Pharmacological Analysis of Positive Chrono- and Inotropic Responses to Denopamine (TA-064) in Dog Cross-Circulated Atrial and Ventricular Preparations

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Abstract—Positive chrono- and inotropic responses to denopamine (TA-064, (−)-(R)-1-(p-hydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)amino]ethanol), a new and orally active cardiotonic agent, were investigated in the canine isolated right atrial or left ventricular preparation which was cross-circulated with blood from another support dog. Denopamine dose-dependently increased the sinus rate, right atrial and left ventricular contractile force. Denopamine was one to two orders of magnitude less potent than isoproterenol. The positive chrono- and inotropic effects of denopamine in isolated, blood-perfused right atria were dose-dependently inhibited by treatment with propranolol and atenolol. The effects of denopamine were only slightly attenuated by ICI 118,551 in doses which completely suppressed the positive chrono- and inotropic effects of procaterol. The increases in sinus rate and atrial contractility induced by denopamine were partially but significantly attenuated by treatment with imipramine in a dose which suppressed the effects of tyramine and potentiated the effects of norepinephrine. These results indicate that denopamine is a highly selective beta-1 adrenoceptor agonist in isolated, blood-perfused dog heart preparations, and they also suggest a mild catecholamine-releasing activity through tyramine-like action in isolated right atria.

Congestive heart failure is characterized by a state of insufficient cardiac output to fulfill the metabolic requirements of the body. One of the therapies for congestive heart failure is augmenting the cardiac pumping function (positive inotropic action). Cardiac glycosides are the drugs used for producing positive inotropic effects, but their effectiveness and safety remain controversial because of their toxicity. Some catecholamines such as dopamine and dobutamine, which have a strong positive inotropic effect, are clinically available only by intravenous administrations. Therefore, potent positive inotropic drugs which are orally active and possess a wide margin of safety are of great interest in the treatment of heart failure. Recently, orally active positive inotropic compounds have been developed which have beta-sympathomimetic (1–4) or phosphodies- terase inhibiting action (5–9), and these agents are structurally unrelated to cardiac glycosides. Denopamine, a newly synthesized phenylethanolamine derivative, is the former type of cardiotonic agent. Ikeo et al. (10) have reported that, administered parenterally or orally, denopamine induced a significant dose-dependent increase in contractile force and dp/dtmax in the left ventricle with a little change in heart rate in anesthetized and conscious dogs. The cardiotonic effects of denopamine with a weak arrhythmogenic activity were also demonstrated in guinea pigs, rabbits, cats, monkeys, rats and pigs (11–14). Kino et al. (15) have revealed that the intravenous infusion of denopamine produced a

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marked increase in $dp/dt_{max}$ in the left ventricle without significant effects on heart rate in both normal and diseased human hearts. The mechanism of the cardiotonic activity of denopamine mainly involves its ability to selectively stimulate cardiac beta-1 receptors (16-18). However, many of the studies were performed by in vivo experiments or by the Langendorff’s method using small animals such as guinea pigs or rabbits in which beta-2 adrenoceptors have a minor role for increasing heart rate or cardiac contractility (19-22). Recently, we have reported the existence of positive chrono- and inotropic responses mediated by beta-2 adrenoceptors in addition to predominant beta-1 adrenoceptors in isolated, blood-perfused right atrial and left ventricular preparations of the dog (23). Therefore, this study was designed to examine the direct cardiac effects of denopamine on isolated, blood-perfused right atrial and left ventricular preparations of the dog which are free from extracardiac modifications such as autonomic nervous reflexes.

Materials and Methods

Preparation of the isolated, blood-perfused right atrial or left ventricular muscle: Experiments were carried out on mongrel dogs of either sex anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Isolated right atria or left ventricles were obtained from 28 recipient dogs weighing 7 to 16 kg, and each preparation was perfused with arterial blood from a second support dog. The details of these preparations have been described in previous papers (24-26). Sodium heparin (500 USP units/kg, i.v.) was administered to each dog at the beginning of the perfusion, and 200 USP units/kg were given each hour thereafter. After heparin (200 USP units/kg, i.v.) was administered, the right atrium or the left ventricle was excised and immersed in cold Ringer’s solution. The wet weight of the isolated right atrial and left ventricular preparations varied from 8 to 16 g and 10 to 18 g, respectively.

In the right atrial preparation, the sinus node artery was cannulated via the right coronary artery, and it was perfused with heparinized blood led from the carotid artery of the support dog with the aid of a peristaltic pump (Harvard Apparatus model 1210). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained at 100 mmHg. The blood flow rate to the isolated atrium was 6 to 11 ml/min. The venous effluent from the preparation was led to a collecting funnel, from which it was returned continuously to the support dog via the external jugular vein. The ventricular margin of the atrium was attached to a rigid stainless steel bar, and the preparation was placed in a glass container which was kept at a constant temperature of 37°C by means of a heating bath circulator (Haake FE 2). The upper part of the atrium was connected to a force-displacement transducer (Nihon Kohden AP 620 G) by a silk thread. The atrial muscle was usually stretched to a resting tension of 2 g. The isometric tension was recorded on a thermo-writing rectigraph (Nihon Kohden WT 685T). A pair of silver electrodes was brought into contact with the epicardial surface of the isolated atrium to record the atrial electrogram. The tachometer was driven by the atrial electrogram.

The left ventricular muscle along the anterior descending branch of the left coronary artery was excised, and the anterior descending artery was cannulated. The left ventricular preparation was perfused with the heparinized blood from the support dog using the same perfusion system for the right atrial preparation. A pair of bipolar silver electrodes was sewn on the ventricular free wall, and the preparation was driven by an electrical stimulator (Nihon Kohden SEN 7103) at a frequency of 2 Hz with square wave pulses of 2 msec duration and twice the threshold voltage (usually 4 V). The left ventricular tension development was measured isometrically by a force-displacement transducer through a fine thread connected to a ventricular surface. The resting tension of the ventricular muscle was 2 g.

The femoral arterial blood pressure and heart rate derived from the ECG lead II of the support dog and the blood flow rate to a preparation were simultaneously recorded.

Drugs: The drugs used in the experiments were denopamine (TA-064, (−)-(R)-1-(p-hydroxyphenyl)-2-[(3,4-dimethoxypheneth-
yl) amino]ethanol, generously donated by Tanabe Seiyaku Co., Ltd., Osaka, Japan), /-isoproterenol hydrochloride (Nikken Kagaku), d,l-norepinephrine hydrochloride (NE, Sankyo), procaterol hydrochloride (Otsuka), tyramine hydrochloride (Wako Pure Chemical), propranolol hydrochloride (Sigma), atenolol (Sigma), ICI 118,551 (generously donated by Imperial Chemical Industries, Macclesfield, England) and imipramine hydrochloride (Fujisawa). All drugs were dissolved in physiological saline before the start of the experiment. The volume of the drug solution injected into the sinus node artery of the isolated right atrium or the anterior descending branch of the isolated left ventricle was 0.01–0.03 ml over a period of 4 sec. The dose of a cardiotonic agent which evoked about 50–60% increase in atrial contractile force with an obvious chronotropic response was roughly selected for the control response, i.e., denopamine at 1 or 3 nmol, EN at 0.1 or 0.1 nmol, procaterol at 1 or 3 nmol and tyramine at 10 or 30 nmol, respectively. Since the effect of each antagonist appeared in less than 1 min and continued for approximately 20–30 min, the positive chrono- and inotropic responses to each agonist were usually assessed 1–1.5 min after treatment with an antagonist.

**Statistical analysis:** All changes in positive chrono- and inotropic responses induced by each dose of substance were expressed as percent changes from their predrug levels. Values presented are means±S.E.M. Data, which were obtained as the maximum responses to each drug, were analyzed by Student's t-test for paired data. A P value less than 0.05 was considered as statistically significant.

**Results**

**Effects of denopamine, isoproterenol, NE and procaterol on sinus rate and tension development in isolated right atria:** When denopamine was injected into the sinus node artery of the spontaneously beating dog right atrium, the drug dose-dependently increased the sinus rate and atrial contractile force, and the positive chrono- and inotropic responses to denopamine at high doses lasted more than 10 min (Fig. 1). Dose-response curves of denopamine, isoproterenol, NE and procaterol for the sinus rate and atrial tension development are shown in Fig. 2. The threshold doses for increasing the sinus rate and

![Fig. 1. Typical tracings of positive chrono- and inotropic responses to 0.3, 3 and 30 nmol of denopamine in an isolated dog right atrium (A) and positive inotropic responses to 1, 10 and 100 nmol of denopamine in an isolated dog left ventricle (B).](image-url)
atrial tension were 0.001 nmol (isoproterenol), 0.01 nmol (NE), 0.03 nmol (procaterol) and 0.1 nmol (denopamine). The rank order of potency for the positive chronotropic and inotropic responses were isoproterenol>NE>denopamine (Fig. 2, Table 1). Denopamine was about 300 times less potent than isoproterenol for increasing sinus rate, whereas denopamine was only 30 times less potent than isoproterenol for increasing atrial contractility (Table 1). Although a selective beta-2 agonist, procaterol, increased the sinus rate more than atrial contractility, the maximal responses were much smaller than those produced by isoproterenol.

**Effects of denopamine, isoproterenol and procaterol on tension development in isolated left ventricles:** When denopamine was administered into the cannulated anterior descending branch of the left coronary artery of the electrically driven left ventricle, positive inotropic effects were induced in a dose-related manner. Typical tracings of increasing doses of denopamine are shown in Fig. 1 (lower panel). Summarized data of dose-responses to denopamine, isoproterenol and procaterol are shown in Fig. 3. The threshold doses for inducing the positive inotropic responses were 0.001 nmol (isoproterenol), 0.03 nmol (procaterol) and 0.1 nmol (denopamine), respectively. The effective dose for increasing ventricular contractility by 100% (ED100) of denopamine was 49 nmol and was about 200 times larger than that of isoproterenol (Table 1).

**Effects of propranolol on positive chronotropic and inotropic responses induced by denopamine and NE:** After treatment with a non-selective beta-blocker, propranolol (0.1–10 nmol), the positive chrono- and inotropic responses to both denopamine (1 or 3 nmol) and NE (0.1 or 0.3 nmol) were dose-dependently suppressed in the isolated, cross-circulated dog right atrium. Summarized data

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### Table 1. ED values of denopamine, isoproterenol and NE for sinus rate, atrial and ventricular developed tension in isolated, blood-perfused canine right atrium and left ventricle

| Parameter | ED100 | Denopamine | Isoproterenol | NE |
|-----------|-------|------------|---------------|-----|
| SR        | ED40  | 18.3±5.5   | 0.06±0.01     | 0.54±0.20 |
| AT        | ED150 | 3.7±1.9    | 0.13±0.05     | 0.39±0.08 |
| VT        | ED100 | 49.0±22.8  | 0.25±0.04     |     |

Data were obtained from 6–9 isolated right atria and 4–6 isolated left ventricles. Each value is presented as a mean±S.E.M. Basal sinus rate, atrial contractile force and ventricular contractile force were 110.4±5.5 beats/min, 2.0±0.3 g and 5.0±0.5 g, respectively. SR, sinus rate; AT, atrial tension development; VT, ventricular tension development; NE, norepinephrine.
Effects of atenolol on positive chrono- and inotropic responses induced by denopamine, NE and procaterol: A selective beta-1 antagonist, atenolol (0.1–10 nmol), dose dependently inhibited the increases in sinus rate and atrial contractility elicited by denopamine and NE, whereas atenolol only slightly attenuated the positive chrono- and inotropic

Fig. 3. Dose-response curves of isoproterenol (■, n=6), denopamine (○, n=4) and procaterol (●, n=6) for tension development of isolated left ventricles. Data are expressed as percentage changes of each basal value. Basal ventricular contractile force was 5.0±0.5 g. Points represent mean values, and vertical bars show S.E.M.

Fig. 4. Effects of treatment with propranolol (0.1–10 nmol) on increases in sinus rate (upper panel) and atrial tension development (lower panel) induced by denopamine (1 or 3 nmol, open columns) and norepinephrine (NE, 0.1 or 0.3 nmol, hatched columns) in 5 isolated, blood-perfused right atria. Data are expressed as percentage changes of each basal value. Vertical bars show S.E.M. *P<0.05, **P<0.01, compared with the respective control (C).
responses to procaterol in the dog right atrium (Fig. 5). Mean percentage inhibitions in the denopamine-induced positive chrono- and inotropic responses by 10 nmol of atenolol were 63.8±5.9 and 55.9±7.9%, respectively; and those in the NE-induced responses by the same dose of atenolol were 79.5±1.6 and 79.4±3.6%, respectively.

![Diagram of Fig. 5](image)

**Fig. 5.** Effects of treatment with atenolol (0.1–10 nmol) on increases in sinus rate (upper panel) and atrial tension development (lower panel) induced by denopamine (1 or 3 nmol, open columns), norepinephrine (NE, 0.1 or 0.3 nmol, hatched columns) and procaterol (1 or 3 nmol, dotted columns) in 5–10 isolated, blood-perfused right atria. Data are expressed as percentage changes of each basal value. Vertical bars show S.E.M. *P<0.05, **P<0.01, compared with the respective control (C).

![Diagram of Fig. 6](image)

**Fig. 6.** Effects of treatment with ICI 118,551 (0.1–10 nmol) on increases in sinus rate (upper panel) and atrial tension development (lower panel) induced by denopamine (1 or 3 nmol, open columns), norepinephrine (NE, 0.1 or 0.3 nmol, hatched columns) and procaterol (1 or 3 nmol, dotted columns) in 5–9 isolated, blood-perfused right atria. Data are expressed as percentage changes of each basal value. Vertical bars show S.E.M. *P<0.05, **P<0.01, compared with the respective control (C).
Effects of ICI 118,551 on positive chronotropic and inotropic responses evoked by denopamine, NE and procaterol: A selective beta-2 antagonist, ICI 118,551 (0.1–10 nmol), dose-dependently inhibited the increases in sinus rate and atrial contractile force elicited by procaterol. ICI 118,551 did not significantly affect the responses to NE, and the antagonist at lower doses also did not significantly modify the positive chronotropic and inotropic responses to denopamine. However, ICI 118,551 at 10 nmol significantly (P<0.05) attenuated the positive chronotropic response to denopamine, and it tended to decrease the positive inotropic response to denopamine (0.05<P<0.1, Fig. 6).

Effects of imipramine on positive chronotropic and inotropic responses induced by denopamine, NE and tyramine: Imipramine, an uptake blocker of catecholamines, at a dose of 300 nmol significantly inhibited the positive chronotropic and inotropic responses to tyramine (10 or 30 nmol) and potentiated the effects of NE (0.1 or 0.3 nmol) in 7–8 isolated, blood-perfused dog right atria. After treatment with the same dose of imipramine, the denopamine-induced increases in sinus rate and atrial contractile force were slightly but significantly depressed (Fig. 7). Mean percentage inhibitions in the positive chronotropic and inotropic responses to tyramine by imipramine were 68.4±7.4 and 69.4±5.1%, respectively; and those in the denopamine-induced responses were 48.0±6.6 and 30.5±10.7%, respectively.

Discussion
The positive chronotropic and inotropic effects of a recently developed orally active cardiotonic agent, denopamine, were investigated in isolated, cross-circulated heart preparations of the dog. It has been reported that denopamine increased LV dp/dtmax, heart rate, cardiac output and myocardial oxygen consumption without changing cardiac metabolism in dogs and rabbits (11, 27). Kino et al. (15) have reported that denopamine showed a positive inotropic effect with minimal or no change in heart rate or left ventricular systolic pressure in both healthy subjects and patients with congestive heart failure who were maximally treated with conventional regimens. These results suggest that denopamine is a good agent for treating patients with impaired cardiac function, especially for long-term management of congestive heart failure. In our study, denopamine dose-dependently increased the sinus rate, atrial and ventricular contractility in isolated dog heart preparations and the effects of denopamine at higher doses lasted more than 10 min. It has been reported that the positive inotropic activity of denopamine was 1/60–1/300 that of isoproterenol in many species (12, 28), and these results are consistent with our findings.

Denopamine produced a potent positive inotropic effect without affecting systemic blood pressure (12), and it has also been shown that the increases in heart rate and myocardial oxygen consumption by denopamine were rather weak (27). Nagao et al. (12) also reported that the positive inotropic effect of denopamine was more pronounced.
than the positive chronotropic effect, compared with those of isoproterenol. Ikeo et al. (10) suggested that the weaker positive chronotropic effect of denopamine in conscious dogs than in anesthetized dogs might be due to the baroreflex which was initiated by a slight increase in systolic blood pressure and an increase in rate of pressure change secondary to its positive inotropic action. In the isolated right atrium which was free from autonomic nervous reflexes, denopamine increased atrial contractility more than the sinus rate compared with a non-selective beta-agonist, isoproterenol. The ED$_{150}$ of denopamine for atrial tension indicates that denopamine is only 30 times less potent than isoproterenol, whereas the ED$_{40}$ of denopamine for the sinus rate is about 300 times larger than that of isoproterenol, suggesting the relatively selective inotropy of denopamine.

The mechanism of action by which denopamine elicited the positive chrono and inotropic responses was reported to mainly involve the stimulation of beta-1 adrenoceptors by this agant (16-18), and this was also confirmed by the present study. We have recently reported that a selective beta-1 agonist, dobutamine, was much more effective in increasing atrial contractility than the sinus rate in isolated, blood-perfused dog right atria (23). Denopamine increased atrial contractility more than the sinus rate. Furthermore, the positive chrono- and inotropic effects induced by denopamine in isolated right atria were dose-dependently inhibited by treatment with a non-selective beta-blocker, propranolol, which inhibited the NE-induced responses. These responses were also blocked by a selective beta-1 antagonist, atenolol, in a dose range which hardly affected the positive responses to procaterol. The positive chrono- and inotropic responses to NE which is known to have a much higher selectivity for beta-1 adrenoceptors than beta-2 adrenoceptors (20, 29-31) were inhibited by 10 nmol of atenolol more strongly than those to denopamine (Fig. 5). Although Lands et al. (32, 33) first subclassified beta-adrenoceptors into beta-1 and beta-2 subtypes with absolute organ-specific distribution such as beta-1 receptors in the heart and beta-2 receptors in the lung, it has been suggested that both receptor subtypes exert a physiological role in the cat, dog and human hearts from pharmacological (20, 34, 35), biochemical (30, 36, 37) and receptor-binding studies (38-40). Recently, we have also reported that positive chronotropic and inotropic responses to beta-adrenoceptor agonist were in part mediated via the beta-2 adrenoceptors in the isolated, blood-perfused dog atrium and that the beta-2 adrenoceptor influenced the sinus rate more than atrial tension (23). Consequently, we have demonstrated that a selective beta-2 antagonist antagonized the sinus rate increase more than the atrial contractility increase when both beta-receptor subtypes were simultaneously stimulated. In the right atrium, the denopamine-induced positive chronotropic response was slightly but significantly reduced by the treatment with 10 nmol of ICI 118,551, which did not affect the responses to NE. Ozaki et al. (41) have reported that denopamine caused vasodilatation of the dog coronary artery which was contracted with prostaglandin F$_{2a}$ by the activation of beta-1 adrenoceptors. In their study, however, a relaxation of the renal artery induced by denopamine was only slightly attenuated by a selective beta-1 antagonist, metoprolol, and the effect was completely inhibited by propranolol. Nagao et al. (12) showed that the small vasodilating effect of denopamine on the femoral artery of the anesthetized dog was shifted after treatment with propranolol. It has been suggested that beta-adrenergic receptors in renal, mesenteric and femoral arteries were of the beta-2 subtype (42). These results may suggest that denopamine also possesses a weak beta-2 adrenoceptor stimulating property.

Nagao et al. (12) have reported that in the reserpinized dog, denopamine exerted increases in cardiac contractile force, heart rate and blood pressure similar to those in the non-reserpinized normal dog, suggesting that the cardiotonic actions of denopamine were not due to the release of endogenous catecholamines. Kino et al. (15) have also reported that the positive inotropic effects of denopamine were not mediated by the release of the intrinsic catecholamines by measuring the
plasma catecholamine levels. However, in this study, the positive chrono- and inotropic responses to denopamine was significantly attenuated by treatment with imipramine. The same dose of imipramine significantly depressed the tyramine-induced responses and significantly potentiated the responses to NE, indicating the dose of imipramine acted as an uptake blocker of endogenously released or externally administered NE. The mean percentage inhibitions by imipramine of the positive responses to denopamine were much smaller than those to tyramine. Therefore, the catecholamine releasing action of denopamine seems to be partly involved in the positive chrono- and inotropic effects of denopamine.

In conclusion, denopamine is a highly selective, but not a specific, beta-1 adrenoceptor agonist in the isolated, blood-perfused canine right atrium and left ventricle, and it is suggested that the positive chrono- and inotropic responses to denopamine are in part mediated by the tyramine-like catecholamine releasing action in the isolated, blood-perfused canine right atrium.

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