CORONARY VASODILATATION AND ADRENERGIC RECEPTORS IN THE DOG HEART AND CORONARY*

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Abstract - The question of whether the coronary blood vessels contain an intrinsic adrenergic mechanism for vasodilatation of physiological significance has been examined in the canine heart-lung preparation with a donor by studying the response of the coronary vessels to epinephrine, norepinephrine, isoproterenol and salbutamol in combination with practolol. To differentiate the vasodilatation mediated through adrenoceptors in the coronary vessels from that resulting from an increase in the myocardial O₂ consumption, a special method of analysis was developed based on the linear relation between the coronary flow and the myocardial O₂ consumption. It was found that all four compounds produced an increase in the coronary flow attributable to an increased myocardial O₂ consumption. Epinephrine and norepinephrine produced a decrease in the coronary flow after practolol which completely abolished the increase in the myocardial O₂ consumption as well as the positive inotropic and chronotropic effects produced by these compounds, while isoproterenol and salbutamol produced an increase. These results indicate that adrenergic β-receptor exists in the coronary subserving a vasodilatation. However, the vasodilatation through this mechanism is of minor importance under physiological conditions and becomes completely masked in the presence of an overwhelmingly strong vasodilatation consequent to an increase in the myocardial O₂ consumption.

Considerable controversy still exists concerning the actions of catecholamines on the vasculature of the myocardium. The general consensus of opinion is that they produce vasodilatation. The problem is to what extent the adrenergic receptor in the coronary is involved in vasodilatation. The recent demonstration of β-adrenergic receptor activity as manifested by relaxation in isolated coronary artery strips (1) and in the non-beating heart (2) do not necessarily mean that β-adrenergic receptor activity in the coronary vessels plays a paramount role in the coronary vasodilatation of the intact beating heart, since catecholamines produce in vivo a marked change in the mechanical and metabolic activities of the myocardium, which can, in its turn, affect the tone of the coronary vessels considerably.

Another problem associated with the above is the question of whether the adrenoceptor in the coronary vasculature mediating the vasodilatation, if such exists, is to be classified as β₁ or β₂. Some data from flow studies in the intact heart have been interpreted as indicating that the coronary beta receptors are of the β₁ type (3), while other data are inter-

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Interpreted as indicating that the coronary beta receptors are of the $\beta_2$ type (4–10).

Experiments carried out with the isolated smooth muscle preparation of the coronary vessels also produced contradictory results: Baron et al. (11) and Drew and Levy (12) maintained that the coronary artery beta receptor resembled that of the myocardium more closely than it did that of other vascular smooth muscle, while Bayer et al. (13) came to the conclusion that the beta receptor of the isolated swine coronary strip could be classified as $\beta_2$.

Using the canine heart-lung preparation supported by a donor dog, the present study was undertaken to determine in in vivo preparations the extent to which the adrenoceptor in the coronary vasculature is responsible for the catecholamine-induced vasodilatation and to clarify the sub-group of the $\beta$-receptor to which the adrenoceptors of the coronary vessels belong. To separate the direct effects of catecholamines on coronary vessels from indirect effects resulting from stimulation of the myocardium, a new method of analysis was devised and was found to be very useful.

MATERIALS AND METHODS

All the experiments were conducted on the canine heart-lung preparation supported by a donor (HLP c donor), a schematic diagram of which is presented in Fig. 1.

Mongrel dogs of either sex weighing between 7–13 kg were anaesthetized with sodium

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**Fig. 1.** Schematic diagram of the canine heart-lung preparation supported by a donor (HLP c donor)

FA: femoral artery. FV: femoral vein.
pentobarbital (35 mg/kg) administered intraperitoneally. The HLP's were prepared according to the Krayer-Mendez modification of the original Starling's method, details of which were described previously (14, 15). The blood to be utilized for HLP was obtained from other large dogs under slight thiopental anaesthesia (20 mg/kg i.v.) on the morning of the experiment. Just before the withdrawal of the blood, 500 u/kg of heparin sodium was injected to the bleeder animals via femoral vein and the blood was collected in a polyethylene beaker, to each 1 l of which 5000 u of heparin was added. To compensate for the loss of glucose during standing, about 500 mg of glucose was added to each 1 l of the blood immediately before the start of the experiment. After complete set up of the preparation, a mixture of heparin (1000 u/hr) and glucose (660 mg/hr) was infused continuously into the venous reservoir to prevent the blood from coagulating and to maintain the blood sugar at a normal level. The total blood volume present in HLP at the beginning varied between 1200-1600 ml. The lungs of the preparation were ventilated artificially with a mixture of 95% O₂ and 5% CO₂ at a tidal volume of 15 ml/kg and a frequency of 18 per minute.

By adjusting the pneumatic resistance, the mean arterial pressure, recorded by a strain-gauge pressure transducer (Nihon Kohden MPU-0.5), was set at a value between 90-100 mm Hg. The blood level in the venous reservoir was kept constant at 100 mm above the opening of the inferior vena cava. The systemic cardiac output (total output of the left ventricle minus coronary flow) was measured by a square-wave electromagnetic flowmeter (Nihon Kohden MF-26) equipped with a cannulating-type flow probe (6 mm²). It was initially set at a value between 400-600 ml/min. The right atrial pressure was measured with another pressure transducer (Nihon Kohden LPU-0.1), which gives a good measure of the myocardial contractility in this preparation. The heart rate was recorded continuously using a linearly-recording cardio-tachometer (Nihon Kohden RT-5) triggered by R waves of ECG. ECG was recorded before and after administration of the drugs, with leads positioned to correspond to the standard limb lead II. Coronary sinus outflow was led out by a Morawitz cannula, measured by another square-wave electromagnetic flowmeter with cannulating-type probe (2 mm²), sent to the donor via femoral vein and was returned as fresh arterial blood to the venous reservoir of the HLP with the aid of a peristaltic pump (Harvard Apparatus Model 1203) from the femoral artery of the donor. Great care was taken to maintain the rate of the blood flowing into the femoral vein always equal to that of the blood coming out of the femoral artery, while maintaining the blood pressure of the donor at the physiological level. When necessary, the blood was transfused to the donor via femoral vein. Artificial respiration of the donor was not conducted. Combination of a donor with HLP was necessary in order to maintain the coronary blood flow at a physiological level, since an increase in the coronary flow inevitably develops in the course of time in the usual HLP, as already pointed out by Hilton and Eichholtz (16). The donor dog was anesthetized with a mixture of chloralose (45 mg/kg) and urethane (450 mg/kg) given intravenously after premedication with morphine hydrochloride administered subcutaneously (1.5 mg/kg). Small supplementary doses of anesthetic were given when
necessary. Every 2 hr 100 u/kg of heparin sodium was injected to the donor.

The oxygen consumption of the myocardium was calculated multiplying the coronary arteriovenous difference of oxygen content of the blood (vol %) by the corresponding coronary flow (ml/min/100 g heart). To determine the O$_2$ content of the blood, the O$_2$ saturation and hemoglobin content of the arterial blood was determined on blood samples withdrawn at times from the site designated as A in Fig. 1, with the aid of a hemoreflector (Kipp and Zonen MO-1) and a hemoglobinometer (Erma Optical Works Model 303), respectively. The O$_2$ saturation of the coronary venous blood was continuously recorded by an oximeter (Kipp and Zonen, CC-Oximeter Model MO-3) on a potentiometric recorder (Yokogawa Electric Works ER2P-10/10). For the calibration of this record, the venous blood samples were also withdrawn at times and their oxygen saturation was determined with a hemoreflector. From these values, O$_2$ content of the blood was calculated in the following way.

\[
O_2 \text{ content (vol. \%)} = O_2 \text{ saturation (\%)} \times \text{Hb content (g/dl)} \times 0.0136
\]

where 0.0136 represents the O$_2$ capacity of the hemoglobin.

Drugs used were 1-epinephrine hydrochloride (Sankyo), d,l-norepinephrine hydrochloride (Sankyo), 1-isoproterenol hydrochloride (Proterenol, Nikken Kagaku), salbutamol hydrochloride (Sankyo), practolol hydrochloride (ICI Japan), propranolol hydrochloride (ICI Japan) and phentolamine mesylate (Ciba-Geigy).

To obtain a steady level of drug action, drugs were infused continuously in most of the experiments by a multi-speed syringe pump into the rubber tubing leading to the venous cannula, except blockers which were injected as a single administration into the venous reservoir of the HLP. The volume of the infusate was kept at less than 0.056 ml/min.

RESULTS

**Coronary blood flow and myocardial oxygen consumption in HLP’s c donor**

Although the coronary blood flow in the ordinary HLP increased gradually in the course of time, reaching the maximum value of more than 200 ml/min/100 g heart after 2 or 3 hr, thus making the preparation unsuitable for the study of the coronary circulation, the coronary blood flow in HLP c donor was found to be remarkably constant over the period of more than 10 hr, indicating that the introduction of the donor was successful in completely eliminating the loss of tone of the coronary vessels inherent in the usual HLP. The coronary blood flow and the myocardial oxygen consumption at the start of the experiments were 19.9 ± 2.3 ml/min/100 g and 2.10 ± 0.22 ml/min/100 g, respectively. In the course of the first 1 or 2 hr, there was an increase in the coronary flow associated with an increase in the myocardial oxygen consumption. However, the coronary blood flow and the myocardial oxygen consumption (given in parentheses) remained almost constant after this period, being 46.0 ± 4.8 (2.90 ± 0.08) ml/min/100 g at 4 hr (n = 5) and 46.0 ± 7.4 (3.00 ± 0.36) ml/min/100 g at 10 hr (n = 5). Oxygen saturation of the arterial blood also remained almost constant. It was over 94.0% during the entire course of the experiments.

It is well known that the coronary blood flow is extremely efficiently regulated in ac-
cordance with the changes in the myocardial oxygen consumption. Fig. 2 depicts the relation between the coronary blood flow and the myocardial oxygen consumption observed in the HLP c donor. Despite a rather wide variation of the heart rate (103-143/min) and the systemic cardiac output (270-446 ml/min/100 g heart), there exists a straight-

![Graph](image)

**Fig. 2.** Relationship between the myocardial oxygen consumption and the coronary blood flow.

line relationship between these two parameters ($r=0.92$). In the present experiment the oxygen consumption of the myocardium is calculated following the Fick's principle as the product of the arteriovenous difference of the blood oxygen content and the coronary blood flow. Therefore, the regression line in this figure is drawn to cut through the origin of this graph.

**Effects of epinephrine, norepinephrine, isoproterenol and salbutamol on the coronary blood flow and the myocardial oxygen consumption**

Continuous infusion of one of the above four sympathomimetics to this preparation (3-10 µg/min of epinephrine and norepinephrine; 0.3-1 µg/min of isoproterenol; 10-30 µg/min of salbutamol) resulted in an increase in the coronary blood flow and an increase in the myocardial oxygen consumption, associated with definite positive inotropic and chronotropic effects. Even with smaller doses of salbutamol (1-3 µg/min), which is believed to be a selective stimulant of adrenergic $\beta_2$-receptor, it was not feasible to produce a definite increase in the coronary flow without provoking an increase in the myocardial oxygen consumption, in agreement with the results obtained by Broadley (6), which show that doses of salbutamol only slightly in excess of those producing only dilatation of the coronary produced stimulation of the myocardium. To give some idea about the temporal relation of these actions of sympathomimetics, the effects of 3 µg/min of epinephrine are illustrated in Fig. 3. Immediately after administration, a marked increase in the systemic cardiac

![Graph](image)

**Fig. 3.** Effects of epinephrine (3 µg/min) on the heart and coronary circulation. Epinephrine was infused for 20 min into the rubber tubing leading to the venous cannula.

SOP: systemic cardiac output (total output of the left ventricle minus the coronary flow). CF: coronary sinus outflow measured with a Morawitz cannula. RAP: right atrial pressure. HR: heart rate/min. $O_2$ consump.: myocardial $O_2$ consumption. HLP c donor No. 106; Dog 9 kg, female; Heart wt. 86.0 g.
output and a marked fall of the right atrial pressure were observed, concomitant with a slight and transient increase in the coronary flow. A more definite and sustained increase in the coronary flow then ensued, keeping pace with a gradual increase in the myocardial O₂ consumption.

![Diagram](image)

**Fig. 4.** Effects of epinephrine (3-10 μg/min), norepinephrine (3-10 μg/min) and isoproterenol (0.3-1 μg/min) on the relationship between myocardial O₂ consumption and coronary blood flow.

- Δ O₂ consumption (%): changes in the myocardial O₂ consumption expressed as percent of the control.
- Δ Cor. flow (%): changes in the coronary blood flow expressed as percent of the control.
- Open circles: norepinephrine.
- Solid circles: isoproterenol.
- Crosses: epinephrine.

Figs. 4 and 5 show the effects of the four sympathomimetics on the relationship between the myocardial oxygen consumption and the coronary blood flow 20 min after the start of the infusion when the coronary blood flow reached a steady high value. The changes in the coronary blood flow produced by these compounds are expressed as a percent of the initial value and plotted against the changes in the myocardial oxygen consumption expressed as percent of the initial value. As can be seen from these figures, the data obtained with isoproterenol and salbutamol fell close on a straight line with the gradient of 1, while almost all the points obtained for epinephrine and norepinephrine lie below this line.

**Effects of phentolamine and practolol**

Prior (10 min) administration of an adrenergic α-blocker, phentolamine (5-10 mg), which produced a decrease in the coronary flow (21.2 ± 1.80%, n = 14) and a slight decrease in the myocardial oxygen consumption (6.32 ± 1.24%, n = 14) in this preparation, resulted in no change in the positive inotropic and chronotropic effects of all the four sympathomimetics. Under this condition the relationship between the increase in the coronary
blood flow and the increase in the myocardial oxygen consumption for isoproterenol remained unchanged (Fig. 6). Epinephrine and norepinephrine produced a greater increase in the coronary flow than in control experiments, relationship between the increase in the coronary flow and the increase in myocardial oxygen consumption now conforming to a regression line with a gradient of 1.

Ten minutes after pretreatment of the preparation with 10–30 mg of practolol, a selective blocker of the adrenergic \(\beta_1\)-receptor, which produced a decrease in the coronary flow (27.5 ± 4.74%, \(n=9\)) associated with a decrease in the myocardial oxygen consumption (23.8 ± 5.03%, \(n=9\)), and which effectively suppressed the increase in the myocardial oxygen consumption as well as the positive inotropic and chronotropic effects of all the four sympathomimetics, there was a decrease in the coronary flow after epinephrine and norepinephrine, while there was a minimal but definite increase in the coronary flow after isoproterenol (Fig. 7). Salbutamol produced an increase in the coronary flow of almost the same magnitude as that produced by this compound before practolol (Fig. 5). Subsequent administration of propranolol (1–3 mg) effectively antagonized this residual increase in the coronary flow produced by isoproterenol and salbutamol, while the coronary flow decrease by epinephrine and norepinephrine induced in the presence of practolol was abolished by phentolamine, in confirmation of the previous reports by Hashimoto et al. (17), Parratt (18), and Gaal et al. (19).
DISCUSSION

In the heart-lung preparation supported by a donor (HLP c donor) a straight-line relationship was found between the coronary blood flow (Y) and the myocardial oxygen consumption (X), corresponding to an equation: \( Y = aX \) (\( a = \text{constant} \)).

Suppose there is a substance which has no direct effect on the coronary vessel, but has a marked stimulating effect on the myocardium, the myocardial oxygen consumption would increase from \( X_1 \) to \( X_2 \), thereby increasing the coronary blood flow from \( Y_1 = aX_1 \) (1) to \( Y_2 = aX_2 \) (2) in conformity to the above equation. Dividing equation (2) by (1), we obtain

\[
\frac{Y_2}{Y_1} = \frac{X_2}{X_1} \quad (3)
\]

Subtracting 1 from both sides of the equation (3) and rearranging, the equation becomes:

\[
\frac{Y_2 - Y_1}{Y_1} = \frac{X_2 - X_1}{X_1} \quad (4)
\]

What the equation (4) means is that the percent increase of the myocardial oxygen consumption should be equal to the percent increase in the coronary flow, if the substance produces an increase in the coronary flow merely by increasing the myocardial oxygen consumption.

As a corollary, it may be inferred that there is a constriction of the coronary blood vessel, if the percent increase in the myocardial oxygen consumption produced by a substance is greater than the percent increase in the coronary blood flow, while a disproportionately greater increase in the coronary blood flow indicates a direct dilatatory effect on the coronary blood vessels.

Epinephrine and norepinephrine produced a disproportionately smaller increase in the coronary blood flow as compared with an increase in the myocardial oxygen consumption, indicating that there was a vasoconstriction under the influence of these two catecholamines. After administration of phentolamine, an adrenergic \( \alpha \)-blocking agent, the coronary flow increase by these two compounds became greater, the percent increase in the flow now becoming equal to the percent increase in the myocardial oxygen consumption, indicating that the activation of the adrenergic \( \alpha \)-receptor was involved in the vasoconstriction. These findings are consistent with the view that there exists an adrenergic \( \alpha \)-receptor in the coronary vasculature, which can influence the coronary vascular resistance under physiological conditions, and are in agreement with those of Pitt et al. (20), who observed a decrease in the coronary flow after phenylephrine, a selective stimulant of adrenergic \( \alpha \)-receptor, and of Broadley (6), who reported on an initial rise of the perfusion pressure susceptible to \( \alpha \)-adrenergic blockade after threshold doses of epinephrine and norepinephrine in the guinea-pig’s heart perfused at a constant flow by the method of Langendorff.

As for the increase in the coronary flow induced by norepinephrine, epinephrine and isoproterenol, it may be concluded that the increase was almost exclusively due to an increase in the myocardial oxygen consumption produced by these compounds. Although
there is no doubt that adrenergic β-mechanism for vasodilatation exists in the coronary vessels as is demonstrated in the present experiment by salbutamol and repeatedly in the past by many researchers (1, 2, 18, 21), the vasodilatation through this mechanism seems to be of minor importance under physiological conditions, since the percent increase in the coronary flow produced by isoproterenol corresponds exactly to the percent increase in the myocardial oxygen consumption. The fact that the percent increase in the coronary flow produced by epinephrine after α-adrenergic blockade never exceeded the percent increase in the myocardial oxygen consumption is also compatible with this conclusion.

Concerning the subtypes of the β-receptor of the coronary blood vessels there has been much controversy. Generally, the experiments conducted with in vivo preparations tended to point to β₂ type receptor, while the results obtained by some investigators with isolated strips of the coronary artery implicated the β₁ type receptor (for references, see "introduction" section). The isolated strip preparations are usually obtained from an artery with a diameter not less than 250 μ. In other words, they represent not the arterioles, the main site of resistance to blood flow, but the large conductance artery. Therefore, great caution is necessary in extrapolating the results obtained with this preparation to the blood flow changes occurring in the in situ heart. The present findings that salbutamol produced an increase in the coronary flow after prior administration of practolol and, therefore, in the complete absence of the positive inotropic and chronotropic effects and that this residual increase was in turn abolished by subsequent administration of propranolol support the idea that the adrenergic receptor in the coronary is of β₂-type. Although an increase in the coronary blood flow produced by isoproterenol after practolol was not so remarkable as that produced by salbutamol, this may have been due to a combined effect of the relatively low selectivity of practolol towards the β₂-receptor of the coronary and the non-selective action of isoproterenol towards β₁ and β₂ receptors. According to Ross and Jorgensen (9), coronary vasodilatation induced by isoproterenol was blocked by approximately 20 times the dose of practolol required to block the increase in myocardial contractility. Broadley (6) also noted the low selectivity of practolol to the coronary β₂-receptor. It is conceivable that a dose of practolol, which completely abolished the positive inotropic and chronotropic effects of isoproterenol, also suppressed to some degree the stimulatory effect of this substance on the coronary vascular β₂-receptor.

Lucchesi and Hodgeman (3) concluded that the coronary β-receptor is of β₁-type, since practolol produced comparable degrees of blockade in both the myocardium as well as in the coronary vascular bed. However, when their results (e.g. Fig. 5) are carefully examined, it is apparent that the blockade by practolol was greater in the myocardium than in the coronary, suggesting that the receptor in the myocardium and that in the coronary are not homogenous.

In summary, it may be concluded that the coronary vasodilatation induced by catecholamines is mainly due to an increase in myocardial oxygen consumption, in confirmation of a previous conclusion obtained by Hashimoto et al. (17), Hardin et al. (22), Berne (23). Although there is no doubt that the adrenergic β-receptor exists in the coronary
blood vessel itself subserving the vasodilatation, as the results obtained by salbutamol in the presence of practolol indicate, the vasodilatation elicitable through this route is of minor significance under physiological conditions and is completely masked in an overwhelmingly stronger dilatation produced as a result of an increase in the myocardial oxygen consumption. In contrast, the vasoconstriction through α-receptor is physiologically meaningful and occurs even in the presence of a large increase in the myocardial oxygen consumption.

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