Maintaining Blood pH at 7.4 during Hypothermia Has no Significant Effect on Work of the Isolated Rat Heart

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Variation in the pH of biologic fluids parallels modifications in the neutral point of water, which is temperature dependent. Therefore, pH adjustment, when organs from homeotherms are subjected to hypothermia as presently practiced in cardiac surgery or organ preservation, appears to be justified. The present study evaluated, during moderate hypothermia (26°C), the effect of variations in perfusate pH on hemodynamic performance of isolated working rat hearts in conditions of increased workload. Perfusates of blood with a pH corrected according to the pH–temperature relationship of neutral water, and blood with pH maintained at 7.4 were used. Hemodynamic function was unaltered by respiratory modifications in blood pH (normal pH blood: pH = 7.59 ± 0.01; PCO₂ = 20 ± 1 mmHg; blood maintained at pH 7.4: pH = 7.39 ± 1; PCO₂ = 37 ± 1 mmHg) and the hypothermic heart perfused with blood at pH 7.4 maintained its ability to do work in response to increased workload. The authors conclude that isolated heart at this degree of hypothermia has the capacity to resist noticeable changes in blood pH with no deleterious effect on its functional characteristics even at high workloads. The results suggest that the range of optimum extracellular pH value is relatively large at a given temperature. Such good tolerance could be related to tissue buffering efficiency and no conclusion concerning the relationship between tolerance of cellular function and intracellular pH changes can be made. (Key words: Acid–base equilibrium: hypothermia. Heart: myocardial function. Hypothermia: acid–base equilibrium.)

HYPOTHERMIA is a standard technique used for protecting the myocardium during cardiac surgery. However, the appropriate acid–base status for a mammalian organ subjected to hypothermia is still under debate,1,2 and little is known about its ability to tolerate acid–base changes. In the past, it was common to attempt to establish an arterial pH of approximately 7.4, irrespective of blood temperature. Recently, because of the concept of acid–base temperature dependence,3 grounded on observations made in ectotherm animals, a new strategy has been proposed. Rather than maintaining a pH of 7.4 during hypothermia, it has been suggested that PCO₂ should be adjusted to provide an increased pH in accordance with the pH–temperatuer relationship of ectotherms and to maintain total CO₂ content of extracellular fluids constant. In a recent editorial, White4 pointed out the opposing nature of the two strategies and predicted that functional characteristics of mammalian tissues would differ during hypothermia, when perfused with blood at pH 7.4 or at pH corrected for the pH–temperature relationship and constant total CO₂. He also emphasized that there has been little experimental work done to specifically evaluate the consequences of these two acid–base regimens. In one study, impairment of the cardiovascular function was observed using the first strategy (maintaining pH 7.4) during systemic hypothermia (27°C) in dogs.5 In contrast, under similar hypothermic conditions (26°C), mechanical performance of an isolated blood perfused rat heart was not altered greatly by moderate shifts from normal blood acid–base status.6

In the present study, the question of whether maintenance of pH 7.4 during moderate hypothermia might generate a basic defect in cardiac muscle function was examined. We determined whether or not correcting pH for the level of hypothermia used (26°C) (i.e., maintaining pH = 7.4) would impair the functional properties of an isolated, blood-perfused rat heart in conditions of increased workload. Thus, hemodynamic performance at 26°C, was studied at different levels of atrial filling pressure during successive perfusions with blood at normal acid–base status (pH = 7.6 at 26°C) and with blood at maintained pH 7.4.

Methods

Male rats of the Wistar strain were used (body weight = 325 ± 15 g, mean ± SEM).

HEART PREPARATION AND PERFUSION SYSTEM

Experiments were performed in a blood perfused working heart apparatus, as described in detail previously.5 Perfusate entered the left atrium and was ejected from the left ventricle via the cannulated aorta into a fluid column. The perfusion system reproduced an artificial systemic circulation and regulated afterload (fixed at 75 mmHg) and preload. The preload could be
adjusted to any chosen value under 25 mmHg and was fixed in the present experiment at 7.7, 14.6, or 21.5 mmHg. The left atrium could be perfused through one of two symmetric circuits that contained different perfusates. The whole perfusion apparatus was enclosed in a thermostatic chamber.

**PERFUSATE**

Erythrocyte-enriched buffer at a final hematocrit of 30%, prepared as previously described, was used. The reconstituted blood was filtered (Swank filter 1L 204®, Laboratoires Fandres, pore size 10 µm) before use.

Equilibration with chosen gas mixtures was carried out using a membrane oxygenator (Hospal). Gas mixtures used to equilibrate the perfusing blood were prepared from pure O₂, CO₂, and N₂ with Worshoff gas mixing pumps. The respective fractional concentrations of O₂ and CO₂ were F₉₂ = 0.10 and F₆₂ = 0.028 for normal pH perfusate (pH = 7.6), F₁₄₂ = 0.10 and F₁₄₂ = 0.06 for perfusate at pH 7.4.

**MEASUREMENTS**

Blood–gas analysis and pH measurements were performed at 26°C (temperature of the experiment) on samples of arterial blood (taken from the perfusion tubing above the left atrium cannula) and coronary venous blood (taken from the pulmonary artery outflow). Blood O₂ content was measured using a coulometric micromethod (Lex-O₂-Con®, Lexington Instrument Corp., Waltham, Massachusetts).

Cardiac performance was assessed by measuring aortic pressure (using a Statham P 23 Db pressure transducer placed just above the aortic cannula), heart rate, aortic flow, and coronary flow.

Myocardial oxygen consumption (MV₉₂, ml·min⁻¹) was calculated as the product of coronary flow (CF) and the arterio-coronary sinus blood O₂ content difference (C(a–v)O₂). External cardiac work was calculated as the product of mean aortic pressure (MAP), cardiac output, and a correction coefficient (1.33 × 10⁻⁴) and was expressed in joules per minute. Aortic flow, coronary flow, MV₉₂, and external work were expressed per gram of both ventricles wet weight. Cardiac efficiency was computed as the ratio of external work to the product of MV₉₂ and the calorific value of oxygen (20.3 J·P⁻¹).

**EXPERIMENTAL PROCEDURE**

Six hearts (mean weight = 1.00 ± 0.06 g) were studied at 26°C. Ventricular performance was assessed at three levels of atrial filling pressure (i.e., 7.7, 14.6, and 21.5 mmHg) and compared with a normal pH perfusate (pH = 7.6) and pH 7.4 perfusate as follows: the heart was removed quickly from the rat and mounted on the perfusion apparatus as previously described. After an equilibration period of 30 min at 7.7 mmHg atrial filling pressure with the perfusate at pH 7.6, the first measurement was taken. Atrial filling pressure then was increased successively to 14.6 and 21.5 mmHg and then returned to 7.7 mmHg, for periods of 15 min. Each new experimental state was maintained for 5 min before data were recorded. After return to 7.7 mmHg atrial filling pressure, the perfusate was changed to blood at pH 7.4. Atrial filling pressure was modified as above, and then finally the perfusate was changed back to blood at pH 7.6. The latter measurements enabled verification of preparation stability.

**STATISTICAL ANALYSIS**

Results were expressed as mean values and standard error of the mean (SEM). Mean values obtained at low atrial filling pressure during the different perfusion periods were compared by analysis of variance. The individual heart variation was taken in account as a concomitant factor in a three-way analysis of variance, under the hypothesis of no interactive effect of the three factors (single heart, pH level, and preload level). Differences between mean values were tested by the Student’s t test, weighted by using the residual variance of the variance analysis and taken as significant when P < 0.05.

**Results**

Table 1 shows the mean values obtained for blood acid–base balance (pH, P₉₂CO₂, [HCO₃⁻]), blood oxygenation (P₂O and C₀₂P), and hematocrit of arterial and coronary venous blood. The rather high blood P₂O value (near 90 mmHg) in reference to the theoretic value for F₂O₂ = 0.10 at 26°C (i.e., near 74 mmHg) is due to a slight and constant air contamination through flexible perfusion tubing between oxygenator outlet and heart inlet.

Comparisons of the mean values for hemodynamic variables obtained during periods of low atrial filling pressure and normal pH (at the beginning, middle, and end of the experiment) were not significantly different, attesting to the stability of the preparation.

Figure 1 shows the effects of pH on cardiac performance at the different levels of atrial filling pressure (AFP). At normal pH, no significant heart rate increase related to increasing filling pressure was evidenced; at pH 7.4, a slight heart rate increase versus increasing AFP was observed, and a significant difference was evidenced between the lowest and the highest preload (135 to 144 beats·min⁻¹, P < 0.001). At the highest
TABLE 1. Arterial and Coronary Venous Blood Gas and Acid–Base Variables of Hypothermic Working Hearts

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<thead>
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<th>Hypothermic Norm</th>
<th>Hypothermic Acidosis</th>
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<tr>
<td></td>
<td>Normal Acid–Base Status</td>
<td>Acidosis</td>
</tr>
<tr>
<td>pH</td>
<td>7.56 ± 0.01</td>
<td>7.39 ± 0.01</td>
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<tr>
<td>P&lt;sub&gt;1&lt;/sub&gt; (mm Hg)</td>
<td>20 ± 1</td>
<td>37 ± 1</td>
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<td>[HCO&lt;sub&gt;3&lt;/sub&gt;]&lt;sup&gt;-&lt;/sup&gt; mmol · L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>22 ± 1</td>
<td>25 ± 1</td>
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<tr>
<td>P&lt;sub&gt;4&lt;/sub&gt; (mm Hg)</td>
<td>94 ± 3</td>
<td>90 ± 3</td>
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<tr>
<td>C&lt;sub&gt;tv&lt;/sub&gt; (ml · dl&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>12.8 ± 0.1</td>
<td>12.8 ± 0.1</td>
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<tr>
<td>Hematocrit (%)</td>
<td>29.5 ± 0.2</td>
<td>29.9 ± 0.2</td>
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Values are mean ± SEM.

filling pressure, even though the cardiac output mean value tended to be higher when hearts were perfused at normal pH, the difference from the cardiac output mean value at pH 7.4 was not statistically significant. Coronary flow tended to be higher at pH 7.4, but a significant difference between flow values at the two pHs was only evidenced at the highest preload.

Figure 2 shows the effects of pH on external work and oxygen utilization of the blood perfused heart at the different levels of atrial filling pressure. External work increased significantly with atrial filling pressure at the two levels of blood pH. No significant difference between values for external work at the two pH levels was evidenced even at the highest workload. Arteriovenous blood oxygen content difference was increased

![Graphs showing effects of pH on heart rate, cardiac output, arterial oxygen content, and myocardial oxygen consumption.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931411/)

**Fig. 1.** Effect of pH on cardiac performance of the blood perfused isolated working rat heart. Each mean represents data obtained from six hearts. A.F.P. = Atrial filling pressure. *P < 0.05 for pH 7.4 versus normal acid–base status.

**Fig. 2.** Effect of pH on external work and oxygen utilization of the blood perfused isolated working rat heart. C<sub>(a-v)</sub>O<sub>2</sub> = arteriovenous difference in blood oxygen content. MVO<sub>2</sub> = myocardial oxygen consumption. *P < 0.05; **P < 0.01 for pH 7.4 versus normal acid–base status.
at the highest workload at both pH levels and was not influenced by perfusate pH. Myocardial oxygen consumption increased with increasing atrial filling pressure; similarly to coronary flow, $\dot{M}_{\text{VO}_2}$ was increased significantly at pH 7.4 at the greatest workload. Since coronary flow increased at pH 7.4, cardiac efficiency tended to be lower at this pH level, but no significant difference was exhibited. Cardiac efficiency was not significantly modified by atrial filling pressure levels at either pH.

**Discussion**

The aim of these experiments was to examine the tolerance of an isolated organ to changes in extracellular pH during hypothermia and to determine if the choice of pH regulation strategy is crucial for organ preservation. The present study demonstrated that maintaining a pH of 7.4 during moderate hypothermia did not impair cardiac performance nor significantly modify left ventricular reserve capacity, which was tested over a range of preload conditions.

The blood perfused working isolated rat heart was chosen for these experiments, since this model provided a good means for perfusion control through a tissue in which small changes in blood oxygen delivery would be critical to its function. By using unconstituted blood perfusate, it was possible to satisfy myocardial oxygen demand\textsuperscript{6} and to investigate the adaptation of the heart during hypothermia to changes in pH that would modify blood oxygen release. The present experiments provided data concerning the adaptability of the heart during hypothermia to changes in workload. It was shown that the preparation did respond to changes in filling pressure: cardiac output, external work, and $\dot{M}_{\text{VO}_2}$ increased with atrial filling pressure, and the coronary vascular bed demonstrated flow autoregulation (coronary flow increased in response to changes in perfusion pressure).

The relationship between the pH of the biologic fluids and temperature first was examined by Stadie et al.,\textsuperscript{7} who investigated the thermodynamic principles upon which such a relationship could be founded. Their experiments with blood and plasma in closed system conditions showed that total CO\textsubscript{2} remains constant and pH decreases as temperature is increased. However, these observations were not applied in practice to cardiovascular surgery or organ preservation, where hypothermia commonly is used and the perfusate pH is maintained at a “normal” pH value of 7.4, irrespective of organ temperature. The theory of acid–base regulation with temperature change has been reconsidered in recent years. In 1975, Rahn et al.\textsuperscript{8} proposed a general concept of hydrogen ion regulation, which explains acid–base regulation in relation to body temperature and applies to all animal species. These authors suggested that preservation of intracellular neutrality, essential for cellular function, is governed by the dissociation of water and can be regulated by imidazole-rich protein buffers. The extracellular environment that receives acid byproducts of cell activity is maintained at constant alkalinity relative to intracellular pH. The difference between extracellular pH and neutrality is constant for each species and ranges from 0.6 to 0.8 pH units. It is unaffected by temperature, and total CO\textsubscript{2} content of both extracellular and intracellular fluids remains constant.

Experiments therefore were undertaken to examine the soundness of this strategy for pH adaptation, using the concept of pH temperature dependence, in homeotherm animals subjected to hypothermia. In hypothermic (27°C) dogs placed on coronary bypass, it was shown\textsuperscript{9,10} that alkaline pH greater than 7.4 induced an important improvement in cardiovascular function. Blayo et al.\textsuperscript{9} have reported results in hypothermic adult humans (27°C) on coronary bypass for whom the $P_{\text{CO}_2}$ in the gas mixture was adjusted to provide normal pH and $P_{\text{CO}_2}$ values measured at 37°C. This procedure resulted in stabilization of arterial and mixed venous blood total CO\textsubscript{2} content and [HCO\textsubscript{3}⁻] without metabolic acidosis. Experimental studies of kidney function after cold preservation (7°C) and transplantation showed a functional improvement when the preservation fluid pH was increased.\textsuperscript{11} A recent work\textsuperscript{12} evidenced that the electrical stability of hypothermic canine heart was increased when blood pH was corrected according to the pH–temperature relationship of neutral water. All of these results suggest that the choice of blood acid–base equilibrium during hypothermia may be crucial for cellular function and that the optimum pH value is that which follows the pH–temperature relationship of neutral water. However, our present work indicates, since isolated heart was shown to be resistant to appreciable changes in pH without hemodynamic impairment, that the range of optimum pH value can be enlarged.

The effects of respiratory-induced pH changes on cardiovascular function have been investigated and shown to alter both coronary vascular resistance and myocardial contractility. In the present experiments, only the first effect was observed: coronary flow was greater when pH decreased. This decrease in coronary vascular resistance might explain the slight (insignificant) decrease in cardiac efficiency observed at the lower pH value. The mechanism of the effect of respiratory acid–base change on coronary vascular resistance has not been elucidated but may be related to blood pH and/or $P_{\text{CO}_2}$ changes.\textsuperscript{13,14} The positive relationship between pH and myocardial contractility is well documented,\textsuperscript{14–16} but it only has
been observed in cases of large variation in acid–base status. In the present experiments we did not observe any modification of external work due to changing blood pH, even at high levels of preload in the present experiments.

One explanation for this absence of functional cardiac change with pH has been provided by the results of Clancy and Gonzalez. Using isolated cat papillary muscles, they showed that acidosis at 25°C (pH from 7.4 to 6.9) was not associated with significant changes in contractility, whereas a negative inotropic effect was observed at 30 and 38°C. It also has been shown that intracellular pH is the important determinant of the effect of blood acidosis on cardiac function. The mechanisms of altered myocardial contractility with intracellular acidosis are not completely clear, but many sites for H⁺ ion action have been considered, particularly for H⁺ interaction with Ca²⁺ ions. In the present work, only blood pH was controlled, and due to the uncertainties pertaining to measurements of intracellular pH, little is known concerning the intracellular response to temperature change and the regulation of pH when Pco₂ is altered during hypothermia. However, the few data obtained in this field indicate that there is, as for extracellular fluids, an inverse relationship between temperature and pH and that both intracellular and extracellular CO₂ content are maintained constant when temperature is changed. The mechanisms involved during hypothermia are probably multiple, including temperature dependence of the pK values of intracellular buffers, changes in ion fluxes (H⁺, OH⁻, or HCO₃⁻), and changes in intracellular buffering. Saborowski et al. demonstrated that an acute decrease in temperature from 38 to 22°C altered the relationship between the pH and the pH in rat myocardium, which suggests an increase in buffering capacity during hypothermia. An increase in intracellular buffering capacity against changes in Pco₂ is a possible explanation for the absence of changes in mechanical performance in the present work.

In conclusion, this investigation studied the separate and combined effects of keeping the pH at 7.4 and increasing preload during moderate hypothermia (26°C) on cardiac performance. Even the combination of the two factors did not deleteriously affect cardiac function. Two main issues arise from this study. First, cardiac function that theoretically may be improved by perfusing the heart with blood at pH adjusted according to the pH–temperature relationship of neutral water was not altered meaningfully during perfusion of blood at pH 7.4 at the temperature studied. Second, the deleterious effect of maintaining blood pH at 7.4 as observed in the whole animal apparently was not a consequence of a specific effect on myocardial function. In the isolated heart, intracellular buffering probably compensated for the change in blood pH, and myocardial function was preserved since pHi was protected. In the whole organism, the possibility that cardiac performance can be modified by changes in the neutral or hormonal status induced by pH change remains.

These results provide information for possible application in the management of organ preservation and the choice of cardioplegic solutions: although the pH–temperature dependence of biologic fluids is unquestionable, the isolated organ appears to have the capacity to resist noticeable changes in blood pH without hemodynamic functional damage.

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References