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Effects of pH, PCO_2, and Adenosine on the Contractility of Pig Coronary Artery

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= ABSTRACT =

Effects of pH, PCO_2, and adenosine on the vascular contractility were investigated in the pig coronary arteries. The helical strips of isolated coronary arteries were immersed in the HEPES or HCO_3^-/CO_2-buffered Tyrode's solution equilibrated with 100% O_2 or 95% O_2-5% CO_2 at 35 °C. The contraction was recorded isometrically using a force transducer.

The amplitudes of contraction induced by ACh, high K^+, and electrical field stimulation (EFS) were decreased by elevating extracellular pH (pHo) and were increased by lowering pHo. A shift from 0% CO_2 to 5% CO_2 at constant pHo (pH 7.4) reduced the contractions induced by ACh, high K^+, EFS. However the contraction induced by 100mM K^+ was less influenced by the change of pHo or CO_2. The contraction induced by ACh in Ca^{2+}-free Tyrode's solution as well as the contraction developed by the addition of extracellular of Ca^{2+} were decreased by lowering pHo and were increased by elevating pHo. High K^+ (25mM)-induced contraction at pH 6.8 was not returned to the level of the contraction at pH 7.4 by the elevation of extracellular calcium $[Ca^{2+}]_o$. Adenosine-induced relaxation was more significant with 5% CO_2 than 0% CO_2 in the high K^+-induced contraction and was more significant with low pHo than high pHo in the contraction induced by EFS.

From the above results, it is suggested that H^+ and CO_2 inhibit Ca^{2+} influx as well as Ca^{2+} release from intracellular Ca^{2+} storage sites and enhance the relaxing effect of adenosine in the pig coronary artery.

Key Words: pH, PCO_2, Adenosine, Pig coronary artery.

INTRODUCTION

Coronary blood flow is controlled by mechanical, metabolic, and neural factors. Coronary blood flow is regulated almost entirely by vascular response to local needs of the cardiac musculature. This mechanism is well operated even though the nerves to the heart are removed. Metabolic factors such as PO_2, PCO_2, H^+, and adenosine etc. are well known as a major controller of coronary blood flow (Berne & Levy, 1986)

It was well known that arterial tone could be influenced by changes in extracellular pH (MacLellan et al, 1974). Especially, cerebral, coronary, and pulmonary artery were more sensitive to hydrogen ion than other kinds of arteries (Wary, 1988). In general, several evidences suggest that the lowering of pH is associated with a decrease in vascular smooth muscle tone. Whereas, in the pulmonary artery, lowering pH produced a vasoconstriction rather than the relaxation (Berne & Levy, 1986). Although ample evidence indicates that hydrogen ion induces dramatic alterations in smooth muscle contractility, the mechanisms responsible for such effect have not been

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defined.

Several actions of hydrogen ion could theoretically account for the inhibitory actions of hydrogen ion on smooth muscle contractility. Low pH has been shown to 1) reduce calcium influx due to increasing of membrane potential (Äberg et al, 1967; van Breemen et al, 1972; Kohlhardt et al, 1976; Löfqvist & Nilsson, 1981), 2) increase in intracellular calcium sequestration (Loutzenhiser et al, 1990), 3) inhibit the activation of contractile apparatus (Portzenhl et al, 1969; Mrwa et al, 1974; Fabiato & Fabiato, 1978), 4) inhibit the activation of receptor due to directly binding of hydrogen ions to the receptor (Flavahan & McGrath, 1981).

A close correlation between cardiac carbon dioxide production and coronary blood flow was well known (Barcroft & Dixon; 1907). Carbon dioxide which is produced by continuous cardiac metabolism can influence on the resistance vessels due to its high membrane permeability and intracellular acidification by it. However, effect of the change in intracellular hydrogen ion concentration on the contractility was still controversy. Spurway and Wary (1987) reported using a phosphorus nuclear magnetic resonance that vascular tone was increased by cytosolic acidification. On the other hand, Busa and Nuccitelli (1984) suggested that cytosolic alkalization resulted in augmentation of binding between calmodulin and Ca^{2+} and prolonged the smooth muscle contraction. Siskind et al (1989) also reported that alkalization of smooth muscle led to a rise in cytosolic Ca^{2+} via Ca^{2+} release from intracellular Ca^{2+} store.

Adenosine is the mediator of metabolically controlled coronary vasomotion, linking cardiac metabolism and coronary vasodilation (Berne & Levy, 1986). Recent evidence suggested that adenosine could play a key role as a metabolic vasodilator. According to the adenosine hypothesis, a reduction in myocardial O_2 tension which is caused by low coronary blood flow, hypoxia, or increased metabolic activity of the heart lead to the myocardial formation of adenosine, which crosses the interstitial fluid space to reach the coronary resistance vessels and induce vasodilation via activating an adenosine receptor. However, it is little known that the interrelation between adenosine-induced relaxation and the change of extracellular or intracellular hydrogen ion concentrations.

The present study was designed to investigate the effects of hydrogen ions, carbon dioxide, and adenosine on the coronary arterial contraction as well as to investigate the interrelation among the metabolic factors in the coronary arterial contraction.

METHOD

Pig hearts were obtained from slaughter house and transported to the laboratory in ice-cold (4°C) oxygenated Tris-buffered Tyrode's solution. Branches of left anterior descending coronary artery (about 1mm OD) were carefully dissected, cleaned of fat and periarterial connective tissue, and recovered for 2 hours at room temperature. For the measurement of mechanical tension, coronary arteries cut spirally with a width of approximately 2 mm and a length of 10 mm. After muscle strips with intact endothelium were fixed with silk in glass hook, muscle strips were suspended in a themostatically controlled (35°C) organ bath (50ml) under 0.25g tension. Changes of tension were measured isometrically by means of force transducer (F-60, Narco Biosystem), then amplified and recorded on physiograph (MK-IV, Narco Biosystem) (Fig. 1).

For electrical field stimulation to muscle strips, platinum plate electrodes (about 0.5cm x 2.5cm) placed near muscle strips. The intensity of electrical stimulation was controlled by suppressing 110 volts with alternating current (60Hz) and muscle strips were stimulated for 15 seconds every 3 minutes at a range of current strengths (0.1 $\sim$ 1.0A) (Fig. 1).

The change of extracellular pH (6.8, 7.4, 8.0) was accomplished by titrating with 0.1N NaOH in the HEPES-buffered Tyrode's solution. The decrease of intracellular pH at constant ex-

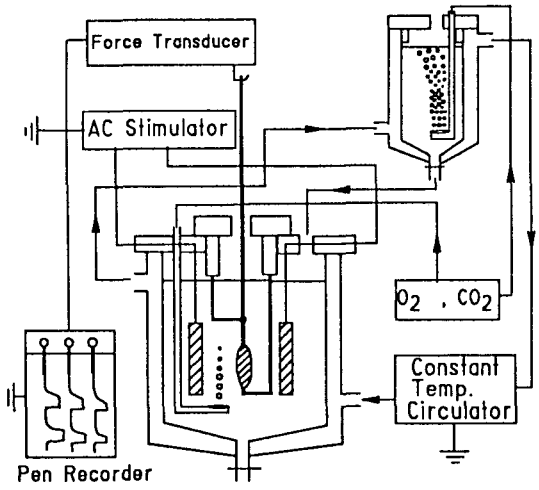

Fig. 1. Experimental setup for measurement of the isometric tension in the vascular smooth muscle strips

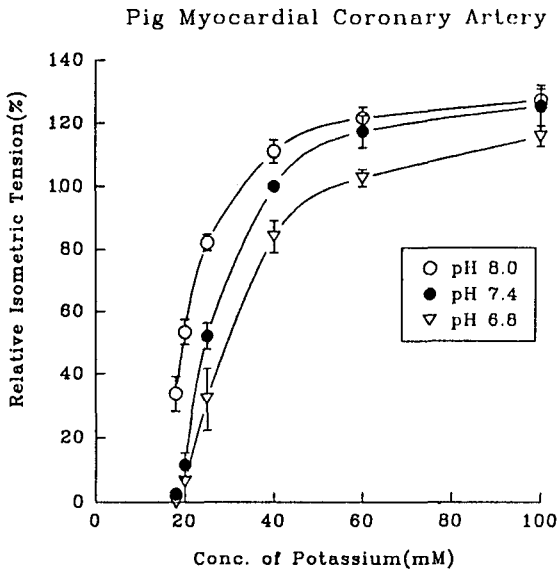

Fig. 2. Effects of the changes of extracellular pH on the contractions induced by high K^+ in the pig coronary artery. Ordinate: Percentage of maximal tension induced by 40mM K^+ at pH 7.4. Points show mean of responses of 6 arteries. Vertical bars show S.E.M..

tracellular pH (pH 7.4) was accomplished by changing from HEPES-buffered Tyrode's solution equilibrated with 100% O_2 to HCO_3^-/CO_2-buffered Tyrode's solutions equilibrated with 5% CO_2.

HEPES-buffered Tyrode's solution was composed as following composition (mM): NaCl 158, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 5, and Glucose 6. This solution was oxygenated by equilibration with 100% O_2. HCO_3^-/CO_2-buffered Tyrode's solution was composed as following composition (mM): NaCl 135, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, $NaHCO_3$ 27, and Glucose 6. This solution was oxygenated by equilibration with 5% CO_2 + 95% O_2. The pH of these solutions was adjusted to 7.4 at 35°C. The high K^+ Tyrode's solution was made by replacing NaCl in the Tyrode's solution with an equivalent concentration of KCl. To measure contractile responses in Ca^{2+}-free solution, tissues were placed in Tyrode's solution without added calcium and with 0.1mM EGTA.

Statistical analysis of the data was assessed by Student's t-test and differences were considered significant at $p<0.05$.

RESULTS

Effects of extracellular pH (pHo) change on the contractions

Effects of extracellular pH (pHo) change on the contraction induced by high K^+, ACh and electrical field stimulation were investigated. Arterial strips were exposed to the range of 15mM~100mM K^+ gradually at pH 6.8, 7.4, 8.0 respectively. Each response is expressed as percent of the maximal contraction induced by 40mM K^+ at pHo 7.4. As shown in Fig. 2, lowering pHo from 7.4 to 6.8 shifted the dose-response curve of high K^+ toward the right and elevating pHo from 7.4 to 8.0 shifted the curve to the opposite direction. However, the contraction induced by 100mM K^+, maximal contraction, at pHo 6.8, 7.4, 8.0 are less influenced by pHo change ($p>0.1$).

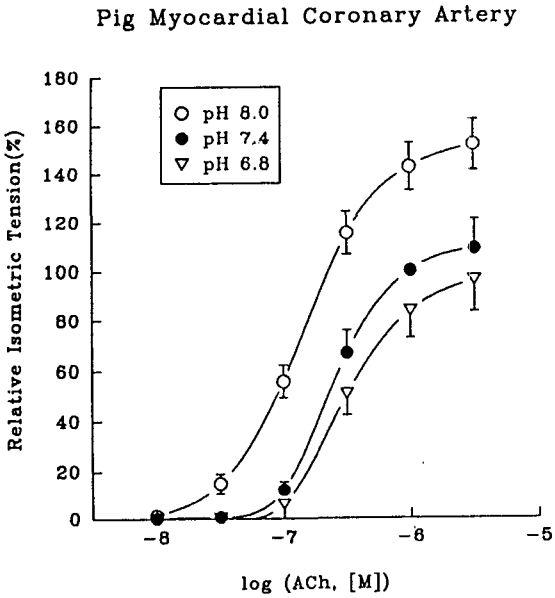

Fig. 3. Effects of the changes of extracellular pH on the contractions induced by ACh in the pig coronary artery. Ordinate: Percentage of maximal tension induced by ACh (10^{-6}M) at pH 7.4. Points show mean of responses of 8 arteries. Vertical bars show S.E.M..

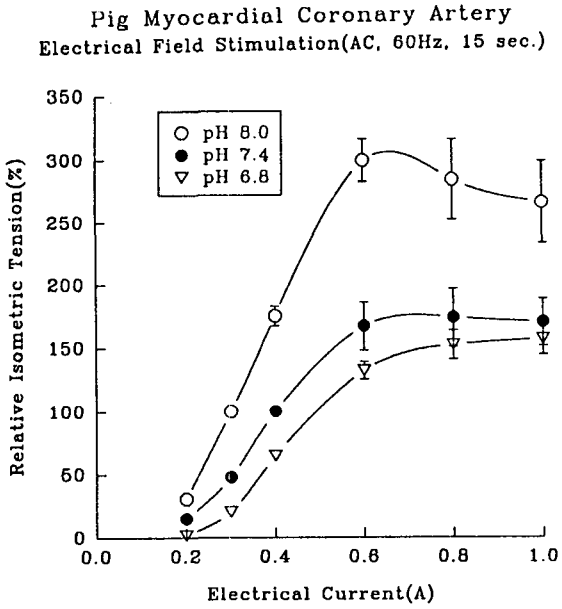

Fig. 4. Effects of the changes of extracellular pH on the contractions induced by electrical field stimulation in the pig coronary artery. Ordinate: Percentage of maximal tension induced by 0.4 A at pH 7.4. Points show mean of responses of 6 arteries. Vertical bars show S.E.M..

ACh was added to the bath cumulatively (from 10^{-8}M to $3x10^{-6}$M) at pHo 6.8, 7.4, 8.0, respectively. Each response is expressed as percent of the maximal contraction induced by 10^{-6}M ACh at pHo 7.4. As shown in Fig. 3, lowering pHo from 7.4 to 6.8 shifted the dose-response curve of ACh toward the right and reduced the maximum tension. Elevating pHo from 7.4 to 8.0 shifted the curve to the opposite direction. The magnitude of tension development was much more affected by elevating pH than lowering pH.

Arterial strips were exposed to the electrical field stimulation (EFS) at pHo 6.8, 7.4, 8.0, respectively. Electrical current for EFS was the range of 0.2∼1.0A. EFS-induced contraction was phasic contraction, so each peak tension was calculated as the amplitude of contraction. Each response is expressed as percent of the maximal contraction induced by 0.4A at pH 7.4. As shown in Fig 4, lowering pHo from 7.4 to 6.8 shifted the dose-response curve of EFS toward the right and reduced the maximum tension. Elevating pHo from 7.4 to 8.0 shifted the curve to the opposite direction. EFS-induced contractions at pH 6.8, 7.4 were current dependent at the range of 0.2∼1.0A, and EFS-induced contraction at pH 8.0 was current dependent at the range of 0.2∼0.6A. However the contraction induced by 1.0A at pH 8.0 was slightly decreased and smaller than contraction induced by 0.6A. The magnitude of tension development was much more affected by elevating pH than lowering pH.

Effects of PCO_2 change on the contractions

In order to evaluate the effect of cytosolic acidification on the contractions, arterial strips were exposed in HCO_3^-/CO_2-buffered Tyrode's

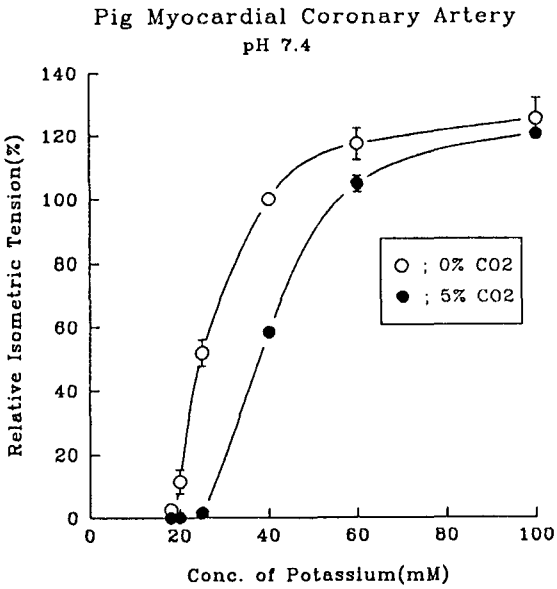

Fig. 5. Effects of the changes of CO_2 on the contractions induced by high K^+ at constant extracellular pH (7.4) in the pig coronary artery. Ordinate: Percentage of maximal tension induced by 40mM K^+ at 0% CO_2 (pH 7.4). Points show mean of responses of 6 arteries. Vertical bars show S.E.M..

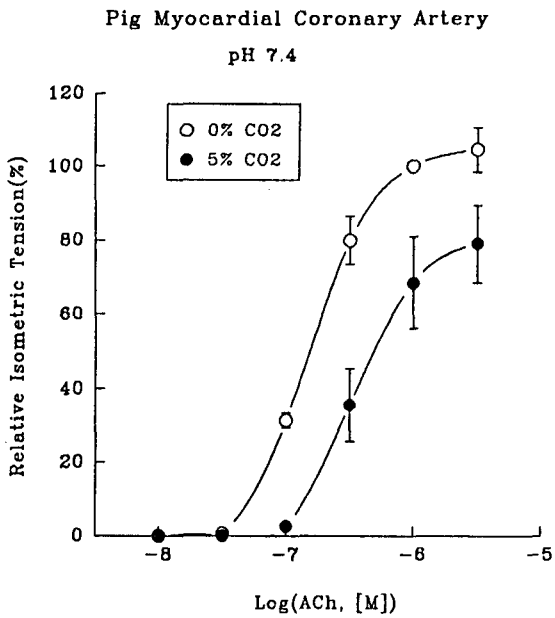

Fig. 6. Effects of the changes of CO_2 on the contractions induced by ACh (10^{-6}M) at constant extracellular pH (7.4) in the pig coronary artery. Ordinate: Percentage of maximal tension induced by ACh (10^{-6}M) at 0% CO_2 (pH 7.4). Points show mean of responses of 6 arteries. Vertical bars show S.E.M..

solution. Effect of PCO_2 change on the contractions induced by high K^+ is shown in Fig. 5. Each response is expressed as percent of maximal contraction induced by 40mM K^+ in 0% CO_2 (pH 7.4). An increase of PCO_2 from 0% to 5% at constant pHo 7.4 shifted the dose-response curves of high K^+ toward the right. However maximal contractile response (100mM K^+) was little affected by the elevation of PCO_2. Effects of PCO_2 change on the contractions induced by ACh is shown in Fig. 6. Each response is expressed as percent of maximal contraction induced by 10^{-6}M ACh in 0% CO_2 (pH 7.4). An increase of PCO_2 also shifted the dose-response curves of ACh toward the right and reduced maximum tension. Effect of PCO_2 change on the contractions induced by electrical field stimulation is shown in Fig. 7. Each response is expressed as percent of maximal contraction induced by 0.4A in 0% CO_2 (pH 7.4). As shown in Fig. 7, an increase of PCO_2 shifted the dose-response curves of EFS toward the right and reduced maximum tension ($p<0.04$).

Effects of pHo change on Ca^{2+} mobilization and Ca^{2+} influx

The effects of pHo change on Ca^{2+} mobilization and Ca^{2+} influx were investigated. Arterial strips were contracted by ACh in the Ca^{2+}-free Tyrode's solution. However, only phasic contraction was produced without external Ca^{2+}. The addition of Ca^{2+} increased the contractile force and produced the tonic contraction. In order to investigate the effect of pH on these contractions, arterial strip was incubated in Ca^{2+}-free Tyrode's solution containing ACh at

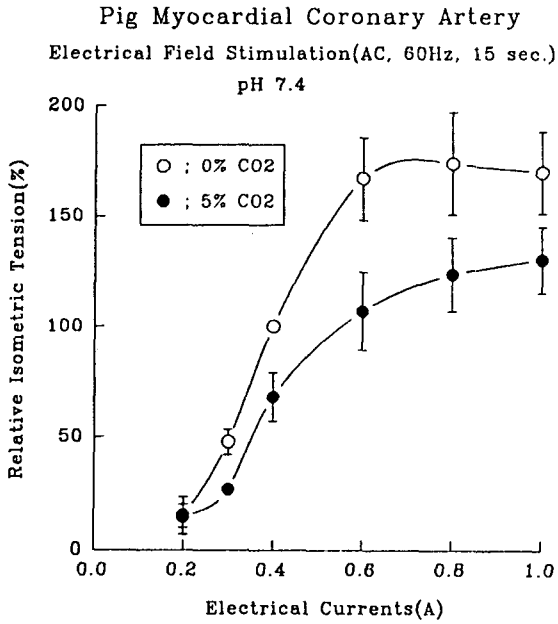

Fig. 7. Effects of the changes of CO_2 on the contractions induced by electrical field stimulation at constant extracellular pH (7.4) in the pig coronary artery. Ordinate: Percentage of maximal tension induced by 0.4 A at 0% CO_2 (pH 7.4). Points show mean of responses of 6 arteries. Vertical bars show S.E.M..

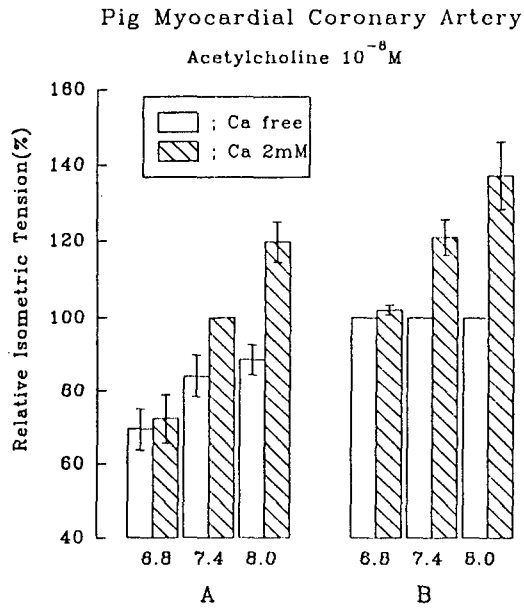

Fig. 8. Effects of the change of extracellular pH on the contractions induced by ACh (10^{-6}M) in the Ca^{2+}-free Tyrode's solution and those contractions after the addition of 2mM Ca^{2+} in the pig coronary artery. Ordinate for A; Percentage of maximal tension induced by ACh (10^{-6}M) at pH 7.4. Oridnate for B ; Percentage of maximal tension induced by ACh (10^{-6}M) in the Ca^{2+}-free Tyrode's solution at each pH. Bars show mean of responses of 10 arteries. Vertical bars show S.E.M..

pH 6.8, 7.4, 8.0, respectively, and Ca^{2+} was added to the bath when the contraction reached peak. In Ca^{2+}-free Tyrode's solution, amplitudes of ACh-induced contraction gradually increased by elevating the pH from 6.8 to 8.0 (Fig. 8A). The higher the pH was, the amplitudes of tonic contraction was much more affected. Namely at low pH (6.8), the addition of Ca^{2+} has little effect on the magnitude of contraction ($p>0.1$). On the other hand, at high pH (pH 8.0), the addition of Ca^{2+} has much more increasing effect on the contractile force (Fig. 8B) ($p<0.02$).

Effect of calcium on the suppressed contraction by low pH

The effect of Ca^{2+} on the contraction which was suppressed by low pH was investigated (Fig. 9). After the incubation of arterial strips in the Ca^{2+}-free and 25mM K^+ Tyrode solution at pH 6.8, cumulative addition of Ca^{2+} increased the amplitude of contraction. However, as shown in Fig. 9, the maximum did not reach the amplitude of contraction that was produced by 25mM K^+ Tyrode solution at pH 7.4.

Effects of adenosine on the contractions at different pH or CO_2

To investigate that hydrogen ion and carbon dioxide have any influence on the relaxing ef-

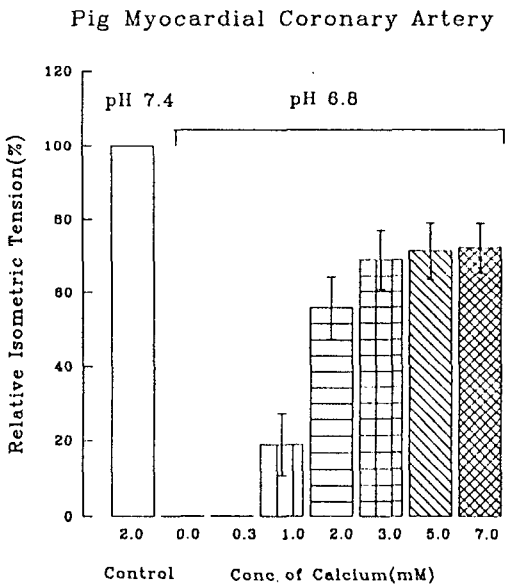

Fig. 9. Effects of calcium concentrations on the contractions induced by 25mM K^+ at extracellular pH 6.8 in the pig coronary artery. Ordinate: Percentrage of maximal tension induced by 25mM K^+ at pH 7.4. Bars show mean of responses of 6 arteries. Vertical bars show S.E.M..

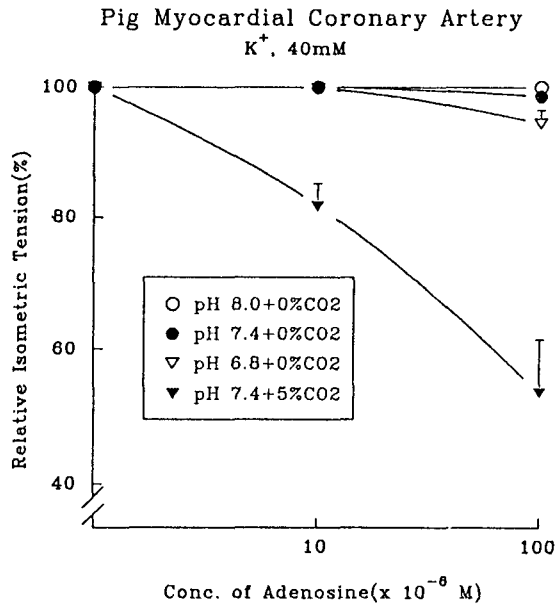

Fig. 10. Effects of adenosine at the different extracellular pH or the changes of CO_2 (at constant extracellular pH 7.4) on the contractions induced by 40 mM K^+ in the pig coronary artery. Ordinate: Percentage of maximal tension induced by 40mM K^+ at 0% CO_2 (pH 7.4). Points show mean of responses of 6 arteries. Vertical bars show S.E.M..

fect by adenosine, arterial strip was contracted by 40mM K^+ or electrical field stimulation at different pH or PCO_2 (at constant pHo) and adenosine was added to the bath. As shown in Fig. 10, the contraction induced 40mM K^+ was relaxed by 10^{-4}M adenosine to 94.5 ± 2.1, 98.6 ± 0.9, 100.0 ± 0.0% at pH 6.8, 7.4, 8.0, respectively. Those contractions were not relaxed significantly by adenosine, also adenosine-induced relaxation was not affected significantly by the change of pHo. On the other hand, 40mM K^+-induced contraction relaxed to 53.6 ± 7.9% by 10^{-4}M adenosine in the Tyrode's solution equilibrated with 5% CO_2/95% O_2 ($p<0.001$).

Effect of adenosine on the contraction induced by electrical field stimulation is shown in Fig. 11. Under 0.4A-induced contraction in the pHo 6.8, 7.4, 8.0, amplitudes of adenosine-induced relaxation were compared as the percent of maximal contraction induced by 0.4A respectively. 0.4A-induced contraction was relaxed by adenosine dose-dependently and those contractions were relaxed by 10^{-6}M adenosine to 15.2 ± 4.9, 32.6 ± 4.9, 37.6 ± 5.6% at pH 6.8, 7.4, 8.0, respectively. Thus adenosine-induced relaxation on the contraction induced by EFS was potentiated by lowering pHo.

DISCUSSION

The coronary blood flow is controlled by interrelation of mechanical, metabolic and neural factors. Among those factors, metabolic fac-

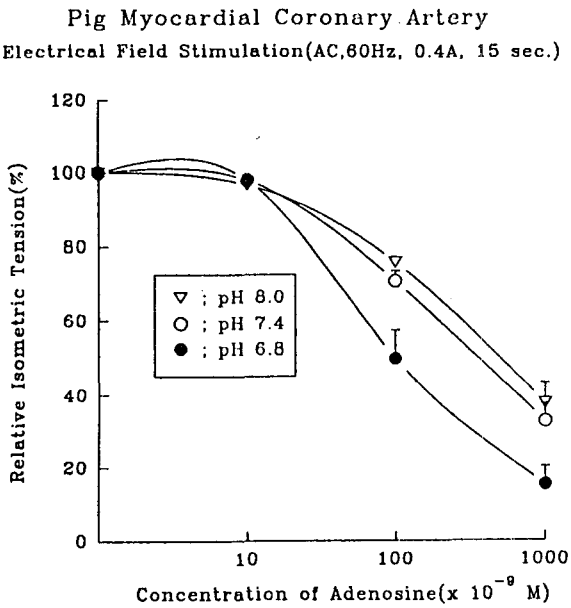

Fig. 11. Effects of adenosine at the different extracellular pH on the contractions induced by electrical field stimulation (0.4 A) in the pig coronary artery. Ordinate: Percentage of maximal tension induced by 0.4 A at 0% CO_2 (pH 7.4). Points show mean of responses of 6 arteries. Vertical bars show S.E.M..

tors such as partial pressure of oxygen and carbon dioxide, hydrogen ions, potassium ions, prostaglandins, adenosine etc. play a key role in the regulation of coronary blood flow.

It is well known that the contractility of several vascular smooth muscle is affected by the change of hydrogen ion concentration. Generally, hydrogen ion suppress the contractility of vascular smooth muscle via acting on the membrane potential, receptor, or calcium channel.

In present study, the amplitude of contraction induced by high K^+ in the coronary artery was decreased by lowering pHo and increased by elevating pHo. This suppressed contraction did not recovered by the addition of Ca^{2+} even though the external Ca^{2+} concentration reached to 7mM and the maximal contraction (100mM K^+) was not influenced by pH change. If hydrogen ion acted on Ca^{2+} channel competitively, the contraction suppressed by lowering pH would be recovered by the addition of external Ca^{2+} concentration and maximal contraction could be influenced by pH change. So it may be suggested that suppression of contraction by lowering pH may be achieved via other mechanism.

Dose-response curve of ACh was shifted toward the right by lowering pH and also maximal contractile response of ACh decreased by lowering pH. This results suggest that hydrogen ion inhibits the activation of ACh receptor. Flavahan & McGrath (1981) reported that hydrogen ions inhibit the activation of receptor due to directly binding of hydrogen ions to the receptor. Furthermore, the phasic and tonic contractions by ACh also were suppressed by lowering pH. Above results suggest that suppression of contraction by lowering pH may be due to the inhibition of intracellular stored Ca^{2+} mobilization and Ca^{2+} influx. Recently Siskind et al (1989) reported that intracellular Ca^{2+} release was reduced in low pH in the A7r5 smooth muscle cell of rat thoracic aorta by using fura-2, fluorescent dye. Loutzenhiser et al (1990) reported that a cytosolic acidification may increase intracellular calcium sequestration directly by modulating calcium uptake in the sarcoplasmic reticulum. Grover & Samson (1986) reported that the calcium uptake by microsomal preparations increases markedly in response to modest decrease of pH in the pig coronary artery. The change of extracellular pH can influence intracellular pH. When pHo was reduced, intracellular pH (pHi) fell. The change in pHi was approximately 40% of the change in pHo (Aickin, 1984). So we could not eliminate the possibility of the intracellular action of hydrogen ion in the suppression of vascular smooth muscle contractility.

It has been observed that carbon dioxide also has an important role in the control of coronary blood flow. In order to evaluate the effect of CO_2 on the contractility, PCO_2 was increased

from 0% to 5% at constant pHo 7.4. The present study demonstrated that contractions induced by high K^+, ACh, and electrical field stimulation were suppressed by elevated PCO_2. These data suggested that the contraction suppressed by elevating PCO_2 might be due to decreased of intracellular pH. Because of its high permeability, CO_2 can diffuse into the interior of the resistance arteries easily. And CO_2 is hydrated and dissociated to form H^+ and HCO_3^- (Jacob, 1940). Intracellular hydrogen ion increases the intracellular calcium and it also inhibits the activation of myosin ATPase and decreases the affinity of Ca^{2+} to calmodulin (Fabiato & Fabiato, 1978; Loutzenhiser et al, 1990).

Adenosine is the mediator of metabolically controlled coronary vasomotions, and adenosine induced the relaxation of several contractions (Berne & Levy, 1986). Adenosine-induced relaxation was possibly explained as decreasing of Ca^{2+} influx due to increasing of intracellular cAMP through the A_2 receptor (Mustafa & Askar, 1985; Mustafa & Ramagopal, 1986). However, it was little known the interrelation between adenosine-induced relaxation and the change of intracellular or extracellular hydrogen ion concentrations. In the present study, 40mM K^+-induced contractions in the HEPES-buffered Tyrode's solution at pH 6.8, 7.4, 8.0 were not changed significantly by adenosine but those in the 5% CO_2/HCO_3^--buffered Tyrode's solution were relaxed significantly by adenosine. The contraction induced by electrical field stimulation was relaxed by adenosine dose-dependently and adenosine-induced relaxation was potentiated by lowering pHo. It is suggested the synergistic effect between adenosine-induced relaxation and relaxation developed by lowering intracellular and/or extracellular pH. Raberger et al (1975) reported that respiratory acidosis increased coronary blood flow and potentiated adenosine-induced relaxation in the dog.

From the above results, it is suggested that H^+ and CO_2 inhibit the Ca^{2+} influx as well as Ca^{2+} release from intracellular Ca^{2+} storage sites and enhance the relaxing effect of adenosine in the pig coronary artery.

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