Differential Effects of Selective \( \beta_1 \)-Agonist Stimulation on Epi- and Endocardial Oxygen Tension in Anesthetized Dogs

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We investigated the effects of selective \( \beta_1 \)-adrenoceptor stimulation on oxygen tension (pO\(_2\)) in the myocardium of anesthetized dogs. A \( \beta_1 \)-selective full agonist, T-0509 (0.01—0.05 \( \mu \)g/kg, i.v.), caused positive inotropic and chronotropic effects, and increased left circumflex blood flow, although it did not change arterial blood pressure. These effects were inhibited by bisoprolol (10 \( \mu \)g/kg), but not by ICI 118551 (30 \( \mu \)g/kg). Under control conditions, subepicardial pO\(_2\) (pO\(_{2\text{epi}}\)) and subendocardial pO\(_2\) (pO\(_{2\text{endo}}\)) were approximately 33 and 27 mmHg, respectively. T-0509 (0.05 \( \mu \)g/kg) decreased pO\(_{2\text{epi}}\) in all cases, with a mean decrease of 2.6 \( \pm \) 0.5 mmHg, and this was significantly inhibited by bisoprolol. T-0509 caused an increase (7 out of 10 dogs) or a slight decrease (3 out of 10) in the pO\(_{2\text{endo}}\); the mean increment was 2.0 \( \pm \) 1.3 mmHg (\( n=10 \)). Isoproterenol (0.01—0.05 \( \mu \)g/kg, i.v.) exerted positive inotropic and chronotropic effects that were sensitive to bisoprolol, and a hypotensive effect that was sensitive to ICI 118551. Isoproterenol caused an increase in blood flow that was sensitive to ICI 118551. Isoproterenol (0.05 \( \mu \)g/kg) decreased pO\(_{2\text{epi}}\) in all cases, with a mean decrease of 2.7 \( \pm \) 0.5 mmHg, which was significantly inhibited by bisoprolol. Isoproterenol caused an increase (5 out of 10) or a slight decrease (5 out of 10) in the pO\(_{2\text{endo}}\); the mean increment was 1.1 \( \pm \) 1.2 mmHg (\( n=10 \)). These results suggest that, while coronary arteries dilate during selective stimulation of \( \beta_1 \)-adrenoceptors or nonselective stimulation of \( \beta \)-adrenoceptors, the oxygen supply is insufficient in pO\(_{2\text{epi}}\) due to a marked increase in oxygen demand. However, pO\(_{2\text{endo}}\) does not readily decrease under these conditions.

Key words T-0509; oxygen tension; \( \beta_1 \)-adrenoceptor; epicardium; endocardium

Myocardial oxygen tension (pO\(_2\)) is determined by a balance of oxygen demand and supply. During stimulation of \( \beta \)-adrenoceptors, myocardial oxygen demand is increased through positive inotropic and chronotropic actions, while the oxygen supply is increased by compensatory coronary vasodilation. Direct stimulation of coronary \( \beta_1 \)- and \( \beta_2 \)-adrenoceptors also contributes to the oxygen supply through a coronary vasodilatation. In addition, \( \beta_2 \)-mediated changes in systemic blood pressure affect the coronary perfusion pressure.

The \( \beta \)-adrenoceptors in canine heart are suggested to be mainly the \( \beta_1 \) subtype, although a \( \beta_2 \) subtype also exists.\(^1\) \( \beta_2 \)-Adrenoceptors in canine heart have been shown to contribute to an increase in myocardial contractility by \( \beta \)-stimulation.\(^2\) Thus, the presumed coexistence of \( \beta_1 \) and \( \beta_2 \) subtypes in the myocardium may cause complex changes in tissue pO\(_2\) through \( \beta \)-stimulation in addition to differential localization of the \( \beta_1 \) and \( \beta_2 \) subtypes in the canine coronary vasculature.

Non-selective \( \beta \) agonists have been used to investigate myocardial oxygen demand and supply in response to \( \beta_1 \)-stimulation.\(^4\) T-0509 has been shown to be a \( \beta_1 \)-selective full agonist both in vitro and in vivo.\(^6\) Using this selective agonist, we have shown that \( \beta_1 \)-selective stimulation does not change coronary sinus pO\(_2\).\(^9\) This suggests that during \( \beta_1 \)-stimulation, oxygen tension is totally maintained in coronary venous blood due to a good balance between the amount of oxygen spent by the increased work of the heart and that supplied by a metabolic increase in coronary blood flow. However, oxygen demand and supply might be ill-balanced at different depths in myocardial tissue. Basically, the endocardial layer is considered to receive a stronger workload and compression than the epicardial layer.\(^10\) In fact, endocardial pO\(_2\) has been shown to be lower than epicardial pO\(_2\).\(^11\) Therefore, differences in workload and compression may change the pO\(_2\) in the two layers differentially in response to \( \beta_1 \)-stimulation.

Intraluminal arteries are also an important determinant of endocardial pO\(_2\), because these arteries send blood from the epicardial surface to the endocardial layer in the ventricular muscle. Endocardial arteries can be auto-regulated under normal conditions, whereas the vasodilator reserve of the endocardial layer disappears during ischemia, and in these circumstances intraluminal arteries are likely to be the limiting sites for perfusion of the endocardial layer.\(^12\) In vitro studies have shown that large coronary arteries possess \( \beta_1 \)-adrenoceptors.\(^9\) In addition, the intraluminal arteries have been found to have \( \beta_1 \) adrenoceptors.\(^13\) Therefore, \( \beta_1 \)-stimulation may cause redistribution of arterial blood into the subendocardium through dilatation of the intraluminal arteries, and may affect the pO\(_{2\text{epi}}\) and pO\(_{2\text{endo}}\).

The aim of the present study was to investigate the effects of selective \( \beta_1 \)-adrenoceptor stimulation on pO\(_{2\text{epi}}\) and pO\(_{2\text{endo}}\) in anesthetized dogs, and to clarify the role of \( \beta_1 \)-adrenoceptors in the myocardium and coronary vasculature. To this end, we attempted stable recording of pO\(_{2\text{epi}}\) and pO\(_{2\text{endo}}\) in open-chest dogs using a polarographic method, and applied a highly selective \( \beta_1 \)-agonist, T-0509, in comparison with isoproterenol. It was found that pO\(_{2\text{epi}}\) decreased, whereas pO\(_{2\text{endo}}\) showed little alteration, in contrast to previous results obtained from recording of pO\(_2\) in coronary sinus blood.

MATERIALS AND METHODS

Chemicals Drugs used were T-0509 ([(-)-(R)-1-(3,4-dihydroxyphenyl)-2-[3,4-dimethoxyphenethyl]amino]-ethanol HCl), ICI 118551 (synthesized at Tanabe Seiyaku, © 1995 Pharmaceutical Society of Japan

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Saitama, Japan), (-)-isoproterenol (+)-bitartrate 2H₂O (Nacalai Tesque, Kyoto, Japan) and (-)-bisoprolol hemifumarate (Merck, U.S.A.). All drugs used in the present study were dissolved in saline.

**Preparation** Ten adult dogs of either sex weighing 10.6 ± 1.4 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The trachea was cannulated, and the dogs were artificially ventilated (SN-480-3, Shinano, Japan). A thoracotomy was performed at the fifth left intercostal space. The left circumflex artery was isolated, and coronary blood flow (CBF) was measured with a flow probe (i.d. 2.0–3.0 mm) connected to an electromagnetic flowmeter (MFV-3100, Nihon Kohden, Japan). A pressure-probe-tipped transducer (SPC-350, Millar Instruments, U.S.A.) was introduced from the left carotid artery and inserted retrogradely into the left ventricle to measure the left ventricular pressure (LVP). LVdP/dt/P was obtained with a differential amplifier (EQ-601G, Nihon Kohden, Japan). Diastolic blood pressure (DBP) was measured with a catheter inserted into right femoral artery. Heart rate (HR) was measured with a cardiotachometer triggered by the pressure pulses. Both the right and left femoral veins were cannulated for injection of drugs and for constant infusion of sodium pentobarbital (3–5 mg/kg/h). All parameters were recorded simultaneously (WR3701, Graphtec, Japan).

**Measurement of Tissue pO₂** A pair of pin-type platinum electrodes, whose tips were covered with an oxygen-permeable membrane (POE-40PDS, Inter Medical, Japan), were used. The two electrodes were fixed 2 mm apart transversely through a plastic guard. The lengths of the tips protruding from the guard were 2.0 and 7.5 mm, and these were used for recording pO₂epi and pO₂endo, respectively. A pair of electrodes were inserted between the first and second diagonal branches of the left descending coronary artery. A reference silver electrode covered with silver chloride was placed on an area of exposed thoracic tissue. The platinum electrodes were polarized at ~0.5 V against the reference electrode, and the resulting polarographic current flow was measured using a current amplifier (PO2100-DW, Inter Medical, Co., Ltd., Japan) with a time constant of approximately 1 s. The oxygen electrode was calibrated with air-saturated saline maintained at 37°C (150 mmHg). Arterial oxygen tension was >80 mmHg.

To confirm the validity of the electrodes, we used 5 dogs for the experiments described below. When oxygen was mixed with the inspired air at a rate of 0.5 l/min, pO₂epi and pO₂endo increased by 6.1 ± 0.7 and 4.1 ± 1.9 mmHg, respectively. After the termination of oxygen addition, the values declined to the baseline. When the left descending artery was ligated for 30 s at the site above the first diagonal branch, pO₂epi and pO₂endo decreased by 30.3 ± 3.4 and 20.4 ± 4.3 mmHg, respectively. From these preliminary data, we confirmed that the changes in tissue pO₂ could be measured using the oxygen electrode. We also found that the measurement site was the area perfused through the left descending coronary artery.

**Protocols** T-0509 and isoproterenol were administered alternately from the lowest to the highest doses. Thereafter, an antagonist (bisoprolol or ICI 118551) was administered, followed by alternate injections of both agonists.

**Statistics** Data were represented as the means ± S.E.M. Student's or Welch's t-test was used to compare the effects of isoproterenol or T-0509 between the control and the antagonist-treated groups. Paired t-test was used to compare the values before and after addition of bisoprolol or ICI 118551. Differences at p < 0.05 were considered to be statistically significant.

**RESULTS**

**Hemodynamic Effects of β-Agonists** The baseline values of DBP, HR, LVdP/dt/P and CBF are shown in Table 1. A selective β₁-agonist, bisoprolol (10 μg/kg, i.v.) significantly inhibited these parameters, whereas a selective β₂-agonist, ICI 118551 (30 μg/kg, i.v.), had no effect.

- T-0509 increased HR and LVdP/dt/P in a dose-dependent manner without any change in DBP (Figs. 1 and 2). In contrast, isoproterenol decreased DBP, whereas it increased HR and LVdP/dt/P to a degree similar to that seen with T-0509 (Figs. 3 and 4). Bisoprolol significantly inhibited the effects of T-0509 and isoproterenol on HR and LVdP/dt/P, although it did not alter the effect of isoproterenol on DBP (Figs. 1 and 3). On the other hand, ICI 118551 did not alter the effect of T-0509 or isoproterenol on HR and LVdP/dt/P, although it significantly inhibited the effect of isoproterenol on DBP (Figs. 2 and 4).

- T-0509 increased CBF in a dose-dependent manner (Figs. 1 and 2), and this effect was significantly inhibited by bisoprolol, but not by ICI 118551. However, the isoproterenol-induced increase in CBF was significantly inhibited by ICI 118551, whereas it was affected only slightly by bisoprolol (Figs. 3 and 4).

**Effects of β-Agonists on Subepicardial and Subendocardial pO₂** Table 1 shows the pO₂epi and pO₂endo values

<table>
<thead>
<tr>
<th></th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Heart rate (beats/min)</th>
<th>LVdP/dt/P (s⁻¹)</th>
<th>Coronary blood flow (ml/min)</th>
<th>pO₂epi (mmHg)</th>
<th>pO₂endo (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>84.7 ± 7.0</td>
<td>165 ± 16.8</td>
<td>60.4 ± 6.8</td>
<td>15.0 ± 2.5</td>
<td>33.4 ± 2.9</td>
<td>27.5 ± 4.1</td>
</tr>
<tr>
<td>Bisoprolol</td>
<td>74.3 ± 9.7</td>
<td>140 ± 16.8*</td>
<td>46.1 ± 5.1**</td>
<td>12.3 ± 2.9</td>
<td>38.2 ± 3.0</td>
<td>31.6 ± 3.1</td>
</tr>
<tr>
<td>ICI 118551</td>
<td>84.5 ± 8.3</td>
<td>152 ± 12.3</td>
<td>58.5 ± 4.8</td>
<td>15.0 ± 3.2</td>
<td>33.5 ± 2.3</td>
<td>26.1 ± 2.6</td>
</tr>
</tbody>
</table>

pO₂epi, subepicardial oxygen tension; pO₂endo, subendocardial oxygen tension. *p < 0.05; **p < 0.01; compared with the value before each antagonist.
before and after addition of β-antagonists. Before addition of drugs, the subepicardial value tended to be higher than the subendocardial value. Bisoprolol or ICI 118551 caused no significant change in these parameters.

T-0509 and isoproterenol decreased the \( \text{PO}_2 \text{epi} \) in a dose-dependent manner in all preparations. T-0509 (0.05 µg/kg, i.v.) decreased the \( \text{PO}_2 \text{epi} \) by 2.6 ± 0.5 mmHg (\( n = 5 \)). Isoproterenol also decreased the \( \text{PO}_2 \text{epi} \) by 2.7 ± 0.5 mmHg (Figs. 3 and 4). Bisoprolol (10 µg/kg, i.v.) significantly inhibited the effects of both agonists on \( \text{PO}_2 \text{epi} \). In contrast, ICI 118551 (30 µg/kg, i.v.) did not alter the effects of either agonist.

T-0509 and isoproterenol caused an increase of \( \text{PO}_2 \text{endo} \) in some cases and a decrease in others (Figs. 1—4). T-0509
Fig. 3. Effects of Bisoprolol (10 µg/kg, i.v.) on Isoproterenol-Induced Changes in Hemodynamic Parameters and Oxygen Tension

$\Delta P_{O_2}^{sub}$ subepicardial $P_{O_2}$; $P_{O_2}^{endo}$ subendocardial $P_{O_2}$. ○, before administration of bisoprolol; ●, after administration of bisoprolol. Each datum represents the mean ± S.E.M. (n = 5). *, p < 0.05; **, p < 0.01; compared with the value before administration of the antagonist.

Fig. 4. Effects of ICI 118551 (30 µg/kg, i.v.) on Isoproterenol-Induced Changes in Hemodynamic Parameters and Oxygen Tension

$P_{O_2}^{epi}$ subepicardial $P_{O_2}$; $P_{O_2}^{endo}$ subendocardial $P_{O_2}$. ○, before administration of ICI 118551; ●, after administration of ICI 118551. Each datum represents the mean ± S.E.M. (n = 5). *, p < 0.05; **, p < 0.01; compared with the value before administration of the antagonist.

(0.05 µg/kg, i.v.) and isoproterenol (0.05 µg/kg, i.v.) increased the $P_{O_2}^{endo}$ in 7 out of 10 and 5 out of 10 preparations, respectively. The average values obtained from all preparations were 2.0 ± 1.3 mmHg for T-0509 and 1.1 ± 1.2 mmHg for isoproterenol. Neither bisoprolol nor ICI 118551 significantly changed $P_{O_2}^{endo}$ in the presence of either agonist.

**DISCUSSION**

We have reported that a selective $\beta_1$-agonist, T-0509, does not change the coronary sinus $P_{O_2}$, even though it produces a positive inotropic and chronotropic effects. It is uncertain whether such a result obtained for cardiac tissue $P_{O_2}$ is similar to that for sinus $P_{O_2}$. In fact, it is
reported that coronary sinus pO₂ differs from tissue pO₂ during ventricular fibrillation. Therefore, in the present study, we investigated the effects of T-0509 on pO₂_epi and pO₂_endo by inserting a pair of oxygen electrodes into the subepicardial (2.0 mm depth) and subendocardial (7.5 mm depth) layers.

In the preliminary study, the tissue pO₂ was shown to increase when oxygen was added to the inspired air, whereas it decreased when the coronary artery was occluded. Thus, the polarographic measurements with oxygen electrodes demonstrated that changes in pO₂ are likely to reflect the direct oxygen demand and supply of the tissue where the electrode is located. The finding that pO₂_epi was larger than pO₂_endo is consistent with a previous study, and also supports the validity of this measurement. The difference in the two layers may be due to the different oxygen demand and supply, since the workload and compression were larger in the endocardial than in the epicardial layer.

In our previous studies, T-0509 increased CBF via β₁-stimulation, although it did not change the sinus pO₂. This agonist produced strong positive inotropic and chronotropic effects, inducing a marked myocardial oxygen demand, while it increased CBF through a metabolic response to these functional changes, such as an increase in adenosine levels. Both of these actions appeared to produce a good balance between oxygen demand and supply in terms of coronary sinus pO₂. In contrast, T-0509 decreased the pO₂_epi, and this effect was inhibited by bisoprolol, suggesting an imbalance between oxygen demand and supply in this layer.

Like T-0509, isoproterenol exerted strong positive inotropic and chronotropic effects and induced a marked myocardial oxygen demand through β₁-adrenoceptors, but not through β₂-adrenoceptors. Isoproterenol caused marked β₂-mediated coronary dilation, although it decreased the coronary perfusion pressure due to a decreased DBP. In the present study, the co-stimulation of β₁-adrenoceptors appeared not to be beneficial for the oxygen balance in the subepicardium, because isoproterenol did not increase, and in fact decreased, the pO₂_epi and this inhibitory effect was not altered by ICI 118551. This result is in contrast to that of the previous study where isoproterenol increased the sinus pO₂.

As mentioned above, coronary sinus pO₂ does not necessarily correspond to the tissue pO₂. If any shunt occurs between arteries and veins, even metabolic dilation of large arteries might not increase the cardiac tissue pO₂, which would be affected by the capillary density. This might result in oxygen insufficiency in the tissue, despite total balance of the sinus pO₂. Another possible explanation for the difference between the sinus and the tissue is heterogeneity of oxygen demand and supply. Indolfi et al. have reported that reverse coronary steal occurs from the right to the left ventricle during bradycardia in the ischemic canine heart. In the present case, the metabolically increased blood flow could be preferentially shifted to other sites from the area between the branches of the left descending artery.

Endocardial blood flow is restricted more severely during cardiac systole since the endocardial layer is more compressed than the epicardial layer. In particular, the decrease in endocardial blood flow during β₁-stimulation would be marked during the shortened diastole. Nevertheless, pO₂_endo was not readily decreased by T-0509. A similar observation was made by Trimble and Downey, although a selective effect of the β₁ subtype could not be confirmed from their results because of their use of a non-selective agonist. They suggest that isoproterenol decreased epicardial flow without changing endocardial flow.

These phenomena suggest that there is some mechanism by which pO₂_endo is resistant to changes in oxygen demand during β₁-stimulation. One possibility is that more workload and compression in endocardial layers during β₁-stimulation cause a marked decrease in blood flow than in epicardial layers, resulting in a stronger relaxation as a compensatory action. Another possibility is a redistribution of tissue blood flow through intraluminal arterioles between the epicardial and endocardial layers. Large conduit arteries have been shown to contain β₁-adrenoceptors in ligand-binding experiments and experiments measuring vascular tension. It has also been reported that resistance arteries contain β₁-receptors. Furthermore, Murphree and Saffitz have reported that intraluminal arteries should possess the β₁ subtype, similar to the surrounding cardiac tissue. When T-0509 is injected, these arteries may contribute to the redistribution of coronary blood, and the pO₂_endo may not be readily reduced. Since ICI 118551 did not inhibit the isoproterenol-induced decrease in pO₂_endo, β₂-mediated vasodilation is unlikely to contribute to the redistribution of coronary blood within cardiac tissues.

In conclusion, while coronary arteries dilate during selective stimulation of β₁-adrenoceptors or nonselective stimulation of β-adrenoceptors, oxygen supply is insufficient in pO₂_epi due to a marked increase in oxygen demand. However, pO₂_endo does not readily decrease under these conditions.

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REFERENCES


