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ORIGINAL ARTICLE

Robert Létienne · Bruno Vié · Aline Puech · Sylvie Vieu
Bruno Le Grand · Gareth W. John

Evidence that ranolazine behaves as a weak β_1- and β_2-adrenoceptor antagonist in the rat cardiovascular system

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Abstract The clinical anti-anginal effectiveness of ranolazine is currently being evaluated. However, the mechanism of its anti-ischaemic action is still unclear. The aim of this work was to establish whether ranolazine exerts functional β-adrenoceptor antagonist activity in the rat cardiovascular system.

Radioligand binding studies were performed in rat hearts and guinea-pig lungs for β_1- and β_2-adrenoceptor affinity, respectively. Ranolazine had micromolar affinity for both β_1- and β_2-adrenoceptors (pK_i5.8 and 6.3, respectively). Developed tension was measured in isolated rat left atria (electrically driven at 4 Hz) and cumulative concentration/response curves to (±)isoprenaline (0.01–1000 nM) constructed. Ranolazine (0.32–10 μM) surmountably but weakly antagonised isoprenaline-induced positive inotropic responses, with an apparent pA_2 of 5.85 (5.69–6.00) and a slope of –0.74 (–0.70 to –0.77). In bivagotomised, atropinised pithed rats, ranolazine per se evoked marked bradycardia at doses above 10 mg/kg i.v. (maximum variation at 80 mg/kg –125±15 bpm, n=6, P<0.001) by a mechanism apparently unrelated to blockade of β_1- or β_2-adrenoceptors. Cumulative incremental doses of (±)isoprenaline (0.63 ng/kg to 0.16 mg/kg i.v.) administered to pithed rats induced concomitant depressor and chronotropic responses. Animals received either vehicle (saline 0.9% i.v., n=12), atenolol (0.04–2.5 mg/kg i.v., n=6 per dose), ICI 118551 (0.01–0.63 mg/kg i.v., n=6 or 7 per dose), (±)propranolol (0.01–0.63 mg/kg i.v., n=6 per dose) or ranolazine (2.5–80 mg/kg i.v., n=6 or 7 per dose) 10 min prior to isoprenaline. Ranolazine dose-dependently and competitively antagonised isoprenaline-induced decreases in diastolic arterial pressure (DAP, dose ratio 12.2 with 80 mg/kg ranolazine) and increases in heart rate (HR, dose ratio 20.3 with 80 mg/kg ranolazine). Collectively, these results demonstrate that ranolazine behaves as a weak β_1- and β_2-adrenoceptor antagonist in the rat cardiovascular system.

Key words Ranolazine · β-Adrenoceptor antagonist · Inotropic responses · Atria · Arterial pressure · Radioligand binding

R. Létienne · B. Vié · A. Puech · S. Vieu · B. Le Grand
G. W. John (✉)
Division of Cardiovascular Diseases II,
Centre de Recherche Pierre Fabre,
17 avenue Jean Moulin, 81106 Castres Cedex, France
e-mail: gareth.john@pierre-fabre.com,
Fax: +33-563-714363

Introduction

Ranolazine has demonstrated myocardial anti-ischaemic activity in a number of in vivo (Allely and Alps 1990; Allely et al. 1993) and in vitro (Gralinski et al. 1994; McCormack et al. 1996) preparations from several different species. Initial clinical findings have suggested that ranolazine is an orally active agent potentially useful for angina pectoris therapy (Cocco et al. 1992). In patients with chronic, stable angina pectoris, the minimum effective anti-anginal dose is approximately 240 mg (Cocco et al. 1992; Thadani et al. 1994). In a double-blind study, Pepine and Wolff (1999) have employed higher ranolazine doses (267 or 400 mg tid and 400 mg bid) and suggested that there is a threshold plasma concentration which must be attained before anti-anginal activity occurs. Ranolazine is currently in phase III clinical studies in stable angina.

The mechanism by which ranolazine protects the ischaemic myocardium remains to be elucidated, although a beneficial effect upon myocardial cell metabolism has been suggested (Clarke et al. 1993, 1996; McCormack 1998; Hara et al. 1999). Using patch clamp recordings in guinea-pig ventricular myocytes, Allen and Chapman (1996) have examined the action of ranolazine on basal L-type calcium current ($I_{Ca,L}$) and on $I_{Ca,L}$ stimulated by activation of the protein kinase A cascade. Ranolazine markedly attenuated $I_{Ca,L}$, but only when facilitated by β-adrenoceptor activation, suggesting β-adrenoceptor antagonist properties of ranolazine. The objective of the present investigation was thus to evaluate whether ranolazine

exerts antagonist activity at β_1- and β_2-adrenoceptors in the rat cardiovascular system, employing both in vitro and in vivo procedures. The results of this study have been presented in part to the British Pharmacological Society (Létienne et al. 2000).

Materials and methods

General procedures for in vitro and in vivo studies were in accordance with French law and local ethical committee guidelines for animal research. Male Sprague-Dawley rats (280–500 g, OFA, Iffa-Credo, France) were housed under carefully controlled conditions (21 °C and 55% relative humidity with a 12-h light/dark cycle). Animals had free access to tap water and received standard rat chow.

Radioligand binding

The assays were performed by standard procedures at CEREP (Celle L'Evescault, France), using either rat heart membranes for β_1-adrenoceptor affinity with atenolol as reference ligand, or guinea-pig lung membranes for β_2-adrenoceptor affinity with ICI 118551 as reference ligand (Abrahamsson et al. 1988).

Freshly dissected rat heart ventricles were placed in ice-cold 50 mM TRIS-HCl buffer (pH 7.4) and finely minced and homogenised. The homogenate was centrifuged at 1,000 *g* for 10 min and the resulting pellet discarded. The supernatant was centrifuged at 100,000 *g* for 60 min. The final pellet was frozen and stored at –70 °C until use.

Freshly dissected guinea-pig lungs were placed in ice-cold 50 mM sodium phosphate buffer (pH 7.5) and minced and homogenised. The homogenate was passed twice through gauze layers and centrifuged at 50,000 *g* for 10 min. The resulting pellet was resuspended in the same buffer and centrifuged again as described above. The final pellet was frozen and stored at –70 °C until use.

Aliquots of rat heart and guinea-pig lung membrane preparations were incubated for 20 min at 22 °C in 500 µl 20 mM TRIS-HCL buffer containing 2 mM $MgCl_2$, 154 mM NaCl, 0.1 mM GTP, 0.5 nM (heart) or 0.4 nM (lung) [^{3}H](–)CGP-12177 and increasing concentrations of the competing drugs. Non-specific binding was determined in the presence of 50 µM alprenolol. After incubation, the samples were filtered rapidly under vacuum through glass fibre filters (Wallac Filtermat A) and rinsed 3 times with ice-cold buffer using a Tomtec cell harvester. Bound radioactivity was measured with a scintillation counter (Betaplate, Wallac) using a solid scintillant (MeltiLex B/HS, Wallac).

In each assay, ranolazine was tested using ten concentrations in a range of 0.01–300 µM in duplicate to obtain displacement curves. In each experiment, the respective reference compound was tested at eight concentrations in duplicate to obtain a competition curve.

Studies in rat isolated left atria

Experiments were carried out upon rats weighing 400–500 g. Animals were anaesthetised with 60 mg/kg i.p. pentobarbitone sodium (Sanofi, France) and heparinised (500 IU i.v.).

Atria were isolated as previously described (Le Grand et al. 1993). The Krebs solution comprised (mM): NaCl 119.0; KCl 5.6; $MgSO_4.7H_2O$ 1.17; $CaCl_2$. $2H_2O$ 2.1; NaH_2PO_4 1.0; $NaHCO_3$ 25.0; D-(+)glucose 10.0; pH 7.4. The bath was maintained at 34 °C and the solution bubbled continuously with 95% O_2/5% CO_2. A tension of 1 g was applied to each atrium. Atria were electrically driven at 4 Hz via two electrodes placed at each end of the atrium (Grass S 48 stimulator). The force of isometric contraction was measured with a tension transducer (GM3, Scaime, Annemasse, France) connected to an amplifier (Gould Instruments, France). The analogue signal was digitised and recorded simultaneously by means of data acquisition software (AcqKnowledge, Biopac, System 5, Goleta, Calif., USA).

Experimental protocol

After a 30-min stabilisation period, a single concentration of ranolazine (0.32, 1, 3.2, or 10 µM; *n*=8, 8, 4, 8 atria, respectively) or vehicle (distilled water; *n*=12) was added to the organ bath. Subsequently, after a 15-min equilibration period, a cumulative concentration/effect curve was constructed for (±)isoprenaline (10 pM to 1 µM). Each concentration was added when developed tension had reached a plateau.

In vivo studies

Rats (280–400 g) were anaesthetised by placing them in a diethyl ether-saturated container for approximately 30 s and then pithed according to the method of Gillespie and Muir (1967). One orbit was perforated and the brain penetrated by a steel rod which was inserted into the spinal cord. Immediately after pithing, rats were tracheotomised and mechanically ventilated (60 cycles/min; 2.5 ml/cycle; Harvard, South Natick, Mass., USA) to maintain blood gases within the physiological range. Rectal temperature was maintained at 37 °C by means of a rectal probe thermometer attached to a homeothermic blanket control unit (Harvard). Bilateral vagotomy was performed and atropine sulphate (1.25 mg/kg i.v.) administered. Catheters were inserted into the penile vein for infusing drugs and into the right carotid artery to measure arterial pressure continuously via a pressure transducer (Statham P10EZ, Viggo-Spectramed, Oxnad, Calif., USA) connected to an amplifier (Gould). Heart rate (HR) was derived from the arterial pressure signal by means of a tachometer (Biotach, Gould).

Experimental protocol

Rats were allowed at least 30 min for haemodynamic parameters to stabilise. Prazosin (0.16 mg/kg i.v.) was administered to exclude putative α_1-adrenoceptor-mediated effects. Pharmacological protocols were carried out in five groups of rats. In group 1, rats (*n*=12) received an i.v. bolus injection of vehicle (sterile saline 0.9%). In group 2, an i.v. bolus injection of atenolol (0.04, 0.16, 0.63 or 2.5 mg/kg; *n*=6 per dose) was administered to block preferentially β_1-adrenoceptors (Abrahamsson et al. 1988). In group 3, rats received an i.v. bolus injection of ICI 118551 (0.01, 0.04, 0.16 or 0.63 mg/kg; *n*=6, 6, 7 and 7, respectively), to block preferentially β_2-adrenoceptors (Bilski et al. 1983). In group 4, an i.v. bolus of (±)propranolol (0.01, 0.04, 0.16 or 0.63 mg/kg; *n*=6 per dose) was administered to block β_1- and β_2-adrenoceptors non-selectively (Bulbring and Tomita 1987). In group 5, animals received an i.v. bolus injection of ranolazine (2.5, 10, 20, 40, 60 or 80 mg/kg; *n*=6, 6, 6, 7, 6 and 6, respectively). Drugs/vehicle were administered as bolus injections over 2–3 min in a 1 ml/kg solution. However, since major haemodynamic effects were observed with the highest dose of ranolazine (80 mg/kg) this dose was infused i.v. over 5 min. After 15 min stabilisation, cumulative dose/response curves to (±)isoprenaline (0.63 ng/kg to 0.16 mg/kg i.v.) were constructed.

Drugs

Atenolol racemate (base), atropine sulphate, (±)isoprenaline hydrochloride, prazosin hydrochloride and (±)propranolol hydrochloride were purchased from the Sigma (St. Louis, Mo., USA). ICI 118551 was obtained from Tocris Cookson (Bristol, UK). Ranolazine hydrochloride was synthesised by the Department of Analytical Chemistry (Centre de Recherche Pierre Fabre, Castres, France). All drugs were dissolved either in distilled water for in vitro studies or in sterile saline (0.9%) for in vivo studies.

Data analysis

For the radioligand binding assays, specific radioligand binding is defined as the difference between total and non-specific binding determined in the presence of excess unlabelled ligand. Results are expressed as the percentage of control specific binding obtained in the presence of ranolazine. IC_{50} values and Hill coefficients (n_H) were determined by non-linear regression analysis of the competition curves. IC_{50} and n_H values were obtained by Hill equation curve fitting. IC_{50}s were converted to K_i values according to the Cheng and Prusoff (1973) equation: $K_i=IC_{50}/1+L/K_d$, where L is the concentration of the radioligand in the assay and K_d the affinity of the radioligand for the receptor) and expressed as the negative logarithm (pK_i).

Concentration/response curves were fitted using an operational sigmoid model (Origin, Microcal Software, Northampton, Mass., USA) from relative maximal effects induced by isoprenaline. From the results of this analysis, the geometric mean concentration of isoprenaline producing 50% of the maximal response (EC_{50}) was calculated with 95% confidence intervals. Relative antagonist potencies (pA_2, the negative logarithm of the concentration of an antagonist causing a two-fold, rightwards surmountable displacement of the control concentration/response curve) were estimated by Schild analysis (Schild 1947). For the in vivo studies, dose/response curves were fitted by the same method as that employed for the in vitro studies and the ED_{50} for isoprenaline calculated with 95% confidence limits. The isoprenaline dose ratio was subsequently calculated to evaluate antagonism of isoprenaline-induced diastolic hypotensive responses and tachycardia. The isoprenaline dose ratio was defined as the quotient of the isoprenaline ED_{50} in presence and absence of antagonist (Arunlakshana and Schild 1959).

All values were expressed as means±SEM. One factor analysis of variance (ANOVA) with or without repeated measurements was used to assess significance among and between groups respectively, followed by Dunnett's test. $P<0.05$ was considered significant.

Results

Affinity of ranolazine for β_1- and β_2-adrenoceptors

The IC_{50}, n_H and K_i values and 95% confidence limits for ranolazine and reference compounds at β_1- and β_2-adrenoceptors are presented in Table 1. Ranolazine did not differentiate between β_1- and β_2-adrenoceptors, possessing almost equivalent affinity at these sites (pK_i at β_1- and β_2-adrenoceptors 5.8 and 6.3, respectively). However, contrary to that for β_1-adrenoceptors, the slope of the inhibition curve for β_2-adrenoceptors was slightly lower than unity. The pK_i for ranolazine at β_2-adrenoceptors should therefore be interpreted with caution (Table 1). ICI 118551 had nanomolar affinity for β_2-adrenoceptors, whereas atenolol showed micromolar affinity for β_1-adrenoceptors. Thus, ranolazine had an affinity for β_1-adrenoceptors in a similar range of concentrations to atenolol.

Effects of ranolazine on β-adrenoceptor-mediated positive inotropic responses

In isolated rat left atria, isoprenaline elicited concentration-dependent positive inotropic responses (Fig. 1A). Maximum developed tension increased significantly from baseline (1.74±0.12 vs. 0.93±0.06 g, $n=12$, $P<0.001$). Under these conditions, the EC_{50} (95% confidence limits) for isoprenaline was 3.2 (2.1–5.1) nM. Figure 1A shows that ranolazine antagonised the inotropic responses induced by (±)isoprenaline concentration dependently without depressing the maximum response. The isoprenaline EC_{50} was 18.9 (15.3–23.3) nM at 10 μM ranolazine. Figure 1B represents the relative potency of ranolazine as estimated by Schild analysis. Two distinct slopes were not discernible and an apparent pA_2 of 5.85 (5.69–6.00) was determined with a slope of –0.74 (–0.70 to –0.77), being less than unity.

Haemodynamic effects of ranolazine in pithed rats

In pithed rats, diastolic arterial pressure (DAP) and HR values did not significantly differ between groups of rats under baseline conditions. Average baseline values of DAP and HR were 49.6±0.6 mmHg and 351±2 bpm, respectively, for all animals ($n=123$).

In pithed rats, ranolazine exerted significant haemodynamic effects. Indeed, Table 2 shows that ranolazine induced marked, significant bradycardia at doses of 10 mg/kg and higher (maximum variation at 80 mg/kg –125±15 bpm,

Table 1 IC_{50}, Hill coefficient (n_H) and K_i values for ranolazine, ICI 118.551 and atenolol at β_1- and β_2-adrenoceptors. Values in *parentheses* are 95% confidence limits. Ranolazine was tested in each assay at ten concentrations (range 0.01–300 μM) in duplicate to obtain competition curves. In each experiment, the respective reference compound was tested at eight concentrations (atenolol 0.01–100 μM, ICI 118551 0.0001–3 μM) in duplicate to obtain a competition curve in order to validate the procedure (*n.d.* not determined)

Compounds	β_1-receptors			β_2-receptors		
	IC_{50} (nM)	K_i (nM)	n_H	IC_{50} (nM)	K_i (nM)	n_H
atenolol	2,110 (1,342–3,366)	603	0.8 (0.6–1.1)	13,900 n.d.	4,300	0.9 n.d.
ICI 118551	205 n.d.	n.d.	0.6 n.d.	3.6 (2.6–4.9)	n.d.	0.6 (0.5–0.8)
ranolazine	5,010 (3,997–6,306)	1,430	1.1 (0.9–1.4)	1,560 (974–2,469)	485	0.7 (0.5–1.0)

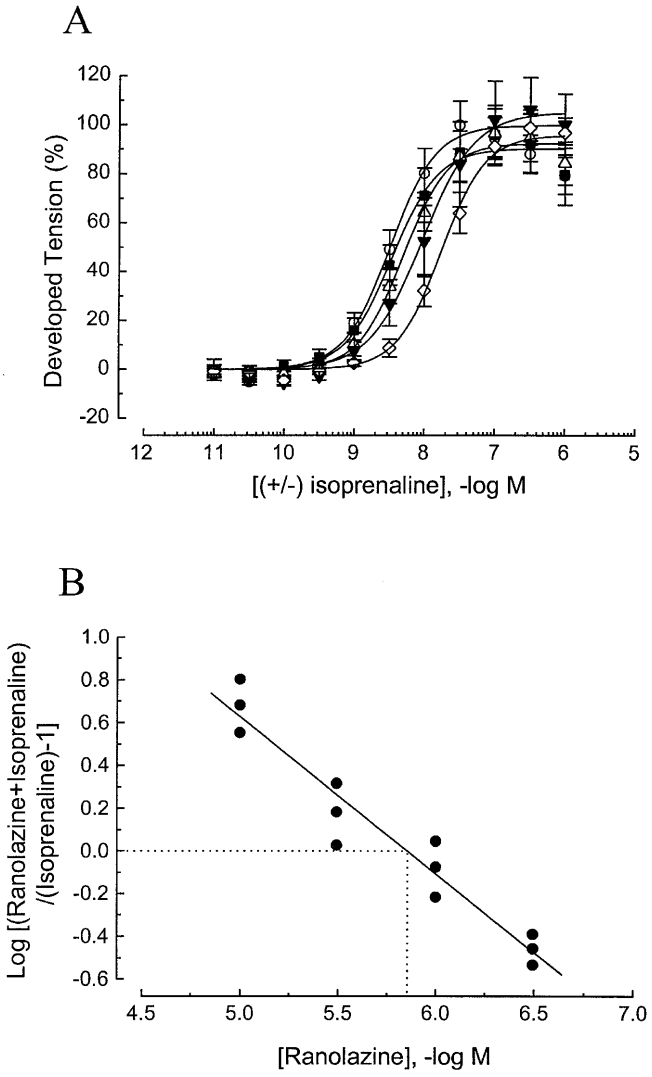

Fig. 1 **A** (±)Isoprenaline-evoked increases in developed tension in absence (*open circles*, *n*=12) or presence of ranolazine at concentrations of 0.32 μM (*filled squares*, *n*=8), 1 μM (*open triangles*, *n*=8), 3.2 μM (*filled inverted triangles*, *n*=4) and 10 μM (*open diamonds*, *n*=8). Values are the percentage of maximal responses induced by (±)isoprenaline in presence of its vehicle (distilled water) means±SEM. **B** Corresponding Schild plot for ranolazine

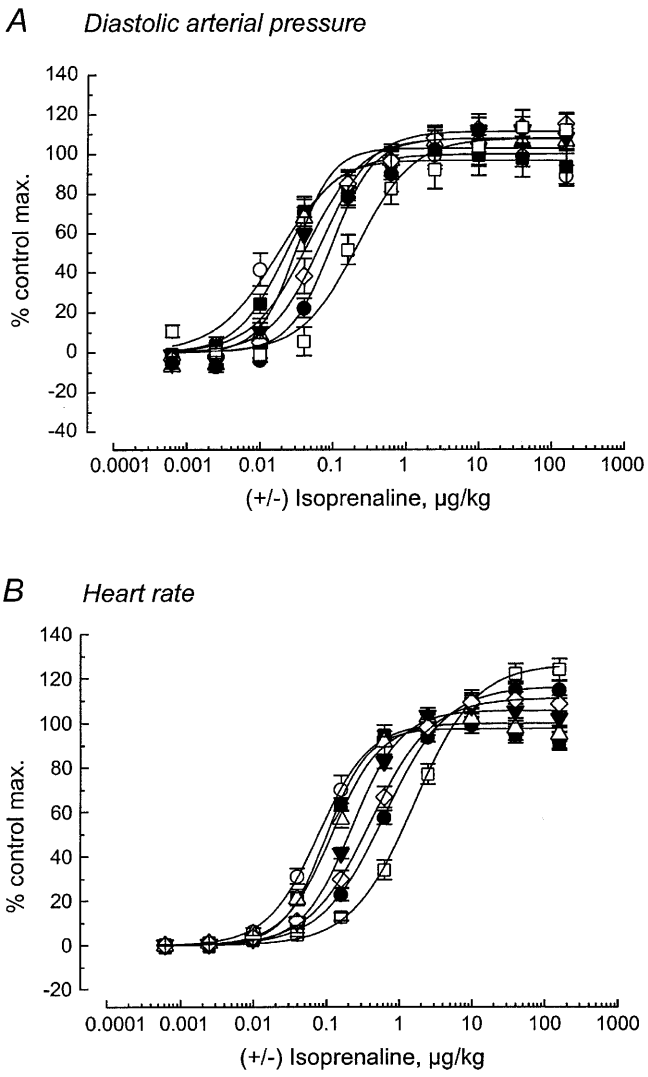

Fig. 2A, B Cumulative dose/response curves to (±)isoprenaline in the absence (*open circles*, *n*=12) or presence of ranolazine at i.v. doses of 2.5 mg/kg, (*filled squares*, *n*=6), 10 mg/kg, (*open triangles*, *n*=6), 20 mg/kg, (*filled inverted triangles*, *n*=6), 40 mg/kg, (*open diamonds*, *n*=6), 60 mg/kg, (*filled circles*, *n*=6) and 80 mg/kg (*open squares*). **A** Maximal decrease in diastolic arterial pressure. **B** Maximal increase in HR induced by (±)isoprenaline in the presence of vehicle (sterile saline 0.9%). Vehicle or ranolazine were administered 15 min. prior to (±)isoprenaline. Ranolazine (80 mg/kg) was infused i.v. over 5 min

Table 2 Haemodynamic effects of i.v. ranolazine in the pithed rat. Data are the maximum changes (Δ) in diastolic arterial pressure (*DAP*) and heart rate (*HR*) following ranolazine administration and prior to (±)isoprenaline challenge (*n* number of animals)

Treatment	Dose (mg/kg)	*n*	ΔDAP (mmHg)	Δ HR (bpm)
vehicle (saline 0.9%)		12	1.2±0.6	0±1
ranolazine	2.5	6	2.0±1.4	–12±2
	10	6	3.4±1.8	–38±3***
	20	6	11.1±1.0	–68±4***
	40	7	16.8±4.4 **	–96±6***
	60	6	19.3±8.8**	–119±9***
	80	6	11.9±7.9	–125±15***

**P<0.01; ***P<0.001 vs. vehicle

n=6, P<0.001, Table 2). The bradycardia was marked and sustained. Indeed, 15 min after ranolazine administration, HR remained significantly lower (321±6 vs. 364±9 bpm, *n*=6, P<0.01 at 60 mg/kg and 300±8 vs. 365±10 bpm, *n*=6, P<0.001 at 80 mg/kg ranolazine, respectively). On the other hand, transient but significant increases in DAP occurred from 40 mg/kg (maximum variation at 60 mg/kg 19.3±8.8 mmHg, *n*=6, P<0.01, Table 2).

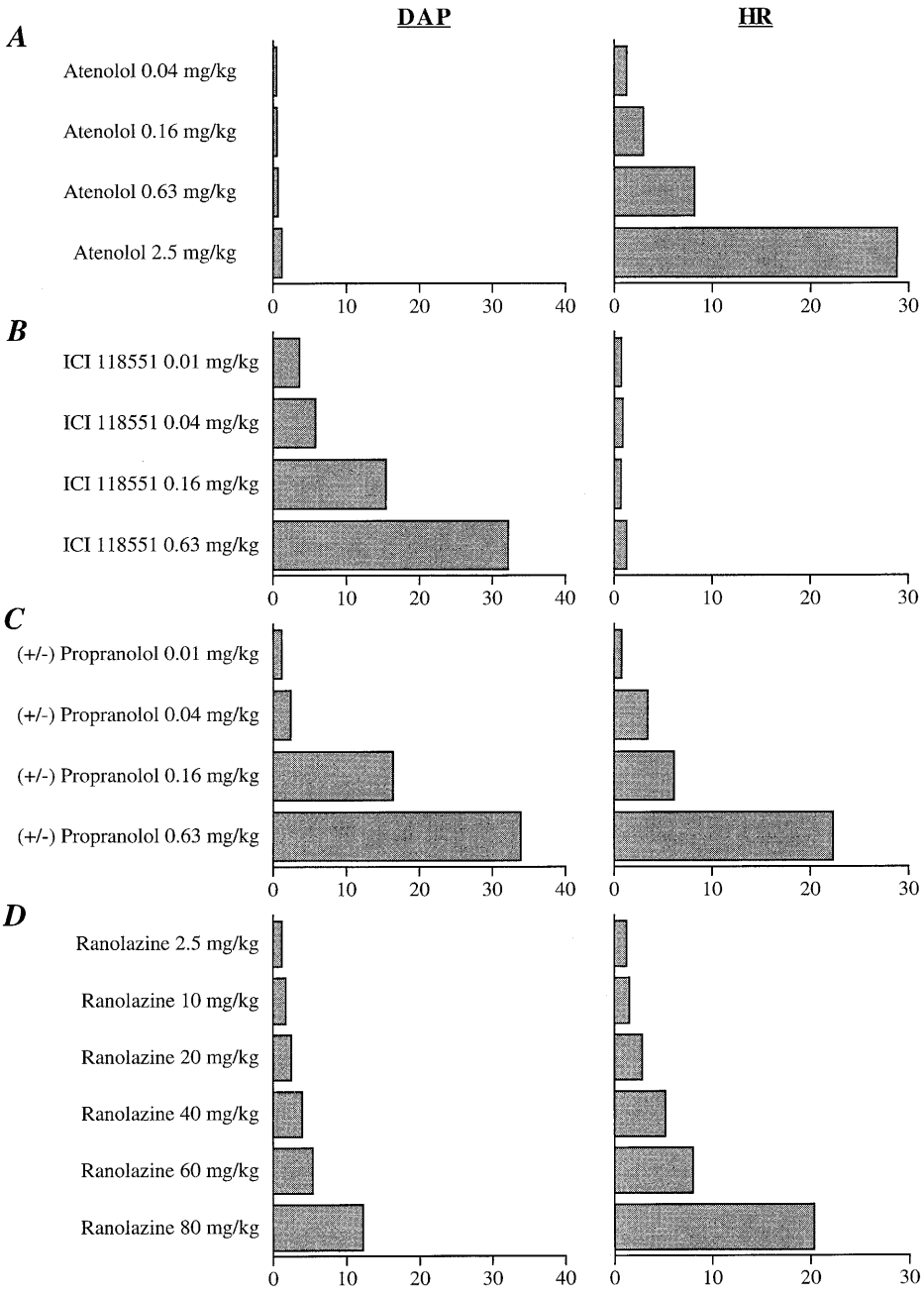

Fig. 3 Comparison of (±)isoprenaline dose ratios in presence of **A** atenolol (0.04–2.5 mg/kg i.v.), **B** ICI 118551 (0.01–0.63 mg/kg i.v.), **C** (±)propranolol (0.01–0.63 mg/kg i.v.) or **D** ranolazine (2.5–80 mg/kg i.v.) calculated for diastolic arterial pressure (*left-hand panels*) and heart rate (*right-hand panels*). Dose ratios were calculated for (±)isoprenaline as indicated in Materials and methods

Involvement of β-adrenoceptor subtypes in isoprenaline-induced depressor and chronotropic responses in pithed rats

In pithed rats, isoprenaline decreased DAP (maximum decrease 46.2±1.5%, n=12, P<0.001) and increased HR (maximum increase 54.1±2.9%, n=12, P<0.001). Under these conditions, the mean ED_{50}s for isoprenaline for the decrease of DAP and the increase of HR were 0.018 (0.012–0.027) and 0.079 (0.065–0.096) μg/kg, respectively.

In the presence of atenolol (0.04–2.5 mg/kg), a relatively selective β_1-adrenoceptor antagonist (Abrahamsson et al. 1988), the hypotensive response induced by isoprenaline was not significantly affected, but the chronotropic responses were surmountably antagonised (dose ratios 1.2 and 28.8 for DAP and HR respectively, with 2.5 mg/kg atenolol, Fig. 3A).

ICI 118551, a relatively selective β_2-adrenoceptor antagonist (Bilski et al. 1983), had no significant effect on the chronotropic responses induced by (±)isoprenaline (dose ratio 1.3 with 0.63 mg/kg ICI 118551) but shifted the hypotensive dose/response curve to the right (dose ratio 32.2, Fig. 3B).

The hypotensive and chronotropic dose/response curves produced by (±)isoprenaline were shifted markedly to the right in the presence of propranolol, a non-selective β-adrenoceptor antagonist (Bulbring and Tomita 1987, dose

ratios 33.9 and 22.3, respectively with 0.63 mg/kg (±)propranolol, Fig. 3C).

Effects of ranolazine on β-adrenoceptor-mediated depressor and chronotropic responses in pithed rats

Figure 2A shows that ranolazine dose-dependently displaced the dose/response curve for isoprenaline-induced decreases in DAP to the right (dose ratio 12.2 with 80 mg/kg ranolazine, Fig. 3D), suggesting that the compound behaves as a competitive antagonist. Similarly (Fig. 2B), ranolazine dose-dependently shifted the dose/response curve for isoprenaline-induced increases in HR to the right (dose ratio 20.3 with ranolazine at 80 mg/kg, Fig. 3D), also suggesting competitive antagonism. Figures 2 and 3 show that ranolazine produced correspondingly greater shifts to the right of the dose/response curves for isoprenaline-induced chronotropic responses than the isoprenaline-induced hypotensive responses.

Discussion

The aim of the present work was to determine whether ranolazine exerts antagonist activity at β-adrenoceptors. Weak (micromolar) binding affinity was observed for ranolazine at both β_1- and β_2-adrenoceptors. In isolated rat atria, ranolazine antagonised isoprenaline-induced positive inotropic responses weakly. Furthermore, in the pithed rat, ranolazine dose-dependently antagonised isoprenaline-induced decreases in DAP and increases in HR, in a competitive manner. Collectively, the data demonstrate that ranolazine behaves as a weak β_1- and β_2-adrenoceptor antagonist.

Radioligand binding affinity of ranolazine for β_1- and β_2-adrenoceptors

Weak radioligand binding affinity was observed for ranolazine at both β_1- and β_2-adrenoceptors, with a slightly higher apparent affinity for β_2-adrenoceptors. In the present study, atenolol did not display higher affinity for β_1-adrenoceptors than ranolazine. This relatively weak β_1-adrenoceptor affinity of atenolol is similar to that seen in other studies (Abrahamsson et al. 1988; Minneman et al. 1979a). In contrast to ranolazine, atenolol is a relatively selective β_1-adrenoceptor antagonist, its β_2-adrenoceptor affinity being about 20-fold lower (Minneman et al. 1979a). ICI 118551 displays subnanomolar affinity for β_2- and submicromolar affinity for β_1-adrenoceptors, as described by Golf et al. (1985) and is thus considered as a relatively selective β_2-adrenoceptor antagonist (Bilski et al. 1983). Since the pK_i for ranolazine at β_2-adrenoceptors was associated with a slope of less than unity, the affinity of ranolazine for β_2-adrenoceptors should be regarded with caution. Consequently, the radioligand binding data do not provide a precise notion of relative affinities of ranolazine at β_1- and β_2-adrenoceptors.

Effects of ranolazine on β-adrenoceptor-mediated positive inotropic responses

The concentration-dependent positive inotropic effects induced by isoprenaline in rat isolated left atria are similar to those found in other studies (Marquez et al. 1981; Ruch et al. 1992). Although radioligand binding and functional studies indicates the coexistence of β_1- and β_2-adrenoceptors in the hearts of various mammals (Brodde 1988; Saito et al. 1989), rat left atria contain relatively more β_1- than β_2-adrenoceptors (Minneman et al. 1979b). Juberg et al. (1985) have shown that β_2-adrenoceptors are unlikely to be coupled to positive inotropic responses in left atria of the rat heart. Therefore, in this model, isoprenaline-induced inotropic responses are mediated mainly by β_1-adrenoceptors. The relative potency of ranolazine estimated by Schild analysis gave an apparent pA_2 of 5.85 and slope of 0.74. However, the displacement to the right of isoprenaline concentration/response curves by ranolazine was weak and the slope of the Schild plot is lower than unity, rendering comparison difficult with binding affinities of the drug at β_1- or β_2-adrenoceptors. Furthermore, in isolated rat left atria, atenolol (0.1–10 μM) competitively antagonised isoprenaline-induced positive inotropic responses, with a pA_2 of 6.99 (data not shown), this value being in close agreement to radioligand binding affinity values determined for atenolol at β_1-adrenoceptors (pK_i 6.2). Thus weak antagonism by ranolazine of isoprenaline-induced inotropic responses may involve both β_1- and β_2-adrenoceptor subtypes.

Haemodynamic effects of ranolazine in pithed rats

In the pithed rat, a relatively low dose (10 mg/kg) of ranolazine induced marked and prolonged bradycardia. β-Adrenoceptor antagonist activity of ranolazine, even at high doses, cannot explain the bradycardia observed in the pithed rat. Propranolol (0.63 mg/kg) did not induce significant bradycardia. In spontaneously beating, isolated rat right atria, ranolazine (up to 10 μM), failed to decrease HR significantly (data not shown). Clearly therefore, ranolazine-induced bradycardia does not appear to be mediated by antagonist activity at β_1- or β_2-adrenoceptors and a direct inhibitory action upon sino-atrial node function also appears unlikely. Further studies are warranted to elucidate the mechanism of ranolazine-induced bradycardia in the pithed rat.

Effects of ranolazine on β-adrenoceptor-mediated depressor and chronotropic responses in pithed rats

The weak antagonist activity of ranolazine at β-adrenoceptors, observed in our in vitro studies, was confirmed subsequently in vivo. In pithed rats, ranolazine dose-dependently antagonised isoprenaline-induced decreases in DAP and increases in HR. The dose/response curves obtained with isoprenaline are comparable to those found in

other studies (Hicks et al. 1987). The relatively selective β_1-adrenoceptor antagonist, atenolol, preferentially antagonised the chronotropic responses induced by isoprenaline, whereas ICI 118551 selectively antagonised the depressor responses. Propranolol, a non-selective β_1- and β_2-adrenoceptor antagonist increased isoprenaline dose ratios for both DAP and HR. It is interesting to note that the pattern of ranolazine-induced anti-isoprenaline actions seems to be half-way between atenolol and propranolol.

Involvement of β-adrenoceptor subtypes in antagonist activity of ranolazine

Collectively, radioligand binding data suggest both β_1- and β_2-adrenoceptor affinities for ranolazine. This is corroborated by the antagonism of isoprenaline-induced positive inotropic responses in isolated rat left atria, although the latter does not define clearly the β-adrenoceptor subtype(s) involved. In the pithed rat preparation, ranolazine exerted β-adrenoceptor antagonist activity at both β_1- and β_2-subtypes; this appeared to be more pronounced for β_1- over β_2-adrenoceptors. In the light of these observations, it appears that ranolazine behaves as a weak β_1- and β_2-adrenoceptor antagonist, with a propensity towards more β_1-adrenoceptor antagonist activity in vivo. However, the possible involvement of other β-adrenoceptor subtypes cannot be excluded. Recent evidence indicates the existence of putative β_4-adrenoceptors based on cardiostimulant effects of isoprenaline (Kaumann 1997). In the rat atrium, the putative β_4-adrenoceptor may be stimulated by (–)CGP 12177 (Sarsero et al. 1999). Thus, in the present study possible antagonist activity of ranolazine at β_4-adrenoceptors cannot be excluded. Interaction of ranolazine with β_3-adrenoceptors is unlikely in the rat heart, in which β_3-adrenoceptors generally are not detected (Steinberg 1999) although such a possibility cannot be excluded in man.

Anti-ischaemic efficacy of ranolazine in vivo

Although several studies have shown ranolazine to be an effective anti-ischaemic agent (Allely et al. 1993; McCormack et al. 1996), other studies have not confirmed this (Black et al. 1994; Cocco et al. 1992; Thadani et al. 1994). A recent study by Pepine and Wolff, (1999) has clarified contradictory findings from earlier studies, the negative results of which are probably due to the use of low doses. Pepine and Wolff (1999) have suggested that higher ranolazine plasma concentrations may be associated with an anti-anginal effect, and that there is a threshold ranolazine plasma concentration required for anti-anginal activity. Moreover, in the currently ongoing phase III clinical trials, angina patients are administered ranolazine at doses of 500, 1000 and 1500 mg bid.

Possible relationship between β-adrenoceptor antagonist activity of ranolazine and the mechanism of its anti-anginal action

The precise molecular mechanism of the anti-anginal action of ranolazine has not yet been identified clearly. It has been suggested that ranolazine shifts ATP production away from fatty acid oxidation towards glucose oxidation during cardiac ischaemia and/or hypoxia (McCormack 1998; Hara et al. 1999). During ischaemia, this switch in metabolic substrate seems to be associated with an indirect stimulation of pyruvate dehydrogenase activity, probably by inhibiting β-oxidation of fatty acids (Clarke et al. 1993, 1996). Interestingly, Schiffelers et al. (1999) have shown that lipolysis is inhibited by atenolol in man. Thus, ranolazine might be expected to inhibit fatty acid oxidation and consequently activate pyruvate dehydrogenase by means of its β-adrenoceptor antagonist activity. In addition, ranolazine attenuates the accumulation of lactate and H^+ in the myocardium during ischaemic episodes, seemingly through activation of pyruvate dehydrogenase (McCormack et al. 1996; Hara et al. 1999). Thus, ranolazine, via its β-adrenoceptor antagonist activity, might reduce cardiac work and preserve tissue ATP levels. Thus, we suggest that the β-adrenoceptor antagonist activity of ranolazine is consistent with inhibition of myocardial fatty acid β-oxidation and that this might contribute, at least partly, to its anti-anginal efficacy. Further studies are required to resolve this issue.

In conclusion, ranolazine has micromolar binding affinity for β_1- and β_2-adrenoceptors. In isolated left rat atria, ranolazine exerts β-adrenoceptor antagonist activity and in the pithed rat preparation, ranolazine exerts both β_1- and β_2-adrenoceptor antagonist activity, with a propensity towards β_1-adrenoceptors. Collectively, these results demonstrate that ranolazine behaves as a weak β_1- and β_2-adrenoceptor antagonist in the cardiovascular system of the rat.

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