EFFECTS OF ANOXIA, ACIDOSIS AND TEMPERATURE ON THE CONTRACTILE PROPERTIES OF TURTLE CARDIAC MUSCLE STRIPS

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Summary

The responses to anoxia and acidosis of cardiac ventricular muscle strips from the anoxia-tolerant turtle Chrysemys picta bellii were investigated at 10 °C and 20 °C. Force–velocity curves were determined by quick isotonic releases at 85 % of the time to peak isotropic force under control, anoxia, lactate acidosis and anoxic lactate acidosis conditions. The isotonic forces during quick releases spanned 5–95 % of the measured isotropic force at each condition. Superfusion solution pH was 7.8 and 7.95 for non-acidosis experiments, and 7.0 and 7.15 for acidosis experiments, at 20 °C and 10 °C, respectively. After normalizing force data to control isotropic force, the values of maximum isotonic force (P0), maximum velocity of shortening (Vmax) and maximal power output (Powermax) were evaluated by fitting the curves using the hyperbolic Hill equation. The maximum rate of force development (dF/dtmax), time-to-peak force (Tpf) and half-relaxation time (T1/2) were also determined. At 20 °C, during acidosis, anoxia and anoxic acidosis, P0 decreased significantly to 81 %, 40 % and 24 % of control values, dF/dtmax decreased significantly to 67 %, 53 % and 23 % of control values, and $T_{1/2}$ decreased significantly to 67 %, 40 % and 14 % of control values, respectively. Vmax, however, was not significantly affected by acidosis, anoxia or even anoxic acidosis. Tpf was significantly shortened by anoxia, but prolonged by acidosis. The effects were similar at 10 °C. Temperature did not affect P0, but Vmax decreased by a factor of 1.6–1.8 at all corresponding conditions when temperature was reduced from 20 °C to 10 °C. We conclude that acidosis and anoxia inhibit isotropic force production and Powermax of turtle cardiac muscle, but have no effect on Vmax, and the insensitivity of Vmax indicates that the rate of cross-bridge cycling is not affected by these conditions. Our observations indicate that the reduced power outputs of the hearts of submerged anoxic turtles at low temperature are due in part to inhibition of force production by anoxia and acidosis, and to a reduction of contraction velocity at low temperature.

Key words: Chrysemys picta bellii, turtle, maximum isotropic force, Vmax, Powermax, dF/dtmax, Tpf, T1/2, pH, cardiac function, shortening velocity, power output, muscle.

Introduction

In contrast to hearts of vertebrates in general, the hearts of various freshwater turtles exhibit unusual tolerance to anoxia and acidosis. This ability correlates with the anoxia-tolerance of the whole animal and the continued functioning of its heart, albeit at reduced rate and pressure development, during long periods of experimental anoxic submergence (Herbert and Jackson, 1985b). During in vivo anoxia, the animal also experiences a severe lactate acidosis, so the heart is subjected to combined anoxia and acidosis. The results of studies on in vitro turtle heart preparations exposed to anoxia or acidosis separately have been inconsistent, however, and have reported no effect on function of perfused hearts exposed to anoxia (Reeves, 1963; Wasser et al. 1990b; Jackson et al. 1995) or acidosis (Wasser et al. 1990b; Jackson et al. 1995), a depressant effect on ventricular strips exposed to anoxia (Bing et al. 1972; Wasser et al. 1990a) or acidosis (Wasser et al. 1990a) and a depressant effect of anoxia on in situ hearts (Farrell et al. 1994). Combined anoxia and acidosis, the condition that more accurately simulates the in vivo state of the anoxic turtle, in contrast, consistently and profoundly reduced turtle cardiac functions in perfused hearts and in muscle strips (Wasser et al. 1990a,b; Jackson et al. 1995). Turtles are tolerant to anoxia over a wide range of body temperatures (Herbert and Jackson, 1985a), although it is not known how common an occurrence anoxic submergence is for these animals in nature except at hibernating temperatures (Ultsch, 1989). Both anoxic submergence and low temperature reduced the heart rate of turtles in vivo. Arterial pressure decreased with temperature from 20 °C to 3 °C and was also reduced by anoxia at 3 °C (Herbert and Jackson, 1985b). These changes resulted from both neural and hormonal influences (White and Ross, 1966; Wasser and Jackson, 1991) and from

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intrinsic cardiac responses to anoxia and acidosis. In situ, Farrell et al. (1994) observed that maximum power output decreased by approximately four- to fivefold between 15°C and 5°C and that at each temperature anoxia reduced power output by a further 50% below the normoxic control value. Although this in situ study included the cardiac effects of both temperature and anoxia, it did not include the intrinsic cardiac response to the combined anoxic acidosis and temperature that is encountered by turtles in their natural environment.

In the present study, we investigated the responses of turtle cardiac mechanical function to anoxia, to acidosis and to their combined effects at both 10°C and 20°C using an isolated muscle strip preparation. This arrangement afforded better control of the tissue environment and of muscle fiber length than is possible in an isolated heart preparation. By applying a computer-controlled servo system, we were able to study not only the force of muscle contraction, as has been done in other muscle strip studies, but also the maximum velocity (Vmax) of muscle shortening. From the relationship between force and velocity, we could also calculate maximum power output (Powermax). Our objective in this study was to determine whether anoxia, acidosis and temperature have different effects on force and velocity. We found that anoxia and acidosis each inhibited the force of muscle contraction and maximum power output, but the greatest inhibition was observed from combined anoxia and acidosis. Vmax of muscle was not affected by anoxia and acidosis. Temperature did not affect isometric force, but Vmax was severely reduced at low temperature.

Materials and methods

Experimental animals

Western painted turtles Chrysemys picta bellii (Gray) (both sexes, mass 700–970 g, purchased from Lemberger Associates, Wisconsin, USA) were housed in a tank at 20°C with both deep water and a dry platform for at least 1 week, and feeding was stopped 2–3 days before each experiment. A 12 h:12 h light:dark cycle was provided.

Preparation of ventricular muscle strips

Hearts, excised rapidly following decapitation, were placed in ice-cold HCO3−-buffered Ringer’s solution (see below). A strip of muscle (approximately 10 mm × 3 mm × 2 mm) was cut with fine scissors from either the right or left ventral ventricle along the fibre orientation of the compact layer under a dissecting microscope. Fibre orientation was the same throughout the muscle length. A portion of trabeculae under the compact layer was usually included in the strip. Any obviously damaged ends were cut off.

Muscle strip mounting and instrumentation

The muscle was mounted vertically in a temperature-controlled chamber (10 cm × 1 cm) as described by Fredberg et al. (1993). The top end of the muscle was glued to a metal clip attached by a straightened steel wire to the lever arm of a motor (see below). The bottom end of the muscle was similarly attached to another piece of wire which went through a hole sealed by a mercury bead at the bottom of chamber. Both the motor and the bottom wire were fixed to translation stages for fine adjustment of the position and length of the muscle. After superfusion at approximately 20 ml min⁻¹ with control HCO3−-buffered Ringer’s solution for 1.5 h to stabilize the preparation, the length–tension relationships were measured to define l0, the maximal length at which the active force–length relationship had a positive slope. Muscle length was adjusted to 90–100% of l0, and all further measurements were made at this length. Resting force was 4.1±0.5% of control isometric force. Voltage pulses, applied to platinum plate electrodes by a Grass stimulator (model SD 9), stimulated contractions at 20 min⁻¹ for experiments at 20°C and at 12 min⁻¹ for experiments at 10°C.

To generate force–velocity curves, a Cambridge servo system 300B (Cambridge Technology Inc., MA, USA) was used to control the force against which the muscle contracted both isometrically and isotonically and also to measure isotonic forces and muscle length changes during a series of quick releases. After the muscle had been activated isometrically, the servo system produced critically damped quick releases to different loads at the same proportion of the muscle contraction cycle so that the velocities of shortening from different contractions were measured at the same level of muscle activation. Each of the switches took 4 ms, and we assume that no significant muscle length change occurred during this short period. A Mac 2ci computer equipped with Labview software (National Instruments Co.) was used both to collect data and to control the switch of the servo system from isometric contractions to isotonic releases. Original force and length data from two isotonic releases are shown in Fig. 1.

Measurements and data acquisition

Experiments were conducted at 20°C and 10°C. These temperatures were chosen because they are temperatures that turtles experience in nature. Near-freezing temperatures, at which anoxia and severe acidosis have been observed in nature (Ultsch, 1989), could have been investigated, but this was not experimentally practical because, at the slow heart rate (0.4 beats min⁻¹) during anoxic submergence at 3°C; Herbert and Jackson, 1985b), at least 4–5 h would be required to collect data at one experimental condition.

The force–velocity measurement was modified from the method described by Walley et al. (1991). For each experimental condition, isotonic quick releases were performed at loads ranging from 5% to 95% of measured isometric force at that condition. For every isotonic contraction, the muscle was released at 85% of the time-to-peak force determined from nine (20°C) or six (10°C) isometric contractions immediately preceding the release. This time was chosen because activation reached its peak shortly before peak isometric force was achieved (Walley et al. 1991), and maximal velocity was recorded at this time in preliminary
Maximum power was achieved can be determined by force using the Hill equation, the force value at which is the product of force and velocity. By expressing the velocity extrapolated isometric force \(P_a\) force–velocity relationship at force=0, maximum velocity is given by \((c/a)-b\); at velocity=0, maximum extrapolated isometric force \(P_0\) was given by \((c/b)-a\). Power is the product of force and velocity. By expressing the velocity and force using the Hill equation, the force value at which maximum power was achieved can be determined by differentiating. Power\(_{\text{max}}\) calculated at this force was \(axb+ac-2\sqrt{axb}c\).

Time-to-peak force \((T_{PF})\) was measured as the time from the onset of contraction to the peak isometric force. The half-relaxation time \((T_{1/2})\) was determined as the time between the attainment of peak force and the point where active force fell by 50%. The maximum rate of force development \((dF/dt_{\text{max}})\) was also determined from the force data.

**Superfusion solutions**

We superfused the cardiac muscle strips with the following \(\text{HCO}_3^-\)-buffered Ringer’s solutions. Control solution composition contained (in mmol\(L^{-1}\)): 120 Na\(^+\), 2.5 K\(^+\), 2.0 Ca\(^{2+}\), 1.0 Mg\(^{2+}\), 88.5 Cl\(^-\), 40 HCO\(_3^-\) and 10 glucose. The acidosis solution contained 35 mmol\(L^{-1}\) lactate and 5 mmol\(L^{-1}\) \(\text{HCO}_3^-\); concentrations of the other ions were unchanged. At 20°C, the solutions were equilibrated with 3% \(\text{CO}_2/97\% \text{O}_2\) for normoxic experiments and with 3% \(\text{CO}_2/97\% \text{N}_2\) for anoxic experiments; gas mixtures were supplied by a precision gas-mixing flowmeter (Cameron Instruments Co., model FG-3/MP). The same gas also flowed in the jacketed space around the line connecting the solution bottle to the muscle chamber to prevent a change of gas composition. The pH of the equilibrated solution was 7.8 for control or anoxia, and 7.0 for acidosis and anoxic acidosis, checked by a pH electrode. For the 10°C experiments, we equilibrated solutions at 20°C with 2.2% \(\text{CO}_2\) balanced either by \(\text{O}_2\) or by \(\text{N}_2\) and then passed the solution through a condenser at 10°C to achieve final pH values of 7.95 for non-acidosis and 7.15 for acidosis experiments in the muscle chamber. The difference in pH between 20°C and 10°C (0.15 pH units) conforms to alphastat regulation (Reeves, 1972) and to the in vivo blood pH change for this turtle (Herbert and Jackson, 1985a). The water temperature in the muscle chamber was controlled at either 20.0±0.2°C or 10.0±0.1°C, and the \(P_{O_2}\) of the anoxic perfusate in the chamber was below 0.4 kPa as measured by an \(\text{O}_2\) electrode.

**Experimental protocol**

We studied seven muscles strips at 20°C and five strips at 10°C. For each group, the experimental conditions were randomized in one of the following orders: (1) control, anoxia, anoxic acidosis, control, acidosis and control conditions; or (2) control, acidosis, anoxic acidosis, control, anoxia and control conditions. The results were independent of the order chosen. At each condition, the muscle was allowed to stabilize for approximately 1 h before the isotonic quick releases were performed. Before the protocol was carried out at 10°C, the muscle strip was stabilized at 20°C in control solution and data were also collected at this temperature.

**Data analysis and statistics**

Force data were normalized to the measured isometric force of the initial control period at 20°C. Velocity data were expressed as optimal muscle lengths per second \((l_0\;\text{s}^{-1})\). Mean values of \(P_0\), \(V_{\text{max}}\), \(\text{Power}_{\text{max}}\) and \(dF/dt_{\text{max}}\) in the control period

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**Fig. 1.** Original experimental recordings of two isotonic quick releases at different loads after the muscle had been maximally activated under control condition. (A) Length; (B) force.
following anoxic acidosis recovered to only 99%, 83%, and 83%, respectively, of the initial control values. Therefore, in each experiment, the values of $P_0$, $V_{\text{max}}$, $Power_{\text{max}}$ and $dF/dt_{\text{max}}$ determined from periods after the recovery from anoxic acidosis were corrected by the percentage change between the values of the initial control and the control immediately before that intervention at the same temperature so that all values could be compared. $P_0$, $V_{\text{max}}$, $Power_{\text{max}}$, $T_{1/2}$ values from control, anoxia, acidosis and anoxic acidosis at the same temperature were compared using one-way analysis of variance (ANOVA). Data from the same condition but at different temperatures were compared using $t$-tests. We accepted a level of $P<0.05$ as statistically significant. Data are presented as means ± S.E.M.

### Results

**Effect of anoxia and acidosis**

The force–velocity relationships from typical experiments at 10°C and 20°C are shown in Fig. 2. At 20°C, $P_0$ decreased significantly to 81%, 40% and 24% of its control value after 1h of acidosis, anoxia or combined anoxic acidosis, respectively. $V_{\text{max}}$, however, was not significantly affected by any of the experimental conditions (Table 1). $Power_{\text{max}}$, consequently, was reduced to 75%, 40% and 14% of the control value in acidosis, anoxia and anoxic acidosis, respectively, primarily as a result of the fall in force. $dF/dt_{\text{max}}$ decreased significantly during each of the three experimental conditions to 67%, 53% and 23% of the control rate. Time-to-peak force ($T_{PF}$) was significantly prolonged by 25% during acidosis, but was significantly shortened by 18% during anoxia. No significant change in $T_{PF}$ was found between control and anoxic acidosis at 20°C. Values of half-relaxation time ($T_{1/2}$) were not significantly changed from control values for any of the interventions.

At 10°C, the effects of the experimental manipulations were qualitatively similar to those at 20°C. Specifically, acidosis, anoxia and anoxic acidosis reduced $P_0$ significantly to 77%, 46% and 25% of the control value, reduced $Power_{\text{max}}$ significantly to 74%, 63% and 26% of the control value, and also reduced $dF/dt_{\text{max}}$ significantly to 70%, 64% and 36% of the control value. $V_{\text{max}}$ values during acidosis, anoxia and anoxic acidosis were not significantly different from the control value of 0.25±0.039 $l_0 s^{-1}$. $T_{PF}$ responded to all experimental maneuvers in the same manner as at 20°C, except that $T_{PF}$ was significantly shorter during anoxic acidosis than under control conditions. $T_{1/2}$ values from control, anoxia, acidosis and anoxic acidosis were not significantly different. Detailed values are presented in Table 2.

Force–power curves at 20°C (Fig. 3) and 10°C (Fig. 4) were plotted in two ways: first, by normalizing force data to

### Table 1. Effect of acidosis and anoxia on muscle mechanical properties at 20°C

<table>
<thead>
<tr>
<th>Condition</th>
<th>$dF/dt_{\text{max}}$</th>
<th>$T_{PF}$ (s)</th>
<th>$T_{1/2}$ (s)</th>
<th>$V_{\text{max}}$ ($l_0 s^{-1}$)</th>
<th>$P_0$</th>
<th>$Power_{\text{max}}$ ($l_0 s^{-1}$)</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.88±0.025</td>
<td>0.52±0.020</td>
<td>0.42±0.018</td>
<td>1.02±0.008</td>
<td>0.084±0.0058</td>
<td>7</td>
</tr>
<tr>
<td>Acidosis</td>
<td>0.67±0.028*</td>
<td>1.10±0.041</td>
<td>0.50±0.011</td>
<td>0.40±0.016</td>
<td>0.83±0.034*</td>
<td>0.063±0.0047*</td>
<td>7</td>
</tr>
<tr>
<td>Anoxia</td>
<td>0.53±0.032*</td>
<td>0.72±0.033</td>
<td>0.45±0.026</td>
<td>0.46±0.044</td>
<td>0.41±0.016*</td>
<td>0.034±0.0042*</td>
<td>6</td>
</tr>
<tr>
<td>Anoxic acidosis</td>
<td>0.23±0.018</td>
<td>0.93±0.051</td>
<td>0.52±0.027</td>
<td>0.35±0.050</td>
<td>0.24±0.031*</td>
<td>0.012±0.0015*</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.

$dF/dt_{\text{max}}$ values are normalized to the control value of $dF/dt_{\text{max}}$.

*Denotes a significant difference ($P<0.05$, ANOVA) from the corresponding control value.

$dF/dt_{\text{max}}$, maximum rate of force development; $T_{PF}$, time-to-peak force; $T_{1/2}$, time to half-relaxation; $V_{\text{max}}$, maximum shortening velocity; $P_0$, maximum isometric force; $Power_{\text{max}}$, maximum power output.

Force was normalized to the measured isometric force of the initial control period at 20°C.
Anoxia and acidosis in turtle heart muscle

Table 2. Effect of acidosis and anoxia on muscle mechanical properties at 10°C

<table>
<thead>
<tr>
<th>Condition</th>
<th>$dF/dl_{max}$</th>
<th>$T_{PF}$ (s)</th>
<th>$T_{1/2}$ (s)</th>
<th>$V_{max}$ (l/s)</th>
<th>$P_0$ (l/s)</th>
<th>$Power_{max}$ (l/s)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control at 20°C</td>
<td>1</td>
<td>0.85±0.024</td>
<td>0.49±0.016</td>
<td>0.41±0.048</td>
<td>1.04±0.022</td>
<td>0.085±0.0075</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td>0.47±0.017†</td>
<td>1.82±0.059†</td>
<td>1.02±0.035†</td>
<td>0.25±0.039†</td>
<td>1.10±0.030</td>
<td>0.038±0.0066†</td>
<td>5</td>
</tr>
<tr>
<td>Acidosis</td>
<td>0.33±0.021*†</td>
<td>2.01±0.084*†</td>
<td>1.07±0.057†</td>
<td>0.24±0.036†</td>
<td>0.85±0.084*</td>
<td>0.028±0.0037*†</td>
<td>5</td>
</tr>
<tr>
<td>Anoxia</td>
<td>0.30±0.012*†</td>
<td>1.57±0.044*†</td>
<td>0.91±0.050†</td>
<td>0.26±0.026†</td>
<td>0.51±0.044*</td>
<td>0.024±0.0043*</td>
<td>5</td>
</tr>
<tr>
<td>Anoxic acidosis</td>
<td>0.17±0.013*†</td>
<td>1.45±0.051*†</td>
<td>0.91±0.041†</td>
<td>0.22±0.026†</td>
<td>0.28±0.012*</td>
<td>0.010±0.0008*</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.
$df/dl_{max}$ values are normalized to the control $df/dl_{max}$ at 20°C.
*Denotes a significant difference ($P<0.05$, ANOVA) from the control value at 10°C.
†Denotes a significant difference ($P<0.05$, t-test) between corresponding values at 10°C and 20°C (see Table 1) for the same experimental manipulations.
Abbreviations are defined in Table 1.

Force was normalized to the measured isometric force of the initial control period at 20°C.

Effects of temperature

Temperature affected $P_0$ and $V_{max}$ differently. Under each of the experimental conditions, including control conditions, $V_{max}$ decreased significantly when temperature was reduced from 20°C to 10°C. In contrast, control $P_0$ at 10°C was not significantly different from that at 20°C. The reduction in $Power_{max}$ at 10°C was therefore related to the reduction in $V_{max}$. Decreased temperature prolonged $T_{PF}$ by 1.6- to 2.2-fold and $T_{1/2}$ by 1.8- to 2.2-fold and decreased $df/dl_{max}$ by a factor of 1.4-2.1 in all experimental conditions (Tables 1, 2). The curvature of the force–velocity curves from different experimental conditions, represented by $dF/dP_0$, varied between 0.3 and 0.8 in individual experiments, and no consistent difference was found among the experimental conditions or between the two temperatures.

Discussion

Effects of acidosis, anoxia and anoxic acidosis

Contraction velocity

An important finding in our study is the insensitivity of the maximum velocity of shortening, at either 20°C or 10°C, to anoxia, to acidosis or to combined anoxia and acidosis. In contrast to these results on turtle cardiac muscle, anoxia has been observed to reduce $V_{max}$ of mammalian cardiac muscle. $V_{max}$ of cat papillary muscle fell by 54% (Tyberg et al. 1970) and 23% (Henderson and Brutsaert, 1973) at the end of 1 h of anoxia. Mild hypoxia (18% O2) also significantly reduced $V_{max}$ in rabbit myocardium (Walley et al. 1991). This suggests a stronger resistance of turtle cardiac contractile proteins to oxygen lack. Walley et al. (1991) also investigated the effect of hypercapnic acidosis on $V_{max}$ in rat cardiac papillary muscle and observed little change at an extracellular pH (pH_e) of 6.86. Consistent with our results on turtle cardiac muscle, Ricciardi et al. (1986) demonstrated that maximum unloaded shortening velocity ($V_0$) in rat cardiac trabeculae was not significantly different from control values during steady exposure to non-
CO₂ acidosis at pHₑ of 6.68. However, in a recent study on skinned cardiac muscle, Ricciardi et al. (1994) showed that severe acidosis (pHᵥ values of 6.6 and 6.2) depressed Vₘₐₓ by 17% and 40%, respectively. The lower of these pH values is probably outside the normal physiological range for mammalian muscle.

Because our goal was to study the turtle myocardial response to changes of pH within the physiological range, no attempt was made to examine extreme acidosis not observed in the living animal. In an earlier study of skeletal muscle from the turtle *Pseudemys scripta elegans*, Vₘₐₓ was not affected by a change of pH in the physiological range 6.6–7.8. However, above or below this range, Vₘₐₓ was depressed (Mutungi and Johnston, 1987). It is likely that Vₘₐₓ of turtle cardiac muscle may also be reduced at extremely low unphysiological pH.

The insensitivity of Vₘₐₓ of turtle myocardium to anoxia and acidosis suggests that the rate of cross-bridges cycling was not affected by these conditions. The velocity of cardiac muscle shortening is dependent on the cycling rate of cross-bridges and is also related to the myosin ATPase activity (de Tombe and ter Keurs, 1990; Walley et al. 1991).

**Force**

In the present study, the maximum isometric force of turtle ventricular muscle strip, derived from fitting the results to the Hill equation, was reduced to 40% and 46% from the control value during anoxia at 20°C and 10°C, respectively; the values are within the range observed by others (Bing et al. 1972; Jackson, 1987). This reduction was much less than that in mammalian heart, where anoxia depressed force to 10–20% (Bing et al. 1972; Tyberg et al. 1970). Lactate acidosis also inhibited the force of turtle cardiac muscle contraction to 81% at 20°C (pH7.0) and 77% at 10°C (pH7.15) in our study. This is smaller than the reduction to 55% of the control value during lactate acidosis reported by Jackson (1987), but corresponds to the less severe acidosis applied in the present study (pH7.0 versus 6.8). During combined anoxia and acidosis, the maximum isometric force fell to 24% at 20°C, comparable to the drop to 16% of the control value observed earlier by Wasser et al. (1990a).

The change in isometric force can be achieved by a change in the number of attached cross-bridges and by a change in the mean force generated by one cross-bridge. Acidosis reduces isometric force production of myocardial contraction by interfering with many steps of the excitation–contraction coupling sequence (Orchard and Kentish, 1990), including the magnitude of the Ca²⁺ current, the release of Ca²⁺ from the sarcoplasmic reticulum, and also the myofibrillar response to Ca²⁺ by increasing the competition of protons with Ca²⁺ for the binding site of troponin C. Therefore, acidosis reduces the number of available thin filament binding sites and thereby decreases the number of attached cross-bridges. Anoxia can reduce force generation through the associated acidosis as well as by other mechanisms. For example, anoxia decreases the free energy released from ATP hydrolysis (Driedzic and Gesser, 1994). Elevated intracellular inorganic phosphate (Pᵢ) levels during anoxia reduce the phosphorylation potential. In addition, Pᵢ reduces force generation by reducing the Ca²⁺-sensitivity of the myofilament and by decreasing force at saturating levels of Ca²⁺ (Driedzic and Gesser, 1994). It is believed that Pᵢ depresses the number of strongly bound (force-generating) cross-bridges and increases the number of unattached and weakly attached cross-bridges (Hibberd et al. 1985; Allen et al. 1995). Mechanisms that depress isometric force production should also inhibit dF/dtₘₐₓ, the maximal rate of force increase, ultimately by reducing the rate of formation of the strongly bound cross-bridges. This is in accordance with reductions in P₀ and dF/dtₘₐₓ of similar magnitude in the present study. Both H⁺ and Pᵢ are also thought to reduce the force produced per cross-bridge (Allen et al. 1995).

**Time-to-peak force**

Anoxia and acidosis had opposite effects on the time-to-peak force (Tₑ). Acidosis prolonged Tₑ, probably by delaying the activation of contraction by H⁺ through the interfering mechanisms discussed above. Anoxia, however, shortened Tₑ, possibly by shortening the action potential (Henderson and Brutsaert, 1973) and thereby reducing the time for contraction. Consistent with our finding, a shortened Tₑ in response to anoxia has been reported from mammalian heart preparations (Tyberg et al. 1970; Nakhjavan et al. 1971; Henderson and Brutsaert, 1973). The absence of a change in Tₑ during anoxic
acidosis at 20 °C may be due to these effects counteracting each other.

Power

$P_{\text{max}}$ shows less error than $P_0$ and $V_{\text{max}}$ because it is derived from interpolation of the force–velocity relationship. $P_{\text{max}}$ reflects changes in both velocity and force and is determined by the number of attached cross-bridges, by the mean force per attached cross-bridge and by the rate of cross-bridge cycling. Anoxia, acidosis and combined anoxia and acidosis all depressed maximum power production by approximately the same magnitude as the reduction in force generation. This suggests that the apparent reduction of power output observed during anoxic submergence in vivo at the same temperature (Herbert and Jackson, 1985b) was due to a reduction of force generation but not of the velocity of shortening. In vivo, however, the depressed cardiac function is not due solely to the intrinsic response of cardiac muscle to anoxia and acidosis but also to extrinsic influences such as increased vagal stimulation (White and Ross, 1966).

The reduction of $P_{\text{max}}$ by anoxia and acidosis, however, has not been observed in isolated perfused turtle hearts during anoxia and acidosis. Reeves (1963) saw no reduction in cardiac power output of perfused turtle heart at 25 °C during 15 h of anoxic exposure in vitro. Similarly, Wassef et al. (1990b) and Jackson et al. (1995) observed that function was maintained in isolated hearts at 20 °C, including maximal power output during either anoxia or lactate acidosis. However, in an in situ turtle heart preparation, a significant (approximately 50 %) reduction in maximum cardiac power output during anoxia was reported by Farrell et al. (1994). As suggested by the latter authors, a possible explanation for the discrepancy in these studies is that true maximum power outputs were not achieved under control conditions in the in vitro studies so that the reduction of $P_{\text{max}}$ by anoxia and acidosis was obscured. In our present muscle strip study, the tissue was set at approximately optimal length so that maximum power was generated and an inhibition by anoxia or acidosis was clearly evident. It is not possible, however, to exclude other unknown factors that could explain the different results from these various studies.

**Effect of temperature on mechanical properties**

In this study, maximal isometric force ($P_0$) of turtle cardiac muscle strips was the same at 10 °C and 20 °C. This finding is consistent with the temperature-independence of skeletal muscle force development in both ectothermic and endothermic vertebrates (reviewed by Bennett, 1984). Variations in isometric force production of cardiac muscle in response to a change in temperature have, however, been observed in a number of studies. In turtle atrial muscle, force increased when temperature decreased from 20 °C to 10 °C (Brown, 1957). Decreasing temperature also caused an increase in force production in rat heart trabeculae (de Tombe and ter Keurs, 1990) and in rabbit papillary muscle (Edman et al. 1974). However, in frog (Rana pipiens) cardiac muscle under K+-induced contracture, $P_0$ was maximal at 20 °C and decreased at both lower and higher temperatures (Reiser and Lindley, 1983).

Opposing effects of temperature on contractile events may explain these disparate results. For example, reduced temperature can decrease both maximal Ca2+-activated force and Ca2+-sensitivity in frog and mammalian hearts (Harrison and Bers, 1990). In contrast, lowered temperature can increase contractile force by enhancing Ca2+ availability by prolonging the action potential (Lennard and Huddart, 1991; Moller-Nielsen and Gesser, 1992) and thereby increasing the Ca2+ influx. Increased intracellular [Na+]i, due to depressed Na+/K+-ATPase activity at low temperature, can further increase Ca2+ entry by Na+/Ca2+ exchange (Page and Storm, 1965). Variations in the responses of the muscle force to changing temperature may depend on which mechanism is dominant and may also represent differences among species or result from different methods of measurement.

Despite the insensitivity of $P_0$ to temperature, time-dependent processes in turtle cardiac muscle were significantly influenced by temperature. $P_0$ was slower at 10 °C by a factor of 1.6- to 2.2-fold in turtle heart, close to the $Q_{10}$ of 2.1–2.8 (mean value 2.3) measured in other cardiac muscles between approximately 25 °C and 35 °C (Bennett, 1984). This suggests that the rate of activation was significantly reduced by low temperature. $V_{\text{max}}$ decreased (as did $P_{\text{max}}$) during all experimental manipulations in our study with a $Q_{10}$ of 1.6–1.8, presumably due to reduced cross-bridge cycling rate at low temperature.

In rat heart trabeculae, the $Q_{10}$ of $V_{\text{max}}$ was 4.6 (de Tombe and ter Keurs, 1990). However, lower $Q_{10}$ values of $V_{\text{max}}$ were observed in other cardiac muscle studies (Yeatsman et al. 1969; Hamrell et al. 1978) and in turtle skeletal muscle, where $Q_{10}$ values of 1.9–2.0 between 0 and 10 °C and 1.2–1.3 between 10 and 20 °C were reported (Mutungi and Johnston, 1987). The speed of muscle shortening correlates with myosin ATPase activity (Barany, 1967; Hamrell and Low, 1978). In rat trabeculae, the $Q_{10}$ of $V_{\text{max}}$ was 4.6, close to the estimated $Q_{10}$ of 5 for myosin ATPase in rat cardiac muscle (Siemankowski et al. 1985). As discussed by Woledge et al. (1985), the $Q_{10}$ of $V_{\text{max}}$ depends on the muscle type and species. The smaller $Q_{10}$ of $V_{\text{max}}$ in turtle heart compared with rat heart suggests that turtle heart may have a different type of myosin ATPase than rat heart. It should be noted, however, that de Tombe and ter Keurs (1990) contend that the inability to measure the true $V_{\text{max}}$ at higher temperature might lead to an underestimate of $Q_{10}$. In our study, because the velocity of shortening was determined 50–70 ms after isotonic release, $V_{\text{max}}$ may be underestimated. At higher temperature, a greater reduction of velocity may occur prior to the data analysis period, and this could lead to an underestimate of the true $Q_{10}$.

We conclude from this study that acidosis and anoxia reduce isometric force production and $P_{\text{max}}$ of turtle cardiac muscle, but have no effect on $V_{\text{max}}$, and that the insensitivity of $V_{\text{max}}$ indicates that the rate of cross-bridge cycling is not affected by these conditions. In contrast, a reduction in

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temperature reduces $V_{\text{max}}$, but has no effect on isometric force. These observations indicate that the intrinsic factors causing reduced power outputs of hearts of submerged anoxic turtles at low temperature are reduction of force generation by anoxia and acidosis and reduction of shortening velocity by low temperature.

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