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研究課題名（和文）心筋虚血再灌流後の好気的代謝復活による心筋傷害：二酸化炭素産生とカルシウム過負荷

研究課題名（英文）Ischemia/Reperfusion-induced Myocardial Damage: Role of Carbon Dioxide

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研究成果の概要（和文）：心筋虚血再灌流の過程ではCl-/HCO3-交換の関与が重要な役割を果たしている。血液心筋保護を用いた開心術における心筋細胞および赤血球CO2の移動（HCO3-としてCl-/HCO3-交換による細胞外排出）を検討した。開心術症例を対象とし大動脈遮断解除時の冠静脈洞液を採取した。赤血球のみのイオン移動を推定するため、in-vitroで無O2高CO2灌流下における血液心筋保護液のイオン濃度を測定した。血液心筋保護法では大動脈遮断中でも好気的代謝が残存しCO2が産生されるが、CO2が赤血球内緩衝系でHCO3-に変化後、Cl-/HCO3-交換系を介し赤血球外に排出され心筋細胞緩衝系に影響している。

研究成果の概要（英文）：We investigated the acid-base characteristic alterations of blood cardioplegia (BCP) during aortic cross-clamping in hearts arrested with BCP and during in vitro simulated ischemia. In 40 patients undergoing cardiac surgery following an aortic cross clamp, hearts were infused with a hypothermic BCP intermittently and lastly with a normothermic BCP prior to aortic cross clamp release. We measured pH, pCO2, [HCO3-], and [Cl-] of the coronary sinus effluent in the final BCP. BCP was assessed under in-vitro gassing at with 95% N2 + 5% CO2 (n = 6), 50% N2 + 50% CO2 (n = 3), or 100% CO2 (n = 6). The coronary sinus effluent, compared with the pre-infused BCP, exhibited significantly lower pH and greater pCO2 with no change in the [HCO3-] level. In vitro, the 100% CO2 gassing groups exhibited a significant increase in [HCO3-] under high pCO2-induced acidification. Under anoxia and CO2 retention during aortic cross-clamping, BCP can be a bicarbonate donor to the myocardium.

研究分野：　医歯薬学

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キーワード：　心筋虚血再灌流　二酸化炭素　ナトリウム/水素交換　ナトリウム/カルシウム交換

１．研究開始当初の背景

開心術における心筋虚血再灌流傷害の病態は細胞内Ca^{2+}過負荷であり、大動脈遮断中（虚血中）の細胞内アシドーシス（H^+産生）がNa^+/H^+交換によるNa^+流入とNa^+/Ca^{2+}交換を介するCa^{2+}流入と関連する。また大動脈遮断中にも残存酸素を利用した好気的代謝で低O_2とCO_2貯留が生じている。細胞内CO_2は拡散によって、またはH^+とHCO_3^-としてそれぞれNa^+/H^+交換とCl^-/HCO_3^-交換によって細胞外に排出される。酸素化血液心筋保護液（OBC）使用時の冠静脈洞（CS）液のイオン濃度を評価した。

２．研究の目的

心筋細胞内 CO_2 の Cl^-/HCO_3^-交換による細胞外排出に対する赤血球 Cl^-/HCO_3^-交換の影響に関する検討

３．研究の方法

開心術症例を対象とし大動脈遮断解除(OBC再灌流)時のCS液を採取した。CS液のイオン濃度は心筋細胞膜と赤血球膜における細胞内外のイオン移動が同時に関与するため、赤血球のみのイオン移動も別箇に考慮する必要がある。そのため虚血に暴露された赤血球のみのイオン移動を推定するため、in-vitroで無O_2高CO_2灌流下（虚血シミュレーション）における血液心筋保護液のイオン濃度を測定した。

４．研究成果

CS 液の Cl^-濃度は虚血前（正常 O_2正常 CO_2）の CS 液では変化なく、大動脈遮断解除時(OBC再灌流)の CS 液では有意に低下し（心筋+赤血球）、虚血シミュレーション（赤血球のみ）でも有意な低下を示し、大動脈遮断中のCl^-/HCO_3^-交換の関与が示唆された。また CS 液の HCO_3^-濃度は虚血前（正常 O_2正常 CO_2）の CS 液ではわずかに上昇しており、大動脈遮断解除時(OBC再灌流)の CS 液では濃度の変化がなく（心筋+赤血球）、虚血シミュレーション（赤血球のみ）では有意な上昇を示した。＜結論＞心筋虚血再灌流の過程では Cl^-/HCO_3^-交換の関与が重要な役割を果たしている。血液心筋保護法では大動脈遮断中でも好気的代謝が残存し CO_2が産生されるが、CO_2が赤血球内緩衝系で HCO_3^-に変化後、Cl^-/HCO_3^-交換系を介し赤血球外に排出され心筋細胞内緩衝系に影響している可能性が示唆された。

発表論文（査読あり）抜粋

Introduction

Blood cardioplegia (BCP) has been demonstrated to have potent protective effects against myocardial damage during ischemia and reperfusion, the mechanisms of which have been shown to provide the myocardium with physiological conditions including greater oxygen-supply capacity, higher osmotic pressure, greater acid-base balance capacity, and other numerous positive effects as compared with crystalloid cardioplegia.[1] Meta-analyses of randomized controlled trials dealing with comparison between blood and crystalloid cardioplegia have revealed the superiority of blood cardioplegia to crystalloid cardioplegia in terms of clinical outcomes or enzyme release.[2,3] Although the cardioprotective effects of BCP have been rationalized by theoretical and experimental hypotheses that the oxygen-supply capacity of erythrocytes contributes substantially more to aerobic metabolism as compared with crystalloid cardioplegia, the interaction between the myocardium and BCP in elimination processes of the carbon dioxide (CO_2) produced during aortic cross-clamping has not been elucidated.

During aerobic metabolism, CO_2 is produced through oxidative decarboxylation in the tricarboxylic acid cycle of the myocardial mitochondria. The CO_2 is simply diffused to the extracellular space (plasma in the capillary vessels) in the form of dissolved CO_2 and enters into the erythrocyte. Because of the absence of carbonic anhydrase (CA) in plasma,[4] the CO_2 is hydrated by erythrocyte CA to yield HCO_3^- and H^+ in the erythrocyte. The HCO_3^- (intra-erythrocyte HCO_3^-) is extruded to the extracellular space in exchange for Cl^- through the erythrocyte Cl^-/HCO_3^- exchange, and the H^+ (intra-erythrocyte H^+) is bound to hemoglobin (Hb) to yield the reduced form of Hb (HHb). Both HCO_3^- (in plasma) and HHb (in erythrocytes) are transported in the blood stream to the lungs for external respiration.

Myocardial ischemia is defined as a pathology characterized by anoxia and metabolite accumulation as a result of coronary artery occlusion (cessation of the bloodstream), causing intra-myocyte acidosis and CO_2 retention. Intra-myocyte acidosis under ischemic conditions is mainly due to ATP breakdown with concomitant anaerobic glycolysis,[3] which subsequently leads to intra-myocyte Na^+ increase and resultant myocardial damage due to intra-myocyte Ca^{2+} overload during reperfusion.[6] This intra-myocyte Na^+ increase is known to be caused by activation of alkalization systems (eg, Na^+/H^+ exchange; Na^+/ HCO_3^- cotransport) in response to increased intra-myocyte H^+ or decreased intra-myocyte HCO_3^-. Activation of the myocyte Cl^-/HCO_3^- exchange, an acidification system (HCO_3^- extrusion and Cl^- intrusion), has been demonstrated to induce intra-myocyte acidosis by reducing HCO_3^- during ischemia.[7] Prevention of intra-myocyte HCO_3^- reduction, which is accomplished by inhibiting the myocyte Cl^-/HCO_3^- exchange, may protect against myocyte damage during ischemia and

reperfusion. The CO_2 retention during ischemia results from failing to transport the CO_2 produced by the remaining aerobic metabolism during cessation of coronary perfusion. This increases the amount of intra-erythrocyte CO_2 and enhances intra-erythrocyte HCO_3^- production, followed by an increase of HCO_3^- extrusion via the erythrocyte Cl^-/HCO_3^- exchange. Therefore, the enhanced HCO_3^- extrusion in the erythrocyte under CO_2 retention may be related to cardioprotective effects of BCP by inhibiting the myocyte Cl^-/HCO_3^- exchange during an aortic cross clamp.

We hypothesized that under anoxia and hypercapnia during BCP cardioplegia, CO_2 retention results in elevation of the extracellular HCO_3^- concentration by activating the erythrocyte Cl^-/HCO_3^- exchange, which may be related to cardioprotective effects of BCP by enhancing buffering capacity against ischemia-induced intra-myocyte acidosis. However, the effect of CO_2 retention under anoxic conditions on acid-base characteristics of BCP (eg, Cl^- and HCO_3^- concentrations) has not been investigated clinically or experimentally. To test this, the present study was designed to examine the characteristics of BCP (1) during an aortic cross-clamping in hearts arrested with BCP in a clinical setting and (2) during in vitro simulated ischemia (anoxia and hypercapnia).

Methods

In the clinical study, 40 consecutive adult patients who underwent cardiac surgery (47 ± 4 years old; 22 men and 18 women) were included in the study. Consecutive patient sampling was used to avoid selection bias in terms of disease type, sex, and age. Inclusion criteria included the following: patients 15 years or older and patients with heart disease requiring cardioplegic arrest during cardiac surgery. Exclusion criteria included the following: patients requiring emergency operations (eg, acute myocardial infarction and acute aortic dissection) and patients with chronic obstructive pulmonary disease. Acid-base characteristics of BCP and the coronary sinus effluent sampled during the infusion of terminal BCP prior to aortic cross clamp release were examined using a blood gas analyzer. Informed consent was obtained from each patient. A catheter for the antegrade infusion of cardioplegic solution was inserted into the ascending aorta and a catheter for retrograde infusion of cardioplegic solution was inserted into the coronary sinus. Circuit blood and coronary sinus effluent were sampled, and then the aorta was cross-clamped. Subsequently, the heart was arrested with an antegrade infusion of Young's solution (2 ml/kg body weight) and then infused with oxygenated potassium BCP (antegrade and retrograde, at 18°C every 30 minutes, 15 ml/kg body weight/infusion). Prior to release of the aortic cross clamp, the heart was normothermically (34°C - 36°C) infused with oxygenated BCP (antegrade, 10 ml/kg body weight), and coronary effluent during the BCP infusion was sampled through the retrograde cardioplegic infusion catheter. Young's solution contained 145.4 mM NaCl, 25.0 mM CH_2OHCH_2 (COOK) $_3$•H_2O, and 99.8 mM $MgSO_2$•$7H_2O$. Oxygenated BCP was prepared by mixing circuit blood with crystalloid stock solution (one-to-one volume ratio) through a Y-shaped tubing set in the roller pump. The crystalloid stock solution contained 120.3 mM NaCl, 24.0 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $CaCl_2$•$2H_2O$, 1.3 mM $MgSO_2$•$7H_2O$, 10.0 mM $MgCl_2$•$6H_2O$, and 25.0 mM $NaHCO_3$.

In the in vitro study, one liter of the pre-infused BCP solution for 15 patients was gassed in a thermostatically-controlled beaker for 10 minutes with 95% N_2 + 5% CO_2 (n = 6), 50% N_2 + 50% CO_2 (n = 3), or 100% CO_2 (n = 6) to assess the effect of simulated ischemia (anoxia and hypercapnia) on acid-base characteristics of BCP (in vitro CO_2 gassing). The temperature of the beaker was kept constant at 34°C.

Using a blood gas analyzer (Radiometer ABL 2, Copenhagen), we measured Hb concentration, oxygen saturation (SO_2), pO_2, pCO_2, pH, Na^+ concentration ($[Na^+]$), K^+ concentration ($[K^+]$), Cl^- concentration ($[Cl^-]$) and calculated the HCO_3^- concentration ($[HCO_3^-]$). Oxygen content (C_O) of the circuit blood, BCP, or coronary sinus effluent was calculated using the following equation:

$$C_O = 1.39 \times \text{Hb concentration} \times SO_2/10 \text{ (ml/l sample volume)}$$

The amount of dissolved oxygen in solution was ignored, because it is much smaller than the amount of Hb-bonded oxygen.

In the clinical study, a pCO_2-pH relation of the coronary sinus effluent sampled before aortic cross clamp release was constructed to assess the effect of BCP on the Henderson-Hasselbalch relationship in the ischemic environment.

Statistical analysis

Data are reported as means ± standard errors of the means. Comparisons between the two groups were performed using the paired Student's t-test. A probability of less than 5% ($p < 0.05$) that a difference between groups occurred by chance was accepted as being statistically significant.

Results

Patient characteristics

Valvular disease was diagnosed in twenty patients, coronary artery disease in seven, congenital disease in ten, and cardiac tumors were found in two. The aortic cross clamp time varied from 23 to 214 minutes (mean time, 107.9 ± 7.9 minutes). There was no hospital mortality or morbidity, and all the patients had uneventful postoperative courses.

Characteristics of the coronary sinus effluent before placement and release of the aortic cross clamp (clinical study)

Both circuit blood and coronary sinus effluent were sampled before placement of the aortic cross clamp (ie, post-ischemic sampling) in 15 of the 40 patients. The coronary sinus effluent exhibited significantly lower SO_2, C_O, pH, and $[K^+]$ but significantly greater pCO_2 and $[HCO_3^-]$ compared with the circuit blood. However, there was no difference between the circuit blood and coronary sinus effluent in terms of $[Na^+]$ or $[Cl^-]$. The composition of the BCP used is shown in Table 2. In the post-ischemic period (ie, prior to aortic cross clamp release), the coronary sinus effluent exhibited significantly lower SO_2, C_O, pH, $[K^+]$, and $[Cl^-]$ values but significantly higher pCO_2, and $[Na^+]$ values compared with the pre-infused BCP (Table 2). The $[HCO_3^-]$ was the same as that of the pre-infused BCP. The pCO_2-pH relationship of the coronary sinus effluent did not shift downward.

Effects of in vitro CO_2 gassing on the characteristics of BCP (Table 3)

In the 95% N_2 + 5% CO_2 gassing group, the pCO_2, pH, $[Na^+]$, and $[K^+]$ values remained the same before and after gassing. This group, however, showed a slight but significant increase in $[HCO_3^-]$ and $[Cl^-]$ with a significant decrease in SO_2 and C_O after gassing. Conversely, the 50% N_2 + 50% CO_2 gassing group and the 100% CO_2 gassing group exhibited a significant increase in $[HCO_3^-]$ and $[Na^+]$ with an elevated pCO_2 and lowered pH after gassing, which was accompanied by a significant decrease in SO_2, C_O, and $[Cl^-]$ (Figure 2). The Hb and $[K^+]$ values remained the same before and after gassing in all groups.

Discussion

In the present study, prior to aortic cross clamp release, the postischemic coronary effluent showed no downward shift of the pCO_2-pH relationship (ie, no decrease in $[HCO_3^-]$), and the simulated ischemic BCP condition (ie, anoxic CO_2 gassing to blood cardioplegia) resulted in a significant increase in $[HCO_3^-]$ under high pCO_2-induced acidification (low pH). This suggests a HCO_3^--supplying effect of blood cardioplegia to the myocardium in response to anoxia and hypercapnia.

In the aerobically perfused myocardium, the characteristics of the coronary sinus effluent reflect the normal interaction between the myocardium and blood, which includes O_2 and CO_2 transport, metabolite elimination, pH regulation, and ion homeostasis. The preischemic baseline characteristics of the coronary sinus effluent in the present (clinical) study demonstrated that a decrease in oxygen content of coronary sinus effluent, as a result of O_2 delivery to the myocardium, was accompanied by an increase in pCO_2, a decrease in pH, and an increase in HCO_3^-. The increase in HCO_3^- of the coronary sinus effluent may be associated with intra-erythrocyte CO_2 hydration in response to myocardial CO_2 production during aerobic metabolism. In the ischemic myocardium under the protection of BCP, however, the myocardium and BCP are exposed simultaneously to anoxia and hypercapnia in a confined coronary vascular bed, which may result in altered interaction between the myocardium and BCP. Coronary sinus effluent sampled prior to aortic cross clamp release in the present (clinical) study showed that a decrease in oxygen content was accompanied by an increase in pCO_2 and a decrease in pH. It was not, however, accompanied by an increase in HCO_3^- suggesting more HCO_3^- consumption during myocardial ischemia than during normal aerobic coronary perfusion to buffer the H^+ produced by myocardial ATP breakdown during ischemia. The amount of HCO_3^- was constant despite a pH decrease associated with an unphysiological CO_2 accumulation, which is evidenced by no downward shift of the pCO_2-pH relationship in the coronary effluent during myocardial ischemia. Such an unphysiological CO_2 accumulation under hypoxic conditions may be indicative of the potential role of BCP as a HCO_3^- supplier to compensate for the ischemia-induced myocyte H^+ production. We investigated acid-base characteristic alterations of BCP in response to simulated ischemic conditions (ie, anoxia, hypercapnia, and acidosis) in the in vitro CO_2 gassing model, because the results can be clearly demonstrated by unphysiologically low O_2 and high CO_2 conditions.

Erythrocyte pH has been reported to increase with a decrease in oxygen saturation (hypoxia),[8] the mechanism of which may be related to H^+ extrusion with the erythrocyte Na^+/H^+ exchange because it is oxygen-sensitive and activated at low pO_2.[9] An increase in erythrocyte pH promotes erythrocyte HCO_3^- formation at a given pCO_2.[10] The 95% N_2 + 5% CO_2 gassing group in the present study, which was examined in an anoxic environment without

hypercapnia, showed an increase in extracellular [HCO_3^-] suggesting the possibility of hypoxia-induced erythrocyte HCO_3^- formation. Also, pCO_2 elevation (hypercapnia) enhances CO_2 supply to erythrocytes because their membranes are permeable to CO_2. This elevation increases the amount of erythrocyte CA-catalyzed CO_2 hydration thus producing HCO_3^- and H^+ in the erythrocyte. The H^+ produced is buffered by being bound to Hb, whereas the HCO_3^- produced is easily extruded to the outside of the erythrocyte because the erythrocyte membrane is known to be permeable to Cl^- and HCO_3^- through the Cl^-/HCO_3^- exchange.[11] In support of these experimental findings, the present study demonstrated that the CO_2 gassing (50% N_2 + 50% CO_2; 100% CO_2) groups exhibited a significant [HCO_3^-] increase and [Cl^-] decrease with concomitant anoxia and hypercapnia after gassing. This suggests the ischemic environment in the confined coronary vascular bed may, by increasing extracellular [HCO_3^-], enhance the ability of blood cardioplegia to alleviate extracellular acidosis, resulting in the intra-myocyte HCO_3^- elevation caused by inhibition of myocyte Cl^-/HCO_3^- exchange (Figure 3).

Relative contribution of the myocardium to the [Cl^-] decrease of coronary sinus effluent during myocardial ischemia has not been determined. In a myocyte, intracellular acid-base balance is regulated by alkalization systems (eg, Na^+/H^+ exchange, Na^+/HCO_3^- cotransport) and acidification systems (eg, Cl^-/HCO_3^- exchange, Cl^-/OH^- exchange).[12-14] Transsarcolemmal Cl^- intrusion and HCO_3^- extrusion via the Cl^-/HCO_3^- exchange has been reported to play an important role in the intracellular acidification during pH regulation and be responsible partly for intracellular acidosis during ischemia. Hypercapnia-induced extracellular acidosis has been reported to activate the Cl^-/HCO_3^- exchange to promote intracellular acidosis by extruding HCO_3^-, thus leading to an increase in intracellular Na^+ through the Na^+/H^+ exchange.[15] Stilbene derivatives, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid (SITS) and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), known as Cl^-/HCO_3^- exchange blockers, have been demonstrated to suppress the development of intracellular acidosis during myocardial ischemia and exert a protective effect against ischemia and reperfusion-induced myocardial injury.[7,16] Activation of the Cl^-/HCO_3^- exchange may result in intracellular Cl^- increase with concomitant intracellular acidosis. This possibility is suggested in several studies dealing with intracellular acidosis in a ventricular papillary muscle tissue subjected to simulated (paraffin oil) ischemia[17] or coronary occlusion-induced ischemia.[18] In the present study, the coronary sinus effluent exhibited a substantial [Cl^-] decrease prior to aortic cross clamp release and the extent of the [Cl^-] decrease was much greater in the coronary sinus effluent than that in the CO_2-gassed BCP suggesting partial involvement of myocardial Cl^- uptake in the [Cl^-] decrease of the coronary sinus effluent during ischemia.

Changes in extracellular cation (Na^+ and K^+) concentrations during aortic cross-clamping may reflect the interaction between the myocardium and BCP. In the present study, the coronary sinus effluent prior to releasing the aortic cross clamp, showed a significant [Na^+] increase and [K^+] decrease. The [Na^+] increase of the coronary sinus effluent may be attributed to the response of erythrocytes to anoxia and hypercapnia, because BCP resulted in a significant [Na^+] increase when gassed in vitro with a 50% N_2 + 50% CO_2 gas or a 100% CO_2 gas. However, the [K^+] decrease of coronary sinus effluent could be partially involved in myocardial K^+ uptake, because [K^+] did not decrease in any CO_2 gassing groups.

References

1. Barner HB. Historical aspects and current review of myocardial protection. In: Salerno TA, ed. Warm heart surgery. London, UK: Arnold, a member of the Hodder Headline Group; 1995, p. 1-15.
2. Guru V, Omura J, Alghamdi AA, Weisel R, Fremes SE. Is blood superior to crystalloid cardioplegia? A meta-analysis of randomized clinical trials. Circulation 2006; 114[suppl I]: I-331-8.
3. Jacob S, Kallikourdis A, Sellke F, Dunning J. Is blood cardioplegia superior to crystalloid cardioplegia? Interact Cardiovasc Thorac Surg 2008; 7: 491-9.
4. Gilmour KM, Perry SF, Bernier NJ, Henry RP, Wood CM. Extracellular carbonic anhydrase in the dogfish, *Squalus acanthias*: a role in CO_2 excretion. Physiol Biochem Zool 2001; 74: 477-92.
5. Denis SC, Gevers W, Opie LH. Protons in ischemia: where do they come from; where do they go to? J Mol Cell Cardiol 1991; 23: 1077-86.
6. Pike MM, Kitakaze M, Marban E. 23Na-NMR measurements of intracellular sodium in intact perfused ferret hearts during ischemia and reperfusion. Am. J Physiol 1990; 259: H1767-73.
7. Lai ZF, Liu J, Nishi K. Effects of stilbene derivatives SITS and DIDS on development of intracellular acidosis during ischemia in

isolated guinea pig ventricular papillary muscle in vitro. Jpn J Pharmacol 1996; 72: 161-74.
8. Jensen FB. Pronounced influence of Hb-O_2 saturation on red cell pH in tench blood in vivo and in vitro. J Exp Zool 1986; 238: 119-24.
9. Motais R, Garcia-Romeu F, Borgese F. The control of Na^+/H^+ exchange by molecular oxygen in trout erythrocytes. A possible role of haemoglobin as a transducer. J Gen Physiol 1987; 90: 197-207.
10. Jensen FB. Red blood cell pH, the Bohr effect, and other oxygenation-linked phenomena in blood O_2 and CO_2 transport. Acta Physiol Scand 2004; 182: 215-27.
11. Geers C, Gros G. Carbon dioxide transport and carbonic anhydrase in blood and muscle. Physiol Rev 2000; 80: 681-715.
12. Sun B, Leem CH, Vaughan-Jones RD. Novel chloride-dependent acid loader in the guinea-pig ventricular myocyte: part of a dual acid-loading mechanism. J Physiol 1996; 495: 65-82.
13. Leem CH, Vaughan-Jones RD. Sarcolemmal mechanisms for pH1 recovery from alkalosis in the guinea-pig ventricular myocyte. J Physiol 1998; 509: 487-96.
14. Leem CH, Lagadic-Gossmann D, Vaughan-Jones RD. Characteristics of intracellular pH regulation in the guinea-pig ventricular myocyte. J Physiol 1999; 517: 159-80.
15. Harrison SM, Frampton JE, McCall E, Boyett MR, Orchard CH. Contraction and intracellular Ca^{2+}, Na^+, and H^+ during acidosis in rat ventricular myocytes. Am J Physiol 1992; 262: C348-57.
16. Kawasaki H, Otani H, Mishima K, Imamura H, Inagaki C. Involvement of anion exchange in the hypoxia/reoxygenation-induced changes in pH_i and $[Ca^{2+}]_i$ in cardiac myocyte. Euro J Pharmacol 2001; 411: 35-43.
17. Lai ZF, Nishi K. Intracellular chloride activity increases in guinea pig ventricular muscle during simulated ischemia. Am J Physiol 1998; 275 (Heart Circ Physiol 44): 1613-9.
18. Tanaka H, Matsui S, Kawanishi T, Shigenobu K. Use of chloride blockers: A novel approach for cardioprotetion against ischemia-reperfusion damage. J Pharmacol Exp Ther 1996; 278: 854-61.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計１件）
Blood Cardioplegia Serves as a Bicarbonate Donor to the Myocardium during Ischemia: Effects of Anoxia and Hypercapnia on Acid-Base Characteristics of Blood Cardioplegic Solution.

Hiroshi Yamamoto, Kazutomo Goh, Katsuaki Magishi, Tadahiro Sasajima, Fumio Yamamoto
Eur Surg Res 2011;47:267–273

〔学会発表〕（計０件）

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