Effect of Acidosis on Contractile Function in the Newborn Rabbit Heart

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ABSTRACT. Effects of respiratory and metabolic acidosis (pH approximately 6.8) on myocardial function were studied in the newborn and adult rabbits. Mechanical function was studied in the isolated arterially perfused heart preparation. Acidosis was induced either by increase of the perfusate PCO_2 or by decrease of the bicarbonate content. During respiratory acidosis, developed tension (DT) decreased to $43 \pm 3\%$ of control ($n = 18$) in the adult and this depression was significantly greater than in the newborn (DT = $92 \pm 4\%$, $n = 6$). Depression of DT by respiratory acidosis was observed even at high extracellular Ca. During metabolic acidosis, mechanical function decreased gradually and DT at 30 min into acidosis in the adult was $78 \pm 3\%$ of control ($n = 6$). This depression of DT in the adult was significantly greater than in the newborn (DT at 30 min = $96 \pm 1\%$ of control, $n = 6$). Statistical analysis using paired t test showed that respiratory acidosis, but not metabolic acidosis, caused significant negative inotropism in the newborn. Myofibrils were isolated and the ATPase was measured at 10^{-8} to 10^{-4} M Ca and at pH of 7.1 (control), 6.5, and 6.0. Reducing pH depressed the ATPase activity similarly in the newborn and adult. Intracellular buffer capacity was determined by titrating muscle homogenate with HCl. Although the initial pH was not different, addition of HCl to the homogenate caused less decrease in pH in the newborn. These data indicate that contractile function in the newborn heart is more resistant to acidosis and this may be due partly to the greater intracellular buffer capacity. (*Pediatr Res* 19: 482-488, 1985)

Abbreviations

DT, developed tension

RT, resting tension

+dT/dt max, maximal rate of tension development

DTT, dithiothreitol

TES, N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid

Several investigators examined the effect of acidosis on cardiac function in the premature animals (1, 2). These *in vivo* studies showed that respiratory acidosis, but not metabolic acidosis, depressed cardiac function. Because preload, afterload, and heart

rate change with development, it is difficult to assess the age-related difference in the effect of acidosis per se in the *in vivo* study. Developmental changes in the effect of acidosis have not been studied in the isolated heart preparation.

Myocardial contractile force is largely dependent on the myofibrillar ATPase activity and the amount of contractile calcium (3). Kentish and Nayler (4) showed in the adult rabbit that acidosis reduced myofibrillar ATPase activity and increased calcium concentration which was required to activate ATPase to the half-maximal value. Age-related changes in the pH dependence of the myofibrillar ATPase have not been studied. Furthermore, competitive interaction between hydrogen (H^+) and calcium (Ca^{2+}) on cardiac contraction has been reported by several investigators (5, 6). The relationship between Ca^{2+} and H^+ in the premature myocardium is not determined. Accordingly, the present study was designed to investigate developmental changes in 1) the effect of acidosis on contractile function, 2) pH dependence of myofibrillar ATPase, and 3) the interaction between H^+ and Ca^{2+} on contractile function in the isolated heart preparation.

METHODS

The experiment utilized the fetus (28th day of gestation), newborn (2-5 day old), and adult (6-12 month old) New Zealand White rabbits. After sacrificing the doe with pentobarbital (50 mg/kg), the fetuses were delivered by cesarean section, placed on a warmed (38°C) water-pad, and used within 2 h after delivery. The fetal, newborn, and adult rabbits were heparinized (0.15 U/g body weight) and anesthetized with pentobarbital (0.05 mg/g body weight, intravenous or intraperitoneal). The heart was then excised from the chest cavity and used for either mechanical function study or biochemical determination.

Experimental preparation. Experiments were performed in the isolated, arterially perfused ventricular (in the fetus and newborn) or septal (in the newborn and adult) preparation as described previously (3, 7-10). In the ventricular preparation, the aorta was cannulated with PE-50 polyethylene cannula and the heart was perfused with oxygenated perfusate at a constant perfusion rate of 2.5 ml/g tissue per minute using a Harvard pump. The base of the right and left ventricle was fixed between two Harmon forceps and the apex was attached to the Statham (UC3) transducer using a silk suture. In the septal preparation, the septal branch of the left coronary artery was cannulated and the right and left ventricular free walls were trimmed away. The base of the septum was suspended to a force transducer. The muscle was stimulated at 36 bpm, and its temperature was maintained at $27 \pm 0.5^\circ\text{C}$. The following parameters of mechanical function were monitored continuously: DT, RT, tension development (+dT/dt max), maximal rate of relaxation, time to peak tension, and half-time to relaxation.

Perfusion solutions. The control Krebs-Henseleit solution contained in mM: NaCl, 118; KCl, 6; CaCl_2 , 1.5; glucose, 6; MgCl_2 ,

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1; NaHCO_3 , 24; and NaH_2PO_4 , 0.436. The control solution was equilibrated with 95% O_2 and 5% CO_2 yielding a final pH of 7.35–7.42. Respiratory acidosis was produced by equilibration with 80% O_2 –20% CO_2 and the perfusate pH was 6.81 ± 0.01 . Metabolic acidosis was produced by decreasing NaHCO_3 to 6 mM (the solution gassed with 95% O_2 –5% CO_2) and pH of the perfusate was 6.78 ± 0.02 . In the metabolic acidosis solution, NaHCO_3 was replaced with NaCl and the total Na^+ concentration was kept to 142.436 mM.

Experimental protocol. Initially, the muscles were perfused with a control solution containing 1.5 mM calcium for 60 min to allow for stabilization of mechanical function. During the initial 40 min of each experiment, the length of the muscle preparation was adjusted so that the tension was equal to 90% of maximal tension. After this initial period, both resting tension and length-tension relationship remained unchanged under control conditions. The following studies were then performed.

1) Effect of respiratory or metabolic acidosis at 1.5 mM $[\text{Ca}]_0$: the muscles were perfused with an acidotic solution (either respiratory or metabolic) for 10 or 30 min and then perfused with a control solution for 30 min.

2) Mechanical function during respiratory acidosis at various $[\text{Ca}]_0$: the muscles were perfused with either a control solution or an acidotic solution containing 1.5 mM $[\text{Ca}]_0$ for 20 min. After the mechanical function reached a new steady state, the muscles were perfused with solutions containing 0.1, 0.3, 0.75, 1.5, 3, 7.5, 15, and 30 mM Ca^{2+} (pH kept either at 7.4 or 6.8). The duration of perfusion was 5 min at each calcium concentration. Mechanical function reached a new steady state 2 min after switching to a new calcium concentration, and all measurements were made at 4 min. Parameters describing mechanical function were expressed as a percentage of control values and a g/g tissue wet weight.

Titration of muscle homogenate. After the heart was excised from the thoracic cavity, the heart was washed in a ice-cold saline. Approximately 1 g of the muscles were then suspended in 11 vol of Tris buffer (1 mM, pH 7.1) containing 150 mM KCl and 1 mM EDTA, which was equilibrated with 100% O_2 . The muscles were homogenized with a Polytron tissue processor (Brinkman, Westbury, NY) at a setting of 4.6 for 4 s. The pH of the homogenate was monitored using a pH electrode, a pH meter, and a recorder (Toa-dempa, Tokyo, Japan). The flasks containing the homogenate were gassed with 100% O_2 . The homogenate was stirred slowly and was titrated using 1 N HCl. The titration was carried out at 27° C and all procedures were finished within 5 min after homogenization. In some experiments, the heart was excised from the animal, perfused via the aorta with the homogenizing solution (approximately 10 ml/g muscle), and then homogenized. This perfusion procedure, however, did not affect the titration curve significantly, suggesting the negligible buffer capacity of the blood and extracellular fluid existed in the muscle at the time of the homogenization.

Myofibrillar isolation. The myofibrillar fraction was isolated by a modified method of Solaro *et al.* (11) as described previously (3). The muscle was homogenized in 10 vol of a solution containing 60 mM KCl, 10 mM TES (pH 7.1), and 1 mM DTT and centrifuged at $750 \times g$ for 15 min. The pellet was washed four times with the same solution. The resulting pellet was suspended in a solution containing 60 mM KCl, 10 mM TES, 1 mM DTT, and 1% Triton X-100 (pH 7.1) and centrifuged. The resulting pellet was suspended in a solution containing 60 mM KCl, 10 mM TES, 1 mM DTT, and 2 mM EGTA (pH 7.1) and then washed four times with the initial solution. The final pellet was suspended in a solution containing 50 mM KCl, 1 mM TES, and 1 mM DTT (pH 7.1).

Protein was measured by the Lowry method (12) using bovine serum albumin as a standard.

Assay of Ca^{2+} -ATPase activity. Myofibrillar ATPase activity was measured at pH 7.1, 6.5, and 6.0. The ATPase activity at pH 7.1 was considered to be a control value since several studies

suggest that intracellular pH under physiological conditions is about 7.1 (13).

The ATPase activity was measured as described previously at 27° C and at low ionic strength (0.0758 M) using a modified method of Solaro *et al.* (11). A desired Ca concentration in the medium was obtained using an EGTA-buffer system (3). The absolute binding constants used in the present study are similar to those cited by Fabiato and Fabiato (13) and these values were adjusted for the ionic strength. The apparent binding constants at various pH were calculated using these absolute binding constants and these values are shown in Table 1. Using these apparent binding constants, the composition of the reaction mixture was obtained from the calculation and programs described by Fabiato and Fabiato (13). The reaction medium contained 30 mM TES, 1 mM $\text{K}_2\text{-EGTA}$, 5 mM azide, and various amounts of CaCl_2 , $\text{Na}_2\text{-ATP}$, MgCl_2 , and KCl. The pH was adjusted with 1 M KOH. Concentrations of free Mg (3.16 mM), Mg-ATP (3.16 mM), total Na (6.5 mM), and total K (54 mM) in the media remained virtually constant. The final volume of the media was 1 ml. The reaction was started by the addition of enzymes (150 μg of myofibrillar protein) and stopped by the addition of 0.5 ml of ice-cold 20% trichloroacetic acid. Inorganic phosphate concentration in the medium was then measured using the method of Fiske and SubbaRow (27). Ca-activated myofibrillar ATPase was calculated as myofibrillar ATPase at various pCa–basal ATPase (no Ca added).

Statistical analysis. Results were expressed as the mean $\pm$ SE. Statistical analysis was performed using the two-tailed *t* test (14). Paired *t* test was used to determine the significance of the inotropic effect of acidosis. The probability was considered to be significant if the *p* value was less than 0.05.

RESULTS

Mechanical function. Mechanical function parameters in the fetus, newborn, and adult under control conditions were similar to the previously reported values (3) except that in the present study DT in the adult (Fig. 3) was slightly less than the previous data.

Respiratory acidosis. Respiratory acidosis caused a rapid decline in DT and $+dT/dt(\text{max})$, followed by a partial recovery of these variables (Fig. 1). Mechanical function reached a steady state after 10 min into acidosis and in a preliminary study it remained stable for at least 60 min into acidosis. Since the responses of DT and $+dT/dt(\text{max})$ to acidosis were similar, only the DT data will be presented. The effect of acidosis in the newborn ventricular preparation was not significantly different from that in the newborn septal preparation. The newborn data shown below are those obtained in the ventricular preparation. In the newborn, DT decreased to $65 \pm 5\%$ of control at 3 min and recovered to $92 \pm 4\%$ at 10 min into acidosis ($n = 6$) (Fig. 2). Statistical analysis using paired *t* test showed that DT at 10 min was significantly less than control in both the newborn and adult. The negative inotropism of acidosis in the adult was greater than in the newborn (Fig. 2). Upon returning to a control solution, an overshoot of DT and $+dT/dt(\text{max})$ was observed and there was no age-related difference in the extent of this overshoot. In the fetus, acidosis depressed DT to $68 \pm 2\%$ at 3 min and to $89 \pm 2\%$ at 10 min ($n = 6$) and these values were

Table 1. Apparent stability constants at various pH

Ligand	Metal	Stability constants		
		pH 7.1	pH 6.5	pH 6.0
EGTA	Ca	4.166×10^6	2.670×10^5	2.698×10^4
EGTA	Mg	5.582×10	1.137×10	3.400
ATP	Ca	4.934×10^3	2.056×10^3	7.942×10^2
ATP	Mg	2.133×10^4	9.048×10^3	3.666×10^3
ATP	K	4.377	2.086	1.079
ATP	Na	6.475	5.002	4.332

Fig. 1. Typical experiments that show the effect of respiratory acidosis (pH 6.8) on tension and first derivatives of tension development (dT/dt). In the newborn and adult, respiratory acidosis caused a rapid decline of mechanical function, followed by a partial recovery.

Fig. 2. Effect of respiratory acidosis on DT. In the adult, the negative inotropic effect of acidosis was significantly greater than in the newborn. * $p < 0.05$, ** $p < 0.01$ (significant difference from the adult value).

not significantly different from those in the newborn and therefore only the newborn heart was utilized as a premature myocardium in the following experiments.

Figure 3 shows DT at various $[Ca]_0$ in the control and acidotic muscles. In the newborn, the responses of DT to various $[Ca]_0$ were similar in the control and acidotic muscles. A double reciprocal plot of DT as a function of $[Ca]_0$ showed that the K_m value at pH 7.4 (2.6 ± 0.2 mM Ca) was not significantly different from the value at pH 6.8 (3.2 ± 0.4 mM). In the adult, acidosis depressed DT at all $[Ca]_0$ studied. In the adult, the K_m value at pH 7.4 (1.3 ± 0.1 mM) was significantly ($p < 0.05$) different from that at pH 6.8 (4.1 ± 0.8 mM). Thus, acidosis shifted the dose (Ca)-response (DT) curve to the right and downward in the adult, suggesting a noncompetitive inhibition between hydrogen

and calcium ions. Figure 3 also shows that DT at high $[Ca]_0$ (and at pH 7.4) in the adult was similar to that in the newborn. This was due to slightly depressed DT in the adult compared to the previously reported value (3). In the present study, however, $+dT/dt(max)$ at high $[Ca]_0$ was similar to the previous value (3) and the maximal $+dT/dt(max)$ in the adult was significantly greater than that in the newborn.

In both control and acidotic adult muscles, increases in RT were observed at high $[Ca]_0$ (Fig. 4). In the newborn, RT did not change from control at high $[Ca]_0$ as described previously (3).

Metabolic acidosis. Metabolic acidosis initially caused minimal increases and then gradual decreases in DT and $+dT/dt(max)$ (Fig. 5). In the newborn, DT at 30 min into acidosis was $96 \pm 1\%$ of control ($n = 6$). This value was not significantly

Fig. 3. Effect of $[Ca]_0$ on the DT in the control and acidotic (respiratory) muscles in the adult (A) and newborn (B). * $p < 0.05$, ** $p < 0.01$ (significant difference from the value at pH 7.4).

different from control and was significantly greater than that in the adult ($80 \pm 3\%$ of control, $n = 6$). On returning to the control solution, DT and $+dT/dt(max)$ decreased further and then returned to the control level in both age groups. If the mechanical function at new steady states during acidosis (the values at 30 min in metabolic acidosis and at 10 min in respiratory acidosis) are compared, the depressant effect of metabolic acidosis was significantly less than that of respiratory acidosis in the adult (but not in the newborn).

Titration of the muscle homogenate. Although the initial pH of the homogenate was not significantly different, addition of HCl to the homogenate caused significant difference in pH between the newborn and adult (Fig. 6). Figure 6 also shows that the buffer capacity of the homogenizing solution per se is very weak.

Myofibrillar ATPase. At pH 7.1, myofibrillar ATPase activity in the adult was greater than that in the newborn as reported previously (3). Reducing the media pH depressed myofibrillar ATPase activity similarly in the newborn and adult (Fig. 7). At pCa 5, myofibrillar ATPase at pH 6.8 was $74 \pm 6\%$ of control (pH 7.1) in the newborn and $73 \pm 6\%$ in the adult. At pH 6.0 (and at pCa 5), myofibrillar ATPase activity was $56 \pm 3\%$ of

control in the newborn and $55 \pm 5\%$ of control in the adult. It must be noted that at pCa range greater than 5 the relative depression of the Ca-activated ATPase by acidosis is greater than at pCa 5 in both age groups.

DISCUSSION

The present study was designed to determine the effect of acidosis on contractile function in the isolated heart of the premature rabbit. The negative inotropism of acidosis in the fetus and newborn was less than in the adult (Fig. 2). In the present study, the control value of DT in the adult was slightly less than the previously reported data (3) and as a result DT in the adult at high $[Ca]_0$ was similar to that in the newborn (Fig. 3). However, $+dT/dt(max)$ was identical to the previous data (3) and the maximal $+dT/dt(max)$ at high $[Ca]_0$ in the adult was significantly greater than in the newborn. The reasons for this discrepancy between DT and $+dT/dt(max)$ are not clear. Since myocardial ATP contents under control conditions and during acidosis were not depressed (data not shown), it is unlikely that oxygenation of the adult muscle was inadequate. It must be noted that the effect of acidosis on DT and $+dT/dt(max)$ were

Fig. 4. Effect of $[Ca]_o$ on RT in the control and respiratory acidotic muscles. In the adult, RT increased at 30 mM $[Ca]_o$ significantly ($p < 0.05$) both at pH 7.4 and 6.8.

Fig. 5. Effect of metabolic acidosis (pH 6.8) on DT. Metabolic acidosis caused a transient increase in DT, followed by a gradual decrease. On returning to the control solution, DT decreased further and then recovered to the control level. * $p < 0.05$, ** $p < 0.01$ (significant difference from the adult value).

similar and for these reasons it is likely that the present data (Fig. 2) indeed show the greater resistance of the premature myocardium to acidosis.

In both the newborn and adult, respiratory acidosis caused a more rapid and greater decline than metabolic acidosis. Similar results regarding the difference between respiratory and metabolic acidosis have been described previously (6, 15–17). Ellis and Thomas (18) showed that during acidosis intracellular pH changed in a similar manner to mechanical function. This suggests that intracellular, rather than extracellular, pH is a controlling factor of the inotropism. Ellis and Thomas (18) also showed a transient increase in intracellular pH during metabolic acidosis and this may explain the transient increase in mechanical function observed in the present study (Fig. 4). The fact that the

negative inotropism of metabolic acidosis in the newborn was minimal may be in agreement with the results of Downing *et al.* (1) obtained in the lamb. However, the duration of acidosis was relatively short in the present study and it remains possible that a longer period of metabolic acidosis may depress contractility significantly.

Myocardial contractile force is largely dependent on the amount of myofibrils, myofibrillar ATPase, and the amount of calcium reaching myofibrils. Although the precise mechanisms for the negative inotropism of acidosis are not clear, decreases in myofibrillar ATPase activity and the amount of contractile calcium have been postulated (4, 5, 6, 19, 20). The age-related difference in the negative inotropism of acidosis might be due to the differences in 1) intracellular pH during acidosis or 2) the

Fig. 6. Myofibrillar ATPase at various pH in the adult (A) and newborn (B). The relative depression of the ATPase at low pH was similar in the two age groups.

Fig. 7. Titration curve of the muscle homogenate. Although the initial pH was not different, addition of HCl caused significant difference (* $p < 0.05$, ** $p < 0.01$) in the pH of the homogenate between the two age groups.

effect of acidosis per se on myofibrillar ATPase and/or the amount of contractile calcium. Kentish and Nayler (4) showed in the adult rabbit that acidosis reduced myofibrillar ATPase activity and increased Ca concentration required to reach half maximal ATPase activity. The present finding in the adult was similar to the result of Kentish and Nayler (4). Furthermore, the relative depression of myofibrillar ATPase was similar in the newborn and adult. Therefore, it is difficult to explain the age-related difference in the effect of acidosis by the myofibrillar ATPase.

One may argue that H^+ may decrease Ca binding to troponin (5) and this effect in the newborn may be different from that in the adult. However, since myofibrillar ATPase represents Ca-activated myosin ATPase in the presence of actin, troponin, and tropomyosin, age-related difference in the competition of hydrogen and calcium ions at the troponin-binding site is unlikely.

Williamson *et al.* (6) showed that the decreased contractile function during acidosis was restored by raising $[Ca]_0$ and postulated a competitive interaction between H^+ and Ca^{2+} on the regulatory sites of myocardial contractility. In contrast, Mattiazzi *et al.* (19) showed that the negative inotropic effect of acidosis could not be completely offset by increasing $[Ca]_0$. The present finding in the adult (Fig. 3) is in agreement with the Mattiazzi's

data. Furthermore, increases in resting tension were observed at high $[Ca]_0$ in both control and acidotic muscles. Jarmakani *et al.* (21) showed that myocardial contracture was caused by increased cytosolic Ca and/or decreased myocardial high energy phosphates. Because no decrease in myocardial ATP content was observed at high $[Ca]_0$ (data not shown), it is likely that myofibrils were saturated with Ca^{2+} .

The hypothesis that contractile force decreases during acidosis in the presence of enough calcium is also supported by the study of Allen and Orchard (22). These authors showed that on induction of acidosis aequorin light emission (an intracellular Ca indicator) did not change initially and it increased gradually with a similar time course to that of the recovery of mechanical function. Furthermore, Fabiato and Fabiato (23) and Donaldson and Hermansen (24) have shown in skinned cardiac fibers that the maximal tension decreased during acidosis at saturating Ca concentrations. Therefore, the age-related difference in the effect of acidosis might not be explained by the difference in the amount of contractile calcium. Of course, it is possible that acidosis depresses functions of intracellular organelles such as sarcoplasmic reticulum (25), mitochondria, and sarcolemma (26) and these effects may alter cellular Ca metabolism. Fabiato and Fabiato (23) suggested that acidosis might depress Ca loading and release from sarcoplasmic reticulum. Since contraction of the premature myocardium may be less dependent on Ca-release from sarcoplasmic reticulum (3), developmental changes in the function of this organelle may explain the difference in the effect of acidosis. Although the relationships between dysfunction of the intracellular organelles and negative inotropism are not clear, the effect of acidosis on the intracellular organelles in the premature myocardium remains to be studied.

Since the age-related difference in the negative inotropism of acidosis might be due to the difference in the intracellular pH, intracellular buffer capacity was estimated by titrating muscle homogenate. The present results show that buffer capacity of the homogenate in the newborn was greater than in the adult (Fig. 6). This method, however, does not estimate intracellular buffering capacity under physiological conditions and therefore has some limitations. First, oxygenation of the muscle homogenate might not be adequate during the titration procedure. In a preliminary study, however, pH of the homogenate remained stable for at least 10 min both in the newborn and adult, suggesting that oxygenation was enough at least in this period. Second, intracellular organelles might not remain intact after homogenization procedure. Although this possibility remains,

the homogenization procedure in the present study was similar to that used previously to isolate sarcoplasmic reticulum and mitochondria (3). These data suggest that intracellular buffer capacity in the newborn muscle is greater than in the adult and this, at least in part, may explain the age-related difference in the inotropic effect of acidosis. The reasons for the difference in buffer capacity are unknown. In addition to the electrolytes and proteins, intracellular organelles may play important roles as buffers (6, 16, 18). In order to determine the age-related difference in the intracellular buffer capacity under more physiological conditions, direct measurement of intracellular pH should be performed.

In summary, the present study showed that the negative inotropic effect of acidosis in the developing heart is less than in the adult and this may be due in part to the greater intracellular buffer capacity.

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