SHORT COMMUNICATION

The kinetics of efflux of 5,5-dimethyl-2,4-oxazolidinedione (DMO) from the myocardium

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Summary

1. The efflux of ¹⁴C-labelled 5,5-dimethyl-2,4-oxazolidinedione (DMO) from the myocardium of the rabbit has been studied. The perfusate pH was 7.38.

2. The effluent curve is complex and appears to be the resultant of movement between at least four compartments.

3. The first two probably represent intravascular and extracellular compartments; the last two have smaller rate constants and may represent intracellular spaces. Intracellular pH (pHᵢ) calculated from the effluent curve was 7.23 ± 0.05. pHᵢ estimated from the steady-state distribution volume of DMO was 7.28 ± 0.02.

4. The existence of two intracellular compartments suggests that DMO is not homogeneously distributed in the myocardium. It is suggested that the apparent greater buffering capacity of cardiac than skeletal muscle can be explained by the greater number and volume of mitochondria in the myocardium, compartmentalization of DMO and assumptions inherent in the DMO method for the measurement of pHᵢ.

Key words: DMO, myocardium, pH.

Introduction

During a respiratory acidosis, intracellular pH (pHᵢ) measured by the DMO method (Waddell & Butler, 1959) is reduced more in skeletal than in cardiac muscle for a given change of extracellular pH (pHₑ) (Clancy & Brown, 1966; Poole-Wilson & Cameron, 1975). The reason for this apparent difference is unclear but Lai, Attebery & Brown (1973) have suggested that there is a more active hydrogen ion pump in cardiac muscle. In this paper, it is demonstrated that DMO is compartmentalized in myocardium and an alternative hypothesis is proposed.

Methods

The experimental preparation was the arterially perfused interventricular septum that has been previously described (Langer & Brady, 1968; Poole-Wilson & Langer, 1975). New Zealand white rabbits weighing between 2 and 4 kg were anaesthetized with sodium pentobarbitone. The heart was removed. A cannula was tied into the septal artery and the triangular piece of tissue suspended between forceps. The third corner was connected to a transducer (Statham Instruments, Calif., U.S.A.) and the tension recorded. The muscle was stimulated to give 35 beats/min. The tissue temperature was measured with a needle thermistor and maintained at 26°C. The perfusate was warmed by a heating coil immediately adjacent to the arterial cannula. The septa weighed between 1.0 and 1.5 g and the mean flow rate of the perfusate was 1.5 ml min⁻¹ g⁻¹ wet wt.

The perfusate contained (mmol/l): Na⁺ 142, K⁺ 4.0, Ca²⁺ 1.5, Mg²⁺ 1.0, Cl⁻ 117, H₂PO₄⁻ 0.4, HCO₃⁻ 28 and glucose 5.6. It was equilibrated with

¹⁴C-labelled 5,5-dimethyl-2,4-oxazolidinedione.
O\textsubscript{2}/CO\textsubscript{2} (95:5) gas mixture. The pH was 7.38 ± 0.01 (n = 11). The tissue was perfused by hydrostatic pressure from a reservoir 150 cm above the preparation.

The muscle was labelled with \[^{14}\text{C}]\text{DMO}\) for 45 min. At time zero, wash-out of the isotope was begun with non-radioactive perfusate and continued for 60 min. A fixed number of effluent drops, usually four, were collected in containers for beta radioactivity counting and the time was noted. The period of time, in which the drops formed, was also recorded with a stopwatch. A portion of each sample collected during a wash-out was counted for radioactivity for 10 min (Beckman, LS-200B). The radioisotopic activity of each sample collected during a wash-out was calculated as counts min\(^{-1}\) min\(^{-1}\) to take account of any small changes of flow rate and plotted semilogarithmically against time. The curves were semilogarithmically represented loss from the vascular space. The mean rate constants for the other components were calculated as counts min\(^{-1}\) min\(^{-1}\) to take account of any small changes of flow rate and plotted semilogarithmically against time. The curves were analysed graphically by the method of Solomon (1960). Assuming a parallel model for the efflux of \[^{14}\text{C}]\text{DMO}\), the content of each compartment was calculated by dividing the intercept on the vertical axis by the rate constant. From these values, the ratio of the amount of intra- and extra-cellular \[^{14}\text{C}]\text{DMO}\) was determined and hence a value for pH\textsubscript{i} calculated by the method of Walker, Goodwin & Cohen (1969). The dissociation constant of DMO at 26°C was taken as 537 nmol/l (Steinmetz, 1969). pH\textsubscript{i} was assumed to be that of the perfusate. In separate experiments, the pH\textsubscript{i} of the septum was estimated from the steady-state distribution volume of \[^{14}\text{C}]\text{DMO}\) after 45 min equilibration. A small piece of muscle (100 mg) was dissolved in 1 ml of Soluene (Packard) and counted after adding scintillant (Poole-Wilson & Cameron, 1975). A correction was made for quenching with an internal standard of \[^{14}\text{C}]\text{hexadecane.}\)

The extracellular space of the septum was 41.2 ± 2.2 ml of water/100 g wet wt. (n = 7). The tissue water was 83.9 ± 0.1 ml/100 g wet wt. (n = 81) (Poole-Wilson & Langer, 1975).

The efflux of \[^{14}\text{C}]\text{DMO}\) is depicted in Fig. 1. After a delay of 45 min, the muscle was relabelled and the experiment repeated; the two curves were superimposable and myocardial function was unaltered. The curve could be resolved graphically into a minimum of four components expressing escape from four phases (0, 1, 2, 3). The first component had a high rate constant and probably represented loss from the vascular space. The mean rate constants for the other components (1, 2, 3) were 0.46 ± 0.02, 0.16 ± 0.01 and 0.037 ± 0.003 min\(^{-1}\) (n = 11) respectively. The radio-isotopic contents of each phase expressed as a percentage of the radioactivity counts in 1 ml of labelled perfusate/g of wet muscle were 39.9 ± 2.7, 17.7 ± 1.8 and 12.8 ± 2.3 (n = 11) respectively. The content of phase 1 was similar to that of the extracellular compartment.

In three experiments, the residual \[^{14}\text{C}]\text{DMO}\) in the muscle was measured; it represented less than 1%, of the total radioactivity counts in the tissue after labelling. The introduction of unlabelled DMO (0.5 mmol/l) during the wash-out (Fig. 1) or before and during the labelling period did not alter the efflux curve; the \(^{14}\text{C}\) does not appear to bind to the septum. The efflux curves in three experiments were not altered by an increase of the temperature of the perfusate to 32°C during the entire wash-out and labelling periods.

Previous experiments have shown that in this preparation the rate constant for the efflux of many substances from the extracellular space is not less than 0.35 min\(^{-1}\) (Shine, Serena & Langer, 1971); the efflux from this compartment therefore would be largely complete in 20 min (10 half-times). The slope of the line through the points on the efflux curve between 20 and 40 min was greater than that between 40 and 60 min (P < 0.001). The part of the curve from 20 min onwards cannot be represented by a single kinetic compartment.

If it is assumed that the amount of \[^{14}\text{C}]\text{DMO}\) in phases 2 and 3 is of intracellular origin and the
amount in phase 1 is of extracellular origin, \( pH_1 \) can be calculated from the equations used by Walker, Goodwin & Cohen (1969) since the distribution of \([1^{14}C]\)DMO is known. The \( pH_1 \) was \( 7.23 \pm 0.05 \) (\( n = 11 \)). \( pH_1 \) calculated from the steady-state distribution volume of \([1^{14}C]\)DMO was \( 7.28 \pm 0.02 \) (\( n = 5 \)). The values obtained from the kinetic analysis and by the steady-state method are therefore similar.

**Discussion**

The method used in this paper for the analysis of the effluent curve of \([1^{14}C]\)DMO from the septum assumes that the radioisotope is distributed in several kinetically independent compartments; the extracellular and intracellular fluid exchange directly with the vascular space (parallel model). If the intracellular isotope exchanges with the extracellular compartment before reaching the vascular compartment, a series model would be more appropriate and the values for the rate constant and content of each compartment would have to be adjusted to take account of reflux of isotope which might occur from the extracellular compartment back into the intracellular fluid (see appendix by A.F. Huxley in Solomon, 1960). The parallel model appears to be more applicable for several reasons. First, the distribution volume of \([1^{14}C]\)DMO in compartment 1 if a parallel model is assumed was not significantly different from that of \([^{51}Cr]EDTA \) in the extracellular compartment. If a correction is made for a series model, the isotopic content of phase 1 is increased and becomes significantly greater than that predicted from the distribution volume of \([^{51}Cr]EDTA \) (\( P < 0.01 \)). Secondly, \( pH_1 \) calculated from the effluent curve did not differ from that obtained by the steady-state method (Waddell & Butler, 1959). A substantial difference (\( P < 0.001 \)) does exist if the corrections for a series model are made. Thirdly, because DMO is 93% ionized at a \( pH \) of 7.38 in the extracellular compartment, only 7% of its content at any moment is available to reflux. It is implicit in the use of the weak acid, DMO, to measure \( pH_1 \) that the cell membrane is relatively impermeable to the ionized molecule. Intracellular un-ionized DMO having traversed the cell membrane will become ionized in the extra-
cellular space; consequently, the possibility of reflux is diminished and a parallel model favoured. Fourthly, studies of the morphology of the extracellular space in the septum show that 36% of the cell surface is less than 250 nm from a capillary and that in these areas only 3% of the extracellular space lies between the two surfaces (Frank & Langer, 1974). Thus the anatomy of the tissue supports the concept that isotope in the extracellular space and intracellular space in the septum show that in these areas only.

The proportion of cell volume occupied by mitochondria is 34% in the myocardium (Page, McCallister & Power, 1971) and only 5% in skeletal muscle (Engel & Stonnington, 1974).

The heterogeneous distribution of hydrogen ions in myocardium has important implications for the measurement of pH under some circumstances. During a respiratory acidosis, pH falls more in skeletal than cardiac muscle for a given change of pH. (Clancy & Brown, 1966; Poole-Wilson & Cameron, 1975); pH was measured by the DMO method. Lai et al. (1973) attributed this difference to the greater activity of a hydrogen ion pump in the myocardium because no changes in physicochemical buffering or metabolism could be demonstrated in an acidosis. Cohen & Iles (1975) suggested the difference might be explained by a greater influx of lactate into cardiac muscle during a respiratory acidosis. An alternative explanation arising out of the present experiments is that the difference is due to a disparity in the intracellular distribution of hydrogen ion in the two tissues and associated problems in the use of the DMO method. If the proportion of cell volume occupied by mitochondria is six times greater in cardiac than in skeletal muscle and the pH of mitochondria were slightly greater than the cytosol (Addanki et al., 1967), the arithmetical mean pH of cardiac muscle would be greater than that of skeletal muscle under control conditions. This difference would pertain even if pH, and consequently pH, were reduced in an acidosis. However, if 'overall' pH, is measured by the DMO method in a multi-compartmental system the values obtained for pH, are not the same as the arithmetical mean pH,.

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References


