Bradycardic Effects of AQ-A 39 (Falipamil) in Situ and in Isolated, Blood-Perfused Dog Hearts

Comparison with Alinidine and Verapamil

Yasuhiro OGIWARA, M.D., Yasuyuki FURUKAWA, M.D.,
Kunio AKAHANE, M.D., Masayuki HANIUDA, M.D.,
and Shigetoshi CHIBA, M.D.

SUMMARY

The cardiovascular effects of a specific bradycardic agent, AQ-A 39, were investigated in intact donor dogs and isolated and cross-perfused dog heart preparations. Intravenous administration of AQ-A 39 (10–1000 $\mu\text{g}/\text{kg}$) to the donor dog caused a dose-dependent heart rate decrease in the donor dog and a decreased atrial rate in the isolated atrium perfused by the donor's blood. The arterial blood pressure of the donor dog and contractile force of the atrial preparation were unchanged or slightly decreased. The direct injection of AQ-A 39 (1–300 μg) into the sinus node artery of the isolated atrium caused dose-dependent negative chronotropic and slight, transient positive inotropic responses. Alinidine and verapamil caused marked negative chronotropic and inotropic responses. The negative chronotropic effect of AQ-A 39 was not modified by atropine. However, it was enhanced slightly but significantly by propranolol, indicating that AQ-A 39-induced bradycardia was antagonized partly by *beta*-adrenoceptor function. These results confirmed that AQ-A 39 selectively reduced sinus rate by a direct action on the sinus node. Furthermore, the potency of the bradycardic action, compared with the decrease in contractility, was greater than for alinidine or verapamil. AQ-A 39 (300 μg) tended to depress norepinephrine (NE)-induced positive chronotropic but not inotropic effects in isolated atria. By contrast, verapamil (3–10 μg) significantly depressed the NE-induced positive inotropic but not the chronotropic effect, and propranolol (10 μg) suppressed both cardiac effects of NE. These data suggest that the AQ-A 39-induced, selective attenuation of the NE-induced chronotropic effect is not due to either calcium channel blockade or *beta*-adrenoceptor antagonism.

Additional Indexing Words:

AQ-A 39 Alinidine Isolated dog atrium Bradycardia Pro-
pranolol Verapamil

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

Address for reprints: Shigetoshi Chiba, M.D., Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390, Japan.

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AQ-A 39, a derivative of verapamil, is a bradycardic agent that predominantly decreases heart rate in comparison with cardiac contractility and atrioventricular conductivity.^{1)–3)} Although AQ-A 39 readily induced a negative chronotropic effect, an inotropic effect was inconsistent. Dammgen et al¹⁾ and Kawada et al³⁾ demonstrated that AQ-A 39, at higher doses, induced a negative inotropic effect in isolated or anesthetized mammalian hearts. On the other hand, Urthaler and Walker⁴⁾ observed that AQ-A 39 elicited a positive inotropic response which was diminished in part by a *beta*-adrenoceptor antagonist, propranolol. Sys et al⁵⁾ also observed that, at lower doses, AQ-A 39 induced a minute positive inotropic action on mammalian ventricular muscles but that at higher doses myocardial contractility decreased. In the open-chest, anesthetized dog, intravenous administration of AQ-A 39 decreased heart rate without affecting left ventricular dP/dt, while alinidine, another bradycardic agent, decreased both heart rate and left ventricular dP/dt.⁶⁾ Although the mechanisms of the bradycardic actions of AQ-A 39 are not completely understood, it was reported that AQ-A 39 inhibited a slow inward current in rabbit heart preparations.^{7),8)} However, the involvement of *beta*-adrenergic or muscarinic receptors was excluded for the mechanisms of the AQ-A 39-induced bradycardia in isolated guinea pig atria.^{2),9)}

Recently, we studied the bradycardic effects of alinidine injected intravenously to the support dog or intraarterially to the isolated atria using isolated, cross-circulated dog heart preparations.¹⁰⁾ Alinidine induced a greater negative chronotropic effect than a negative inotropic effect in isolated dog atria. Thus, in the present study, we examined the cardiovascular effects of AQ-A 39 administered intravenously in the anesthetized donor dog. The chronotropic and inotropic effects of AQ-A 39, alinidine and verapamil injected intraarterially were then compared in the isolated, perfused atrial preparation.

It was reported that AQ-A 39 attenuated catecholamine-induced positive chronotropic, but not inotropic effects in isolated mammalian hearts.⁹⁾ However, the mechanism of the AQ-A 39-induced selective attenuation of catecholamine-induced positive chronotropic effect is unclear. Thus, we also investigated the selective attenuation of the catecholamine-induced positive chronotropic effect in the isolated, blood-perfused dog atrial preparations.

METHODS

The isolated right atria were obtained from 24 mongrel dogs weighing 8 to 23 kg. Each dog was anesthetized intravenously with 30 mg/kg of sodium

pentobarbital. After administration of 200 USP units/kg of sodium heparin, the right atrium was excised and immersed in cold Tyrode's solution. The wet weight of the isolated right atrium varied from 6 to 12 g. Twenty-four support dogs (9 to 27 kg) were also anesthetized with sodium pentobarbital and administered sodium heparin (500 USP units/kg i.v.) at the beginning of the perfusion. Support dogs were artificially ventilated with room air using a Harvard respirator (model 607). The isolated right atrium was perfused through the cannulated sinus node artery *via* the right coronary artery with heparinized arterial blood led from the left carotid artery of the support dog with the aid of a peristaltic pump (Harvard Apparatus, model 1201). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained constant at 100 mmHg. The blood flow rate to the atrial preparation was 4 to 7 ml/min. The ventricular margin of the isolated atrium was fixed to a stainless steel bar and placed in a cup-shaped glass container, which was maintained at a constant temperature of 37°C by means of a heating bath circulator (Haake, FE2). The upper part of the isolated atrium was connected to a force-displacement transducer (Nihon Kohden, AP620G) by a silk thread to record the isometric developed tension. The atrial muscle was usually stretched to a resting tension of 2 g. A bipolar electrode was sewn on the epicardial surface of the atrium to record the atrial electrogram, and the spontaneous atrial rate of the atrium was derived from the electrogram with a cardiometer (Nihon Kohden, AP600G). The perfused blood was collected in a blood-reservoir and returned to the support dog *via* the left jugular vein. Sodium heparin (200 USP units/kg) was given intravenously at 1-hr intervals.

The effects of AQ-A 39 on the ventricular contractility were studied in 3 isolated and electrically paced left ventricular preparations. The left ventricular muscle with the anterior descending branch of the left coronary artery was excised and the anterior descending branch was cannulated. The isolated preparation was perfused with heparinized arterial blood led from the donor dog, using the same circulating circuit which was used in the atrial preparation. Bipolar electrodes were sewn on the ventricular free wall. The ventricular muscle was driven electrically with rectangular pulses through the electrodes using an electrical stimulator (Nihon Kohden, SEN 7103). The stimulus pulse was 2 msec duration, 6 V and 2 Hz. The left ventricular developed tension was measured isometrically by a force-displacement transducer through a fine thread connected to the ventricular surface.

The heart rate derived from the electrocardiograph (lead II) and the femoral arterial blood pressure of the donor dog, plus the isometric developed tension and spontaneous atrial rate of the isolated preparation, were recorded

on a thermal-writing rectigraph (Nihon Kohden, WT685G). Details of the isolated, blood-perfused dog right atrial and left ventricular preparations have been described in previous papers.¹¹⁾⁻¹³⁾

The drugs used in the experiments were AQ-A 39 hydrochloride (Dr. Karl Thomae GmbH, Biberach), atropine sulfate (Wako Pure Chemical Ind., Osaka), *d,l*-propranolol hydrochloride (Sigma Chemical Co., St. Louis, MO), acetylcholine chloride (Daiichi, Tokyo), norepinephrine hydrochloride (Sankyo, Tokyo), isoproterenol hydrochloride (Nikken Chemicals, Tokyo), alinidine hydrobromide (Boehringer Ingelheim, Hyogo) and verapamil hydrochloride (Knoll A.G., Ludwigshafen). Drugs were dissolved in physiological saline before starting the experiments. Drugs were injected into the sinus node artery of the isolated atrium and into the anterior descending branch through a rubber tube with a microsyringe (Terumo Co., Tokyo) or administered into the external jugular vein of the donor dog. The amount of drug solution injected into the sinus node artery was 0.01 to 0.03 ml over a period of 4 sec.

Summarized data are expressed as percentage of changes in maximum responses to each substance. Analysis of variance was used for the multiple comparisons of data and Student's *t*-test for paired data was used for comparison before and after antagonist treatment. *P* values of less than 0.05 were considered statistically significant.

RESULTS

Cardiovascular effects of intravenous administration of AQ-A 39 in intact dogs and on isolated, blood-perfused atrial preparations:

Figure 1 shows the effects of intravenous injection of AQ-A 39 (300 μ g/kg) into the external jugular vein of a donor dog. AQ-A 39 decreased the heart rate of the donor dog consistently but did not change atrial contractility. About 1.5 min after an intravenous injection of AQ-A 39, the atrial rate of the isolated atrium decreased because the drug injected into the donor dog perfused the isolated atrium. The developed tension of the isolated atrium also increased slightly and transiently. At doses of 10–100 μ g/kg, i.v., AQ-A 39 dose-dependently decreased both the heart rate of the donor dog and the atrial rate of the isolated atrium (Fig. 2). The durations of the decreases in heart rate and atrial rate at 300 μ g/kg i.v. were approximately 30 and 20 min, respectively. AQ-A 39 at 1,000 μ g/kg i.v. decreased the heart rate and atrial rate by approximately 20% from the control levels. On the other hand, these i.v. doses of AQ-A 39 revealed a biphasic response, consisting of a transient decrease followed by an increase in mean arterial blood pressure

Fig. 1. Effects of intravenous administration of AQ-A 39 (300 µg/kg) on the heart rate and systemic blood pressure of a donor dog (upper panel) and on the atrial rate and developed tension in an isolated dog atrium (lower panel) perfused with arterial blood of the donor dog.

Fig. 2. AQ-A 39 dose-response curves for percentage changes in the heart rate and mean arterial blood pressure (MABP) of the donor dog (left panel) and in the atrial rate and developed tension of the isolated, blood-perfused atrium (right panel) in 6 experiments. AQ-A 39 was injected intravenously into the donor dog at doses of 10–1,000 µg/kg. The control levels of heart rate and MABP of the donor dog were 134 ± 11 (mean $\pm$ SEM) beats/min and 85 ± 12.5 mmHg, respectively. The control levels of atrial rate and developed tension of the isolated atrium were 92 ± 9 beats/min and 1.8 ± 0.2 g, respectively. Vertical bars show SEM. See text for explanation of ph 1 and ph 2.

(MABP). In Fig. 2 (left panel), the transient decrease and increase in MABP were indicated as "ph 1" and "ph 2", respectively. After these temporary, biphasic changes in MABP, MABP was returned to the predrug level and maintained constant during the AQ-A 39-induced bradycardia.

In the isolated atrial preparation, AQ-A 39 at doses of 10–1,000 $\mu\text{g}/\text{kg}$ i.v. caused a transient increase in developed tension, indicated as "ph 1" in Fig. 2 (right panel). After this temporary positive inotropic effect, though, there were no significant changes in contractility up to 300 $\mu\text{g}/\text{kg}$ i.v. However, AQ-A 39 at 1,000 $\mu\text{g}/\text{kg}$ i.v. elicited a slight negative inotropic effect ($p < 0.05$) as indicated by "ph 2" in Fig. 2 (right panel).

Effects of AQ-A 39 on pacemaker activity and atrial contractility in the isolated, blood-perfused dog atrium:

The effects of a direct injection of a dose of 10 or 100 μg of AQ-A 39 into the sinus node artery of an isolated atrium are shown in Fig. 3. Ten micrograms of AQ-A 39 elicited a negative chronotropic and a small positive inotropic effect, while 100 μg of AQ-A 39 produced a marked negative chronotropic response and a slight negative inotropic response which followed a transient positive inotropic reaction. Data comparing the effects of AQ-A 39, alinidine and verapamil on pacemaker activity and contractility in isolated atria are presented in Fig. 4. The verapamil effects on the isolated and blood-perfused preparation are cited from Chiba.¹⁴⁾ When doses of 1–300 μg of AQ-A 39 were injected directly into the sinus node artery of the isolated, blood-perfused dog atrium, a negative chronotropic effect was produced in a dose-dependent manner (Fig. 4). The drug also induced a small negative inotropic effect following a transient positive inotropic effect. Another specific bradycardic agent, alinidine, at doses of 1–300 μg , elicited dose-dependent negative chronotropic and inotropic responses (Fig. 4, open circles). Verapamil also produced marked negative inotropic and chronotropic effects in a dose-related fashion. The order of the depth of bradycardia was ve-

Fig. 3. The effects of injections of AQ-A 39 (10 or 100 μg) into the sinus node artery of an isolated and perfused dog atrium on atrial rate and developed tension.

Fig. 4. Dose-response curves of percent changes in atrial rate and developed tension after direct injection of AQ-A 39, alinidine and verapamil into the sinus node artery of the isolated and perfused dog atrium in 5-7 experiments. The control levels of atrial rate and developed tension were 99 ± 9 (mean $\pm$ SEM) beats/min and 1.9 ± 0.2 g, respectively. Vertical bars show SEM.

rapamil $\gg$ AQ-A 39 $\geq$ alinidine. Unlike AQ-A 39 and alinidine, verapamil caused sinus arrest in 4 out of 7 preparations at $30 \mu\text{g}$ and in all 3 preparations at $100 \mu\text{g}$. The threshold doses for the negative inotropic effects of verapamil, alinidine and AQ-A 39 were approximately 0.3, 3 and $30 \mu\text{g}$, respectively. The negative inotropic effect of AQ-A 39 was quite small in comparison with those of alinidine and verapamil. Furthermore, unlike alinidine and verapamil, the percentage of attenuation of decreases in chronotropic effects of AQ-A 39 was always greater than for inotropic effects.

In 3 electrically paced isolated and blood-perfused dog left ventricular preparations, AQ-A 39 ($1-300 \mu\text{g}$) caused only a slight, transient positive inotropic response (data not shown).

Pharmacological analyses of chronotropic and inotropic responses to AQ-A 39 in the isolated atrium:

Ten micrograms of atropine, a potent muscarinic antagonist, completely inhibited the negative chronotropic and inotropic effects of $0.3\ \mu\text{g}$ of acetylcholine. However, the negative chronotropic and inotropic effects of $300\ \mu\text{g}$ of AQ-A 39 were not significantly affected in 6 isolated atria (Fig. 5). Atropine ($10\ \mu\text{g}$) treatment did not alter the basal atrial rate or the developed tension.

Treatment with $10\ \mu\text{g}$ of propranolol, a *beta*-adrenoceptor antagonist, consistently inhibited norepinephrine (NE) ($0.03\ \mu\text{g}$)-induced positive chronotropic and inotropic effects in isolated atria. This dose of propranolol slightly but significantly ($p < 0.05$) potentiated the negative chronotropic response to AQ-A 39 (Fig. 6). The small negative inotropic response to AQ-A 39 was not enhanced significantly, and the transient positive inotropic response to AQ-A 39 was not affected.

Fig. 5. Effects of atropine treatment ($10\ \mu\text{g}$) on decreases in atrial rate and developed tension in response to AQ-A 39 ($300\ \mu\text{g}$). Data are taken from 6 isolated and blood-perfused dog atria.

Fig. 6. Effects of propranolol ($10\ \mu\text{g}$) on chronotropic and inotropic responses to AQ-A 39 ($300\ \mu\text{g}$). Data reflect results from 6 isolated and blood-perfused dog atria. * $p < 0.05$ compared to the respective control.

Influences of AQ-A 39 on positive chronotropic and inotropic responses to catecholamines:

When NE (0.03 and 0.1 μg) or isoproterenol (ISP; 0.01 and 0.03 μg) were injected into the sinus node artery of the isolated atrial preparation, positive chronotropic and inotropic effects were dose-dependently observed. When the sinus rate and atrial contractile force were decreased by 25 ± 6.0 and $14 \pm 6.0\%$, respectively, by 300 μg of AQ-A 39, the positive chronotropic effects of ISP and NE were also depressed slightly (Fig. 7). The effects of a small dose of NE (0.3 μg) were significantly attenuated by 300 μg of AQ-A 39. On the other hand, the positive inotropic effects of ISP and NE were not affected.

The effects of verapamil (3 or 10 μg) on the positive cardiac responses to NE (0.03 and 0.1 μg) were also tested in 4 experiments. When verapamil reduced both the sinus rate (about 15%) and the atrial contractile force (about 50%), it did not depress the positive chronotropic effect of NE. However, the positive inotropic effect of NE, expressed in g but not in percentage changes, was depressed significantly ($p < 0.05$).

DISCUSSION

It has been reported that AQ-A 39 selectively decreased heart rate at

Fig. 7. Influences of AQ-A 39 (300 μg) on positive chronotropic and inotropic responses to either isoproterenol [0.01 (low) and 0.03 (high) μg], or norepinephrine [0.03 (low) and 0.1 (high) μg] in the isolated and blood-perfused dog atrium. * $p < 0.05$ compared to the respective control.

doses which did not produce any other prominent cardiovascular effects.^{1)-3),9)} Kobinger and Lillie²⁾ reported that 5 mg/kg i.v. of AQ-A 39 markedly increased the heart period and the duration of diastole, but the decrease in blood pressure was small in chloralose anesthetized cats. In conscious dogs, they also observed that an intravenous injection of AQ-A 39 markedly decreased the heart rate but had no effect on the blood pressure. In the present study, intravenous administration of AQ-A 39 to the anesthetized donor dog caused dose-dependent decreases in both the heart rate of the donor dog and the atrial rate of the isolated atrium. On the other hand, an intravenous injection of AQ-A 39 had no significant tonic effect on blood pressure, after the conclusion of transient biphasic effects. Furthermore, AQ-A 39 (up to 300 μ g/kg i.v.) caused bradycardia with no significant inotropic changes, after the transient increase in atrial developed tension in the isolated atrium. Thus, AQ-A 39 selectively decreased the heart rate, in comparison with the changes in blood pressure and cardiac contractility in anesthetized dogs. The decreases in heart rate of the donor dog and in atrial rate of the isolated atrium were approximately of the same degree, referenced as percentage decreases from control values. Therefore, we confirmed that a site of the bradycardic actions of AQ-A 39 may be the sinus node in the dog heart.

Verdouw et al¹⁵⁾ reported that AQ-A 39 (total dose 5.25 mg/kg i.v.) in the anesthetized pig markedly decreased the heart rate and slightly decreased the myocardial contractility; systemic vascular resistance was not affected. Siegl et al⁶⁾ observed that AQ-A 39 decreased the vascular resistance and increased the stroke volume in both non-paced and paced dog preparations, but a significant change in left ventricular $dP/dt_{\max}$ was not observed. From those results, they suggested that the augmented stroke volume in the paced preparation was probably due to a reduction in afterload by AQ-A 39. In the present study, the blood pressure of the donor dog was not affected significantly by AQ-A 39, except for transient effects immediately after the drug injection. Furthermore, the atrial contractility of the preparation did not consistently change with AQ-A 39. Although we did not measure the long-term effects on total vascular resistance, if the vascular resistance was markedly decreased by AQ-A 39, the blood pressure would probably decrease. Therefore, the decrease in total vascular resistance with AQ-A 39 appears to be relatively small in the anesthetized dog.

Direct injection of AQ-A 39 into the sinus node artery of the isolated atrial preparation produced a negative chronotropic effect; no significant negative inotropic effect following a transient positive one. On the other hand, alinidine caused marked negative chronotropic and inotropic effects. Kobinger and Lillie²⁾ observed the predominant bradycardic effect of AQ-A 39

and alinidine in isolated guinea pig atria and concluded that the responses to AQ-A 39 and alinidine were similar. Kawada et al^{3),16)} reported that both AQ-A 39 and alinidine produced negative chronotropic effects in isolated, blood-perfused heart preparations from dogs and that both drugs at higher doses caused negative inotropic effects in the isolated, blood-perfused papillary muscle. In the present study, AQ-A 39 always elicited a negative chronotropic effect, but a decrease in contractility was not consistent. On the other hand, alinidine showed both negative chronotropic and inotropic effects. Thus, using isolated and blood-perfused canine atrial preparations, we demonstrated that AQ-A 39 differed from alinidine with respect to its actions on cardiac contractility.

It was reported that the AQ-A 39-induced bradycardia partly reflects actions on slow calcium channels in electrophysiological experiments.^{7),8)} This is consistent with its structural similarities to verapamil. However, the chronotropic and inotropic responses to verapamil were different from those of AQ-A 39 in isolated canine atria. Both drugs caused similar negative chronotropic effects, but verapamil produced a marked depression of cardiac contractility and sinus arrest. Therefore, the calcium antagonistic effects of AQ-A 39 may be disregarded in the dog heart.³⁾

This study confirmed that the negative cardiac effects of AQ-A 39 in the dog heart are not mediated by modification of muscarinic cholinergic receptors. As reported in the guinea-pig,²⁾ the negative chronotropic and inotropic effects of AQ-A 39 were not significantly affected by an adequate dose of atropine. Sys et al⁵⁾ reported that low concentrations of AQ-A 39 could produce a positive inotropic effect in the cat papillary muscle. Urthaler and Walker⁴⁾ also demonstrated a positive inotropic effect of AQ-A 39 in isolated dog ventricles. In their experiments, the positive inotropic effect of AQ-A 39 was significantly reduced by propranolol treatment. Thus, they concluded that this positive inotropic effect was due, in part to neuronal norepinephrine release.

The negative chronotropic effects of AQ-A 39 in the present experiments were significantly enhanced by propranolol treatment, suggesting that the AQ-A 39-induced bradycardia is antagonized, in part, by *beta*-adrenoceptor activity. The slight negative inotropic effect of AQ-A 39 also tended to be potentiated by propranolol, but not significantly. On the other hand, the transient positive inotropic effect was not affected by propranolol, suggesting that it is independent of *beta*-adrenoceptors. From these results, it is considered that AQ-A 39-induced cardiac responses are modified, at least in part, by actions of *beta*-receptors in the dog heart.

It has been reported that AQ-A 39 and alinidine depressed isoproterenol-

induced tachycardia, but that propranolol suppressed both positive chronotropic and inotropic responses to isoproterenol in guinea pig atria.⁹⁾ Wenger and Siegl¹⁷⁾ also reported that AQ-A 39 had a greater selectivity for inhibiting the chronotropic responses to *beta*-adrenergic stimulation than verapamil or timolol in the anesthetized dog. In the present study, the positive chronotropic responses to low doses of norepinephrine were significantly attenuated by AQ-A 39 treatment. However, the positive inotropic responses to these catecholamines were not depressed at all. We recently observed that alinidine attenuated increases in sinus rate in response to either catecholamines or dibutyryl cyclic AMP, without affecting increases in contractile force in response to those agents in isolated and perfused dog atria.¹⁰⁾ The selective attenuation of the NE-induced positive chronotropic effect of AQ-A 39 was similar to the attenuation induced by alinidine. By contrast, verapamil significantly depressed the NE-induced positive inotropic response, but not the chronotropic response. Propranolol, though, inhibited both positive chronotropic and inotropic responses to NE. These results suggest that the selective attenuation of the NE-induced positive chronotropic effect by AQ-A 39 or alinidine is related to a specific bradycardic property, which is independent of *beta*-adrenoceptor blockade and calcium channel antagonism in the isolated dog atrium.

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