Weak Arrhythmogenic Property of the New Cardiotonic Agent Denopamine in Dogs: Comparison with Catecholamines

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Abstract—We studied the arrhythmogenic activity of denopamine, in relation to its positive inotropic action, in dogs and compared it with those of catecholamines. The positive inotropic activities of the compounds as expressed in terms of the ED₅₀ (μg/kg, i.v.), that increased LV dp/dt max of anesthetized dogs by 100% of the control were 8.0 for denopamine, 0.27 for norepinephrine, 0.03 for isoproterenol, 3.8 for dobutamine and 16 for dopamine. On the other hand, the doses of these drugs at which the “non-sinus/total rate” increased significantly (about 30% of total beats, μg/kg, i.v.) were more than 1000 for denopamine, 1.0 for norepinephrine and isoproterenol, and 300 for dobutamine and dopamine in coronary ligated dogs. The ratios of these doses to the ED₅₀ are more than 126 for denopamine, 80 for dobutamine, 33 for isoproterenol, 19 for dopamine and 3.8 for norepinephrine. Arrhythmogenic activity of denopamine was also weaker than those of dobutamine and dopamine in halothane anesthetized dogs. The arrhythmogenic activity of dobutamine was weak as reported, but that of denopamine was the weakest among the drugs tested.

Positive inotropic agents generally exhibit arrhythmogenic activity. Especially, catecholamines augment ectopic automaticity and cause arrhythmia by stimulating the adrenergic β-receptor (1, 2). Arrhythmogenic activities of norepinephrine, isoproterenol and dopamine have been reported (3). This action is one of the limiting factors when inotropic drugs are used. Dobutamine has been selected as a positive inotropic agent with weak arrhythmogenic activity (3).

Denopamine (TA-064) is a novel, orally active cardiotonic agent. It has no catechol moiety in its structure, but it is a highly selective β₁-adrenoceptor agonist (4). In addition to lacking the α-adrenoceptor agonistic property, the positive inotropic action of denopamine is more pronounced than its positive chronotropic action (4).

In this study, the arrhythmogenic action of denopamine was studied in coronary ligated conscious dogs as well as in halothane anesthetized dogs by intravenous administration. Similarly, the effect of denopamine on cardiac function was studied in pentobarbital anesthetized dogs. We calculated the ratio of arrhythmogenic to positive inotropic action of denopamine and compared it with those of some catecholamines. Denopamine showed the weakest arrhythmogenicity among the compounds tested.

Materials and Methods

1. Animals

Forty-seven mongrel dogs, weighing 10–23.3 kg, were used. The dogs used for the coronary ligation study were free of microfillarias.

2. Arrhythmogenic activity test in coronary ligated dogs

Preparation: Two-stage ligation of the left anterior descending coronary artery was performed as described by Harris (5). Under pentobarbital sodium anesthesia (30 mg/kg, i.v.), the dog was intubated and artificially respirated (room air, 15 ml/kg/stroke, 20
strokes/min). Thoracotomy was performed at the left 4th intercostal space under sterile conditions. The left anterior descending coronary artery was separated from surrounding tissues within 1 cm from the bifurcation of the left circumflex coronary artery, proximal to the first branch. The artery was constricted with a steel wire (0.9 mm in diameter). The artery was ligated 30 min after the constriction and the thorax was closed.

Thereafter, a cannula, made of vinyl tubing (Extension tube®), was inserted into the left jugular vein for drug administration. The cannula was filled with sodium heparin solution (1000 units/ml) after the operation. Electrodes for electrocardiogram recording were set under the skin of every extremity.

After the operation, antibiotics (penicillin G and streptomycin) were injected intramuscularly. The cannula and the lead wires were fixed at the back of the dog and were protected from spoiling by a dog jacket.

Procedure: Three or 4 days after the operation, dogs which scarcely showed spontaneous arrhythmia were used. Experiments were performed with the dogs in a standing position. Standard limb lead electrocardiograms were recorded on an electrocardiograph (Fukuda, FD-32) with instrumented electrodes. Non-sinus beats judged from the electrocardiogram were defined as arrhythmia.

Experiments were carried out in 2 groups. Because denopamine is a long-lasting drug, a group of 6 dogs was allotted to denopamine administration only. The actions of isoproterenol, dobutamine and dopamine were studied in the other group of 6 dogs, by shifting the schedule of administration, because the actions of these compounds are short-lived. In both cases, effects of norepinephrine, at doses of 0.5–4 µg/kg, i.v., were studied to test the response of the preparation before the start of experiment with every test drug. Drugs were administered through the implanted venous cannula at doses elevated stepwise at 5 min intervals. If many arrhythmic beats were observed, electrocardiograms were recorded at 5 min intervals until the arrhythmia subsided, and then the next dose of the drug was administered. As a rule, electrocardiograms were recorded for 2 min before and after each dose, and the numbers of both total and arrhythmic beats and the arrhythmic ratio (number of arrhythmia/total beats) for 2 min were obtained.

In the experiments with denopamine, the effect of norepinephrine was re-examined 3 hr after the administration of the maximum dose of denopamine.

3. Arrhythmogenic activity test in halothane anesthetized dogs

Anesthesia was produced with intravenous administration of ketamine (20 mg/kg) and maintained after endotracheal intubation with 1.5% halothane in oxygen volatilized by a precision vaporizer and delivered through a semiclosed anesthetic circuit. All dogs were volume ventilated to maintain an arterial Pco2 within the physiological range. Standard limb lead electrocardiograms were recorded on a polygraphy (Nihon Kohden, RM-45). Non-sinus beats judged from the electrocardiogram were defined as arrhythmia.

As in the case of coronary ligated dogs, a group of 6 dogs was allotted to denopamine administration only. The actions of dobutamine and dopamine were studied in the other group of 5 dogs by shifting the schedule of administration. The action of denopamine was also studied in this group after the experiment with dobutamine and dopamine. In all cases, the effects of norepinephrine, at doses of 1–4 µg/kg, i.v., were studied to test the response of the preparation before the start of experiment with every test drug. Drugs were administered into the left femoral vein at doses elevated stepwise at 5 min intervals as in the other experiments. As a rule, electrocardiograms were recorded for 1 min before and after each dose, and the numbers of both total and arrhythmic beats and the arrhythmic ratio (number of arrhythmia/total beats) for 1 min were obtained.

4. Effects on cardiac function in anesthetized dogs

Experiments were carried out under pentobarbital sodium anesthesia (30 mg/kg, i.v.) and artificial respiration (room air, 15 ml/kg/stroke, 20 strokes/min). During the
experiment, sodium pentobarbital at a rate of 4 mg/kg/hr was infused into the right femoral vein to keep the level of anesthesia constant.

A polyethylene cannula was inserted into the right femoral artery, and aortic pressure was measured with a pressure transducer (Nihon Kohden, MPU-0.5). Heart rate was measured with a cardiotachometer (Nihon Kohden, AT-600G) triggered by aortic pressure pulses. A polyethylene cannula was also inserted into the right femoral vein, and central venous pressure was measured with a pressure transducer (Nihon Kohden, LPU-0.1). The zero level of central venous pressure was referenced at the center of the height of the thorax. Left ventricular pressure was measured with a catheter tip transducer (Millar, PC-350), which was inserted from the left common carotid artery. LV dp/dt and $\frac{LV \ dp/dt}{P}$ were calculated with a differentiation amplifier (Nihon Kohden, EO-601G).

The early diastolic phase of left ventricular pressure was measured at 5 msec intervals, and Weiss' time constant $T$ (6) was obtained with the aid of a calculator and a X-Y plotter (Hewlett Packard, 9845B). Variables, except $T$, were simultaneously recorded on a recticorder (Nihon Kohden, WT-685G).

5. Drugs

The compounds used in this study are as follows: denopamine (Organic Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd.), norepinephrine (Nor-adrenaline®, Sankyo), isoproterenol hydrochloride(Sooner-L®, Kaken Seiyaku), dopamine hydrochloride (Nakarai Yakuhin), and dobutamine (Dobutrex®, Shionogi).

Denopamine was dissolved with an equivalent amount of 0.1 N HCl and diluted with 0.9% NaCl solution to the administration volume of 0.1 ml/kg. Other drugs were dissolved in 0.9% NaCl solution to make an administration volume of 0.1 ml/kg.

6. Statistics

Results were expressed by means, means ±S.E.M. or geometric means (95% confidence limits). Statistical analysis was performed using the paired $t$-test or analysis of variance. $P<0.05$ was considered to be statistically significant.

Results

1. Arrhythmogenic activity in coronary ligated dogs

Many types of arrhythmia were observed in this series of experiments as reported by Moran et al. (7). Though the arrhythmogenic activity of denopamine was weak as will be described later, the types of denopamine-induced arrhythmia were not different from those induced by other compounds. In our preparation, norepinephrine produced a dose-dependent increase in the arrhythmic ratio, which was about 85% in each trial at a dose of 4 $\mu$g/kg, i.v.

Figure 1 shows the effects of isoproterenol, dobutamine and dopamine. As shown in this figure, the response to norepinephrine was similar in each drug group, and isoproterenol (1 and 3 $\mu$g/kg, i.v.), dobutamine (300 $\mu$g/kg, i.v.) and dopamine (300 $\mu$g/kg, i.v.) also increased the arrhythmic ratio significantly.

Figure 2 shows the effect of denopamine. The pre-test response to norepinephrine in this group was also similar to those shown in Fig. 1. Under these conditions, denopamine, up to 30 $\mu$g/kg, i.v., did not increase the arrhythmic ratio at all. Denopamine, at doses above 100 $\mu$g/kg, i.v., tended to increase the arrhythmic ratio, but the increase was not statistically significant even at the highest dose of 1000 $\mu$g/kg, i.v.

In this preparation, the post-test response to norepinephrine, tested 3 hr after the administration of denopamine, tended to decrease compared to the pre-test response. The arrhythmic ratio at a norepinephrine dose of 4 $\mu$g/kg, i.v., decreased significantly ($P<0.05$) from the pre-test response at the same dose.

2. Arrhythmogenic activity in halothane anesthetized dogs

As in the case of coronary ligated dogs, the
types of arrhythmia observed in this preparation have already been reported (8, 9). Types of denopamine-induced arrhythmia observed in a small number of cases were similar to those observed in other drug groups.

Figure 3 shows the effects of dobutamine, dopamine and denopamine, and Fig. 4 shows the effect of denopamine. As in coronary ligated dogs, norepinephrine induced an increase in arrhythmic ratio in a dose-dependent manner. The numbers of dogs developing arrhythmia by norepinephrine were similar in each drug group, but norepinephrine-induced increase in arrhythmic ratio tended to decrease after the experiments of dobutamine and dopamine.

Dobutamine and dopamine induced arrhythmia in a dose-dependent manner at a dose of 30 μg/kg, i.v., or more. On the other hand, denopamine, results shown in Figs. 3 and 4, scarcely induced arrhythmia in both cases. Even if development of arrhythmia was observed, effect of denopamine was not
dose-related, and the arrhythmic ratio was less than 10%.

3. Effects on cardiac function

As shown in Table 1, pre-test values of the cardiac parameters were not significantly different among the drug groups. Responses to norepinephrine, at doses of 0.125–1 μg/kg, i.v., were also not different among the drug groups (data not shown).

Figure 5 shows dose-response relationships of the cardiovascular effects of the test drugs, including pooled data of the norepinephrine effects.

Norepinephrine increased, and isoproterenol decreased the mean aortic pressure in dose-dependent manners. However, other three drugs caused biphasic responses; they increased and then decreased the mean aortic pressure. Dopamine produced a marked increase in mean aortic pressure, while denopamine exhibited only a small increase. Denopamine and dobutamine, at higher doses, caused considerable dose-related decreases.

Heart rate was decreased by norepinephrine. Other drugs caused dose-dependent increases in heart rate except for dopamine, which decreased the heart rate after a transient increase. Isoproterenol and denopamine showed almost maximum
responses at the largest doses. The increase in heart rate caused by the largest dose of denopamine (81.8±7.8 beats/min) was significantly smaller than that caused by the largest dose of isoproterenol (107.8±7.0 beats/min, P<0.05).

All drugs increased central venous pressure, especially at high doses of dopamine, but denopamine and dobutamine caused biphasic responses: decreases followed by increases. Denopamine, at doses of 3 and 10 μg/kg, i.v., decreased central venous pressure significantly (P<0.01).

T was shortened by all drugs. Compared to the effects on LV $dp/dt_{\text{max}}$, the effect of denopamine on T was relatively stronger than that of dobutamine. The effect of dopamine on T was the weakest of all.

All drugs caused an increase in LV $dp/dt_{\text{max}}$ dose-dependently as in LV $dp/dt_{\text{max}}$. The maximum responses of LV $dp/dt_{\text{max}}$ to denopamine and dobutamine were smaller, but not significantly, than those to iso-
Fig. 4. Arrhythmogenic action of denopamine was studied in halothane anesthetized dogs. Administration of drugs and recording of electrocardiograms were performed as described in the legend for Fig. 3. Each column and vertical bar indicate the mean ±S.E.M. of 6 experiments. Numerals in the figure represent the number of dogs that developed arrhythmia. □: sinus rate, ▮▮▮: non-sinus rate, NE: norepinephrine, DEN: denopamine.

Fig. 5. Cardiovascular actions of denopamine and some catecholamines were studied in anesthetized dogs. Drugs were administered through the venous cannula at doses elevated stepwise at 5 min intervals. Each symbol indicates the mean of 6 experiments except the case of norepinephrine (N=24). When biphasic responses were observed, the secondary phases were shown in broken lines. MAP: mean aortic pressure, HR: heart rate, CVP: central venous pressure, T: Weiss' time constant, □: denopamine, □: isoproterenol, ●: norepinephrine, ▽: dobutamine, △: dopamine.
proterenol. Maximum responses of LV dp/dt max were not obtained in these dose ranges of norepinephrine and dopamine. The relative potencies of the effect on LV dp/dt max were in the decreasing order of isoproterenol > norepinephrine > dobutamine > denopamine > dopamine.

Table 2 shows geometric means and 95% confidence limits of the dose at which LV dp/dt max increases by 100% (ED100). Table 2 also shows the doses at which the arrhythmic ratio increased significantly in coronary ligated dogs (cf. Figs. 1 and 2) and the ratios of these doses to ED100. The relative arrhythmogenic activities of norepinephrine, dopamine, isoproterenol, dobutamine and denopamine decreased in this order.

Discussion

We studied arrhythmogenic activities of denopamine and some catecholamines in coronary ligated conscious dogs and in halothane anesthetized dogs and studied the positive inotropic actions of these compounds in pentobarbital anesthetized dogs. The order of relative arrhythmogenic activity in coronary ligated dogs, compared to their positive inotropic activities, was norepinephrine > dopamine > isoproterenol > dobutamine > denopamine. Arrhythmogenic activity of denopamine was also weaker than those of dobutamine and dopamine in halothane anesthetized dogs. Thus the arrhythmogenic activity of denopamine was the weakest among the drugs tested.

Hoshiyama et al. reported that the order of the activity to induce a cardiac automaticity in isolated cat papillary muscle was isoproterenol > norepinephrine = dopamine > dobutamine > denopamine (10). Sato et al. also reported that the activity of denopamine to induce a cardiac automaticity was weaker than that of isoproterenol and that denopamine did not affect the cardiac action potential duration in isolated guinea pig papillary muscle (11). Moreover, they found that denopamine-induced increase in IC50 for a given positive inotropy was smaller than that induced by isoproterenol in partially depolarized guinea pig papillary muscle. All of these effects of denopamine in isolated tissues were not dose-dependent. Mechanisms of these phenomena are not clear, but such a dose-unrelated effect of denopamine was also observed in halothane anesthetized dogs. In addition to these in vitro studies, Tokutake et al. reported that denopamine-induced rises of interstitial K+ and the ST segment of the epicardial electrocardiogram in the ischemic heart of anesthetized dogs were less pronounced than those of iso-

| Compound       | ED100 (µg/kg) (A) | Arrhythogenic dose (µg/kg) (B) | Arrhythogenic index (B/A) |
|----------------|-------------------|--------------------------------|--------------------------|
| Denopamine     | 7.95 (3.96–15.98) | >1000                          | >125.8                   |
| Norepinephrine | 0.268 (0.211–0.339) | 1.0                             | 3.8                      |
| Isoproterenol  | 0.027 (0.020–0.038) | 1.0                             | 33.3                     |
| Dobutamine     | 3.76 (1.34–10.56)  | 300                             | 79.8                     |
| Dopamine       | 15.85 (8.68–28.96) | 300                             | 18.9                     |

*ED100 is the dose which causes a 100% increase in LV dp/dt max obtained graphically from each dose-response curve. Values in the parentheses are 95% confidence limits. *Values are obtained in conscious coronary ligated dogs (cf. Figs. 1 and 2).
proterenol at a similar inotropic action (12). These findings can explain the reason why the arrhythmogenic activity of denopamine was weak.

The arrhythmogenic activity of isoproterenol, relative to its positive inotropic effect in pentobarbital anesthetized dogs, was weaker than those of norepinephrine and dopamine in coronary ligated dogs. This order cannot be explained by the findings in isolated tissues. Norepinephrine and dopamine markedly elevated aortic pressure and decreased heart rate transiently in anesthetized dogs. It has been reported that vagolytic drugs suppressed the norepinephrine-induced arrhythmia in conscious coronary ligated dogs (13). Therefore, the hypertensive action and baroreflex-induced bradycardia with norepinephrine and dopamine seemed to augment arrhythmogenicity in our preparations.

Recently, the effects of denopamine on cardiac intracellular cyclic AMP levels and adenylate cyclase activity were studied, and denopamine was classified into a group of β₁ agonists with relatively diminished capacity to activate adenylate cyclase (14, 15). Although dobutamine has α- and β₂-adrenoceptor stimulating actions (3), it also belongs to this group (16). This characteristic of denopamine and dobutamine is interesting in relation to their weak arrhythmogenicity.

The maximum increases in LV dp/dt p max and heart rate with denopamine were smaller, the former insignificantly and the latter significantly, than those with isoproterenol. In this sense, denopamine seems to be a selective positive inotropic agent as has been reported (4). The result also suggest that denopamine is a partial agonist with a high intrinsic activity at least in its chronotropic action. This property of denopamine may also contribute to its weak activity to induce a cardiac abnormal automaticity.

The arrhythmogenic activity of norepinephrine tended to decline by pretreatment with denopamine. Since denopamine exhibited a partial agonistic property in anesthetized dogs, as described above, the β-adrenoreceptor blocking action of denopamine might be involved in this decline. However, the differences between denopamine and isoproterenol in their maximum responses of positive inotropic and chronotropic actions were small. Norepinephrine-induced rhythm disturbance in coronary ligated dogs was reported to be normalized by atrial electrostimulation at a pacing rate higher than ventricular arrhythmia (13). This explanation seems to be applicable to the present result, because the basal heart rate was increased by denopamine pretreatment.

Effects of denopamine on central venous pressure and T were not similar to those of the other compounds tested. Thus further studies are called for on the effects of the venous system and the lusitropic action of denopamine.

In conclusion, denopamine possesses positive inotropic action, but the arrhythmogenic activity of denopamine was relatively weaker than those of some catecholamines. This property is mainly attributable to its weak evoking action on a cardiac abnormal automaticity and to its lack of an effect on action potential duration observed in in vitro preparations. The lack of hypertensive action of denopamine may also contribute to this property. Arrhythmogenicity of denopamine should be studied further in various clinical situations.

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