Cardiac Effects of a Selective Rho-Associated Kinase Inhibitor, Y-27632, Assessed in Canine Isolated, Blood-Perfused Heart Preparations

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ABSTRACT—Chronotropic, inotropic and coronary effects of Y-27632 ((+)-(R)-trans-4-(1-aminoethyl)-N-(4-pyridyl) cyclohexanecarboxamide dihydrochloride, monohydrate), a specific inhibitor of Rho-associated coiled-coil forming protein serine/threonine kinase (ROCK), were assessed using canine isolated, blood-perfused heart preparations. Y-27632 slightly enhanced sinoatrial automaticity and significantly increased coronary blood flow, while it decreased ventricular contraction. The concentrations of Y-27632 needed to cause the currently observed changes were similar to those inhibiting ROCK in a previous in vitro study. These results suggest that the constitutional ROCK in the heart mainly regulates the ventricular contractility and coronary vascular tone rather than the sinoatrial automaticity.

Keywords: Y-27632, Sinus nodal automaticity, Ventricular contraction

Y-27632 is a specific inhibitor of Rho-associated coiled-coil forming protein serine/threonine kinase (ROCK), which has been used to study physiological and pathophysiological roles of Rho/ROCK-mediated calcium sensitization (1, 2). In the field of cardiovascular pharmacology, Y-27632 has been shown to inhibit smooth-muscle contraction (1), dramatically correct the blood pressure of hypertensive rat models (1), suppress coronary vasospasm (3), inhibit ET-1-induced hypertrophic responses (4), and suppress neointimal formation of balloon-injured arteries (5). However, the information regarding the role of constitutional ROCK in the regulation of the sinus nodal automaticity and ventricular contraction is still lacking. In this study, we assessed the chronotropic and inotropic effects of Y-27632 in comparison with the coronary vasodilator action, using canine isolated, blood-perfused heart preparations (6, 7).

The experiment was performed in accordance with the rules and regulations of the Committee for Research at the Yamanashi Medical University. Animals were obtained through the Animal Laboratory for Research of Yamanashi Medical University. Experiments were carried out using the canine isolated sinoatrial node and papillary muscle preparations cross-circulated with heparinized arterial blood of the donor dog (6, 7).

The preparation was obtained from a beagle dog (CSK Research Park, Nagano) of either sex, weighing approximately 10 kg. The dog was anesthetized with pentobarbital sodium (30 mg/kg, i.v.), given heparin calcium (500 U/kg, i.v.), and exsanguinated. The heart was excised and plunged into cold Tyrode’s solution kept at about 4°C. The sinoatrial node preparation consists of the entire right atrium (6). The sinus node artery was cannulated through the right coronary artery. Bipolar recording electrodes were attached on the atrial epicardium close to the sinus node. The papillary muscle preparation consists of the anterior papillary muscle of the right ventricle attached to the interventricular septum (7). The anterior septal artery, which is a sole nutrient artery of the preparation, was directly cannulated. Bipolar stimulating electrodes were attached onto the His-bundle region.

HBD dogs (Kitayama Labes, Yoshihi Farm, Gifu) of either sex, weighing 20 – 25 kg, were used for the blood-donor dog. The dog was anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and supplemented with 4 – 5 mg /kg per hour. After intubation, dogs were artificially ventilated with room air (SN-480-3; Shinano, Tokyo). The systemic blood pressure and surface lead II ECG were continuously monitored using a polygraph system (RM-6000; Nihon Kohden, Tokyo). At the start of cross-circulation, heparin calcium (500 U/kg, i.v.) was given followed by an additional dose of 200 U/kg per hour.

The preparations were placed in a double-wall glass
jacket maintained at 38°C by circulating warm water and perfused with arterial blood from the carotid artery of the blood-donor dog. Perfusion pressure was kept at 120 mmHg with a peristaltic pump (7553-00; Cole-Parmer, Chicago, IL, USA) and Starling’s pneumatic resistance placed parallel to the perfusion circuit. Venous blood from the preparations and excess blood passing through the pneumatic resistance were collected in a blood reservoir and returned to the jugular vein of the blood-donor dog.

The spontaneously beating rate of the sinoatrial node preparation (i.e., sinoatrial rate) was measured with a heart rate counter (AT-601G, Nihon Kohden) triggered by the atrial electrogram. The papillary muscle preparation was electrically driven through the stimulating electrodes at a cycle length of 500 ms using a stimulator (SEN-7203, Nihon Kohden) and an isolation unit (SS-201J, Nihon Kohden). The stimulation pulses were rectangular in shape, 1–2 V amplitude (about 20% above the threshold voltage), and 5-ms duration. Developed tension of the papillary muscle under a resting tension of 2 g was measured isometrically using a force displacement transducer (DRM-200S; Dia Medical, Tokyo) and an amplifier (DRM-T20, Dia Medical). The coronary blood flow through the nutrient artery of the papillary muscle preparation was continuously measured with an electromagnetic flowmeter (MFV-3200, Nihon Kohden).

Because relatively small amount of a drug was administered to the preparations compared to those needed in a whole animal model, multiple drug doses were studied in the same preparation (6, 7). The effluent blood through each preparation immediately after the drug injection was discarded to eliminate the drug effects on the donor dog. Once the preparations were stabilized, Y-27632 in doses of 1 to 10 μg or its vehicle saline was injected into each nutrient artery of the preparations using a small microsyringe in the volume of 10–30 μl over 4 s. The effects of Y-27632 on each parameter were assessed.

The following drugs were purchased: pentobarbital sod-

Fig. 1. Typical tracings of the effects of Y-27632 on the sinoatrial rate (SAR) (A), developed tension (DT) of the papillary muscle, and coronary blood flow (CBF) through the anterior septal artery (B).

Fig. 2. Dose-response curves of Y-27632 and vehicle saline for the percent changes in the sinoatrial rate (SAR) (A), developed tension (DT) of the papillary muscle (B) and coronary blood flow (CBF) through the anterior septal artery (C) (mean ± S.E.M., n = 4). *P<0.05, compared with the respective pre-drug control values.
um (Tokyo-Kasei, Tokyo) and heparin calcium (Mitsui, Tokyo). Y-27632; (+)-(R)-trans-4-(1-aminomethyl)-N-(4-pyridyl) cyclohexanecarboxamide dihydrochloride, monohydrate (MW: 338.3) was generously provided by Welfide Co., Ltd. (Osaka), which was dissolved with saline in concentrations of 0.1 and 1 mg/ml. The data are presented as the mean ± S.E.M. The statistical comparisons of mean values were carried out by the paired t-test. A P-value of less than 0.05 was considered significant.

One hour after the start of cross-circulation, the sinoatrial node preparation showed the sinoatrial rate of 87 ± 2 beats /min (n = 4), whereas the papillary muscle preparation showed the developed tension of 2.9 ± 0.7 g and coronary blood flow of 4.3 ± 0.6 ml/min (n = 4). Typical tracings of the chronotropic, inotropic and coronary vasodilator effects are depicted in Fig. 1, and their percent changes are summarized in Fig. 2. Administration of Y-27632 increased the sinoatrial rate in a dose-related manner, but the extent of the positive chronotropic action was relatively small even after the administration of the highest dose. Meanwhile, the drug decreased the developed tension of the papillary muscle and increased the coronary blood flow in a dose-related manner. The vehicle saline did not affect any of these parameters.

Given the limited information regarding the cardiovascular profile of Y-27632, we assessed its effects on the sinoatrial automaticity, ventricular contraction and coronary blood flow using the well-established canine isolated, blood-perfused heart preparations (6, 7). As clearly shown in the results, Y-27632 slightly enhanced the sinoatrial automaticity and significantly increased the coronary blood flow, while it decreased the ventricular contraction. Although similar vasodilator actions of Y-27632 have been reported (1, 3), this is the first report describing the positive chronotropic and negative inotropic effects of Y-27632. While the negative inotropic effect of Y-27632 might be related to the decreased myofibrillar calcium sensitivity via the inhibition of the RhoA-ROCK pathway, the mechanism of the slight but significant positive chronotropic action remains to be elucidated.

The relation between the doses of Y-27632 needed to cause the observed changes in this study and those used for inhibiting ROCK can be estimated as follows. As the coronary blood flow was in the range of 2.5 – 5.0 ml/min, the drug concentration assessed in this study can be roughly estimated to be 0.2 – 4.0 μg/ml (0.6 – 11.8 μM). The concentration of Y-27632 exerting the ROCK inhibition has been shown to be 0.1 – 10.0 μM in the cellular level (1). Moreover, Y-27632 was demonstrated to be >200 times more selective for ROCK than for conventional protein kinase C, cyclic AMP dependent protein kinase or myosin light-chain kinase (1). Thus, the present results suggest that the constitutional ROCK may regulate the sinus nodal automaticity and ventricular contractility in addition to the coronary vascular tone. Indeed, a recent study has shown that α1-adrenergic stimulation increases the myofibrillar calcium sensitivity mainly through the upregulated Gq-RhoA-ROCK signaling pathway in the failing myocardium (8).

In summary, the present study indicates that Y-27632 itself can induce potent negative inotropic and coronary vasodilator actions in addition to the modest positive chronotropic effect. The constitutional ROCK may play an important role in the regulation of cardiac functions.

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