

## Cardiovascular Effects of a Phosphodiesterase III inhibitor in the Presence of Carvedilol in Dogs

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**ABSTRACT.** The aim of this study was to determine whether dobutamine, dopamine, or milrinone (a phosphodiesterase [PDE] III inhibitor) would support cardiac function that had been attenuated by administration of the  $\beta$ -blocker, carvedilol (0.2, 0.4, or 0.8 mg/kg). Hemodynamic and cardiac parameters including the heart rate (HR), left-ventricular fractional shortening (FS), and arterial pressure were measured in six healthy dogs without cardiac disease. Carvedilol did not affect FS or arterial pressure, but decreased the HR significantly. The positive inotropic and chronotropic responses to dobutamine and dopamine were attenuated by carvedilol, whereas arterial pressure was unaffected. Milrinone did not affect the HR and decreased arterial pressure, whereas FS was significantly greater both in the control and carvedilol-treated groups. Although milrinone affected the negative chronotropic effects of carvedilol, milrinone increased FS and prevented the decrease in arterial pressure. These results suggest that inhibition of PDE III preserves cardiac contractility and hemodynamic function in the presence of carvedilol.

**KEY WORDS:**  $\beta$ -blocker, cardiac function, heart failure, inotropic agent.

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Clinical studies on humans have shown that treatment of congestive heart failure with  $\beta$ -blockers can reduce mortality [4, 5, 10, 16] by improving myocardial energy metabolism, reducing arrhythmia, reducing damage to cardiac myocytes, up-regulating  $\beta_1$  receptor expression, and improving diastolic function [5]. However, cardiac function must be monitored carefully in heart failure patients who are undergoing treatment with  $\beta$ -blockers because such treatment is associated with side effects that include bradycardia, hypotension, and A-V node blocking [2, 21].

It has been established that sympathomimetic drugs such as dobutamine and dopamine may prevent acute heart failure and circulatory collapse. However, the down-regulation of myocardial  $\beta$ -receptors that occurs during heart failure leads to an attenuation of the chronotropic and inotropic effects of  $\beta$ -receptor agonists such as adrenaline, noradrenaline, dobutamine, and dopamine [3, 7, 9]. Milrinone, an inhibitor of phosphodiesterase (PDE) III, increases the concentration of intracellular cyclic adenosine monophosphate (cAMP) by retarding the degradation of this molecule [1, 22]. The modulation of intracellular cAMP by milrinone produces inotropic and chronotropic effects on the heart and relaxation of vessels in the absence of  $\beta$ -receptor stimulation [1, 12, 23]. Therefore, milrinone may produce beneficial effects even though myocardial  $\beta$ -receptors are down-regulated during heart failure. Presumably, the beneficial effects of milrinone also apply in situations in which  $\beta$ -receptors have been blocked by the administration of a  $\beta$ -

blocker [11].

The objective of this study was to determine whether dobutamine, dopamine, or milrinone could support cardiac function that had been attenuated by the administration of a  $\beta$ -blocker. To achieve the objective, the hemodynamic and cardiac responses of healthy dogs to dobutamine, dopamine, and milrinone were evaluated after treating the animals with the nonselective  $\beta$ -blocker, carvedilol.

### MATERIALS AND METHODS

The Guidelines for Institutional Laboratory Animal Care and Use of the School of Veterinary Medicine and Animal Science at Kitasato University, Japan, were followed during this study.

The experiments were performed on six mature, mixed-breed dogs (7–15 kg) of either sex. The dogs were fed commercially available dry food. All dogs were acclimated to the laboratory and handling and were trained to remain calm on the examination table.

**Instrumentation:** Six dogs were given butorphanol (0.1–0.2 mg/kg) and diazepam (5 mg/kg) and were then anesthetized with ketamine (5–10 mg/kg) and isoflurane. The dogs were then intubated. Anesthesia was maintained using isoflurane (1.5–2.0%) with 100% O<sub>2</sub>. Body temperature was measured with an anal probe and was maintained between 37 and 38°C with a heating pad. The heart rate (HR) was monitored. Tygon catheters (Norton Elastic and Synthetic Division, Akron, OH, U.S.A.) were implanted into the abdominal aorta via the femoral artery and were exteriorized between the scapulae. Following surgical catheterization, the dogs were placed on an antibiotic regimen (ampicillin

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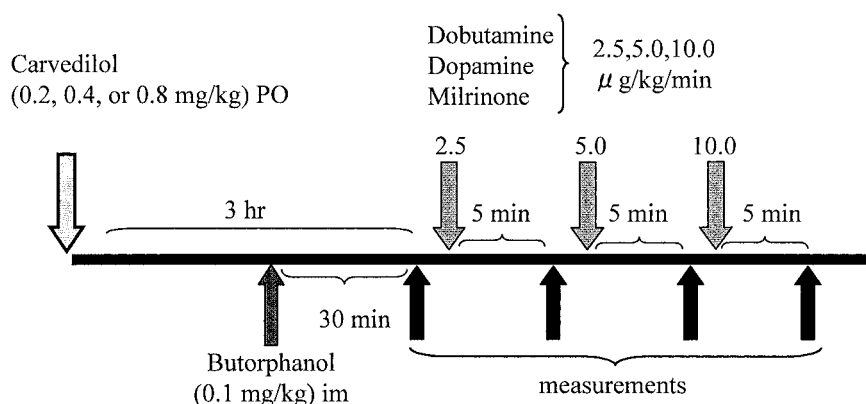


Fig. 1. Experimental protocol. The interval of each carvedilol dosage was taken 3 days at least. Heart rate, arterial pressure (systolic, diastolic, mean), left ventricular dimension by echocardiography were measured.

sodium) for 5 days. The catheter was jacketed and was flushed with heparin every other day.

**Cardiovascular effects of carvedilol:** Carvedilol was administered at a dose of 0.2, 0.4, or 0.8 mg/kg at 09:00 hr. Experiments commenced at 12:00 hr because the plasma concentration of carvedilol peaks 2–3 hr after administration [25]. Dobutamine, dopamine, or milrinone was infused at 2.5, 5.0, and 10 µg/kg/min for 5 min each via the saphenous vein [18] (Fig. 1).

The HR was calculated from an electrocardiogram (ECG). Systolic (SAP), diastolic (DAP), and mean (MAP) arterial pressure were measured via a catheter that was located within the femoral artery and connected to a fluid-filled transducer (Nippon Koden, Tokyo, Japan). The ECG and arterial pressure were analyzed using computer software (HEM; Notocord, Croissy-Sur-Seine, France). The fractional shortening (FS) and the left ventricular internal diameter were measured by echocardiography (SONOS 5500; Hewlett Packard, Tokyo, Japan). All measurements were performed after the dogs had been sedated by injection of 0.1 mg/kg butorphanol.

**Data analysis:** All data are expressed as the mean  $\pm$  SD. Comparisons between values at baseline and at various

times after drug administration were made using ANOVA followed by Dunnett's *post hoc* test. A value of  $P < 0.05$  was considered to be statistically significant.

## RESULTS

Carvedilol significantly decreased the HR ( $P < 0.05$ ) but did not affect SAP, DAP, MAP, FS, or the left ventricular internal diameter (Table 1).

**Heart rate response to dobutamine, dopamine, and milrinone:** Summary data for the effects of carvedilol on the changes in HR caused by dobutamine, dopamine, and milrinone are presented in Fig. 2. Dobutamine (10 µg/kg/min) caused a significant ( $85 \pm 69\%$ ) increase in the HR in control animals; this response was attenuated by carvedilol. Specifically, the change in the HR in response to 10 µg/kg/min dobutamine and 0.2, 0.4, and 0.8 mg/kg carvedilol was  $6 \pm 4$ ,  $10 \pm 7$ , and  $1 \pm 10\%$ , respectively. Dopamine (10 µg/kg/min) caused a significant ( $48 \pm 45\%$ ) increase in the HR in control animals; this response was attenuated by carvedilol. Specifically, the change in the HR in response to 10 µg/kg/min dopamine and 0.2, 0.4, and 0.8 mg/kg carvedilol was  $28 \pm 36$ ,  $21 \pm 9$ , and  $19 \pm 15\%$ , respectively.

Table 1. Baseline hemodynamic changing after carvedilol

	n	Baseline	Carvedilol (mg/kg)		
			0.2	0.4	0.8
Heart rate (beats/min)	6	$85 \pm 11$	$73 \pm 10$	$61 \pm 8^*$	$54 \pm 7^*$
Systolic arterial pressure (mmHg)	6	$120 \pm 7$	$120 \pm 11$	$126 \pm 9$	$121 \pm 11$
Mean arterial pressure (mmHg)	6	$86 \pm 5$	$86 \pm 4$	$90 \pm 15$	$83 \pm 5$
Diastolic arterial pressure (mmHg)	6	$73 \pm 7$	$72 \pm 3$	$72 \pm 10$	$72 \pm 16$
Fractional shortening (%)	6	$35 \pm 2$	$37 \pm 5$	$39 \pm 4$	$35 \pm 2$
LV IDd (mm)	6	$33.3 \pm 0.4$	$31.8 \pm 0.3$	$30.6 \pm 0.3$	$30.4 \pm 0.3$

\* is  $P < 0.05$  vs baseline, LV IDd: left ventricular internal diameter diastolic.

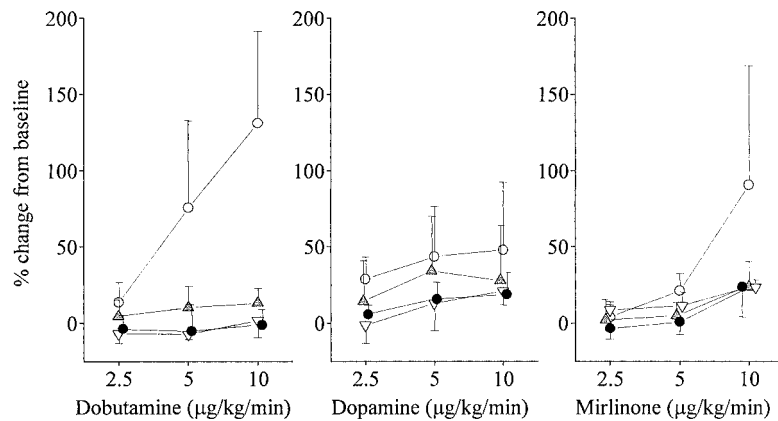


Fig. 2. Heart rate response to dobutamine (left), dopamine (middle), and milrinone (right) with carvedilol in conscious dogs.  $\circ$ : control,  $\blacktriangle$ : carvedilol 0.2 mg/kg,  $\nabla$ : carvedilol 0.4 mg/kg,  $\bullet$ : carvedilol 0.8 mg/kg.

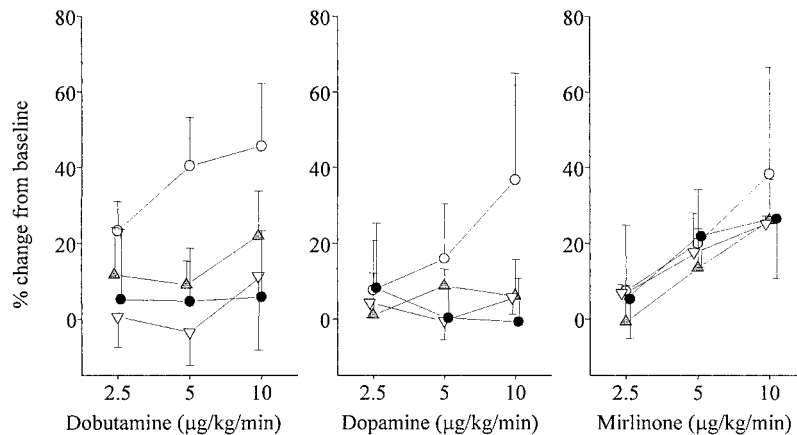


Fig. 3. Fractional shortening response to dobutamine (left), dopamine (middle), and milrinone (right) with carvedilol in conscious dogs.  $\circ$ : control,  $\blacktriangle$ : carvedilol 0.2 mg/kg,  $\nabla$ : carvedilol 0.4 mg/kg,  $\bullet$ : carvedilol 0.8 mg/kg.

Milrinone (10  $\mu\text{g/kg/min}$ ) caused a significant ( $91 \pm 78\%$ ) increase in the HR in control animals; this response was attenuated by carvedilol. Specifically, the change in the HR in response to 10  $\mu\text{g/kg/min}$  milrinone and 0.2, 0.4, and 0.8 mg/kg carvedilol was  $24 \pm 17$ ,  $23 \pm 7$ , and  $24 \pm 20\%$ , respectively.

**Fractional shortening response to dobutamine, dopamine, and milrinone:** Summary data for the effects of carvedilol on the changes in FS caused by dobutamine, dopamine, and milrinone are presented in Fig. 3. Dobutamine significantly increased FS in control animals ( $46 \pm 17\%$ ) and in dogs that were treated with 0.2 mg/kg carvedilol ( $22 \pm 12\%$ ). However, the dobutamine-induced increase in FS was reduced to  $11 \pm 20$  and  $6 \pm 18\%$  by 0.4 and 0.8 mg/kg carvedilol, respectively. Dopamine caused a significant increase in FS in the controls ( $37 \pm 28\%$ ); this response was attenuated by carvedilol. Specifically, the increase in FS was reduced to  $6 \pm 10$ ,  $6 \pm 5$ , and  $-1 \pm 12\%$

by 0.2, 0.4, and 0.8 mg/kg carvedilol, respectively. Milrinone caused a significant increase in FS in the controls ( $38 \pm 28\%$ ) as well as in the presence of carvedilol (FS= $26 \pm 11$ ,  $25 \pm 3$ , and  $27 \pm 16\%$  in the presence of 0.2, 0.4, and 0.8 mg/kg carvedilol, respectively).

**Systolic arterial pressure response to dobutamine, dopamine, and milrinone:** SAP was not affected by dobutamine, dopamine, or milrinone.

## DISCUSSION

In conscious, calm animals, carvedilol at a dose of 0.4 or 0.8 mg/kg decreases the HR in healthy dogs and dogs with mild mitral regurgitation, whereas FS and blood pressure are unaffected [25]. Therefore, such doses of carvedilol would appear to be insufficient to block  $\beta$ -receptors sufficiently in healthy animals to attenuate cardiac function. However,  $\beta$ -blockers can aggravate the cardiac symptoms

of humans with severe heart failure due to a depression of cardiac function [4, 7, 14, 20]. Similarly,  $\beta$ -blockers can lead to a worsening of the cardiac symptoms in dogs with severe heart failure, and administration of  $\beta$ -blockers to such dogs is contraindicated. Therefore, carvedilol administration should be increased by gradual titration while cardiac function is monitored closely.

Although the present study was performed using healthy dogs, carvedilol attenuated the increase in HR, FS, and left ventricular contractility that was induced by the administration of dobutamine or dopamine, whereas the increase in FS induced by milrinone was unaffected by carvedilol. The administration of milrinone during carvedilol administration for the treatment of severe heart failure in humans may be beneficial for some patients [8, 11, 20]. For example, in patients with severe heart failure (New York Heart Association grades IIIb and IV), the combined administration of carvedilol and milrinone improved symptoms to grades II or IIIa [14]. When the effects of administering dobutamine and milrinone to patients who were receiving carvedilol for the treatment of grade II to IV heart failure were compared, the dose of milrinone that appeared to be sufficient to produce a cardiotonic effect was lower than the dose of dobutamine required produce a similar effect [15]. These data support the hypothesis that PDE III inhibitors preserve cardiac function.

In conclusion, the results of our study indicate that the combined administration of carvedilol and a PDE III inhibitor might reduce or prevent the adverse cardiac effects of carvedilol. This study has the following limitations. First, whether the effects of carvedilol on dogs are comparable to the effects of this drug on humans is not known. Second, in dogs with heart failure,  $\beta$ -receptors are down-regulated [6, 13, 24] and intracellular cAMP concentrations are reduced [17, 19]. Therefore, the responses of animals with heart failure to  $\beta$ -receptor agonists and PDE III inhibitors may differ from those of healthy animals. Further studies are necessary to determine the clinical efficacy of carvedilol and milrinone treatment in dogs with heart failure.

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