The Ionic Dependence of Cardiac
Excitability and Contractility

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ABSTRACT In contrast to the large volume of data supporting the dependence of cardiac excitability and phasic contractility on external Na, Van der Kloot and Rubin (1962) and Singh (1962) have reported the persistence of both electrical and phasic mechanical activity in frog atrial and ventricular preparations soaked in isotonic sucrose solutions. The acute ionic dependence of excitability and contractility in small frog atrial trabeculae has been investigated with the conclusion that excitability and phasic contractions may continue for extended periods of time in sucrose media if the extracellular ionic concentrations remain above 2% of normal. This behavior is attributed to the slow exchange properties of the cell surfaces of the frog cardiac trabeculae and the antagonistic effects of Na, K, and Ca ions on both membrane excitability and fiber contractility.

Since the work of Overton (1902), a vast quantity of data has accumulated indicative of an intimate link of Na with excitability. Considerable variation in the Na dependence of the spike potential amplitude has been noted but, with few exceptions, the persistence of excitability in nerve, skeletal muscle, and cardiac muscle has been shown to be dependent upon a critical external Na concentration. Coraboeuf and Otsuka (1956) and Delèze (1959) found that the height of the action potential overshoot in guinea pig ventricle is independent of external Na until the tissue has been perfused with reduced Na perfusate for some 20 min, but conduction block did eventually occur in Na-free media and the rate of depolarization was proportionally dependent upon external Na. Brady and Woodbury (1960) and Casteels (1962) have shown that a minimum of 10% normal Na is necessary to maintain the excitability of frog ventricle in the presence of normal concentrations of K and Ca. In the absence of K and Ca, Casteels found excitability to persist in excess of 2 hr in 4.7 mM Na, but this reduced quantity of Na was essential to excitability.

On the other hand, reports of Van der Kloot and Rubin (1962) and Singh (1962) show that frog ventricles or atria display excitability for periods in
excess of 2 hr when soaked in isotonic sucrose media. These experiments are interpreted to imply that excitability and contractility are retained in the complete absence of external Na.

Our study strongly indicates that the persistence of excitability and contractility in the nonperfused preparation results from a combination of (a) long diffusion pathways in the unstirred extracellular space and (b) competitive influences of the constituent perfusate cations on excitability and contractility. Furthermore, the isotopic exchange data of Van der Kloot and Dane (1964) support this interpretation.

The most suitable preparation with which to study the physiological effects of external ions is one with a minimum of extracellular space. The presence of an unstirred layer of fluid adjacent to the cell membrane not only increases the time required to change surface concentrations but also, since the cells themselves are stores of ions contained within semipermeable membranes, the unstirred external fluid layer establishes a space across which a gradient of ion concentration can exist even though the ultimate external environment is maintained at zero concentration. A single fiber would be most favorable for this study but since such preparations are exceedingly difficult to obtain from cardiac tissue, a frog atrial trabecula containing somewhat less than 100 fibers was used. In such a preparation, maximum diffusion distances are not in excess of 50 μ. The behavior of this tissue in response to changes in perfusate concentrations is contrasted to that of larger preparations of the frog heart.

METHODS

A bull frog (Rana catesbiana) atrium was excised and pinned to a dissecting dish containing normal Ringer’s solution, so that the trabecular structure was exposed. With the aid of a dissecting microscope and Lawton ophthalmological scissors, a suitable trabecula was carefully tied with monofilament silk thread and dissected. One end of the muscle was tied to the hook in the perfusing chamber (Fig. 1) and the other attached to a capacitance tension transducer designed to operate in the range 0 to 500 mg (Schilling, 1960). The length of the preparation varied from 3.0 to 5.0 mm, and the weight from 0.2 to 0.5 mg.

The perfusion chamber was 10 mm in length, 1.5 mm in width, and 1.5 mm in depth. The solution in the chamber could be quickly switched from normal Ringer’s solution to a test solution. A flow rate of 2.5 ml/min was maintained throughout each experiment.

Normal Ringer’s solution contained the following ionic concentrations in mM: NaCl, 106.00; KCl, 2.70; CaCl₂, 1.8; MgCl₂, 0.49; NaHCO₃, 10.0; NaH₂PO₄, 0.32; glucose, 1 g per liter. In Na⁺-free solutions, KHCO₃ (2.4 mM) and K₂HPO₄ (0.08 mM) were used as buffers. All solutions of ionic strength below normal were made isotonic by adding appropriate amounts of sucrose on a basis of 90% sucrose per molar equivalent of NaCl (Merck Index, 1960). All solutions were oxygenated with 98%
O₂ and 2% CO₂. Sodium bicarbonate—buffered solutions maintained a pH of 7.2. The KHCO₃ solutions maintained a pH around 6.8 to 7.0. Isotonic sucrose solutions tended towards a pH of 5 after 30 min oxygenation as did unbuffered Ringer's solution. In the unbuffered Ringer solution, contractile tension slowly declined but excitability persisted even after the pH had fallen below 5.6. Since conduction block occurred in isotonic sucrose solutions in a matter of seconds, the pH change was assumed not to have a first order effect on excitability.

A pair of stimulating electrodes made from platinum wire (5 mil diameter) was placed at one end of the muscle, one wire on either side of the trabecula. This arrangement reduced shock artifact to tolerable levels. Stimuli were rectangular pulses of 3 to 5 msec duration, coupled to the electrodes with a transformer. With the use of the wick drain as the indifferent electrode, the reference drifted only a few millivolts at most during a solution change. When necessary, a correction of the zero reference level was made by noting the drift when the solution was changed with the electrode withdrawn from the cell.

The general characteristics of the electrical and mechanical responses to solution changes depended little upon stimulus rate and since contractile responses in normal solutions were near maximal at a stimulus interval of 2.5 sec, this interval was maintained throughout the experiments.

Intracellular recordings were made with a flexibly mounted glass microelectrode of less than 0.5 μ tip diameter (Woodbury and Brady, 1956). Tension and electrical responses were recorded on a dual-trace oscilloscope and a two-channel chart recorder.

RESULTS

Effects of Separately Varied Cation Species on Transmembrane Potential and Tension. Fig. 2 shows the electrical and mechanical responses of the trabecula when the Na concentration of the perfusate is abruptly lowered. Large alterations of both the transmembrane potential and isometric contractile tension...
are visible within the first response following the solution change. The amplitude and duration of the action potential fall quickly with inexcitability and contracture occurring within a few beats. The action potential duration shortens with a half-time of 2 to 3 sec. Within 20 sec a contracture develops which is several fold greater than normal twitch tension. Stimuli, increased in amplitude and duration, will evoke local nonpropagated electrical responses with accompanying small twitchlike contractile responses superimposed on the contracture. Recovery to normal can usually be recorded from the same impalement upon returning to control solutions.

Figure 2. Action potential and tension responses in reduced Na solutions. A, Na-free medium. B, 2% Na. Coincident action potentials and contractile responses are indicated by similar lines. Time of response is relative to the onset of the solution change. In B, the response of 2.5 sec is primarily stimulus artifact. In this response, the stimulus was increased several fold both in amplitude and duration. Positions of the superimposed action potentials are correct within a few millivolts.

Fig. 3 shows a plot of consecutive tension amplitudes in a series of contractions, including the responses after returning to normal Na. As the contracture develops, the height of the twitches declines so that by the time conduction block is about to occur, little extra tension is generated by the twitch. With the return to normal solution, excitability returns within seconds, the contracture subsides, and twitch tension returns to normal after a small undershoot.

Fig. 4 illustrates the dependence of trabecular action potentials and contractions on external K and Ca. Note the tendency towards an elongation of action potential duration in reduced Ca and, particularly, in reduced K contrasting to the shortening effect of reduced Na. Twitch heights increase in low K, but no contracture develops similar to that in low Na solutions.
Reducing Ca tends to abolish contractility entirely. The steady-state level of these effects occurs in 30 to 60 sec after solution change, as shown in Fig. 5. In each case, only one concentration was varied at a time. The apparent linearity of the relations is only approximate and no particular significance is attributed to this pattern.

![Figure 3](image)

**Figure 3.** Time course of resting tension (dashed line) and peak contractile tension (solid line) following exposure of the tissue to a Na-free solution and recovery in normal solution. During the blocked period the solid line shows the continuation of contracture tension.

![Figure 4](image)

**Figure 4.** Sequence of transmembrane potentials and contractile tensions following exposure to reduced ionic media. A, potassium-free. B, calcium-free. Dashed line above tension responses in A shows final maximum height of twitch tension in this solution. Coincident action potentials and tension responses are shown by similar lines.

**Effects of Ringer’s Solution Dilution with Isotonic Sucrose**  Fig. 6 shows tracings of action potentials and tension responses in solutions diluted to various ionic concentrations with isotonic sucrose. Note the comparative stability of both electrical and mechanical responses even in solutions diluted to 2.0% of normal ionic concentration. Several other effects of Ringer’s solution dilution are noteworthy. The hyperpolarization of the membrane reflecting the reduction of K is still evident. As a consequence, the excitation threshold is
elevated, requiring larger stimuli to elicit propagated responses. On the other hand, the level of contracture tension (represented by the rise in diastolic tension) is not nearly as dramatic as in Figs. 2 and 3, where only Na was reduced. Of special interest, however, is the persistence of excitability and contractile tension in excess of 4 min (Fig. 6D) in the 2% solution. In this

Figure 5. A, transmembrane potential duration as a function of external cation concentration for variation of individual species concentrations. B, contractile tension height as a function of external cation concentration. Each point is a single measurement. The lines were drawn by eye.
preparation, conduction block occurred after 5.5 min perfusion with isotonic sucrose.

Fig. 7 illustrates the continuous, approximately linear increase in contractile tension and action potential duration as an isotonic sucrose solution is approached. The level of contracture in isotonic sucrose was predictable.

![Graph of contractile tension and action potential duration](image)

**Figure 6.** Sequence of responses of transmembrane potential and tension responses upon perfusion of atrial trabeculae with Ringer's solution diluted with isotonic sucrose. Normal in each case is the response in normal Ringer's solution. A, dilution to 50%. B, 10%. C, 2%. D, same as C, but with a different impalement of the same trabecula at a later time. The transmembrane potentials within each sequence were recorded from the same fibers. The correspondence of membrane potentials and tension responses is indicated by similar line tracings. The time of the recording following the solution change is given in the inset in each case. In D, both the first small spike and the following larger spike of the 2 and 4 min membrane potential records are stimulus artifacts. They persist after conduction block occurs.
from the continuous rise in peak tension as the ionic concentrations were reduced. However, the transient response of the action potential duration in solutions diluted to 2% or less was first to lengthen and then to shorten to zero as conduction block occurred. Action potential duration in these solutions plotted in Fig. 7 is the maximum durations before shortening began.

It should be mentioned that small, local, nonpropagated responses can be elicited with very large stimuli in isotonic sucrose solutions after conduction block has occurred but such responses would be expected from the electrical cable properties of the cells and the nonpropagating local twitches seen by Huxley and Taylor (1958) in response to locally applied current.

**DISCUSSION**

**Response Time to Solution Changes**  The differences in exchange characteristics of the perfused and nonperfused frog ventricles are clearly evident in a comparison of the Na efflux data of Johnson (1957) and Van der Kloot and Dane (1964). The half-time for Na\(^{2+}\) exchange for the last 4% of exchangeable Na in the perfused ventricle was about 14 min. In the same range of isotope washout in the nonperfused preparation, the data of Van der Kloot and Rubin show a half-time for Na\(^{2+}\) washout of greater than 40 min. Of further significance is the time at which this level of activity of the washout data was reached, about 3 min for the perfused ventricle and 90 min for the nonperfused preparation. In contrast, the acute sensitivity of the trabecular preparation to the reduction of external Na is readily apparent in Fig. 2, where the action potential duration shortened to zero and conduction block occurred within 30 sec. However, it should be pointed out that this response

![Figure 7. Tension amplitude and transmembrane potential duration plotted as a function of cation content of Ringer's solution diluted with isotonic sucrose. The lines are a least square fit of the plotted points.](https://jgp.rupress.org/content/49/6/788/F7)
time is still considerably larger than expected on the basis of free diffusion in a cylindrical preparation of this size. The half-time for the loss of a freely diffusing ion, say Ca (diffusion constant, \(6 \times 10^{-6} \text{ cm/sec}\)), from a cylinder of 50 \(\mu\) radius is 0.3 sec (Crank, 1956). The half-time for the fall in contractile tension of an atrial trabecula in a Ca-free medium (Fig. 4B) was 4 sec; i.e., more than tenfold longer than calculated. Part of the increase can be attributed to an unstirred shell of solution about the trabecula resulting from the laminar flow of perfusate through the chamber. Also, in histological sections of this tissue (unpublished data) the fibers appear tightly packed so that diffusion from the interstices may be limited to very narrow and relatively long channels. Another factor slowing diffusion rates in this preparation may be the spongy structure of the frog heart. The relative volume of fluid trapped in this network may be appreciable. In any case, if a sizeable extracellular space is added to this system as in a nonperfused frog ventricle or atrium, or even a strip of atrium or ventricle, substantial ion concentration changes at the cell surfaces may require surprisingly long soaking periods. For example, the contractile tension in an excised atrial trabecula fell to 50% of its initial value in 8 sec when it was perfused with a K-free, Ca-free Ringer solution. Only a trace of contractile tension remained after 30 sec, even with 10 times threshold stimulation. In the nonperfused ventricle, 7 to 10 min were required for contractions to fall to a small fraction of the initial twitch tension (Van der Kloot and Rubin, 1962).

In addition to the extracellular diffusion factors, it should be kept in mind that the cells themselves are stores of sizeable quantities of the ions in question, and that the cell membranes are moderately permeable to these ions. In response to a fall in an external ion concentration, it would not be unexpected that some loss to the extracellular space might occur. In this respect, Brady and Woodbury (1960) found that the overshoot of the frog ventricular action potential in low Na solutions reached a steady-state level near zero potential almost independent of the external Na concentration in perfusates with Na below 50% of normal. This was interpreted to indicate that in the course of establishment of the steady-state level (which undoubtedly involves both active and passive processes) sufficient intracellular Na was lost to maintain a nearly even balance of Na concentration across the cell membrane. Specifically, in those experiments, the zero level of the overshoot in 20% Na would require a loss of approximately one-third of the intracellular Na in 2 to 5 min. This represents a membrane Na efflux of the order of 10 \(\mu\)M/cm\(^2\)/sec which is comparable with the Na fluxes in frog ventricle reported by Johnson (1957). However, the actual quantity of Na lost is questionable in view of Orkand and Niedergerke's (1964) recent report showing that a reduction of Na, disproportionate to \([\text{Na}]^+/[\text{Ca}]\), would give higher membrane action potentials.
than if Ca were also reduced. Nevertheless, using the loss of excitability and contractility of the whole heart as end points of the measurement of ion depletion at the cell membrane surface, it should not be surprising to find a continuation of activity beyond the period calculated for block to occur on the assumption of freely diffusible ions.

**Antagonistic Effects of Na, K, and Ca**

It has been well established that depletion of K in the perfusate of the frog heart tends to hyperpolarize the cell membrane, increase the action potential duration, and to elevate contractile tension. On the other hand, Ca depletion, while also tending to elongate the action potential duration, reduces contractility; but the reduction of Na decreases the action potential duration and elevates contractility (Lüttgau and Niedergerke, 1958). It is not unexpected, then, that the simultaneous depletion of all three cations will result in some competitive steady state for both the action potential and twitch tension. The persistence of an action potential and twitch tension in 2% ionic concentration (diluted with isotonic sucrose), as in Fig. 6C and D, is evidence of this point. As shown by Casteels (1962), however, a disproportionate reduction of either K or Ca in the low Na medium leads to inexcitability.

Another question concerns the time required for the ionic concentrations in the extracellular space of a nonperfused frog ventricle to fall to a low level when the tissue is soaked in an isotonic sucrose solution. Assume the frog ventricle to be a sphere 3 mm in diameter. When placed in a stirred solution at zero concentration the sphere will require 23 min for its freely diffusing Ca to fall to 2% of the initial quantity (Crank, 1956). Although we cannot assume that a frog ventricle is a sphere of uniformly distributed and diffusible Ca, its chamber constitutes a sizeable quantity of extracellular fluid which is a maximum distance from the bathing solution. Its slow exchange with the bathing solution when the chamber of the ventricle is not directly perfused is evident from the washout characteristics of the perfused and nonperfused preparations of Johnson (1957) and Van der Kloot and Dane (1964), mentioned earlier. Furthermore, the diffusion characteristics of the isolated atrial trabeculae suggest that the effective diffusibility of electrolytes in the interstices is reduced by a factor of 10, so that the equivalent diffusion time in the non-perfused ventricle may, indeed, be several hours.

It is concluded that in the presence of long diffusion distances in the nonperfused frog heart soaked in isotonic sucrose, sufficient electrolytes, in balanced proportions, remain in the vicinity of the excitable membranes to maintain excitability and contractility, perhaps indefinitely. However, in accordance with the ionic hypothesis of excitation, a complete absence of external Na or its reduction to less than 5 to 10% of normal in the presence of normal K and Ca renders the frog heart incapable of propagating membrane depolarization.
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