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# OPC-18790, a novel positive inotropic agent, has both arterial and venous vascular dilating actions in the dog

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**Abstract**

OPC-18790, (±)6-[3-(3,4-dimethoxybenzylamino)-2- hydroxypropoxy]-2(1*H*)-quinolinone, is a novel positive inotropic agent with a moderate vasodilating action. We examined the vasodilating action of OPC-18790 in detail in the pentobarbital-anesthetized dogs using a colored microsphere technique for resistance vessels and a mean circulatory filling pressure method for capacitance vessels. Intravenously (i.v.) infused OPC-18790 increased the first derivative of left ventricular pressure (LVdP/dt max), cardiac output, heart rate and decreased total peripheral resistance but did not affect mean blood pressure. OPC-18790 significantly increased arterial blood flow distribution to heart and decreased vascular resistance in heart. OPC-18790 at 300 μg/kg i.v. and nitroglycerin at 50 μg/kg i.v. did not affect mean circulatory filling pressure in intact anesthetized dogs, but both compounds decreased mean circulatory filling pressure in spinally anesthetized dogs. OPC-18790 also decreased resistance to venous return but nitroglycerin did not. These results suggest that OPC-18790 has both arterial and venous vasodilating actions in addition to its positive inotropic action. These actions may produce an improvement of cardiohemodynamics in heart failure.

*Keywords:* Positive inotropic agent; Systemic hemodynamics; Blood flow distribution; Mean circulatory filling pressure; OPC-18790

## 1. Introduction

Several quinolinone-derivative positive inotropic agents, including vesnarinone (Tominaga et al., 1984) and OPC-18790 (6-[3-(3,4-dimethoxybenzylamino)-2-hydroxypropoxy]-2(1*H*)-quinolinone; Fujioka et al., 1992) have recently been developed. Among the many new positive inotropic agents which have been evaluated clinically, it has been demonstrated that only vesnarinone is orally effective in prolonging the survival of heart failure patients (Feldman et al., 1993). OPC-18790 has a moderate vasodilating action in addition to a cardiovascular profile similar to that of vesnarinone (Hosokawa et al., 1992). Furthermore, because OPC-18790 is soluble in water, a property not found in vesnarinone, it can be administered intravenously (i.v.).

Initial clinical studies showed that i.v. administered OPC-18790 improved left ventricular functions without changing heart rate (Mishima et al., 1992; Cody et al., 1993; Hoit et al., 1994). The efficacy of OPC-18790 and dobutamine were compared, and OPC-18790 differed from dobutamine, in that it exhibited no increase in heart rate, a greater pre-load reduction, and an increase in cardiac performance at a lower estimated metabolic cost (Abraham et al., 1994). Recent studies in which OPC-18790 was assessed by measuring the left ventricular pressure-volume relationships following inferior vena cava occlusion at baseline and at peak drug effect showed not only that OPC-18790 has inotropic properties, but that it also reduces pre-load at a low dose and produces mixed vasodilation in heart failure patients with a left ventricular ejection fraction of $< 35\%$ (Feldman et al., 1993). The mechanisms of action of OPC-18790 were demonstrated to be both an inhibition of peak III phosphodiesterase (Hosokawa et al., 1992; Sugioka et al., 1994; Endoh et al., 1994) and a prolongation of the action potential duration as a result of its inhibition of $K^+$ channels (Takase et al., 1996). These mechanisms are the same as those of vesnarinone. OPC-18790 exerts a positive inotropic action with no chronotropic action in isolated heart preparations

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(Hosokawa et al., 1992; Itoh et al., 1995a) or with a small extent of positive chronotropic action in vivo preparations (Ishikawa et al., 1995, Itoh et al., 1995b). This discrepancy may be attributable to reflex tachycardia resulting from the vasodilating action, but the characteristics of vasodilating action of OPC-18790 has not yet been elucidated in detail. In this study, we investigated in detail the vasodilating action of OPC-18790 in pentobarbital-anesthetized dog preparations using a colored microsphere technique for resistance vessels and a mean circulatory filling pressure method for capacitance vessels.

## 2. Materials and methods

This study was performed in accordance with the Guidelines for Animal Experimentation in Otsuka Pharmaceutical.

### *2.1. Effects of OPC-18790 on hemodynamics in nerve-intact and denervated dogs*

Mongrel dogs of either sex weighing 9.5–16.0 kg were anesthetized with pentobarbital sodium at an initial dose of 30 mg/kg i.v. followed by maintenance at a rate of 4 mg/kg/h i.v. by the use of an infusion pump (STC-521; Terumo, Tokyo, Japan). Following endotracheal intubation and ventilation with a respirator (SN-480-3, Shinano, Tokyo, Japan) with a tidal volume of 20 ml/kg and a rate of 18 strokes/min, a femoral artery was cannulated and connected to a pressure transducer (MPU-0.5; Toyo-Boldwin, Tokyo, Japan) for blood pressure measurement and a femoral vein was cannulated for the injection of test drugs. A 4F Millar microtip transducer (MPC-500; Millar Instruments, TX, USA) for measurement of left ventricular pressure (LVP) was inserted through the left carotid artery into the left ventricular chamber. The chest was opened by a midline section in all dogs. In cardiac-denervated dogs, afferent branches of stellete ganglia and vagal nerves at mid-cervical level in either side were ligated with silk sutures and cut. A lactate Ringer's solution was infused via a femoral vein at a rate of 6 ml/kg/h throughout the experiment. LVP, the first derivative of LVP (LVdP/dt), blood pressure and heart rate, which was counted using a tachometer triggered by blood pressure pulse waves, were all measured using a polygraph system (Polygraph; NEC-San-ei, Tokyo, Japan) and recorded by a thermal-pen recorder (Recti-Horiz 8K, NEC-San-ei). LVdP/dt max was used as an index of cardiac contractility. OPC-18790 was administered at doses of 10 to 1000 $\mu$g/kg i.v. in both the nerve-intact and denervated dogs.

### *2.2. Effects of OPC-18790 on blood flow distribution*

Mongrel dogs of either sex weighing 8.0–14.0 kg were anesthetized and ventilated using same methods as described above. The chest was opened through the left fourth intercostal space and the heart was exposed and suspended in the cardiac cradle. A right femoral artery was cannulated for blood pressure measurement and for sampling the reference blood. A right femoral vein was cannulated for infusion of a test drugs. A 4F Millar microtip transducer for measurement of LVP was inserted through the left atrium into the left ventricular chamber. A 4F polyethylene catheter was inserted into the left atrium for the injection of microspheres. An electro-magnetic flow probe (Nihon-Kohden, Tokyo, Japan) was placed on the origin of aorta in order to measure cardiac output in these experiments. Cardiac output was corrected according to body surface area and total peripheral resistance was calculated as follows:

$$\text{Body surface area}\,(\text{m}^2) = 0.12 \times (\text{kg body weight})^{2/3}$$

$$\text{Total peripheral resistance}\,(\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}\cdot\text{m}^2) = (\text{mean blood pressure} \times 8 \times 10^4)/\text{cardiac output}$$

Blood flow distribution was measured using non-radioactive colored microspheres (Hale et al., 1988). The microspheres (E-Z Trac, Los Angeles, CA, USA), each 15 $\mu$m in diameter, were agitated in an ultrasonic mixer before injection, and approximately $6 \times 10^6$ microspheres (red, yellow and blue) were injected via the left atrial catheter. A reference arterial blood sample was withdrawn through the femoral catheter at a rate of 7 ml/min ($Qr$) beginning 10 s before the start of the injection and continuing for 2 min. After the experiment, tissue samples (1–10 g wet weight) for the measurement of blood flow were taken and digested in an alkaline solution, and the microspheres were recovered and counted. Tissue blood flow ($Qm$) was calculated from the equation presented below:

$$Qm\,(\text{ml/min/g}) = (Cm \times Qr)/Cr$$

where $Cm$ is the number of microspheres recovered from 1 g of tissue, $Qr$ is reference blood sample withdrawal rate (7 ml/min), and $Cr$ is the number of microspheres in the reference blood sample. Regional vascular resistance was calculated as follow:

$$\text{Regional vascular resistance}\,(\text{mm Hg}\cdot\text{min}\cdot\text{g/ml}) = \text{mean blood pressure}/Qm\,(\text{ml/min/g})$$

The dogs were divided into three groups. OPC-18790 was administered at a dose of 3 $\mu$g/kg/min (low dose) for 30 min and subsequently at 10 $\mu$g/kg/min (high dose) for 30 min. Amrinone was administered at a dose of 30 $\mu$g/kg/min (low dose) for 30 min and subsequently at 100 $\mu$g/kg/min (high dose) for 30 min. Physiological saline as the vehicle was administered at a rate of 0.15 ml/min for 30 min and subsequently at 0.5 ml/min for 30 min. Hemodynamics measurements and regional blood flow measurements were performed just before start of

drug infusion, 30 min after the start of the low dose and 30 min after the start of the high dose.

### 2.3. Effects of OPC-18790 on mean circulatory filling pressure

Mean circulatory filling pressure was measured according to the method of Hirakawa et al. (1992). Mongrel dogs of either sex weighing 11.0–18.0 kg were anesthetized and ventilated using the same methods described above. The chest was opened through the left fourth intercostal space. The left carotid artery was cannulated for blood pressure measurement. The left jugular vein was cannulated for injection of a test drug. A polyethylene catheter was inserted into the right atrium for measurement of right atrial pressure. A 4F Millar microtip transducer for measurement of LVP was inserted into the left ventricular chamber. An electro-magnetic flow probe was placed on the origin of the aorta.

An arterio-venous shunt was made between femoral arteries and veins of both sides using vinyl catheters and roller pump (Tokyo Rika Kikai, RP-1000, Tokyo, Japan) was connected midway in the shunt circuit. Electrodes were placed on the left atrium and the left ventricular apex. To determine mean circulatory filling pressure, heart was arrested by fibrillation with electrical stimulator (20 V, 10 Hz; Sen-3301, Nihon-Kohden, Tokyo, Japan) and at the same time the arterio-venous shunt was opened and blood was rapidly transferred from the arteries to the veins by operating the constant-flow pump. Mean circulatory filling pressure was measured at the point at which mean blood pressure and right atrial pressure reached equilibrium. Immediately after determining mean circulatory filling pressure, the heart was defibrillated (Cardiolife-mini, Nihon-Kohden, Tokyo, Japan). Resistance to venous return and pressure gradient for venous return were calculated as follows:

Pressure gradient for venous return (mm Hg)

= (mean circulatory filling pressure)

− (right atrial pressure)

Resistance to venous return ($dyn \cdot s \cdot cm^{-5} \cdot m^2$)

= (pressure gradient for venous return) $\times 8 \times 10^4$

/cardiac output

OPC-18790 was administered at a dose of 300 μg/kg i.v. and nitroglycerin was administered at a dose of 50 μg/kg i.v. Hemodynamics measurements were performed before and 2 min after the drug injection.

To eliminate reflex mechanisms, some animals were spinally anesthetized by an injection of dibucaine at a dose of 0.6 mg/kg into the Cisterna magna at the level of the first segment of the cervical cord. To maintain mean blood pressure at 80 mm Hg, the dog was given an i.v. infusion of epinephrine.

### 2.4. Drugs

The drugs used in the experiments were OPC-18790 (Otsuka Pharmaceutical, Tokyo, Japan) and amrinone (synthesized by Otsuka Pharmaceutical). Nitroglycerin (Nihon-kayaku, Tokyo, Japan), epinephrine (Daiichi Pharmaceutical, Tokyo, Japan) and dibucaine hydrochloride (Wako Chemicals, Osaka, Japan) were obtained from commercial sources.

### 2.5. Statistical analysis

Data was expressed as the mean ± S.E.M. Differences among the groups were analyzed by analysis of variance (ANOVA) with Student's *t*-test (Tukey's multiple comparison) using the software SAS (Statistical Analysis System, SAS Institute, Japan). $P$ values of $< 0.05$ were considered to indicate significant differences.

## 3. Results

### 3.1. Positive inotropic effect of OPC-18790 and its modification by cardiac denervation

Basal values in intact group for LVdP/dt max, heart rate and mean blood pressure were 3800 ± 380 mm Hg/s,

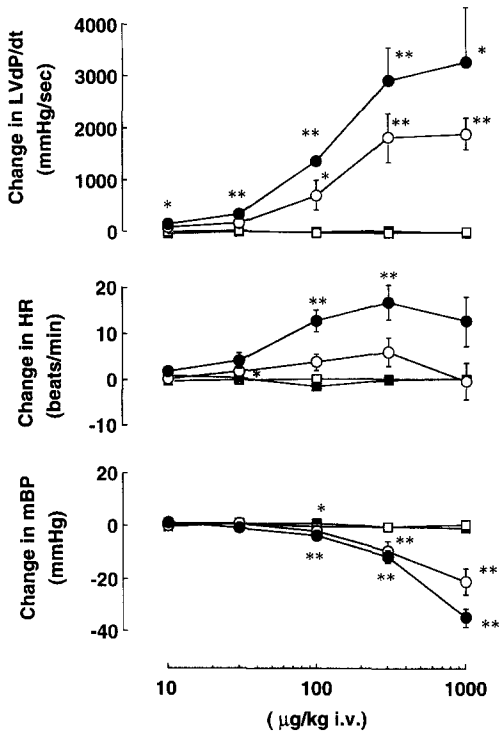


Fig. 1. Hemodynamic effects of i.v. bolus injection of OPC-18790 and vehicle in intact anesthetized dogs and cardiac-denervated anesthetized dogs. Filled circles represent OPC-18790 in nerve-intact dogs, filled squares represent the vehicle in nerve-intact dogs, open circles represent OPC-18790 in cardiac-denervated dogs and open squares represent the vehicle in cardiac-denervated dogs. All values represent mean ± S.E.M. of 4 experiments. HR, heart rate; MBP, mean blood pressure; LVdP/dt, the first derivative of left ventricular pressure. * $P < 0.05$, ** $P < 0.01$ vs. vehicle-treated group.

Table 1
Basal parameters for systemic hemodynamics in anesthetized dogs for the measurement of regional blood flow distribution

| | Vehicle ($n = 8$) | OPC-18790 ($n = 7$) | Amrinone ($n = 4$–6) |
|---|---|---|---|
| HR (beats/min) | 151 ± 5 | 160 ± 6 | 148 ± 5 |
| mBP (mm Hg) | 106 ± 6 | 116 ± 4 | 111 ± 5 |
| LVEDP (mm Hg) | 5.6 ± 0.6 | 7.9 ± 0.7 | 8.4 ± 0.6 |
| LVdP/dt max (mm Hg/s) | 2980 ± 80 | 2830 ± 190 | 3170 ± 350 |
| CO (ml/min/m$^2$) | 2032 ± 131 | 2038 ± 136 | 2067 ± 164 |
| TPR (dyn · s · cm$^{-5}$ · m$^2$) | 4319 ± 350 | 4683 ± 349 | 4465 ± 448 |

There were no significant differences among the three groups. All values represent the mean ± S.E.M. HR, heart rate; mBP, mean blood pressure; LVEDP, left ventricular end diastolic pressure; LVdP/dt, the first derivative of left ventricular pressure; CO, cardiac output; TPR, total peripheral resistance.

175 ± 15 beats/min and 119 ± 5 mm Hg, respectively. In the cardiac-denervated group, basal values for LVdP/dt max, heart rate and mean blood pressure were 2250 ± 312 mm Hg/s, 113 ± 3 beats/min and 111 ± 10 mm Hg, respectively, indicating that cardiac contractility and heart rate were attenuated by cardiac denervation. OPC-18790 at doses of 10–1000 μg/kg i.v. dose-dependently increased LVdP/dt max (by as much as 80–100% at the highest dose) in both intact and denervated dogs (Fig. 1). OPC-18790 produced biphasic changes in heart rate, i.e. an increase followed by transient reduction. However, the transient reduction of heart rate was small (data not shown). The maximal increase in heart rate was about 10% of the basal value in the intact group, and the increase tended to be smaller in denervated dogs (Fig. 1). OPC-18790 dose-dependently lowered mean blood pressure but there was no significant difference between the responses in the intact and the denervated group (Fig. 1).

### 3.2. Effects of OPC-18790 on blood flow distribution

Basal hemodynamic values are shown in Table 1. There were no statistically significant differences between the placebo (saline), OPC-18790 and amrinone groups. OPC-18790 at doses of 3 and 10 μg/kg/min for 30 min increased LVdP/dt max, cardiac output and heart rate and decreased total peripheral resistance and left ventricular end diastolic pressure in a dose-dependent manner but did not affect mean blood pressure (Fig. 2). Amrinone at doses of 30 and 100 μg/kg/min for 30 min also increased LVdP/dt max, cardiac output and heart rate and decreased total peripheral resistance, left ventricular end diastolic

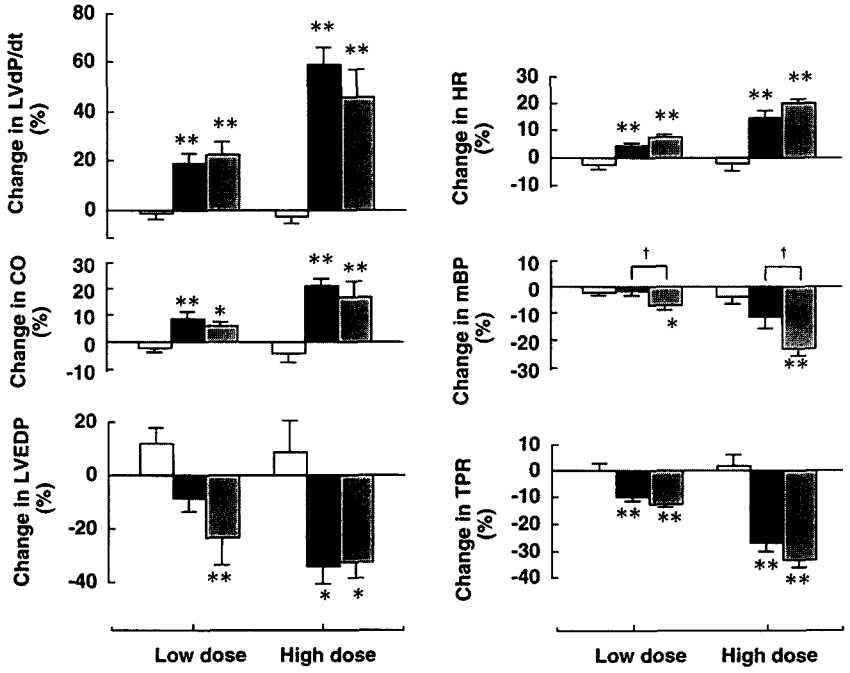


Fig. 2. Effects of i.v. infused OPC-18790, amrinone and vehicle on hemodynamics in anesthetized dogs. Filled columns represent OPC-18790, hatched columns represent amrinone and open columns represent the vehicle. All values represent mean ± S.E.M. of 4–8 experiments. * $P < 0.05$, ** $P < 0.01$ vs. control group. † $P < 0.05$ vs. amrinone group (for abbreviations, see Table 1).

Table 2
Effects of OPC-18790, amrinone, and vehicle on regional blood flow and regional vascular resistance in anesthetized dogs

| | Vehicle ($n = 7$–8) | | | OPC-18790 ($n = 6$–7) | | | Amrinone ($n = 6$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Basal (ml/min/g) (mm Hg min g/ml) | 0.15 ml/min (% change) | 0.5 ml/min (% change) | Basal (ml/min/g) (mm Hg min g/ml) | 3 μg/kg/min (% change) | 10 μg/kg/min (% change) | Basal (ml/min/g) (mm Hg min g/ml | 30 μg/kg/min (% change) | 100 μg/kg/min (% change) |
| *LV outer* | | | | | | | | | |
| Flow | 0.93 ± 0.09 | −1 ± 4 | 13 ± 7 | 1.02 ± 0.13 | 16 ± 6 | 79 ± 16 [aa] | 0.96 ± 0.13 | 45 ± 22 [a] | 67 ± 14 [a] |
| Vascular resistance | 117 ± 8 | 0 ± 5 | −14 ± 4 | 125 ± 14 | −14 ± 3 | −47 ± 6 [aa] | 138 ± 33 | −30 ± 8 [aa] | −53 ± 3 [aa] |
| *LV inner* | | | | | | | | | |
| Flow | 1.18 ± 0.11 | −3 ± 5 | −9 ± 7 | 1.23 ± 0.16 | 3 ± 6 | 61 ± 15 [aa] | 1.26 ± 0.17 | 28 ± 18 | 34 ± 12 |
| Vascular resistance | 93 ± 5 | 3 ± 6 | −10 ± 4 | 106 ± 14 | −3 ± 5 | −41 ± 8 [aa] | 99 ± 19 | −20 ± 11 | −39 ± 8 [a] |
| *RV* | | | | | | | | | |
| Flow | 0.58 ± 0.05 | 7 ± 6 | 16 ± 6 | 0.62 ± 0.11 | 16 ± 10 | 82 ± 18 [aa] | 0.68 ± 0.15 | 43 ± 20 | 90 ± 14 [aa] |
| Vascular resistance | 202 ± 31 | −6 ± 7 | −16 ± 4 | 214 ± 184 | −12 ± 5 | −48 ± 6 [aa] | 206 ± 46 | −30 ± 7 [a] | 59 ± 4 [aa] |
| *Small intestine* | | | | | | | | | |
| Flow | 0.44 ± 0.05 | −13 ± 5 | −4 ± 9 | 0.46 ± 0.08 | 26 ± 27 | 14 ± 11 | 0.57 ± 0.1 | −1 ± 12 | −1 ± 18 |
| Vascular resistance | 265 ± 32 | 15 ± 6 | 7 ± 10 | 304 ± 56 | −7 ± 12 | −19 ± 7 | 243 ± 56 | 1 ± 13 | −11 ± 14 |
| *Stomach* | | | | | | | | | |
| Flow | 0.43 ± 0.11 | −9 ± 10 | −8 ± 16 | 0.52 ± 0.19 | 8 ± 12 | 13 ± 26 | 0.57 ± 0.16 | 13 ± 19 | 2 ± 22 |
| Vascular resistance | 461 ± 137 | 21 ± 18 | 23 ± 16 | 436 ± 112 | −2 ± 10 | −4 ± 15 | 264 ± 59 | −8 ± 13 | −6 ± 20 |
| *Kidney cortex* | | | | | | | | | |
| Flow | 4.71 ± 0.07 | 9 ± 4 | 22 ± 6 | 5.72 ± 1.06 | 9 ± 5 | 27 ± 9 | 5.3 ± 0.78 | 22 ± 8 | 56 ± 18 |
| Vascular resistance | 29 ± 6 | −9 ± 4 | −20 ± 5 | 24 ± 3 | −9 ± 4 | −28 ± 6 | 23 ± 3 | −22 ± 6 | −46 ± 9 [a] |
| *Spleen* | | | | | | | | | |
| Flow | 0.62 ± 0.06 | 1 ± 9 | 11 ± 11 | 1.08 ± 0.12 | 7 ± 4 | 32 ± 14 | 0.76 ± 0.15 | 21 ± 10 | 26 ± 11 |
| Vascular resistance | 175 ± 19 | 1 ± 10 | −8 ± 10 | 116 ± 14 | −7 ± 4 | −28 ± 8 | 202 ± 58 | −20 ± 8 | −36 ± 7 |

All values represent the mean ± S.E.M. LV, left ventricle; RV, right ventricle.
[a] $P < 0.05$, [aa] $P < 0.01$ compared with the change of vehicle group.

Table 3
Effects of OPC-18790 and nitroglycerin on hemodynamic parameters in nerve-intact dogs for measurement of MCFP

| | MCFP (mm Hg) | HR (beats/min) | mBP (mm Hg) | LVdP/dt max (mm Hg/s) | CO (ml/min/m$^2$) | RAP (mm Hg) | LVEDP (mm Hg) | TPR (dyn·s·cm$^{-5}$·m$^2$) | PGVR (mm Hg) | RVR (dyn·s·cm$^{-5}$·m$^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Vehicle (n = 6)* | | | | | | | | | | |
| Basal | 7.9±0.7 | 137±10 | 103±6 | 2030±210 | 1574±161 | 1.8±0.4 | 3.7±1.1 | 5516±624 | 6.0±0.5 | 292±22 |
| ΔChange | −0.5±0.4 | 1±0 | −1.0±1 | −40±20 | −61±24 | 0.0±0.0 | 0.0±0.1 | 149±125 | −0.4±0.2 | 7±10 |
| *OPC-18790 300 μg/kg i.v. (n = 6)* | | | | | | | | | | |
| Basal | 8.2±0.5 | 132±7 | 100±3 | 2170±130 | 1810±137 | 1.8±0.1 | 5.5±1.4 | 4554±400 | 7±0.4 | 287±29 |
| ΔChange | −0.7±0.4 | 13±4 [a] | −13±2 [a,b] | 910±140 [aa,bb] | 293±53 [a,bb] | −0.3±0.2 | −1.5±0.3 [a] | −1132±139 [a] | −0.6±0.2 | −47±10 [aa,bb] |
| *Nitroglycerin 50 μg/kg i.v. (n = 6)* | | | | | | | | | | |
| Basal | 8.8±0.7 | 148±8 | 107±7 | 2500±290 | 2036±231 | 1.3±0.5 | 5.3±1.9 | 4576±885 | 7±0.7 | 257±25 |
| ΔChange | −1.5±0.5 | 4±2 | −26±4 [aa] | −420±130 | −339±173 | −0.2±0.1 | −1.3±0.5 [a] | −636±645 | −1.1±0.4 | 41±12 |

All values represent the mean±S.E.M. MCFP, mean circulatory filling pressure; HR, heart rate; mBP, mean blood pressure; LVdP/dt, the first derivative of left ventricular pressure; CO, cardiac output; RAP, right atrial pressure; LVEDP, left ventricular end diastolic pressure; TPR, total peripheral resistance; PGVR, pressure gradient for venous return; RVR, resistance to venous return.
[a] $P < 0.05$, [aa] $P < 0.01$ compared with the change from basal values of control group. [b] $P < 0.05$, [bb] $P < 0.01$ compared with the change from basal values of nitroglycerin group.

Table 4
Effects of OPC-18790 and nitroglycerin on hemodynamic parameters in spinally anesthetized dogs for measurement of MCFP

| | MCFP (mm Hg) | HR (beats/min) | mBP (mm Hg) | LVdP/dt max (mm Hg/s) | CO (ml/min/$m^2$) | RAP (mm Hg) | LVEDP (mm Hg) | TPR (dyn·s·$cm^{-5}$·$m^2$) | PGVR (mm Hg) | RVR (dyn·s·$cm^{-5}$·$m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Vehicle (n = 6)* | | | | | | | | | | |
| Basal | 12 ± 0.8 | 132 ± 11 | 85 ± 4 | 2630 ± 200 | 2875 ± 418 | 2.8 ± 0.4 | 6.1 ± 0.8 | 2561 ± 299 | 9.0 ± 0.6 | 248 ± 23 |
| ΔChange | −0.5 ± 0.2 | −0 ± 0 | −1 ± 0 | −10 ± 10 | −40 ± 19 | 0.0 ± 0.0 | 0.0 ± 0.1 | 30 ± 30 | −0.4 ± 0.1 | 11 ± 5 |
| *OPC-18790 300 μg/kg i.v. (n = 6)* | | | | | | | | | | |
| Basal | 12 ± 0.7 | 140 ± 4 | 86 ± 3 | 2600 ± 230 | 2592 ± 171 | 2.2 ± 0.3 | 4.8 ± 0.4 | 2716 ± 189 | 9.0 ± 0.4 | 266 ± 12 |
| ΔChange | −1.9 ± 0.2 [aa] | 22 ± 4 [aa,bb] | −13 ± 2 [aa] | 540 ± 40 [aa,bb] | 516 ± 242 [a,bb] | −0.3 ± 0.2 | −0.7 ± 0.2 [a,bb] | −730 ± 123 [aa,bb] | −1.3 ± 0.2 [aa] | −59 ± 14 [aa,bb] |
| *Nitroglycerin 50 μg/kg i.v. (n = 6)* | | | | | | | | | | |
| Basal | 12 ± 0.2 | 134 ± 5 | 83 ± 2 | 2580 ± 160 | 2642 ± 130 | 3.3 ± 0.1 | 4.6 ± 0.8 | 2551 ± 154 | 9 ± 0.2 | 258 ± 13 |
| ΔChange | −1.9 ± 0.3 [aa] | −2 ± 3 | −18 ± 2 [aa] | −490 ± 70 [aa] | −389 ± 91 | −0.5 ± 0.1 [a] | −1.5 ± 0.1 [aa] | −225 ± 100 | −1.4 ± 0.2 [aa] | 9 ± 7 |

All values represent the mean ± S.E.M. (abbreviations as in Table 3).
[a] $P < 0.05$, [aa] $P < 0.01$ compared with the change from basal values of control group. [b] $P < 0.05$, [bb] $P < 0.01$ compared with the change from basal values of nitroglycerin group.

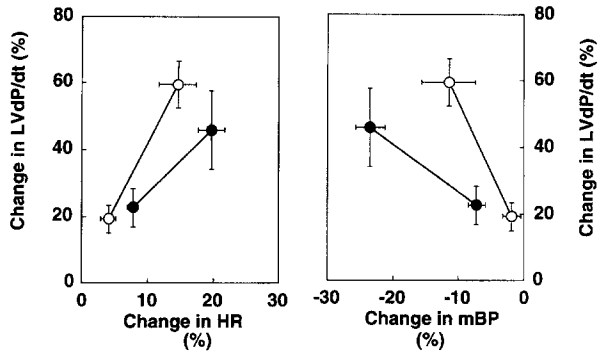


Fig. 3. Relationships between cardiac contractility and heart rate (left side) and between cardiac contractility and mean blood pressure (right side) during infusion of OPC-18790 (3 and 10 μg/kg/min) and amrinone (30 and 100 μg/kg/min) in anesthetized dogs. Open circles represent OPC-18790 and filled circles represent amrinone. All values represent mean ± S.E.M. of 6–7 experiments (for abbreviations, see Table 1).

pressure and mean blood pressure significantly (Fig. 2). At the same time, both OPC-18790 and amrinone significantly increased left and right ventricular blood flow distribution and decreased vascular resistance in heart (Table 2). Amrinone significantly decreased renal vascular resistance and tended to increase blood flow to kidney and spleen. OPC-18790 tended to increase blood flow to spleen but the change was not statistically significant (Table 2). As shown in Fig. 3, OPC-18790 exerted a smaller increase in heart rate than amrinone at the same inotropic level, and OPC-18790 also exerted less change in mean blood pressure at the same inotropic level.

### 3.3. Effects of OPC-18790 on capacitance vessels

To evaluate the action of OPC-18790 on capacitance vessels, we chose the method of mean circulatory filling pressure and compared the results obtained with those for nitroglycerin. The basal values of hemodynamic parameters and the change exerted by saline, OPC-18790 (300 μg/kg i.v.) and nitroglycerin (50 μg/kg i.v.) in intact anesthetized dogs are shown in Table 3. OPC-18790 increased LVdP/dt max, cardiac output and heart rate, by 42, 16 and 9%, respectively, and decreased mean blood pressure, left ventricular end diastolic pressure and total peripheral resistance, resistance to venous return by 13, 29, 25 and 16%, respectively, but did not affect mean circulatory filling pressure, pressure gradient for venous return or right atrial pressure. Nitroglycerin decreased mean blood pressure and left ventricular end diastolic pressure by 24 and 34%, respectively, and tended to decrease LVdP/dt max, mean circulatory filling pressure, pressure gradient for venous return and cardiac output, but these changes were not statistically significant.

We then used spinally anesthetized dogs to evaluate the direct action on capacitance vessels with reflex mechanisms eliminated. The basal values of hemodynamic parameters and the change exerted by saline, OPC-18790 (300 μg/kg i.v.) and nitroglycerin (50 μg/kg i.v.) in spinally anesthetized dogs are summarized in Table 4. In spinally anesthetized dogs, basal values of mean circulatory filling pressure was relatively higher than in nerve-intact dogs, perhaps, because epinephrine was continuously infused to maintain blood pressure. OPC-18790 increased LVdP/dt max, cardiac output and heart rate and decreased mean blood pressure, left ventricular end diastolic pressure, total peripheral resistance, resistance to venous return. Furthermore, OPC-18790 significantly decreased mean circulatory filling pressure and pressure gradient for venous return in spinally anesthetized dogs. Nitroglycerin significantly decreased mean blood pressure, left ventricular end diastolic pressure, right atrial pressure, LVdP/dt max, mean circulatory filling pressure and pressure gradient for venous return and tended to decrease cardiac output and total peripheral resistance but again the changes were not statistically significant. Both OPC-18790 and nitroglycerin decreased mean circulatory filling pressure significantly by about 16%. But OPC-18790 produced greater reduction of total peripheral resistance by 27% than nitroglycerin by 8% in spinally anesthetized dogs.

## 4. Discussion

In this study, we demonstrated that an i.v. bolus injection of OPC-18790 in nerve-intact dogs produced a preferential increase in LVdP/dt max in a dose-dependent manner, with moderate increase in heart rate and decrease in mean blood pressure (Fig. 1). I.v. infused OPC-18790 also increased LVdP/dt max and cardiac output in a dose-dependent manner, with a moderate increase in heart rate and no significant decrease in mean blood pressure (Fig. 2). These cardiovascular actions of OPC-18790 correspond with our previous experiments using in vivo dog preparations (Hosokawa et al., 1992; Ishikawa et al., 1995; Itoh et al., 1995b). However, it was observed in isolated heart preparations of dogs (Hosokawa et al., 1992), guinea pigs (Itoh et al., 1995a) and rabbits (Takase et al., 1996) that OPC-18790 lacks a direct positive chronotropic action in contrast to pure phosphodiesterase III inhibitors, such as amrinone and milrinone. In dogs denervated by stellate ganglionectomy and vagotomy on both sides, the increase in heart rate by OPC-18790 was markedly attenuated with little effect on mean blood pressure (Fig. 2). This observation suggests that the moderate increase in heart rate by OPC-18790 in intact whole animal preparations is primarily mediated by the reflex mechanism. Reflex tachycardia due to the drop in blood pressure may be one of the explanations for the increase in heart rate. Although baroreflex mechanism was eliminated in spinally anesthetized dogs which were continuously administered epinephrine to maintain blood pressure, OPC-18790 at a dose of 0.3 mg/kg i.v. increased heart rate due to phos-

phodiesterase inhibition. In isolated right atrium preparations (Hosokawa et al., 1992) and denervated dogs in this study (Fig. 1), the positive chronotropic action may be canceled by an ancillary action of OPC-18790 that leads to a negative chronotropic effect. However, in nerve-intact anesthetized dogs and epinephrine infused spinally anesthetized dog, where sympathetic tone is relatively elevated and basal cAMP production rate is more elevated than that of isolated preparation, the positive chronotropic effect resulting from phosphodiesterase inhibition may overcome the negative chronotropic component of OPC-18790. The mechanism of negative chronotropic action is not well understood. Since OPC-18790 inhibits both inward rectifying $K^+$ current ($I_{K1}$) and delayed rectifier $K^+$ currents ($I_K$) and prolongs action potential duration in guinea-pig ventricular myocytes (Takase et al., 1996), these effects may also contribute to the negative chronotropic action in sino-atrial node preparations. When the cardiovascular actions of i.v. infused OPC-18790 were compared with those of amrinone in intact anesthetized dogs, amrinone exerted a substantial (statistically significant) decrease in mean blood pressure. This means that amrinone is less selective in separation of inotropic and vasodilating actions than OPC-18790. As shown in Fig. 3, the relationship between positive inotropic actions and positive chronotropic actions of OPC-18790 is different from that with amrinone, and this may be attributable to both differences in direct chronotropic action and differences in vasodilating action.

Both OPC-18790 and amrinone increased cardiac output (Fig. 2). We examined the distribution of cardiac output using a colored microsphere technique. Both compounds significantly increased ventricular coronary blood flow, which is reasonable because both compounds have positive inotropic action in addition to direct arterial vasodilating action, and the positive inotropic action requires an increase in blood flow. Both OPC-18790 and amrinone also tended to increase other important tissue blood flows, such as those to spleen and kidney, and decreased tissue vascular resistance in these tissue (statistically not significant). The effects of OPC-18790 were smaller than those of amrinone (Table 2). There were no organs to which blood flow was decreased despite the drop in mean blood pressure. These results indicate that OPC-18790 dilated arterial blood vessels, especially in ventricular myocardium. The reduction in total peripheral resistance also indicates a dilation of arterial blood vessels. The vasodilating action of OPC-18790 may be caused by the inhibition of phosphodiesterase (Kauffmann et al., 1987) and/or an $\alpha$-adrenoceptor blocking action (our unpublished data of radioligand-binding study). However, as shown in Fig. 3, the selectivity between the positive inotropic effect and the vasodilating effect of OPC-18790 differed from that with amrinone. OPC-18790 has a weaker vasodilating action than amrinone at the same inotropic state. It is not unclear why OPC-18790 has a weaker vasodilating action than amrinone. The potential mechanism of positive inotropic action of OPC-18790 is thought to be not only an inhibition of phosphodiesterase III but also a prolongation of action potential duration resulting from the inhibition of $K^+$ channels (Takase et al., 1996). Therefore, OPC-18790 may be more selective toward positive inotropic action than a pure phosphodiesterase III inhibitor.

Venous tone plays an important role in the regulation of cardiac output. To examine the effects of OPC-18790 on total venous capacitance vessels, we measured mean circulatory filling pressure as an index of total venous tone (Guyton et al., 1954). Mean circulatory filling pressure is proportional to total blood volume and inversely dependent on venous compliance: an increase in mean circulatory filling pressure reflects an increase in venous tone and a decrease in mean circulatory filling pressure reflects a decrease in venous tone in intact dogs (total blood volume being constant). In the present study, OPC-18790 at a dose of 300 $\mu$g/kg i.v. decreased total peripheral resistance ($P < 0.05$) but did not affect mean circulatory filling pressure in nerve-intact dogs. Nitroglycerin, known as a venodilator, also produced no significant decrease in mean circulatory filling pressure. It has been reported that nitroglycerin decreased mean circulatory filling pressure when the autonomic reflex system was impaired by pre-treatment with hexamethonium or spinal anesthesia (Nagata et al., 1989; Ogilvie and Zborowska-Sluis, 1991), and that the venodilating action of nitroglycerin was masked by baroreceptor reflex activation initiated by a decrease in mean blood pressure in nerve-intact whole animals (D'Oyley et al., 1989; Nagata et al., 1989). In fact, OPC-18790 and nitroglycerin both decreased mean circulatory filling pressure ($P < 0.01$) in spinally anesthetized dogs. These results indicate that OPC-18790 has a direct venodilating action like nitroglycerin and that baroreceptor reflex-activated venoconstriction masked its effects in nerve-intact animals. Although both OPC-18790 and nitroglycerin decreased pressure gradient for venous return and mean circulatory filling pressure, where venous capacitance vessels were dilated and venous return should be reduced due to venous blood pooling, OPC-18790 increased cardiac output but nitroglycerin tended to decrease it. The increase in cardiac output produced by OPC-18790 may be the results from the increase in cardiac contractility and heart rate, and the decrease in resistance to venous return and total peripheral resistance. It has been reported that milrinone, phosphodiesterase III inhibitor, showed similar effects (Hirakawa et al., 1992).

In heart failure patients, it has been reported that the both arterial resistance and venous tone are increased due to increase in circulating cathecholamines and plasma renin activity, and impairment of arterial baroreceptor reflex mechanism (Ferguson et al., 1991). Therefore, it is possible that OPC-18790, like nitroglycerin, may produce a greater venodilation in heart failure patients than it did in this study. Actually, it has been demonstrated that OPC-18790 produces a greater reduction in pre-load in severe

heart failure patients (Abraham et al., 1994). In this study, both nitroglycerin and OPC-18790 dilated venous capacitance vessels (mean circulatory filling pressure). However, only OPC-18790 lowered venous resistance (resistance to venous return) in spinally anesthetized dogs. Furthermore, OPC-18790 dilated arterial resistance vessels (total peripheral resistance). Hemodynamic intervention comprised not only of an increase in cardiac contractility but also of a reduction in pre- and after-loads may be more desirable for treatment of heart failure because reduction of pre- and after-loads is useful by influencing the myocardial energetic (Chatterjee et al., 1988). Thus, the cardiohemodynamic profile of OPC-18790, specifically a positive inotropic action with minimal chronotropic action and a reduction of pre- and after-loads, meet the requirement.

In conclusion, we demonstrated that OPC-18790 produced a positive inotropic action with a little chronotropic action which is partly due to baroreflex-mediated response and produced both arterial and venous vasodilating actions in anesthetized dogs. These cardiovascular properties are thought to be useful for treatment of heart failure patients.

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