J Huazhong Univ Sci Technol［Med Sci］
DOI 10.1007/s11596-008-0621-8 28(6):706-710,2008

# The Effect of Hypercapnic Acidosis Preconditioning on Rabbit Myocardium*

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**Summary:** This study observed the protective effect of hypercapnic acidosis preconditioning on rabbit heart suffered from ischemia-reperfusion injury. Hypercapnic acidosis was established in animals with mechanical hypoventilation before ischemia-reperfusion. Thirty-two rabbits were randomly divided into 4 groups, with each having 8 aminals in term of the degree of acidification: hypercapnic acidosis group A (group A), hypercapnic acidosis group B (group B), hypercapnic acidosis group C (group C), ischemia and reperfusion group (group IR). Animals in group IR were ventilated normally (tidal volume: 15 mL/kg, breathing rate 35 bpm). The $PETCO_2$ was maintained at the level of 40–50 mmHg for 30 min. Animals in groups A, B, C received low-frequency, low-volume ventilation to achieve hypercarbonic acidosis and the target levels of $PETCO_2$ were 75–85 ,65–75, 55–65 mmHg, respectively, with levels being maintained for 5 min. The animals then were ventilated normally to lower $PETCO_2$ to 40–50 mmHg. The left anterior branch artery of all the animals was ligated for 30 min and reperfused for 180 min. Then the infarct size was calculated. The cardiomyocytes were morphologically observed and ECG and hemodynamics were monitored on continuous basis. Acid-base balance was measured during procedure. Our results showed that the infarct size was (48.5±11.5)% of the risk area in the control group and (42.4±7.9)% in group C ($P$>0.05). Mean infarct size was significantly smaller in group B (34.5%±9.4%) ($P$<0.05 vs control group) and group A (31.0%±9.1%) ($P$<0.01 vs control group). It is concluded that HA-preconditioning can effectively protect the myocardium.
**Key words：** hypercapnic acidosis; preconditioning; heart; infarct size

Myocardial ischemia preconditioning (IPC), defined as a series of repetitive brief ischemic episodes prior to prolonged ischemic event, reduces myocardial infarct size and improves functional recovery[1]. The exact mechanisms of this process remain to be elucidated but have been hypothesized to include activation of potassium adenosine triphosphate (KATP) channel, which could be activated by acidosis as many studies showed[2-4].

IPC results in transient acidosis. On the other hand, a single period of acidification or repeated acidification of the heart in the absence of ischemia can protect myocardium against prolonged ischemic injury, implying that acidosis *per se* may be a trigger of IPC[5-6].

Acidosis can be caused by hypoventilation. Hypoventilation was originally adopted to treat the patients with acute respiratory distress syndrome (ARDS), for it could improve the survival of those patients. Later, mounting evidence suggested that hypoventilation not only attenuated lung injury in patient with ARDS but also directly attenuated lung, brain and isolated heart injury after ischemia-reperfusion[5-9]. Hypercapnic acidosis caused by hypoventilation was hypothesized to be a protective factor.

Temporary acidosis during reperfusion after myocardial ischemia limits myocardial infarct size in dogs[10]. Nomura and his coworkers found that hypercapnic reperfusion (pH 6.8) for a short period after ischemia improved functional recovery after cold cardioplegic ischemia in isolated neonatal lamb hearts[11]. Whether hypercapnic acidosis might further provide myocardial protection *in vivo* if initiated prior to myocardiacl ischemia is not clear. Therefore, our hypothesis is that hypercapnic acidosis preconditioning caused by hypoventilation before myocardial ischemia would be cardio-protective. It may provide a new convenient method for anesthesiologists to protect the heart undergoing cardio pulmonary bypass (CPB) by adjusting the respiratory parameters to induce hypercapnic acidosis if our hypothesis can be confirmed.

## 1 MATERIALS AND METHODS

### 1.1 Experimental Methods

The experimental protocol was approved by the

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*This project was supported by a grant from the Special Foundation for Doctoral Program of the Ministry of Education of People's Republic of China (No. 20050533022).

Animal Care and Use Committee of our university. A total of 32 New Zealand white rabbits of either sex (2.0–2.5 kg) were anaesthetized with ketamine at 50 mg/kg IM. Anesthesia and muscle relaxation were maintained with a continuous infusion of a mixture of ketamine (3 mg/kg/h) and pancuronium 0.1 mg/kg/h. Depth of anesthesia was assessed by the absence of pedal and palpebral reflexes following tracheotomy.

Body temperature, recorded through a thermistor inserted into the esophagus was maintained between 38–39°C by means of a servo-controlled heating element incorporated into the operating table. Pre-cordial electrocardiograph was recorded by use of bipolar chest leads. A fluid-filled catheter was placed in the right femoral artery and was connected to a transducer for measurement of arterial pressure. The right internal jugular vein was catheterized with a catheter (1-mm in internal diameter) to infuse fluids and drugs. Hetastarch at 5 mL/kg/h was infused continuously via this intravenous cannula. Fentanyl 50 μg was intravenouly injected before thoractomy to provide adequate analgesia.

The rabbits were randomized, by using a computer-generated randomization list, into four groups: control group, $PETCO_2$ 80 mmHg group, $PETCO_2$ 70 mmHg group, and $PETCO_2$ 60 mmHg group. All groups underwent a 30-min coronary artery occlusion and 3-h reperfusion. Before this extended ischemia-reperfusion period, the animals in the control group were ventilated with tidal volume at 15 mL/kg and respiratory rate at 35 bpm for 40 min. The $PETCO_2$ was monitored in the range of 40–50 mmHg. The animals in $PETCO_2$ 80 mmHg group were hypoventilated with tidal volume at 12 mL/kg and respiratory rate at 25 bpm. The PET $CO_2$ target value in $PETCO_2$ 80 mmHg group was 75–85 mmHg. It was maintained for 5 min till the $PETCO_2$ achieved target value of 75–85 mmHg, and then normal ventilation was provided. The artery was not occluded until the $PETCO_2$ returned to 40–50 mmHg. The animals in the $PETCO_2$ 70 mmHg group and $PETCO_2$ 60 mmHg group were also hypoventilated with tidal volume at 12 mL/kg and respiratory rate at 25 bpm. The $PETCO_2$ target value in the $PETCO_2$ 70 mmHg group was 65–75 mmHg. The $PETCO_2$ target value in the $PETCO_2$ 60 mmHg group was 55–65 mmHg. The $PETCO_2$ target values in these two groups were maintained for 5 min after the values were achieved, and then the animals were ventilated normally at a $PETCO_2$ of 45–50 mmHg. The artery was not occluded until the $PETCO_2$ returned to 40–50 mmHg. End-tidal gas concentrations were measured continuously by the $PETCO_2$ monitor. The animal's lungs were ventilated mechanically with 100% oxygen during hypoventilation period. $FiO_2$ was adjusted to 40% except for this period.

Before this preconditioning period a left thoracotomy was performed in the fourth intercostal space and the pericardium was opened. A 2/0 silk thread was then passed around the circumflex branch of the left coronary artery, with its ends being threaded through a small polyethylene tube. The rabbits were allowed 20 min to reach a steady state after surgical preparation. Coronary occlusion was produced by pulling the snare and clamping it with a mosquito hemostatic forceps. The artery was occluded when the $PETCO_2$ returned to 40–50 mmHg. Reperfusion was produced by releasing the clamp after 30-min occlusion. Myocardial ischemia was confirmed by ST-segment elevation of the ECG as well as observation of regional cyanosis over the myocardial surface. Reperfusion was confirmed by reactive hyperemia over the surface after the snare was released. Arterial blood gases were determined at three time points: baseline, post hypoventilation and pre-ischemia.

At the end of the reperfusion period, the coronary artery was briefly re-occluded and 50% Uniperse blue was injected into the jugular vein to delineate area at risk *in vivo*. With this technique, the previously non-ischemic area appeared blue whereas the area at risk remained unstained. Anaesthetized rabbits were then scarified via injection with 10-mL air and the heart was excised and cut into five or six 2-mm-thick transverse slices.

After removal of the right ventricular tissue, each slice was identified under microscope and weighed. Each slice was incubated for 15 min in tetrozolium chloride to differentiate infarcted (pale) from viable (red) myocardial areas. The extent of the left ventricle (LV) area, area at risk, and infarct size were weighed. Total weights of area at risk and area of necrosis were calculated and expressed as weight (g) or as percentages of total LV weight. It was decided prospectively that hearts with a risk region <10% of the LV weight would be excluded from the study.

**1.2 Statistical Analysis**

Statistical analysis was performed by using one-way analysis of variance. Statistical calculations were performed by using SPSS12.0. Student-Newman-Keuls tests were used for multiple comparisons. All values are expressed as $\bar{x} \pm s$. A $P<0.05$ was considered to be statistically significant.

## 2 RESULTS

**2.1 Hemodynamic Data**

Hemodynamic data including heart rate and arterial pressure are summarized in table 1. HA significantly increased mean arterial pressure and heart rate ($P<0.05$, vs baseline). There was a significant difference in the effect between $PETCO_2$ 80 mmHg group, PETCO2 70 mmHg group, group PET $CO_2$ 60 mmHg group and control group ($P<0.05$ vs control). Mean arterial pressure decreased when the PET $CO_2$ returned to 40–50 mmHg. No serious arrhythmias, such as ventricular fibrillation (VF) or ventricular tachycardia (VT) developed before the ischemia.

**2.2 Arterial Blood Gas**

Arterial blood gas data, including pH, $PCO_2$, $SAO_2$, and $PO_2$, are given in table 2. The baselines showed no statistically significant differences in these parameters among the groups. pH and $PCO_2$ of each HA group at baseline time were significantly lower than that of the control group ($P<0.05$ vs control). $PCO_2$ and pH of each HA group at T2 were significantly higher than those of T1 ($P<0.05$, vs baseline). There were no significant differences in $SAO_2$ among the groups at each time points. $PO_2$ of each group continued to increase. $PO_2$ of each group at T2 and T3 was significantly higher than the baseline ($P<0.05$ vs baseline).

**Table 1 Hemodynamic measurements in different experimental groups ($n$=8)**

| Groups | | Times | | |
|---|---|---|---|---|
| | | T1 | T2 | T3 |
| Control | MAB(mmHg) | 81±13 | 84±15 | 77±13 |
| | HR(bpm) | 198±29 | 203±36 | 211±24 |
| $PETCO_2$80mmHg | MAB(mmHg) | 80±14 | 106±11*# | 79±12 |
| | HR(bpm) | 192±23 | 279±29*# | 226±26# |
| $PETCO_2$70mmHg | MAB(mmHg) | 78±12 | 114±11*# | 83±13 |
| | HR(bpm) | 199±27 | 311±17*# | 245±23# |
| $PETCO_2$60mmHg | MAB(mmHg) | 76±12 | 112±13*# | 81±14 |
| | HR(bpm) | 196±21 | 282±24*# | 233±29# |

MAB: mean artery pressure; HR: heart rate

$T_1$: baseline; $T_2$: at the end of hypoventilation; $T_3$: preischemia

*$P$<0.05 as compared with control; #$P$<0.05 as compared with baseline

**Table 2 Arterial blood gas measurements in different experimental groups ($n$=12)**

| Groups | | Times | | |
|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ |
| $PETCO_2$80mmHg | pH | 7.56±0.07 | 6.94±.12*# | 7.46±0.05 |
| | PCO2(mmHg) | 45.2±0.8 | 81.6±2.4*# | 44.7±0.9 |
| | $SAO_2$（%） | 95.3±2.4 | 99.7±0.2 | 99.8±0.1 |
| | $PO_2$（mmHg） | 80.7±8.8 | 102.4±7.9* | 135.6±2.3* |
| $PETCO_2$70mmHg | pH | 7.55±0.08 | 7.12±0.11*# | 7.50±0.06 |
| | PCO2(mmHg) | 46.0±0.7 | 69.5±3.2#* | 45.1±0.8 |
| | $SAO_2$（%） | 96.5±1.2 | 99.5±0.3 | 99.6±0.2 |
| | $PO_2$（mmHg） | 81.2±7.9 | 117.6±8.9* | 145.1±2.1* |
| $PETCO_2$60mmHg | pH | 7.59±0.06 | 7.29±.11*# | 7.48±0.07 |
| | PCO2(mmHg) | 45.3±0.6 | 60.4±2.6#* | 45.5±0.7 |
| | $SAO_2$（%） | 98.1±2.1 | 99.4±0.2 | 99.6±0.1 |
| | $PO_2$（mmHg） | 80.2±9.3 | 122.5±0.1* | 156.0±0.2* |
| Control | pH | 7.52±0.06 | 7.48±0.07 | 7.51±0.09 |
| | PCO2(mmHg) | 44.6±0.7 | 45.4±0.9 | 43.5±0.6 |
| | $SAO_2$（%） | 96.2±1.3 | 99.4±0.2 | 99.6±0.2 |
| | $PO_2$（mmHg） | 81.2±7.3 | 131.4±1.5* | 160.5±0.6* |

$T_1$: baseline; $T_2$: at the end of hypoventilation; T3: preischemia

*$P$<0.05 as compared with baseline; # $P$<0.05 as compared with control

### 2.3 Infarct Size

The areas of risk and infarct sizes are presented in table 3. In $PETCO_2$ 80 mmHg group, HA significantly decreased infarct size as compared with the control group ($P$<0.01). In $PETCO_2$ 70 mmHg group, HA significantly decreased infarct size when compared with the control group ($P$<0.05). The infarct size in $PETCO_2$ 80 mmHg group was significantly smaller than that of $PETCO_2$ 60 mmHg group ($P$<0.05). The infarct sizes of group $PETCO_2$ 80 mmHg and group $PETCO_2$ 70 mmHg were not significantly different. The infarct size of group $PETCO_2$ 70 mmHg and group $PETCO_2$ 60 mmHg were not significantly different. The infarct size of $PETCO_2$ 60 mmHg group and control group were not significantly different.

**Table 3 Area at risk, infarct size and risk zone infracted**

| Groups | Area at risk (g) | Infarct size (g) | Necrosis/Area at risk (%) |
|---|---|---|---|
| A | 1.02±0.29 | 0.32±0.11 | 31.0±9.1*# |
| B | 1.07±0.33 | 0.36±0.15 | 34.5±9.4* |
| C | 1.04±0.28 | 0.41±0.16 | 42.4±7.9 |
| Control | 0.97±0.31 | 0.52±0.21 | 48.5±11.5 |

*$P$<0.05 as compared with control; #$P$<0.05 as compared with group C

## 3 DISCUSSION

The present study showed that hypoventilation successfully caused a hypercapnic acidosis (HA). HA with $PETCO_2$ 65–75 mmHg and 75–85 mmHg significantly decreased myocardial infarct size following a subsequent prolonged ischemic insult. $PETCO_2$ of 55–65 mmHg had no effect.

The result demonstrated that HA preconditioning could effectively protect the ischemia-reperfusion heart. It provides a new convenient method for anesthesiologists to protect the heart undergoing cardio-pulmonary bypass (CPB) surgery by adjusting the respiratory parameters. At the same time, it suggests that acidosis caused by ischemia is an important component of IPC, perhaps the trigger of the IPC. The other investigators exposed the isolated heart to $NH_4Cl$ for 10 min one to several times to acidify the heart and demonstrated that it could also effectively protect the heart from subsequent ischemia- reperfusion injury[5, 6]. They believed that acidosis *per se* may be an important component of IPC because the brief ischemic episodes of IPC result in transient acidosis and activation of $H^+$-sodium exchange, thereby accentuating intracellular calcium overload.

The present study demonstrated HA caused by low tidal volume and low respiratory frequency could protect the heart from ischemia-reperfusion injury of heart *in vivo*, and the protective effect was positively correlated with the end tidal carbon dioxide and pH when the range of end tidal carbon dioxide was below 85 mmHg and pH value was greater than 6.94. This end tidal carbon dioxide concentration and pH value range were suggested by the prior studies to be safe for animals and human beings[7, 8, 11]. Serious hypercapnia would cause low blood pressure, serious ventricular arrhythmias such as ventricular fibrillation or ventricular tachycardia[12]. It did not cause serious arrhythmias when the therapeutic hypercapnia was applied for ARDS and/or lung ischemic injury when the range of end tidal carbon dioxide was below 85 mmHg[13, 14]. Our results were consistent with these findidngs. No VF or VT developed in the control group and the hypercapnic acidosis groups during the preischemia period.

Ketamine and fentannyl were used as a basal anesthetic in this study. They might influence our results because ketamine has been shown to block preconditioning[15], whereas δ opiates such as fentannyl confers cardio-protection by mimicking ischemic preconditioning[16]. In this study, all groups received the same anesthetic protocol, and therefore, the drugs used could not

explain the differences among the groups.

The end points of injury used to demonstrate the protective effect include infarct size, stunning, and arrhythmias[17]. Infarct size reduction has been considered to be the gold standard for the efficacy of a preconditioning stimulus in protecting heart, and the infarct size is always diminished following IPC, myocardial stunning is not a good measure for the efficacy of acute IPC and, therefore, this study used the infarct size as the end point of injury.

The KATP channels play an important role in controlling membrane potential and cellular excitability [18-20]. The activation of KATP channels can confer cardio-protection. It is known that the KATP channels are made of the pore-forming Kir6 subunit and the sulfonylurea receptor (SUR) subunit[21]. One important property of the KATP channels is pH sensitivity. C-terminal histidine residue (His175) is likely to be the protonation site in Kir6.2 and to be responsible for the pH-dependent channel activation and previous studies showed that intracellular $H^+$ and ATP allosterically modulated the Kir6.2 channel[3,22]. This partly explains why the hypercapnic acidosis can protect the myocardium from ischemia-reperfusion injury as suggested by this study.

The KATP channels are stimulated by a brief exposure to moderate acidification. The activation degree increased when the pH was decreased and the maximal activation occurred at pH 6.5 to 6.8[3,4,23], which was consistent with the results of the present study. In this study the animals were assigned into different groups according to the degree of acidification (pH values) and the result demonstrated the pH worked in a dose-dependent manner. The protective effect increased with decreasing pH when PH value was greater than 6.94 and the end tidal $CO_2$ was less than 85 mmHg.

Acidification-protecting effect on KATP channel activity is limited by the acidification degree. The KATP channels were stimulated by a brief exposure to moderate acidification but inhibited with a longer exposure to lower pH. Khuri *et al* found that tissue with pH<6.2 during ischemia was associated with poor outcome in patients and in experimental animals with hypothermic myocardial ischemia[24,25]. The inhibitory effect was much more apparent at pH 5.7 and was only partially reversible. There must exist an upper limitation of hypercapnic acidification although this upper limitation was not defined in this study. The protective effect on the myocardium would become a harmful one when the pH was beyond this limitation. The range of pH value used in the present study was greater than 6.94, considering the adverse-effect of excessive carbon dioxide on the cardiovascular system such as VF or VT. Further study is needed to identify this limitation.

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(Received May 18, 2008)