showed that membrane potential not only affects the amplitude of the spontaneous oscillations, but can also influence their oscillatory period. This is evident in the inset of Fig. 8 where the oscillations at -2 mV are clearly faster than those at -79 mV. The voltage dependence of the oscillatory frequency was examined further with spectral analysis as illustrated in Fig. 9. Current records (*A*) and corresponding power spectra (*B*) are shown for runs at -72 and -34 mV. It is clear that steady depolarization increases the peak oscillatory frequency and broadens the spectral distribution. Fig. 9C shows that this trend holds true over a wide range of membrane potential. Results from the preparation illustrated in panels *A* and *B* and a companion experiment were analyzed as follows. The total power of each spectrum was computed by performing a running integral of power density vs. frequency. In this manner, the area under each power spectrum could be divided into four equal parts, marked off along the frequency axis by quarter-power ($\nu_{1/4}$), half-power ($\nu_{1/2}$), and three-quarter-power ($\nu_{3/4}$) frequencies. In the plot, the median frequency $\nu_{1/2}$ is

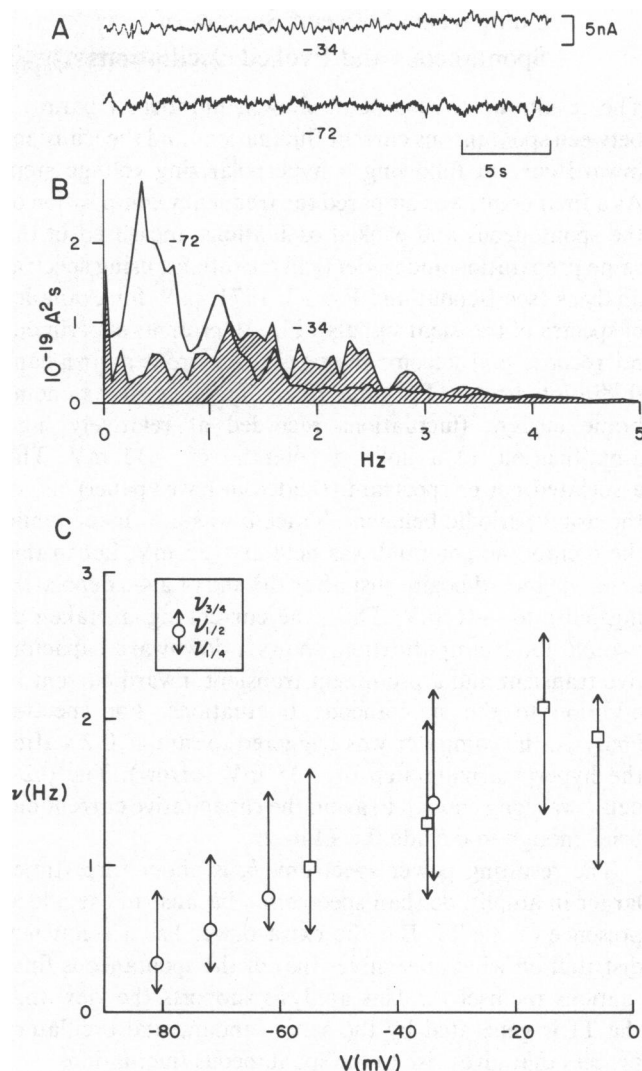


FIGURE 9 Influence of membrane potential level on the periodicity of oscillatory fluctuations. *A*, current signals recorded at holding potentials of -72 and -34 mV. *B*, corresponding power spectra. *C*, collected results from the experiment illustrated in *A* and *B* (open circles, preparation 154-4) and from another experiment (open squares, preparation 155-4). Both preparations were intoxicated by 2 μ M strophanthidin. Open symbols indicate the median spectral frequency $\nu_{1/2}$ while arrows indicate the spread between $\nu_{1/4}$ and $\nu_{3/4}$, as defined in the text.

represented by open symbols, and the broadness of the spectrum is indicated by the arrowheads at $\nu_{1/4}$ and $\nu_{3/4}$. The results from the two preparations show a roughly threefold increase in the half-power frequency between -80 and -10 mV. Similar results were obtained in other experiments, although in some cases the voltage dependence was less pronounced. An acceleratory effect of varying the membrane potential to less negative values has also been observed in the timing of TI. For example, in Fig. 5 of Kass et al. (1978a), the latency between the hyperpolarizing step and the peak of the transient inward current decreased threefold as the level of step varied between -80 and -10 mV, in close agreement with the voltage dependence of fluctuation frequency shown here.

Comparison between Spectra of Spontaneous and Evoked Oscillations

The results up to this point show a number of parallels between spontaneous current fluctuations and the transient inward current following a hyperpolarizing voltage step. As a final point, we compared the frequency composition of the spontaneous and evoked oscillations, measured in the same preparation under identical conditions, using spectral analysis (see Bendat and Piersol, 1971, p. 9, for examples of spectra of transient signals). Fig. 10 presents experimental records and accompanying spectra from a strophanthidin-intoxicated Purkinje fiber. Trace *a* shows membrane current fluctuations recorded at relatively high amplification, at a holding potential of -35 mV. The associated power spectrum (shaded in lower panel) shows the usual periodic behavior. Trace *b* was also taken while the membrane potential was held at -35 mV, but in this case, the record begins just after the end of a 4-s depolarizing pulse to -16 mV. Thus, the current signal, taken at twofold lower amplification, shows a downward capacitive transient and a prominent transient inward current in addition to the spontaneous fluctuations. For spectral analysis, the computer was triggered to sample 0.2 s after the hyperpolarizing step to -35 mV (arrow). The 0.2-s delay was long enough to avoid the capacitive current but brief enough to include the TI itself.

The resulting power spectrum, *b*, is about three times larger in amplitude than spectrum *a* because of the added presence of the TI. But the extra power has a frequency distribution which resembles that of the spontaneous fluctuations themselves. This analysis supports the view that the TI is generated by the same fundamental oscillatory process that gives rise to the spontaneous fluctuations.

DISCUSSION

The electrical fluctuations described in this paper are distinguished from other forms of sustained activity by several fundamentally different characteristics. They persist under voltage clamp, unlike oscillations originating from voltage-dependent channel gating (for example, Vassalle, 1966; Hauswirth et al., 1969; Noma and Irisawa, 1976; Brown et al., 1976; Katzung and Morgenstern, 1977). The current fluctuations described here give rise to power spectra with clear resonant peaks, and not the single time-constant components expected from random opening and closing of independent ion channels (see for example Noma et al., 1979). As we discuss below, several lines of evidence suggest that the periodic fluctuations arise from an oscillator at the cytoplasmic level.

Oscillatory current fluctuations must be considered along with the regenerative ionic currents as a source of instabilities in membrane potential. These mechanisms can interact and potentiate each other because the periodicity of the current fluctuations may overlap with resonances of the sarcolemmal membrane impedance (Fig. 1), and

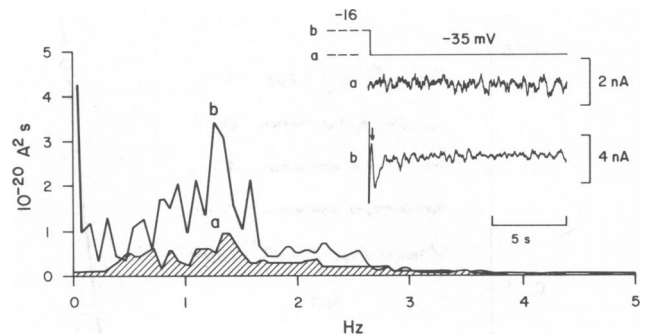


FIGURE 10 Direct comparison between spontaneous and evoked current oscillations. Inset, *a*, spontaneous current fluctuations recorded while membrane potential was held at -35 mV. *b*, TI and spontaneous fluctuations, also recorded at -35 mV, but following the termination of a 4-s pulse to -16 mV. Note that twofold higher amplification was used for trace *a* to improve resolution. Vertical arrow in *b* marks the initiation of sampling for spectral analysis. The 0.2-s delay following the hyperpolarizing step allowed ample time for the capacity transient to subside. Graphed below are the corresponding power spectra. Each spectrum was based on four blocks of data, each lasting 12.8 s. Spectrum *b* (TI fluctuations) has a large peak near 0 Hz that corresponds to a slow "creep" in membrane current following the hyperpolarizing step (see Eisner and Lederer, 1979). Preparation 155-4, $2 \mu\text{M}$ strophanthidin.

because hyperpolarizing potential changes enhance the current oscillations (Fig. 10). Even small voltage fluctuations can play a significant role in beat-to-beat variations in pacemaker rhythm (Clay and DeHaan, 1979). Larger depolarizations resulting from oscillatory currents can reach threshold and produce extra systoles in certain types of cardiac arrhythmias (for reviews, see Ferrier, 1977; Cranefield, 1977; Tsien et al., 1978).

Oscillatory current fluctuations were deliberately accentuated in our experiments by using strophanthidin or zero-potassium media, which inhibit the sodium pump and produce Ca-overload (see also Lederer, 1976; Lederer and Tsien, 1976; Aronson and Gelles, 1977; Eisner and Lederer, 1979). But similar fluctuations can appear quite prominently in some heart cells in the absence of overt intervention (Akselrod et al., 1979; Mehdi and Sachs, 1978; Stern et al., 1981). More work needs to be done to assess the relative importance of periodic and random sources of current noise in different cardiac preparations under a range of experimental conditions. Measurements of light scattering associated with contractile fluctuations have already pointed out interesting variations among diverse cardiac tissues (Kort and Lakatta, 1981). Future studies on channels underlying the action potential and their noise spectra may call for procedures to first minimize oscillatory current fluctuations.

Role of Intracellular Ca in Oscillatory Fluctuations

The experiments in this paper provide support for the involvement of Ca ions in the oscillatory current fluctuations (see also Irisawa and Noma, 1977; Mehdi and Sachs,

1978; Eisner and Lederer, 1979). Some of the evidence parallels earlier work on evoked current oscillations (Kass et al., 1978a). The spontaneous fluctuations are decreased in magnitude and oscillatory frequency by removing extracellular Ca or applying the Ca channel blocker D600. Cross-correlational analysis shows that current fluctuations are closely related to fluctuations in contractile force, which presumably express variations in myoplasmic Ca. Each of these results is subject to various interpretations: for example, (a) as indicating a fluctuating Ca influx, regulated by oscillatory changes in an intracellular mediator other than Ca, or (b) as indicating oscillatory variations in intracellular free Ca, controlling both force and sarcolemmal membrane current (carried by other ions, not necessarily Ca). These possibilities were considered in an earlier study of evoked oscillations (Kass et al., 1978b), where we used measurements of TI reversal potentials in different Na_o and Ca_o to argue in favor of the second possibility.

Intracellular injection of the Ca buffer EGTA represents a different approach to the same question. We found that the evoked and spontaneous oscillations in both membrane current and contractile force were strongly inhibited by intracellular EGTA. These results provide a rather direct argument against an oscillatory Ca influx as proposed above, as this hypothesis predicts that EGTA should suppress variations in force, but not the current associated with the Ca influx itself.

Is intracellular Ca a primary oscillatory variable? It seems unlikely that Ca merely tracks primary oscillations in some other intracellular ion or metabolite such as H^+ or cyclic AMP. Electromechanical oscillations are slowed, not just changed in amplitude, by exposure to 0-Ca solution or to D600, replacement of Ca by Sr, or by lightly buffering intracellular Ca with a limited injection of EGTA. Likewise, in skinned cardiac preparations, cyclic contractions decrease in frequency when EGTA concentration is increased (Fabiato and Fabiato, 1975, Fig. 3; Endo and Kitizawa, 1978, Fig. 13) or when Ca is replaced by Sr (A. Fabiato, personal communication). The simplest interpretation is that myoplasmic calcium is actually a primary oscillatory variable, and that a rate-limiting step in the oscillatory cycle is slowed down by weakly buffering Ca or replacing it with Sr.

Relationship between Spontaneous and Evoked Oscillations

Our spectral analysis shows that current fluctuations have the same frequency distribution as the larger damped current oscillations evoked by a hyperpolarizing step. This supports the view that both phenomena are generated by oscillations in intracellular Ca, possibly arising from Ca uptake and release from the sarcoplasmic reticulum (see above). If one accepts the idea of localized cytoplasmic oscillators, there are a number of ways in which a hyperpo-

larizing voltage change could lead to a large damped oscillation over and above the spontaneous fluctuations. The voltage step could act by synchronizing independent oscillatory units, momentarily resetting their phase, to yield a large summated response (Lederer, 1976; Kass et al., 1978a). Alternatively, the voltage change could temporarily increase the amplitude of each of the continuously active oscillator units. Finally, the hyperpolarization could lead to recruitment of quiescent oscillatory units that do not contribute to sustained oscillations because of damping: continuous fluctuations would then be attributed to a small number of spontaneously active units that happened to respond to elevated ambient Ca with a stable oscillatory limit cycle.

Spontaneous Activity Related to Ca_i in Neurons and Other Cells

Some intriguing similarities exist between oscillatory fluctuations in heart cells and spontaneously recurrent potential changes in other cells (see Berridge and Rapp, 1979, for review). In many neurons, as in skeletal and cardiac muscle, subsurface cisterns and external membranes are closely apposed, with structural specializations consistent with some sort of functional coupling (Rosenbluth, 1962; Henkart et al., 1976). The subsurface cisterns are a form of endoplasmic reticulum and have been shown to sequester Ca in squid giant axons (Henkart et al., 1978). In frog sympathetic ganglion neurons, which also have subsurface cisterns (Dickinson and Reese, 1974), Kuba and Nishi (1976) recorded spontaneously recurrent hyperpolarizations under the influence of caffeine. Because the spontaneous potential changes were abolished by injecting EDTA or removing extracellular Ca, and reversed at potentials negative to E_K , Kuba and Nishi suggested that a Ca-activated K channel (see Meech, 1978) might be periodically activated by cycles of Ca release and reuptake by intracellular stores. A similar mechanism has been proposed by Nelson and Henkart (1979) to account for oscillatory membrane potential changes in L cells (Nelson et al., 1972; Okada et al., 1977). In L cells as well as sympathetic neurons, these very interesting observations need to be followed up by experiments to see if oscillatory activity persists when membrane potential is clamped, and to find out whether conductance changes really track cytoplasmic free Ca.

In such respects, cardiac Purkinje fibers are a rather favorable system for studying surface membrane activity related to intracellular Ca. Voltage-clamp experiments can be carried out with an acceptable degree of longitudinal non-uniformity (Lederer and Tsien, 1976, Fig. 2) and force measurements provide a convenient if imperfect bioassay of intracellular Ca. However, other experimental approaches may be needed for analysis of the "cytoplasmic Ca oscillator" itself. Little is known about the membrane properties of subsurface cisterns or the factors that regulate Ca movements between the SR and the cytosol.

Ca-dependent oscillations in calcium content have been observed in vesicles of cardiac SR, but only on a time scale of minutes (Katz et al., 1980). Such biochemical studies tend to average over a large number of vesicles whose behavior may not be synchronous on a time scale of seconds. The best hope for analyzing faster Ca movements may rest with single cardiac cells, intact as well as skinned, and methods for studying mechanical or electrical fluctuations in localized regions of the cell.

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