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The Effects of pH on Ca^{2+}-Activated Force in Frog Skeletal Muscle Fibers

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Abstract. Ca^{2+}-activated isometric force was recorded in skinned (sarcolemma mechanically removed) segments of frog skeletal muscle fibers immersed in bathing solutions of different pH (5.0–10.5) and Ca^{2+} concentrations. Force in maximally activated fibers was near zero at pH 5.5, increased as pH increased to 7.5, remained relatively constant until pH 9.0 and then rapidly declined to zero by pH 10.5. The Ca^{2+} concentration at which 50% of maximum force was developed decreased 25-fold as pH increased from 5.5–7.5. The data also indicate that, while the fibers remain viable with acidosis, they deteriorate rapidly with alkalosis. These observations may be relevant clinically, since they parallel known effects of acidosis on cardiac contractility. The possible sites of action of H^+ on the Ca^{2+}-activated force generating mechanisms are discussed.

Key words: pH – Skinned fibers – Ca^{2+}-activated force – Muscle fibers – Frog.

Introduction

Associated with contractial activity in intact striated muscle cells are variations in intracellular pH [10], Since changes in myoplasmic pH affect muscle function, it is important to know the pH dependence of Ca^{2+}-activated force development. The effects which changes in pH have on the relationship between sarcoplasmic $[Ca^{2+}]$ and developed force have been studied in both chemically and mechanically skinned skeletal and cardiac muscle fiber preparations [2, 4, 6, 14, 17].

All of these studies indicate that the Ca^{2+} concentration at which 50% of maximum force is developed increases as pH decreases (i.e., the Ca^{2+}-sensitivity of these preparations decreases with decreasing pH). Maximum Ca^{2+}-activated force may also be pH dependent [4, 6, 14, 17], although one preliminary report [2] indicated that it may be independent of pH between 5.8 and 7.8. The present study is intended to expand the previous results by determining the acid and alkaline pH limits of Ca^{2+}-activated force development in mechanically skinned frog skeletal fibers and to correlate the data with published biochemical measures of concentration.

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Methods

Preparation. Fibers from frog (*Rana pipiens*) *semitendinosus* muscle were skinned (sarcolemma mechanically removed) [12] by the procedure described by Donaldson and Kerrick [3]. Fiber segments between 1.5 and 2.5 mm long were mounted in the forceps of a photodiode force transducer and were prestretched to sarcomere lengths between 2.2 and 2.4 μm as measured with a Zeiss phase-contrast microscope. Isometric force was measured by a method similar to that of Hellam and Podolsky [8]. Transducer complicance was less than 1 μm/mg and fibers shortened by less than 6% of their mounted lengths.

Bathing Solutions. Mounted fibers were immersed sequentially in solutions of different ionic composition. Bathing solutions contained 12 mmol/l Na^+, 78 mmol/l K^+, 1 mmol/l Mg^{2+}, 2 mmol/l $MgATP^{2-}$, 15 mmol/l creatine phosphate, 15 units/ml creatine phosphokinase, 2 mmol/l Cl^-, 7 mmol/l ethyleneglycolbis-(β-aminoethyl ether)-N,N,N′,N′ tetraacetic acid (EGTA), with propionate as the major anion. The ionic equilibria and ionic strengths of these solutions were calculated as described in Kerrick and Donaldson [9]. Before every experiment the pH of each solution was checked (±0.01 pH units), afterward, the solutions were covered with a thin layer of silicone oil. Room temperature was controlled at 22° ±1°C.

Protocol. Ca^{2+}-activated isometric force was recorded in skinned fiber preparations immersed in bathing solutions of different pH values and Ca^{2+} concentrations. Submaximal Ca^{2+}-activated force data were collected with a "stepping protocol" (Fig. 1) [3]. The percentage of maximum Ca^{2+}-activated force was obtained by dividing the force of each submaximal contracture by the maximum Ca^{2+}-activated force developed by the fiber at the same pH.

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Maximum-force ratios were obtained by a "bracketing" (MFR_B) [3] protocol as illustrated in Fig. 1, or a modified bracketing protocol ($MFR_{R/D}$) illustrated in Fig. 3. For each fiber the maximum force developed at each pH was normalized to that developed at pH 7.0 so that the results from different fibers could be compared. MFR_B is defined as the ratio of the maximum Ca^{2+}-activated force generated at any test pH to the average of that developed in the two bracketing contractures at pH 7.0. This procedure could only correctly by used below pH 7.5 because contractures at alkaline pH were followed by incomplete relaxation and reduced contractility (see Figs. 2–3).

The term *residual force* refers to the steady-state force present while a fiber is immersed in a relaxing solution. Residual force increases whenever a fiber fails to relax completely. Large changes in residual force were always associated with contractures at alkaline pH (see Figs. 2–3). Soaking a fiber in a pH 7.0 relaxing solution for more than 2 h had no significant effect on residual force (data not shown). Note that whenever residual force changed during a contracture, the Ca^{2+}-induced increment in force upon activation differs from the Ca^{2+}-dependent decrement in force during relaxation.

We therefore adopted the protocol illustrated in Fig. 3 to obtain the maximum force ratio data at all pH values tested. The extent of deterioation during exposure to alkaline pH was minimized by first activating fibers at neutral pH (arrow 1) before immersing them in the alkaline test solution containing less Ca^{2+} (arrow 2). Because of residual force changes, the Ca^{2+}-dependent portion of the test contracture (arrows 2–3) was assumed to be the difference between (1) the force present just prior to the solution change at arrow 3 minus (2) the steady-state force present during subsequent immersion in a pH 7.0 relaxing solution (arrows 3–4). $MFR_{R/D}$ is obtained by dividing this force decrement upon relaxation (at neutral pH) by the increment (or decrement) in force associated with the next Ca^{2+}-induced contracture at pH 7.0 (arrows 4–5).

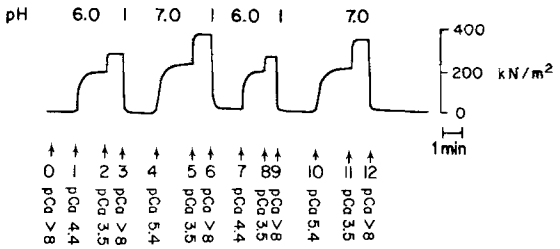

Fig. 1. Chart recording of submaximal and maximal Ca^{2+}-activated contractures at two pH values illustrating the stepping and bracketing protocols. Arrows indicate times at which changes in bathing solutions were made and the changes are indicated below the arrow number. See text for details. Force increases upwards and the abscissa shows time. Fiber diameter, 85 μm

The $[Ca^{2+}]$ needed to produce maximum force varies with pH; thus, for purposes of comparison, the maximum-force ratios (Table 1) were obtained at two supramaximum Ca^{2+} concentrations. Solutions containing 3.2×10^{-4} mol/l Ca^{2+} were used between pH 5.0 and 7.5 and those containing 1×10^{-4} mol/l Ca^{2+} for pH's

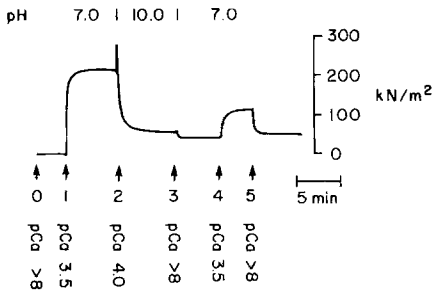

Fig. 3. Chart recording of maximum Ca^{2+}-activated contractures at two pH values illustrating the irreversible effects of alkaline pH on fiber contractility. Arrows indicate time at which changes in bathing solutions were made. Changes are indicated below the arrow number. See text for details. Force increases upwards and the abscissa shows time. Fiber diameter, 60 μm

Table 1. Mean maximum-force ratios by two methods at various pH values

pH	MFR_B[a]		$MFR_{R/D}$[b]		Number of fibers
	S.E.M.	Mean maximumforce ratio	Mean maximumforce ratio	S.E.M.	
5.0	0.05	0.05	0.01	0.02	12
5.5	0.02	0.54	0.60	0.02	18
6.0	0.02	0.73	0.76	0.01	18
6.5	0.02	0.83	0.87	0.01	17
7.0	–	1.00	1.00	–	–
7.5	0.04	0.95	1.28	0.07	21
8.0			1.22	0.05	21
8.5			1.42	0.07	7
9.0			1.15	0.06	7
9.5			0.85	0.07	16
10.0			0.22	0.06	10
10.5			0.04	0.04	16

[a] Maximum force generated at a test pH/average force generated in two adjacent maximal contractures at pH 7.0

[b] Ca^{2+}-activated force at a test pH (total force minus residual force)/Ca^{2+}-activated force at pH 7.0 in the next contracture (total force minus residual force)

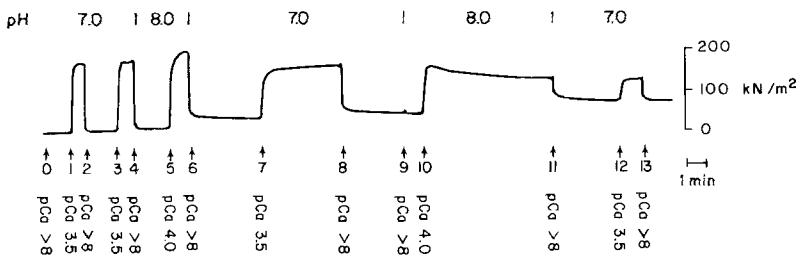

Fig. 2
Chart recording of maximum Ca^{2+}-activated contractures at pH 7.0 and 10.0 illustrating the modified bracketing protocol. Arrows indicate times at which solution changes were made, and the changes are indicated below each arrow number. See text for details. Force increases upwards and the abscissa shows time Fiber diameter, 80 μm

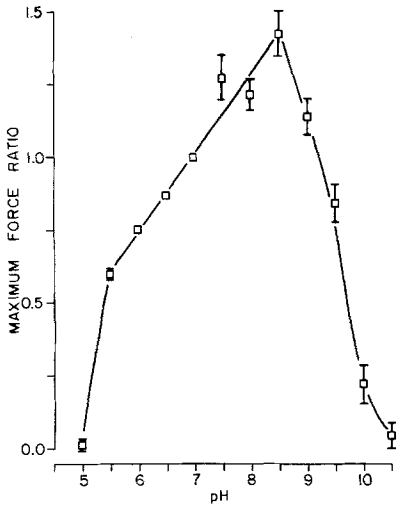

Fig. 4. Relationship between maximum force ratio, $MFR_{R/D}$, and myoplasmic pH. See text for ionic conditions and Table 1 for standard errors of the means and sample sizes. The solid curve is fit to the data by hand

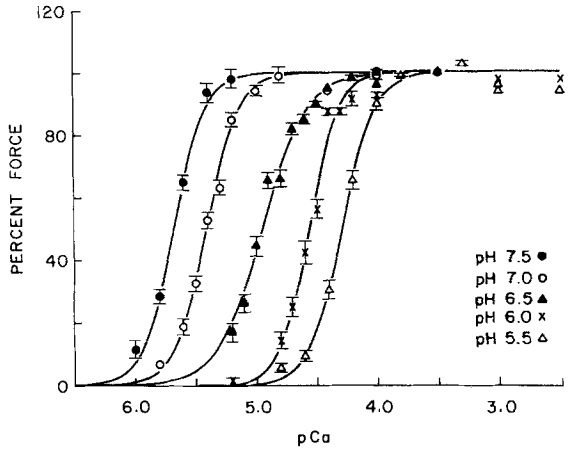

Fig. 5. Relationship between the percentages of maximum force and pCa ($-\log_{10}[Ca^{2+}]$) at various pH values. Symbols represent the standard errors of the means of the individual points at each pCa and pH. All the individual data points at each pH were fit to the following form of the Hill equation:

$$\%F = 100 \times \frac{[Ca^{2+}]^n}{K^n + [Ca^{2+}]^n}$$

where $\%F$ refers to percentage of maximum force and n and K are constants. The computer drawn lines represent the results of the nonlinear least squares regression fitting analysis. The values of the two constants, $\overset{*}{n}$ and $p\overset{*}{K}$, used to draw these lines are listed in Table 2. Details of the method used to obtain their approximate joint 95% confidence limits will be provided upon request

between 8.0 and 10.5. These solutions were verified to be maximally activating by comparing the force developed in solutions containing either a greater or lesser amount of Ca^{2+} at the same pH.

Results

The mean values (± S.E.M.) of the two maximum force ratios (MFR_B and $MFR_{R/D}$) at various pH values are shown in Table 1. Note that these two ratios differ most at pH 7.5. This difference is due to the fact that following activation at alkaline pH fibers did not completely relax and redeveloped significantly less Ca^{2+}-activated force. Therefore, no attempt was made to obtain bracketing data above pH 7.5.

The $MFR_{R/D}$ ratios of Table 1 are plotted against pH in Fig. 4. Below pH 7.5 maximum force decreased with decreasing pH until at pH 5.0 no force was developed. As mentioned earlier, no deleterious effects on the fiber performance were observed over this pH range. Maximum force did not change significantly between pH 7.5 and 9.0 but after increasing the pH from 9.0 to 10.5, it decreased to zero. This effect was assumed to be on Ca^{2+} activation, since the protocol for $MFR_{R/D}$ was designed to minimize the contribution of irreversible effects resulting from exposure to alkaline pH (i.e., marked decrease in maximum force and appearance of residual force).

Irreversible deterioration occurred faster and to a greater extent in alkaline solutions containing high Ca^{2+} than in relaxing solutions (no added Ca^{2+}) at the same pH. For instance, following a total of 8 min of exposure to 1×10^{-4} mol/l Ca^{2+} at pH 8.0, Ca^{2+}-activated force at pH 7.0 decreased by 67% (Fig. 2), whereas following a continuous 20-min exposure to a pH 8.0 relaxing solution, it decreased by only 25% (data not shown). The loss of mechanical activity in alkaline solutions was also accelerated when chloride instead of propionate was the major anion [11].

The relationship between pCa and percent maximum force at various pH values is shown in Fig. 5. These data indicate that for a 100-fold increase in $[H^+]$, a 25-fold increase in $[Ca^{2+}]$ is needed to maintain a constant level of submaximal activation. Table 2 contains the results of nonlinear least squares regression

Table 2. $\overset{*}{n}$, $-\log_{10}(\overset{*}{K})$ and their respective approximate 96% confidence limits obtained by a nonlinear least squares fit of the hill equation[a] to the individual pCa versus % force data points at various pH values

pH	−95%	$\overset{*}{n}$	+95%	−95%	$-\log_{10}(\overset{*}{K})$	+95%
7.5	2.79	3.43	4.23	5.66	5.68	5.71
7.0	2.78	3.27	3.83	5.39	5.41	5.43
6.5	2.05	2.34	2.68	4.93	4.95	4.97
6.0	3.06	3.59	4.22	4.54	4.56	4.58
5.5	2.67	3.21	3.89	4.26	4.29	4.32

[a] See legend of Fig. 5.

analysis of the data in this figure. These are the values of the parameters $\overset{*}{n}$ and $p\overset{*}{K}$ used to draw the theoretical curves shown in Fig. 5.

Discussion

The data indicate that frog skeletal muscle can undergo Ca^{2+}-regulated contraction and relaxation cycles over a wide pH range and that the maximum force developed and the longevity of the preparation depend upon pH. Alkaline pH enhances force development only transiently since it also results in a rapid irreversible loss of contractility, whereas acid pH produced a reversible depression of both force development and Ca^{2+} sensitivity.

Published data showing that acid pH reduces maximum force in glycerine-extracted rabbit soleus muscle bundles [1] and dog papillary muscle fibers [17], mechanically skinned frog skeletal [6], rat ventricle [6] and rabbit skeletal and cardiac fibers [4] are qualitatively in consonance with these data. In contrast, one preliminary study indicates maximum force may not be affected by pH between 5.8 and 7.8 in frog skeletal fibers [2]. These differences are probably due to the normalization procedures used, since our study has shown that muscle fibers deteriorate at different rates depending on the major anion and pH of the bathing medium.

Actin activation of myosin MgATPase and maximum Ca^{2+}-activated force development are similarly pH-dependent [5,13,17]; hence, the pH-dependence of maximal Ca^{2+}-activated force may be related to the pH-dependence of the actin-myosin interaction [20,22]. Alternatively, pH may modulate the strength of the force-generating cross-bridges, but to our knowledge, no data exist that would enable us to evaluate this possibility.

The pH-dependence of the pCa-force relationship reported in this study is quantitatively similar to the pH-dependence of Ca^{2+} binding to the low-affinity sites of the calcium-binding subunit of rabbit skeletal [15] and bovine cardiac [16] troponin-C, suggesting that protons may alter the pCa-force relationship by competing with Ca^{2+} for activating sites on frog TnC as well.

The experiments show that the major effects of alkaline pH (7.5–9.0) on Ca^{2+}-activated force are the irreversible increase in residual force and loss of contractility. These observations are similar to the effect of low [KCl]s on the mechanical activity of skinned fibers [7,21]. The mechanism responsible for these effects is not known.

It is well documented that acidosis decreases cardiac contractility and that changes in intracellular pH are much more potent in this regard than are extracellular changes [18,19]. Our data show that independent of any effects pH may have on intracellular $[Ca^{2+}]$[6], energy production [18] or other cellular mechanisms, intracellular acidosis depresses mechanical function reversibly at the level of actomyosin interaction. It also shifts the $[Ca^{2+}]$-percent maximal force relationship so that a higher $[Ca^{2+}]$ is required to reach a given level of activation. On the other hand, alkaline pH irreversibly alters the force-generating properties of myofibrils.

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