Muscle Mechanics and Energetics: A Comparative View

SPECIES-INDEPENDENT METABOLIC RESPONSE TO AN INCREASE OF $[\text{Ca}^{2+}]_i$ IN QUIESCENT CARDIAC MUSCLE

Marie-Louise Ward, Patricia J Cooper,† Peter J Hanley* and Denis S Loiselle

Department of Physiology, Faculty of Medicine and Health Science and Bioengineering Institute, University of Auckland, Auckland, New Zealand.

SUMMARY

1. The aim of the present investigation was to contrast the $\text{Ca}^{2+}$ dependence of cardiac energy metabolism in two species with differential reliance on extracellular $\text{Ca}^{2+}$ for excitation–contraction coupling.

2. We measured energy expenditure as the rate of oxygen consumption ($V_O_2$) of isolated, Langendorff-perfused hearts of rats and guinea-pigs during KCl arrest. In parallel experiments, we indexed intracellular $\text{Ca}^{2+}$ concentration ($[\text{Ca}^{2+}]_i$) of isolated right-ventricular trabeculae, using the $\text{Ca}^{2+}$ fluorophore fura-2 and ratiometric spectrofluorometry. By varying $[\text{Na}^{+}]_o$ and ratiometric spectrofluorometry. By varying extracellular $\text{Na}^{+}$ concentration ($[\text{Na}^{+}]_o$), $V_O_2-[\text{Na}^{+}]_o$ and $[\text{Ca}^{2+}]_i-[\text{Na}^{+}]_o$ relationships were constructed for each species.

3. Reduction of $[\text{Na}^{+}]_o$, during KCl arrest caused pronounced species-dependent elevations of both $V_O_2$ and $[\text{Ca}^{2+}]_i$. Despite the species dependence of both $V_O_2$ and $[\text{Ca}^{2+}]_i$ on $[\text{Na}^{+}]_o$, a single species-independent $V_O_2-[\text{Ca}^{2+}]_i$ relationship obtained.

4. We infer that elevation of the metabolic rate of the arrested heart above its basal value is determined primarily by $[\text{Ca}^{2+}]_i$, and is not species dependent.

Key words: cardiac basal metabolism, extracellular $\text{Na}^{+}$, fura-2 fluorescence, intracellular $\text{Ca}^{2+}$, myocardial oxygen consumption.

INTRODUCTION

Contraction of cardiac muscle is a consequence of $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release from the sarcoplasmic reticulum.1,2 In this process, an influx of $\text{Ca}^{2+}$ via voltage-dependent $\text{Ca}^{2+}$ channels in the sarcolemmal membrane (the so-called dihydropyridine receptors) induces the release of $\text{Ca}^{2+}$ from specialized $\text{Ca}^{2+}$ release channels of the sarcoplasmic reticulum membrane (the so-called ryanodine receptors). Thus, the $\text{Ca}^{2+}$ that activates contraction arises from two sources: the surface membrane (extracellular) and the sarcoplasmic reticulum (internal stores). The relative dependence on these two sources of $\text{Ca}^{2+}$ varies across species, resulting in a broad spectrum of reliance on intra- and extracellular stores to initiate contraction. For the experimentalist, it is a happy circumstance that rat and guinea-pig, species of comparable size, occupy extreme ends of this spectrum. In the words of Mitchell et al. ‘...the balance is towards release from stores in rat cells and towards entry through the surface in guinea-pig cells’.3

This species-difference in ionic behaviour of active hearts is reflected in an equally striking species-difference in the metabolic behaviour of quiescent hearts. As we have recently reported,4 whereas arrested hearts of either species show poteniated rates of oxygen consumption ($V_O_2$) when extracellular $\text{Na}^{+}$ concentration ($[\text{Na}^{+}]_o$) is reduced, the response of the guinea-pig is considerably blunted vis-à-vis that of the rat. We have attributed this metabolic difference to a species-difference in Gibbs Free Energy of the sarcosomal $\text{Na}^{+}$-$\text{Ca}^{2+}$ exchanger. We inferred that, in order to achieve a given increment of intracellular $\text{Ca}^{2+}$ concentration ($[\text{Ca}^{2+}]_i$) via reverse-mode activity of the exchanger, a greater reduction of $[\text{Na}^{+}]_o$ is required in guinea-pig myocardium. Underlying that interpretation was the untested assumption that the dependence of $V_O_2$ on $[\text{Ca}^{2+}]_i$ in the arrested guinea-pig heart is comparable with that known to prevail in quiescent rat myocytes.5,6 An equally plausible hypothesis would be the existence of a species difference in metabolic response to $[\text{Ca}^{2+}]_i$.

In order to distinguish between these competing hypotheses, we have measured, in parallel experiments, $[\text{Ca}^{2+}]_i$ (indexed as ratiometrically derived fura-2 fluorescence) and $V_O_2$ as functions of $[\text{Na}^{+}]_o$ in quiescent myocardial tissues of rat and guinea-pig.

METHODS

The experimental methods were approved by the University of Auckland Animal Ethics Committee and have been described in detail recently.4

Correspondence: Denis Loiselle, Department of Physiology, University of Auckland, Private Bag 92019, Auckland, New Zealand.

Email: ds.loiselle@auckland.ac.nz

Presented at Muscle Mechanics and Energetics: A Comparative View, Melbourne, October 2002. The papers in these proceedings have been peer reviewed.

†Present address: Institut für Normale und Pathologische Physiologie, der Universität Marburg, Marburg, Germany.

‡Present address: Laboratory of Physiology, University of Oxford, Oxford OX1 3PT, UK.

‡A preliminary account of this work has been presented to the International National Union of Physiological Sciences, Christchurch, New Zealand, August 2001, and has appeared in Abstract form (Ward ML, Cooper PJ, Hanley PJ, Loiselle DS. Species-independence of $\text{Ca}^{2+}$-dependent rate of oxygen consumption of quiescent myocardium. Proc. Physiol. Soc. New Zealand 2001; 20 (Suppl. 1): 88P).

Received 13 November 2002; revision 19 February 2003; accepted 23 February 2003.
Briefly, Wistar rats and outbred (multicolour) guinea-pigs from the Auckland colony, of either sex and weighing less than 350 g, were killed by decapitation. For the whole-heart experiments (i.e. for measurement of rate of oxygen consumption, \( V_O_2 \) and left ventricular pressure (\( P_L V_2 \))), the excised heart was washed in chilled saline and immediately mounted in the Langendorff perfusion rig. In these experiments, \( V_O_2 \) was calculated as the product of coronary flow and the arteriovenous difference in content of oxygen. The latter was determined using a fuel-cell device (OxyCon; Department of Physiology, University of Tasmania, Hobart, Tasmania, Australia) or, in selected experiments, \( P_L O_2 \) electrodes (Microelectrodes, Londonderry, NH, USA) situated in the aortic (inflow) and pulmonary arterial (outflow) catheters.

For trabeculae experiments (i.e. for estimation of intracellular \( Ca^{2+} \) concentration, [\( Ca^{2+} \)], the right ventricle was opened and a suitable trabecula (if present) was removed and placed in an organ bath that was mounted on the stage of the inverted microscope and formed part of a Caïms spectrofluorometric system.\(^{11}\) One end of the trabecula was attached (using a monofilament ‘snare’) to a fixed hook and the other to a force transducer (AE-801; SensoNor, Horten, Norway). The preparation was loaded with fura-2/AM using 1 mmol/L probenecid to enhance retention of the dye in the extracellular spaces.

Experiments were conducted at 37°C and pH 7.4 using a modified Krebs–Henseleit solution (equilibrated with 95% \( O_2 /5% CO_2 \) of the following composition (in mmol/L): \( NaCl 118; KCl 4.8; MgSO_4 1.18; KHPO_4 1.18; NaHCO_3 24.8; CaCl_2 2.5; glucose 10. \) Whole-heart perfusion solutions were supplemented with insulin (10 U/L) and the colloid replacement Haemaccel (Hoechst, Auckland, New Zealand). For achievement of cardiac arrest, the KCl concentration of the perfusate was increased to 20 mmol/L. Low-\( Na^+ \) solutions were made by equimolar substitution of LiCl for NaCl.

At the conclusion of an experiment, preparations (whole hearts or trabeculae) were dried at 70°C for 24 h to determine dry weight; \( V_O_2 \) is expressed as \( \mu mol/min per g dry weight \).

**Curve fitting**

Sigmoidal relationships among \( V_O_2 \), the fura-2 fluorescence ratio and [\( Na^+ \)], were fitted according to a four-parameter version of the Hill Equation:

\[
y(x) = \frac{y_{\text{max}}}{1 + (x/K_m)^n}
\]

In this expression, the dependent variable (\( y \)) takes on values between 0 and \( y_{\text{max}} \), whereas \( K_m \) is the value of \( x \) that achieves half-maximal elevation of \( y \) above \( y_{\text{max}} \). The parameter \( n \) determines the steepness of the relation at \( K_m \); its sign determines whether \( y \) increases or decreases with \( x \).

**RESULTS**

Arrest of Langendorff-perfused whole hearts by elevating [\( K^+ \)], to 20 mmol/L under standard conditions of [\( Na^+ \)], (143 mmol/L) lowered the rates of oxygen consumption to 17 and 22% for rat and guinea-pig, respectively, of that observed during spontaneous beating under isovolumic conditions (Fig. 1). Sufficient reduction of [\( Na^+ \)], during cardioplegia then caused an abrupt increase of instantaneous \( V_O_2 \), the time-course of which was characterized by a transient peak followed by a sustained plateau (see Fig. 1, inset). Following Fiolet et al.,\(^{3,8}\) we recorded the peak values of instantaneous \( V_O_2 \) and have plotted their average values as a function of [\( Na^+ \)], in Fig. 1. As is evident from either the raw data (mean±SEM) or the curves fitted according to eqn 1, a sigmoidal dependence of \( V_O_2 \) on [\( Na^+ \)], is obtained. We have previously attributed this behaviour to reversal of the sarcolemmal \( Na^+/-Ca^{2+} \) exchanger.\(^{4}\) Note that [\( Na^+ \)], must be lowered a great deal more in the guinea-pig heart than in the rat heart before the metabolism of the KCl-arrested myocardium is potentiated. Indeed, the respective K\(_m\) values of the two relationships are 67 and 25 mmol/L for rat and guinea-pig,\(^{4}\) respectively.

It is of note that the plateau values of \( V_O_2 \) during arrest (achieved at 3 mmol/L [\( Na^+ \)], in both species) approximated (or even exceeded) the mean values recorded during the preceding period of spontaneous isovolumic beating (Fig. 1). Thus, it is of importance to consider whether the supply of oxygen to these saline-circulated hearts was compromised during periods of low-\( Na^+ \) perfusion. The mean values of arterial partial pressure of oxygen (\( P_A O_2 \)) were 84.2±1.3 and 86.4±1.5 kPa for guinea-pig (\( n = 35 \)) and rat (\( n = 39 \)), respectively. The corresponding venous values (\( P_V O_2 \)) varied with [\( Na^+ \)]. During normonatraemic arrest (143 mmol/L [\( Na^+ \)], \( P_A O_2 \) averaged 72.9±2.1 and 76.1±1.9 kPa for guinea-pig (\( n = 12 \)) and rat (\( n = 13 \)), respectively. These values decreased with extracellular \( Na^+ \) concentration to 17.3±2.0 and 14.4±6.6 kPa for guinea-pig (\( n = 5 \)) and rat (\( n = 3 \)), respectively, when [\( Na^+ \)], was reduced to 3 mmol/L. Furthermore, there was no indication of hypoxia-induced vasodilation of the coronary vasculature, such as occurs in the KCI-arrested heart when arterial \( P_A O_2 \) is reduced below 150 mmHg.\(^{8}\) In fact, there was no effect of either increasing [\( K^+ \)], or reducing [\( Na^+ \)], on coronary vascular flow in the hearts of either species. Thus, we can be confident that the extreme plateau values of \( V_O_2 \) observed during low-\( Na^+ \) perfusion of K\(^+\)-arrested hearts cannot be attributed to oxygen insufficiency. Indeed, the minimum venous \( P_V O_2 \) values observed in vitro exceeded those that would normally prevail in the arterial blood of either species in vivo (13 kPa).

Armed with confidence that the whole-heart oxygen consumption results of Fig. 1 reflect response-to-demand rather than a transient peak followed by a sustained plateau (see Fig. 1, inset). Following Fiolet et al.,\(^{3,8}\) we recorded the peak values of instantaneous \( V_O_2 \) and have plotted their average values as a function of [\( Na^+ \)], in Fig. 1. As is evident from either the raw data (mean±SEM) or the curves fitted according to eqn 1, a sigmoidal dependence of \( V_O_2 \) on [\( Na^+ \)], is obtained. We have previously attributed this behaviour to reversal of the sarcolemmal \( Na^+/-Ca^{2+} \) exchanger.\(^{4}\) Note that [\( Na^+ \)], must be lowered a great deal more in the guinea-pig heart than in the rat heart before the metabolism of the KCI-arrested myocardium is potentiated. Indeed, the respective K\(_m\) values of the two relationships are 67 and 25 mmol/L for rat and guinea-pig,\(^{4}\) respectively.

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compromise-of-supply, we can focus on the effects of low-Na+ superfusion of isolated trabeculae. These data are shown in Fig. 2 where R, the fura-2 (340/380) fluorescence ratio for each species, is plotted as a function of [Na+]o. Once again, the data for each species show a sigmoidal dependence on [Na+]o and, once again, the rightward shift of the relationship for the guinea-pig is considerable. In fact, in the guinea-pig heart, there is no discernible increase of [Ca2+]i (indexed as the 340/380 nm fura-2 fluorescence ratio) until [Na+]o has been reduced to approximately 40 mmol/L, a value that approximates the $K_m$ of the relationship for the rat heart. As can be seen in the inset of Fig. 2, the temporal response of [Ca2+]i to a reduction of [Na+]o (in this case, to 31 mmol/L) was comparable with that of $V_\text{O}_2$ (cf. Fig. 1 inset). Note, too, the small increase in resting fluorescence upon elevation of [K+]o from 6 to 20 mmol/L. This behaviour is characteristic of rat trabeculae; it is not seen in the guinea-pig.

At this juncture, we have determined, for each species, the dependence of $V_\text{O}_2$ on [Na+]o in isolated perfused whole hearts (Fig. 1) and, in separate experiments, the dependence of [Ca2+]i (indexed as the 340/380 fura-2 fluorescence ratio) on [Na+]o in isolated superfused trabeculae (Fig. 2). We are now in a position to ask whether the dependence of cardiac basal oxygen consumption in intracellular Ca2+ concentration differs between the two species. Despite the pronounced species dependence of both the $V_\text{O}_2$–[Na+]o and [Ca2+]i–[Na+]o relationships (Figs 1,2), a single species-independent relationship is revealed when $V_\text{O}_2$ is plotted as a function of the fura-2 fluorescence ratio (Fig. 3). Note that we have plotted $V_\text{O}_2$ as a function of log10R in deference to the logarithmic nature of pCa (where pCa = $-\log_{10}$([Ca2+]i)). Note, further, that we have not normalized the metabolic data within species. Nevertheless, the fit of a single Hill curve (eqn 1) to the combined data of both species is excellent, the standard error of estimate (1.9 μmol/min per g dry weight) being only approximately 5% of the full-scale values, whereas the coefficient of determination (i.e. $r^2$) is 0.9906.

**DISCUSSION**

The objective of the present investigation was to determine whether the observed6,8,10 and inferred6 Ca2+ dependence of the metabolic rate of quiescent myocardial tissue is species dependent. On the one hand, species-independence may have been predicted on the assumption that the Ca2+-dependent activation of ATP-dependent sarcoplasmic reticular Ca2+ pumps, the ‘triggering’ by Ca2+ of actin-activated myosin ATPase activity, the activation by Ca2+ of mitochondrial dehydrogenases11,12 and the mitochondrial P : O ratio are each ‘biological constants’, independent of species. On the other hand, differences between rat and guinea-pig in the rate of loss of intracellular Ca2+ during prolonged quiescence13, the rate of gain of Ca2+ by the SR following caffeine-depletion,14 the extent of diastolic Ca2+ loading of the sarcoplasmic reticulum15 and the sensitivity of the myofilaments to [Ca2+]i (see Fig. 21 in Bers16), may equally have led to the opposite prediction; namely, species dependence of metabolic rate on [Ca2+]i.

In the event, there can be little dispute. Despite the aforementioned differences (and others; reviewed by Cooper et al.), as well as the striking separation of both the $V_\text{O}_2$–[Na+]o and [Ca2+]i–[Na+]o relationships, the dependence of the suprabasal metabolic rate of the K+-arrested heart on intracellular Ca2+ concentration is, nevertheless, independent of species. This result, in turn, has two implications. The various ATPases of the cardiac myocytes of these two rodent species must be activated to an equivalent extent by a given increase of [Ca2+]i, and the resulting metabolic demand must
be met by an equivalent increase in the rate of oxygen consumption.

How, then, are the species-dependent responses of $\dot{VO}_2$ and $[Ca^{2+}]$, to a reduction of $[Na^+]$, to be explained? In an earlier study, we attributed these differences to species differences in Gibbs Free Energy of the sarcoslemmal Na$^+$–Ca$^{2+}$ exchanger$^4$ caused, in turn, by a species difference in intracellular concentration of some ion, probably Na$^+$. In this view, the much smaller increase of $[Ca^{2+}]$, in the guinea-pig, in response to a given decrement of $[Na^+]$, is the consequence of a substantially larger transmembrane gradient of Gibbs Free Energy that favours Na$^+$, dependent Ca$^{2+}$ efflux on the exchanger. Inasmuch as lowering extracellular Na$^+$ was merely a method of varying intracellular Ca$^{2+}$, we would now predict that any intervention that elevates [Ca$^{2+}$], during cardioplegia will have equivalent metabolic responses in the two species.

A critical appraisal

Whereas the prima facie evidence of Fig. 3 is convincing, we admit that it does not square with all published evidence. The disparities are both qualitative and quantitative. For example, Sham et al.$^{11}$ report the resting intracellular concentrations of Ca$^{2+}$ to be 82 and 139 mmol/L for rat and guinea-pig, respectively, whereas we detected no difference in fura-2 fluorescence in standard (143 mmol/L) [Na$^+$]. A second concern arises from the results of Ebus and Stienen,$^{18}$ who measured, as a function of pCa, both force development and ATPase activity of chemically skinned trabeculae of the rat. They found that the ATPase–pCa relationship was well described by the sum of two Hill curves, with $K_m$ values of 6.21 and 5.44. They attributed the former, which accounted for approximately 20% of total energy flux, to the ATPase activity of the sarcoplasmic reticulum Ca$^{2+}$ pump and the latter to the actomyosin ATPase activity of the cross-bridges. We see no hint of two components underlying the $\dot{VO}_2$–[Ca$^{2+}$] relationships of either species (Fig. 3), perhaps because we investigated the response to only seven (rat) or nine (guinea-pig) levels of [Na$^+$]. Finally, we are open to the criticism that we report fura-2 fluorescence ratios of either axis.

ACKNOWLEDGEMENTS

This research was made possible through the generous support of The National Heart Foundation of New Zealand and the New Zealand Lottery Grants Board (Medical), as well as by the award of a Health Research Council of New Zealand Postgraduate Scholarship to Dr Peter Hanley. M-LW is the recipient of a PhD Scholarship from the Auckland Medical Research Foundation.

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