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The Kinetic Analysis of Simultaneous Calcium Exchange and Sorbitol Washout in the Rat Heart (38386)

SOMSONG PENPARGKUL,[1] F. NORMAN BRIGGS,[2] AND JAMES SCHEUER[3]

Departments of Medicine and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

One approach to the study of how calcium controls cardiac contractility is to measure the kinetics of calcium exchange in contracting myocardium (1–3). Since the extracellular calcium concentration influences the amount of calcium involved in excitation–contraction coupling (4), knowledge of calcium movements in the extracellular space is of great importance. If only a small fraction of the calcium in the extracellular space is related to the control of excitation–contraction coupling, changes in this component could easily be hidden by extracellular events. In order to more clearly evaluate the relation of calcium movements in the interstitial space to intracellular calcium exchange, rat hearts were simultaneously loaded with $^{45}Ca^{2+}$ and ^{3}H sorbitol and the washout curves analyzed.

The influence of quinidine was also examined to determine if a decrease in contractility alters the kinetics of the washout of the extracellular space.

Methods and Materials. The hearts of 200- to 250-g Wistar rats were perfused in an isolated working rat heart apparatus (5). Two reservoirs and two atrial bubble chambers, one for isotopic and one for nonisotopic fluids, could be abruptly switched into and out of the perfusion system. The perfusion medium contained 143 m*M* sodium, 123 m*M* chloride, 25 m*M* bicarbonate, 6 m*M* potassium, 1.2 m*M* magnesium, 1.2 m*M* phosphate, 2.0 m*M* calcium, 0.5 m*M* disodium EDTA, and 5 m*M* glucose with insulin 0.1 unit/ml, and was bubbled with 5% CO_2–95% O_2 and perfusion was conducted at 37°. Except for the first 15-min control period, fluid was not recirculated.

After the heart was mounted the atrioventricular node was crushed so that the spontaneous heart rate was less than 200 beats/minute. The right ventricle was paced with a square-wave stimulus of 1.2–1.6 V with a duration of 4 msec.

Aortic, coronary flows, ventricular systolic pressures, and maximum *dP/dt* were monitored under conditions of constant heart rate, atrial pressure, and aortic pressure.

One quinidine and one control study was performed on any given day. The quinidine study was performed first in order to establish the rate at which the quinidine heart was captured. The control study was performed at the same heart rate.

After the hearts were perfused for a 15-min control period, a perfusion medium containing ^{45}calcium[4] 200 μCi/liter and tracer ^{3}H-sorbitol[5], (38.5 mCi/mg) 250 μCi/liter was switched into the system, and the perfusion was continued for 10 min. A nonradioactive perfusion medium was then abruptly switched into the system for a radioactive washout period of 10 min. Integrated 5-sec samples were obtained during the first minute, 15-sec samples during the second minute, 30-sec samples during the third and fourth minute, and 1-min samples between the fifth and tenth minutes. For quinidine experiments, quinidine was included during both the radioactive loading and washout phases.

Radioactivity was measured by liquid scintil-

[1] Present address: Somsong Penpargkul, M. D., Ph. D., Department of Medicine, Montefiore Hospital and Medical Center, 111 East 210th Street, Bronx, New York 10467.

[2] Present address: F. Norman Briggs, Ph.D., Department of Physiology, Virginia Commonwealth University, Medical College of Virginia, Richmond, Virginia 23219.

[3] Present address: James Scheuer, M. D., Department of Medicine, Montefiore Hospital and Medical Center, 111 East 210th Street, Bronx, New York 10467.

[4] Obtained from New England Nuclear, Boston, MA.

[5] Obtained from New England Nuclear, Boston, MA.

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lation counting using a dual-channel spectrometer. Crossover and quenching were evaluated and corrected for in each experiment.

The rate of exchange of calcium and washout of sorbitol was calculated using the specific activity of the initial perfusion medium and the cpm in the effluent.

$$\frac{\text{cpm/ml of effluent} \times \text{ml/min effluent}}{\text{cpm/ml of initial perfusate} \times \text{g}} = \text{ml/min per g exchange or washout}$$

The mean exchange rate for calcium and washout rate for sorbitol for each point in time was calculated for eight control and eight quinidine-treated hearts and plotted on a semilogarithmic paper. To obtain the slope and volume of the slowest component (component 3) the last five data points of the washout curve were used in a computer program to develop a regression line. The next slowest component (component 2) of the washout was obtained by subtracting calculated values for component 3 from the observed mean values for calcium and sorbitol efflux rates. The slope and intercept of the second component was obtained by visual inspection of the resulting curve. Half-times were estimated graphically and rate constants calculated. The volume of component 3 at the onset of washout was calculated from the value of the zero time intercept of component 3 divided by the rate constant of this component.

Results. Mechanical performance. The heart rates in the control and quinidine experiments averaged 202±2 beats/minute. The mechanical performance of the hearts reached a steady state in 10–15 min.

All hearts treated with quinidine ($2.1–2.5 \times 10^{-5}$ *M*) showed an immediate increase in left ventricular pressure and maximum *dP/dt* which reached a peak in 15 sec and returned to the baseline in 1 min. After 15 sec the mean increase in LVP and *dP/dt* were 16 and 26%, respectively. These values gradually fell so that 5 min after the addition of quinidine they had reached 85 (LVP) and 68% (*dP/dt*) of the prequinidine values. Hearts performed at that level for the remainder of the loading and unloading periods. Table I shows the mean values for cardiac function during the washout period. Coronary flow was significantly greater in the presence of quinidine. Cardiac output was unchanged.

^{45}Ca exchange and ^{3}H sorbitol washout. Figure 1 shows the semilogarithmic plots of the ^{45}Ca exchanges and ^{3}H sorbitol washouts in control and quinidine-treated hearts. The slope of the slowest component (component 3) of the washout is indicated.

Figure 2 shows the kinetics of washout when that due to component 3 has been subtracted. Since all of the data could not be fit by this second component there must have been a faster component of the washout, component 1.

The rate constants for washout of sorbitol and exchange of ^{45}Ca are shown in Table II. There were no significant differences between the values.

In each heart the space represented by the total amount of calcium recovered during washout exceeded the space represented by the total amount of sorbitol recovered. This difference could be accounted for by the greater volume for calcium than for sorbitol found by analysis of component 3. When analyzed inidvidually, the distribution for calcium in compartment 3 was greater than for sorbitol in each heart (Control: sorbitol 0.11 ml/g, ^{45}Ca 0.18 ml/g ($P < 0.05$); quinidine: sorbitol 0.13 ml/g. ^{45}Ca 0.18 ml/g ($P < 0.05$).

TABLE I. Cardiac Performance During Washout Studies.[a]

	Number of hearts	Heart weight (mg)	Dry/wet heart weight (%)	Coronary flow[b] (ml/min)	Cardiac output[b] (ml/min)	PLVSP[c] (mm Hg)	Maximum LV *dP/dt* (mm Hg/sec)
Control	8	765 ± 25	18.9 ± 0.2	17.4 ± 1.7	52.5 ± 1.9	142 ± 5	2733 ± 266
Quinidine	8	787 ± 48	18.7 ± 0.1	21.2 ± 2.8	49.0 ± 2.6	124 ± 2	1860 ± 90
P Value		NS	NS	<0.05	NS	<0.01	<0.01

[a] Values are mean ± SE.
[b] Values are per g wet weight.
[c] PLVSP = peak left ventricular systolic pressure.

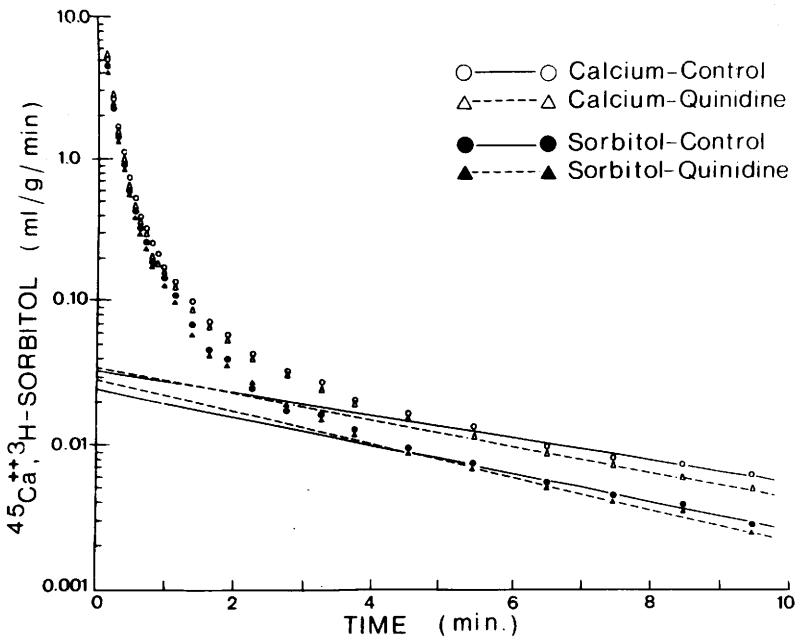

FIG 1. The efflux of ^{45}calcium and ^{3}H sorbitol from paced perfused working hearts. The curves show the mean points for eight hearts exposed to quinidine. The regression lines for the last five points were determined by computer and the rate constants are presented in Table II. This regression line and intercept represent compartment 3. The data are presented in milliliters per gram per minute based on the calculation cpm released/specific activity of the initial perfusion medium in cpm/ml.

The negative inotropic effect of quinidine is documented in Table I. The data in Table II show that neither the kinetics of washout of sorbitol nor exchange of calcium was altered by quinidine in either compartment 2 or 3. The net volume of distribution of calcium into compartment 3 (^{45}Ca space − sorbitol space) was also not measurably altered by quinidine.

Discussion. In the present experiments each identifiable kinetic of calcium exchange was associated with an identical kinetic associated with the washout of the extracellular marker sorbitol. Therefore, with the experimental design employed in these experiments calcium exchange rates were determined by processes in the vascular and/or the interstitial spaces. Therefore, exchange of calcium between intracellular compartments such as the sarcoplasmic reticulum must either be so rapid that this subcellular compartment becomes kinetically indistinguishable from the interstitium or so little calcium is involved that it could not be identified kinetically in the current experiments.

The earliest component of washout most probably includes the vascular space, cardiac chambers, and apparatus dead space, and would be expected to be limited by the rate of perfusion. With a coronary flow of 17–20 ml/min and an apparatus dead space of 0.45 ml this compartment would have been cleared in about 1.2 sec. That portion of the sorbitol washout and calcium exchange which was extravascular could be resolved as the sum of two exponential processes. Since the rate constants for these two processes were not significantly different for calcium and sorbitol, one may conclude that the factors determining the rate of calcium exchange in these compartments are to be found in the interstitial fluid space.

The observation that the interstitial fluid volume of rat heart is kinetically heterogeneous has been made previously by Morgan *et al.* (6), and Fisher and Young (7). Young (8) concluded that the faster of the two components is dependent upon ultrafiltration between the interstitial and vascular space. This component of washout was absent in the quiescent heart, and it was dependent upon the force of myocardial contraction. Since quinidine had a relatively slight effect on peak left ventricular pressure in the current experiments, the force of contraction was modified very little. It is, therefore, not surprising that

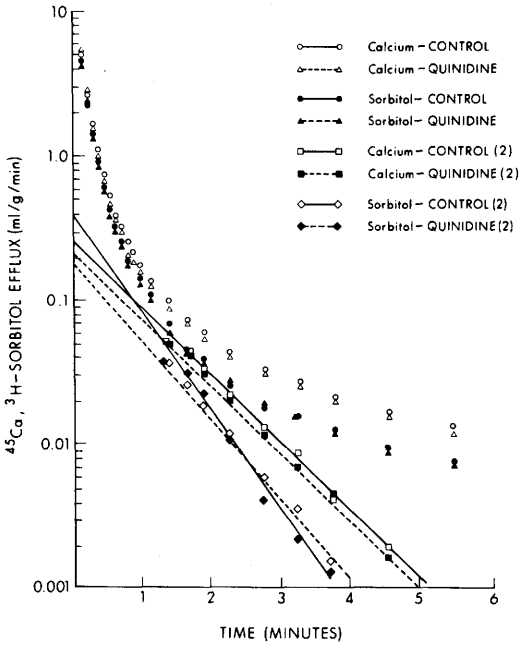

FIG. 2. The gross data points for the first 6 min as shown in Fig. 1 are presented along with points derived from subtraction of compartment 3 from these points. In this case the regression lines, which represent compartment 2, were estimated by eye.

quinidine had no affect on the rate constants of phase 2 for either sorbitol or calcium.

Young (8) has reported that the rate constant for washout of the slow component of the interstitial fluid is modified by molecular size. However, the relation between permeability coefficients and rate constants was not sufficiently distinct to detect differences between efflux rates of substances with diffusion constants as similar as sorbitol (0.67×10^{-5} cm^2 sec^{-1}) and calcium (1.89×10^{-5} cm^2 sec^{-1}). We also could detect no difference between rates at which calcium and sorbitol are washed out of phase 2.

Although quinidine decreased the contractility (*dP/dt* max) of the heart, no corresponding change in the kinetics or distribution of calcium could be detected. Since the decay of contractile performance in calcium-free perfusion systems is very rapid (1–3), it is likely that the calcium responsible for a change in contractility would be represented in our 10-min exchange and washout experiments. The rapidity of the quinidine effect on performance supports this assumption. Equilibration of contractile-related calcium occurs within 5 min in rabbit atria (9). The fact that we observed no difference in calcium kinetics in two contractile states indicates that any intracellular or membrane events that may be responsible for the difference in contractile performance are hidden by the extracellular calcium exchange rates that we observed. This might occur if the change in calcium pool involved in causing this decreased inotropic state was so small that it could not be detected with our experimental design, or more probably, that intracellular exchange related to contractility is more rapid than calcium exchange between the interstitial and vascular space. The possibility that there is an intracellular calcium pool which is in rapid equilibrium with an extracellular pool may be the basis for the observation that the calculated volumes of distribution for calcium (0.18 ml/g) in compartment 3 are slightly greater than the volume of distribution of the extracellular marker sorbitol (0.11 ml/g).

Summary. In isolated working rat hearts, the washout of ^{3}H sorbitol as well as the exchange of $^{45}Ca^{2+}$ could be described as the sum of three processes, two of which were found to be exponential in nature. The volume of distribution of $^{45}Ca^{2+}$ was greater than that of the sorbitol. This greater volume of distribution was clearest for the compartment with the longest time constant. Addition of quinidine in doses which produced a 32% decline in maximum *dP/dt* had no effect on the rate constants for the washout of sorbitol or the exchange of $^{45}Ca^{2+}$. The difference between the volume of distribution of calcium and sorbitol was also not measurably changed by the negative inotropic intervention.

The results suggest that the kinetics of $^{45}Ca^{2+}$ exchange are determined by those processes which limit the washout of the extracellular space. The studies with quinidine further suggest that if this agent displaces Ca^{2+} from binding or storage sites, the amount affected is too small to be detected by kinetic analysis of calcium exchange in the rat heart.

TABLE II. The Effect of Quinidine on the Kinetics of ^{14}C Sorbitol and ^{45}Ca Washout.

	Rate constant (min^{-1})			
	Control		Quinidine	
Component	Sorbitol	Calcium	Sorbitol	Calcium
2	1.25	0.87	1.07	0.87
3	0.19	0.18	0.22	0.19

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