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ARTICLE

Celiprolol induces β_3-adrenoceptors-dependent relaxation in isolated porcine coronary arteries

Mohammed Amine Abdelkrim, Lionel Martignat, Marc Gogny, Jean-Claude Desfontis, Jacques Noireaud, and Mohamed Yassine Mallem

Abstract: In porcine coronary arteries (PCAs), celiprolol, a selective β_1-adrenoceptors antagonist, induces vasodilatation by an endothelium- and nitric oxide (NO)-dependent pathway. However, the mechanisms of that vascular effect have not been precisely established. β_3-Adrenoceptors have been shown to be involved in the relaxation per se of various vascular beds, including coronary vessels. Thus, we evaluated (*i*) the presence of β_3-adrenoceptors in the PCA and (*ii*) their role in celiprolol-induced vasodilatation. PCA rings were placed in organ baths and preconstricted with KCl. All experiments were performed in the presence of nadolol (a β_1/β_2-adrenoceptor antagonist). Cumulative concentration–response curves to SR 58611A and ICI 215001 (2 β_3-adrenoceptor agonists) and to celiprolol were constructed. We also used semiquantitative reverse transcription – polymerase chain reaction, which clearly showed the presence of β_3-adrenoceptor transcripts. SR 58611A, ICI 215001, and celiprolol induced concentration-dependent relaxations in PCA rings. SR 58611A-induced relaxation was almost abolished after removal of endothelium or pretreatment with L-NAME (a NO synthase inhibitor). The vasorelaxations induced by SR 58611A and celiprolol were inhibited in the presence of SR 59230A and L-748337 (2 selective β_3-adrenoceptor antagonists). We showed (*i*) that PCAs possess functional β_3-adrenoceptors mediating endothelium- and NO-dependent relaxation, and (*ii*) that celiprolol exerts a β_3-adrenoceptor agonistic activity in this vascular bed.

Key words: celiprolol, β_3-adrenoceptors, vasorelaxation, endothelium, nitric oxide, porcine coronary arteries.

Résumé : Dans l'artère coronaire porcine (ACP), le céliprolol, un antagoniste sélectif des récepteurs adrénergiques (RAs) β_1, induit une vasodilatation impliquant une voie de signalisation endothéliale et dépendante du monoxyde d'azote (NO). Les mécanismes de cet effet vasculaire ne sont pas précisément établis. Les RAs β_3 sont impliqués dans la relaxation per se de différents lits vasculaires incluant les vaisseaux coronariens. Nous avons donc évalué (*i*) la présence de RAs β_3 dans l'ACP et (*ii*) leur rôle dans la vasodilatation induite par le céliprolol. Des anneaux d'ACP ont été placés dans des cuves à organes isolés, précontractés avec du KCl et en présence de nadolol (antagoniste des RAs β_1/β_2). Des courbes cumulatives concentrations réponses au SR 58611A, au ICI 215001 (agonistes du RA β_3) et au céliprolol ont été construites. La méthode de réaction en chaîne par polymérisation de transcription inverse a montré la présence de transcrits du RA β_3. Le SR 58611A, l'ICI 215001 et le céliprolol induisent une relaxation concentration-dépendante dans les anneaux d'ACP. La relaxation induite par le SR 58611A est presque totalement abolie dans les anneaux désendothélialisés ou après prétraitement avec du L-NAME (inhibiteur de la NO synthase). Les vasorelaxations induites par le SR 58611A et le céliprolol sont inhibées en présence de SR 59230A et de L-748337 (antagonistes du RA β_3). Ainsi, les ACPs possèdent des RAs β_3, induisant une relaxation endothéliale NO dépendante et le céliprolol y exerce un effet agoniste via les RAs β_3.

Mots-clés : céliprolol, récepteurs adrénergiques β_3, vasorelaxation, endothélium, monoxyde d'azote, artères coronaries porcines.

Introduction

Celiprolol, a third generation β-adrenoceptor blocker, is a selective β_1-adrenoceptor blocker with ancillary properties that include partial β_2-adrenoceptor agonism and the ability to relax vascular smooth muscle directly (Toda 2003). The vasodilatory property of celiprolol involving its ability to interact with β_2-adrenoceptor has been described in human, rat, and guinea-pig blood vessels (Dhein et al. 1992; Kakoki et al. 1999; Sauvaget et al. 2010). However, many experimental works have shown that celiprolol-induced vasodilatation is ascribable to nitric oxide (NO) release from endothelium aside from that mediated through β_2-adrenoceptor stimulation. Thus, in rat thoracic aorta, celiprolol causes vasorelaxation also through 5-hydroxytryptamine (5-HT) receptor activation (Kakoki et al. 1999). In the arterioles of the rat cremaster muscle, propranolol only partially attenuates the celiprolol-mediated relaxation (Perrone and Barrett 1991). Furthermore, in porcine and dog coronary arteries, celiprolol produces a vasodilatation by a mechanism that is insensitive to β-adrenoceptor blockade (Noda et al. 2001; Asanuma et al. 2003).

β_3-Adrenoceptors have been shown to be involved in the relaxation per se of various vascular beds (Trochu et al. 1999; Mallem et al. 2004). Furthermore, vasodilatation induced by nebivolol, another third generation β-adrenoceptor blocker, involves β_3-adrenoceptors (for references see Rozec et al. 2006). However, no study has specifically explored the participation of β_3-adrenoceptors in the vasodilatory effect of celiprolol. Only Noda et al. (2001) addressed that possibility but failed to find any evidence that β_3-adrenoceptors were involved in the celiprolol-induced effect using cyanopindolol, a non-

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specific β_3-adrenoceptor antagonist in porcine coronary arteries (PCAs). Hence, the aim of the present study was to investigate whether PCAs possess functional β_3-adrenoceptors and to evaluate whether celiprolol-induced vasorelaxation involves activation of β_3-adrenoceptors in this vascular bed.

Materials and methods

Biological materials

Porcine hearts were collected at a local abattoir (Montfort sur Meu, France). From the freshly excised heart, the left anterior descending coronary arteries were isolated and placed in ice-cold Tyrode solution of the following composition (mmol/L): NaCl, 137; KCl, 5.4; $CaCl_2$, 2.5; Na_2HPO_4, 1.2; $MgCl_2$, 1.2; HEPES, 20; and D-glucose, 15 (pH adjusted to 7.4 with 5 mol/L NaOH). After collection, PCAs were maintained in ice-cold Tyrode solution for transport to the laboratory (45 min).

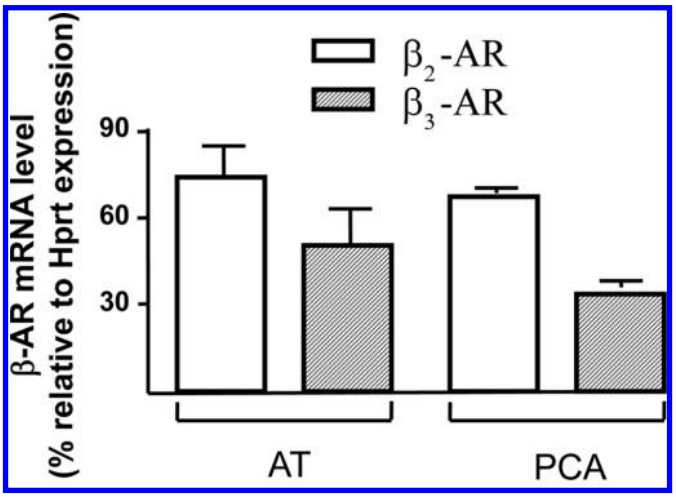
Semiquantitative reverse transcription – polymerase chain reaction (RT-Q-PCR) for mRNA amplification

Total RNAs were extracted from porcine adipose tissues (taken as a positive control for β_2- and β_3-adrenoceptor expressions) and PCAs rings, using the NucleoSpin RNA II kit (Macherey Nagel EURL, Hoerd, France). First-strand cDNA was synthesized, 1 h at 37 °C, using M-MLV reverse transcriptase (Promega, Charbonnières, France) and random hexamer primers (Promega). RT-Q-PCR was performed on 2 μL of cDNA, with 5× Hot Firepol EvaGreen qPCR Mix (Solis BioDyne, Tartu, Estonia), on an ABI Prism 7300 real-time sequence detection system (Applied Biosystems, Courtaboeuf, France). Cycling parameters were initiation at 50 °C for 2 min and denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation 95 °C for 20 s and annealing at 68 °C for 30 s. The following pig-specific primer pairs were used: hypoxanthine-guanine phosphoribosyltransferase (forward 5′-TGGTCAAGCAGCATAATCCAAAGA-3′, reverse 5′-AGTCAAGGGCATAGCCTACCACAA-3′), β_2-adrenoceptor (ENSSSCT00000015773, forward 5′-CCCGATGATAGCACTGATTCACAG-3′, reverse 5′-GGAAAGGGGCGCTTAGAAAGTAGA-3′), and β_3-adrenoceptor (ENSSSCT00000017229, forward 5′-GCTCATCATGGGAACCTTCACTCT-3′, reverse 5′-CTAGCCAGTTAAGGGCGAGGAAAG-3′). All experiments were normalized with a unique reference sample. Relative RT-PCR data were processed according to a standard method generating reference hypoxanthine-guanine phosphoribosyltransferase gene normalized expression values.

Contraction–relaxation studies

In the laboratory, arteries were cleaned of fat and connective tissues and cut into rings (3–4 mm long). In some experiments, the rings were denuded of endothelium by gently rubbing the intimal surface with a pair of small, fine forceps. Rings were suspended on stainless-steel wires in a 5 mL organ bath containing Krebs solution of the following composition (mmol/L): NaCl, 118.3; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 20; EDTA, 0.016; D-glucose, 11.1; and $CaCl_2$, 2.5. The temperature of the bath was maintained at 37 ± 0.5 °C, and the Krebs solution was continuously oxygenated with a 95% O_2, 5% CO_2 gas mixture. PCA rings were progressively stretched to a resting tension of 2 g. Isometric tension was recorded using a force displacement transducer (EMKA Technologies, Paris, France) and displayed on a computer (Acqknowledge version 4.1, MP150 BIOPAC system, Cerom, France). After a 1 h equilibration period, with the Krebs solution being changed every 15 min, the rings were contracted with 80 mmol/L KCl. Thereafter, the rings were partially contracted with KCl (20–30 mmol/L, concentration adjusted to produce a similar tone level of 50% of the maximal response), and the presence of a functional endothelium was evaluated by the response (≥60% relaxation) to a single concentration of bradykinin (1 μmol/L). In endothelium-denuded rings, endothelium removal was confirmed by the absence of bradykinin-induced relaxation.

Fig. 1. Semiquantitative reverse transcription – polymerase chain reaction of β_2- and β_3-adrenoceptor (β_2-AR and β_3-AR) mRNA expression in porcine coronary artery (PCA) rings and adipose tissue (AT). β-Adrenoceptor mRNA levels were expressed as % relative to hypoxanthine-guanine phosphoribosyltransferase (Hprt) expression.

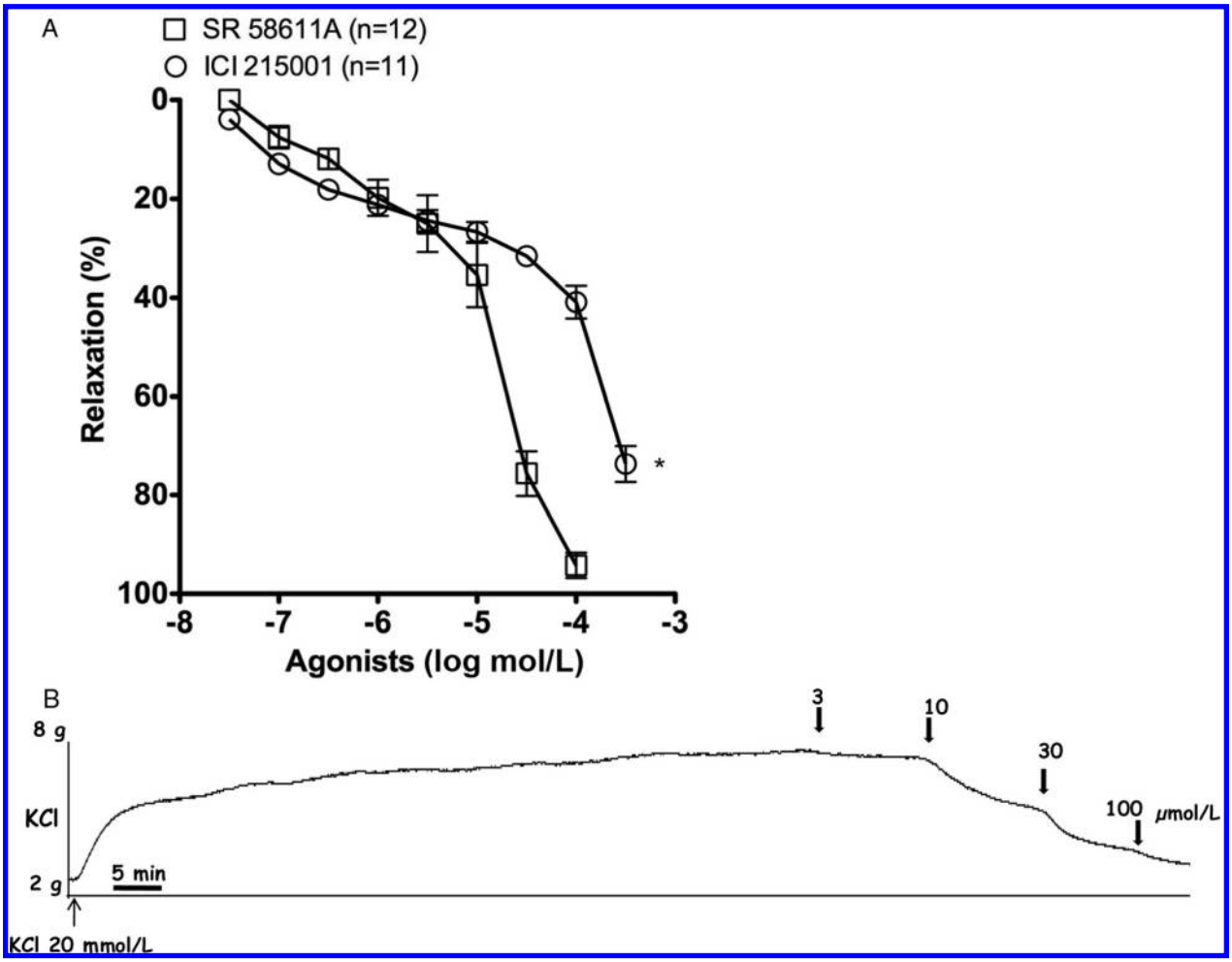


All functional studies presently reported were carried out using rings pre-equilibrated for 30 min in Krebs solution containing nadolol (a β_1- and β_2-adrenoceptor antagonist, 10 μmol/L). After equilibrating for 30 min, rings were contracted again with KCl (20–30 mmol/L, see above). Once the contraction reached a plateau, cumulative concentration–response curves (CCRCs) to SR 58611A (0.03–100 μmol/L) and ICI 215001 (0.03–300 μmol/L) (2 β_3-adrenoceptor agonists) and to celiprolol (0.1–300 μmol/L) were then constructed. The relaxation produced by each concentration of the drugs was measured after a steady state was reached. Values were expressed as the percentage change in the maximal tension of rings after addition of KCl. Some rings were equilibrated for 30 min in Krebs solution containing L-748337 (3 and 10 μmol/L) or SR 59230A (10 μmol/L) (2 β_3-adrenoceptor antagonists) or L-NAME (a nonselective NO synthase inhibitor, 100 μmol/L) before plotting CCRCs to SR 58611A or celiprolol. It should be noted that since celiprolol was previously reported to induce endothelium- and NO-dependent relaxation in PCAs (Noda et al. 2001), the effects of endothelium removal and NO synthase inhibition upon the celiprolol-induced response have not been determined in the present work.

Drugs

Nadolol, SR 59230A (3-(2-ethylphenoxy)-1-[[(1*S*)-1,2,3,4-tetrahydronaphthalen-1-yl]amino]-(2*S*)-2-propanol oxalate salt) and L-NAME (*N*ω-nitro-L-arginine methyl ester hydrochloride) were obtained from Sigma-Aldrich (France). SR 58611A ((*RS*)-*N*-[(2*S*)-7-ethoxycarbonylmethoxy-1,2,3,4-tetrahydronaphtalen-2-yl]-(2*R*)-2 (3-chlorophenyl)-2 hydroethanamine hydrochloride) was a generous gift from Sanofi Synthelabo Recherche (France). L-748337 (*N*-[[3-[(2*S*)-2-hydroxy-3-[[2-[4-[(phenylsulfonyl)amino]phenyl]ethyl] amino]propoxy]phenyl]methyl]-acetamide) and ICI 215001 ((*S*)-4-[2-hydroxy-3-phenoxypropylaminoethoxy]phenoxyacetic acid hydrochloride) were provided by Tocris bioscience (Bristol, UK). Celiprolol hydrochloride was purchased from LGC standards (Molsheim, France). All drugs were prepared as stock solutions in distilled water, with the exception of nadolol, which was dissolved in HCl and neutralized with NaOH to pH 7.4, and of L-748337, ICI 215001, SR 58611A, and SR 59230A, which were dissolved in dimethylsulphoxide (Sigma-Aldrich). The final concentration of the solvents in the organ bath was less than 0.1% *v*/*v* and was used as a control for the effect of the active drugs.

Fig. 2. (A) The effects of SR 58611A (a preferential β_3-adrenoceptor agonist) and ICI 215001 (a partial β_3-adrenoceptor agonist) in the presence of nadolol (a β_1- and β_2-adrenoceptor antagonist) in intact porcine coronary artery rings. Cumulative concentration–response curves to SR 58611A (0.03–100 μmol/L) and ICI 215001 (0.03–300 μmol/L) were constructed after 30 min of pretreatment with and in the presence of nadolol (10 μmol/L). Each point is the mean of *n* experiments obtained from *n* pig hearts, and vertical lines show the standard error of the mean. When no error bar is shown, the error is smaller than the symbol. * indicates a significant difference (at $P < 0.001$) between ICI 215001 and SR 58611A. (B) A typical recording of relaxant effects of SR 58611A (3–100 μmol/L) in an intact porcine coronary artery ring in the presence of nadolol (10 μmol/L) and preconstriction with KCl (20–30 mmol/L). SR 58611A induced a concentration-dependent relaxation (characterized by its slow kinetics and long duration) at concentrations up to 100 μmol/L.



Statistical analysis

Results were expressed as a mean ± SE of *n* experiments, where *n* is the number of pig hearts (one PCA/pig heart). Different CCRCs were compared on R software (R Development Core Team 2007), using the linear mixed-effects model (Pinheiro and Bates 2000; Thorin et al. 2010; Kabbesh et al. 2012). The linear mixed-effects model must follow a normal distribution of residuals. Normality of residuals was systematically verified and validated. A level of $P < 0.05$ was considered to be statistically significant. For RT-Q-PCR experiments, statistical comparisons between groups of porcine adipose tissues versus PCA samples were performed using a nonparametric Mann–Whitney test.

Results

β_3-Adrenoceptor mRNA expression in PCA rings

RT-Q-PCR results clearly indicated the expression of β_2-adrenoceptor mRNA in both adipose tissues and PCAs (Fig. 1). There were no statistical differences between the 2 tissue types regarding β_2-adrenoceptor mRNA expression. More interestingly, we also found the unambiguous expression of β_3-adrenoceptor transcripts in PCAs. The β_3-adrenoceptor mRNA level in PCAs, hence, represented 35.8% ± 6.4% ($n = 6$) of the hypoxanthine-guanine phosphoribosyltransferase gene basal transcription. Thus, in PCAs, β_3-adrenoceptor mRNA expression was about half of β_2-adrenoceptor mRNA expression (68.05 ± 5.16, $P < 0.005$).

Relaxant effects of SR 58611A and ICI 215001

Both β_3-adrenoceptor agonists, SR 58611A and ICI 215001, induced a concentration-dependent relaxation of PCA rings contracted with KCl (20–30 mmol/L) after pretreatment and in the presence of 10 μmol/L nadolol, with SR 58611A having a greater efficacy than ICI 215001 (Figs. 2A and 2B). The maximal responses obtained for the highest concentration of SR 58611A and ICI 215001 used were 94.25% ± 2.37% ($n = 12$) and 73.70% ± 3.63% ($n = 11$), respectively, ($P < 0.001$).

After pretreatment and in the presence of 10 μmol/L nadolol, CCRCs to SR 58611A were concentration-dependently shifted to the right by pretreatment with 3 and 10 μmol/L L-748337 ($P < 0.01$), and the SR 58611A-induced relaxation was significantly antagonized by pretreatment with 10 μmol/L SR 59230A ($P < 0.01$) (Fig. 3).

The relaxant effect of SR 58611A was almost abolished after removal of the endothelium or pretreatment with 100 μmol/L

Fig. 3. The effects of L-748337 and SR 59230A (2 β_3-adrenoceptor antagonists) upon relaxations induced by SR 58611A (a preferential β_3-adrenoceptor agonist) in the presence of nadolol (a β_1- and β_2-adrenoceptor antagonist) in intact porcine coronary artery rings. Cumulative concentration–response curves to SR 58611A (0.03–100 μmol/L) were constructed after 30 min pretreatment with and in the presence of nadolol (10 μmol/L) and in the presence of L-748337 (3 and 10 μmol/L) or SR 59230A (10 μmol/L). Each point is the mean of *n* experiments obtained from *n* pig hearts, and vertical lines show the standard error of the mean. When no error bar is shown, the error is smaller than the symbol. *, $, and # indicate that the relaxation effect of L-748337 (10 μmol/L), SR 59230A, and L-748337 (3 μmol/L), respectively, was significant different (at $P < 0.01$) from that of the SR 58611A control alone and in the presence of nadolol.

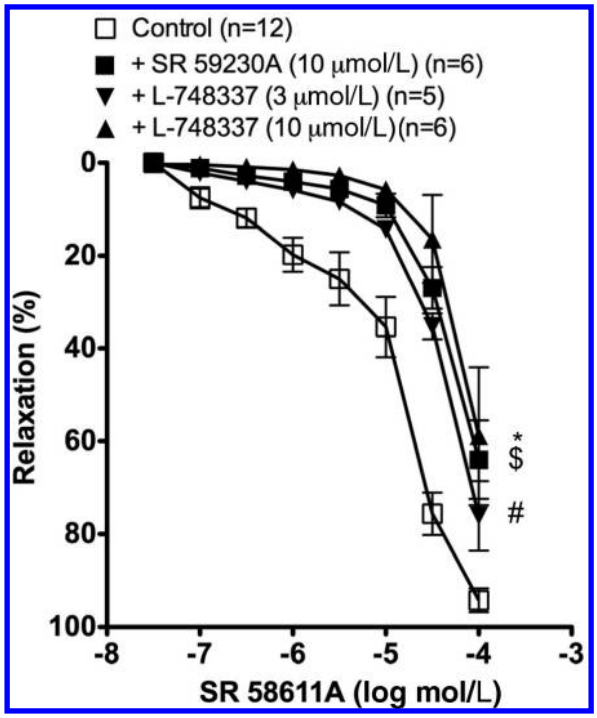
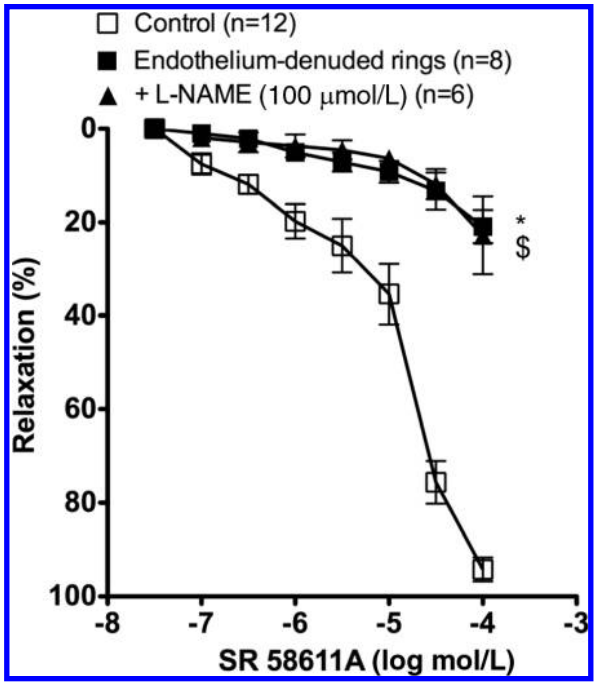


Fig. 4. The effects of L-NAME (a nitric oxide synthase inhibitor) or endothelium removal upon the relaxations induced by SR 58611A (a preferential β_3-adrenoceptor agonist) in the presence of nadolol (a β_1- and β_2-adrenoceptor antagonist) in intact porcine coronary artery rings. Cumulative concentration–response curves to SR 58611A (0.03–100 μmol/L) were constructed after 30 min of pretreatment with and in the presence of nadolol (10 μmol/L) in endothelium-intact (control), endothelium-denuded rings, or endothelium-intact rings pretreated with 100 μmol/L L-NAME. Each point is the mean of *n* experiments obtained from *n* pig hearts, and vertical lines show the standard error of the mean. When no error bar is shown, the error is smaller than the symbol. * and $ indicate that the relaxation effect following removal of endothelium and pretreatment with L-NAME, respectively, was significantly different (at $P < 0.0002$) from that of the SR 58611A control.



L-NAME (Fig. 4). For the highest concentration used, the maximal responses obtained following pretreatment with L-NAME or removal of endothelium were 22.86% ± 8.33% (*n* = 8) and 20.95% ± 3.54% (*n* = 6), respectively, which are significantly different ($P < 0.0002$) from the control.

Relaxant effect of celiprolol

After pretreatment and in the presence of nadolol, celiprolol (0.1–300 μmol/L) induced a concentration-dependent relaxation of PCA rings. The maximal response obtained for the highest concentration used was 72.38% ± 5.76% (*n* = 8). This effect was significantly antagonized by L-748337 (3 μmol/L) and SR 59230A (10 μmol/L) with a rightward shift of the CCRC ($P < 0.0001$), compare with the control (Fig. 5).

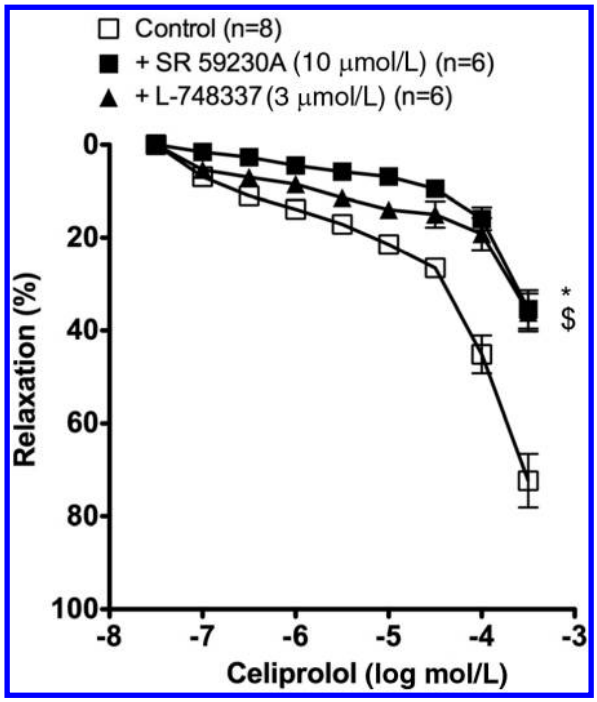
Discussion

In the present study, we show for the first time, by both molecular and functional approaches, the presence of β_3-adrenoceptors in PCA. Furthermore, our study provides evidence of the involvement of β_3-adrenoceptor in the celiprolol-induced vasorelaxation in this vascular bed.

The predominantly expressed β-adrenoceptor in PCA is the β_1-adrenoceptor (Nishimura et al. 1987; Toda and Okamura 1990). In the present study, by using RT-Q-PCR analysis, we clearly demonstrated that both the β_2- and β_3-adrenoceptors are expressed in PCA. A quantitative determination of mRNA levels for the different β-adrenoceptor subtypes has not been systematically performed before in the coronary arteries of the pig or of other animal species. To the best of our knowledge, only one study reported β_3-adrenoceptor transcripts in endothelial cells from human coronary microarteries (Moniotte et al. 2001), suggesting that β_3-adrenoceptors may also play a role in small coronary blood vessels. Although we have quantified only mRNA expression and not the receptor protein, the presence of β_3-adrenoceptor on endothelial cells of PCA is supported by the combination of RT-Q-PCR analysis and functional data. They indicate that the β_3-adrenoceptor agonist SR 58611A relaxed PCAs through the endothelium-, NO-dependent pathway, as very weak relaxation was observed in endothelium-denuded PCA or in the presence of L-NAME.

In our study, pharmacological evidence for the presence of functional β_3-adrenoceptor in PCA was reinforced by using β_3-adrenoceptor antagonists. The relaxant effect of SR 58611A was inhibited by pretreatment with 2 selective β_3-adrenoceptor antagonists, SR 59230A and L-748337 (Manara et al. 1995, 1996; Candelore et al. 1999). L-748337 is actually a selective competitive antagonist of β_3-adrenoceptors in both rat (Mallem et al. 2004) and human (Rozec et al. 2005) blood vessels but seems to have a higher affinity in vitro for β_3-adrenoceptors in humans than in rats (Candelore et al. 1999). In the same recombinant cell preparations, SR 59230A showed similar affinity for human and rat β_3-adrenoceptors but also exhibited

Fig. 5. The effects of celiprolol (a third generation β-adrenoceptor blocker) in the presence of nadolol (a β_1- and β_2-adrenoceptor antagonist) and of L-748337 and SR 59230A (2 β_3-adrenoceptor antagonists) in intact porcine coronary artery rings. Cumulative concentration–response curves to celiprolol (0.03–300 μmol/L) after pretreatment and in the presence or not of L-748337 (3 μmol/L) or SR 59230A (10 μmol/L) were constructed after 30 min of pretreatment with and in the presence of nadolol (10 μmol/L). Each point is the mean of *n* experiments obtained from *n* pig hearts, and vertical lines show the standard error of the mean. When no error bar is shown, the error is smaller than the symbol. * and $ indicate that relaxation effect from the presence of SR 59230A and L-748337, respectively, was significantly different (at $P < 0.0001$) from that of the celiprolol control.



an agonist effect that was dependent on the level of receptor expression (Candelore et al. 1999) and the concentration used (Strosberg and Pietri-Rouxel 1996). In our study, we used SR 59230A at a concentration (i.e., 10 μmol/L) that is above the reported affinity of this drug for β_3-adrenoceptors (4 nmol/L). To rule out nonselective action of SR 59230A, relaxant response to SR 58611A was also evaluated in the presence of 1 μmol/L SR 59230A. The latter also inhibited the SR 58611A-induced relaxation, although, to a lesser extent than that observed in the presence of 10 μmol/L (data not shown).

Although CCRCs to SR 58611A in the presence of β_3-adrenoceptor antagonist did not reach a maximum response, the competitive nature of the β_3-adrenoceptor antagonism is supported by the concentration-dependent rightward shift of CCRCs to SR 58611A in the presence of L-748337. Ideally, several concentrations of L-748337 should have been tested to rigorously define the quantitative relationship between such displacement and the antagonist concentration. However, an accurate value of EC_{50} could not be determined as CCRCs were incomplete due to the solubility limit for SR 58611A. Nevertheless, the data obtained in the present study are highly suggestive that β_3-adrenoceptors may represent another target, in addition to β_1/β_2-adrenoceptor, that might participate in the β-adrenoceptor-mediated relaxation in PCAs.

We found that relaxations to SR 58611A and ICI 215001 occurred only with higher concentrations in PCAs and were somewhat slower than with bradykinin and isoprenaline (unpublished observations). This is in agreement with that found in other studies using blood vessels in vitro (Trochu et al. 1999; Mallem et al. 2003, 2004; Rozec et al. 2005) and further confirms the low sensitivity of the β_3-adrenoceptor in vascular preparations.

SR 58611A-induced relaxation was markedly reduced after endothelium removal or inhibition of NO synthase by L-NAME, confirming the endothelial localization of the target mechanism activated by SR 58611A, i.e., β_3-adrenoceptors. This finding is in accordance with previous data demonstrating the endothelial localization of β_3-adrenoceptors in rat aorta (Rautureau et al. 2002; Mallem et al. 2004) and human blood vessels (Dessy et al. 2004; Rozec et al. 2005).

As previously pointed out, some β-adrenoceptor antagonists, such as nebivolol, have been reported to interact with β_3-adrenoceptor. Thus it is not unreasonable to question whether celiprolol can similarly target the β_3-adrenoceptor (Gosgnach et al. 2001). In addition, we pursued this line of investigation to test the hypothesis that β_3-adrenoceptor agonism exerted by celiprolol can partially explain its vasorelaxant activity in PCAs. The role of β_3-adrenoceptor in the celiprolol-mediated relaxation has not been yet extensively studied. However, Noda et al. (2001) investigated the role of β_3-adrenoceptors in celiprolol-mediated relaxation by using a nonspecific β_3-adrenoceptor antagonist, cyanopindolol. In our study, RT-Q-PCR analysis in combination with a pharmacological approach using selective antagonists allowed accurate assessment of the celiprolol-induced β_3-adrenoceptor response in the PCA. We showed that celiprolol was still able to cause a concentration-dependent relaxation in the presence of nadolol and that in the presence of selective β_3-adrenoceptor antagonists, this relaxation was significantly reduced.

Thus, the present study showed that (*i*) PCAs possess functional β_3-adrenoceptors mediating endothelial and NO-dependent relaxation and (*ii*) celiprolol exerts a β_3-adrenoceptor agonistic activity in this vascular bed. However, whether the potential beneficial effects of celiprolol are due to β_3-adrenoceptor activation in conductance coronary vessels or coronary microvessels or both cannot be solved by the present experiments. Therefore, further studies are needed for full assessment and understanding of the coronary vasodilatory effect of celiprolol.

Acknowledgements

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