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Antiarrhythmic and cardiohemodynamic effects of a novel Ca^{2+} channel blocker, AH-1058, assessed in canine arrhythmia models

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Abstract

The antiarrhythmic profile and cardiohemodynamic effect of a novel Ca^{2+} channel blocker, 4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-[(*E*)-3-(3-methoxy-2-nitro)phenyl-2-propenyl]piperidine hydrochloride (AH-1058), were analyzed using the epinephrine-, digitalis- and two-stage coronary ligation-induced canine ventricular arrhythmia models. Intravenous administration of AH-1058 (100 μg/kg) effectively suppressed each of the ventricular arrhythmias accompanied by weak hypotensive effects. The results contrast well with those of a typical Ca^{2+} channel blocker, verapamil, which suppresses only the epinephrine-induced ventricular arrhythmia with severe hypotension. These results indicate that AH-1058 may possess a more selective inhibitory action on Ca^{2+} channels in the heart than on those in the vessels. Furthermore, the antiarrhythmic actions of AH-1058 were slower in onset and longer-lasting, than those in our previous studies using other antiarrhythmic drugs, including Na^+ and Ca^{2+} channel blockers. The antiarrhythmic effects of AH-1058 did not correlate with its plasma concentrations when administered either intravenously or orally. These results suggest that AH-1058 can become a long-acting Ca^{2+} channel blocker with unique antiarrhythmic properties, and that AH-1058 may be used in certain pathological processes, for which selective inhibition of the cardiac Ca^{2+} channels is essential.

Keywords: AH-1058; Ca^{2+} channel blocker; Ventricular arrhythmia

1. Introduction

4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-[(*E*)-3-(3-methoxy-2-nitro)phenyl-2-propenyl]piperidine hydrochloride (AH-1058), is a novel cyproheptadine-derived Ca^{2+} channel blocker, and its chemical structure is quite different from that of typical Ca^{2+} channel blockers, including verapamil, diltiazem and nifedipine, as shown in Fig. 1. In previous electrophysiological in vitro studies using guinea pig cardiomyocytes, AH-1058 blocked L-type Ca^{2+} channels without affecting Na^+ and K^+ channel currents (Takahara et al., 1999). Regarding the transmembrane action potential, AH-1058 shortens the action potential duration without affecting the maximum upstroke velocity of the action potential (Tanaka et al., 1999). Moreover, in a recent study using the canine isolated blood-perfused heart preparation, AH-1058 exerted negative chronotropic, inotropic and dromotropic effects simultaneously with its coronary vasodilator action (Takahara et al., 2000), each of which may reflect the Ca^{2+} channel blocking activity of AH-1058.

The purpose of the present study was to assess the antiarrhythmic profile as well as electrophysiological properties of AH-1058, using three types of canine in vivo arrhythmia models: namely, epinephrine-, digitalis- and two-stage coronary ligation-induced arrhythmia models (Hashimoto et al., 1982, 1985; Shibuya et al., 1983). In our

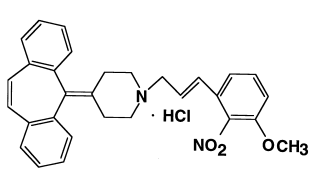

Fig. 1. Chemical structure of AH-1058.

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previous studies with more than 50 antiarrhythmic drugs (Shibuya et al., 1983; Komori et al., 1985, Hashimoto et al., 1991; Matsuzaki et al., 1993), the epinephrine-induced ventricular arrhythmia was suppressed by drugs possessing a Ca^{2+} channel blocking property or a β-adrenoceptor blocking action, while digitalis- and two-stage coronary ligation-induced arrhythmias were inhibited by Na^{+} channel blockers. In the present study, we compared the current results with AH-1058 with those for Na^{+} and Ca^{2+} channel blockers in our previous studies, to characterize the electrophysiological profile of AH-1058 in vivo.

2. Materials and methods

All experiments were performed according to the Guidelines for Animal Experiments, Ajinomoto (Tokyo, Japan).

2.1. Epinephrine-induced arrhythmia

Six beagle dogs (group 1) were anesthetized initially with thiopental sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, 1.0% halothane vaporized with 100% oxygen was inhaled with a volume-limited ventilator (SN-408-3, Shinano, Tokyo, Japan). Epinephrine was infused into the left femoral vein at a rate of 1.5 μg/kg/min for 18 min using a syringe pump (Shibuya et al., 1983). It has already been reported that the ventricular arrhythmias were observed for more than 20 min when an antiarrhythmic drug was not used (Awaji et al., 1995). Three minutes after the start of epinephrine infusion, AH-1058 in a dose of 100 μg/kg was intravenously administered into the right femoral vein. The lead II electrocardiogram (ECG), the right atrial electrogram and mean blood pressure were recorded for 15 min using a polygraph system (RM-6000, Nihon Kohden, Tokyo, Japan).

2.2. Digitalis-induced arrhythmia

Six beagle dogs (group 2) were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and intubated with a cuffed endotracheal tube. Ouabain in a dose of 40 μg/kg was intravenously injected, and 10 μg/kg of ouabain was supplemented every 20 min until stable ventricular tachycardia was produced (Hashimoto et al., 1985). It has already been reported that this arrhythmia was stable at least for 1 h when an antiarrhythmic drug was not used (Awaji et al., 1995). After a stable ventricular tachycardia was induced, AH-1058 in a dose of 100 μg/kg was intravenously administered via the right femoral vein. The lead II ECG, the right atrial electrogram and mean blood pressure were recorded for 1 h using a polygraph system (RM-6000, Nihon Kohden).

2.3. Two-stage coronary ligation-induced arrhythmia

Twelve beagle dogs were initially anesthetized with thiopental sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, 1.0% halothane was inhaled with a volume-limited ventilator (SN-408-3, Shinano). The chest was opened and the left anterior descending artery was ligated in a two-stage manner (Hashimoto et al., 1982). It has already been reported that the arrhythmia lasted for at least 2 days after coronary ligation when an antiarrhythmic drug was not used (Awaji et al., 1995). AH-1058 in a dose of 100 μg/kg was intravenously administered without anesthesia at 24 and 48 h after the coronary ligation (group 3, $n = 5$). The lead II ECG, the left atrial electrogram and mean blood pressure were recorded for 4 h using a polygraph system (RM-6000, Nihon Kohden). AH-1058 was also given orally in a dose of 300 μg/kg to another series of the coronary ligation-induced arrhythmia model at 24 and 48 h after ligation (group 4, $n = 7$). The ECG was recorded for 8 h after the drug administration.

2.4. Evaluation of antiarrhythmic effects

The severity of ventricular arrhythmias was expressed as the arrhythmic ratio: the number of ventricular ectopic beats (/min) divided by that of total QRS complexes (/min). For example, the arrhythmic ratio is zero during the sinus rhythm, while it is one during ventricular tachycardia. The ventricular ectopic beats were evaluated from the shape of the QRS complex and its sequential relation to the atrial electrogram. The arrhythmic ratio was almost one before the drug injection in each arrhythmia model. When the ratios after drug administration decreased significantly from the 0-time value, the antiarrhythmic effects of the drug were judged as significant, as previously reported (Hashimoto et al., 1982, 1985; Shibuya et al., 1983).

2.5. Determination of plasma concentration of AH-1058

Arterial blood, 3 ml, was drawn at 0, 1, 3, 5, 7, 10, 15, 30, 60, 120 and 240 min after intravenous administration of AH-1058 in group 3, while venous blood was drawn at 0, 1, 2, 4, 6, 8 and 24 h after oral administration of AH-1058 in group 4. The blood samples were centrifuged at $1500 \times g$ for 15 min. The concentration of AH-1058 in the supernatant plasma was measured using a high-performance liquid chromatographic technique. The pharmacokinetic parameters were analyzed using commercially available software, WinNonlin-Pro (Pharsight, Cary, NC, USA).

2.6. Drugs

The following drugs were used: AH-1058 (Ajinomoto), thiopental sodium (Tanabe Seiyaku, Osaka, Japan),

halothane (Takeda Chemical Industries, Osaka, Japan), pentobarbital sodium (Tokyo Kasei, Tokyo, Japan), heparin calcium (Mitsui Pharmaceuticals, Tokyo, Japan), epinephrine (Dai-ichi Seiyaku, Tokyo, Japan) and (−)-ouabain octahydrate (Aldrich Chemical, Milwaukee, WI, USA). AH-1058 was dissolved in polyethylene glycol 400/saline (70:30, vol/vol) for intravenous administration or polyethylene glycol 400 (100%) for oral administration.

2.7. Statistics

All values are expressed as means ± S.E. Analysis of variance (ANOVA) for repeated measures was employed for overall statistical analysis, followed by contrasts for statistical analysis comparing basal values (zero time) and other values. Statistical analysis was performed using commercially available software, SuperANOVA (Abacus Concepts, Berkeley, CA, USA). Differences with a *P*-value of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effects of AH-1058 on epinephrine-induced arrhythmia model (group 1)

Three minute after the start of the intravenous infusion of epinephrine, almost all the beats became of ventricular origin. The effects of AH-1058 on the epinephrine-induced arrhythmia model are summarized in Fig. 2A. AH-1058 in a dose of 100 μg/kg significantly decreased the total heart rate from 1 min, atrial rate from 3 min and blood pressure from 1 min, and increased the number of conducted beats from 4 min after drug administration. The arrhythmic ratio decreased significantly from 4 min after drug administration. These significant changes lasted throughout the 15-min observation period.

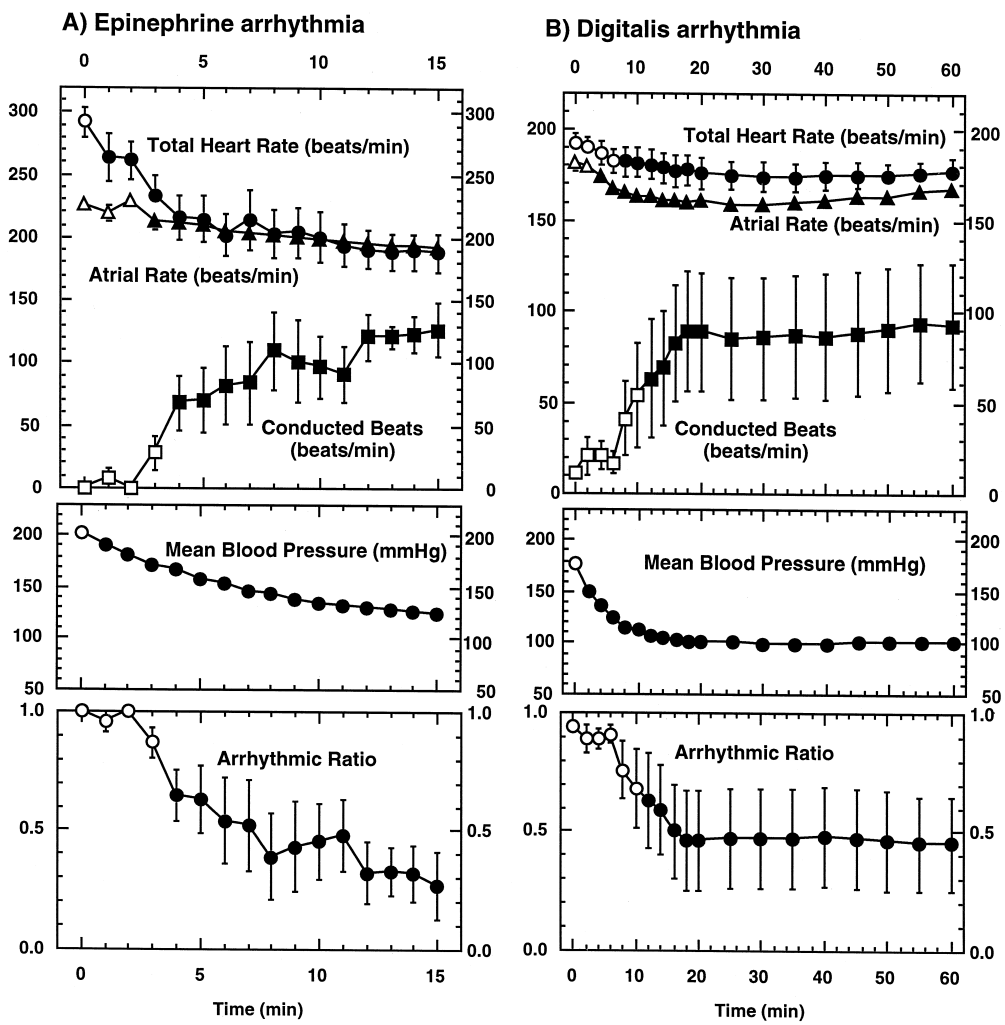

Fig. 2. Effects of intravenous administration of AH-1058 (100 μg/kg) on the arrhythmias induced by epinephrine (A, group 1, $n = 6$) and digitalis (B, group 2, $n = 6$) in dogs. Data are expressed as means ± S.E. Closed symbols represent significant change from the 0-time values ($P < 0.05$).

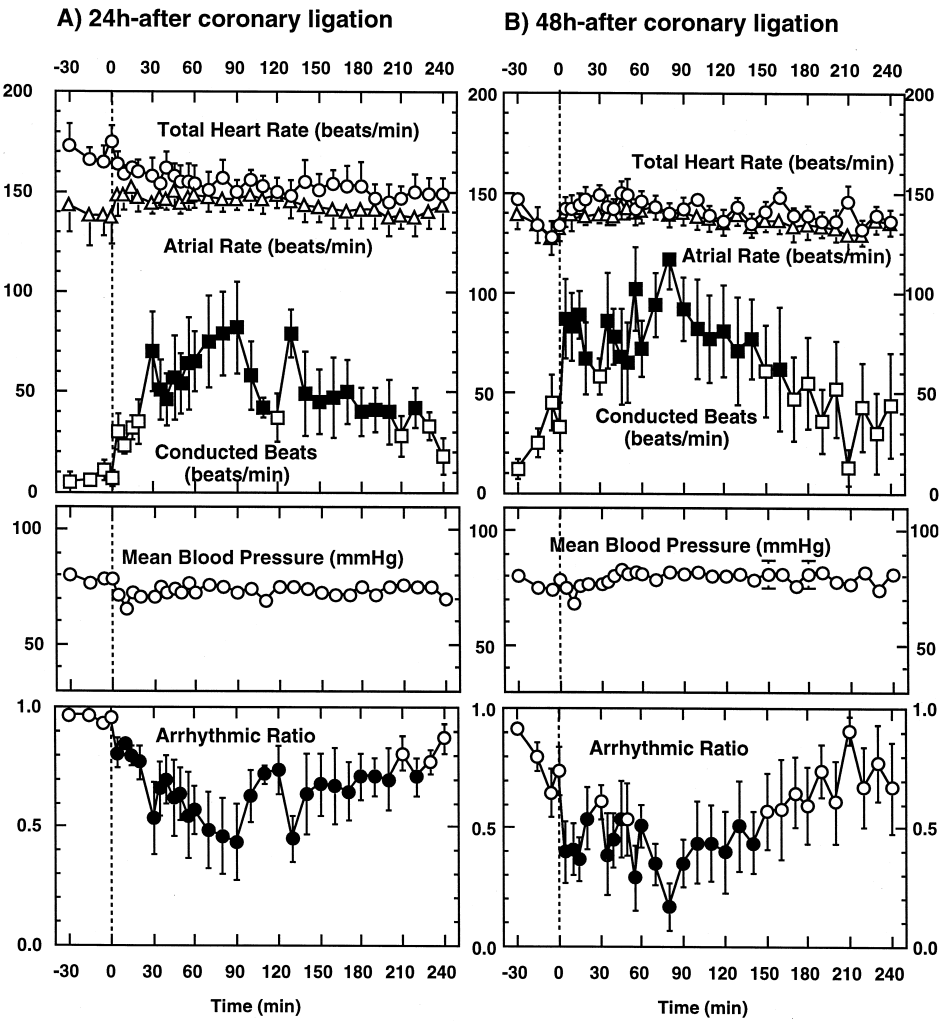

Fig. 3. Effects of intravenous administration of AH-1058 on the arrhythmias induced by two-stage coronary ligation in dogs (group 3). AH-1058 (100 μg/kg) was administered at 24 h (A, $n = 5$) or 48 h (B, $n = 5$) after coronary ligation. Data are expressed as means ± S.E. Closed symbols represent significant change from the pre-administration values ($P < 0.05$).

3.2. Effects of AH-1058 on digitalis-induced arrhythmia model (group 2)

After intravenous injection of a total dose of 70–90 μg/kg of ouabain, almost all the beats became of ventricular origin. The effects of AH-1058 on the digitalis-induced arrhythmia model are summarized in Fig. 2B. AH-1058 in a dose of 100 μg/kg significantly decreased the total heart rate from 8 min, atrial rate from 4 min and blood pressure from 2 min, and increased the number of conducted beats from 12 min after drug administration. The arrhythmic ratio decreased significantly from 12 min after drug administration. These significant changes lasted throughout the 60-min observation period.

3.3. Effects of AH-1058 on two-stage coronary ligation-induced arrhythmia model (group 3 and group 4)

One to two days after the coronary ligation, all dogs showed continuously occurring multifocal ventricular ectopic beats. The arrhythmic ratios at 24 and 48 h after ligation were 0.98 ± 0.01 and 0.86 ± 0.05 ($n = 12$), respectively. The effects of intravenous administration of AH-1058 on the coronary ligation-induced arrhythmia

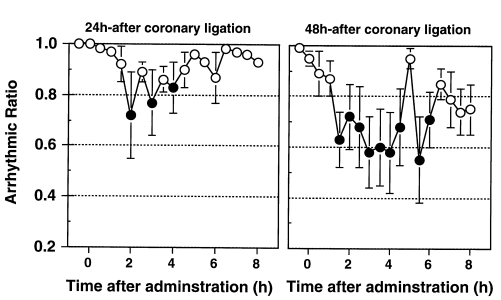

Fig. 4. Effects of oral administration of AH-1058 on the arrhythmias induced by two-stage coronary ligation in dogs (group 4). AH-1058 (300 μg/kg) was administered at 24 h (A, $n = 7$) or 48 h (B, $n = 7$) after coronary ligation. Data are expressed as means ± S.E. Closed symbols represent significant change from the pre-administration values ($P < 0.05$).

model are summarized in Fig. 3, while the antiarrhythmic effects of oral administration of AH-1058 are shown in Fig. 4. In the 24-h arrhythmia model, AH-1058 (100 μg/kg, i.v., $n = 5$, group 3) significantly increased the number of conducted beats from 25 to 220 min (except for 120 and 210 min) and decreased the arrhythmic ratio from 5 to 220 min (except for 210 min), while little change was observed in the total heart rate, atrial rate and mean blood pressure (Fig. 3A). In the 48-h arrhythmia model, AH-1058 (100 μg/kg, i.v., $n = 5$, group 3) significantly increased the number of conducted beats from 5 to 160 min (except for 30 and 150 min) and decreased the arrhythmic ratio from 5 to 140 min (except for 30 and 50 min) with little change in the total heart rate, atrial rate and mean blood pressure (Fig. 3B). Oral administration of AH-1058 (300 μg/kg, $n = 7$, group 4) also decreased the arrhythmic ratio (Fig. 4). In the 24-h model, significant changes were detected at 2, 3 and 4 h after the drug administration, while in the 48-h model, these were detected from 1.5 to 6 h.

3.4. Plasma concentration of AH-1058

The time course of the plasma drug concentrations after i.v. and p.o. administration of AH-1058 is shown in Fig. 5. The drug concentrations in the 24- and 48-h models after i.v. administration fitted well the two-compartment theory model, and the time course curves of the two models were similar. The elimination half-life ($t_{1/2\beta}$) after the i.v. administration of 100 μg/kg of AH-1058 was 0.21 ± 0.03 h in the 24-h model and 0.19 ± 0.02 h in the 48-h model. On the other hand, after the p.o. administration of 300 μg/kg of AH-1058, the plasma drug concentration was higher in the 48-h model than in the 24-h model. The maximum plasma concentration (C_{max}) and the time corresponding to C_{max} (T_{max}) were 10.1 ± 2.6 ng/ml and 1.3 ± 0.2 h in the 24-h model, while these were 14.7 ± 2.0 ng/ml and 1.6 ± 0.2 h in the 48-h model, respectively. Bioavailability calculated from the area under the plasma concentration–time curve was 29.4% in the 24-h model, while it was 40.6% in the 48-h model, possibly reflecting the influence of, and the recovery from, the surgical invasion.

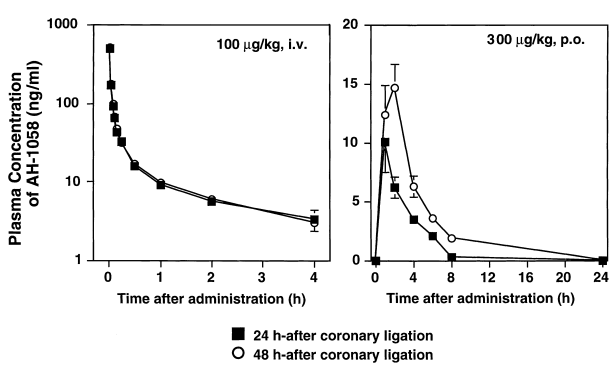

Fig. 5. Time course for the plasma concentration of AH-1058 in the two-stage coronary ligated dogs (groups 3 and 4). AH-1058 was intravenously (100 μg/kg) or orally (300 μg/kg) administered at 24 and 48 h after coronary ligation. Data are expressed as means ± S.E.

4. Discussion

The present study was designed to characterize the antiarrhythmic profile as well as cardiohemodynamic effects of AH-1058, using well-established canine arrhythmia models (Hashimoto et al., 1982, 1985; Shibuya et al., 1983). As clearly shown by the results, AH-1058 effectively suppressed each of the epinephrine-, digitalis- and two-stage coronary ligation-induced ventricular arrhythmias, which is quite different from antiarrhythmic profile of typical Ca^{2+} channel blockers including verapamil, diltiazem and nifedipine that suppress only the epinephrine-induced arrhythmia (Hashimoto et al., 1991).

Since the epinephrine-induced ventricular arrhythmia has been shown to be suppressed by drugs possessing Ca^{2+} channel blocking properties or a β-adrenoceptor blocking action (Shibuya et al., 1983; Komori et al., 1985; Hashimoto et al., 1991; Matsuzaki et al., 1993), the present in vivo finding that AH-1058 suppressed the epinephrine-induced ventricular arrhythmias is consistent with the previous observation in vitro that AH-1058 blocks L-type Ca^{2+} channels without affecting Na^+ and K^+ channel currents (Takahara et al., 1999, 2000; Tanaka et al., 1999) and that AH-1058 hardly affects β-adrenoceptor binding (unpublished data).

In the in vitro studies using the heart with digitalis intoxication or severe ischemia, it has been shown that intracellular Ca^{2+} overload is closely associated with the generation of arrhythmias, including delayed after depolarization leading to triggered activity, which has been reported to be effectively suppressed by verapamil (Rosen et al., 1973; El-Sherif et al., 1983; Wit and Rosen, 1983; Kimura et al., 1984; LeMarec et al., 1985; Rosen, 1988; Sugiyama and Hashimoto, 1999). However, in our previous studies (Hashimoto et al., 1991), intravenous administration of verapamil did not suppress the canine digitalis- or two-stage coronary ligation-induced arrhythmias because of the onset of lethal hypotension. Contrary to what we had expected, based on this previous knowledge, AH-1058 suppressed both digitalis- and coronary ligation-induced arrhythmias. Since AH-1058 lacks the Na^+ channel blocking action (Takahara et al., 1999, 2000; Tanaka et al., 1999) and hardly affected the blood pressure of the coronary ligation-induced arrhythmia model in this study, AH-1058 may possess a selective inhibitory action on Ca^{2+} channels in the heart compared with that in the vessels, which might explain the unique antiarrhythmic effects of AH-1058. However, this hypothesis and/or another possible mechanism, including a central nervous system-mediated action (Robkin, 1994), remain to be elucidated.

Another unique and important property of AH-1058 is its pharmacodynamics. While most of the clinically available antiarrhythmic drugs exert their maximum antiarrhythmic effects within 10 min of their administration (Hashimoto et al., 1982, 1984, 1987, 1988; Akiyama and Hashimoto, 1989), the antiarrhythmic action of AH-1058 was slow in onset, namely the peak effect appeared more than 10 min after administration, and was longer-lasting than that of other antiarrhythmic drugs (Hashimoto et al., 1991). Using the coronary ligation-induced arrhythmia model in the conscious state, we examined the relationship between the antiarrhythmic action of AH-1058 and its plasma drug concentrations. The antiarrhythmic effects of AH-1058 did not necessarily correlate with its plasma concentrations when administered intravenously as well as orally. Since no active metabolite modulating Na^+ or Ca^{2+} channel function could be found on extensive laboratory examinations and similar slow kinetics were reported from the in vitro study (Tanaka et al., 1999), the present results may be explained by the fast elimination rate of AH-1058 from plasma, about seven times faster than that of verapamil (Komori et al., 1985), and/or its high lipophilicity allowing it to be readily distributed to the cardiac tissues.

In summary, AH-1058 suppressed each of the epinephrine-, digitalis- and two-stage coronary ligation-induced ventricular arrhythmias with a minor hypotensive action, and the effects were slow in onset and long-lasting. Monitoring of the plasma drug concentration of AH-1058 may not be helpful for predicting the extent of its antiarrhythmic action. The results of this experimental study suggest that AH-1058 can be applied to certain pathological processes in which selective inhibition of the cardiac Ca^{2+} channels is essential.

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