Negative Chronotropic and Inotropic Effects of U-92032, a Novel T-Type Ca\(^{2+}\) Channel Blocker, on the Isolated, Blood-Perfused Dog Atrium

Manoj Lakhe, Yasuyuki Furukawa*, Takanori Yonezawa, Yoshito Nagashima, Masamichi Hirose and Shigetoshi Chiba

Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390, Japan

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ABSTRACT—We investigated the effects of U-92032 ((7-((bis-4-fluorophenyl)methyl)-1-piperazinyl)-2-2(2-hydroxyethylamino)-4-(1-methylethyl)-2,4,6-cycloheptatrien-l-one), a novel T-type Ca\(^{2+}\) channel blocker, on sinus rate and atrial contractile force in the isolated, blood-perfused atrium of the dog. U-92032 (1 to 300 nmol) induced negative chronotropic and inotropic responses in a dose-dependent manner, and the percentage decrease in sinus rate was less than that in atrial contractile force. Atropine did not affect the negative responses to U-92032. These results suggest that U-92032, a T-type Ca\(^{2+}\) channel blocker, simultaneously decreases the sinus rate and atrial force as do L-type Ca\(^{2+}\) channel blockers in the isolated dog atrium.

Keywords: U-92032, T-type Ca\(^{2+}\) channel, L-type Ca\(^{2+}\) channel

The spontaneous depolarization of sinoatrial (SA) nodal pacemaker cells is mainly caused by the activation of T-type (I\(_{Ca,T}\)) and L-type (I\(_{Ca,L}\)) Ca\(^{2+}\) currents, hyperpolarization-activated inward current (I\(_f\)) and delayed rectifier K\(^+\) current (I\(_k\)) (1). Although there are many reports on the cardiac effects of I\(_{Ca,L}\), I\(_f\) and I\(_k\) blockers in mammalian cardiac tissues, there is little information on the effects of I\(_{Ca,T}\) blockers on the heart. The T-type Ca\(^{2+}\) channels are found in cardiac tissues, atrial myocytes (2), SA nodal cells (3), Purkinje fiber cells (4) and ventricular myocytes (5). Inorganic Ca\(^{2+}\) channel blockers such as divalent cations show little preferential block of T-type versus L-type Ca\(^{2+}\) channels. Likewise most organic Ca\(^{2+}\) channel blockers do not offer better selectivity. Although tetramethrin (3) and niguldipine (6) blocked T-type Ca\(^{2+}\) channels of the cardiac cells preferentially, their effects on two types of Ca\(^{2+}\) channels still overlapped. It has been recently reported that U-92032 at a low concentration (1 \(\mu\)M) blocked selectively the T-type Ca\(^{2+}\) channels, but at a high concentration (10 \(\mu\)M), it blocked both T-type and L-type Ca\(^{2+}\) channels in guinea pig atrial cells (7). Therefore, to elucidate the role of I\(_{Ca,T}\) in the regulation of heart rate and myocardial contractility, we studied the effects of U-92032, a novel I\(_{Ca,T}\) blocker, on the SA nodal pacemaker activity and myocardial contractility in isolated, blood-perfused dog atrium.

An isolated right atrial preparation was perfused with heparinized arterial blood from an anesthetized support dog. The details of the preparation have been described in a previous paper (8). Support dogs weighing 12 to 23 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and ventilated artificially through a cuffed tracheal tube with room air by using a respirator (model 607; Harvard Apparatus Co., Inc., Millis, MA, USA). Sodium heparin (500 USP units/kg, i.v.) was administered to each dog at the beginning of the perfusion of the isolated atrial preparation, and 200 USP units/kg were given each hour thereafter.

Isolated right atrial preparations were obtained from other Mongrel dogs weighing from 9 to 13 kg. Each dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The right atrium was excised and immersed in cold Ringer’s solution. The sinus node artery of the isolated right atrium was cannulated, and each preparation was perfused with heparinized blood from the carotid artery of the anesthetized support dog by the aid of a peristaltic pump (model 1210, Harvard Apparatus). A pneumatic resistance was placed in parallel with the perfusion system so that the perfusion pressure could be maintained constant at 100 mmHg. The rate of blood flow to the atrial preparation was 2 to 8 ml/min. The venous effluent from the preparation was led to a collecting funnel and returned to the support dog through the external jugular

* To whom correspondence should be addressed.
vein. The preparation was anchored to a stainless steel bar and placed in a cup-shaped glass container kept at 37°C. The upper part of the cardiac preparation was connected to a force-displacement transducer (AP 620G; Nihon Kohden, Tokyo) by a silk thread. The cardiac tissue was usually stretched to a resting tension of 2 g. Isometric tension was recorded on a thermo-writing rectigraph (RTA-1200, Nihon Kohden). A pair of bipolar silver electrodes was brought into contact with the epicardial surface of the isolated preparation in order to record the atrial electrogram. The atrial rate was derived from the electrogram with a cardio-tachometer (AT-600G, Nihon Kohden). The femoral arterial blood pressure of the support dog, heart rate derived from lead II of the ECG and the rate of blood flow to an atrial preparation were monitored simultaneously.

We examined the effects of U-92032 at a dose of 1–300 nmol on the SA nodal pacemaker activity and atrial contractility in 9 isolated right atria. We also studied the effects of atropine (10 nmol) on the negative cardiac responses to U-92032 (100 nmol) in 5 isolated atria.

Drugs used in the present experiments were U-92032 ((7-((bis-4-fluorophenyl)methyl)-1-piperazinyl)-2-(2-hydroxyethylamino)-4-(1-methyl-ethyl)-2, 4, 6-cycloheptatrien-1-one) (generously synthesized by Kissei Yakuhin Kogyo, Matsumoto), acetylcholine chloride (Daiichi Seiyaku, Tokyo), atropine sulfate (Wako Pure Chemicals, Tokyo) and norepinephrine hydrochloride (Sankyo, Tokyo). U-92032 was dissolved in ethanol and then diluted with saline to obtain low concentrations. Other drugs were dissolved in saline before starting the experiments. The amount of drug solution injected was 1 to 30 µl.

Data are shown as the maximal change in response to each substance and are expressed as the mean±S.E.M. The analysis of variance was applied for multiple comparisons, and the paired Student's t-test was used for comparison between two groups. P values of less than 0.05 were considered statistically significant.

When U-92032 in a dose of 1–300 nmol was injected into the sinus node artery of the isolated, blood-perfused right atrium of the dog, U-92032 induced negative chronotropic and inotropic effects dose-dependently (P<0.001) in 9 isolated, perfused dog atria. The percentage changes in sinus rate in response to U-92032 were smaller than those in atrial contractile force; e.g., U-92032 at a dose of 300 nmol decreased the sinus rate and atrial contractile force by 8.7±2.6% and 28.0±4.4%, respectively. The threshold dose of the chronotropic and inotropic response was 30 nmol. Atropine at a dose of 10 nmol did not affect the negative chronotropic and inotropic responses to U-92032 at 100 nmol in 5 isolated, perfused dog atria, when it abolished the negative chronotropic and inotropic responses to acetylcholine (1 or 3 nmol).

In the present study, we demonstrated that U-92032, an I_{Ca-T} blocker, decreased the sinus rate and atrial contractile force, and the negative cardiac responses to U-92032 were not inhibited by atropine in the isolated, blood-perfused dog atrium. These results suggest that U-92032, an I_{Ca-T} blocker does not selectively decrease the sinus rate without affecting the myocardial contractile force in the dog heart.

It has been suggested that I_{Ca-T} has an important role for generation of the pacemaker potential in SA nodal cells (1, 3), although I_{Ca-T} has been found in mammalian atrial myocardial cells (2), Purkinje fibers (4) and ventricular myocytes (5). The characteristics of I_{Ca-T} in each cardiac tissue are similar. U-92032 at low concentrations (0.1–1 µM) blocked I_{Ca-T} selectively in guinea pig atrial cells, but at high concentrations (more than 1 µM), it blocked both I_{Ca-T} and I_{Ca-L} (7). Additionally, U-92032 at 6 µM also selectively blocked I_{Ca-T} but not nifedipine-sensitive non-inactivating currents in a mouse neuronal cell line, N1E-115 cells (9). Thus, U-92032 at a low dose would be expected to work as an I_{Ca-T} blocker. Because of the limited solubility of U-92032, we tested the cardiac effects of U-92032 at a dose range of 1–300 nmol in isolated, blood-perfused dog atrium. Because we injected a drug into the sinus node artery of the isolated dog atrium, it is difficult to determine the drug concentration in the perfused blood. However, our injected doses of U-92032 roughly correspond to 1–100 µM. The threshold dose of
U-92032 for negative chronotropic and inotropic effects was not different and the percentage decreases in sinus rate induced by U-92032 at low doses were less than those in atrial contractile force (Fig. 1). Therefore, it is conceivable that the inhibition of \( I_{Ca-T} \) by U-92032 at the doses used in the present study does not decrease the sinus rate selectively in isolated perfused dog atrium. However, the possibility of the inhibition of \( I_{Ca-L} \) by U-92032 cannot be completely excluded in the present study. \( I_{Ca-L} \) blockers, verapamil and nicardipine, decreased both the sinus rate and atrial contractile force in isolated perfused dog atrium (10). Thus, to define the selective control of the sinus rate by the inhibition of \( I_{Ca-T} \), we need further studies using other \( I_{Ca-T} \) blockers including tetramethrin and nifludipine.

To control the sinus rate, we have previously investigated the cardiac effects of \( I_K \) and \( I_f \) blockers in addition to \( I_{Ca-L} \) blockers on isolated, blood-perfused dog heart preparations. Zatebradine, an \( I_f \) blocker different from \( I_K \) and \( I_{Ca-L} \) blockers, directly and selectively decreased the sinus rate without decreases in the atrial contractile force and attenuated the increase in the sinus rate induced by adrenergic interventions (11, 12). Thus, because of non-selective depression by U-92032 of the chronotropic and inotropic effects, U-92032 may not be useful as a bradycardic agent in the heart.

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