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Effect of acidosis on Ca^{2+} sensitivity of skinned cardiac muscle with troponin C exchange

Implications for myocardial ischemia

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By using a novel approach for the study of the effects of pH variation in skinned myocardium, the present experiments were aimed to provide new insights into the mechanism of ischemia. Ca^{2+} sensitivity is decreased by acid pH, but the effect is more than double in cardiac myofilaments than that in fast-twitch skeletal muscle fibers. With the technique of troponin C exchange in myocytes, we find here that the effect of pH is the same with cardiac or skeletal troponin C. These results rule out a direct H^+-Ca^{2+} competition on the Ca^{2+}-binding sites of troponin C as a significant mechanism of ischemia. The findings provide conclusive evidence in favor of the idea that acidosis modulates the protein-protein interactions in the regulatory complex in cardiac muscle.

Starling's law; Pump failure; Contractility; Activation; Regulation; (Vertebrate muscle)

1. INTRODUCTION

The effects of ischemia in the heart are of interest because cardiac performance is markedly depressed [1]. Obstructed blood flow in ischemic heart causes the accumulation of metabolic products in myocardial cells including a drop in pH (i.e., accumulation of protons) [2]. Acidotic pH decreases the Ca^{2+} sensitivity of cardiac myofilaments [3]. This down-regulation is an important mechanism for depressed contractility in ischemia and is studied here.

Ca^{2+} sensitivity for contraction is largely a reflection of Ca^{2+}-binding properties of the low-affinity site on cardiac troponin C [4], and a natural possibility was that acidosis affected the H^+-Ca^{2+} interaction on that site [1]. Consistent with this idea, lowering the pH was found to reduce somewhat the affinity of purified skeletal troponin C for Ca^{2+} binding [5].

On the other hand, it was found that the effect of pH on the regulation of myofilaments was different in cardiac muscles from neonatal and adult rat hearts [6]. But no discernible differences in troponin Cs are manifest in the developing heart [7]. Also, the pH effect on Ca^{2+} affinity of purified troponin C was further modified when in complex with TnI [5], suggesting alternatively that a significant action of acidosis on troponin C may be mediated through other subunits. A more direct approach was needed to gain further insights into the effects of pH on myocardial contractility.

The pH effect on Ca^{2+} sensitivity appears to be greater in skinned cardiac muscle than in fast-twitch skeletal muscle fiber of rabbit [8]. The primary structures, and the Ca^{2+}-binding abilities, of cardiac and skeletal troponin Cs are also distinct [4]. Further, it is known that troponin C in cardiac muscle can be functionally exchanged with skeletal troponin C [9,10], suggesting a novel approach in the present study for investigating the role of the

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troponin C moiety in the mechanism of acidosis. The results provide conclusive evidence that acidotic pH affects the entire regulatory complex in myocytes to induce the decrease in Ca^{2+} sensitivity by troponin C.

2. METHODS

Thin trabeculae (50–60 μm by 1–2 mm) were isolated from the right ventricle of freshly excised heart from adult Syrian golden hamster. Skinning was performed with detergent treatment (0.5% lubrol-X) for 30 min [9]. Detergent was added to the relaxing solution which contained in addition 100 mM K-propionate, 20 mM imidazole, 5.6 mM $MgCl_2$, 5 mM ATP, 5 mM EGTA, 20 mM phosphocreatine and approx. 250 U/ml creatine phosphokinase. Force transducer and the sarcomere length adjustments (to 2.2 μm, unless otherwise indicated; sarcomere length adjustment made by laser diffraction) were as before [9,11]. Activating solutions were made by adjusting the EGTA to Ca-EGTA ratio. The pH was 7.00 ± 0.01 or 6.5, and total salt was 190 mM. The design of the solutions was by computer. Activations were at 20°C.

For fast-twitch fibers, strips of psoas muscle were stored in skinning solution containing 150 mM K-propionate, 5 mM Mg-acetate, 5 mM ATP, 5 mM EGTA, 1 mM dithiothreitol and 50% (v/v) glycerol, at −20°C. Individual fiber segments were isolated after 24 h. Because of elongated slack-length in these segments, the sarcomere length was adjusted to 2.6 μm.

Extraction of up to 80% troponin C in cardiac muscle was made by incubation for 30 min at 30°C in a solution containing 5 mM EDTA, 10 mM imidazole, pH 7.2 [9]. The extraction period for fast-twitch fibers was only 5–15 min. The protocol, described recently, for 100% troponin C extraction was avoided to prevent the loss of an additional cofactor essential for activation [12]. Purified troponin Cs (bovine cardiac TnC and rabbit skeletal muscle TnC) were used to reload the extracted specimens. The batches of purified troponins in this study were collected by Dr Stylianos Scordilis [9]. The level of troponin C in the skinned specimen was checked routinely by SDS-PAGE runs as described before [13].

The data were fitted with a computer using the method of least squares and are given as ± SE. ΔpK is defined as the shift in the p*Ca*-force curve and measured at the point where force is half maximal.

3. RESULTS

Fig.1 compares the effects of varying the pH from 7.0 to 6.5 on p*Ca*-force relations of skinned cardiac and skeletal muscles of hamster. The p*Ca*-force relation (fitted with the Hill equation) in each instance is shifted to the right with acid pH, but the shift is more than twice as great in the myocardium (ΔpK = 0.60 ± 0.04 p*Ca* unit, n = 8; sarcomere length, 2.2 μm) than in fast muscle (0.24 ± 0.03 p*Ca* unit, n = 4; 2.6 μm). A control experiment was made on fast-twitch fibers (2.2 μm) using the skinning procedure of cardiac muscle, but the results were similar to the above.

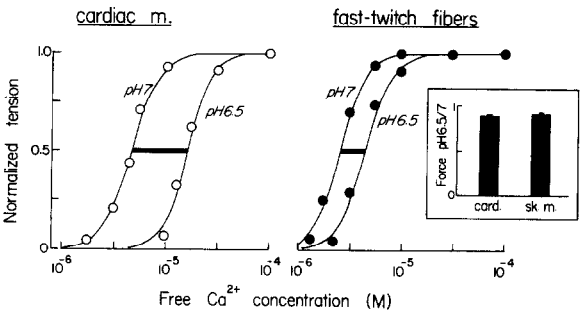

Fig.1. Effect of acidosis on the Ca^{2+} sensitivity of fast-twitch fibers and cardiac muscle. Data are on typical preparations. (Inset) Effect of pH on force with p*Ca* 4.

The steady isometric force levels achieved with maximal calcium activations (p*Ca* 4) were lower in acid pH in both tissues (inset to fig.1); the difference between the results in the two tissues was small (force in pH 6.5/pH 7 = 0.89 ± 0.03 (8) in myocardium, 0.92 ± 0.01 (4) in skeletal muscle) and not significant (by t-test). This was an important rationale for using pH 6.5 for further studies into the effects of acidosis on cardiac regulation.

Fig.2 shows the effects of acid pH on the p*Ca*-force relation of myocardium with troponin C exchange. The decrease in Ca^{2+} sensitivity with pH after cardiac troponin C-loading (ΔpK = 0.59 ± 0.08 p*Ca* unit, n = 3) was found to be equal to that in the native (unextracted) trabeculae (0.60 ± 0.04 p*Ca* unit as above). We next measured the effect of pH in trabeculae containing skeletal troponin C.

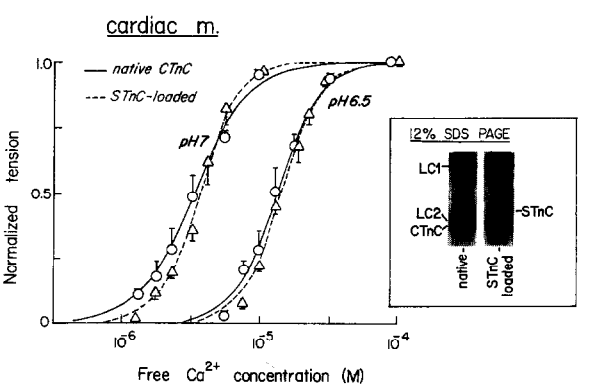

Fig.2. Effect of troponin C exchange on Hill curves of cardiac muscle. The gel lanes are typical. CTnC and STnC are cardiac and (fast) skeletal troponin C, respectively.

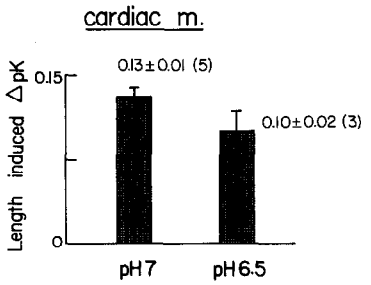

Fig.3. Length-induced shifts in cardiac Ca^{2+} sensitivities at pH 7 and 6.5. ΔpK is the separation in p*Ca*-force curves for sarcomere lengths of 2.4 μm and 1.9 μm.

The decrease in Ca^{2+} sensitivity at pH 6.5 was also found to be 0.59 ± 0.08 (5) p*Ca* unit. Thus presumably the final influence of pH on skeletal troponin C in myocardium was over twofold higher than the effect in skeletal muscle.

3.1. *Sarcomere length-induced shift of cardiac sensitivity in acid pH*

Stretching the sarcomere length in cardiac muscle between 1.9 and 2.4 μm increases the Ca^{2+} sensitivity [14], a phenomenon that underlies the Starling's law of the heart [10]. Because pH is shown to modulate the overall mechanism for Ca^{2+} sensitivity of the myofilaments, it appeared worthwhile to check whether the length-dependency of the Ca^{2+} sensitivity was also modified. The results are summarized in fig.3.

The p*Ca*-force relations of the skinned trabeculae were measured at 1.9 μm and 2.4 μm. At pH 7, the force curve was shifted by 0.13 p*Ca* unit to the left at the longer sarcomere length (i.e. fiber is more sensitive at 2.4 μm). In acid pH, the length-induced shift in myocardium appeared to be somewhat less, but the observed difference from pH 7 was not significant by *t*-test.

4. DISCUSSION

The present study shows that acidosis down-regulated the Ca^{2+} sensitivity of the myocardium to the same extent with cardiac and skeletal troponin Cs. These results indicate that acidotic pH acts on different subunits (than troponin C) in the regulatory complex in the myocyte to modify the trigger mechanism ultimately controlled by the troponin C moiety.

4.1. *Effects of pH on subunit interactions in the regulatory complex*

By troponin C exchange in cardiac muscle, Gulati et al. [15] demonstrated that the Ca^{2+}-binding property of troponin C almost completely dominates the characteristic sensitivity and cooperativity in the activation mechanisms of the myocyte. The length-dependence of Ca^{2+} sensitivity is also characteristically different (greater) in cardiac muscle than in fast-twitch fibers and this distinction too is governed by the troponin C moiety [10]. Thus it is expected that decreased Ca^{2+} sensitivity with acid pH in myocytes is an effect of (or on) troponin C as well. The present study shows that, despite a two-fold difference in the effects of pH with troponins in their native milieus (fig.1), in cardiac muscle the effect was similar with cardiac and skeletal troponin Cs. This is conclusive evidence that the initial effect of pH in the myocyte is on subunit(s) of the regulatory complex other than troponin C. Further, consistent with the expected role of troponin C in the trigger mechanism, the results suggest that acidosis produces the effect on regulation by modifying the interactions among the various regulatory subunits.

Other evidence, though less direct, in support of this idea in fibers comes from studies with untreated skinned slow-twitch muscle that has troponin C of the cardiac isoform [16] but the pH effect on Ca^{2+} sensitivity is of the fast-twitch type [17,18]. This is currently explained by the fact that TnI isoforms are homologous in fast-twitch and slow-twitch fibers [19]. Also, as mentioned above, the results on cardiac muscles from neonatal and adult rats too can be considered to be consistent with the idea that the major effect of pH on troponin C is mediated through other subunit(s) [6].

4.2. *Implications for the mechanism of ischemia*

For ischemic heart, of the cellular metabolites accumulated, the consequences of increases in the proton and P_i concentrations are the best studied because both produce significant decreases in the Ca^{2+} sensitivity of cardiac myofilaments. Regarding acidosis, in fact, knowledge of the association of altered pH with diminution of cardiac performance is more than a century old [20], but the suggestion that pH may cause a part of this effect by reducing the Ca^{2+} affinity of regulatory proteins is

quite recent [1]. The present study now advances this suggestion and provides convincing evidence that the mechanism of acidosis operates by modification of the protein-protein interactions in the regulatory complex and not, as thought earlier, by facilitating a direct H^+-Ca^{2+} exchange on cardiac troponin C.

Further, the bigger effect of acid pH in cardiac muscle than in skeletal muscle may also be an additional protective mechanism for slowing down the metabolism during ischemia in focal regions of the heart. Such a mechanism would limit the continued accumulation of deleterious substances, retarding irreversible damage of the myocyte and protecting the heart in mild ischemia from early pump failure.

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