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Cardiovascular effects of Y-27632, a selective Rho-associated kinase inhibitor, assessed in the halothane-anesthetized canine model

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Abstract

Y-27632, (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate, is a selective Rho-associated kinase inhibitor, which has been suggested to possess multiple clinical applications based on the in vitro observations. Since information regarding in vivo cardiovascular effects of Y-27632 is still limited, we assessed them using the halothane-anesthetized, closed-chest canine model. Administration of Y-27632 in a dose of 0.01 mg/kg, i.v. significantly decreased total peripheral vascular resistance together with an increase of cardiac output without affecting other cardiovascular parameters. Moreover, additional administration of Y-27632 in a dose of 0.1 mg/kg, i.v. significantly decreased blood pressure and left ventricular end-diastolic pressure, increased the heart rate and cardiac contractility, enhanced atrioventricular conduction and shortened the repolarization process as well as the effective refractory period. These results indicate that Y-27632 exerts a potent arterio-venodilator action with cardiostimulatory effects possibly through the sympathetic reflex in the in vivo canine model.

Keywords: Y-27632; Vasodilator; Rho-kinase; Monophasic action potential

1. Introduction

Y-27632, (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate, is a specific inhibitor of Rho-associated coiled-coil forming protein serine/threonine kinase (ROCK) (Uehata et al., 1997; Ishizaki et al., 2000). Using Y-27632, previous studies have indicated that the Rho/ROCK-mediated pathway plays an important role in various cellular functions, such as actin cytoskeleton organization, cell adhesion, motility and neovascular formation in addition to vascular and bronchial smooth muscle contraction (Hall, 1998; Horwitz and Parsons, 1999; Takai et al., 2001). A recent multicenter phase II clinical study in Japan have indicated that another Rho/ROCK inhibitor fasudil can exert anti-anginal effects (Shimokawa et al., 2002). Therefore, inhibition of Rho/ROCK is now expected to be a novel mechanism for the treatment of various pathological conditions including hypertension, arteriosclerosis, bronchial asthma, glaucoma, cancer and ischemic heart disease (Uehata et al., 1997; Ito et al., 1999; Sawada et al., 2000; Iizuka et al., 2000; Rao et al., 2001; Shimokawa, 2002).

On the other hand, the role of Rho/ROCK in the physiological regulation of cardiac function has been analyzed using transgenic mice, in which overexpression of RhoA suppresses the sinus and atrio-ventricular nodal functions (Sah et al., 1999). In addition, another study has demonstrated that overexpression of RhoA markedly reduced the basal current generated by Kv1.2 expressed in Xenopus oocytes (Cachero et al., 1998). More recently, using the canine-isolated, blood-perfused heart preparations, we have confirmed that intracoronary administration of Y-27632 can exert the positive chronotropic, negative inotropic, negative dromotropic and repolarization-promoting effects at doses that could induce the coronary vasodilator actions (Sugiyama et al., 2002a,b). Since information regarding the in vivo cardiohemodynamic and electrophysiological effects of Y-27632 is important for future clinical application, in the present study, we simultaneously

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assessed them using the halothane-anesthetized in vivo canine model (Sugiyama and Hashimoto, 1998; Sugiyama et al., 1999, 2001a,b; Satoh et al., 1999; Takahara et al., 2000).

2. Materials and methods

All experiments were performed according to Guidelines for Animal Experiments, University of Yamanashi Faculty of Medicine.

2.1. Cardiohemodynamic parameters

Five female Beagle dogs weighing 11.1 ± 0.3 kg were initially anesthetized 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). The tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. To prevent blood clotting, heparin calcium (100 IU/kg) was intravenously administered. A heparinized catheter was placed in the aorta for continuous monitoring of the systemic blood pressure. A thermodilution catheter (TC-704; Nihon-Kohden, Tokyo, Japan) was positioned at the right side of the heart via the right femoral vein, and the cardiac output was measured by a standard thermodilution method by using a cardiac output computer (MFC-1100; Nihon-Kohden). Total peripheral vascular resistance (TPR) was calculated using the basic equation: TPR = mean blood pressure/cardiac output. A pigtail catheter was positioned at the left ventricle through the left femoral artery to measure ventricular pressure. The maximal upstroke velocity of the left ventricular pressure ($LVdP/dt_{max}$) and left ventricular end-diastolic pressure (LVEDP) were obtained to estimate the contractility and preload of the left ventricle, respectively.

2.2. Electrophysiological parameters

The surface lead II ECG was obtained from the limb electrodes. Corrected QT interval (QTc) was calculated using Bazett's formula. A quad-polar electrodes catheter was positioned at the non-coronary cusp of the aortic valves via the right femoral artery to record His bundle electrogram. A bidirectional steerable monophasic action potential recording/pacing combination catheter (1675P; EP Technologies, Sunnyvale, CA, USA) was positioned at the endocardium of the interventricular septum in the right ventricle via the left femoral vein to obtain the monophasic action potential signals. The signals were amplified with a direct-current preamplifier (300; EP Technologies). The duration of the monophasic action potential signal was measured as an interval, along a line horizontal to the diastolic baseline, from the upstroke to the desired repolarization level, and the interval (ms) at 90% repolarization was defined as MAP_{90}.

The heart was electrically driven using a cardiac stimulator (SEC-3102; Nihon-Kohden) with the pacing electrodes of the monophasic action potential recording/pacing combination catheter in the right ventricle. Stimulation pulses were rectangular in shape, 1–2 V (about twice the threshold voltage) and of 1-ms duration. MAP_{90} was measured during the sinus rhythm ($MAP_{90(sinus)}$) and at the pacing cycle length of 400 ms ($MAP_{90(CL400)}$) and at 300 ms ($MAP_{90(CL300)}$). The effective refractory period was assessed by a programmed electrical stimulation to the right ventricle. The pacing protocol consisted of five beats of basal stimuli in a cycle length of 400 ms followed by an extra stimulus of various coupling intervals. Starting in the late diastole, the coupling interval was shortened in 5- to 10-ms decrements until refractoriness occurred. The duration of the terminal repolarization phase (TRP) of the ventricle, namely, phase 3 repolarization of the action potential, was calculated by the difference between the $MAP_{90(CL400)}$ and the effective refractory period (ERP) at the same site ($TRP = MAP_{90(CL400)} - ERP$), which shows the electrical vulnerability of the ventricular muscle (Franz, 1994; Kirchhof et al., 1998; Sugiyama and Hashimoto, 1998, 2002; Sugiyama et al., 1999, 2001a,b; Satoh et al., 1999).

2.3. Experimental protocol

The cardiohemodynamic and electrophysiological parameters were continuously monitored using a polygraph system (RM-6000; Nihon-Kohden), and analyzed with a real-time full automatic data analysis system (MP/VAS 3 for Macintosh, ver 1.0, Physio-Tech, Tokyo, Japan). Each measurement of ECG, the monophasic action potential, atrio-His and His-ventricular interval was the mean of three consecutive recordings. The cardiovascular variables were assessed in the following order. The cardiac output was measured twice. Next, the ECG, His bundle electrogram, systemic and left ventricular pressure and the monophasic action potential signals were recorded under a sinus rhythm. Then, the monophasic action potential signals were recorded during the ventricular pacing at a cycle length of 400 and 300 ms. Finally, the effective refractory period was assessed by the programmed electrical stimulation, as described above. After the basal assessment, Y-27632 in a dose of 0.01 mg/kg was administered over 10 min and each parameter was assessed 5, 10, 15, 20 and 30 min after the start of the drug infusion. Next, Y-27632 in a dose of 0.1 mg/kg was additionally administered over 10 min, and each parameter was observed 5, 10, 15, 20, 30, 45 and 60 min after the start of the drug infusion.

2.4. Drugs

Y-27632 (MW: 338.3) was generously provided from Welfide (Osaka, Japan) and dissolved with saline. The following drugs were purchased: thiopental sodium (Tanabe,

Osaka, Japan), halothane (Takeda, Osaka, Japan) and heparin calcium (Mitsui, Tokyo, Japan).

2.5. Statistics

Data are expressed as the mean ± S.E.M. The statistical comparisons within a parameter were evaluated by one-way, repeated-measures analysis of variance (ANOVA) followed by Contrasts for mean values comparison. A P value <0.05 was considered statistically significant.

3. Results

Typical tracings of blood pressure, left ventricular pressure, surface lead II ECG, His bundle electrogram and the monophasic action potential signal during the sinus rhythm before the administration of the drug (Control) are depicted in Fig. 1 (left).

3.1. Effects on the heart rate and blood pressure

Typical tracings of the effects of Y-27632 on blood pressure and the time courses of changes in heart rate and blood pressure are shown in Figs. 1 (right) and 2. The pre-drug control value of the heart rate was 119 ± 9 beats/min, and those of the systolic, mean and diastolic blood pressures were 149 ± 5, 121 ± 4 and 101 ± 3 mm Hg, respectively. The heart rate and blood pressure were hardly affected by Y-27632 in a dose of 0.01 mg/kg. After the start of 0.1 mg/kg of Y-27632 infusion, the heart rate increased and the peak change was +58%. Meanwhile, the systolic, mean and diastolic blood pressure decreased and the peak changes were −6%, −15% and −27%, respectively.

3.2. Effects on cardiac output and total peripheral vascular resistance

The time courses of changes in cardiac output and total peripheral vascular resistance are summarized in Fig. 2. The pre-drug control values of cardiac output and total peripheral vascular resistance were 1.70 ± 0.22 l/min and 77 ± 12 mm Hg min/l, respectively. Cardiac output was increased by both doses of Y-27632, and the peak changes at the low and high doses were +17% and +111%, respectively. Meanwhile, total peripheral vascular resistance was decreased by both doses of Y-27632, and the peak changes at the low and high doses were −15% and −59%, respectively.

3.3. Effects on the LVdP/dt$_{max}$ and LVEDP

Typical tracings of the effects of Y-27632 on LVP are depicted in Fig. 1 (right) and the time courses of changes in the LVdP/dt_{max} and LVEDP are summarized in Fig. 2. The pre-drug control values of LVdP/dt_{max} and LVEDP were 2.38 ± 0.14 mm Hg/ms and 10.1 ± 0.4 mm Hg, respectively. A slight increase in LVdP/dt_{max} and decrease in LVEDP were observed after the administration of the low

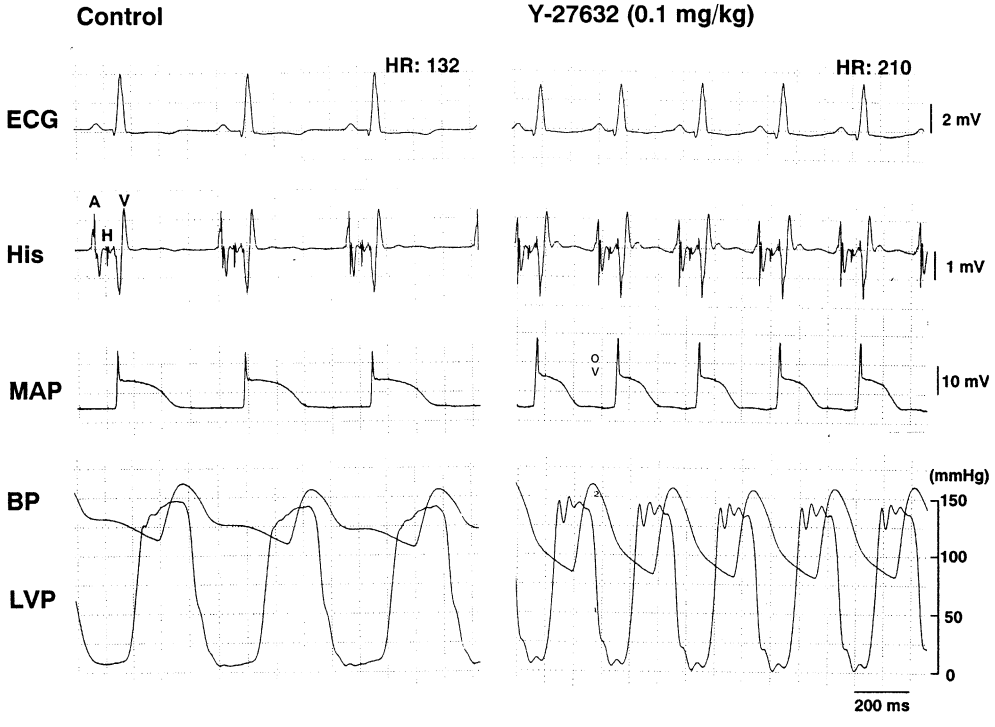

Fig. 1. Typical tracings of the surface lead II electrocardiogram (ECG), His bundle electrogram (His), monophasic action potentials recorded from the right ventricle (MAP), blood pressure (BP) and left ventricular pressure (LVP) during sinus rhythm at pre-drug control (Control) and 10 min after the start of Y-27632 infusion (0.1 mg/kg).

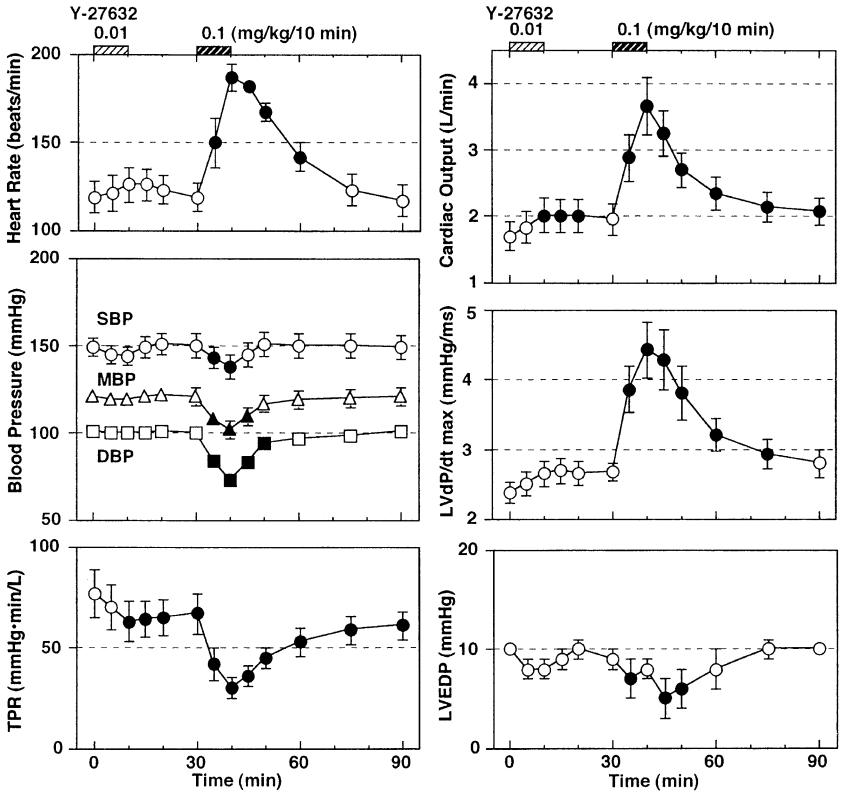

Fig. 2. Time courses of the effect of Y-27632 on heart rate (upper left panel); systolic blood pressure (SBP; circles), mean blood pressure (MBP; triangles) and diastolic blood pressure (DBP; squares) (middle left panel); total peripheral vascular resistance (TPR) (lower left panel); cardiac output (upper right panel); maximum upstroke velocity of left ventricular pressure (LVdP/dt_{max}) (middle right panel); left ventricular end-diastolic pressure (LVEDP) (lower right panel). Data are presented as the mean ± S.E.M. ($n = 5$). Closed symbols represent statistically significant differences from each pre-drug control (0 time) value by $P < 0.05$.

dose, but these changes did not reach statistical significance. After the administration of the high dose, LVdP/dt_{max} was increased, and the peak change was +86%. Meanwhile, LVEDP decreased, and the peak change was −42%.

3.4. Effects on the ECG

Typical tracings of the effects of Y-27632 on ECG and the time courses of changes in the ECG parameters are shown in Figs. 1 (right) and 3. The pre-drug control values of PR interval, QRS width, QT interval and QTc were 106 ± 11, 68 ± 3, 291 ± 21 ms and 404 ± 15 ms/s$^{1/2}$, respectively. Any of these ECG parameters was hardly affected by the low dose of Y-27632. After the administration of the high dose, PR and QT intervals were shortened, and the peak changes were −15% and −25%, respectively. No significant change was detected in QRS width or QTc during the experimental period.

3.5. Effects on the His bundle electrogram and the monophasic action potential during sinus rhythm

Typical tracings of the effects of Y-27632 on His bundle electrogram and the monophasic action potential and the time courses of changes in atrio-His and His-ventricular intervals and $MAP_{90(sinus)}$ during sinus rhythm are also shown in Figs. 1 (right) and 3. The pre-drug control values of atrio-His and His-ventricular intervals and $MAP_{90(sinus)}$ were 81 ± 10, 29 ± 2 and 249 ± 15 ms, respectively. No significant change was detected in atrio-His and His-ventricular intervals and $MAP_{90(sinus)}$ after the administration of the low dose. After the administration of the high dose, the $MAP_{90(sinus)}$ interval was shortened, and the peak change was −25%. There was a tendency for the atrio-His interval to be shortened at this dose, although it did not achieve statistical significance. No significant change was detected in His-ventricular interval during the experimental period.

3.6. Effects on monophasic action potential, effective refractory period and terminal repolarization period during ventricular pacing

The time courses of changes in $MAP_{90(CL400)}$, $MAP_{90(CL300)}$, the effective refractory period and the terminal repolarization period are summarized in Fig. 3. The pre-drug control values of $MAP_{90(CL400)}$, $MAP_{90(CL300)}$, the effective refractory period and the terminal repolarization period were 244 ± 10, 227 ± 6, 206 ± 11 and 38 ± 2 ms, respectively. No significant changes were detected in $MAP_{90(CL400)}$, $MAP_{90(CL300)}$ and the terminal repolarization

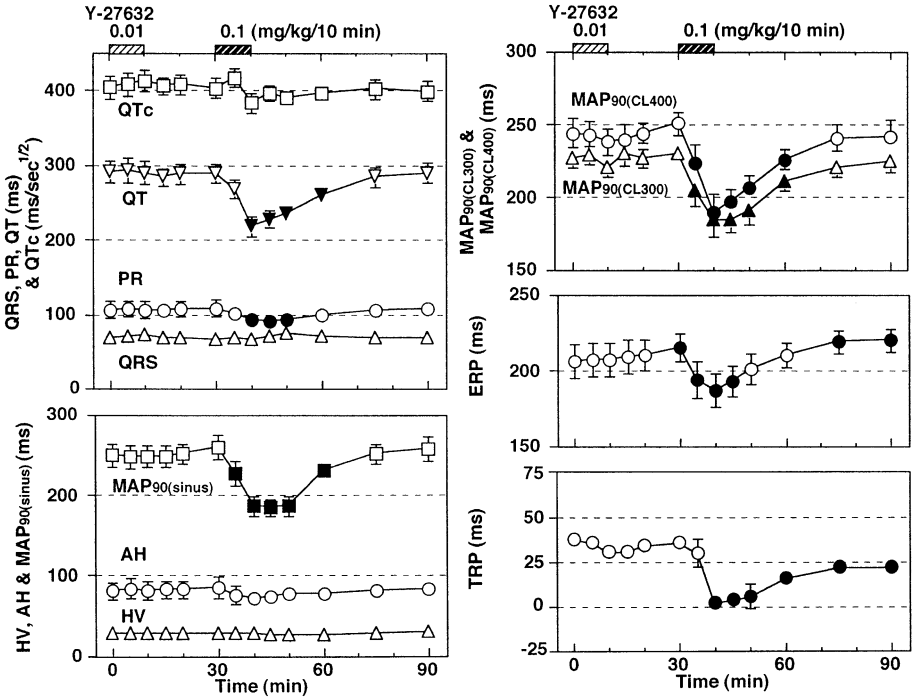

Fig. 3. Time courses of the effect of Y-27632 on PR interval (circles), QRS width (triangles), QT interval (triangles) and QTc (squares) (upper left panel); atrio-His interval (AH; circles) and His-ventricular interval (HV; triangles) and duration of monophasic action potential at a level of 90% repolarization (MAP_{90}) during sinus rhythm recorded from the right ventricle ($MAP_{90(sinus)}$; squares) (lower left panel); MAP_{90} during the electrical ventricular pacing at a cycle length of 400 ms ($MAP_{90(CL400)}$; circles), MAP_{90} during the electrical ventricular pacing at a cycle length of 300 ms ($MAP_{90(CL300)}$; triangles) (upper right panel); effective refractory period of the right ventricle (ERP) (middle right panel) and terminal repolarization period (TRP) (lower right panel). Data are presented as the mean ± S.E.M. ($n=5$). Closed symbols represent statistically significant differences from each pre-drug control (0 time) value by $P<0.05$.

period after the administration of the low dose, while the effective refractory period was significantly prolonged, and the peak change was +5%. After the administration of the high dose, $MAP_{90(CL400)}$, $MAP_{90(CL300)}$, the effective refractory period shortened, and the peak changes were +21%, +18% and +9%, respectively. The effective refractory period then returned to the basal level and prolonged for 45–60 min after the administration of the high dose. The peak change was +7%. The terminal repolarization period was shortened significantly after the administration of the high dose, and the peak change was −91%.

4. Discussion

Given the limited information, the in vivo cardiovascular effects of Y-27632 were assessed using the halothane-anesthetized canine model to clarify the safety margin of the drug on the cardiovascular system (Sugiyama and Hashimoto, 1998; Sugiyama et al., 1999; Satoh et al., 1999; Takahara et al., 2000). Y-27632 in a dose of 0.01 mg/kg, i.v. slightly, but significantly decreased the total peripheral vascular resistance and increased the cardiac output. Moreover, a 10 times higher dose of Y-27632 exerted positive inotropic, chronotropic and dromotropic effects together with a hypotensive action. In addition, Y-27632 shortened MAP_{90} as well as the effective refractory period, and the extent of shortening of MAP_{90} was greater than that of the effective refractory period, leading to the shortening of the electrically vulnerable period (Nattel and Zeng, 1984; Franz and Costard, 1988; Franz, 1994; Sugiyama and Hashimoto, 2002). Since information regarding pharmacokinetic property of Y-27632 is still lacking, peak plasma concentrations were roughly estimated based on our previous experience with this animal model (Sugiyama and Hashimoto, 1998; Sugiyama et al., 2001a,b), which could be around 10–20 ng/ml (=0.03–0.06 μM) and 100–200 ng/ml (=0.3–0.6 μM) after the administration of 0.01 and 0.1 mg/kg of Y-27632, respectively. Since the range of concentrations of Y-27632 in vitro that could significantly inhibit the Rho/ROCK was 0.1–10 μM (Uehata et al., 1997), the present results might reflect the effects of subtherapeutic to therapeutic in vitro dose ranges of Y-27632.

As shown in the results, previously described vasodilator action in vitro was confirmed in the present in vivo study (Uehata et al., 1997). For example, Y-27632 reduced the total peripheral vascular resistance at both doses, which would reflect the arteriolar dilator action, resulting in the decrease of the afterload to the left ventricle. Meanwhile, the drug also decreased LVEDP after the high dose administration, which would be associated with the venodilator action, leading to the reduction of the preload to the left ventricle. These profiles of vasodilator effects of Y-27632

are quite similar to those of phosphodiesterase III inhibitor toborinone (Sugiyama et al., 2001a), although molecular mechanisms are different between the drugs. These results at least indicate that Rho/ROCK may play an important role in the physiological regulation of the vascular tone of venulae as well as the arterioles in vivo, as previously reported for the isolated saphenous vein smooth muscles (McGregor et al., 2002).

The cardiotonic actions of Y-27632 in this model, namely, positive inotropic, chronotropic and dromotropic effects, were not necessarily in accordance with its direct cardiac effects obtained from our previous in vitro studies, in which the drug at equivalent doses induced significant negative inotropic, negative dromotropic and slight positive chronotropic effects in the canine isolated, blood-perfused heart preparations (Sugiyama et al., 2002a,b). Since the in situ heart, as used in this study, is physiologically regulated by both neuronal and humoral control (Sugiyama et al., 1999, 2001c; Shiina et al., 2000), the cardiostimulatory effects of Y-27632 observed after the high dose would be largely induced by the sympathetic reflex resulting from its hypotensive action.

Since the current canine model has been used for assessing the potential QT-prolonging properties of drugs (Sugiyama and Hashimoto, 1998; Sugiyama et al., 1999; Satoh et al., 1999), the effects of Y-27632 on the ventricular repolarization phase also deserve a comment. Intravenous administration of Y-27632 shortened the QT interval and MAP_{90}, indicating the abbreviation of the ventricular repolarization process. This result is essentially in accordance with a study that overexpression of RhoA in the heart reduces Kv1.2 current of Xenopus oocytes, which prolonged the repolarization period (Cachero et al., 1998). Moreover, the current result is directionally similar to our recent study with the canine isolated, blood-perfused heart preparations that effective coronary vasodilating doses of Y-27632 shortened monophasic action potential duration by 6% (Sugiyama et al., 2002b), extent of which was less great than the present in vivo results. Since β-adrenoceptor stimulation has been shown to increase the slowly activating component of delayed rectifier K^+ current (I_{Ks}), which enhances repolarization process of the ventricular cells (Sanguinetti et al., 1991; Salata et al., 1998), the shortening of the repolarization process by Y-27632 in this study would be associated with the hypotension-induced sympathetic nerve activation in addition to its direct repolarization-promoting effect.

Y-27632 prolonged the effective refractory period when its hypotensive action disappeared at both doses, whereas the high dose infusion shortened it soon after the administration. It has been reported that using the same animal model as used in this study, a drug-induced increase of adrenergic tone can shorten the effective refractory period without affecting the intraventricular conduction time, which was totally abolished by the pretreatment of the animals with β-blocker esmolol (Sugiyama et al., 2001c). These present and previous results suggest that Y-27632 itself and/or its metabolites may prolong the effective refractory period, which might have been reversed by the hypotension-induced increase of adrenergic tone. Thus, further investigation will be needed to better understand the biphasic action of Y-27632 on the effective refractory period.

In summary, Y-27632 exerts a potent arterio-venodilator action, leading to the potent positive chronotropic, inotropic, dromotropic and repolarization-promoting effects via the increase of sympathetic tone in the canine in vivo model. The data shown in this study will provide convenient guidelines for comparing the possible effectiveness and potential adverse effects of the new Rho/ROCK inhibitors.

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