Sodium Exchange in Dog Ventricular Muscle

Relation to frequency of contraction and its possible role in the control of myocardial contractility

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ABSTRACT Sodium exchange was studied in the arterially perfused papillary muscle of the dog. Three kinetically defined phases accounted for all the myocardial sodium: phase 0 (vascular)- λ_0 (exchange constant) = 3.6 min^{-1} ; phase 1 (interstitial)- λ_1 = 0.62 min^{-1} ; phase 2 (intracellular)- $\lambda_2 < 0.020 \text{ min}^{-1}$ in quiescent muscles. The phase 2 exchange rate was proportional to frequency of contraction and increased by approximately 0.004 min^{-1} for each 1 beat/min increment in rate in muscles demonstrating stable function. A sudden increase in frequency of contraction was followed by a marked increase in phase 2 sodium exchange if muscle function did not deteriorate. This increased exchange required 14 min to achieve a steady state. During this time active tension increased (positive staircase) and then declined to become stable as the sodium exchange stabilized. In muscles in which increased frequency of contraction produced a progressive decrease in active tension and contracture, sodium exchange failed to increase. The characteristics of sodium exchange are compared to those previously defined for calcium and potassium in the perfused dog papillary muscle. It is proposed that alteration in sodium exchange is a primary determinant of calcium and potassium movements and thereby plays a significant role in the control of myocardial contractility.

A previous study (1) of the isolated, arterially perfused dog papillary muscle indicated that a small, transient net loss of intracellular potassium occurred upon an abrupt increase in the frequency of contraction. This study was in agreement with that of Hajdu (2) in frog ventricle and of Sarnoff (3) in the blood-perfused isolated dog heart. It seemed possible that this transient K^+ loss might be based upon a transient lag in the ability of the myocardial Na^+

pump to supplement its Na^+ -pumping to the full extent required by the increased frequency of stimulation. The possibility of this " Na^+ -pump lag" has been discussed by Woodbury (4) on the basis of studies on frog skeletal muscle by Hodgkin and Horowicz (5). It is the primary purpose of this study to define the basic properties of Na^+ exchange in the perfused dog papillary at fixed rates of stimulation and, further, to determine the characteristics of Na^+ exchange upon abrupt increments in frequency of contraction.

METHODS

The experimental technique utilized the isolated, arterially perfused dog papillary muscle preparation previously described (6) in detail. The perfusion and isotope monitoring techniques were exactly as described in the potassium study (1) except that the radiation to the window of the G-M probe was collimated by a thicker lead cylinder (3 cm wall thickness).

The perfusate used in all experiments was of the following composition: NaCl , 130 mM; KCl , 4 mM; CaCl_2 , 5 mM; NaHCO_3 , 14 mM; NaH_2PO_4 , 0.435 mM; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.0 mM; and glucose, 5.56 mM. The perfusate was equilibrated to 98% O_2 -2% CO_2 at 24°C and contained approximately 0.04 ml O_2 /ml at a pH of 7.3-7.4 at the 150 mm Hg perfusion pressure used. Perfusion rate was approximately 1 ml/g tissue/min.

For isotopic labeling of the tissue, the perfusate was labeled with isotopic ^{24}Na (supplied by Iso/Serve, Inc., Cambridge, Mass.) to a specific activity varying between 15 and 150 $\mu\text{C}/\text{ml}$ depending upon the requirements of the experiment. All readings throughout the course of the experiment were corrected every 10 min for radioactive decay of ^{24}Na .

Following perfusion, the wet weight of the muscles was determined. They were then dried in porcelain crucibles at 90 - 100°C until weight-stable (4-6 hr) and the percentage tissue water calculated. The tissue was then digested in concentrated HNO_3 and the solution uniformly distributed in planchets for counting of total isotopic activity.

Total tissue sodium at various perfusion times was derived from analysis of multiple samples from the right ventricular wall (prior to perfusion) and from septal tissue after 1, 2, and 3 hr perfusion. 393 samples were analyzed from 21 hearts. After percent tissue water was found, the samples were ashed at 600°C for 12 hr. The ash was then dissolved in ion-free H_2O and analyzed for sodium content by flame spectrophotometry (model B flame spectrophotometer, Beckman Instruments, Inc., Palo Alto, Calif.).

RESULTS

Na^+ Content of Perfused Myocardium As expected from the previous K^+ study (1), there was a net gain of Na^+ and water in the myocardial tissue perfused at 23 - 24°C with a cell-free and protein-free medium over the course of 3 hr. This gain, as derived from analysis of septal tissue for total Na^+ con-

tent, resulted in a Na^+ content of 100.4 ± 1.11^1 mmoles/liter tissue water and a water content of $82.8 \pm 0.12\%$ after 2 hr of perfusion. Though septal tissue samples indicated a gain of 10 mmoles of Na^+ between the 2nd and 3rd hr, isotopic studies of the perfused papillary muscle indicated that there was very little net change in sodium content after 2 hr. This is shown by the isotopic labeling curve illustrated in Fig. 1. This muscle was perfused for a total of 143 min, during the last 60 min of which the perfusate contained ^{24}Na . It should be noted that near asymptotic value was reached by 40 min with little further gain in counts for the next 20 min. Analysis of total isotopic

FIGURE 1. Linear plot of ^{24}Na uptake for the last 60 min of a total 143 min perfusion of dog papillary muscle.

activity at the termination of perfusion in the muscle whose labeling curve is illustrated in Fig. 1 indicated a Na^+ content of 95 mmoles/liter tissue water. All muscles in which kinetics were evaluated were labeled to virtual asymptotic levels prior to washout. Therefore, there was little net change in sodium during the period when kinetics were being defined.

Kinetically Defined Phases of Na^+ A portion of the semilogarithmic plot of tissue and effluent activity during washout of ^{24}Na from a papillary muscle isotopically labeled for 65 min and washed out for 45 min, is illustrated in Fig. 2. This muscle was contracting at a rate of 6 beats/min. Graphical analysis of the effluent curve as described by Solomon (7) indicates that it may be resolved into three exponentially defined phases (0-2). This basic pattern was defined in 15 muscles. It is to be noted that the tissue and effluent

¹ 1 SE of mean.

plots became parallel after 15 min of washout. This indicates that all the tissue Na^+ which was labeled in 65 min was accounted for in the phases defined during washout. Since the isotopic activity of the muscle had reached virtual asymptote prior to the washout and since total tissue Na^+ values derived from asymptotic isotopic activity agree with flame photometry values, the total Na^+ content is accounted for in the three kinetically defined phases.

FIGURE 2. Semilogarithmic plot of the initial portion of a 45 min washout of a papillary muscle which had been previously labeled with ^{24}Na for 65 min. Tissue activity (x) in counts/min is plotted with simultaneous effluent activity (●) in counts/min/min. The resolution of the effluent curve into phases 0 to 2 with their respective time constants (λ) is indicated. The muscle was contracting at 6 beats/min.

The following phase rate constants (λ) were defined (respective time constants, (τ), in parentheses).

$$0 = 3.6 \pm 0.19^1 \text{ min}^{-1} (\tau = 0.29 \text{ min})$$

$$1 = 0.62 \pm 0.01 \text{ min}^{-1} (\tau = 1.61 \text{ min})$$

$$2 = 0.089 \text{ min}^{-1} (\tau = 11.2 \text{ min}) \text{ (mean frequency of contraction} = 22/\text{min}).$$

^{24}Na Exchange and Frequency of Contraction That portion of sodium exchange represented by phase 2 is markedly dependent upon the frequency of contraction. This is illustrated in Fig. 3 which summarizes the results from 10 muscles. The shaded area represents the range covered by the 10 experiments, and its extrapolated ordinate intercept indicates that a quiescent muscle should demonstrate a λ_2 of less than 0.020 min^{-1} . The slope in Fig. 3

indicates that λ_2 increases by approximately 0.004 min^{-1} for each 1 beat/min increment in rate between 0 and 35/min.

It was demonstrated in the 10 muscles that the rate constants of phases 0 and 1 were independent of the frequency of contraction. This implies that the portions of the muscle represented by phases 0, 1, and 2 exchanged in a "parallel manner" from a kinetic standpoint. This will be discussed more fully below. Moreover, it suggested the possibility that isotopic washout curves could be produced in which resolution of phases 1 and 2 would become

FIGURE 3. The relation between phase 2 ^{24}Na exchange and frequency of contraction. The shaded portion covers the range of values. The broken lines indicate extrapolation to zero frequency of contraction.

less distinct. This would be expected as λ_2 increased secondary to higher rates of stimulation. Such an experiment is illustrated in the washout effluent curve in Fig. 4. This muscle was beating at 54/min. Stable function was maintained by hyperperfusion at 3 ml/g tissue/min.² When compared to the effluent curve in Fig. 2, it is evident that the "knee" of the curve is much less distinct. Though this is partly attributable to the increase in λ_1 , it is largely due to the 8.5-fold increase in λ_2 . The rate of contraction was nine times greater in the experiment illustrated in Fig. 4 as compared to that in Fig. 2.

² It had been determined previously that perfusion rates above the standard 1 ml/g tissue/min had no significant effect on phase 2 sodium exchange; i.e., physiological rates of perfusion were not limiting for λ_2 . It should be noted in Fig. 4 that λ_0 and λ_1 are significantly above their respective means. This indicates that the Na^+ exchange of the systems represented by phases 0 and 1 can be accelerated by increasing the perfusion rate above physiological range.

Na⁺ Content of Phases 1 and 2 The total isotopic activity ascribable to a particular kinetic phase can be derived from the zero time intercepts of the phase plotted from the tissue washout as recorded by the G-M probe. The intercepts are representative of the total ionic content of a phase if the tissue had been previously labeled to asymptotic values. (The intercept ratio has to be corrected according to the relative values of λ_1 and λ_2 as indicated by Huxley (8).) The accuracy of the phase extrapolations to zero time is improved if the rate constant for phase 2 is as small as possible and remains consistent over a prolonged washout. This was accomplished by using a concentration

FIGURE 4. Semilogarithmic plot of effluent activity (●) during washout of a papillary muscle previously labeled with ^{24}Na . The muscle was contracting at 54 beats/min. The resolution of the effluent curve into phases 0 to 2 with their respective rate constants (λ) is indicated. The effluent curve should be compared to that of Fig. 2 (see text).

of 10 mM K^+ in the perfusate. This enabled the muscle to be stimulated during the labeling period and to be held quiescent during washout. (It might be expected that the high $[\text{K}^+]_o$ would increase λ_2 for Na^+ somewhat over the value predicted from Fig. 3 but this did not present a problem in the analysis.)

The muscle was perfused for a total of 158 min. Total tissue Na^+ content by flame photometry was 92 mmoles/liter tissue water, $\lambda_1 = 0.53 \text{ min}^{-1}$, $\lambda_2 = 0.063 \text{ min}^{-1}$, and the ratio of $\frac{\text{Phase 1 intercept}}{\text{Phase 2 intercept}} = 2.9$. This ratio corrects to 3.8 according to Huxley's formulation (8).

The Na^+ content of phase 0 was not calculated directly from washout curves because of difficulty in checking its exact intercept value. This is because the count for the first few seconds after commencing washout is influenced by some residual loading solution from the perfusion system.

Abrupt Changes in Frequency of Contraction The preceding results indicate some of the properties of sodium exchange at fixed frequencies of contraction. The experiment illustrated in Fig. 5 indicates the relationship of Na^+ exchange to muscle function when frequency of contraction was increased from

FIGURE 5. Semilogarithmic plot of tissue (x) activity in counts/min and effluent (●) activity in counts/min/min from a papillary muscle during the 21st to 45th min of washout of ^{24}Na . The frequency of contraction was increased from 0 to 12/min at 22.5 min and maintained at this level for the remainder of the experiment. The mechanical function of the muscle is indicated. The broken line represents the regression from the slope established after the 37th min of washout. The asterisk indicates the calculated point on the regression line for 94% equilibrium of ^{24}Na in the effluent following increase in frequency of contraction. See text for analysis.

0 to 12/min. This muscle had been completely quiescent for 7 min prior to onset of stimulation at 12/min. Analysis of the slope of tissue and effluent activity for the 3 min immediately prior to onset of stimulation indicated a $\lambda_2 = 0.013 \text{ min}^{-1}$. (It should be noted that this value falls in the middle of the shaded area at its ordinate intercept in Fig. 3.)

At the start of stimulation (at 22.5 min after start of washout) phases 0 and

1 were virtually clear of isotopic activity so that the plot is representative of phase 2 Na^+ . Upon commencing stimulation both tissue and effluent activity changed significantly indicating a marked alteration in the course of isotopic washout. It should be noted that 14 min were required to establish a $\lambda_2 = 0.066 \text{ min}^{-1}$. This is slightly above the rate of phase 2 Na^+ exchange which would be predicted at 12 beats/min from the relation illustrated in Fig. 3. The increase in effluent activity indicates an increase in the rate of exchange of ^{24}Na from that portion of the tissue represented by phase 2. This is confirmed by the tissue plot, which, however, is less accurate because of the low order counts recorded by the probe after 24 min of washout. At the 35th minute a steady state is established at a $\lambda_2 = 0.066 \text{ min}^{-1}$. The tissue slope becomes parallel confirming that exchange has arrived at a steady state in a kinetically homogeneous system.

It was during the period prior to establishing steady state that the active tension development of the muscle varied. A positive tension staircase increased to a maximum of 14.8 g over the first 2–3 min. The tension then remained relatively stable for the next 3–4 min but then began to decline and continued to decrease for the next 9 min. After this it remained stable for the remainder of the experiment. It should be noted that there was no increase in diastolic tension nor any tension alternans during the 9 min period of declining tension. It is evident in Fig. 5 that the systolic tension finally stabilized at approximately the same time sodium exchange had reached a steady state.

This experiment demonstrates that, in the perfused papillary muscle in which perfusion rate remains constant, a significant period of time is required for sodium exchange to adjust to an increased frequency of contraction. Coincident with the adjustment in phase 2 Na^+ exchange there occur a series of changes in active tension development.

The experiment illustrated in Fig. 6 indicates the same parameters of Na^+ exchange and tension development but in a muscle in which rate was increased abruptly from 22 to 38/min. The muscle was perfused at a rate of 1.16 ml/g/min which will support rates of contraction up to 25–30 beats/min but will not sustain muscle function at higher frequencies for any length of time. The phase 2 exchange constant with the muscle contracting at 22/min was 0.110 min^{-1} which is close to the expected value (Fig. 3). Muscle function was stable at this rate of contraction. In contrast to the experiment illustrated in Fig. 5, when the frequency of contraction was increased to 38/min, there was no increase in phase 2 sodium exchange. In fact the exchange rate began to decline after some 8 min following initiation of increased frequency of contraction. On the basis of the relationship illustrated in Fig. 3 the slopes should have increased, eventually, to the extent indicated by the broken line in Fig. 6.

The sequence of active tension development also differed quite markedly

when compared to Fig. 5. Upon increase in frequency, there was a positive tension staircase as in Fig. 5, but this was followed by a *progressive* decline in systolic tension with no evidence of stabilization. A tension alternans was present throughout the period of stimulation at 38/min. Associated with the

FIGURE 6. Plots as in Fig. 5. The frequency of contraction was increased from 22 to 38/min at the 15th min of ^{24}Na washout and maintained for the remainder of the experiment. Note the continuous decline in actively developed tension and the progressive increase in diastolic tension. The broken line indicates the slope of tissue and effluent activity which should have been established at a frequency of contraction of 38/min. See text for analysis.

progressive decline in actively developed tension, there was a continuous increase in diastolic tension to the extent of 60% of the actively developed tension at the termination of the experiment.

This experiment demonstrates a progressive deficiency in the ability of the muscle to exchange phase 2 sodium at higher frequencies of contraction. This deficiency was coincident with deterioration in muscle function, which was evident from the progressively falling systolic tension and rising diastolic tension.

DISCUSSION

The Kinetically Defined Na⁺ Phases It was demonstrated that papillary muscle Na⁺ content as derived from near asymptotic isotopic activity agreed well with direct tissue analysis of septal samples perfused for the same period of time. It was also noted that the curves of effluent isotopic activity and isotopic activity recorded directly from the tissue during washout tended to become parallel during their phase 2 portions (Fig. 2). Persoff (9) has shown that this indicates that all of the substance which has been labeled is accounted for in the washout. Therefore the sodium content of the dog papillary muscle is completely accounted for in the three exponentially defined phases of exchange. This three phase pattern is strikingly similar to that found by Conn and Wood (10) for the in situ dog heart.

PHASE 0 The exchange constant for phase 0 ($\lambda_0 = 3.6 \text{ min}^{-1}$) is similar to that previously found for ¹³¹I-labeled albumin (6), calcium (11), and potassium (1) in the perfused papillary muscle. This suggests that phase 0 is predominantly representative of sodium in the vascular space. This is supported by the increase in λ_0 produced by more rapid perfusion (Fig. 4). The vascular compartment had been previously measured as 7% of the wet weight of the perfused muscle (6). At a Na⁺ concentration of 144 mM in the perfusate this would account for approximately 12 mmoles/liter tissue water.

Before proceeding to discussion of phases 1 and 2 it is necessary to discuss the method of kinetic analysis used. This has been previously presented with reference to K⁺ exchange in the perfused papillary muscle (1). Anatomically, intracellular Na⁺ would be expected to exchange with the vascular system in a series arrangement through the interstitial space. Under the kinetic conditions imposed by this "open series two compartment system" the phase exchange constants, λ_1 and λ_2 , are quite accurate representations, respectively, of interstitial and cellular exchange, K_1 and K_2 , if flux at the capillary is sufficient to prevent the development of a significant concentration gradient in the interstitium. If such a gradient develops then λ_1 and λ_2 do not accurately represent the true exchange constants, K_1 and K_2 , but each is a composite of K_1 and K_2 in varying proportion depending on the magnitude of the gradient. The following ratios will determine the existence of a concentration gradient:

$$K_2/K_1 = \text{cellular exchange constant/interstitial exchange constant}$$

$$C_i V_i / C_o V_o = \text{cellular ionic content/interstitial ionic content}$$

$$A_i / A_o = \text{cellular surface area/capillary surface area.}$$

These ratios are made up of components (12) of the basic efflux equation $m_e = KCV/A$. The smaller each ratio, the more accurately will λ_1 and λ_2

represent K_1 and K_2 .³ In the previous study (1) it was concluded that the ratios were sufficiently small in the case of K^+ ion that λ_1 and λ_2 could be considered accurate representations of K_1 and K_2 . This was based on a study in which a papillary muscle was labeled simultaneously with ^{42}K and sucrose- ^{14}C and subsequently washed out. Sucrose washed out with a rate constant of 0.53 min^{-1} (in agreement with previous sucrose studies (6)) and potassium with a λ_1 of 0.63 min^{-1} . Since sucrose remains predominantly extracellular all the ratios above approach zero. In addition the study of Page and Bernstein (13) indicated that interstitial exchange of K^+ (and Na^+) should be similar to that of sucrose. Since the rate constant for sucrose exchange was actually somewhat less than that of λ_1 for K^+ it was concluded that despite a $C_iV_i/C_oV_o \sim 20$ for K^+ there was no evidence for a significant interstitial concentration gradient and $K_1 \gg K_2$ for potassium. (Obviously, some gradient has to exist if there is to be facilitated ionic movement from cell to capillary but this will be kept small by a relatively rapid interstitial clearance of the substance under consideration.) Therefore, it was thought to be realistic to consider K^+ flux from a parallel kinetic viewpoint. This was supported by analysis of phase K^+ content.

The experimental findings indicate that Na^+ may be considered in the same way. λ_1 for Na^+ is 0.62 min^{-1} or nearly equal to λ_1 for K^+ and again greater than that for sucrose. In addition C_iV_i/C_oV_o of Na^+ can be estimated at approximately $1/50$ that of K^+ . Cellular exchange of Na^+ is certainly more rapid than cellular K^+ exchange in a beating muscle, but since large increases in rate (which certainly increase cellular exchange) fail to alter λ_1 , K_1 must exceed K_2 for Na^+ by enough to prevent a concentration gradient from building in the interstitium. It should also be noted that λ_1 for calcium is 0.59 min^{-1} (11). This, again, is nearly equal to λ_1 for Na^+ , K^+ and to the rate constant for sucrose. This indicates that interstitial clearance is rapid enough in the vascularly perfused papillary muscle for the exchange of these ions, including Na^+ , to be considered parallel from a kinetic viewpoint. Consequently, all calculations of content and fluxes will assume a parallel system to be operative.

PHASE 1 On the basis of the preceding discussion it is assumed that phase 1 sodium exchange represents interstitial sodium exchange. The corrected phase 1: phase 2 Na^+ content ratio in the muscle analyzed was 3.8 with a total Na^+ content of 92 mmoles/liter tissue water. The total Na^+ content in a series of perfused septal samples was estimated at 100.4 ± 1.11 mmoles. Since phase 0 was estimated to account for 12 mmoles, phase 1

³ The relation of K_1 and K_2 in an open series two compartment system to experimentally measurable parameters has been mathematically derived. The complete derivation is not pertinent to the present study, but will be forwarded on request.

content would account for 70 mmoles and phase 2 for 18 mmoles in muscles with total Na^+ content of approximately 100 mmoles. If the interstitial Na^+ concentration equals that of the perfusate (144 mM), then the interstitial Na^+ space is 48.5% of total tissue water or 40% of wet weight of tissue perfused for approximately 2 hr. The interstitial K^+ space was found to be 42% of wet weight using a method of effluent analysis (1) in the perfused papillary muscle.

An approximation of the efflux from the phase 1 compartment can be derived from the relation (12):

$$m_e = \lambda_1 C_o V / A \quad (1)$$

where

$$m_e = \text{efflux}$$

λ_1 = exchange constant for phase 1 (assumed to represent interstitial Na^+)

C_o = interstitial concentration of Na^+

V = volume of compartment

A = surface area of compartment

If the surface area of the interstitium is equivalent to the estimated capillary surface area of $1000 \text{ cm}^2/\text{cm}^3$ (14), equation 1 indicates a flux of $590 \mu\text{moles}/\text{cm}^2/\text{sec}$ or $35.4 \mu\text{moles}/\text{g}$ wet tissue/min.

PHASE 2 This phase represents all the remaining sodium in the muscle. This is assumed to be intracellular. No other phases beyond phase 2 are demonstrable (Fig. 2) in muscles which are labeled to virtual asymptote. This indicates that intracellular Na^+ is distributed homogeneously from a kinetic viewpoint although intracellular exchanges more rapid than λ_2 could not be identified.

The total Na^+ content of phase 2 was calculated at 18 mmoles/liter tissue H_2O in muscles perfused for 2 hr. Intracellular volume is calculated to account for 43% of total tissue water. Therefore, intracellular Na^+ concentration would be 42 mM. This is in the range of approximately 1.5 times normal based on the study of Conn and Wood (10). They found a cell sodium of about 13 mmoles/kg in the isolated dog heart perfused with blood at 38°C . The gain in cellular sodium agrees well with a one for one Na^+ for K^+ exchange in the perfused papillary preparation. It can be estimated from the previous K^+ study (1) that intracellular K^+ concentration decreased by approximately 15 mM during 2 hr of perfusion. This loss corresponds well to the estimated gain in intracellular Na^+ concentration found in the present study. These values indicate a partial inadequacy of the Na^+ pump in the perfused papillary muscle with the establishment of increased cellular Na^+ content when steady state is reached. The increased $[\text{Na}]_i$ would tend to

elevate computed cellular fluxes relative to in vivo values, all other factors remaining equal.

The data summarized in Fig. 3 indicate the marked dependence of phase 2 sodium exchange upon frequency of contraction. Extrapolation of the shaded area indicates that the quiescent muscle has a $\lambda_2 < 0.020 \text{ min}^{-1}$. In the muscle which had a 7 min period of quiescence λ_2 had decreased to 0.013 min^{-1} . The slope in Fig. 3 indicates that λ_2 increases by very nearly 0.004 min^{-1} for each 1 beat/min increment in rate for the range between 0 and 35/min. This is a restricted range of frequency within which the $0.004 \text{ min}^{-1}/\text{beat}$ factor is realistic. The experiment illustrated in Fig. 4 indicates that this factor increases greatly at higher rates. This is most likely related, at least in part, to increases in $[\text{Na}]_i$ which are known to have a marked influence on the flux values (15, 16). The factor of $0.004 \text{ min}^{-1}/\text{beat}$ for λ_2 is, therefore, empiric and restricted to the frequency range described.

The efflux from the phase 2 (assumed intracellular) compartment can be derived from equation 1. The cellular V/A ratio of $4.23 \times 10^{-4} \text{ cm}$ previously derived (1) is used. The maximum efflux from the resting muscle cell ($\lambda_2 = 0.020 \text{ min}^{-1}$) is computed to be $5.9 \mu\text{moles}/\text{cm}^2/\text{sec}$ or $0.33 \mu\text{mole}/\text{g}$ wet tissue/min. Since each beat increases the exchange by 0.004 min^{-1} , each impulse is associated with a flux of $71 \mu\text{moles}/\text{cm}^2$ or $0.066 \mu\text{mole}/\text{g}$ wet tissue or $0.081 \text{ mmole}/\text{liter}$ tissue water. This indicates $2.3 \mu\text{moles}/\text{g}$ wet tissue/min flux at the cell membrane at a contraction frequency of 30/min. Computed flux at the capillary membrane was calculated to be more than 15-fold greater. This is consistent with the proposal that the presence of a significant interstitial concentration gradient is unlikely in the perfused papillary muscle.

Sodium Flux The sodium flux required to depolarize the cell can be estimated from the relation $M = CV/F$ where M is the molar concentration of Na^+ , C is the membrane capacitance, V is the voltage, and F is the Faraday, C is assumed to be $11 \mu\text{f}/\text{cm}^2$ (17), and $V = 110 \text{ mv}$ for mammalian ventricular muscle. The Na^+ flux associated with each stimulus is calculated to be $12.5 \mu\text{moles}/\text{cm}^2$. The experimental value of $71 \mu\text{moles}/\text{cm}^2$ obtained in this study is nearly six times greater than this. It is of particular note that the experimental value obtained in the dog papillary muscle is remarkably similar to the value computed by Noble (18) for Purkinje fibers. Na^+ gain during one action potential was computed to be $77 \mu\text{moles}/\text{cm}^2$. The value of $71 \mu\text{moles}$ in the present study indicates that some $58 \mu\text{moles}$ is in excess of what is required to change the charge on the membrane capacity. As indicated by Noble (18) this excess is electrogenically "wasted," at least in part, as a result of Na^+ and K^+ currents overlapping each other over a portion of the action potential duration. It is also likely that the "excess Na^+ " is partially balanced by accompanying Cl^- movement. Hutter and Noble (19) and

Carmeliet (20) have demonstrated that Cl^- ions contribute little at resting potential, but Carmeliet has shown a significant contribution of Cl^- ions as electrical charge carriers during the depolarized state in cardiac muscle.

Finally, the total Na^+ flux from phase 1 (interstitium) and phase 2 (cell) at a rate of 30/min is approximately $38 \mu\text{moles/g/min}$. At the perfusion rates used (1 ml/g/min) the vascular Na^+ turnover is $144 \mu\text{moles/g/min}$. This indicates that capillary turnover is not limiting for Na^+ exchange in the perfused papillary muscle under the experimental conditions of the study.

Abrupt Increments in Rate: Relation of Na^+ Exchange and Tension Development

The experiment illustrated in Fig. 5 shows that 14 min were required for phase 2 Na^+ exchange (assumed cellular) to arrive at a steady state (as measured from venous effluent and direct tissue counting) following an increase in the frequency of contraction. The rate of ^{24}Na efflux in the venous drainage increased abruptly upon stimulation and this is confirmed by the rapid decline in tissue activity. Following the peak in effluent activity, at the 25th min, various mechanisms can be proposed to explain the subsequent course of events. Though the decline in effluent activity appears exponential between the 25th and 35th min, the nonparallelism of the tissue activity does not support the establishment of a single new rate constant in a kinetically homogeneous system during this period. It is possible that the increased frequency introduced, transiently, another "compartment" which dominated the effluent curve. This is a possibility raised from a consideration of the study of Dick and Fry (21) on Na^+ efflux from frog toe muscles in which a somewhat similar transient in effluent activity was introduced by producing changes in external Na^+ concentration. A less complex explanation, however, is that the increased rate of contraction induced an increased rate of ^{24}Na exchange and that this exchange rate then gradually declined over the 25–35 min period. This is consistent with the flattening of the tissue slope and the decline in effluent activity during this period.

The distinction between kinetic mechanisms is somewhat semantic. The crucial points are that cellular Na^+ exchange increased following an increase in frequency of contraction and it required 14 min to reach a steady-state exchange compatible with the altered contractile rate. It would be expected that some time would be required for an increased flux arising at the cell membrane to become fully manifest in the venous effluent. Though interstitial exchange has been stated to be rapid enough to prevent a significant concentration gradient from developing, it is, by no means, instantaneous. The time required for flux events at the cell membrane to become fully manifest in the venous effluent is probably best computed from the phase 1 exchange constant ($\lambda_1 = 0.62 \text{ min}^{-1}$). (This assumption seemed to be valid in explaining

the transient in effluent activity following high K^+ perfusion of the papillary in the previous K^+ study (1).) If a new steady-state exchange were established instantaneously at the cell membrane upon a change in frequency of stimulation, it should require 4.5–5 min to achieve near full representation of the event in the venous effluent. This point is indicated by the asterisk on the regression line drawn from the slope established for effluent activity after the 37th min of washout in Fig. 5. It is likely then that there is an abrupt increase in Na^+ exchange at the cell membrane upon increase in frequency of contraction and that it requires approximately 9 min to reach a steady state consistent with the established augmented frequency of contraction.

The development of active tension in the muscle increases and then declines largely during the period of increased Na^+ exchange and then stabilizes after a new steady state has been attained. The possible significance of this will be discussed below in relation to the transient events previously described for calcium and potassium exchange which occur secondary to changes in frequency of contraction.

The major difference in the muscle illustrated in Fig. 6 is that phase 2 (cellular) Na^+ exchange failed to increase upon an increase in rate of contraction and never established a λ_2 consistent with the increased frequency. This muscle demonstrated an initial positive staircase but then actively developed tension rapidly and progressively declined in association with the appearance of tension alternans and marked contracture. The possible significance of this sequence will also be discussed below.

Correlation of Sodium, Potassium, and Calcium Movements and Myocardial Function

Fig. 7 represents the proposed temporal relationship among Ca^{++} , K^+ , and Na^+ exchange following abrupt increases in frequency of contraction in the perfused dog papillary muscle. The pattern represented is for muscles with functional response similar to that of the muscle represented in Fig. 5. The $K^+ - Ca^{++}$ relation was previously defined (1, 6, 22). The curves in Fig. 7 are uncorrected for time required for interstitial equilibration since phase 1 rate constants are very nearly equal for Na^+ , K^+ , and Ca^{++} .

It is noted that there is a transient net K^+ loss which continues for some 15–20 min. This K^+ loss was shown to be intracellular in origin. This loss occurs during the time when cellular Na^+ exchange is increasing and adjusting to the new contractile rate. This suggests that, despite the increased rate of Na^+ exchange, there is some intracellular Na^+ accumulation occurring over this period. At the approximate time net K^+ loss ceases, Na^+ exchange slows to reach a steady state. This sequence is consistent with recent findings which demonstrate that the membrane ATPase activation believed to be important in Na pumping, is sensitive to Na^+ concentration on the “inside”

of cellular membranes (23, 24). The correlation of Na^+ and K^+ movements and rates of exchange is consistent with the proposal that the rate of Na^+ pumping by the myocardial cell is sensitive to small changes in concentration of Na^+ at the intracellular side of the membrane. Opit and Charnock (25) have proposed that the membrane sodium-pumping cycle has an inherent feedback control in which its energy-producing ATPase cycle is "stimulated by raising Na^+ concentration and depressed as this concentration falls and the K^+ concentration rises." The period of rapid Na^+ exchange would then correlate with an increased Na^+ concentration at the inside of the membrane while the Na^+ -pumping mechanism was adjusting to increased requirements secondary to the increased rate of stimulation. This proposed increase of intracellular Na^+ is supported by the transient net loss of K^+ occurring at this time. The cessation of K^+ loss indicates that Na^+ concentration at the

FIGURE 7. Temporal relation among net change in tissue calcium, rate of net K^+ loss, and rate of cell Na^+ exchange in dog papillary muscle following an increase in frequency of contraction. See text for analysis.

inside of the membrane is no longer increasing and coincides with Na^+ exchange reaching a steady state.

It is proposed that the Ca^{++} ion plays a role in another feedback mechanism which, however, would be dependent upon the Na^+ -pumping system discussed. Previous studies (6, 22) of Ca^{++} exchange in the dog papillary muscle indicated a net increase in myocardial Ca^{++} associated with increments in frequency of contraction. This net gain was reversed in the face of continued increment in rate in muscles in which the rate increase did not result in *progressive* fall of contractile tension and onset of contracture; i.e., function similar to that of the muscle represented in Fig. 5. The course of the net Ca^{++} movement is shown in Fig. 7. It can be seen that net uptake of Ca^{++} continues while the rate of K^+ loss is increasing. This would be coincident with the period of increased Na^+ concentration at the inside of the membrane contributing to the increase in Na^+ pump activity. It seems possible that a portion of the increased intracellular Na^+ is derived from a displacement of Na^+ from a region of the cell membrane where it is in competition for sites with the Ca^{++} ion. There is much evidence (11, 26–29) to indicate that there

is a region of heart muscle in which Na^+ and Ca^{++} ions compete for sites. It is suggested that this region is a portion of membrane of the longitudinal sarcotubular system which is not in direct continuity with the extracellular space. It would follow that this membrane forms those vesicular structures which store Ca^{++} (30–32). It is likely that this Ca^{++} storage system has been defined kinetically as phase 2 for Ca^{++} exchange ($\lambda = 0.116 \text{ min}^{-1}$) in the dog papillary muscle (11). If Na^+ is displaced from the competitive area on this membrane, it would then be immediately replaced by Ca^{++} from the vesicular storage area until Na^+ is pumped back. This increased Ca^{++} would then contribute to increased tension development as demonstrated for the first 6–7 min following the increased frequency of contraction (Fig. 5). This Ca^{++} which moves from the storage area to the membrane would be replaced to the storage region from the interstitial space at a rate determined by the phase 2 Ca^{++} exchange constant. (0.116 min^{-1} , $t_{1/2} = 6.0 \text{ min}$). Therefore, there would be a beat to beat equilibrium of Ca^{++} established between the competitive membrane and the storage area but a considerably longer period would be required for the storage area to come to equilibrium with interstitial Ca^{++} . This might explain the additional time required for the “ Ca^{++} transient” to complete its course (22–25 min) when compared to the “ K^+ and Na^+ transients” (14–18 min) (see Figs. 5 and 7).

According to the foregoing hypothesis, tissue Ca^{++} content (probably localized to the sarcotubular system) increases during the period when Na^+ is displaced to the inside of the membrane. This is associated with an increase in contractile tension. As Na^+ pumping increases, net K^+ loss diminishes, Na^+ is returned to the sites of competition with Ca^{++} , Ca^{++} is displaced, and contractile tension falls to a level primarily determined by the $\text{Na}:\text{Ca}$ ratio at the sarcotubular membrane. It would follow from this hypothesis that the amplitude and maintenance of the classical Bowditch rate–tension staircase (33) depend, primarily, on the activity of the membrane Na^+ pump relative to the frequency of contraction established.

If the Na^+ pump is unable, for any reason, to increase its activity relative to the demands of contractile frequency, the sequence demonstrated in Fig. 6 might be expected. Na^+ ions would accumulate at the inside of the membrane to the capacity of the sites involved and this would, through the secondary Ca^{++} movements, induce a positive staircase. Continued deficiency in Na^+ pumping would allow more and more Na^+ to accumulate intracellularly and more Ca^{++} to accumulate at the sarcotubular membrane and in the storage area. As the capacity of the storage area (estimated at 3.2 mmoles Ca^{++} /liter tissue H_2O (22)) is exceeded, it has been demonstrated (11, 22) that Ca^{++} increments become firmly bound by some portion of the tissue and seem to correlate with progressively rising diastolic tension leading to failure of the muscle. This is the pattern demonstrated in Fig. 6.

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