EFFECTS OF GRADED CORONARY CONSTRUCTION ON REGIONAL OXYGEN AND CARBON DIOXIDE TENSIONS IN OUTER AND INNER LAYERS OF THE CANINE MYOCARDIUM

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This study was undertaken to investigate the effects of graded coronary constriction on regional gas tensions of the myocardium. In 12 open chest dogs, tissue carbon dioxide (PtCO₂) and oxygen (PtO₂) tensions were measured simultaneously in outer and inner layers of the myocardium using a mass spectrometer. In normal condition, higher PtO₂ and lower PtCO₂ were observed in outer layer than in inner layer. With application of coronary constriction, increase in PtCO₂ and decrease in PtO₂ were observed in both layers of the myocardium, but the response to the ischemic stimuli by applying coronary constriction in inner layer was different from that in outer layer. Severe coronary constriction, more than 90% in its diameter, was necessary to produce significant changes in both gas tensions in both layers of the myocardium. Decrease in PtO₂ was found in the condition of less severe coronary constriction and to be greater in inner layer than in outer layer of the myocardium. In terms of the changes in PtCO₂, inner layer was also more susceptible to the ischemic stimuli than outer layer. The greater and earlier elevation of PtCO₂ in inner layer than in outer layer is regarded as one of the possible mechanisms of the reduction of myocardial contraction in the early stage of myocardial ischemia.

Mass spectrometer has been used to measure different gas tensions in blood and tissue.¹² This technic has been shown to provide a useful method for the continuous and simultaneous measurement of intramyocardial oxygen and carbon dioxide tensions. Especially, an increase of carbon dioxide tension was reported to be a new sensitive indicator for the severity of regional ischemia.³ However, no attempts have been made to measure both of oxygen and carbon dioxide tensions simultaneously in outer and inner layers of the myocardium during acute coronary insufficiency.

It is well documented that the inner layer of the myocardium is the most vulnerable area to ischemia, because of its greater energy demand due to greater shortening of contractile unit⁴ and of its greater extravascular compression.⁵ Experimental studies demonstrated that the

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degree of coronary constriction sufficient to produce any changes in myocardial tissue flow\(^6\) and substrate levels\(^7\) in inner layer is different from those in outer layer.

This study was undertaken to investigate the dynamic aspects of the changes in intramyocardial gas tensions in these two layers following graded coronary constriction.

**METHODS**

Twenty mongrel dogs weighing 10–13 Kg were anesthetized with an intravenous injection of 20 mg/Kg of sodium pentobarbital. The animal was ventilated with room air and supplementary oxygen using a volume respirator to maintain arterial oxygen tension more than 100 mmHg and pH from 7.35 to 7.45. The heart was exposed by a left thoracotomy through 5th intercostal space, the pericardium was incised and pericardial cradle was made. The sinus node was crushed and heart-rate was kept constant at 150 beats/min with pace-maker applied to right atrium. The left anterior descending coronary artery was isolated from the adjacent tissue at the level just below the first diagonal branch for the cannulation. After the ligation of anterior descending artery, its distal part was cannulated with an autoperfused cannula (3 mm i.d.) which received blood from carotid artery. This perfusing system contained a rubber tubing section, 0.5 cm in length, for applying a constrictor and a cannulating type of electromagnetic flow transducer (Nihon-Koden, MF-26). After the placement of an autoperfused cannula, anticoagulation was achieved with heparin.

An electric pressure transducer was placed between the coronary constrictor and a cannulating site of the coronary artery to measure coronary perfusion pressure. Aortic pressure was measured with electric pressure transducer and aortic flow was measured with an electromagnetic flow transducer placed around the aortic root (Fig. 1).

Flow and pressure were recorded with multi-writing pen recorder. Arterial blood gas tensions were measured intermittently.

Regional gas tensions were measured with a mass spectrometer (MS-8, Scientific Research Instruments). Two mass spectrometer probes for

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**TABLE I CHANGES IN HEMODYNAMIC PARAMETERS**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>70</th>
<th>75</th>
<th>80</th>
<th>82.5</th>
<th>85</th>
<th>87.5</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic Pressure (mmHg)</td>
<td>(S)</td>
<td>102±5</td>
<td>102±8</td>
<td>103±6</td>
<td>104±6</td>
<td>109±3</td>
<td>102±6</td>
<td>102±7</td>
<td>100±7</td>
</tr>
<tr>
<td></td>
<td>(D)</td>
<td>76±5</td>
<td>72±8</td>
<td>77±5</td>
<td>79±5</td>
<td>82±6</td>
<td>77±5</td>
<td>80±6</td>
<td>76±6</td>
</tr>
<tr>
<td>Aortic Flow (ml/min)</td>
<td></td>
<td>1050±61</td>
<td>1033±69</td>
<td>1053±58</td>
<td>1047±56</td>
<td>972±63</td>
<td>1015±50</td>
<td>989±48</td>
<td>943±53</td>
</tr>
<tr>
<td>Coronary Flow (ml/min)</td>
<td></td>
<td>20.2±2.1</td>
<td>18.9±2.6</td>
<td>18.1±1.8</td>
<td>16.6±1.5</td>
<td>15.3±1.3</td>
<td>13.6±1.3</td>
<td>10.3±1.2</td>
<td>7.4±1.4</td>
</tr>
<tr>
<td>Coronary Perfusion Pressure (mmHg)</td>
<td></td>
<td>81.1±4.2</td>
<td>75.3±6.5</td>
<td>74.9±5.4</td>
<td>67.9±5.4</td>
<td>60.5±4.8</td>
<td>52.8±4.1</td>
<td>42.1±5.2</td>
<td>29.9±2.6</td>
</tr>
</tbody>
</table>

\(S=\) systolic pressure, \(D=\) diastolic pressure.

\*, **, ***: Significant at \(p < 0.05, < 0.005, < 0.001\) by a paired comparison with control, respectively.
the measurement of intramyocardial gas tensions were placed in the area supplied by the anterior descending coronary artery. One probe was inserted into outer layer at a depth of approximately 2 mm and the other into inner layer at a depth approximately 10 mm from the epicardial surface. The technical details of the procedures and calibration of the equipment have been reported previously. After the experiment, the position of each probe was confirmed by dissection. Gas tensions in outer and inner layers were employed when each probe was correctly placed within outer and inner one-thirds of the left ventricular wall, respectively.

Thirty minutes after the insertion of the probes, PO₂ (Pito₂) and PCO₂ (PtCO₂) in both myocardial layers were recorded continuously under each condition from the control state to the total occlusion of the perfusion cannula. Despite of careful insertion of the probes into the myocardium, the incidence of hematoma was detected in several dogs by the recording of excessively high Pito₂ value. Since such preparations were excluded from this study, 12 of 20 experiments were used for the statistical analysis. Coronary constriction was performed by constricting the cannulating tube with a screw-type constrictor. Constriction was kept for 10 minutes at each step and its severity was increased to more advanced one. Observations were made on hemodynamic parameters and gas tensions at the end of each constriction period.

Before the experiment, the degree of constriction was determined in relation to the turn of the screw as follows; The screw in the constrictor device was turned in a stepwise fashion which caused different degree of constriction of the perfusion cannula. The picture of cross section of the cannula was taken on each step of its turn. The picture was enlarged to 20 times of the original for the measurement of the cross sectional area with planimeter.

The area on each step of constriction was diverted to luminal diameter as follows;

\[ \text{Diameter} = \sqrt{\frac{\text{area on each step}}{\pi}} \times 2 \]

and expressed in this study by percent decrease of luminal diameter. After each experiment, the relation between the turn of screw and the degree of coronary constriction was ascertained in the same manner.
Fig. 2. Changes in $\Delta P_{tO_2}$ in outer and inner layers of the myocardium with graded coronary constriction.

*, **: Significant difference of the values between two layers at $P < 0.05$, $< 0.01$, respectively.

Fig. 3. Changes in $\Delta P_{tCO_2}$ in outer and inner layers of the myocardium with graded coronary constriction.

*, **: Significant difference of the values between two layers at $P < 0.05$, $< 0.01$, respectively.
TABLE III  CRITICAL LEVELS OF CORONARY CONSTRICTION, CORONARY FLOW, AND CORONARY PERFUSION PRESSURE IN OUTER AND INNER LAYERS OF THE MYOCARDIUM

<table>
<thead>
<tr>
<th></th>
<th>Constriction (%)</th>
<th>Coronary Flow (ml/min)</th>
<th>Coronary Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_{O_2} )</td>
<td>87.1 ± 2.5</td>
<td>10.3 ± 2.2 (51.3 ± 8.3%)</td>
<td>44.7 ± 6.1 (54.8 ± 6.2%)</td>
</tr>
<tr>
<td>In</td>
<td>81.0 ± 2.0</td>
<td>14.4 ± 1.5 (73.5 ± 3.8%)</td>
<td>52.8 ± 4.7 (65.4 ± 5.1%)</td>
</tr>
<tr>
<td>( P_{CO_2} )</td>
<td>O 85.8 ± 1.7</td>
<td>10.7 ± 1.6 (52.3 ± 6.1%)</td>
<td>38.7 ± 4.1 (47.6 ± 4.4%)</td>
</tr>
<tr>
<td>In</td>
<td>84.8 ± 1.6</td>
<td>12.2 ± 1.5 (61.4 ± 4.9%)</td>
<td>43.7 ± 3.5 (54.8 ± 4.5%)</td>
</tr>
</tbody>
</table>

*O=outer layer, In=inner layer. *: Significant difference of critical levels between two layers at \( p < 0.05 \).

All values were expressed as mean ± 1 standard error (SE) and difference was tested for statistical significance by the paired Student's t-test.

RESULTS

Ten steps of different degree of constriction were applied from 50% constriction to total occlusion, but significant changes in parameters were observed only under the conditions of more than 75% constriction and accordingly the data corresponding to these conditions were presented here.

Hemodynamic parameters (Table I)

Aortic pressure was not altered significantly by applying constriction. A significant decrease of aortic flow was observed only in the condition of total occlusion. Perfusion pressure and flow of coronary artery were decreased in parallel with graded constriction, but more than 80% constriction was necessary to produce any significant changes in both parameters. In total occlusion, coronary perfusion pressure was kept nearly 20 mmHg, while no coronary flow was detected. Heart-rate was kept constant at 150/min with pace-maker and no significant arrhythmias or conduction disturbance were observed during the whole experimental period.

Myocardial gas tensions (Table II)

In control condition, \( P_{O_2} \) was higher in outer layer than in inner layer (\( p < 0.01 \)), while \( P_{CO_2} \) was lower in outer layer than in inner layer (\( p < 0.01 \)). With application of coronary constriction, an increase in \( P_{CO_2} \) and a decrease in \( P_{O_2} \) were observed in both layers, but there was considerable variation in the response to the ischemic stimuli in each layer.

\( P_{O_2} \) in inner layer decreased progressively in proportion to the severity of the constriction and attained to the lowest value in the total occlusion. On the other hand, \( P_{O_2} \) in outer layer revealed no significant decrease until 90% constriction. Further constriction to 100% produced no more decrease in \( P_{O_2} \) in outer layer. The difference of \( P_{O_2} \) between two layers was observed only in control condition (\( p < 0.01 \)), but also at every step of coronary constriction (\( p < 0.01 \)). \( P_{O_2} \) was always lower in inner layer than in outer layer, but outer to inner ratio of \( P_{O_2} \) has shown no significant change at every step of coronary constriction (Table II). It was revealed that the magnitude of change of \( P_{O_2} \) from control level (\( \Delta P_{O_2} \)) during coronary constriction was also different in each layer (Fig. 2). With rather slight coronary constriction till 82.5%, \( \Delta P_{O_2} \) in outer layer revealed an inconsistent change. In 7 of 12 dogs, \( \Delta P_{O_2} \) increased slightly, despite of an increase in coronary constriction. On the other hand, \( \Delta P_{O_2} \) in inner layer has shown a decreasing trend with increasing severity of coronary constriction. At each step of constriction from 75 to 82.5%, there was a statistically significant difference between \( \Delta P_{O_2} \) of both layers (Fig. 2).

A significant increase in \( P_{CO_2} \) was detected in inner and outer layers at the step of 85% and 90% constriction, respectively. It was clear from these data that more than 90% constriction was necessary for the appearance of significant changes in both gas tensions in both layers of the myocardium.

At every step of constriction, higher values of \( P_{CO_2} \) were observed in inner layer than in outer layer. (Table II). In total occlusion, \( P_{CO_2} \) in inner layer increased to the value more than 100 mmHg in 7 of 12 dogs, while \( P_{CO_2} \) in outer layer remained less than 50 mmHg in 5 dogs. Outer to inner ratio of \( P_{CO_2} \) has shown a statistically significant decrease at the step of 87.5% constriction and resulted in the lowest value in

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total occlusion (Table II). The magnitude of change of PtCO₂ from control level (ΔPtCO₂) was greater in inner layer than that in outer layer.

Especially, in the advanced condition of coronary constriction, there was a statistically significant difference between ΔPtCO₂ in both layers (Fig.3).

PtCO₂/PtO₂ ratio in outer layer was 1.04 ± 0.14 in control condition and the ratio has shown an increasing tendency in parallel with the severity of coronary constriction and resulted in the significant increase in condition more than 87.5% constriction. The ratio in inner layer was higher than in outer layer not only in control condition, but also at every step of constriction. The ratio in inner layer also increased gradually and attained to the significantly higher value than that in control condition in conditions more than 85% constriction (Table II).

Critical coronary constriction, which was defined here as the constriction to produce a significant change in intramyocardial gas tensions, was compared between outer and inner layer. When critical constriction was assessed by the change in PtO₂, there was significant difference between both layers. A significant decrease in PtO₂ appeared in inner layer in condition of 81.0 ± 2.0% constriction, while in outer layer in condition of 87.5 ± 2.5%. There was a statistically significant difference between these two values (p < 0.05). When coronary blood flow and coronary constriction, in which PtO₂ has begun to decrease in each layer, were determined, there was also a significant difference between the values in each layer (Table III).

When critical constriction was assessed by the change in PtCO₂, on the other hand, there was no significant difference between the values in two layers.

Significant increase in PtCO₂ appeared in both layers of the myocardium in condition of the nearly same severity of coronary constriction and of the decrease in coronary blood flow and perfusion pressure (Table III).

DISCUSSION

Critique of the method

Before the discussion of our results, it is necessary to consider the method of coronary perfusion and the mass spectrometric method for the determination of intramyocardial gas tensions, which were used in the present study. There are several objections to using an artificial perfusion system for the study of coronary circulation. It is desirable to study coronary circulation without any artificial interventions, but the requirement for the exact measurement of the degree of coronary constriction forced us to use this system. We had done a preliminary observation on the perfusion system. There was no significant difference in resting coronary blood flow in both situations with and without the perfusion system and reactive hyperemic response was also kept in the coronary artery perfused with the cannula. From the findings above mentioned, it is considered to be acceptable to apply the perfusion system without any significant disturbance of the autoregulatory mechanism of coronary circulation. The rate of changes in coronary blood flow and perfusion pressure during graded coronary constriction in this study are essentially similar to those reported in the study which was done by applying the occluder of the snare type to the coronary artery in situ?

Therefore, it was presumed that the expression of the degree of coronary constriction in the present study was reasonable to estimate the stenotic lesions of the coronary artery in situ.

In some experimental studies, the local tissue oxygen tension was measured with the polarographic electrode method, but monitoring of only this parameter of myocardial perfusion is not sufficient for a full understanding of myocardial ischemic state. It seems more reasonable that simultaneous measurements of PtO₂ and PtCO₂ permit some more exact information about the ischemic state of the myocardium?.

The mass spectrometric technique has certain disadvantages; 1) a degree of local myocardial injury resulted from the placement of the probe and 2) moderately slow response time to sudden change in gas tension.

Severe injury of myocardium on insertion of the probe usually results in excessive intramyocardial hemorrhage and consequently induces the recording of excessively high baseline myocardial oxygen tension (> 50 mmHg). In this study, the probe was inserted asatraumatically as possible to avoid the excessive bleeding in the myocardium and any data which showed abnormally high intramyocardial oxygen tension were not employed.

When the insertion of probe was appropriate at the beginning of the study, the levels of both gas tensions have shown the slight changes within 2% from the initial value during the observation.

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period of 3 hours. To allow for the slow response time pertinent to the mass spectrometry, the determination of intramyocardial gas tensions was performed at each step 10 minutes following a given constriction.

**Gas tensions in control condition**

Significant gradient in PtO₂ across the myocardium has been reported in normal heart studied with the polarographic method⁹,¹¹ and the mass spectrometry¹² PtO₂ in the present study was in good agreement with them in the other studies using the mass spectrometry, but it was slightly higher than the values measured with the polarographic method. There was also a significant difference in PtCO₂ between outer and inner layers of the myocardium, but PtCO₂ in the present study were slightly lower than the values in the other study. In the present study, respiration was maintained with the mixture of room air and pure oxygen to prevent hypoxemia and accordingly PaCO₂ in control condition (26.3 ± 2.8 mmHg) and in total occlusion (24.4 ± 4.8 mmHg) were found to be lower than normal value. Since PtCO₂ is linearly related to PaCO₂, lower PaCO₂ resulted in lower PtCO₂ and in lower PtCO₂/PtO₂ ratio of the present experiment. In addition to lower PtO₂ and higher PtCO₂ in inner layer than in outer layer, PtCO₂/PtO₂ ratio was also found to be higher in inner layer than in outer layer. The studies on regional myocardial blood flow using radioactive microsphere have revealed that subendocardial blood flow is approximately equal to or higher than blood flow to the outer layer.¹³,¹⁴

Despite of good perfusion to the inner layer, our results reveal that oxygen uptake and carbon dioxide production might be normally greater in inner layer than in outer layer.

Khoury and co-workers reported that no marked difference of PtO₂ and PtCO₂ across the myocardium was found in their study with the mass spectrometry but they measured gas tensions in outer and inner layers not simultaneously in the same dog, but separately in different dogs. Since there was some individual difference in the intramyocardial gas tensions due to the variation in hemodynamic state and the difference of the both gas tensions in two layers was statistically significant, but not so marked in control condition, it is necessary to measure gas tensions in the different layer of the same dog simultaneously.

**Gas tensions on graded coronary constriction**

Several reports have described autoregulation of coronary flow during coronary constriction or reduced perfusion pressure in normal dogs. In the present study, compensatory vasodilatory mechanism was preserved and normal resting flow was maintained till 80% constriction.

From the observation of the changes in intramyocardial gas tensions, it was revealed that no detectable changes were found during the compensatory period. Under the condition more than 82.5% constriction, adaptive vasodilation failed and PtO₂ in inner layer has begun to decrease. However, PtO₂ in outer layer was maintained within normal ranges, when coronary blood flow was reduced to below normal by coronary constriction and it has begun to decrease at the step of 90% constriction. Khuri and associates have demonstrated that decrease in PtO₂ and increase in PtCO₂ were observed coincidentally with application of coronary stenosis sufficient to reduce resting flow and the rate of changes in these gas tensions was proportional to the severity of coronary stenosis but their observation was made on the gas tensions in only one layer of the myocardium.

Winbury and associates have found from the study of changes in myocardial oxygen tension that decrease in PtO₂ in endocardium was greater than that in epicardium during coronary stenosis. From these findings, it could be concluded that the compensatory vasodilatory capacity in inner layer works to the nearly maximal extent even in normal condition and vasodilatory reserve is more limited in inner layer than in outer layer, as reported by other studies on the distributional changes in myocardial blood flow. In addition to PtO₂ changes, ΔP/O₂ during the initial period of graded constriction between 75 and 82.5% suggests the possible improvement of myocardial perfusion in outer layer during the period, probably due to the steal perfusion from subendocardium to the subepicardium.

Outer/inner ratio of PtO₂ has shown a trend to increase on graded constriction, but it was not statistically significant. It is due to individual variation of the response to the ischemic stimuli in each dog and to the small magnitude of changes in PtO₂. When critical coronary constriction was estimated by the changes in PtO₂ a marked difference was demonstrated between the values in the two layers. This result indicates that PtO₂ appears to be a good sensitive indicator to detect the initial stage of myocardial ischemia, though PtO₂ has not shown a decrease.
proportional to the severity of coronary constriction in the advanced stage of myocardial ischemia.

Under normal condition, PtCO\textsubscript{2} is derived from 2 different sources, 1) oxydative respiration produced in molar equivalent to the rate of myocardial O\textsubscript{2} consumption and 2) diffusion from arterial blood. The elevation of PtCO\textsubscript{2} under ischemic condition suggests an augmented anaerobic metabolism with an increase in lactic acid production. An increase in intracellular formation of hydrogen ions results in the elevation of PtCO\textsubscript{2}. The level of PtCO\textsubscript{2} is also dependent on the removal rate of CO\textsubscript{2} through coronary venous system and accordingly decrease of CO\textsubscript{2} removal during myocardial ischemia induces increase in PtCO\textsubscript{2}. In the present study, increase in PtCO\textsubscript{2} was observed in both layers of the myocardium under advanced coronary constriction, but PtCO\textsubscript{2} in both layers on complete occlusion were found to be lower than those reported by Loisance. This is probably due to the difference of time interval from the complete occlusion to the measurement of PtCO\textsubscript{2}. The onset of any detectable increase in PtCO\textsubscript{2} was also different in each layer. An increase in PtCO\textsubscript{2} in inner layer was detected earlier than that in outer layer, just like a decrease in PtO\textsubscript{2}. An increase in PtCO\textsubscript{2} in each layer was revealed to be proportional to the severity of coronary constriction. ΔPtCO\textsubscript{2}, however, was always higher in inner layer than in outer layer.

This findings reflect the more increased production of CO\textsubscript{2} through anaerobic metabolism and lesser washout due to decreased tissue flow readily occurred in inner layer than in outer layer. These results indicate that PtCO\textsubscript{2} is a more useful indicator to detect the severity of myocardial ischemia than PtO\textsubscript{2}. In early stage of myocardial ischemia, however, PtCO\textsubscript{2} is less useful than PtO\textsubscript{2}. A definite difference of PtCO\textsubscript{2}/PtO\textsubscript{2} ratio between outer and inner layer was observed throughout the experiment, but the increasing rate of the ratio on graded constriction was always higher in inner layer than in outer layer. This finding also suggests that the inner layer is more vulnerable to the ischemic stimuli than the outer layer.

Greater and earlier increase of PtCO\textsubscript{2} in inner layer, which induces local acid-base changes, might be the possible mechanism of the reduction of the myocardial contraction in the inner layer under early ischemic condition. Further studies on the relationship between intramyocardial gas tensions and local myocardial contractile function will provide more meaningful evidence on the mechanism of the depression of cardiac function in the ischemic state of the myocardium.

REFERENCES


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