Cardiovascular Effects of a New Phenoxyalkylamine Derivative, 2-isopropyl-5-[3-(2-methoxyphenoxy)propylamino]-2-(3,4,5-trimethoxyphenyl)valeronitrile fumarate (HV-525), in Cross-Circulated Dog Atrial Preparations

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SUMMARY

A newly developed phenoxyalkylamine derivative, 2-isopropyl-5-[3-(2-methoxyphenoxy)propylamino]-2-(3,4,5-trimethoxyphenyl)valeronitrile fumarate (HV-525), was investigated in intact dogs and in isolated dog atria perfused with anesthetized donor dog’s arterial blood. When 0.3 mg/kg of HV-525 was intravenously administered to the donor dog, a depressor effect without significant changes in heart rate was observed in donor dogs and a decrease in developed tension was observed in the isolated atrium. At 1 mg/kg, HV-525 caused a depressor response in donor dogs and decreases in developed tension and atrial rate in isolated atria. The decrease in systemic blood pressure seen following 1 mg/kg of HV-525 was between 15–40 mmHg. These effects continued for about 60 min. When HV-525 was administered into the cannulated sinus node artery of the isolated atrium, dose related negative inotropic and chronotropic actions were observed. Occasionally, HV-525 induced slight, brief positive chronotropic and inotropic effects followed by long-lasting negative effects. The threshold dose for inducing the negative chronotropic effect was approximately 3 μg while the negative inotropic one was approximately 1 μg. A large dose of 100 μg of HV-525 caused a profound deceleration but not atrial arrest. The order of potencies for inducing a negative chronotropic effect in dog atria was verapamil > propranolol > HV-525 > lidocaine > quinidine > phenytoin > disopyramide > procainamide, and that for inducing a negative inotropic effect was verapamil > propranolol > HV-525 > lidocaine > phenytoin > disopyramide > procainamide > quinidine. HV-525 did not induce a significant effect on sinoatrial conduction time. HV-525 at the doses studied, uniformly suppressed the frequency-force rela-
tionship, while verapamil, one of the phenoxyalkylamine derivatives, caused a marked depression of high frequency-induced contraction. Thus, it is concluded that HV-525 has mild depressant properties on the cardiovascular system and may have characteristics different from those of verapamil.

Additional Indexing Words:
Isolated dog atrium SA node Pacemaker activity SA conduction time Atrial contractility

THE calcium channel blocking agents (Ca antagonists) are a 4th class of antiarrhythmic drugs which block the slow inward current that is carried predominantly by calcium.1) The calcium current has many effects and plays an integral role in cardiac electrophysiology, which may contribute to the antiarrhythmic actions of this group of drugs. Among the Ca antagonists, verapamil, one of the phenoxyalkylamine derivatives, has shown predominant antiarrhythmic properties in experimental and clinical situations.1)–3) Recently, a new phenoxyalkylamine derivative, 2-isopropyl-5-[3-(2-methoxyphenoxy)propylamino]-2-(3,4,5-trimethoxyphenyl)valeronitrile fumarate (HV-525) was synthesized for its potential antiarrhythmic properties. In the present experiments, we made an attempt to study the cardiovascular effects of this new compound in comparison with previously reported effects of other antiarrhythmic agents such as quinidine, procainamide, lidocaine, disopyramide, propranolol and verapamil in blood-perfused isolated dog atrial preparations.4),5)

Methods

Eighteen mongrel dogs of either sex, weighing 8–17 kg were anesthetized with sodium pentobarbital, 30 mg/kg, i.v. After i.v. administration of sodium heparin (500 units/kg), the right atrium was quickly excised and plunged into cold physiological saline at approximately 4–10°C. The sinus node artery of the isolated atrium was cannulated via the right coronary artery and perfused with arterial blood led from the carotid artery of the donor dog by aid of a peristaltic pump (Harvard Apparatus, model 1210). The perfusion pressure was constantly maintained at 100 mmHg by use of pneumatic resistance which was set up in parallel with the perfusion circuit. The perfusion flow rate was 2–6 ml/min. The atrium was suspended in a bath filled with blood at a constant temperature of 37°C by use of a circulator thermostat pump (Haake FE2). A bipolar stimulating electrode was placed on the atrial epicardium near the upper part of the sulcus terminalis. A recording electrode
was attached to the caudal portion of the epicardium at a distance of 1.5 cm from the stimulating electrode. The atrial rate (sinus cycle length; SCL) was measured with a cardiotachometer triggered by a signal from the atrial depolarization of the electrogram. Developed tension was isometrically measured with a force displacement transducer (Nihon Kohden AP620G) connected to a part of the atrial muscle with a silk thread.

Sinoatrial conduction time (SACT) was estimated using constant atrial pacing consisting of a train of 8 cycles at a rate 10 beats/min faster than the spontaneous rate (Narula et al, 1978).6) Kobayashi et al7) reported that there was a good correlation between the SACT measured by the continuous atrial pacing method and by the premature atrial stimulation method (Strauss et al, 1973)8) using the isolated dog atrial preparation. SACT was calculated by subtracting the control SCL from the first atrial return cycle. This interval represents the total conduction time into and out of the sinus node. Pacing stimuli were provided by an electric stimulator (Nihon Kohden SEN-7130) with a strength of approximately twice threshold voltage and a duration of 2 msec. SACT was digitized by a pulse counter (Nihon Kohden ET612J) and a printer (Citizen CBM Co.). This procedure was repeated 5 times and SACT was gained using 20% trimmed mean.

The donor dogs, weighing 12–25 kg, were anesthetized with 30 mg/kg of sodium pentobarbital i.v. and artificially ventilated with room air by use of a Harvard respirator. Sodium heparin, 500 units/kg, was administered intravenously at the beginning of the perfusion and 200 units/kg added at hourly intervals. Details of the preparation were previously reported.4),5),7),9),10)

Drugs used in this study were 2-isopropyl-5-[3-(2-methoxyphenoxy)propylamino]-2-(3,4,5-trimethoxyphenyl)valeronitrile fumarate (Fig. 1) (HV-525, provided by Hokuriku Seiyaku Co., Ltd.), norepinephrine hydrochloride (Sankyo), propranolol hydrochloride (Sumitomo Chemicals), verapamil hy-

![Chemical structure of HV-525](https://example.com/chemical_structure_hv525.png)

**Fig. 1.** Chemical structure of 2-isopropyl-5-[3-(2-methoxyphenoxy)propylamino]-2-(3,4,5-trimethoxyphenyl)valeronitrile fumarate (HV-525).
drochloride (Knoll AG) and atropine sulfate (Takeda). The data are expressed as mean±SEM. Paired t-test was performed for statistical analyses.

RESULTS

1. Effects of intravenous HV-525 on intact dogs and on isolated atria

When HV-525 was administered into the jugular vein of the donor dog,

Fig. 2. Cardiovascular effects of 1 mg/kg of HV-525 on systemic blood pressure (SBP) and heart rate (HR) in a donor dog (A), and on atrial rate (AR) and developed tension (DT) in an isolated atrium (B), when administered intravenously to a donor dog.

Fig. 3. Effects of 1 mg/kg of HV-525 on systemic blood pressure (SBP) and heart rate (HR) in a donor dog (A), and developed tension (DT) in an isolated atrium (B) electrically paced (2.5 Hz, 2 volts, 5 msec duration).
a depressor response with slight sinus bradycardia or tachycardia was induced in the intact dog and negative chronotropic and inotropic responses in the isolated atrium were produced in a dose-related manner. Fig. 2 shows an example of a tracing of cardiovascular responses of a donor dog and an isolated atrium. In a paced isolated atrial preparation, the intravenous administration of 1 mg/kg of HV-525 to the donor dog caused a depressor response with bradycardia in the intact animal and a negative inotropic response in the isolated atrium as shown in Fig. 3. A relatively small dose of 0.3 mg/kg, i.v., of HV-525 induced a clear depressor response in donor dogs and a slight decrease in developed tension without any changes in atrial rate

![Graph](image)

Fig. 4. Summarized data of cardiovascular effects of 0.3 and 1.0 mg/kg of HV-525 on intact dogs and isolated atria when injected intravenously into 5 donor dogs. SBP = systemic blood pressure (control mean arterial pressure was 95±15 mmHg in 5 donor dogs); HR = heart rate (control heart rate was 166±22 beats/min in 5 donor dogs); AR = atrial rate (control atrial rate was 112±8 beats/min in 5 atria); and DT = developed tension (control developed tension was 1.4±0.3 g in 5 atria). Each bar shows SEM.
in isolated atria. A relatively large dose of 1 mg/kg, i.v., of HV-525 induced a profound depressor response in donor dogs and decreases in developed tension and atrial rate in isolated atria. Summarized data are shown in Fig. 4.

2. Chronotropic, dromotropic and inotropic effects of intraarterial HV-525 on isolated and perfused dog atria injected into the cannulated sinus node artery

When HV-525 was injected into the cannulated sinus node artery of the isolated atrial preparation, negative chronotropic and inotropic effects were dose-dependently induced. Fig. 5 shows a typical tracing of responses to increasing doses of HV-525 in an isolated atrium. In 2 experiments, sinoatrial conduction time (SACT) was measured at 5 min intervals. Fig. 6 shows data from one of the experiments in an isolated preparation. In this case, SACTs show unstable values, although atrial rate and developed tension show monophasic responses; another one shows no significant change in SACT. Summarized data of the effects of HV-525 on chronotropism and inotropism are shown in Fig. 7. However, in 2 of 7 preparations, HV-525 initially induced brief positive chronotropic and inotropic effects followed by long-lasting negative chronotropic and inotropic effects. The positive chronotropic and inotropic responses to 10 μg of HV-525 became smaller and smaller following repetitive administrations in one experiment.

Fig. 5. Chronotropic and inotropic effects of increasing doses of HV-525 injected directly into the cannulated sinus node artery of the isolated, blood-perfused dog atrial preparation. AR = atrial rate.
Fig. 6. Chronotropic, dromotropic and inotropic effects of 100 µg of HV-525 when injected into the cannulated sinus node artery of an isolated atrial preparation. AR=atrial rate; SACT=sinoatrial conduction time; DT=developed tension.

Fig. 7. Summarized data of chronotropic and inotropic effects of HV-525 on 5 isolated dog atria. AR=atrial rate; DT=developed tension. Control atrial rate was 103±5 beats/min (mean±SEM), and control developed tension was 1.8±0.3 g in 5 preparations.
3. Effects of HV-525 and propranolol on norepinephrine-induced responses

When norepinephrine was injected into the sinus node artery of the isolated atrium, positive chronotropic and inotropic responses were induced in a dose-related manner. Norepinephrine-induced responses were completely inhibited by treatment with propranolol. On the other hand, HV-525 slightly suppressed norepinephrine-induced responses even in large doses of over 30 µg. Fig. 8 shows an example of the effect of 100 µg of HV-525 on the positive chronotropic and inotropic responses to 0.01 µg of norepinephrine in an isolated atrium. Before treatment with HV-525, 0.01 µg of norepinephrine induced increases of 20±5% (mean±SE, n=4) in atrial rate and 45±12% in developed tension, respectively. After 100 µg of HV-525, 0.01 µg of norepinephrine induced increases of 18±7% in atrial rate and 52±14% in developed tension, respectively, indicating that no significant difference in values was observed before and after HV-525. These norepinephrine-induced responses were completely inhibited by treatment with 1 µg of propranolol in the same preparation.

4. Effects of HV-525 and verapamil on frequency-force relationship of isolated dog atrial muscle

The frequency-force relationship of the isolated atrial muscle was examined in a frequency range of 2.0 to 3.5 Hz. The positive staircase phenomenon was induced in all non-treated preparations of dog atrial muscle in this range as reported before. After verapamil treatment, the positive staircase was inverted to a negative staircase phenomenon, but HV-525, even in large doses, did not induce a negative staircase as shown in Fig. 9. Summarized data are shown in Fig. 10.

![Fig. 8. Effects of 100 µg of HV-525 on responses to 0.01 µg of norepinephrine in an isolated atrium.](image-url)
Fig. 9. Effects of 300 μg of HV-525 on frequency-force relationship in an isolated dog atrial muscle. EP = electric pacing.

Fig. 10. Summarized data of effects of verapamil and HV-525 on frequency-force relationship in isolated dog atria.

**Discussion**

In the present study, we examined the cardiovascular effects of a newly synthesized compound, 2-isopropyl-5-[3-(2-methoxyphenoxy)propylamino]-2-(3,4,5-trimethoxyphenyl)valeronitrile fumarate (HV-525) in cross-perfused atrial muscle preparations which were perfused with donor's arterial blood. Intravenous HV-525 usually induced a hypotensive effect with sinus bradycardia or tachycardia in intact dogs and slight negative chronotropic and inotropic effects in isolated atria. These response patterns were similar to those to verapamil in the same type of experiments. Intraarterial administration of HV-525 usually induced negative chronotropic and inotropic effects, indicating that verapamil was roughly 10–30 times more potent than...
HV-525. Since we investigated several antiarrhythmic drugs using similar methods in the past, it is possible to compare their chronotropic and inotropic potencies. Fig. 11 shows summarized data of the comparisons of chronotropic and inotropic effects of 8 different antiarrhythmic agents in isolated and blood-perfused dog atrial muscle preparations. As shown in this figure, HV-525 has a chronotropic potency similar to that of lidocaine or quinidine, but it has a more potent negative inotropic effect than lidocaine or quinidine. The order of potencies for inducing a negative chronotropic effect in isolated dog atria was verapamil > propranolol > HV-525 > lidocaine > quinidine > phenytoin > disopyramide > procainamide, and that for inducing a negative inotropic effect was verapamil > propranolol > HV-525 > lidocaine >
phenytoin > disopyramide > procainamide > quinidine.

Occasionally, HV-525, induced positive chronotropic and inotropic effects when injected directly into the sinus node artery. Recently, Thomas et al\(^1\) reported that minor changes in the nifedipine molecule result in drugs that have pharmacological properties diametrically opposite to those of the classical calcium channel blockers of the dihydropyridine type. BAY K 8644 (a dihydropyridine derivative), the prototype of these new compounds, turned out to have positive inotropic and vasoconstricting effects by increasing the transmembrane calcium current through the slow channel.\(^1\) HV-525 used in this study, a phenoxyalkylamine derivative, may both inhibit and promote transmembrane calcium influx. However, as HV-525-induced positive effects were not usual, more precise experiments are needed to analyze its ability to promote calcium influx by verapamil derivatives.

HV-525 did not change the positive staircase to a negative staircase phenomenon, differing in this respect from verapamil. This means that HV-525 may have a different pharmacologic action on the heart from that of verapamil. A large amount of HV-525 slightly inhibited the norepinephrine-induced positive chronotropic and inotropic effects, although a small dose of propranolol completely blocked the norepinephrine-induced effects. Thus, HV-525 did not have adrenergic beta-receptor blocking properties. From these results, it is concluded that HV-525 has slight, direct negative chronotropic and predominant negative inotropic activity on the heart along, with marked depressor action.

**REFERENCES**