Interaction of Metabolic and Respiratory Acidosis with α and β-adrenoceptor Stimulation in Rat Myocardium

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ABSTRACT

Background: The effects of acute respiratory *versus* metabolic acidosis on the myocardium and their consequences on adrenoceptor stimulation remain poorly described. We compared the effects of metabolic and respiratory acidosis on inotropy and lusitropy in rat myocardium and their effects on the responses to α- and β-adrenoceptor stimulations.

Methods: The effects of acute respiratory and metabolic acidosis (pH 7.10) and their interactions with α *and* β-adrenoceptor stimulations were studied in isolated rat left ventricular papillary muscle (n = 8 per group). Intracellular pH was measured using confocal microscopy and a pH-sensitive fluorophore in isolated rat cardiomyocytes. Data are mean percentages of baseline ± SD.

Results: Respiratory acidosis induced more pronounced negative inotropic effects than metabolic acidosis did both in isotonic (45 ± 3 *versus* 63 ± 6%, $P < 0.001$) and isometric (44 ± 5 *versus* 64 ± 3%, $P < 0.001$) conditions concomitant with a greater decrease in intracellular pH (6.85 ± 0.07 *versus* 7.12 ± 0.07, $P < 0.001$). The response to α-adrenergic stimulation was not modified by respiratory or metabolic acidosis. The inotropic response to β-adrenergic stimulation was impaired only in metabolic acidosis (137 ± 12 *versus* 200 ± 33%, $P < 0.001$), but this effect was not observed with administration of forskolin or dibutiryl-cyclic adenosine monophosphate. This effect might be explained by a change in transmembrane pH gradient only observed with metabolic acidosis. The lusitropic response to β-adrenergic stimulation was not modified by respiratory or metabolic acidosis.

Conclusion: Acute metabolic and respiratory acidosis induce different myocardial effects related to different decreases in intracellular pH. Only metabolic acidosis impairs the positive inotropic effect of β-adrenergic stimulation.

What We Already Know about This Topic

- Adrenergic agents are commonly administered to improve cardiac function in settings of respiratory or metabolic acidosis, yet whether these conditions alter therapeutic efficacy is not well described

What This Article Tells Us That Is New

- Using isolated rat left ventricular papillary muscle, acute respiratory and metabolic acidosis did not alter the response to α-adrenoceptor agonists
- Acute metabolic, but not respiratory acidosis, reduced the inotropic response to β-adrenoceptor agonists

ACUTE acidosis (or acidemia) may result from an excess of hydrogen ions, usually related to inappropriate delivery of oxygen to tissues and shock (metabolic acidosis), or to an excess in carbon dioxide (respiratory acidosis), related to a limitation of alveolar ventilation. Respiratory acidosis can be a consequence of ventilatory distress or a therapeutic objective when permissive hypercapnia is performed in the critically ill patient under mechanical ventilation.[1,2] Both metabolic and respiratory acidosis have been reported to decrease myocardial function,[3–5] although the magnitude of their respective myocardial effects remains poorly described.

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Since 1880, acidosis has been known to induce a negative inotropic effect in the heart[6] through alteration of electrical activity, pumps and channels that regulate intracellular calcium and other ion homeostasis, and modifications of myofilament calcium sensitivity.[3,4,7–11] However, most studies of the effects of acidosis in the heart actually studied myocardial ischemia in which a marked decrease in adenosine 5′-triphosphate is the main event precluding any accurate analysis of the direct effect of acidosis.[12] Moreover, there are some controversies concerning intracellular pH changes induced by extracellular metabolic acidosis.[9–11] In the context of acidosis, the sympathetic system is an important adaptive mechanism for maintaining cardiac output. The inotropic response to β-adrenergic stimulation is decreased in acute metabolic acidosis[13] but has not been previously studied in respiratory acidosis. Although myocardial relaxation is an important and active process that may interfere with contraction, the lusitropic consequences of acidosis remains unknown. The consequences of acidosis on α-adrenergic stimulation also remain unknown.

Therefore, the aim of this study was to compare the direct effects of acute metabolic and respiratory acidosis on inotropy and lusitropy in rat myocardium and their effects on the responses to α- and β-adrenoceptor stimulation. We used isolated left ventricular papillary muscle to assess the inotropic and lusitropic effects and isolated cardiomyocytes to measure intracellular pH.

Materials and Methods

All animals were cared for according to the *Guiding Principles in the Care and Use of Animals* and under the supervision of authorized researchers in an authorized laboratory (agreement number B 75-13-08) in accordance with the regulations of the official edict of the French Ministry of Agriculture. Food and water were given *ad libitum.* Ten-week-old male Wistar rats (Iffa Credo, St Germain sur l'Arbresle, France) were divided into a control group (pH = 7.40), a respiratory acidosis group, and a metabolic acidosis group (both pH 7.10).

Isolated Left Ventricle Papillary Muscle

Shortly after the induction of general anesthesia with pentobarbital, the heart was removed *in bloc*, dissected, and weighed. The left ventricular papillary muscles were carefully excised and suspended vertically in a Krebs-Henseleit bicarbonate buffer solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.1 mM KH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM CaCl,[2] 4.5 mM glucose) maintained at 29°C with a thermostatic water circulator and bubbled with 95% oxygen and 5% carbon dioxide as previously described.[14] The papillary muscles were stimulated at 12 Hz for a 60-min stabilization period at the initial muscle length (L_{max}) at the apex of length–active isometric tension curve. The electromagnetic lever system has been described previously.[14] All analyses were made from digital records of force and length obtained with a computer. Conventional mechanical variables at L_{max} were calculated from three twitches. The first twitch was isotonic and was loaded with the preload corresponding to L_{max}. The second twitch was abruptly clamped to zero load just after the electrical stimulus with a critical damping. The third twitch was fully isometric at L_{max}. We determined the maximum unloaded shortening velocity (V_{max}) using the zero-load technique, and determined maximum shortening ($_{max}Vc$) and lengthening ($_{max}Vr$) velocities and time to peak shortening of the twitch with preload only. In addition, the maximum isometric active force normalized for cross-sectional area (AF), the peaks of the positive (+dF/dt) and the negative (–dF/dt) force derivatives at L_{max} normalized for cross-sectional area, and the time to peak force from the isometric twitch were recorded. Because changes in the contraction phase induce coordinated changes in the relaxation phase, indexes of contraction–relaxation coupling have been developed to study lusitropy.[15] The R1 coefficient ($R1 = {}_{max}Vc/{}_{max}Vr$) studies the coupling between contraction and relaxation under low load and thus lusitropy, in a manner that is independent of inotropic changes. R1 tests sarcoplasmic reticulum (SR) calcium uptake function.[15] The R2 coefficient ($+dF.dt^{-1}/-dF.dt^{-1}$) studies the coupling between contraction and relaxation under high load and thus lusitropy, in a manner that is less dependent on inotropic changes.[14] The cross-sectional area was calculated from the length and weight of papillary muscle, assuming a density of 1.

The effects of metabolic and respiratory acidosis were compared with those of a control group maintained at a pH of 7.40. We studied the effects of acidosis at two calcium concentrations, 0.5 and 2.5 mM. Moreover, we also studied the effects of respiratory and metabolic acidosis on the AF-calcium relationship by varying the extracellular concentration from 2.5 mM to 0.125 mM in separate papillary muscle groups. Last, in two separate groups of muscles (n = 5), we studied the kinetics of recovery from exposure to metabolic or respiratory acidosis (pH 7.10 during 30 min), over a 30-min period after return to control conditions (pH 7.40) at an extracellular concentration of 2.5 mM.

Acute Respiratory versus Metabolic Acidosis

In the acidosis group, we studied a pH of 7.10 because previous studies showed that myocardial consequences occurred below a pH of 7.20 and because this value seemed clinically relevant. A separate 200-ml jacketed reservoir with the same Krebs-Henseleit bicarbonate buffer solution was prepared. In the respiratory acidosis group, the bathing solution was bubbled with two gases (95% oxygen–5% carbon dioxide and 50% oxygen–50% carbon dioxide). The proportions of these two gases were adjusted until a stable pH of 7.10 was obtained, the solution being maintained at 29°C. In the metabolic acidosis group, we added hydrochloric acid while using a continuous infusion to obtain a pH of 7.10. Final concentrations of sodium and calcium were adjusted. Electrolyte concentrations were measured with standard electrodes (Ektachem 500, Johnson & Johnson, Les Ulis, France). Oxygen and carbon dioxide tensions and pH were

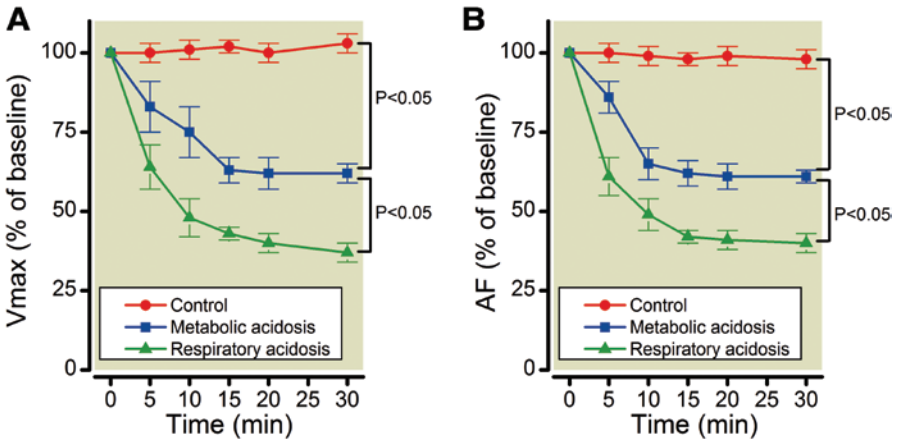

Fig. 1. Comparison of the time course of the inotropic effects of metabolic acidosis (pH = 7.10, n = 8) and respiratory acidosis (pH = 7.10, n = 8) compared with a control group (pH = 7.40, n = 8) in isotonic (*A*) and isometric (*B*) conditions at a calcium concentration of 2.5 mM. Data are mean percent of baseline ± SD. V_{max} = maximum unloaded shortening velocity; AF = isometric active force for cross-sectional area.

measured with standard electrodes (IL-BG3, Instrumentation Laboratory, Saint Mandé, France). Perfusion of the new solution (metabolic or respiratory acidosis) was started quickly and the effects were recorded after a 15-min stabilization period. The optimal time for equilibration of the effects of acidosis was determined by analysis of time-curves responses. A preliminary study showed that a 20-min equilibration period achieved a plateau both in respiratory and metabolic acidosis conditions (fig. 1). pH was continuously monitored (Eutech Instruments, Nijkerk, The Netherlands).

α and β-adrenoceptor Stimulations

To assess the inotropic response to α and β-adrenoceptor stimulations, the extracellular calcium concentration was decreased from 2.5 to 0.5 mM, as previously described.[15] It has been established that the contractility is nearly maximum at a calcium concentration of 2.5 mM in the rat myocardium, precluding a reliable assessment of positive inotropic agents.

α-adrenoceptor stimulation was induced with increasing concentrations of phenylephrine (10^{-8}–10^{-4} M), in the presence of propranolol (10^{-6} M).[15] The effect of phenylephrine was expressed by the percentage of baseline value of the maximal effect of phenylephrine on AF and V_{max} (Eff_{max}) and the concentration of phenylephrine producing 50% of the maximal effect (C_{50}).[15]

β-adrenoceptor stimulation was induced with increasing concentrations of isoproterenol (10^{-8}–10^{-4} M), a nonselective β-adrenoceptor agonist, in the presence of phentolamine (10^{-6} M).[15] The effect of isoproterenol was expressed by the percentage of baseline value of the maximal effect of isoproterenol on AF and V_{max} (Eff_{max}) and the concentration of isoproterenol producing 50% of the maximal effect (C_{50}).[15]

We also studied the effects of stimulation of adenylate cyclase using forskoline (5×10^{-5} M), which directly activates adenylyl cyclase, and the direct effect of 3′-5′-cyclic adenosine monophosphate (cAMP) using dibutyryl- cAMP (5×10^{-5} M), a fat-soluble and diffusible analog of cyclic adenosine monophosphate resistant to hydrolysis in the intracellular involvement, as previously described.[16] Last, to study the recovery of the inotropic response to β–adrenergic stimulation, we exposed a separate group of muscles to metabolic acidosis (pH = 7.10 during 30 min) and then again to control conditions (pH = 7.40). All drugs were purchased from Sigma-Aldrich Chimie (l'Isle d'Abeau-Chesnes, France) and added volumes never exceeded 2% of the total.

Myocardial Cell Isolation Procedure

Ventricular cardiomyocytes were isolated from rat hearts using enzymatic digestion by collagenase A (Roche Diagnostics, Meylan, France) on a Langendorf apparatus, as previously described.[17] After anesthesia was induced by intraperitoneal injection of pentobarbital sodium, the heart was quickly dissected and connected to the Langendorf apparatus by aortic cannulation. A retrograde perfusion was applied with a calcium-free buffer HEPES solution (117 mM NaCl, 5.7 mM KCl, 1,5 mM KH_2PO_4, 4.4 mM $NaHCO_3$, 1.7 mM $MgCl_2$, 11.7 mM glucose, 10 mM creatine, 21 mM HEPES and 20 mM taurine, all from Sigma-Aldrich) bubbled with oxygen at 37°C and pH 7.40.[18] The same buffer with collagenase A (1.2–1.4 mg/ml), 100 μM EGTA and 240 μM $CaCl_2$ (both from Sigma-Aldrich) was perfused for 60–80 min. After enzymatic digestion the heart was removed and the ventricles were suspended in the calcium-free buffer. A light mechanical dissection completed the enzymatic digestion and the cell suspension was filtered at 300 μm. Extracellular calcium was progressively added to the HEPES solution to reach 0.5 mM, cells were fixed on laminin-coated strips, and incubated overnight in the same buffer with penicillin 50 UI/ml and streptomycin 5 μg/ml.

Measurement of Intracellular pH

Fixed cells were exposed to a pH-sensitive fluorophore carboxy-seminaphtorhodafluor-1 acetoxylmethyl ester at a

final concentration of 10 μM for 10 min. The seminaphtorhodafluor-1 acetoxylmethyl estersolution was flushed away and normal superfusion was performed (control *vs.* hypercapnic acidosis *vs.* metabolic acidosis) using a solution containing: 135 mM NaCl, 5.4 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 10 mM HEPES, 5 mM glucose and maintained at 29°C with a thermostatic water circulator. The solutions were continuously bubbled with oxygen. The pH was adjusted to 7.40 with NaOH in the control group. In the hypercapnic acidosis group, carbon dioxide was infused to obtain a pH of 7.10. In the metabolic acidosis group, NaOH was added to obtain a pH of 7.10. Intracellular seminaphtorhodafluor-1 acetoxylmethyl ester was excited by an Argon laser at 514 nm and fluorescence was imaged confocally at 580 and 640 nm using a Leica ×40, 1.25 numerical aperture, oil immersion, plano-apochromatic objective lens, as previously described.[3] Seminaphtorhodafluor-1 acetoxylmethyl ester calibration was performed by abolishing all transsarcolemmal pH gradients using the H+K+ antipor nigericin, as previously described.[19] In brief, the calibration solutions covered a pH range of 6.50–9.50 (6.50; 7.50; 8.50; and 9.50). Cells were exposed to a calibration solution containing 135 mM KCl, 15 mM NaCl, 1 mM KH_2PO_4, 0.5 mM $MgSO_4$, 1 mM EGTA, 10 mM HEPES (for pH = 7.5 and 8.5), 10 mM CAPSO (3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid) (for pH = 9.5) or 10 mM PIPES (for pH = 6.5) and 20 μM nigericin for approximately 5 min. pH calibration data were averaged for 50 cells from three animals. The emission ratio of 590/640 nm obtained in control, metabolic acidosis, and respiratory acidosis cells, was converted to a pH value using the following equation:

$$pH = pKa - \log((R-R_{min})/(R_{max}-R)) - \log F_{640max/min}$$

where R is the fluorescence emission ratio 590 nm/640 nm; R_{max} is the emission ratio at low pH (6.5); R_{min} is the emission ratio at high pH (9.5); $\log F_{640max/min}$ is the ratio of fluorescence measured at 640 nm for high pH to that for low pH.

Statistical Analysis

Data are expressed as mean ± SD. Comparison of two means was performed using the Student *t* test, and comparison of several means was performed using repeated measure two way ANOVA and the and Newman–Keuls test. Concentration response curves were determined by fitting the data to the Hill sigmoid pharmacological model according to the following equation: $Eff_o = Eff_{max}/(1 + (C_{50}/C)^n)$ in which Eff_o is the observed effect, Eff_{max} the maximum effect, C_{50} the concentration that results in 50% of Eff_{max}, *C* the studied concentration, and n the Hill coefficient.[15] Iterative nonlinear regression curve fitting was used to obtain the best fit (Matlab 1.2c software; The MathWorks, South Natick, MA). One-way ANOVA and Newman–Keuls test were used to compare Eff_{max} and C_{50} between groups. All *P* values were two-tailed and a *P* value of less than 0.05 was considered significant. Statistical analysis was performed using NCSS 2007 software (Statistical Solutions Ltd., Cork, Ireland).

Table 1. Comparison of Gases and Electrolytes in the Krebs-Henseleit Solution in the Three Experimental Groups

	Control (n = 8)	Metabolic Acidosis (n = 8)	Hypercapnic Acidosis (n = 8)
pH	7.41 ± 0.03	7.10 ± 0.01*	7.10 ± 0.02*
P_{CO_2}, mmHg	36 ± 3	34 ± 1	71 ± 3*†
P_{O_2}, mmHg	647 ± 33	645 ± 40	629 ± 24
Bicarbonates, mM	22.7 ± 0.3	11.2 ± 0.2*	22.0 ± 0.2†
Sodium, mM	144 ± 1	144 ± 1	144 ± 1
Potassium, mM	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.1*†
Calcium, mM	2.44 ± 0.02	2.39 ± 0.03*	2.42 ± 0.01†
Chloride, mM	121 ± 1	124 ± 1*	123 ± 1*†

Values are mean ± SD.

*$P < 0.05$ *vs.* control group. †$P < 0.05$ *vs.* metabolic acidosis.

P_{O_2} = partial pressure of oxygen; P_{CO_2} = partial pressure of carbon dioxide.

Results

We studied 115 rats. There was no significant difference among groups for weight (330 ± 25 g), heart weight (85 ± 8 mg), or ratio of heart weight to body weight (0.3 ± 0.1 mg/g).

Effects of Acute Respiratory and Metabolic Acidosis

A mean pH of 7.10 was obtained in the two acidosis groups, associated with a high value of carbon dioxide in the respiratory acidosis group and a low bicarbonate value in the metabolic acidosis group (table 1).

We studied 111 left ventricular papillary muscles, L_{max} 4.2 ± 1.0 mm, cross-sectional area 0.45 ± 0.14 mm^2, ratio of resting force to total force 0.17 ± 0.05. There were no significant differences among groups in baseline values of V_{max} (4.28 ± 1.08 L_{max}/s), AF (93 ± 20 mN/mm^2), R1 (0.70 ± 0.10), R2 (2.30 ± 0.80), time to peak shortening (177 ± 20 ms), and time to peak force (156 ± 23 ms). Both respiratory and metabolic acidosis induced a negative inotropic effect, as shown by a decrease in V_{max} and AF (table 2). The negative inotropic effect was significantly more pronounced with respiratory acidosis (fig. 1; table 2). Respiratory acidosis induced a negative lusitropic effect under low load (increase in R1), which was not observed with metabolic acidosis (table 2). Respiratory acidosis also induced a positive lusitropic effect under high load (decrease in R2), which was not observed with metabolic acidosis (table 2).

After exposure to metabolic or respiratory acidosis over a 30-min period (Ca 2.5 mM), a complete recovery was obtained within 5 min (metabolic acidosis n = 5, AF: 103 ± 6% of baseline); (respiratory acidosis n = 5, AF: 118 ± 8% of baseline) after return to a pH of 7.40.

The calcium–AF relationship was obtained in isolated papillary muscles as extracellular calcium concentration was modified over the 2.5–0.125 mM range. The curve was significantly shifted to the right and the shift was more pronounced with respiratory than metabolic acidosis (fig. 2).

Table 2. Effects of Respiratory (pH = 7.10) and Metabolic (pH = 7.10) Acidosis, Compared with Control Conditions (pH = 7.40) on Main Mechanical Variables of Isolated Papillary Muscles at Two Different Calcium Concentrations

	Calcium 2.5 mM					
	Control (n = 8)		Metabolic Acidosis (n = 8)		Respiratory Acidosis (n = 8)	
	Baseline	Effect, %	Baseline	Effect, %	Baseline	Effect, %
V_{max}, L_{max}/s	4.57 ± 0.99	95 ± 12	4.79 ± 0.92	73 ± 4*†	4.72 ± 0.89	56 ± 8*†‡
AF, mN/mm^2	93 ± 14	99 ± 2	95 ± 15	76 ± 8*†	94 ± 14	56 ± 8*†‡
R1	0.76 ± 0.08	101 ± 2	0.73 ± 0.14	103 ± 14	0.62 ± 0.13	126 ± 22*†‡
R2	2.51 ± 0.90	96 ± 4	1.76 ± 0.53	115 ± 10*†	1.48 ± 0.45	104 ± 8‡
TPS, ms	185 ± 10	100 ± 2	186 ± 13	97 ± 2*	168 ± 17	99 ± 5
TPF, ms	144 ± 14	100 ± 1	156 ± 27	100 ± 8	139 ± 20	105 ± 8
V_{max}, L_{max}/s	2.17 ± 0.63	101 ± 6	2.62 ± 0.63	63 ± 6*†	2.64 ± 0.77	45 ± 3*†‡
AF, mN/mm^2	53 ± 13	100 ± 4	59 ± 9	64 ± 3*†	56 ± 10	44 ± 5*†‡
R1	0.68 ± 0.09	100 ± 7	0.64 ± 0.09	109 ± 10	0.69 ± 0.09	124 ± 20*†‡
R2	1.45 ± 0.34	98 ± 4	1.42 ± 0.18	95 ± 7	2.13 ± 0.36†‡	83 ± 20*†‡
TPS, ms	189 ± 13	99 ± 2	180 ± 14	97 ± 4	195 ± 14‡	86 ± 4*†‡
TPF, ms	182 ± 13	99 ± 2	161 ± 14†	104 ± 8	180 ± 15‡	98 ± 15

Values are mean percent of baseline ± SD.

* P < 0.05 vs. baseline; † P < 0.05 vs. Control; ‡ P < 0.05 vs. metabolic acidosis.

AF = isometric active force normalized for cross-sectional area; R1 = $_{max}Vc/_{max}Vr$; R2 = (+dF.dt^{-1}/–dF.dt^{-1}); TPF = time to peak force; TPS = time to peak shortening; V_{max} = maximum unloaded shortening velocity.

Interaction with α–adrenergic Stimulation

Phenylephrine induced a significant positive inotropic effect in isotonic and isometric conditions in the control group. Respiratory and metabolic acidosis did not significantly modify the inotropic response to α-adrenergic stimulation (table 3; fig. 3).

Interaction with β–adrenergic Stimulation

Isoproterenol induced a significant positive inotropic effect in isotonic and isometric conditions in the control group. Metabolic acidosis impaired the inotropic effect of β–adrenergic stimulation both in isotonic and isometric conditions. In contrast, respiratory acidosis did not significantly modify the positive inotropic effect of β-adrenergic stimulation (table 4; fig. 4).

Isoproterenol induced a significant lusitropic effect in isotonic (R1) and isometric (R2) cond itions in the control group. Respiratory and metabolic acidosis did not significantly modify the positive lusitropic effects of β -adrenergic stimulation (table 4).

Because metabolic acidosis impaired the positive inotropic effect of ß-adrenergic stimulation, we performed additional studies to test the direct stimulation of adenylate cyclase using forskolin and the direct effect of cAMP. When forskolin or dibutyryl-cAMP were administered, there were no significant differences between respiratory acidosis and control group in isotonic (data not shown) and isometric conditions (fig. 5).

Last, in a separate group, we verified that, after exposure to metabolic acidosis then again to control conditions (pH = 7.40), a complete recovery of the inotropic response to β–adrenergic stimulation was observed both in isotonic (V_{max}: 174 ± 33 *vs.* 182 ± 17%, *P* = 0.70) and isometric conditions (AF: 181 ± 27 *vs.* 178 ± 4%, *P* = 0.63) as compared with control groups.

Intracellular pH

In the HEPES solution, the extracellular pH obtained was 7.40 ± 0.1 in the control group, 7.10 ± 0.01 in the metabolic acidosis group, and 7.10 ± 0.01 in the respiratory acidosis group. The measurement of intracellular pH in control conditions provided a mean value of 7.22 ± 0.07. Typical images obtained in isolated myocardial cells using confocal microscopy are shown in figure 6. The decrease in intracellular pH was more pronounced with respiratory than with metabolic acidosis (fig. 7A) but the consequences on transmembrane pH gradients were more pronounced in metabolic acidosis (fig. 7B).

Discussion

Acute acidosis is frequently encountered in critically ill patients and is the cause of increased morbidity and mortality.[20–22] Metabolic and respiratory acidosis correspond to different mechanisms potentially leading to different intracellular abnormalities but these two forms of extracellular acidosis impair myocardial function.[7,21,22] Acidosis induces various and complex effects within the myocardium,[23] including increase in diastolic intracellular calcium concentration[7] without change in L-type Ca^{2+} current,[24] decrease in SR calcium uptake through the

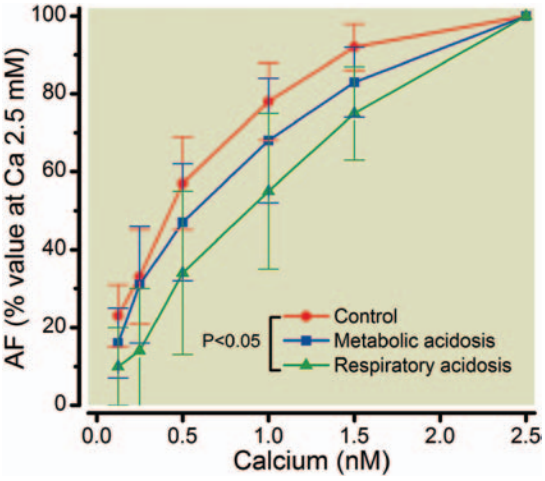

Fig. 2. Comparison of the AF-calcium concentration relationship in control metabolic acidosis (pH =7.10, n = 8), respiratory acidosis (pH = 7.10, n = 8), and in the control group (pH = 7.40, n = 8). Data are mean ± SD. Only respiratory acidosis group significantly differs from control group ($P < 0.05$), shifting the relationship to the right. The absolute values of AF at Ca 2.5 mM significantly differed between control (90 + 15 mN/mm²), metabolic (68 + 12 mN/mm²), and respiratory acidosis (51 + 13 mN/mm²). The absolute depression at the Ca concentration of 2.5 mM is shown in figure 1. AF = isometric active force for cross-sectional area.

Table 3. Comparison of Inotropic Effects of α-Adrenergic Stimulation in Isotonic and Isometric Conditions, in Control Conditions (pH = 7.40) and in Metabolic (pH = 7.10) or Respiratory (pH = 7.10) Acidosis

	Control (n = 8)	Metabolic Acidosis (n = 8)	Respiratory Acidosis (n =8)
V_{max}			
Baseline, L_{max}/s	2.56 ± 0.47	1.70 ± 0.62†‡	1.16 ± 0.37†‡
Eff_{max}, %	179 ± 8*	177 ± 12*	168 ± 9*
C_{50}, µM	2.5 ± 2.6	4.4 ± 0.4	4.8 ± 4.4
AF			
Baseline, mN/mm²	51 ± 12	34 ± 4†‡	26 ± 5†‡
Eff_{max}, %	168 ± 9*	177 ± 11*	175 ± 11*
C_{50}, µM	4.2 ± 3.4	4.2 ± 0.2	3.1 ± 1.9

Values are mean ± SD.

* $P < 0.05$ *vs.* baseline; † $P<0.05$ *vs.* control group; ‡ $P<0.05$ *vs.* metabolic acidosis.

AF = isometric active force normalized for cross-sectional area; C_{50} = concentration of phenylephrine that result in 50% of Eff_{max}; Eff_{max} (%) = maximum effect in percentage of baseline value; L_{max} = initial muscle length; V_{max} = maximum unloaded shortening velocity.

coupling of SR calcium adenosine 5′-triphosphatase and phospholamban,[25,26] a decrease in calcium-induced release compensated by an increase in SR calcium content secondary to an increase in cytoplasmic calcium,[7] and a rise in intracellular sodium caused by both activation of sodium-hydrogen exchange[23] and decrease in sodium–calcium exchange.[9] Activation of calcium/calmodulin-dependent protein kinase II during acidosis compensates for the direct inhibitory effect of acidosis on SR calcium uptake.[27] The net result is an increase in calcium transient that markedly contrasts with the decrease in twitch contraction, indicating that the decrease in myofilament calcium sensitivity is the primary cause of contractile dysfunction during acidosis.[7] One of the main effects of acidosis is the decrease in calcium sensitivity of troponin I,[7,13] a target protein of protein kinase A-dependent phosphorylation during stimulation of the β-adrenergic pathway. Troponin T is only partly responsible for the effect of pH on calcium sensitivity for force development as compared with troponin I,[28] and transgenic mice expressing a single histidine modified troponin I are less susceptible to respiratory acidosis with a preserved myocardial contractility.[22] Acidosis alters myocardial

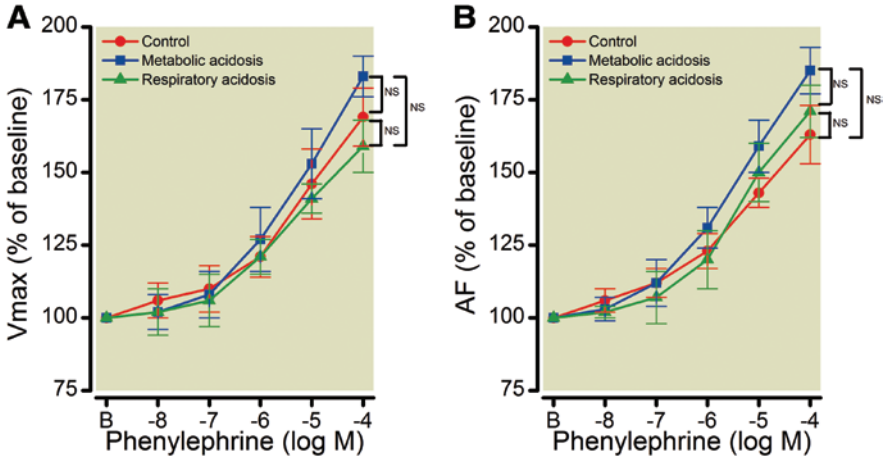

Fig. 3. Comparison of the positive inotropic effects of α-adrenergic stimulation in control conditions (pH = 7.40, n = 8) and in metabolic (pH = 7.10, n = 8) or respiratory acidosis (pH = 7.10, n = 8) in isotonic (*A*) and isometric (*B*) conditions. Data are mean ± SD. AF = isometric active force normalized for cross-sectional area; NS = not significant; V_{max} = maximum unloaded shortening velocity. *P* values refer to the between group comparison of the maximum effect (see table 3).

Table 4. Comparison of Inotropic and Lusitropic Effect of ß-adrenergic Stimulation in Isotonic and Isometric Conditions in Control Conditions (pH = 7.40) and in Metabolic (pH = 7.10) or Respiratory (pH = 7.10) Acidosis

	Control (n=8)	Metabolic Acidosis (n=8)	Respiratory Acidosis (n=8)
Vmax			
Baseline, L_{max}/s	2.11 ± 0.32	1.70 ± 0.32†	1.16 ± 0.31†‡
Eff_{max}, %	194 ± 15*	135 ± 15*†	195 ± 16*‡
C_{50}, µM	4.4 ± 0.4	2.7 ± 2.1†	1.9 ± 0.8†
AF			
Baseline, mN/mm²	54 ± 15	40 ± 6†	24 ± 4†‡
Eff_{max}, %	186 ± 8*	137 ± 12*†	200 ± 33*‡
C_{50}, µM	4.2 ± 0.2	3.8 ± 2.8	3.4 ± 2.3
R1			
Baseline	0.69 ± 0.08	0.69 ± 0.17	0.78 ± 0.18
Eff_{max}, %	64 ± 9*	65 ± 6*	59 ± 7*
C_{50}, µM	2.2 ± 2.0	2.6 ± 3.0	0.3 ± 0.5
R2			
Baseline	1.41 ± 0.39	1.31 ± 0.24	1.76 ± 0.21†‡
Eff_{max}, %	77 ± 8*	78 ± 4*	85 ± 9*
C_{50}, µM	2.8 ± 2.0	1.7 ± 1.2	1.4 ± 2.3

Values are mean ± SD.

* $P < 0.05$ vs. baseline. † $P < 0.05$ *vs*. control group; ‡ $P < 0.05$ *vs*. metabolic acidosis.

AF = isometric active force normalized for cross sectional area; C_{50} = concentration of isoproterenol that result in 50% off Eff_{max}; Eff_{max} (%) = maximum effect in percentage of baseline value; R1 = $_{max}Vc/_{max}Vr$; R2 = $(+dF.dt^{-1}/-dF.dt^{-1})$; V_{max} = maximum unloaded shortening velocity

contractility less in neonatal than in adult heart,[29] and this difference seems to be explained by differences in expression of troponin I isoforms, which exhibit different reaction to acidosis.[30] We observed that respiratory acidosis induced a greater decrease in inotropy (fig. 1), a greater decrease in intracellular pH (fig. 5), and a greater shift to the right of the force-calcium relationship (fig. 2), thus confirming that calcium myofilament sensitivity is probably the main target of the myocardial effects of acidosis.

We are assuming that extracellular hydrogen ion mediates action of metabolic acidosis extracellularly and thus that the change in extracellular bicarbonate concentration (table 1) has no direct effect. We also observed some statistical differences in extracellular potassium, chloride, and calcium concentrations (table 1) but these differences were very small and should be considered as nonphysiologically significant. With regard to calcium, it should be noted that the AF/Ca^{2+} relationship is flat and maximum at these concentrations in the rat myocardium.[14,15]

We observed that respiratory acidosis induced an increase in R1, suggesting an impairment in SR calcium uptake, and a decrease in R2, suggesting a decrease in myofilament calcium sensitivity. These lusitropic effects were not observed in metabolic acidosis (table 2). These results are consistent with the effects of acidosis on SR calcium uptake in isolated myocytes[31] and myofilament calcium sensitivity in skinned cardiac trabeculae.[8] Nevertheless, although our results on R1 are reliable because R1 is not significantly modified by changes in major negative inotropic changes, our results on R2 should be analyzed with caution because R2 is slightly decreased by negative inotropic changes[15] and acidosis induced marked negative inotropic effect. Differences in the lusitropic effects observed in metabolic *versus* respiratory acidosis are consistent with differences observed in decreasing intracellular pH (fig. 5).

In the clinical context of acidosis, the sympathetic system may represent an important adaptive mechanism for maintaining cardiac output and many critically ill patients with acidosis receive catecholamines that stimulate adrenoceptors.

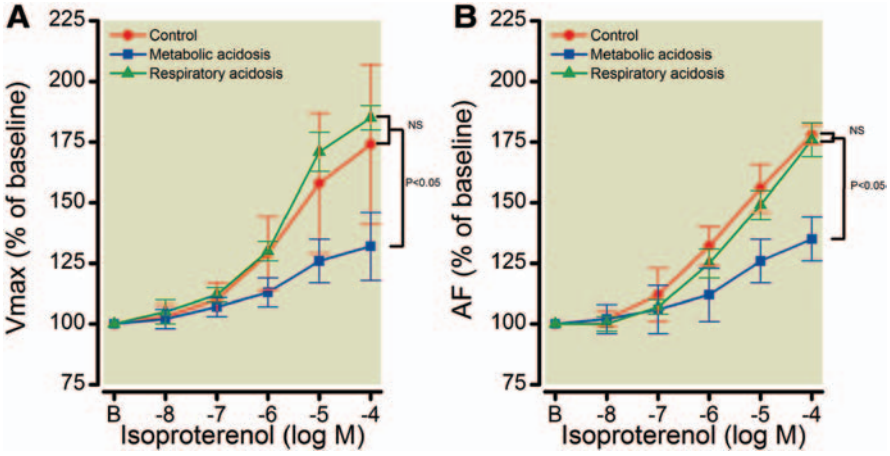

Fig. 4. Comparison of the positive inotropic effects of β-adrenergic stimulation in control conditions (pH = 7.40, n = 8) and in metabolic (pH = 7.10, n = 8) or respiratory acidosis (pH = 7.10, n = 8) in isotonic (*A*) and isometric (*B*) conditions. Data are mean percent of baseline ± SD. NS = not significant; V_{max}= maximum unloaded shortening velocity. AF = isometric active force normalized for cross-sectional area; *P* values refer to the between group comparison of the maximum effect (see table 4).

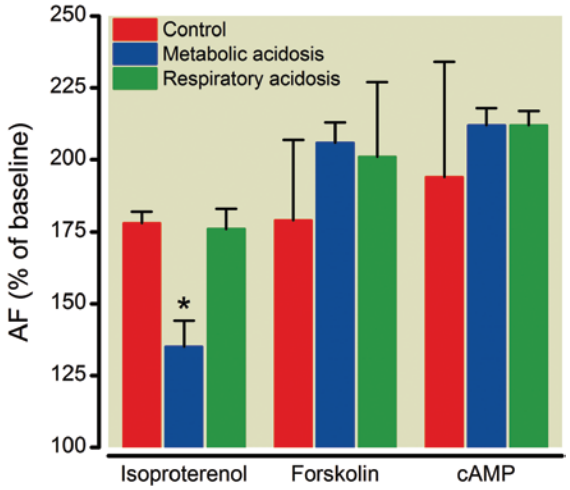

Fig. 5. Comparison of the positive inotropic effects of β-adrenoceptor stimulation with isoproterenol, direct stimulation of adenylate cyclase using forskolin, and administration of dibutiryl cAMP in control conditions (pH = 7.40) and in metabolic (pH = 7.10) or respiratory acidosis (pH = 7.10) in isometric conditions. Data are mean percent of baseline ± SD (n = 8 in each group). AF = isometric active force normalized for cross-sectional area; cAMP = 3′-5′ cyclic adenosine monophosphate; NS = not significant. *$P < 0.05$ *versus* control.

Stimulation of α1-adrenoceptors activates a protein of the Gq/11 family, which stimulates phospholipase C leading to the formation of inositol triphosphate and diacylglycerol from phosphatidyl inositol 4.5 bisphosphate. Inositol triphosphate then binds to its receptor in the SR, causing a change in protein conformation and an increase in cytosolic calcium, inducing a positive inotropic effect. In addition, stimulation of the α1-adrenoceptor increases the calcium sensitivity of troponin C, induces an intracellular alkalinization *via* activation of the Na/H exchanger, and protein phosphorylation of myosin light chain.[32] The effect of acidosis on α1-adrenoceptor stimulation has not been previously studied. We observed that this inotropic effect was not modified by respiratory or metabolic acidosis. Although, the contribution of α-adrenoceptor to inotropy is smaller in humans than in rats, it may participate in the compensation of the negative inotropic effect induced by acidosis. Our study demonstrates that no significant interaction occurs between acidosis and α-adrenoceptor stimulation within the myocardium.

The inotropic response to ß-adrenoceptor stimulation is known to be altered in metabolic acidosis in conjunction with an abnormal production of cAMP by adenylyl cyclase in isolated tissues,[13,33] and this has been confirmed in isolated human failing myocardium.[34] Our study confirms these results (fig. 4). In contrast, we observed that the pharmacological response to the direct stimulation of adenylyl cyclase by forskolin or administration of dibutyryl-cAMP was not significantly modified by metabolic acidosis (fig. 5), suggesting that the metabolic acidosis-induced dysfunction of the β-adrenergic signaling pathway lies between the membrane receptor and adenylyl cyclase. The protonated form of isoproterenol dominates (pKa 8.65) and pH changes may alter its protonation, which may in turn alter binding to the

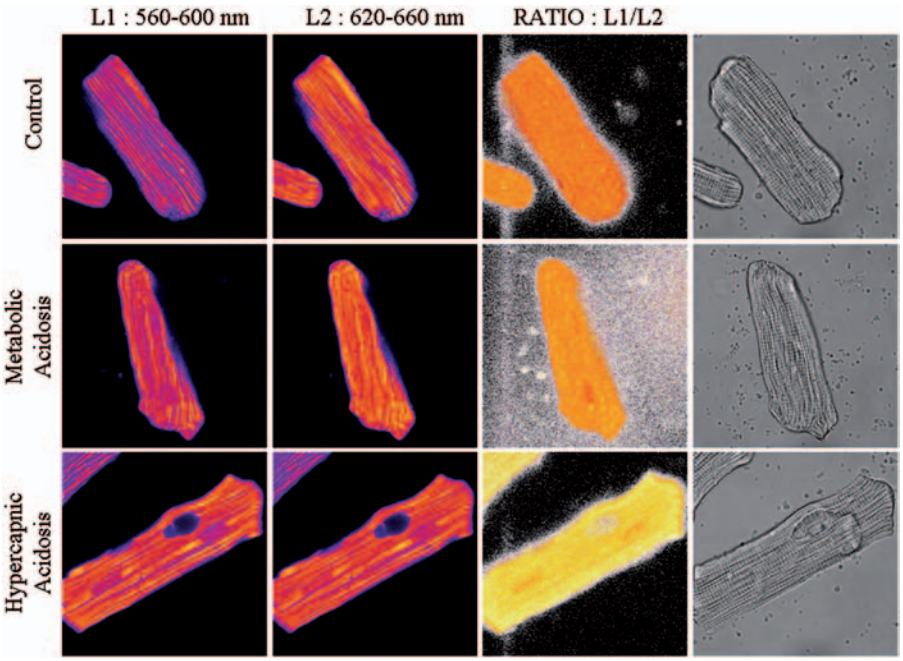

Fig. 6. Typical images obtained using confocal microscopy in isolated rat cardiomyocytes exposed to the pH-sensitive fluorophore carboxy-seminaphtorhodafluor-1 acetoxylmethyl ester-1 and an Argon laser at 514 nm in control conditions (pH 7.40) and in metabolic and respiratory acidosis (pH 7.10). The fluorescence was recorded at 580 nm (L1) and 640 nm (L2). The color shift of the emission ratio (ratio: L1/L2) from *orange* to *yellow* indicates the magnitude of the decrease in intracellular pH. The *left panels* show the aspect of myocardial cells without fluorescence.

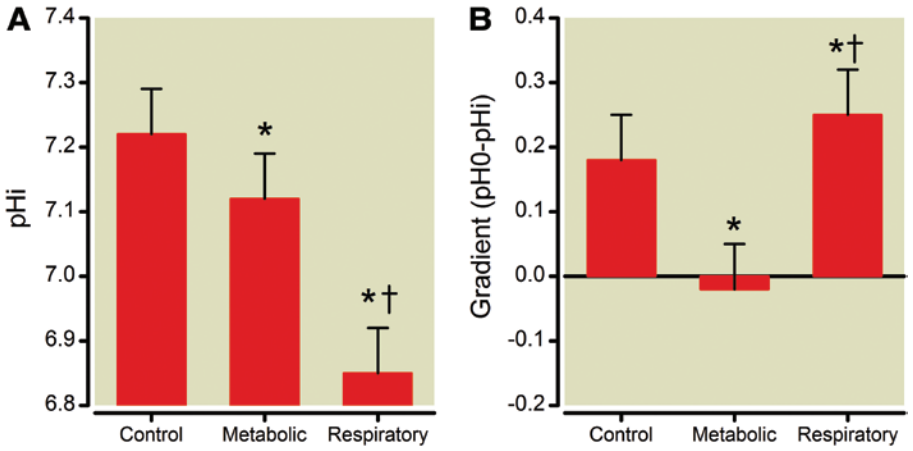

Fig. 7. pHi (*A*) and transmembrane pH gradients (*B*) in isolated rat cardiomyocytes in control conditions (pH 7.40) and in metabolic and respiratory acidosis (pH 7.10). Values are mean + SD (n = 72 to 124 cells). pHi = intracellular pH; pHo = extracellular pH. **P* < 0.05 *versus* control; †*P* < 0.05 *versus* metabolic acidosis.

β-adrenoceptors. The difference observed in response with identical change in pH would seem to exclude a major change in ligand protonation, contributing to our observations. Moreover, during acidosis, the number of β-adrenoceptors and their affinity are not significantly modified.[35] Only extra-physiological acidosis (pH < 6.00) may result in a decreased expression of membrane protein of β-adrenoceptors.[13] These results suggest that the metabolic acidosis-induced dysfunction of the β-adrenergic signaling pathway lies between the membrane receptor and G protein and/or coupling between the receptor and G protein. Although metabolic acidosis induced a lesser decrease in intracellular pH than respiratory acidosis did, its effect on transmembrane pH gradient differs markedly: only metabolic acidosis markedly modified this gradient (fig. 5). This difference in transmembrane pH gradient was noted by Steenergen *et al.*[36] although this study tested a very low extracellular pH (6.70) and used a method of intracellular pH measurement (dimethyl-2.4-oxizolidine dione distribution method) that is now thought as not reliable. This effect on transmembrane pH gradient is a good candidate for explaining the precise mechanisms involved because our study limits the possible targets to the transmembrane domain of the β-adrenergic signaling pathway. Ghanouni *et al.*[37] have suggested that the pH transmembrane gradient may facilitate agonist activation of the β2-adrenoceptor. Further biochemical studies are needed to exactly determine the mechanism involved. The inotropic response to ß-adrenoceptor stimulation was not altered in respiratory acidosis despite important decrease in intracellular pH. This result contrasts with those obtained with other inotropic agents such as levosimendan, which are susceptible to acidosis.[38]

Although the inotropic response to ß-adrenoceptor stimulation was altered in metabolic acidosis, the lusitropic response was preserved under isotonic (R1) and isometric (R2) conditions (table 4). Such discrepancy between inotropic and lusitropic effects of ß-adrenoceptor stimulation has been consistently reported in other situations, such as protamine exposure[16] and diabetes.[39] It has been suggested that only a small cAMP production resulting from ß-adrenoceptor stimulation is sufficient to induce a normal positive lusitropic effect.[40] It is known that the concentration of cAMP required to induce a maximal lusitropic effect is smaller than that required to induce a maximal inotropic effect.[40] These results might be important because lusitropy plays an important role in the maintenance of cardiac output.

The interpretation of our results should take into account some limitations of the experimental model. This study was conducted *in vitro* and the alterations on cardiac function *in vivo* also depend on many other factors (changes in venous return, afterload, autonomic nervous system, and compensatory mechanisms). The model of papillary muscle was studied at 29°C and low-frequency stimulation was necessary to insure stability of the preparation.[41] Rat myocardium differs significantly from human myocardium (heart rate 250–300 beats/min, changes in the force-frequency and action potential, predominant involvement of the SR Ca uptake over the sodium–calcium exchanger and the predominance of iso enzyme V1 myosin). Also, we studied acute acidosis, and our results may not apply to chronic acidosis. We considered the variation of intracellular pH in myocardial cells as a whole whereas it is known that there are noticeable H+ gradients within the cell and spatial regulation of intracellular pH.[42] Moreover, intracellular pH measured in myocardial cells may not duplicate pH changes in papillary muscles. There was a significant difference in baseline R2 between groups (table 2) which could have contributed to the observed results during respiratory acidosis. Last, the effects of acidosis on cardiac contractility are decreased in the neonatal heart,[29] and the inotropic response to β-adrenoceptor stimulation is decreased in aging myocardium.[43] Thus our results may not apply to both extreme ages.

In conclusion, acute metabolic and respiratory acidosis induces different myocardial effects. The direct negative

inotropic effect is more pronounced with respiratory acidosis and this difference is probably related to a greater decrease in intracellular pH during respiratory acidosis. Last, only metabolic acidosis impaired the positive inotropic effect of β-adrenergic stimulation, whereas no interference occurs with α-adrenergic stimulation.

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