1. Introduction

Ranolazine, a piperazine derivative, is used as an anti-anginal drug because of its blocking activity of partial fatty acid oxidation (pFOX), which inhibits fatty acid beta-oxidation and activates pyruvate dehydrogenase, thereby diverting the cardiac energy source from lipids to glucose, which requires less oxygen and helps maintain myocardial function during ischemia (Chaitman, 2002; Conti, 2003; Sabbah et al., 2002; Yao et al., 2009). Ranolazine is used to treat patients with chronic stable angina in clinical practice. The results from clinical trials show that ranolazine reduces the frequency of anginal attacks and increases exercise tolerance in these patients (Chaitman et al., 2004a, 2004b; Scirica et al., 2007; Song et al., 2004; Wu et al., 2004).

Ranolazine has been reported to have weak effects on antagonizing \( \alpha_1 \)-, \( \beta_1 \)-, and \( \beta_2 \)-adrenergic receptors (Allely et al., 1993; Letienne et al., 2001), but cause no significant bradycardia and/or lower systemic blood pressure in patients with chronic angina in clinical practice (Chaitman et al., 2004a; Louis et al., 2002; Pepine and Wolff, 1999). Recently Zhao (Zhao et al., 2011) has reported that ranolazine significantly attenuated the change in blood pressure induced by either phenylephrine or isoproterenol in dogs treated with hexamethonium (an autonomic ganglionic blocking agent) but did not cause this effect in normal conscious dogs. However, the direct effect of ranolazine on vascular tone remains unknown. Therefore, in the present study, we investigate the vascular effect of ranolazine and the roles of endothelium, adrenergic receptor and Ca\(^{2+}\) ion in isolated rat intrarenal arteries.

Ranolazine is mainly used to treat patients with chronic stable angina in clinical practice. However, ranolazine does not lower significantly systemic blood pressure. The direct effect of ranolazine on vascular tone remains unknown. In the present study, we investigated the vascular effects and mechanisms of action of ranolazine in isolated rat intrarenal arteries. Rings of intrarenal arteries were mounted in a small vessel myography using two stainless steel wires for the measurement of isometric tension. L-type Ca\(^{2+}\) currents were recorded in isolated single renal arterial smooth muscle cells using patch clamp techniques in whole-cell mode. Ranolazine induced concentration-dependent relaxations in rings contracted with phenylephrine, but ranolazine failed to cause any relaxation in rings pre-contracted by U46619, 5-HT or endothelin-1. Ranolazine also induced relaxations in norepinephrine pre-contracted rings. Yohimbine failed to induce relaxation in rings pre-contracted by norepinephrine. Propranolol did not affect ranolazine-induced relaxation but the relaxant effect of ranolazine was much less than that of prazosin. Ranolazine-induced relaxations were slight but significantly attenuated by endothelial denudation. Partial inhibition was observed in endothelium-intact arteries exposed to a combination of iiberotoxin and apamin. Ranolazine at higher concentration (>30 \( \mu \)M) inhibited Ca\(^{2+}\)-induced contraction in a noncompetitive manner. Ranolazine reduced L-type Ca\(^{2+}\) currents at potentials between −30 and 50 mV in isolated renal artery myocytes. Therefore it can be said that ranolazine has significant \( \alpha_1 \)-adrenergic receptor and weak calcium channel antagonistic effects in rat intrarenal arteries.
2. Materials and methods

2.1. Vessel preparation (Leung et al., 2005)

This study was approved by the Experimental Animal Ethics Committee, Guangdong General Hospital. Sprague–Dawley rats weighing ~250–300 g were sacrificed by cervical dislocation. After the abdominal cavity was opened, the kidneys were removed and placed in ice-cold Krebs solution (in mM): 119 NaCl, 4.7 KCl, 2.5 CaCl2, 1 MgCl2, 25 NaHCO3, 1.2 KH2PO4, and 11 D-glucose. The intrarenal arteries were dissected from both kidneys, and each artery was cleaned off adhering connective tissues and cut into two ring segments, ~2 mm in length. Each segment was mounted in a Multi Myograph System (Danish Myo Technology, Aarhus, Denmark), and changes in arterial tone were recorded. Briefly, two tungsten wires (each 40 μm in diameter) were inserted through the segment’s lumen with each wire being fixed to the jaws of a myograph. The organ chamber was filled with 5 ml Krebs solution which was constantly bubbled with 95% O2–5% CO2 and maintained at 37 °C. Each ring was stretched initially to 2 mm, an optimal tension, and then allowed to stabilize at this baseline tone for 90 min before the start of each experiment. Each experiment was conducted using rings from different rats. In some arteries, the endothelium was mechanically removed by rubbing the luminal surface of the ring with small stainless steel wire. Functional removal of the endothelium was verified if the relaxant effect of acetylcholine was absent. In experiments a high-K+ solution was used and an equimolar amount of K+ replaced Na+ to retain constant ionic strength.

2.2. Force measurement protocols

To test the contractile ability, each ring was stimulated by 60 mM KCl at 30-min intervals until two consecutive and repeatable contractions were obtained. After the washout of high KCl solution, rings were contracted by 1 μM of phenylephrine. 1 μM of acetylcholine was used to test the integrity of the endothelium. Rings that produced over 60% relaxation in response to acetylcholine were considered to be endothelium-intact. Acetylcholine did not cause the relaxation in endothelium-denuded rings. Consequently, rings were rinsed with Krebs solution until baseline tone was restored or readjusted. The effect of ranolazine on renal arteries was tested.

2.2.1. Effect of ranolazine on agonist-induced contractions

Phenylephrine (α1-adrenoceptor agonist, 1 μM), endothelin-1 (endothelin-1 receptor agonist, 10 nM), U46619 (thromboxane A2 receptor agonist, 100 nM) or 5-HT (Serotonin receptor, 2 μM) were used individually to produce a sustained contraction in renal arteries. Concentration–response curves were constructed by cumulative addition of ranolazine (10 nM–100 μM) into the bathing solution without washout of the previously added drug. A time-matched vehicle (DMSO) was used as control to ensure a genuine relaxing effect of ranolazine.

2.2.2. Role of the adrenoceptor in the ranolazine-induced effect

The relaxant effect of ranolazine was evaluated in rings contracted by norepinephrine (non-selective α-adrenoceptor agonist) to observe the possible role of the adrenoceptor in the ranolazine-induced effect. The ranolazine-induced effect was determined in rings contracted by phenylephrine in the presence of propranolol (non-selective β-adrenoceptor antagonist). The concentration-dependent relaxant effects of ranolazine and prazosin (selective α1-adrenoceptor antagonist) was examined in rings contracted by 1 μM phenylephrine for comparison.

2.2.3. Role of endothelium in ranolazine-induced relaxation

To determine the role of the endothelium, the concentration-dependent relaxation caused by ranolazine was studied in both endothelium-intact and denuded arteries contracted by phenylephrine. Rings with endothelium were pre-incubated with 100 μM L-NAME (inhibitor of NO synthase) 30 min prior to addition of phenylephrine. After a sustained contraction was obtained, ranolazine was applied cumulatively.

2.2.4. Role of Ca2+ in ranolazine-induced relaxation

After 60 mM KCl was used to produce a sustained contraction, concentration–response curves were constructed by cumulative addition of ranolazine (10 nM–100 μM) into the bathing solution to determine the role of extracellular Ca2+. The ability of ranolazine to modulate Ca2+ influx was evaluated by studying concentration dependent responses to CaCl2 (0.1 to 5 mM) in the presence of ranolazine (10 to100 μM). For this set of experiments, rings were rinsed 3 times in a Ca2+-free solution containing 50 μM Na2-EGTA, then incubated in Ca2+-free, 60 mM K+ solution (with or without ranolazine, 30-min incubation) before the cumulative addition of CaCl2.

2.3. Effect of ranolazine on L-type Ca2+ current (Tsang et al., 2004)

Single smooth muscle cells were enzymatically isolated from intrarenal arteries by collagenase. L-type Ca2+ currents in isolated renal artery myocytes were recorded in the whole-cell patch configuration using patch-clamp amplifier MultiClamp 700B. Data acquisition and command voltages were controlled with a software program using a data acquisition system Digidata 1440A. Currents were recorded from holding potentials of −80 mV during 300-ms step pulses to different potentials. Analysis of whole-cell ionic currents was performed by using pClamp10.0 software. BaCl2 was used as Ca2+ channel charge carrier and K+ channels were inhibited by intracellular CsCl. The extracellular bathing solution contained (in mM): NaCl 125, BaCl2 10.8, CsCl 5.4, glucose 10, and Na-Hepes 10 (pH 7.4). The pipette solution contained (in mM): CsCl 120, MgCl2 1, Mg-ATP 3, EGTA 10, and Cs-Hepes 10 (pH 7.3). Experiments were performed at 22 °C.

2.4. Chemicals

Ranolazine, acetylcholine, 9,11-dideoxy-11a,9α-epoxy-methanoprostaglandin F2α (U46619), 5-hydroxy tryptamine (5-HT), Nω-nitro-arginine methyl ester (L-NAME), norepinephrine, ibetoxotoxin, apamin, yohimbine, propranolol and prazosin were purchased from Sigma (St. Louis, MO). U-46619 and propranolol were dissolved in DMSO and others in distilled water. Further dilution was made from a stock solution.

2.5. Data analysis

Data are represented as means ± S.E.M. Increases in contractile force were expressed as a percentage of the mean value of two consecutive responses to 60 mM K+. Cumulative concentration–response curves were analyzed by nonlinear curve fitting using the Sigmaplot 10.0 software. The negative logarithm of the dilator (or constrictor) concentration that caused half (pD2 or pEC50) of the maximal response (Emax) was obtained. For statistical analysis, a two-tailed Student’s t-test or one-way analysis of variance followed by a Newman–Keuls post test was used when more than two groups were compared. Individual concentration–response curves were also compared using a two-way analysis of variance followed by Bonferronic posttests. Statistical significance was accepted when P<0.05.

3. Results

3.1. Ranolazine-induced relaxation in rat renal artery

Phenylephrine (1 μM), U46619 (100 nM), 5-HT (2 μM) or endothelin-1 (10 nM) was used individually to produce a sustained contraction in renal arteries with endothelium. Ranolazine was able to induce concentration-dependent relaxations in rings contracted with phenylephrine (pD2: 5.89 ± 0.06, n = 8) (Fig. 1A), but ranolazine failed to
cause any relaxation in rings pre-contracted by U46619 (Fig. 1B), 5-HT (Fig. 1C) or endothelin-1 (Fig. 1D).

3.2. Role of adrenergic receptor

In norepinephrine pre-contracted rings with endothelium, ranolazine also induced concentration-dependent relaxations with pD2 of 5.99 ± 0.12 (n=5) (Fig. 2A). Selective α2-receptor antagonist yohimbine failed to induce the relaxation in rings pre-contracted by norepinephrine (Fig. 2B). Treatment with propranolol (10 μM) did not affect ranolazine-induced relaxation in phenylephrine contracted rings with endothelium (pD2: 6.10 ± 0.07, n=5 in control and 5.99 ± 0.11, n=5 in propranolol; P>0.05; Fig. 2C). The relaxant effect of ranolazine on phenylephrine precontraction was much less than that of selective α1-adrenoceptor antagonist prazosin (pD2: 5.89 ± 0.06, n=5 in ranolazine and 8.13 ± 0.17, n=5 in prazosin; P<0.05; Fig. 2D).

3.3. Role of endothelium

Ranolazine relaxed endothelium-intact or endothelium-denuded rings contracted with phenylephrine. The relaxations were slight but significantly attenuated by endothelial denudation (pD2: 5.89 ± 0.06 with endothelium and 5.56 ± 0.08 without endothelium, n=8, P<0.05, Fig. 3A). However, treatment with eNOS inhibitor L-NAME did not affect the relaxant effect of ranolazine in rings with endothelium (pD2: 5.92 ± 0.06, n=7). A slight inhibition of ranolazine-induced relaxation was observed in endothelium-intact arteries exposed to a mixture of iberiotoxin and apamin (pD2: 5.83 ± 0.05 in control and 5.50 ± 0.07 in iberiotoxin/apamin, n=6, P<0.05; Fig. 3B), suggesting a positive role of endothelium-derived hyperpolarizing factor.

3.4. Effect of ranolazine on Ca2+ -induced contraction

For the purpose of studying the role of Ca2+ influx, the effect of ranolazine was tested on contractions in membrane depolarized endothelium-denuded rings. In Ca2+-free, 60 mM of K+-containing solution, cumulative addition of CaCl2 induced contractions with a pD2 of 3.45 ± 0.10 (n=5). Ranolazine was found to inhibit CaCl2-induced contraction in a noncompetitive manner with progressive reduction of maximal contraction with increasing concentrations (10 μM to 100 μM) (Fig. 4A), which showed that ranolazine at higher concentration (>30 μM) produced remarked the relaxant effect in KCl (60 mM)-contracted rings (Fig. 4B).

3.5. Effect of ranolazine on L-type Ca2+ current

To confirm that inhibition of the high K+ response was mediated partly through inhibition of Ca2+ influx via Ca2+ channels, whole-cell L-type Ca2+ currents were recorded in isolated single smooth muscle cells (Fig. 4C) and characterized using 10.8 mM Ba2+ ions as divalent charge carriers with patch-clamp technique. The inward current was inhibited by 1 μM nifedipine. A step pulse was applied from −60 mV to +60 mV from a holding potential of −80 mV to examine the current–voltage relationship. Fig. 4D shows that ranolazine at 30 μM significantly inhibited Ca2+ currents at the potentials between −20 and +30 mV.

3.6. Inhibitory effect of nifedipine on contractions induced by activator

For comparison, the effects of L-type Ca2+ channel blocker nifedipine were tested on rings contracted by 2 μM 5-HT, 100 nM U46619, 10 nM endothelin-1, or 60 mM K+ (n=6; Fig. 5A–D). Nifedipine at 1 μM completely abolished CaCl2-induced contraction in the presence of 60 mM K+. Receptor-dependent contractions were also markedly suppressed by nifedipine. The results suggest that calcium influx through L-type calcium channels contribute to the contractions evoked by U46619, 5-HT and endothelin-1 in this artery. But their contributions to the constrictions are much smaller than that of 60 mM KCl.
The aim of this study was to investigate the vascular effect of ranolazine in isolated rat intralobar renal arteries with and without a functional endothelium. These findings are found: one, ranolazine has significant antiadrenergic effects. It produces more relaxation in arteries contracted with phenylephrine than those chemical with other receptor-dependent agonists; two, iberiotoxin- and apamin-sensitive endothelium-derived hyperpolarizing factor (EDHF) may contribute to a small part to ranolazine-induced relaxation in arteries contracted by phenylephrine; and three, a higher concentration (>30 μM), ranolazine partially relaxes 60 mM KCl-induced contractions involving inhibition of Ca²⁺ influx through L-type Ca²⁺ channels.

Ranolazine only causes relaxations in rings contracted with phenylephrine, but fails to induce any relaxation in rings pre-contracted by U46619, serotonin or endothelin-1. It also induces relaxant effects in nonselective α-adrenoceptor agonist norepinephrine pre-contracted rings with endothelium. Selective α₂-adrenoceptor antagonist yohimbine produces no relaxation in rings pre-contracted by norepinephrine. Propranolol does not affect ranolazine-induced relaxation, indicating that phenylephrine induced the contraction by α₁-adrenoceptor in renal artery rings. The relaxant effect of ranolazine was much less than that of classic α₁-adrenoceptor antagonist prazosin. Ranolazine is shown to have weak effect of α₁-adrenoceptor blocker in renal artery. Previously published results similarly indicate that ranolazine bound to both α- and β-adrenergic receptors with micromolar affinity. The Ki values for ranolazine to bind α- and β-adrenergic receptors ranged from a low of 8.2 μM for binding to α₁A receptors in membranes from rat salivary gland to a high of 19.5 μM for binding to α₁B in membranes from rat liver (Zhao et al., 2011). Furthermore, ranolazine is reported to partially block the effect of isoproterenol (ISO) to increase developed tension in rat isolated atria, and to attenuate ISO-induced increases in heart rate (HR; β₁ receptor) and decreases in blood pressure (BP; β₂ receptor) in rats. These results suggest that ranolazine has weak effects to antagonize α₁-, β₁-, and β₂-adrenergic receptors. However ranolazine

**Fig. 3.** Role of endothelium in ranolazine-induced relaxations. A, ranolazine caused endothelium dependent relaxation in phenylephrine pre-contracted rings with endothelium (open circles) and without endothelium (filled circles). 100 μM L-NAME did not affect the relaxant effect of ranolazine in rings with endothelium (open triangles). B, ranolazine induced concentration-dependent relaxations in phenylephrine pre-contracted rings with endothelium in the absence (open circles) and presence of 50 nM iberiotoxin plus 50 nM apamin (filled circles).

**Fig. 2.** Role of adrenergic receptor in ranolazine-induced relaxations. A, ranolazine induced concentration-dependent relaxations in norepinephrine pre-contracted rings. B, yohimbine produced no relaxation in rings pre-contracted by norepinephrine. C, propranolol (10 μM) failed to cause the relaxant effect of ranolazine. D, the relaxant effect of ranolazine was much less than that of α₁-adrenoceptor antagonist prazosin.
do not cause significant bradycardia or lower systemic blood pressure in patients with chronic angina in clinical practice. A reflex regulation of the cardiovascular system in response to the change in BP may potentially mask the effect of ranolazine. Recently Zhao (Zhao et al., 2011) reported that within the therapeutic concentration range of 2–6 μM (Antzelevitch et al., 2004a, 2004b), ranolazine alone did not exert a significant antiadrenergic effect in the conscious dog while after blockade of autonomic transmission with hexamethonium, a significant antiadrenergic effect of ranolazine (4–5 μM) could be demonstrated. Similar potency was found in ranolazine (1–10 μM) between antiadrenergic

![Fig. 4. Role of extracellular Ca\(^{2+}\) in ranolazine-induced relaxations. A, ranolazine inhibited CaCl\(_2\)-induced contraction in a noncompetitive manner with progressive reduction of maximal contraction with increasing concentrations (10 to 100 μM). B, ranolazine induced concentration-dependent relaxation in 60 mM K\(^{+}\)-contracted rings. C, traces from recording of Ca\(^{2+}\) channel currents (in control, in the presence of 30 μM ranolazine or 1 μM nifedipine). D, inhibition of current–voltage curve of L-type Ca\(^{2+}\) currents by ranolazine (filled circles) and nifedipine (filled triangles) in smooth muscle cells isolated from rat renal arteries.](https://example.com/fig4)

![Fig. 5. Inhibitory effect of nifedipine (1 μM) on contractions induced by 5-HT (A), U46619 (B), or endothelin-1 (C) in normal Krebs solution, and by CaCl\(_2\) in 60 mM K\(^{+}\) solution (D).](https://example.com/fig5)
effect in dogs treated with hexamethonium and relaxant effect in rings contracted with phenylephrine.

The clinical importance of the endothelial NO pathway is well accepted (Serban et al., 2010). NO protects against the development of hypertension, and selective endothelial dysfunction are believed to be an early and pathogenic event in hypertension. However, our study results show that endothelium plays a small role in ranolazine-induced relaxation as the relaxing effect is only slightly attenuated by its removal. Further experiments suggest that EDHF may be involved based on the following observations. A combined treatment with iberiotoxin plus apamin, a recipe used to inhibit EDHF-mediated response in rat arteries, significantly inhibits ranolazine-induced relaxation. By contrast, inhibition of NO production by L-NAME has no effect, thus ruling out the involvement of NO.

Voltage-sensitive Ca2+ channels are activated by depolarization in vascular smooth muscle cells when the extracellular K+ concentration rises. Our results show that ranolazine at (> 30 μM) markedly reduced the contractile responses to high K+ as well as CaCl2-induced contractions in Ca2+-free, high K+ solution, thus indirectly suggesting that ranolazine exerts a direct muscle relaxation, probably by acting as a calcium antagonist. In fact, ranolazine inhibited the L-type Ca2+ currents as recorded on single smooth muscle cells isolated from the renal arteries. Similar potency was found in ranolazine between relaxing CaCl2-induced tension and inhibition of the L-type Ca2+ currents, indicating that inhibition of Ca2+ entry via L-type Ca2+ channels contributes vascular relaxation induced by ranolazine at higher concentration. We have thus provided the first line of evidence showing direct antagonism of vascular L-type Ca2+ channels by ranolazine.

We have also observed inhibitory effect of nifedipine on contractions induced by different activators and receptor-dependent contractions were also markedly suppressed by nifedipine. Nifedipine at 1 μM completely abolished CaCl2-induced contraction in the presence of 60 mM K+. The results imply that calcium influx through L-type calcium channels contribute to the contractions evoked by U46619, 5-HT and endothelin-1 in this artery, but their contribution to the contractions was much smaller than that to 60 mM KCl. So, ranolazine at 30 μM slightly inhibits the constriction evoked by 60 mM KCl, while at less than 30 μM produce no effect on the constrictions evoked by U46619, 5-HT and endothelin-1.

In conclusion, our results show that ranolazine has significant antiadrenergic and slight Ca2+ channels antagonistic effects in rat intrarenal arteries.

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