Acid-Base Changes in Ischemic Myocardium and Intervention with Hypothermia or Bicarbonate

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Effects of ischemia and reperfusion on acid-base changes in relation to myocardial contractility, and the effects of correcting H⁺ were studied by lowering PCO₂ or increasing HCO₃⁻ levels. The hearts were perfused by working heart mode and whole heart ischemia was induced by use of a one way valve followed by myocardial warming (37°C, normothermia), cooling (18°C, hypothermia) or warming plus 2.1 mM NaHCO₃ for 15 min. The hearts were then reperfused for 20 min. Coronary effluent was collected through pulmonary artery cannulation and used for the measurement of acid-base changes.

There were close correlations between the decrease in coronary flow and LV pressure, LV dP/dt. Close correlations were also observed between the decline in LV pressure and the rise in PCO₂, H⁺, and the decline in HCO₃⁻. A highly significant correlation was seen between H⁺ and lactate production. Myocardial contractility decreased to the same extent in 3 groups during ischemia, whereas its recovery rate in both the hypothermia and HCO₃⁻-treated groups were significantly higher than in the normothermia group. The increment of H⁺ was significantly less in both the hypothermia and HCO₃⁻-treated groups than in normothermia.

These results indicate that lactate production is the major H⁺ producing source and the correction of H⁺ could minimize the ischemic insult and at the same time contribute to the reperfusion injury.

A reduction in O₂ supply below that required to meet the need of the tissue results in several characteristic changes. Some evidence suggests that the decrease in myocardial contractility may be closely related to altered metabolic changes in response to ischemia. Above all things, tissue acidosis is one of the greatest contributing factors for depressed myocardial contractility. However, the precise correlation and time course between myocardial contractility and the levels of metabolic products, particularly H⁺ and CO₂, have not yet been fully described.

Another question arises as to whether the ischemic insult is diminished when the accumulation of metabolic products is reversed during ischemia. It is of value to study this problem from the therapeutic point of view. Therefore, this experiment was designed to investigate the following two subjects: 1) Whether or not the functional alteration is diminished during either ischemia or reperfusion when the level of hydrogen ion is controlled by hypothermia or addition of bicarbonate during ischemic perfusion. 2) What is the relationship between myocardial contractility and metabolic products that accumulate due to a lack of oxidative metabolism.

Key words:
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Fig.1. Experimental protocol: three different experimental conditions were made, i.e. A) The heart was allowed to function for a 5 min control (preischemic) period as a working heart preparation. Ischemia was induced at 0 time indicated and hearts were perfused for 15 min with warming (37°C) followed by aerobic reperfusion for an additional 20 min. B) All experimental procedures were the same as A except myocardial cooling (18°C) during ischemia. C) All experimental procedures were the same as A except that 2.1 mM NaHCO₃ was added to KHB buffer during ischemia (See Method).

and/or washout of vascular spaces, such as H⁺, CO₂, and lactate particularly during the early phase of ischemia?

METHOD

1) Experimental groups and protocol (Fig. 1)
The hearts were removed and perfused as working heart preparation with an afterload of 60 mmHg and a preload of 7 mmHg previously described by Neely et al¹¹ for 5 min as baseline hemodynamic and metabolic variables. The perfusate was modified Krebs Henseleit bicarbonate (KHB) buffer containing 11 mM glucose and was gassed with a mixture of oxygen (95%) and carbon dioxide (5%). Whole heart ischemia was then induced by use of a one-way ball valve in the aortic outflow tract which prevented retrograde perfusion of the coronary arteries during diastole¹² under electrical pacing (380 beats/min).

During the ischemic perfusion for 15 min, 3 different experimental conditions were used i.e. A) the hearts were warmed with the heart chamber kept in 37°C (normothermia, n = 5), B) they were cooled in 18°C (hypothermia, n = 5) and C) they were warmed in 37°C and perfused with KHB buffer which contained 2.1 mM NaHCO₃ (normothermia plus HCO₃⁻, n = 5). The hearts in each group were then reperfused by aerobic conditioning without pacing for an additional 20 min.

2) Hemodynamic and metabolic parameters
Following the cannulation of the aorta and pulmonary vein by use of the Langendorf technique, an 18 gauge catheter (Intramedicut Catheter Kit, Argyle ®) was inserted via the left atrium into the left ventricle (LV) to measure LV pressure, LV dP/dt and LV end-diastolic pressure. These hemodynamic parameters were monitored by the polygraph (Fukuda, MIC-9800) and recorded by the thermal recorder (Fukuda, RF-80).

Aortic flow was measured by use of an electromagnetic flowmeter (Nihon-Kohden, FF-030T). Coronary effluent was monitored by an electromagnetic flowmeter (Nihon-Kohden, FF-010T) through pulmonary artery cannulation. Coronary effluent was collected at 5 min intervals and used
Fig. 2. Variations in coronary flow (top) and aortic flow (bottom) during preischemic, ischemic and reperfused periods. Closed squares represent the normothermia and closed circles represent the hypothermia respectively. Open circles represent the normothermia plus bicarbonate. Each point represents the mean ± standard error of the mean for 5 hearts in this and all subsequent figures.

Fig. 3. Variations in LV dP/dt during preischemic, ischemic and reperfused periods. These data were obtained from the hearts described in Fig. 2.

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for the measurement of venous PO₂, PCO₂, HCO₃⁻ by use of the Blood-Gas Analyzer (Corning 175) and lactate production by the enzymatic method, respectively. Hydrogen ion was calculated from the value of PCO₂ and HCO₃⁻. Cardiac output was estimated as the sum of coronary flow and aortic flow.

3) Statistical analysis

Hemodynamic data, venous PCO₂, bicarbonate, H⁺ and lactate production were compared by Student's t-test. The level of statistical significance was p < 0.05. The correlation coefficients were computed by the exponential function.

RESULTS

1. Mean changes for coronary flow (top) and aortic flow (bottom) during preischemic, ischemic and reperfused periods (Fig. 2).

Coronary flow decreased to the same extent during ischemia among the 3 groups and recovered to more than 80% of the preischemic level in all groups.

Aortic flow decreased to zero in 3 groups at the end of ischemia; however, it recovered to around 50% of the preischemic level during reperfusion in both hypothermia and normothermia and plus HCO₃⁻ groups when compared with normothermia.

2. Mean changes for cardiac output and LV pressure during preischemic, ischemic and reperfused periods (data not shown).

Cardiac output decreased to the same extent during ischemia in the 3 groups; however, the recovery rate of cardiac output in both hypothermia and normothermia plus HCO₃⁻ was significantly higher than in normothermia. LV pressure showed a significantly higher recovery during reperfusion in both hypothermia and normothermia plus HCO₃⁻ than in normothermia.

3. Mean changes for LV dP/dt during preischemic, ischemic and reperfused periods (Fig. 3).

LV dP/dt decreased to the same extent in all groups during ischemia, whereas the recovery rate of LV dP/dt during reperfusion in both hypothermia and normothermia plus HCO₃⁻ was significantly higher than in normothermia.

4. Mean changes for PCO₂ (Fig. 4, left) and HCO₃⁻ (Fig. 4, right) in venous sample during preischemic, ischemic and reperfused periods. The venous PCO₂ increased sharply during the

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Fig. 5. Variations in H⁺ levels as indicated by the values of PCO₂ and bicarbonate (left) and lactate production (right) during preischemic, ischemic and reperfused periods.

Fig. 6. Correlation between LV pressure and PCO₂ (top, left), LV pressure and bicarbonate (top, right), LV pressure and H⁺ (bottom, left), LV dP/dt and H⁺ (bottom, right) respectively. Each point represents one LV pressure, one dP/dt and PCO₂, bicarbonate, H⁺ in one heart at a specific time during ischemic perfusion.

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ischemic period in normothermia. However, there was a significant reduction in PCO$_2$ increment in both hypothermia and normothermia plus HCO$_3^-$]. There was no significant difference among the 3 groups during reperfusion.

The venous HCO$_3^-$ decreased to about one third of the preischemic level at the end of ischemia in normothermia, whereas the decrease rate in both hypothermia and normothermia plus HCO$_3^-$ was significantly inhibited. However, there was no significant difference among the 3 groups during reperfusion.

5. Mean changes for venous H$^+$ calculated by PCO$_2$ and HCO$_3^-$ (Fig. 5, left) and lactate production (Fig. 5, right) during preischemic, ischemic, and reperfused periods.

The venous H$^+$ increased dramatically during ischemia in normothermia at about 2.4 times that of the preischemic level, whereas this increment was markedly inhibited in both hypothermia and normothermia plus HCO$_3^-$. The lactate production rose in a similar manner to venous H$^+$ in normothermia, while it was inhibited significantly in both hypothermia and normothermia plus HCO$_3^-$.  

6. Correlation between LV pressure and PCO$_2$ (Fig. 6, top, left), LV pressure and HCO$_3^-$ (Fig. 6, top, right), LV pressure and H$^+$ (Fig. 6, bottom, left), LV dP/dt and H$^+$ (Fig. 6, bottom, right) during ischemia.

There were close correlations between the 2 respective parameters, i.e. the rise in venous PCO$_2$ and the decline in venous HCO$_3^-$ correlated fairly closely with the decline in LV pressure ($r = 0.83$ and 0.80 respectively). The rise in venous hydrogen ion correlated closely with the decline in
LV pressure and LV dP/dt (r = 0.88 and 0.86 respectively).

7. Correlation between coronary flow and $H^+$ (Fig. 7, top, left), coronary flow and LV pressure (Fig. 7, top, right), $H^+$ and lactate production (Fig. 7, bottom).

Close correlations were seen between the 2 respective parameters during ischemic perfusion, i.e. the decline in coronary flow correlated with the rise in venous hydrogen ion (r = 0.84) and correlated closely with the decline in LV pressure (r = 0.94). The highly significant correlation was seen particularly between hydrogen ion and lactate production (r = 0.98).

**DISCUSSION**

Since Tennant has reported the importance of pH change in myocardial failure13 many investigators have emphasized the harmful effect of acidosis on myocardial contractility although there have been some reports refuting this.14 However, a hydrogen ion producing process had not yet been established. Furthermore, despite great interest, information as to whether the ischemic insult may or may not be protected when acidosis is reversed is scarce. The reason for this is that technically it is possible to treat metabolic acidosis, whereas there is little methodology for inhibiting the increment of CO₂ production in the ischemic myocardium.

The characteristic linear relationship between pH and temperature has been demonstrated in the blood15 and hydrogen ion has been shown to be regulated by temperature.16 In addition, hypothermia causes an increase in solubility of carbon dioxide.8 These findings led to experiments which supplied information about the correction of hydrogen ion level during ischemia by either hypothermia or addition of bicarbonate to the buffer.

The present investigation showed that the hypothermia during ischemia inhibited not only the rise in CO₂ tension but the fall in bicarbonate, causing significant reduction in hydrogen ion level. Hypothermia during ischemia also promoted the recovery rate in myocardial contractility during reperfusion following 15 min of severe ischemia. In this case, it was unlikely that the washout in hydrogen ion was increased because there was no significant difference in coronary flow rate during ischemia between normothermia and hypothermia. It was therefore more understandable that the energy requirement decreased by hypothermia resulted in the fall in CO₂ production. However, the mechanism for the inhibition of the fall in bicarbonate level which occurred simultaneously during ischemia in hypothermia is still unknown.

When bicarbonate was added to the KHB buffer, not only was the decrease in bicarbonate inhibited but the increase in PCO₂ was suppressed. This correction of acid-base change resulted in significant recovery in myocardial contractility during reperfusion following 15 min of severe ischemia.

As for the relation between myocardial contractility and metabolic products during ischemia, the present study strongly indicated that the accumulation of hydrogen ion, CO₂ and the decrease in bicarbonate level could influence the contractile performance of the heart as well as the coronary flow.

It is well known that lactate in itself is a potent inhibitor of glycolysis at the level of glyceraldehyde 3-phosphate dehydrogenase17 which results in a deleterious effect on myocardial contractility. In the present study, the most striking correlation was observed between hydrogen ion and lactate production, indicating that lactate production was one of the major factors for hydrogen ion sources. Gevers18 proposed a possible mechanism related to the hydrogen ion producing process. Among them, lactate accumulation19,20 which was caused by the breakdown of glycogen to lactate21 and ATP hydrolysis22 were well documented. Thus, the result of this investigation has in part supported this hypothesis.

Although hydrogen ion has been recognized as an important synergistic messenger23 overload of hydrogen ion causes crucial effects on myocardial contractility. Ketz et al3 Cingolani et al4 and Carmeliet et al9 reported the mechanism of depressed myocardial contractility secondary to acidosis, and their common proposal mechanism is the influence of hydrogen ion on the process of $Ca^{2+}$ release in the myofilament. We observed an inhibitory effect on hydrogen ion production by diltiazem, a $Ca^{2+}$ channel blocker, during whole heart ischemia in isolated perfused rat heart, which suggested that the interaction between hydrogen ion and $Ca^{2+}$ would exist.24

Finally, the data showed close correlation between myocardial contractility and the levels of PCO₂, bicarbonate, $H^+$ in the coronary effluent and lactate production during global ischemia.
of an independent fashion. In addition, exposure of the heart to hypothermia or addition of bicarbonate to the buffer during ischemic perfusion led the myocardial contractility to significant recovery during reperfusion when compared with normothermia. Thus, the correction of $\mathrm{H}^+$ levels during ischemia could protect the ischemic insult and at the same time contribute to the preservation of reperfusion injury. The precise interaction between $\mathrm{H}^+$ and $\mathrm{Ca}^{2+}$ in the ischemic myocardium will require further investigation.

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