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Effects of Ranolazine on Vasomotor Responses of Rat Aortic Rings

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Background and Aims. Ranolazine is a piperazine derivative that was approved in 2006 for the treatment of chronic stable angina. Compared with first-line drugs currently used to treat angina, beneficial effects of ranolazine occur without changing hemodynamic parameters such as heart rate and blood pressure. In the present study the effects of ranolazine on vasomotor responses of rat aortic rings were examined.

Methods. Pharmacological evaluation was performed by analyzing the vasomotor responses of ranolazine on aortic rings of adult male Wistar rats precontracted with phenylephrine (10^{-5} M). In each experiment we used a pair of rings (with and without endothelium) from the same aorta and superfused in the same bath.

Results. Ranolazine (10^{-6}−10^{-4}M) induced a concentration-dependent relaxation of phenylephrine-precontracted rings. The relaxation was only partially dependent on the presence of the endothelium (56.78 ± 6.81% in rings with endothelium and 47.88 ± 4.70% in rings without endothelium). In rings with endothelium, L-NAME induced a shift to the right of the concentration-response curve to ranolazine. Blocking the cyclooxygenase pathway induced a leftward shift of the concentration relaxation curve to ranolazine in both types of rings and increased the ranolazine-induced relaxation in rings without endothelium.

Conclusions. Ranolazine has a vasodilatory effect that is predominantly endothelium-independent. The synthesis/release of nitric oxide by the endothelium may, however, contribute to its relaxing action. These effects of ranolazine may contribute to its beneficial effects in patients with stable angina.

Key Words: Nitric oxide, Stable angina, Vasodilation, Smooth muscle, endothelium.

Introduction

Ranolazine (*N*-(2,6-dimethylphenyl)-2-[4-[2-hydroxy-3-(2-methoxyphenoxy)-propyl]piperazin-1-yl] acetamide) is a piperazine derivative that was approved by the U.S. Food and Drug Administration (FDA) in January 2006 for use in chronic angina therapy.

The effects of ranolazine have been tested in multiple clinical trials where it has been shown to safely and effectively reduce angina symptoms and to increase exercise tolerance in patients with symptomatic coronary artery disease) (1−7).

Action of ranolazine is in large part due to preservation of myocardial blood flow during events that induce ischemia, through its effects of inhibition of the late inward sodium current (I_{NaL}) (8), prevention of myocardial cellular sodium overload, and consequent calcium overload, and thereby prevention of compression of intramyocardial nutritive blood vessels by diastolic myocardial stiffness (8). In animal models, the drug was shown to be a potent inhibitor of the I_{NaL} through a concentration-voltage and frequency-dependent inhibition (9,10). Ranolazine and TTX, inhibitors of the late Na^+ current, attenuated the palmitoyl-L-carnitine-induced ventricular contractile dysfunction and the increase of coronary resistance in guinea pig isolated heart (11).

In addition to its action to reduce late I_{Na}, ranolazine also reduces Herg K^+ current (9) and is a weak β_1- and

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β_2-adrenergic receptor antagonist (12), and it has been reported that it is a weak α adrenergic receptors antagonist (13,14).

Because the effects of ranolazine on vascular reactivity are not yet clearly established, in this study we analyzed the effects of ranolazine on the vasomotor responsiveness of rat aortic rings.

Materials and Methods

In Vitro *Measurements of Vascular Responses*

Experiments were performed in adult male Wistar rats weighing 250−300 g. Animals were killed by cervical dislocation followed by decapitation. All animals were cared for in compliance with the guidelines of Animal Care (NOM-062-ZOO, Mexico) (15). Immediately thereafter, a midsternal thoracotomy was performed, and the thoracic aorta was excised and placed in a dissecting chamber filled with aerated Tyrode's solution. Under a stereoscopic microscope the aorta was cleaned of connective and adipose tissue. From the central portion of the aorta, 2-mm-long rings were cut carefully to avoid damage to the endothelium. In every other ring, the endothelium was removed afterwards by gently rubbing the intimal surface.

For each experiment a pair of rings from the same aorta (one with intact endothelium, the other without a functional endothelium) was used. Each of these rings was suspended horizontally in the same miniature organ chamber (volume 0.5 mL) between a stainless steel post fixed to the bottom of the bath and a stainless steel hook attached to an isometric force transducer (Grass, FT 03, Grass Instruments, Quincy, MA). The vessels were continuously superfused with prewarmed (37°C) aerated (95% O_2 and 5% CO_2) modified Tyrode's solution (composition in mM: NaCl, 137; KCl, 2.7; $MgCl_2$, 0.69; $NaHCO_3$, 11.9; NaH_2PO_4, 0.4; $CaCl_2$, 1.8 and glucose, 10; pH was adjusted to 7.4). The rings were initially stretched until resting tension reached 2 g and allowed to equilibrate for 1 h; during this period the resting tension was continuously monitored (Grass, Model 79 Polygraph, Grass Instruments) and, if needed, readjusted to 2 g by further stretching.

Before starting an actual experiment, the functional integrity of both the smooth muscle layer and the endothelium was confirmed using phenylephrine and carbachol responsiveness as described elsewhere (16,17).

Experimental Protocol

In a first series of experiments, the effects of successively increasing concentrations of ranolazine (10^{-11}−10^{-3} M) on the basal tension were analyzed in the absence or in the presence of either the cyclooxygenase inhibitor indomethacin (10^{-6} M) or the competitive inhibitor of nitric oxide synthase Nω-nitro-L-arginine methyl ester L-NAME (300 μM).

To investigate a possible relaxant action of ranolazine, the effects of successively increasing concentrations of ranolazine (10^{-11}−10^{-3} M) on phenylephrine (10^{-5} M) precontracted aorta rings were analyzed. These effects were determined in the absence and in the presence of either indomethacin (10^{-6} M) or L-NAME (300 μM).

Reagents

Glucose was from Merck (Darmstadt, Germany). Ranolazine was prepared as described in the literature (18,19). All other chemicals were from Sigma (St. Louis, MO). Indomethacin was dissolved in 4% sodium carbonate. Ranolazine, L-phenylephrine hydrochloride, carbachol (carbamoylcholine chloride) and Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) were dissolved in deionized water.

Data Analysis

Ranolazine-induced relaxations are expressed as the percent of the maximal tension induced by phenylephrine (10^{-5} M). IC_{50} (−log of the mean molar concentration of ranolazine producing 50% of the maximal response) was determined with the software package Graph Pad Prism (v.5) (San Diego, CA). Data are expressed as mean ± SD for tension development and as mean ± SE for IC_{50} values.

Comparisons of means were made by one-way analysis of variance (ANOVA) and differences between groups were evaluated using Student-Newman-Keuls method (Graph Pad Prism (v.5) software; St. Louis, MO); p value of 0.05 or less was considered significant.

Results

Effects of Ranolazine on Basal Tension

In order to analyze the effects of ranolazine on basal tension, rat aortic rings, either with or without endothelium, were exposed to successively increasing concentrations of ranolazine (10^{-11}−10^{-3} M). No change was observed in basal tension in either of these rings. Similarly, when exposure to increasing concentration of ranolazine was repeated in the presence of either indomethacin or L-NAME, no change in basal tension was observed in either type of rings.

Afterwards, the rings were washed with Tyrode solution for 30 min. Rings were then exposed successively to phenylephrine (10^{-5} M) and carbachol (10^{-5} M). Under these conditions, the contractile response to phenylephrine in both types of rings was markedly depressed in comparison with that recorded during the control phenylephrine- challenge. In rings with endothelium, tension reached, respectively, 1.233 ± 0.33 and 2.511 ± 0.379, whereas in rings without endothelium, the contractile responses amounted to 2.078 ± 0.527 and 2.69 ± 0.358 g, respectively. The relaxation induced by carbachol was, however, similar to that observed during the control challenge.