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European Journal of Pharmacology 300 (1996) 203–210

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Effects of zatebradine on ouabain-, two-stage coronary ligation- and epinephrine-induced ventricular tachyarrhythmias

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Received 5 October 1995; revised 19 December 1995; accepted 22 December 1995

Abstract

To determine whether a hyperpolarization-activated current (I_f) participates in ventricular tachyarrhythmias, we investigated the effects of zatebradine, an I_f inhibitor, on the ventricular tachyarrhythmias induced by ouabain, two-stage coronary ligation and epinephrine infusion in the dog heart. We determined atrial rate, ectopic ventricular rate, total heart rate and arrhythmic ratio (the number of ectopic ventricular beats divided by total heart beats). Zatebradine (0.15, 0.5 and 1.5 mg/kg, i.v.) dose dependently decreased the arrhythmic ratio, ectopic ventricular rate and atrial rate of the ouabain-induced ventricular tachyarrhythmias in pentobarbital-anesthetized dogs. The inhibition by zatebradine of the ventricular arrhythmias needed larger doses than the inhibition of the atrial rate. Zatebradine weakly depressed the ectopic ventricular rate but not the arrhythmic ratio of the ventricular arrhythmias induced by two-stage coronary ligation 24 h after the ligation in conscious dogs. Although neither the ectopic ventricular rate nor the arrhythmic ratio of the epinephrine-induced ventricular arrhythmias was affected by zatebradine, after treatment with zatebradine, the arrhythmias elicited by epinephrine developed more slowly. Together with the previously reported spectra of the effects of the antiarrhythmic agents in three ventricular tachyarrhythmia models, our results suggest that zatebradine may improve automaticity-related ventricular tachyarrhythmias due to I_f inhibition or to other undetermined mechanisms in the heart.

Keywords: Automaticity; Hyperpolarization-activated current (I_f); Ventricular arrhythmia; Zatebradine; Heart, dog

1. Introduction

The hyperpolarization-activated current (I_f) is one of the major currents with pacemaker potential in cardiac pacemaker cells (DiFrancesco, 1990; Irisawa et al., 1993). I_f has been identified in mammalian automatic cells including sinoatrial nodal cells (Brown et al., 1979; DiFrancesco, 1981; Noma et al., 1980) and ventricular myocytes (Yu et al., 1993). I_f blockers such as zatebradine block I_f in isolated rabbit sinoatrial nodal cells and Purkinje fibers (Van Bogaert et al., 1990; Goethals et al., 1993; DiFrancesco, 1994), although zatebradine at a high concentration inhibits delayed rectifier K^+ currents and slow inward Ca^{2+} currents (Doerr and Trautwein, 1990; Goethals et al., 1993; Thollon et al., 1994). Zatebradine also decreases heart rate and tachycardia during exercise (Guth et al., 1987) and protects against the deterioration of regional contractility during acute ischemia in both conscious (Guth et al., 1987) and anesthetized (Dammgen et al., 1985) dogs. Thus, it is suggested that I_f participates in sinoatrial nodal pacemaker activity in the heart in situ. However, there is a paucity of reports of the effects of an I_f inhibitor on the automaticity of Purkinje fibers or abnormal automaticity in the heart in situ. Abnormal automaticity is one of the mechanisms that cause ventricular tachyarrhythmias (Hoffman and Cranefield, 1964; Rosen, 1988).

Therefore, to investigate the role of I_f on ventricular automaticity including Purkinje fibers, we studied the effects of zatebradine on the ventricular tachyarrhythmias induced by ouabain (Hashimoto et al., 1984; Le Marec et al., 1986), two-stage coronary ligation (Harris and Kökernot, 1950; Friedman et al., 1973; Lazzara et al., 1973; Hashimoto et al., 1982) and epinephrine infusion (Hashimoto and Hashimoto, 1972; Shibuya et al., 1983) in dog hearts in situ. These experimentally induced ventricular tachycardias are thought to be induced at least in part

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SSDI 0014-2999(95)00880-2

by abnormal automaticity (Dangman and Danilo, 1990; Hashimoto et al., 1991).

2. Materials and methods

The animal experiments were approved by the Yamanashi Medical University Animal Experimentation Committee and animals were obtained through the Animal Laboratory for Research of Yamanashi Medical University.

2.1. Digitalis-induced arrhythmia

Six Beagle dogs of either sex, weighing 8–12 kg, were anesthetized with pentobarbital sodium, 30 mg/kg, i.v. Ouabain, 40 μg/kg, was injected intravenously, followed by an additional 10 μg/kg every 20 min until a stable ventricular arrhythmia was produced. Arrhythmia was usually produced by 40–50 μg/kg and lasted more than 1 h (Hashimoto et al., 1984).

Catheter tip electrodes were inserted into the right atrium for recording of the atrial electrogram. The lead II electrocardiogram (ECG), atrial electrogram, and femoral arterial blood pressure were continuously recorded. Zatebradine was injected cumulatively at doses of 0.15, 0.5 and 1.5 mg/kg, i.v. at 5-min intervals through the cannula in the jugular vein. Venous samples were drawn from the cannula 5 min before and 1, 3, 8, 13, 30 and 60 min after an initial dose of zatebradine.

2.2. Two-stage coronary ligation-induced arrhythmia

Six Beagle dogs of either sex, weighing 8–12 kg, were anesthetized with thiopental sodium, 30 mg/kg, i.v. After intubation, 1.0% halothane vaporized with 100% oxygen was administered by means of a volume-limited ventilator to maintain anesthesia. By using an aseptic technique, the chest was opened through the 5th intercostal space and the left anterior descending coronary artery was dissected free for two-stage coronary ligation (Harris and Kökernot, 1950; Hashimoto et al., 1982). After a second complete ligation was made following the first partial occlusion, bipolar electrodes were sutured on the left atrial appendage and the chest was closed. Arterial and venous cannulae were inserted into the carotid artery and into the jugular vein through a cervical incision leading to a skin hole on the upper back. The dogs were allowed to recover from the anesthesia and experiments were conducted 24 and 48 h later.

Experiments were done without anesthesia. The lead II ECG and atrial electrogram from implanted electrodes were recorded using Nihon Kohden (Tokyo, Japan) telemetry systems and the instantaneous and mean blood pressure were also recorded continuously. Zatebradine (0.15, 0.5 and 1.5 mg/kg) was injected cumulatively at 5-min intervals through the cannula in the jugular vein. Venous blood samples were taken from the jugular vein before and 1, 3, 8, 13, 30 and 60 min after an initial dose of zatebradine.

Identical experiments were conducted 24 and 48 h after coronary ligation for a given animal. Summarized data for 48 h after coronary ligation were obtained from 5 animals because of a recording system trouble.

2.3. Epinephrine-induced arrhythmia

Nine Beagle dogs of either sex, weighing 7–13 kg, were anesthetized initially with thiopental sodium. After intubation, 1.0% halothane, vaporized with 100% oxygen, was administered with a volume-limited ventilator (20 ml/kg, 15 strokes/min). Each cervical vagus nerve was cut at the neck. Epinephrine was infused through the femoral vein at a rate of 1.5 μg/kg/min using a syringe pump for 18 min. Ventricular arrhythmia occurred approximately 1 or 2 min after the beginning of epinephrine infusion and was maintained during the entire infusion period (Shibuya et al., 1983). After 3 min of epinephrine infusion, zatebradine (0.15, 0.5 and 1.5 mg/kg) was injected cumulatively at 5-min intervals through the cannula in the femoral vein. Venous samples were drawn from the cannula before and 3, 8 and 13 min after an initial dose of zatebradine. Catheter tip electrodes were inserted into the right atrium for measurement of the atrial electrogram. The lead II electrocardiogram (ECG), atrial electrogram, and femoral arterial blood pressure were continuously recorded. After 30 min recovery time, to investigate the effects of zatebradine on the induction of the epinephrine-induced tachyarrhythmias, we again infused the same dose of epinephrine for 18 min and determined the cardiac responses in 7 anesthetized dogs.

2.4. Plasma zatebradine assay

Venous blood samples were centrifuged and the plasma was stored at about −80°C in a freezer until the plasma zatebradine concentration was measured. The plasma zatebradine assay was carried out by an automated high-performance liquid chromatography using a column-switching technique (Roth et al., 1981). Zatebradine was concentrated on a pre-column (VYDAC 201SC) and loaded onto a reversed-phase analytical column (ODS Hypersil, HP) by backflushing. It was then separated on the analytical column and quantified. A fluorescence detector (RF-10A, Shimazu, Kyoto, Japan) was used (excitation wavelength: 285 nm; emission wavelength: 315 nm) to determine the zatebradine concentration.

2.5. Evaluation of antiarrhythmic effects

The severity of ventricular arrhythmia was determined from the ectopic ventricular rate and the arrhythmic ratio. The arrhythmic ratio was expressed as the number of

ventricular ectopic beats divided by the total heart rate. The total heart rate is the number of beats counted from the 5-s strip of the ECG, i.e., the number of ventricular ectopic beats plus the number of conducted beats. The ventricular beats were judged by the different shape of the ventricular complex from the normal QRS complex. The arrhythmic ratio before zatebradine was almost 1 and there were no spontaneous improvements in these ratios (Hashimoto et al., 1982, 1984; Shibuya et al., 1983).

2.6. Drugs

Drugs used in the present study were zatebradine (1,3,4,5-tetrahydro-7,8-dimethoxy-3[3-[[2-(3,4-dimethoxyphenyl)-ethyl]methylamino]propyl]-2*H*-3-benzazepin-2-one-hydrochloride, generously donated by Nippon Boehringer Ingelheim, Hyogo, Japan), epinephrine hydrochloride (epinephrine, Daiichi, Tokyo, Japan) and ouabain (Takeda, Osaka, Japan).

2.7. Statistical analysis

All data were expressed as means ± S.D.M. One-way or two-way analysis of variance with Bonferroni's test was used for the statistical analysis of multiple comparisons of data. P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of zatebradine on digitalis-induced arrhythmia

Fig. 1 presents a representative experiment into the antiarrhythmic effects of zatebradine on the digitalis-induced ventricular arrhythmia in an anesthetized dog. Administration of ouabain at a dose of 40 μg/kg, i.v., caused ventricular arrhythmias. After we confirmed that almost all the beats were of ventricular origin, i.e., the arrhythmic ratio was almost 1, we administered zatebradine at an initial dose of 0.15 mg/kg, i.v., to the animal. Zatebradine dose dependently decreased the ectopic ventricular rate, total heart rate, and the arrhythmic ratio. Finally, digitalis-induced arrhythmia disappeared after 1.5 mg/kg, i.v., of zatebradine was injected.

Before zatebradine administration, the total heart rate, ectopic ventricular rate and atrial rate were 240 ± 31 (mean ± S.D.), 227 ± 45 and 229 ± 38 beats/min, respectively, in 6 animals treated with ouabain. The arrhythmic ratio was 0.94 ± 0.10. Mean femoral arterial blood pressure was 199 ± 45 mm Hg. Digitalis-induced arrhythmia has been previously confirmed to last more than 1 h (Hashimoto et al., 1984).

Zatebradine at a dose of 0.15 mg/kg, i.v., consistently decreased the atrial rate ($P < 0.001$) but did not significantly affect the ectopic ventricular rate and total heart rate

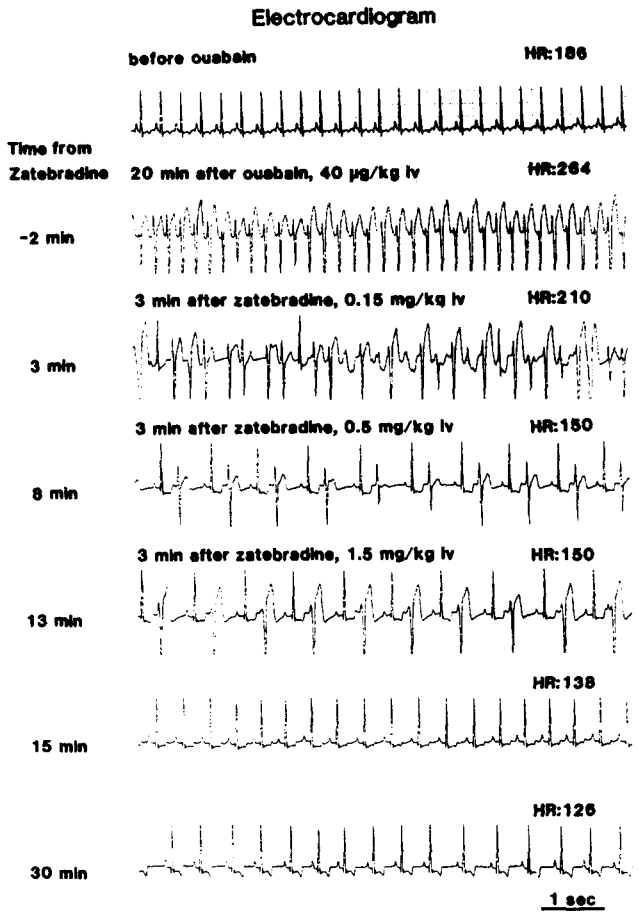

Fig. 1. Effects of zatebradine (0.15, 0.5 and 1.5 mg/kg, i.v.) on the ouabain-induced ventricular tachyarrhythmia in a pentobarbital-anesthetized dog. Twenty minutes after treatment with ouabain at a dose of 40 μg/kg, i.v., zatebradine (0.15, 0.5 and 1.5 mg/kg, i.v.) was given cumulatively at intervals of 5 min. HR: total heart rate.

(Fig. 2). Increasing the dose of zatebradine dose dependently ($P < 0.001$) attenuated the total heart rate and the ectopic ventricular rate. The arrhythmic ratio also decreased dose dependently ($P < 0.001$) but the minimum ratio was 0.55 ± 47 (Fig. 3). That is, in 2 of 6 experiments, the tachyarrhythmias were abolished, in other 2 of 6 experiments the arrhythmias were partially inhibited, but in the other 2 experiments zatebradine had no effect. Ouabain increased mean arterial blood pressure and the increased arterial blood pressure gradually decreased ($P < 0.001$) during experiments. The inhibitory effects of zatebradine on the rate responses were maintained during the whole observation period. Plasma concentrations of zatebradine are shown in Table 1.

3.2. Two-stage coronary ligation-induced arrhythmia

Twenty-four hours after two-stage coronary ligation, the total heart rate, ectopic ventricular rate and atrial rate were 152 ± 12, 151 ± 11 and 116 ± 20 beats/min, respectively, in 6 conscious dogs. The arrhythmic ratio was 0.99 ± 0.02. Mean arterial blood pressure was 90 ± 11 mm Hg.

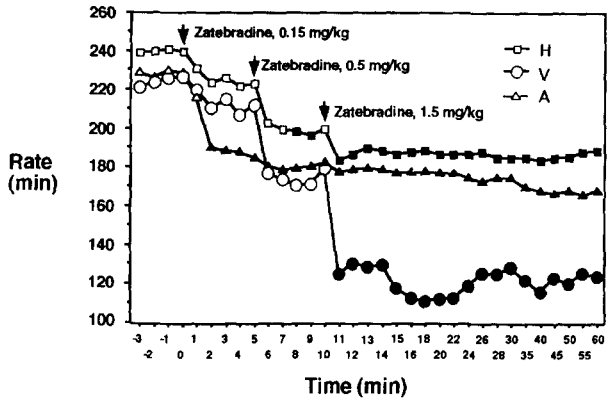

Fig. 2. Effects of zatebradine at doses of 0.15, 0.5 and 1.5 mg/kg, i.v. on the total heart rate (□), ectopic ventricular rate (○) and atrial rate (△) in 6 ouabain-induced ventricular tachyarrhythmia models. Twenty minutes after treatment with ouabain at a dose of 40 μg/kg, i.v., zatebradine (0.15, 0.5 and 1.5 mg/kg) was given cumulatively at intervals of 5 min through the cannula in the jugular vein. Closed symbols present significant ($P < 0.05$) differences from the respective value at the beginning of the initial dose (0.15 mg/kg, i.v.) of zatebradine. H, total heart rate; V, ectopic ventricular rate; A, atrial rate. Standard deviations are omitted. Abscissa shows time (min) from the injection of the initial dose of zatebradine and scale is changed.

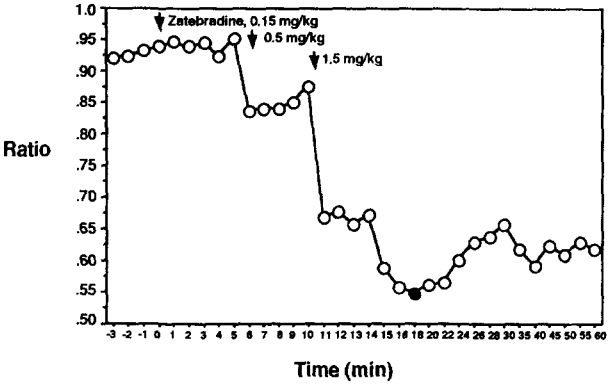

Fig. 3. Effects of zatebradine (0.15, 0.5 and 1.5 mg/kg, i.v.) on the arrhythmic ratio in 6 ouabain-induced ventricular tachyarrhythmia models. Twenty minutes after treatment with ouabain at a dose of 40 μg/kg, i.v., zatebradine (0.15, 0.5 and 1.5 mg/kg, i.v.) was given cumulatively at intervals of 5 min. A closed symbol presents a significant ($P < 0.05$) difference from the value at the beginning of the initial dose (0.15 mg/kg, i.v.) of zatebradine. Standard deviations are omitted. Abscissa shows time (min) from the injection of the initial dose of zatebradine and scale is changed.

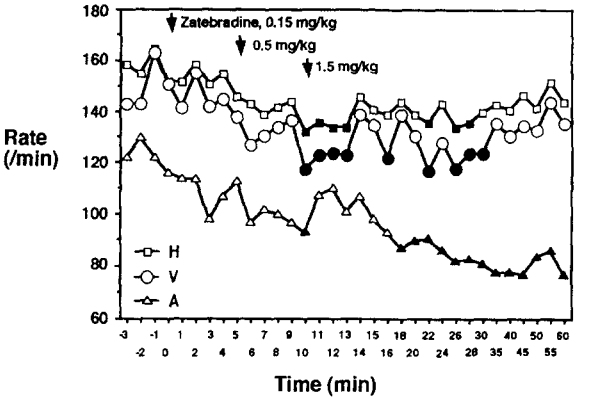

Fig. 4. Effects of zatebradine (0.15, 0.5 and 1.5 mg/kg, i.v.) on the total heart rate (H), ectopic ventricular rate (V) and atrial rate (A) in 6 conscious dogs 24 h after two-stage coronary ligation. After consistent ventricular arrhythmias were confirmed, zatebradine (0.15, 0.5 and 1.5 mg/kg, i.v.) was given cumulatively at intervals of 5 min. Closed symbols present significant ($P < 0.05$) differences from the respective value at the beginning of the initial dose (0.15 mg/kg, i.v.) of zatebradine. Standard deviations are omitted. Abscissa shows time (min) from the injection of the initial dose of zatebradine.

Zatebradine in doses of 0.15, 0.5 and 1.5 mg/kg, i.v., decreased the atrial rate dose dependently ($P < 0.001$) and at the highest dose the atrial rate decreased to 77 ± 14 beats/min in 6 conscious dogs (Fig. 4). Zatebradine also decreased the total heart rate and ectopic ventricular rate slightly but significantly ($P < 0.01$). The attenuation of the ventricular rate by 1.5 mg/kg of zatebradine gradually reversed, but that of the atrial rate did not during the experiment. The arrhythmic ratio was not significantly decreased by zatebradine (Fig. 5). Zatebradine did not affect the mean arterial blood pressure throughout the experiments. Plasma concentrations of zatebradine are shown in Table 1.

Forty-eight hours after two-stage coronary ligation, the total heart rate, ectopic ventricular rate and atrial rate were 133 ± 14, 121 ± 20 and 108 ± 27 beats/min, respectively, in 5 conscious dogs. The arrhythmic ratio was 0.91 ± 0.08 and mean arterial blood pressure was 91 ± 13 mm Hg.

Zatebradine in doses of 0.15, 0.5 and 1.5 mg/kg, i.v., decreased the atrial rate dose dependently ($P < 0.001$) in 5 conscious dogs (Fig. 6). However, it tended to attenuate the total heart rate and ectopic ventricular rate but this

Table 1
Plasma concentrations of zatebradine in ouabain-, two-stage coronary ligation- and epinephrine-induced ventricular arrhythmia models

Group	Expt. No	Plasma concentration of zatebradine (ng/ml)					
		1	3	8	13	30	60 (min)
Ouabain	6	804 ± 386	259 ± 190	959 ± 431	2860 ± 1015	802 ± 428	386 ± 161
Two-stage (24 h)	6	678 ± 305	231 ± 71	859 ± 230	2826 ± 532	987 ± 256	484 ± 178
Two-stage (48 h)	6	656 ± 287	247 ± 84	1108 ± 238	3950 ± 752	1486 ± 429	710 ± 218
Epinephrine	7	–	119 ± 42	442 ± 57	1515 ± 194	–	–

Data are shown as means ± S.D.M. Zatebradine at a dose of 0.15 mg/kg, i.v., was given at time 0 and then 0.5 and 1.5 mg/kg, i.v., of zatebradine were cumulatively given 5 and 10 min, respectively, after the initial dose.

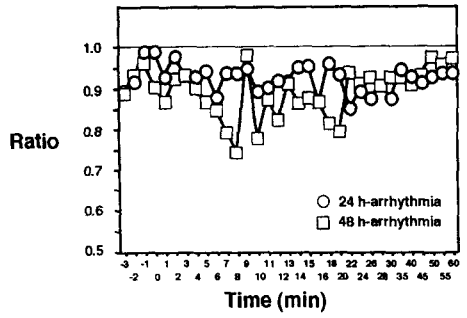

Fig. 5. Effects of zatebradine on the arrhythmic ratio in 6 conscious dogs 24 h and 48 h after two-stage coronary ligation. Standard deviations are omitted. Abscissa shows time (min) from the injection of the initial dose of zatebradine. Zatebradine at doses of 0.15, 0.5 and 1.5 mg/kg, i.v., was given cumulatively at intervals of 5 min.

effect was not statistically significant. The arrhythmic ratio was not affected, either (Fig. 5). Zatebradine did not change the mean arterial blood pressure during experiments. Plasma concentrations of zatebradine are presented in Table 1.

3.3. Epinephrine-induced arrhythmia

Infusion of epinephrine at a rate of 1.5 μg/kg/min for 18 min induced ventricular tachyarrhythmias in dogs whose rate of sinus rhythm was 144 ± 11 beats/min under halothane anesthesia ($n = 9$). Before zatebradine administration, total heart rate, ectopic ventricular rate and atrial rate were 273 ± 35, 273 ± 35 and 207 ± 27 beats/min, respectively, and the arrhythmic ratio was 1. Mean arterial blood pressure was 177 ± 8 mm Hg. Epinephrine-induced arrhythmias lasted more than 18 min in halothane-anesthetized dog hearts (Shibuya et al., 1983).

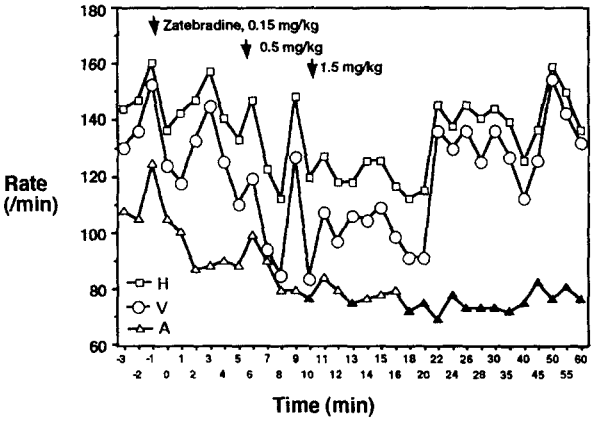

Fig. 6. Effects of zatebradine (0.15, 0.5 and 1.5 mg/kg, i.v.) on the total heart rate (H), ectopic ventricular rate (V) and atrial rate (A) in 6 conscious dogs 48 h after two-stage coronary ligation. After consistent ventricular arrhythmias were confirmed, zatebradine (0.15, 0.5 and 1.5 mg/kg) was given cumulatively at intervals of 5 min through the cannula in the jugular vein. Closed symbols present significant ($P < 0.05$) differences from the respective value at the beginning of the initial dose (0.15 mg/kg, i.v.) of zatebradine. Standard deviations are omitted. Abscissa shows time (min) from the injection of the initial dose of zatebradine.

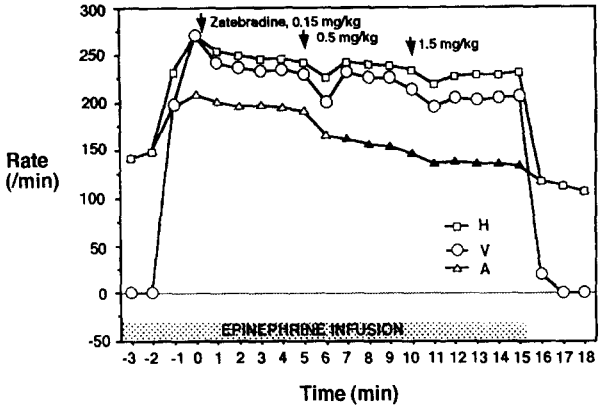

Fig. 7. Effects of zatebradine at doses of 0.15, 0.5 and 1.5 mg/kg, i.v., on the total heart rate (H), ectopic ventricular rate (V) and atrial rate (A) of the epinephrine-induced ventricular tachyarrhythmia model in 9 halothane-anesthetized dogs. Zatebradine at doses of 0.15, 0.5 and 1.5 mg/kg, i.v., was given cumulatively at intervals of 5 min, 3 min after the beginning of epinephrine infusion at a rate of 1.5 μg/kg/min, i.v. Standard deviations are omitted. Abscissa shows time (min) from the injection of the initial dose of zatebradine. Closed symbols present significant ($P < 0.05$) differences from the respective value at the beginning of the initial dose (0.15 mg/kg, i.v.) of zatebradine.

Administration of zatebradine (0.15, 0.5 and 1.5 mg/kg, i.v.) dose-dependently ($P < 0.001$) decreased the atrial rate, with 1.5 mg/kg, i.v., of zatebradine decreasing the atrial rate to 133 ± 19 beats/min (Fig. 7). However, zatebradine attenuated neither the total heart rate nor the ectopic ventricular rate. The summarized data for the arrhythmic ratio did not change significantly, although zatebradine transiently decreased the arrhythmic ratio to less than 0.5 in 4 of 9 experimental animals. After administration of zatebradine at a dose of 1.5 mg/kg, i.v., ventricular fibrillation occurred in 2 of 9 dogs. Mean blood pressure decreased with time ($P < 0.001$) during epinephrine infusion after zatebradine treatment. Plasma concentrations of zatebradine are presented in Table 1.

Thirty minutes after cessation of the epinephrine infusion, to investigate the effects of zatebradine on the epinephrine-induced arrhythmia, we again infused the same dose of epinephrine in 7 halothane-anesthetized dogs. The second epinephrine infusion induced ventricular fibrillation in 1 of 7 dogs. Before infusion of epinephrine, the total heart rate was 83 ± 10 beats /min in 6 sinus rhythm dog hearts. In the 6 dogs, after zatebradine treatment the ventricular tachyarrhythmias induced by epinephrine developed more slowly (Fig. 8a), i.e., 2 min after epinephrine infusion, the ectopic ventricular rate was significantly ($P < 0.05$) less after zatebradine treatment than before zatebradine. However, epinephrine infusion still induced tachyarrhythmias and the ventricular rate 3 min after epinephrine infusion was not significantly different from the ventricular rate in animals before zatebradine. After zatebradine treatment, the atrial rate was much less ($P < 0.001$) than the rate before zatebradine administration (Fig. 8b). However, the maximum increase (58 ± 27 beats/min)

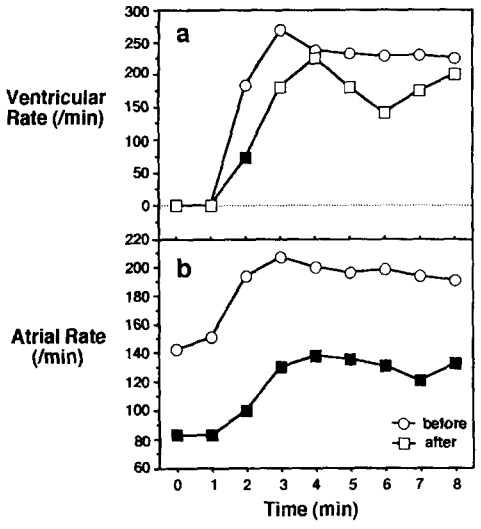

Fig. 8. Effects of zatebradine on the epinephrine-induced ventricular tachyarrhythmias in 6 halothane-anesthetized dogs. Ectopic ventricular rate (a) and atrial rate (b) during epinephrine infusion at a rate of 1.5 μg/kg/min before (○) and after (□) zatebradine treatment. Closed symbols present significant ($P < 0.05$) differences from the respective control value.

in atrial rate induced by epinephrine infusion in zatebradine-treated dogs was not significantly different from the maximum increase (67 ± 36 beats/min) in zatebradine non-treated dogs.

4. Discussion

In the present study, we demonstrated that zatebradine, an I_f inhibitor, partially inhibited digitalis-induced ventricular tachyarrhythmias in pentobarbital-anesthetized dogs and attenuated the ectopic ventricular rate induced by digitalis and 24 h after two-stage coronary ligation in conscious dogs. However, zatebradine did not inhibit the ventricular tachyarrhythmias induced by epinephrine, although the tachyarrhythmias developed more slowly. In three models, zatebradine consistently decreased atrial rate.

Zatebradine at doses used in the present study dose dependently reduced the arrhythmic ratio only of the ouabain-induced ventricular tachyarrhythmia, and not of the others (Figs. 3 and 4, Figs. 6 and 7). Hashimoto and his colleagues (Hashimoto et al., 1991) have extensively investigated the effects of antiarrhythmic agents on the epinephrine-, digitalis- and two-stage coronary ligation ventricular tachyarrhythmia models: Class I antiarrhythmic agents, Na^+ channel blocking drugs, suppress ventricular tachyarrhythmias in all three models, although there are some variations. Lidocaine suppressed only digitalis-induced ventricular tachyarrhythmias, but tocainide and disopyramide inhibited digitalis- and two-stage coronary ligation-induced arrhythmias but not epinephrine-induced arrhythmias (Hashimoto et al., 1982, 1985; Shibuya et al., 1983). Thus, the spectrum of the antiarrhythmic effects of zatebradine is similar to that of lidocaine, although the potency of the antiarrhythmic effect of zatebradine is weaker.

Zatebradine is an I_f blocker (Van Bogaert et al., 1990; Goethals et al., 1993; DiFrancesco, 1994) with weak inhibitory (not efficacious) effects on K^+ currents and I_{Ca} (Doerr and Trautwein, 1990; BoSmith et al., 1993; Furukawa et al., 1993; Goethals et al., 1993). Zatebradine did not affect dV/dt_{max} of the action potential in isolated guinea-pig papillary muscles (Thollon et al., 1994). It is, therefore, conceivable that the inhibitory effects of zatebradine on the digitalis-induced ventricular tachyarrhythmias and the attenuation of the ventricular rate induced by digitalis and 24 h after two-stage coronary ligation are due to (1) inhibition of I_f by zatebradine, (2) inhibition of I_{Ca}, (3) inhibition of I_K or (4) other undetermined mechanisms including intracellular mechanisms and indirect effects of decreases in sinus rate induced by zatebradine. In the present study, we injected zatebradine at doses of 0.15, 0.5 and 1.5 mg/kg intravenously to dogs. Zatebradine decreased dose dependently the atrial rate in the three tachyarrhythmia models (Figs. 2 and 4, Figs. 6 and 7). A 50% inhibition dose of zatebradine for the chronotropic response to sympathetic nerve stimulation was approximately 0.15 mg/kg, i.v., in anesthetized dogs (Furukawa et al., 1995). Additionally, zatebradine (0.05–1.5 mg/kg, i.v.) selectively inhibited the positive chronotropic response but not the inotropic or the dromotropic response to sympathetic nerve stimulation in anesthetized dogs (Furukawa et al., 1995). Verapamil inhibited the positive chronotropic and inotropic responses. Thus, it is likely that zatebradine in the doses used mainly works as an I_f inhibitor with a weak I_K inhibition in dog hearts in situ, although at a high dose it may have an additional undetermined action. Together with the spectrum of the antiarrhythmic effects of zatebradine, therefore, we suggest that zatebradine may work as a new type of antiarrhythmic agent due to inhibition of I_f and/or undetermined mechanisms on the heart.

Twenty-four hours after two-stage coronary ligation, zatebradine decreased the ectopic ventricular rate but did not attenuate the arrhythmic ratio (Figs. 4 and 5). The mechanisms of the two-stage coronary ligation-induced arrhythmias are suggested to be automaticity and triggered activity and the predominant rhythm of the ventricular tachycardia at 24 h appears to be automatic (Le Marec et al., 1985, 1986). Purkinje fibers and ventricular preparations from the heart 1–4 days after coronary ligation show a diminished maximum diastolic potential from −62 to −52 mV (Dangman and Hoffman, 1983; Le Marec et al., 1985, 1986). I_f works voltage dependently on the mammalian SA nodal and Purkinje cells at membrane potentials up to approximately −50 mV and −70 mV, respectively (Brown et al., 1979; DiFrancesco, 1981, 1990). Yu et al. (1993) found that the threshold potential for the activation of I_f was −89 ± 16 mV in canine Purkinje fibers. It is,

thus, uncertain whether I_f participates in the ectopic ventricular rate.

Triggered activity rather than automaticity is predominant in ventricular arrhythmias in the conscious dog heart 48 h after coronary ligation (Le Marec et al., 1986). The relative lack of effect of zatebradine on ventricular arrhythmias 48 h after coronary ligation (Fig. 6) may support the notion that zatebradine decreases the automaticity-related ectopic ventricular rate 24 h after coronary ligation due to inhibition of I_f.

Ouabain causes ventricular tachyarrhythmias in pentobarbital-anesthetized dogs (Figs. 1–3). In the present study, zatebradine decreased the arrhythmic ratio as well as the number of ectopic ventricular beats induced by ouabain. However, zatebradine did not abolish the arrhythmias completely even though the doses used in the present study are adequate to block I_f in the dog heart (Guth et al., 1987; Furukawa et al., 1995). The mechanisms of the digitalis-induced arrhythmias are proposed to be triggered activity and automaticity in the anesthetized dog heart (Le Marec et al., 1985, 1986; Wit and Rosen, 1992). It has been recently suggested that zatebradine prevents tricyclic antidepressant-induced ventricular tachyarrhythmias in a dog model when the beating rate decreases (Ansel et al., 1994). Ouabain-induced tachyarrhythmias are frequency dependent, but there has been no electrophysiological study examining whether zatebradine inhibits ouabain-induced electrophysiological changes such as a transient inward current in cardiac tissues. A transient inward current is caused by increases in intracellular Ca^{2+}. Therefore, zatebradine might suppress the digitalis-induced ventricular tachyarrhythmias by inhibition of I_f or by undetermined mechanisms including intracellular mechanisms and indirect effects induced by bradycardia in the heart.

Zatebradine decreased the atrial rate readily but it did not decrease the arrhythmic ratio or the number of the ectopic ventricular beats in epinephrine-induced arrhythmias of the halothane-anesthetized dog (Fig. 7), indicating that zatebradine is ineffective on epinephrine-induced ventricular arrhythmias in the heart in situ, even though it works as an I_f inhibitor. After zatebradine treatment, tachyarrhythmias developed more slowly, suggesting that I_f blockers delay the induction of ventricular tachyarrhythmia by I_f inhibition or/and sinus bradycardia in the dog heart. Other mechanisms of the epinephrine-induced arrhythmias are also the abnormal automaticity and triggered activity (Hashimoto and Hashimoto, 1972; Hashimoto et al., 1982; Wit and Rosen, 1992). Calcium channel antagonists suppress epinephrine-induced ventricular arrhythmias but not the digitalis- or two-stage coronary ligation-induced arrhythmias (Hashimoto et al., 1991). Zatebradine blocked the positive chronotropic but not the inotropic response to β-adrenoceptor agonists and not the positive chronotropic and inotropic responses to Bay k 8644 in isolated dog atria (Sawaki et al., 1995). Thus, even after I_f was blocked by zatebradine in halothane-anesthetized dogs, activation of I_{Ca} by epinephrine would increase atrial rate and cause ventricular tachyarrhythmias.

In summary, we demonstrated that zatebradine, an I_f inhibitor, has a mild antiarrhythmic effect on digitalis-induced ventricular tachyarrhythmias in the anesthetized dog and suggest that zatebradine may improve automaticity-related ventricular arrhythmias by inhibition of I_f or by other undetermined mechanisms in the dog heart in situ.

Acknowledgements

We gratefully acknowledge the skilled technical assistance of Miss A. Ozawa and also thank Nippon Boehringer Ingelheim (Hyogo, Japan) for the generous supply of zatebradine and for the assay for measuring plasma concentration of zatebradine.

References

Ansel, G.M., J.P. Meimer and S.D. Nelson, 1994, Prevention of tricyclic antidepressant-induced ventricular tachyarrhythmia by a specific bradycardic agent in a canine model, J. Cardiovasc. Pharmacol. 24, 256.

BoSmith, R.E., I. Briggs and N.C. Sturgess, 1993, Inhibitory actions of ZENCA ZD7288 on whole-cell hyperpolarization activated inward current (I_f) in guinea-pig dissociated sinoatrial node cells, Br. J. Pharmacol. 110, 343.

Brown, H.F., D. DiFrancesco and S.J. Noble, 1979, Cardiac pacemaker oscillation and its modulation by autonomic transmitters, J. Exp. Biol. 81, 175.

Dammgen, J.W., K.A. Lamping and G.J. Gross, 1985, Actions of two new bradycardic agents, AQ-AH 208 and UL-FS 49, on ischemic myocardial perfusion and function, J. Cardiovasc. Pharmacol. 7, 71.

Dangman, K.H. and P. Danilo Jr., 1990, Intact animal models of automatic rhythms, in: Cardiac Electrophysiology: A Textbook, eds. M.R. Rosen, M.J. Janse and A.L. Wit (Futura, New York) p. 223.

Dangman, K.H. and B.F. Hoffman, 1983, Antiarrhythmic effects of ethmozin in cardiac Purkinje fibers: suppression of automaticity and abolition of triggering, J. Pharmacol. Exp. Ther. 227, 578.

DiFrancesco, D., 1981, A new interpretation of the pace-maker current in calf Purkinje fibers, J. Physiol. 314, 359.

DiFrancesco, D., 1990, The hyperpolarization-activated current, I_f, and cardiac pacemaking, in: Cardiac Electrophysiology: A Textbook, eds. M.R. Rosen, M.J. Janse and A.L. Wit (Futura, New York) p. 117.

DiFrancesco, D., 1994, Some properties of the UL-FS 49 block of the hyperpolarization-activated current (I_f) in sino-atrial node myocytes, Pflüg. Arch. 427, 64.

Doerr, T. and W. Trautwein, 1990, On the mechanism of the 'specific bradycardic action' of the verapamil derivative UL-FS 49, Naunyn-Schmied. Arch. Pharmacol. 341, 331.

Friedman, P.L., J.R. Stewart and A.L. Wit, 1973, Spontaneous and induced cardiac arrhythmias in subendocardial Purkinje fibers surviving extensive myocardial infarction in dogs, Circ. Res. 33, 612.

Furukawa, Y., S. Takayama, S. Sawaki, Y. Inoue and S. Chiba, 1993, A bradycardic agent, UL-FS 49, increases atrial force and decreases ventricular force in isolated, blood-perfused heart preparations of dogs, J. Pharmacol. Exp. Ther. 265, 801.

Furukawa, Y., H. Nakano, T. Oguchi, M. Kasama, Y. Hoyano and S. Chiba, 1995, Selective inhibition by zatebradine and discrete parasympathetic stimulation of the positive chronotropic response to sympathetic stimulation in anesthetized dogs, J. Pharmacol. Exp. Ther. 272, 744.

Goethals, M., A. Raes and P.P. Van Bogaert, 1993, Use-dependent block of the pacemaker current I_f in rabbit sinoatrial node cells by zatebradine (UL-FS 49): on the mode of action of sinus node inhibitors, Circulation 88, 2389.

Guth, B.D., G. Heusch, R. Seitelberger and J. Ross Jr., 1987, Elimination of exercise-induced regional myocardial dysfunction by a bradycardiac agent in dogs with chronic coronary stenosis, Circ. Res. 75, 661.

Harris, A.S. and R.H. Kökernot, 1950, Effects of diphenylhydantoin sodium (Dilantin sodium) and phenobarbital sodium upon ectopic ventricular tachycardia in acute myocardial infarction, Am. J. Physiol. 163, 505.

Hashimoto, K. and K. Hashimoto, 1972, The mechanism of sensitization of the ventricle to epinephrine by halothane, Am. Heart J. 83, 652.

Hashimoto, K., H. Sato, T. Shibuya and S. Imai, 1982, Canine-effective plasma concentrations of antiarrhythmic drugs on the two-stage coronary ligation arrhythmia, J. Pharmacol. Exp. Ther. 223, 801.

Hashimoto, K., S. Komori, M. Ishii and J. Kamiya, 1984, Effective plasma concentrations of aprindine in canine ventricular arrhythmias, J. Cardiovasc. Pharmacol. 6, 12.

Hashimoto, K., M. Ishii, S. Komori and H. Mitsuhashi, 1985, Canine digitalis arrhythmia as a model for detecting Na-channel blocking antiarrhythmic drugs: a comparative study using other canine arrhythmia models and the new antiarrhythmic drugs, propafenone, tocainide and SUN 1165, Heart Vessels 1, 29.

Hashimoto, K., A. Haruno, T. Matsuzaki, A. Sugiyama and K. Akiyama, 1991, Effects of antiarrhythmic drugs on canine ventricular arrhythmia models: which electrophysiological characteristics of drugs are related to their effectiveness?, Cardiovasc. Drug. Ther. 5, 805.

Hoffman, B.F. and P.F. Cranefield, 1964, The physiological basis of cardiac arrhythmias, Am. J. Med. 37, 670.

Irisawa, H., H.F. Brown and W. Giles, 1993, Cardiac pacemaking in sinoatrial node, Physiol. Rev. 73, 197.

Lazzara, R., N. El-Sherif and B.J. Scherlag, 1973, Electrophysiological properties of canine Purkinje cells in one-day-old myocardial infarction, Circ. Res. 33, 722.

Le Marec, H., K.H. Dangman, P. Danilo Jr. and M.R. Rosen, 1985, An evaluation of automaticity and triggered activity in the canine heart one to four days after myocardial infarction, Circulation 71, 1224.

Le Marec, H., W. Spinelli and M.R. Rosen, 1986, The effects of doxorubicin on ventricular tachycardia, Circulation 74, 881.

Noma, A., H. Irisawa, S. Kokubun, H. Kotake, M. Nishimura and Y. Watanabe, 1980, Slow current systems in the A-V node of the rabbit heart, Nature 285, 228.

Rosen, M.R., 1988, Mechanisms for arrhythmias, Am. J. Cardiol. 61, 2A.

Roth, W., K. Beschke, R. Jauch, A. Zimmer and F.W. Koss, 1981, Fully automated HPLC – a new chromatograph for pharmacokinetic drug monitoring by direct injection of body fluids, J. Chromatogr. 222, 13.

Sawaki, S., Y. Furukawa, Y. Inoue, T. Oguchi and S. Chiba, 1995, Zatebradine attenuates cyclic AMP-related positive chronotropic but not inotropic responses in isolated, perfused right atria of the dog, Clin. Exp. Pharmacol. Physiol. 22, 29.

Shibuya, T., K. Hashimoto and S. Imai, 1983, Effective plasma concentrations of antiarrhythmic drugs against sustained halothane-epinephrine arrhythmia in dogs, J. Cardiovasc. Pharmacol. 5, 538.

Thollon, C., C. Cambarrat, J. Vian, J.-F. Prost, J.L. Peglion and J.P. Vilaine, 1994, Electrophysiological effects of S 16257, a novel sinoatrial node modulator, on rabbit and guinea-pig cardiac preparations: comparison with UL-FS 49, Br. J. Pharmacol. 112, 37.

Van Bogaert, P.P., M. Goethals and C. Simoens, 1990, Use- and frequency-dependent blockade by UL-FS 49 of the I_f pacemaker current in sheep cardiac Purkinje fibres, Eur. J. Pharmacol. 187, 241.

Wit, A.L. and M.R. Rosen, 1992, Afterdepolarizations and triggered activity: distinction from automaticity as an arrhythmogenic mechanism, in: The Heart and Cardiovascular System, 2nd edn., eds. H.A. Fozzard, E. Haber, R.B. Jennings, A.M. Katz and H.E. Morgan (Raven Press, New York) p. 2113.

Yu, H., F. Chang and I.S. Cohen, 1993, Pacemaker current exists in ventricular myocytes, Circ. Res. 72, 232.