Positive Inotropic and Negative Chronotropic Effects of OPC-8490, a Newly Developed Cardiotonic, in Isolated, Blood-Perfused Canine Heart Preparations

Yasuyuki Furukawa, M.D., Kunio Akaane, M.D., Masayuki Haniuda, M.D., and Shigetoshi Chiba, M.D.

SUMMARY
When a new cardiotonic, OPC-8490 (3, 4-dihydro-6-[4-(4-oxo-4-phenylbutyl)-1-piperazinylcarbonyl]-2(1H)-quinolinone citrate), was administered into the jugular vein of a support dog, at doses of 0.1, 0.3 and 1 μmol/kg, decreases in heart rate and arterial blood pressure were dose-dependently induced in intact support dogs. One and a half min after administration, positive inotropic and slight negative chronotropic responses were observed in isolated right atria perfused with arterial blood of support dogs. Administration of OPC-8490 into the sinus node artery of the isolated atrium induced positive inotropic and biphasic chronotropic effects, an initial brief positive (Ph1) followed by a long-lasting negative (Ph2) chronotropic effect in a dose-related manner. OPC-8490 at 1000 nmol caused a triphasic, Ph1 followed by Ph2 and slight positive (Ph3) chronotropic effects and an inotropic effect. In the left ventricular muscle preparation driven electrically at 2 Hz, 10−3000 nmol of OPC-8490 increased contractile force in a dose related manner. OPC-8490-induced responses were not significantly modified by propranolol or atropine. When isoproterenol (0.04 nmol/min) increased the basal sinus rate and contractile force of isolated atria, the Ph1 was suppressed and the Ph3 became clear, although the negative phase (Ph2) was not changed. The positive inotropic effect was not significantly changed. When intramural vagal nerve stimulation decreased sinus rate and contractile force, the positive inotropic and negative (Ph2) chronotropic effects were depressed. Verapamil significantly depressed the positive inotropic but not the chronotropic responses to OPC-8490. The positive inotropic effect of OPC-8490 was depressed by pinacidil but not changed by ouabain, although the chronotropic responses to OPC-8490 were not changed. These results suggest that cardiac responses to OPC-8490 involve several mechanisms including cyclic AMP dependent, Ca channel-dependent, potassium current-inhibitory and/or other mechanisms in the dog heart.

From the Department of Pharmacology, Shinshu University School of Medicine, Matsumoto, Japan.

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RECENTLY, a number of inotropic agents that are structurally different from catecholamine or digitalis have been investigated for the treatment of congestive heart failure.1) Previously, one quinolinone derivative, OPC-8212 was developed and pharmacologically investigated.2),3) Yanagisawa et al4) reported that OPC-8212 is a new positive inotropic drug with only minimal effects on heart rate and vasodilation. They clearly demonstrated that the positive inotropic effect of OPC-8212 was accompanied by accumulation of cyclic AMP in the canine ventricle. It has been well recognized that drugs acting by an increase in intracellular cyclic AMP levels simultaneously produce positive chronotropic and inotropic effects.5)-7) Thus, it is assumed that OPC-8212 has other properties in addition to phosphodiesterase inhibition to produce a positive inotropic and negative chronotropic effect. More recently, another quinolinone derivative, OPC-8490 was developed.8) This study was designed to analyze cardiac responses to OPC-8490 pharmacologically using propranolol, atropine, isoproterenol, vagal nerve stimulation, verapamil, pinacidil and ouabain in isolated, perfused canine atria and ventricles.

METHODS

Forty mongrel dogs of either sex weighing 8 to 20 kg were anesthetized with sodium pentobarbital, 30 mg/kg intravenously. The right atrium (n=15) was quickly excised and immersed in Tyrode's solution at 4 to 10°C. The isolated atrium was perfused with arterial blood through the cannulated sinus node artery. The blood was introduced from the carotid artery of the heparinized support dog under a constant perfusion pressure of 100 mmHg by aid of a peristaltic pump (Harvard Apparatus, 1210). The atrium was suspended in a bath filled with blood at a constant temperature of 37°C. The spontaneous atrial rate of the isolated atrium was recorded by a tachometer (Nihon Kohden, RT-2) which was triggered by the atrial electrogram. A bipolar stimulating electrode located on vagal nerve fibers was attached to the atrial epicardium near the upper part of the sulcus terminalis. A recording electrode was placed on the caudal portion of the epicardium at a distance of 1.5 cm from the stimulating electrode. The upper part of the sulcus terminalis of the isolated atrium was connected directly to the force displacement transducer (Grass, FTO3B) by a silk thread and isometric
developed tension was continuously measured.

The effects of OPC-8490 were studied in 5 isolated ventricular preparations. The left ventricle was quickly excised and immersed in Tyrode's solution. The muscle was perfused through the cannulated anterior descending branch of the left coronary artery. The blood was led from the carotid artery of the support dog as in the atrial preparation. The ventricular muscle was electrically driven at 2 Hz with rectangular pulses using an electronic stimulator (Nihon Kohden, MSE-3). The stimulus strength was about twice the threshold voltage (5 msec duration and 1-5 volts). The support dogs (n=20) were anesthetized with sodium pentobarbital, 30 mg/kg intravenously, and ventilated through a cuffed endotracheal tube with room air and additional oxygen (Harvard Respirator, Model 607). Sodium heparin (500 units/kg, i.v.) was given before cannulation of the carotid artery and then hourly thereafter (200 units/kg, i.v.). The heart rate of the support dog was also measured by a tachometer which was triggered by the R waves of the cardiotachograph. The blood pressure of the support dog was continuously recorded. Details of the cross-circulated cardiac muscle preparations are described in previous papers.9)-11)

The drugs used in this study were OPC-8490 (3, 4-dihydro-6-[4-(4-oxo-4-phenylbutyl)-1-piperazinylcarbonyl]-2(1H)-quinolinone citrate, Ohtsuka Co.) (Fig. 1), propranolol hydrochloride (Sigma), norepinephrine hydrochloride (Sankyo), carbachol (carbamylcholine chloride, Aldrich), atropine sulfate (Takeda), isoproterenol hydrochloride (Nikken), verapamil hydrochloride (Knoll), ouabain (Takeda) and pinacidil (Shionogi). The drug was injected into the external jugular vein of the support dog, into the sinus node artery of the isolated atrium or into the anterior descending branch of

![Chemical structure of OPC-8490](image)

**Fig. 1.** Chemical structure of OPC-8490.
the left coronary artery of the isolated left ventricle. Data were analyzed by paired t-test.

**Results**

*Cardiovascular effects of an intravenous administration of OPC-8490 to support dogs on both heart rate and systemic blood pressure of the support dogs and on atrial rate and tension development of isolated atria:*

When OPC-8490 at doses of 0.1, 0.3 and 1 μmol/kg was injected into the jugular vein of a support dog, dose-dependent decreases in heart rate and systemic blood pressure were observed in the support dog (Fig. 2). One
Effects of OPC-8490 injected into the sinus node artery of the isolated atrium on SA node pacemaker activity and atrial contractility:

OPC-8490 at doses of 10 and 100 nmol induced biphasic, negative (Ph1) following transient positive (Ph2) chronotropic and positive inotropic effects in an isolated, blood-perfused dog atrium. A typical tracing is shown in Fig. 3. At a dose of 1000 nmol, OPC-8490 evoked a positive inotropic and a triphasic, transient positive (Ph1) followed by negative (Ph2) with small positive (Ph3) chronotropic effects. These triphasic chronotropic effects of OPC-8490 (1000 nmol) were observed in 4 of 6 experiments. Figure 4 shows the summarized data, but it does not show a small positive chronotropic effect (Ph3) followed by a negative chronotropic effect (Ph2).
Fig. 5.

Fig. 5. Effects of 10 nmol of propranolol on OPC-8490- and norepinephrine-induced chronotropic and inotropic responses. Ph1 = phase 1; Ph2 = phase 2. Control sinus rate was 108 ± 4.4 beats/min, and developed tension was 3.4 ± 0.4 g in 5 isolated atrial preparations. Each bar shows SEM.

Fig. 6. Effect of 4 nmol of atropine on OPC-8490- and carbachol-induced chronotropic and inotropic responses in 5 isolated atrial preparations. Ph1 = phase 1; Ph2 = phase 2. Each bar shows SEM.

Effects of OPC-8490 injected into the anterior descending branch of the left coronary artery of the isolated left ventricle:

An injection of OPC-8490 produced a positive inotropic effect in a dose-related manner. At 20, 100, 300, 1000 and 3000 nmol, OPC-8490 induced increases of 6 ± 0.6, 7 ± 2.2, 18 ± 3.9, 35 ± 12.2 and 58 ± 18.7%, respectively, in tension development of isolated ventricular preparations.

Effects of propranolol on OPC-8490-induced cardiac effects:

OPC-8490 (100 or 300 nmol)-induced transient positive (Ph1) chronotropic and positive inotropic responses were not inhibited by propranolol (10 nmol) which significantly (p < 0.01) inhibited positive chronotropic and inotropic responses to norepinephrine (0.3 nmol) in 5 isolated atria. Sum-
EFFECTS OF OPC-8490

Fig. 7. Fig. 8.

Fig. 7. Chronotropic and inotropic effects of OPC-8490 before and after treatment with 100 nmol of isoproterenol. Each bar shows SEM. Ph1 = phase 1; Ph2 = phase 2; Ph3 = phase 3.

Fig. 8. Effects of vagal nerve stimulation on OPC-8490-induced chronotropic and inotropic responses in isolated atria. Ph1 = phase 1; Ph2 = phase 2. Each bar shows SEM.

Effects of atropine on OPC-8490-induced negative chronotropic effects:

When atropine (4 nmol) inhibited the negative chronotropic and inotropic responses to carbachol (0.5 nmol), it did not inhibit the OPC-8490 (100 or 300 nmol)-induced negative chronotropic effect (Ph2) in 5 isolated atria (Fig. 6).

Effects of continuous infusion of isoproterenol on OPC-8490-induced cardiac responses:

When isoproterenol was infused at a rate of 0.04 nmol/min into the sinus node artery, atrial rate and atrial developed tension were increased about 50%, from 100±6 (mean±SE) beats/min to 155±16 beats/min and about 100%, from 2.9±0.9 g to 5.9±1.0 g, respectively, in 5 isolated perfused atria. During a continuous infusion of isoproterenol, the OPC-8490-induced transient positive (Ph1) chronotropic response was suppressed significantly.
Fig. 9. Effect of verapamil on OPC-8490-induced chronotropic and inotropic responses in 5 isolated atrial preparations. Each bar shows SEM.

Fig. 10. Effect of pinacidil on OPC-8490-induced chronotropic and inotropic responses in 5 isolated atrial preparations. Each bar shows SEM.

(p<0.05) and the negative phase (Ph2) of the chronotropic effect was not changed (Fig. 7). Although 100 nmol of OPC-8490 induced a biphasic, transient positive and negative chronotropic effect in the control experiment, during isoproterenol infusion a positive chronotropic effect (Ph3) appeared in the secondary negative chronotropic response (Ph2) similar to the chronotropic response to 1000 nmol OPC-8490.

Effects of vagal nerve stimulation on OPC-8490-induced cardiac responses:
Endogenous acetylcholine evoked by repeated intramural vagal nerve
stimulation caused negative chronotropic and inotropic effects and maintained virtually the same levels during stimulation as those reported previously. When the extent of vagal stimulation was increased by changing the stimulation frequency from 5 to 10 Hz or the stimulation voltage from a low to a high level, the greater negative chronotropic and inotropic responses were obtained in a frequency- or voltage-related manner. We precisely determined a submaximum voltage as a high voltage which induced only negative chronotropic and inotropic responses but not positive cardiac responses, which were blocked by propranolol, after cessation of stimulation.

When vagal stimulation decreased basal sinus rate and atrial contractile force from 106±5 (mean±SE, n=6) to 90±5 (n=6) and 74±6 (n=4) beats/min, and from 3.2±0.5 to 2.0±0.5 and 1.4±0.6 g, respectively, the negative (Ph2) chronotropic effect of 100 nmol of OPC-8490 was significantly attenuated by a greater degree of vagal stimulation (p<0.05), but the transient positive (Ph1) chronotropic effect was not changed (Fig. 8). Vagal stimulation tended to attenuate the OPC-8490-induced positive inotropic response, although not significantly.

Effects of verapamil, pinacidil or ouabain on OPC-8490-induced cardiac actions:

When verapamil (6 nmol) decreased basal sinus rate and atrial contractile force from 98±7 (mean±SE) to 84±6 beats/min and from 3.8±0.5 to 2.8±0.3 g, respectively, the positive inotropic effect of OPC-8490 (100 nmol) was significantly (p<0.05) attenuated, although the transient positive (Ph1) followed by negative (Ph2) chronotropic effect was not significantly attenuated. Summarized data are shown in Fig. 9.

Pinacidil produced negative chronotropic and inotropic effects by itself. After 0.3 μmol of pinacidil, OPC-8490-induced positive inotropic responses were significantly depressed but not modified for OPC-8490-induced chronotropic responses. Summarized data are shown in Fig. 10.

Although the effects of ouabain on the OPC-8490-induced cardiac responses were also investigated in 4 isolated perfused atria, the OPC-8490-induced cardiac responses were not significantly changed (data not shown).

**DISCUSSION**

A number of cardiotonic agents have been developed in recent years, including amrinone13,14 and milrinone15 (bipyridine derivatives), MDL 17,04316 and MDL 19,20517 (limizolone derivatives), sulmazol18 (a benzimidazole derivative), forskolin19 (a diterpene derivative) and Bay k 864420 (a dihydropyridine derivative).
In the present study, a quinolinone derivative, OPC-8490 was investigated in isolated, blood-perfused dog atrial preparations. An intravenous injection of OPC-8490 induced hypotension with bradycardia in intact dogs and a negative chronotropic and a positive inotropic effect in isolated atria. Even with direct application of OPC-8490 to the SA node, clear positive inotropic and negative chronotropic effects were produced in a dose-related manner, although an extremely brief positive chronotropic effect usually accompanied them.

OPC-8490-induced positive inotropic effects were not significantly modified by propranolol and atropine, indicating that these effects are not mediated by adrenergic and cholinergic mechanisms. Although it was reported that OPC-8490 has phosphodiesterase inhibitory properties and causes an increase in cyclic AMP in myocardial tissues, these effects were not potentiated by isoproterenol infusion in blood-perfused atrial preparations.

A recently developed quinolinone derivative, OPC-8212 produced a positive inotropic effect associated with a concomitant elevation of cyclic AMP levels in canine isolated right ventricular muscle. Yanagisawa et al showed that carbachol markedly depressed the mechanical and cyclic AMP responses to OPC-8212. Moreover, they reported that the positive inotropic effect of isoproterenol was enhanced by OPC-8212 in a concentration-dependent manner, suggesting that OPC-8212 produces its effect on canine ventricular muscle by inhibiting cyclic AMP phosphodiesterase activity. They also observed that OPC-8212 produced positive inotropic but only minimal positive chronotropic effects. Since drugs acting by increasing intracellular cyclic AMP levels produce simultaneously positive inotropic and chronotropic effects, OPC-8212 may have other cardiac activity with phosphodiesterase inhibitory properties.

In the present study, OPC-8490-induced positive inotropic effects were slightly depressed by vagal stimulation. The muscarinic receptor-mediated antagonism of the cyclic AMP-dependent mechanism might be due to inhibition of adenylate cyclase activity through activation of the inhibiting guanine nucleotide regulatory protein. Thus, it is postulated that OPC-8490 may have phosphodiesterase inhibitory properties but not only properties. Differing from the effects of OPC-8212, OPC-8490-induced positive inotropic effects were not modified by isoproterenol infusion, suggesting a relatively small cyclic AMP-dependent mechanism of OPC-8490 activity.

Iijima and Taira (1987) reported that pinacidil increased the background potassium current in isolated guinea pig hearts. In this study, OPC-8490-induced positive inotropic effects were significantly depressed by pinac-
cidil, suggesting that OPC-8490 partially exerts its action on potassium-dependent mechanisms. Moreover, OPC-8490 may not exert its action on Na⁺-K⁺-ATPase activity, because OPC-8490-induced effects were not modified by ouabain treatment.

On chronotropism, OPC-8490 induced a biphasic chronotropic effect (Ph1 and Ph2) in a relatively small dose, and a triphasic chronotropic effect (Ph1, Ph2 and Ph3) in a large dose. These chronotropic responses were not modified by propranolol and atropine, indicating no adrenergic and cholinergic components to its action. The transient positive chronotropic responses (Ph1) disappeared during isoproterenol infusion. Since isoproterenol caused a marked increase in atrial rate, the transient increase may have been masked by tachycardia or may involve unknown mechanisms. On the other hand, the third positive chronotropic effect (Ph3) became clear even with a small dose during isoproterenol treatment. This may have been due to the inhibitory action of OPC-8490 on phosphodiesterase activity. Differing from OPC-8212, OPC-8490 usually produced a negative chronotropic effect (Ph2), although the mechanisms are as yet unknown.

From the present results, it is suggested that a new cardiotonic agent, OPC-8490, has positive inotropic properties with negative chronotropic effects probably by several different modes of action such as a relatively small degree of phosphodiesterase inhibitory activity, an increase in inward calcium current activity, a modification of potassium current actions and others.

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