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SHORT COMMUNICATION

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Comparison of the α-adrenoceptor-mediated effects of β_3-adrenoceptor ligands in rat pulmonary artery

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Abstract We have recently shown that the β-adrenoceptor ligands CGP 12177, bupranolol, and SR 59230A (aryloxypropanolamines), but not BRL 37344 and CL 316243 (phenylethanolamines), exhibit significant affinity for α_1-adrenoceptors and that CGP 12177 displays partial agonist properties at α-adrenoceptors in rat pulmonary artery. In this study, bupranolol and SR 59230A were further evaluated for their potential α-adrenoceptor mediated effects (i.e., agonist and/or antagonist properties) in rat intralobar pulmonary artery and compared with BRL 37344 and CL 316243. Bupranolol induced a relaxation in phenylephrine-precontracted arteries, but had no effect in prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) -precontracted ones. SR 59230A also elicited a relaxation in phenylephrine-precontracted arteries. In $PGF_{2\alpha}$ -precontracted arteries, SR 59230A induced a contractile response that was insensitive to the irreversible α-adrenoceptor antagonist phenoxybenzamine. BRL 37344 at high concentrations, but not CL 316243, produced slight relaxation in both phenylephrine- and $PGF_{2\alpha}$ -precontracted arteries. The contractile response to phenylephrine was antagonized by bupranolol and SR 59230A in a competitive manner (pA_2: 6.38 and 7.08 respectively). The concentration–response curve to phenylephrine was also shifted to the right by BRL 37344 (mean pK_b: 4.45), but not by CL 316243 (100 μM). This study indicates that the aryloxypropanolamine derivatives bupranolol and SR 59230A exhibit competitive antagonist, but no agonist properties on α_1-adrenoceptors, SR 59230A also inducing α-adrenoceptor-independent contraction. Among the phenylethanolamines, BRL 37344 but not CL 316243, also exerts an antagonist effect on α_1-adrenoceptors, with a much lower potency than the aryloxypropanolamines studied.

Keywords α-adrenoceptor · β-adrenoceptor · BRL 37344 · Bupranolol · CL 316243 · SR 59230A · Pulmonary artery

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Introduction

The aryloxypropanolamines CGP 12177 (β_1/β_2-adrenoceptor antagonist, with agonist properties on β_3-adrenoceptor and low affinity state of β_1-adrenoceptor), bupranolol (non-selective β-adrenoceptor antagonist), and SR 59230A (β_3-adrenoceptor antagonist) are extensively used for characterization of β-adrenoceptor subtypes. An interference of these compounds with the α_1-adrenoceptor signaling pathway was initially postulated from experiments showing that CGP 12177, bupranolol, and SR 59230A induced relaxation in phenylephrine-precontracted, but not in prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) -precontracted rat aorta (Brahmadevara et al. 2003). Recently, we have shown that these aryloxypropanolamines exhibited a significant affinity (pK_i>5) for α_1-adrenoceptors (Leblais et al. 2004a). These findings were soon after confirmed by Brahmadevara et al. (2004), who reported the binding affinity of various β-adrenoceptors ligands for α_1-adrenoceptor, and the antagonist effect of CGP 12177 on phenylephrine-induced contraction in rat aorta. One important observation of our previous work was that, in $PGF_{2\alpha}$ -precontracted rat pulmonary arteries, CGP 12177 induced a contractile response

that was decreased by α-adrenoceptor antagonists, indicating that CGP 12177 also exhibited agonist properties for α-adrenoceptors (Leblais et al. 2004a). In the present study, bupranolol and SR 59230A were further evaluated and compared with CGP 12177 for their potential α-adrenoceptor-mediated effects (i.e., agonist and/or antagonist properties) in rat pulmonary artery. These aryloxypropanolamines were also compared with the phenylethanolamines BRL 37344 and CL 316243, two selective β_3-adrenoceptor agonists that display a low affinity ($pK_i < 4$) for α_1-adrenoceptors (Leblais et al. 2004a). Preliminary accounts of this work have previously been presented in abstract form (Leblais et al. 2004b).

Materials and methods

Measurements of isometric tension Intralobar pulmonary arteries (2nd–3rd order branch; internal diameter: 440–870 μm) were isolated from male Wistar rats (11–18 weeks old; from Elevage Janvier, Le Genest St Isle, France) and mounted in a wire myograph, as previously described (Leblais et al. 2004a). Briefly, arteries were bathed in physiological salt solution (PSS; containing (in mM): NaCl 119; KCl 4.7; $CaCl_2$ 1.5; $MgSO_4$ 1.17; KH_2PO_4 1.18; $NaHCO_3$ 25; glucose 5.5), maintained at 37°C, and gassed with a mixture of 95% O_2-5% CO_2 (pH 7.4). After equilibration, PSS containing 80 mM KCl (equimolar substitution with NaCl) was added. After washouts, cumulative concentration–response curves (CRC) to bupranolol, SR 59230A, BRL 37344 or CL 316243 were performed in arteries precontracted with phenylephrine (30 nM) or $PGF_{2\alpha}$ (3 μM) to a similar amplitude (15–20% of 80 mM KCl response). To irreversibly alkylate α-adrenoceptors, some arteries were pretreated with phenoxybenzamine (PBZ, 1 μM) for 15 min before the addition of $PGF_{2\alpha}$. This treatment totally abolishes the contractile effect of phenylephrine in rat pulmonary artery (Leblais et al. 2004a). To determine the α-adrenoceptor antagonist potency of ligands, cumulative CRC were produced for phenylephrine in arteries precontracted with $PGF_{2\alpha}$ in the absence or presence of bupranolol, SR 59230A, CGP 12177, BRL 37344 or CL 316243. In experiments with SR 59230A and CGP 12177 (which produced a contractile effect by themselves), the concentration of $PGF_{2\alpha}$ was decreased (up to 1 μM) to obtain a similar contraction amplitude to that obtained with 3 μM of $PGF_{2\alpha}$ in experiments with bupranolol, BRL 37344, and CL 316243 (which did not exert a contractile effect by themselves).

Drugs BRL 37344 sodium salt ((±)-(R*,R*)-[4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]phenoxy]acetic acid sodium), (±)-CGP 12177 hydrochloride (4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazol-2-one), CL 316243 (disodium 5-[(2R)-2-([(2R)-2-(3-chlorophenyl)-2-hydroxyethyl]amino) propyl]-1,3-benzodioxole-2,2-dicarboxylate), $PGF_{2\alpha}$ Tris salt, phenoxybenzamine hydrochloride, (−)-phenylephrine hydrochloride, and SR 59230A oxalate salt (3-(2-ethylphenoxy)-1[(1S)-1,2,3,4-tetrahydronaphth-1-ylamino]-(2S)-2-propanol oxalate) were supplied by Sigma Chemical (St Quentin-Fallavier, France). Bupranolol was obtained from Schwarz Pharma (Monheim, Germany). All drugs were prepared as stock solution in distilled water, except SR 59230A, which was dissolved in dimethylsulf-

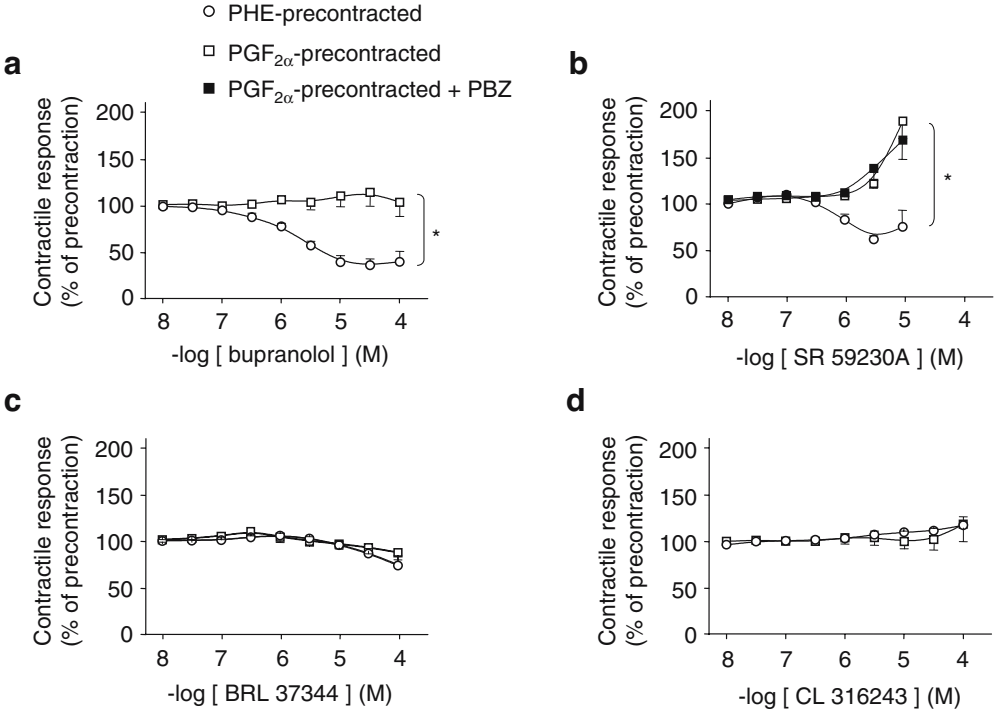

Fig. 1 Effect of **a** bupranolol, **b** SR 59230A, **c** BRL 37344, and **d** CL 316243 in rat pulmonary arteries precontracted with phenylephrine (*PHE*, 30 nM; *open circles*) or $PGF_{2\alpha}$ (3 μM; *open squares*). Some arteries were pretreated with phenoxybenzamine (*PBZ*, 1 μM for 15 min; *closed squares*). Each point is the mean ± SEM of 4–8 experiments. The response is expressed as the percentage of precontraction, 100% corresponding to the contraction induced by phenylephrine or $PGF_{2\alpha}$. *$P<0.001$

oxide so that the final concentration of the solvent was less than 0.05%.

Data analysis Isometric tension was expressed either as a percentage of the tone induced by the precontractile agent or as a percentage of the response to 80 mM KCl, as appropriate. EC_{50} values (concentration producing 50% of the maximum response) were estimated using the Boltzmann equation fit and converted to pD_2. Concentration ratios (r) were determined from individual EC_{50} values in the presence and absence of antagonist. pA_2 values were calculated by linear regression and obtained from the x-intercept of the plot of log (r−1) against log (molar antagonist concentration), when the slope was not different from unity (Arunlakshana and Schild 1959). In some cases, pK_b values were estimated according to the equation $pK_b = \log(r-1) - \log(\text{molar antagonist concentration})$, where r is the ratio of the mean EC_{50} values in the presence and absence of antagonist.

Data are given as means±SEM. They were compared using a Student's *t* test or a two-way analysis of variance (ANOVA) as appropriate. Differences were considered statistically significant when $P<0.05$.

Results

Effects on phenylephrine- or $PGF_{2\alpha}$ -precontracted pulmonary arteries

In rat pulmonary arteries precontracted with 30 nM phenylephrine, bupranolol and SR 59230A induced a concentration-dependent relaxant effect, with a maximum relaxation of 64.8±6.1% (n=4) and 40.7±3.7% (n=5) of initial tone respectively (Fig. 1a, b). When arteries were precontracted with 3 μM $PGF_{2\alpha}$ (a concentration producing a contraction of similar amplitude to that induced by 30 nM phenylephrine), bupranolol did not modify the active tension (102.6±15.2% at 100 μM, n=4), whereas SR 59230A significantly enhanced it (Fig. 1a, b). The contraction induced by SR 59230A was not prevented in arteries pretreated with the irreversible α-adrenoceptor antagonist PBZ (1 μM) (Fig. 1b). A contractile effect of SR 59230A was also observed in non-precontracted arteries (data not shown).

BRL 37344 and CL 316243 exerted similar effects in either phenylephrine- or $PGF_{2\alpha}$ -precontracted arteries (Fig. 1c, d). BRL 37344 elicited a slight relaxation at

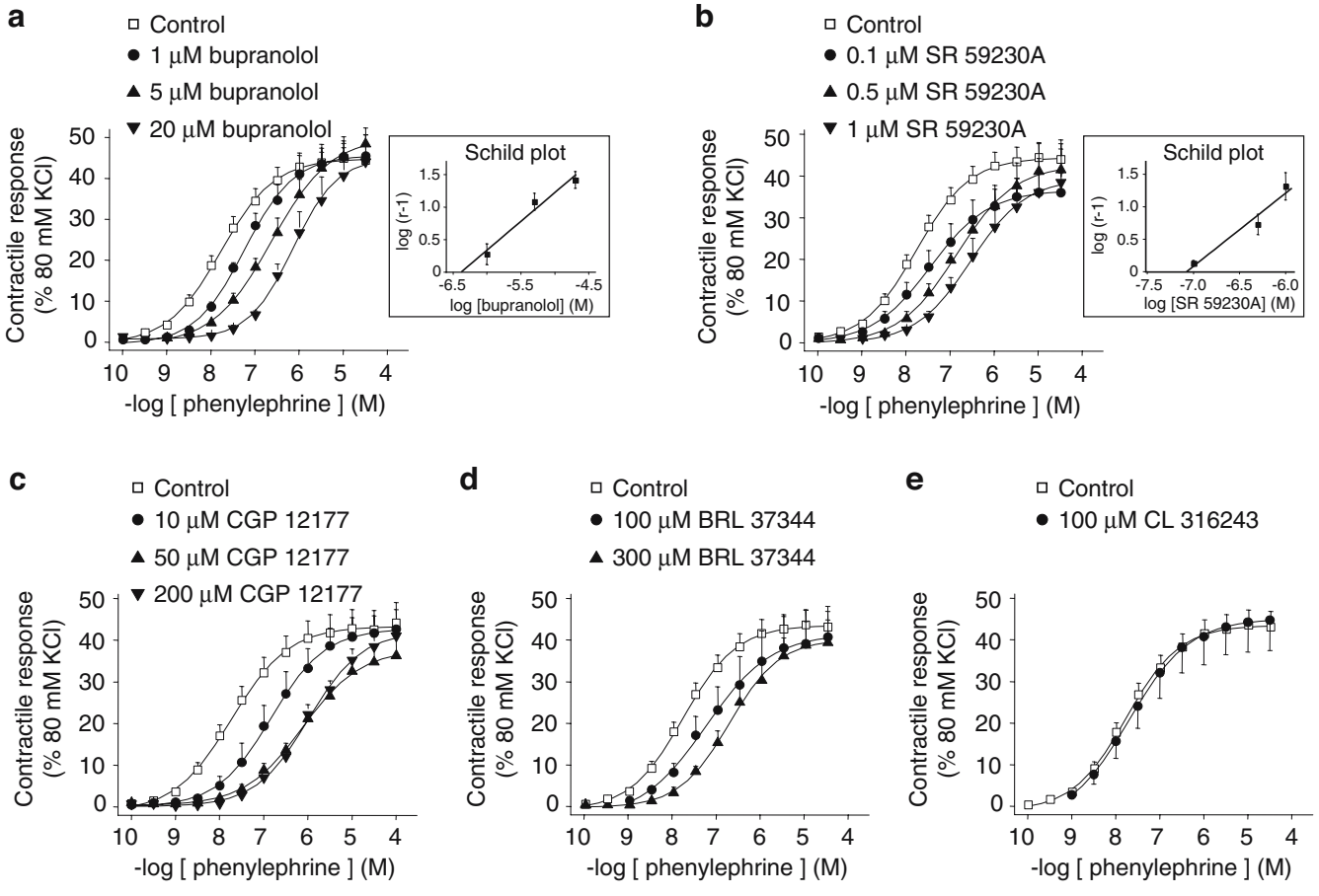

Fig. 2 Effect of **a** bupranolol, **b** SR 59230A, **c** CGP 12177, **d** BRL 37344, and **e** CL 316243 on contractile response to phenylephrine in rat pulmonary arteries precontracted with $PGF_{2\alpha}$. Each point is the mean ± SEM of 4–19 experiments. The response is expressed as the percentage of the contraction induced by 80 mM KCl. Corresponding Schild plots are shown as insets in **a** and **b**

concentrations ≥30 μM (at 100 μM: relaxation of 28.0±10.6% (n=4) and 14.1±7.7% (n=5) in phenylephrine- and $PGF_{2\alpha}$ -precontracted arteries respectively) (Fig. 1c). CL 316243 did not significantly modify the tone induced by either phenylephrine or $PGF_{2\alpha}$ (Fig. 1d).

Antagonism of phenylephrine-induced contraction in pulmonary arteries

In rat pulmonary artery, increasing concentrations of bupranolol and SR 59230A produced rightward shifts of the CRC to phenylephrine, with no significant depression in the maximum response (Fig. 2a, b). Schild plot analysis of the antagonist effect of bupranolol and SR 59230A produced a straight line with a slope that was not different from unity (0.89±0.17 and 1.12±0.25 respectively) and pA_2 values of 6.38 and 7.08 respectively. The other aryloxypropanolamine CGP 12177 (10 and 50 μM) also shifted the CRC to phenylephrine to the right without affecting its maximum response (Fig. 2c). Increasing the concentration of CGP 12177 to 200 μM did not produce a further shift of the CRC to phenylephrine. The estimated pK_b values of the antagonist effect of 10 and 50 μM CGP 12177 were 5.99 and 5.90 respectively.

The phenylethanolamine BRL 37344 (100 and 300 μM) produced rightward shifts of the CRC to phenylephrine, with no reduction in the maximum response (Fig. 2d). The estimated pK_b values of the antagonist effect of 100 and 300 μM BRL 37344 were 4.43 and 4.49 respectively. CL 316243 up to 100 μM did not significantly antagonize the contractile response to phenylephrine (pD_2 values: 7.74±0.09 (n=19) and 7.57±0.15 (n=4), in the absence and presence of 100 μM CL 316243 respectively; Fig. 2e).

Discussion

The present study shows that in rat pulmonary artery, SR 59230A, a compound used as a β_3-adrenoceptor antagonist, induced relaxation in phenylephrine-precontracted preparations over the same concentration range that it induced contraction in $PGF_{2\alpha}$ -precontracted ones. The profile of contractile response to SR 59230A was similar to that of CGP 12177 (Leblais et al. 2004a) in terms of precontractile agent-dependence. However, whereas phenoxybenzamine markedly diminished CGP 12177-induced contraction (Leblais et al. 2004a), it did not affect the contractile effect of SR 59230A. This indicates that SR 59230A exerted a contractile response through α-adrenoceptor-independent mechanism(s) in rat pulmonary artery, the nature of which remains to be determined. These mechanism(s) could be tissue-specific since no contractile effect of SR 59230A was reported in rat aorta precontracted with $PGF_{2\alpha}$ (Brahmadevara et al. 2003). Nevertheless, this study shows that in rat pulmonary artery, unlike CGP 12177, SR 59230A did not exhibit α-adrenoceptor agonist properties. Both SR 59230A and CGP 12177 induced relaxation after precontraction with phenylephrine in rat pulmonary artery (this study) and aorta (Brahmadevara et al. 2003). Moreover, both compounds also antagonized the contractile response to phenylephrine in rat pulmonary arteries. The antagonism exerted by SR 59230A appears clearly competitive. The lower potency of SR 59230A to cause relaxation (active concentration range: 0.3 to 10 μM) than to antagonize phenylephrine effects (pA_2: 7.08) might be explained by its dual effect, i.e., α_1-adrenoceptor antagonism and α-adrenoceptor-independent contraction. Overall, these data indicate that in rat pulmonary artery, SR 59230A induces contraction through α-adrenoceptor-independent mechanism(s) and exerts a competitive antagonist property on the α_1-adrenoceptor. In the case of CGP 12177, the displacement of the CRC to phenylephrine obtained with 10 and 50 μM of CGP 12177 was consistent with a competitive antagonism, while no further antagonism was observed with a higher concentration of the compound. We have previously shown that CGP 12177 exhibits not only a partial α_1-adrenoceptor agonist effect, but also a contractile effect through α-adrenoceptor-independent mechanism(s), especially at concentrations greater than 30 μM (Leblais et al. 2004a). This complex pharmacological profile of CGP 12177 might explain its particular inhibitory effect on phenylephrine-induced contraction in rat pulmonary artery. This deserves further investigation.

Bupranolol, a nonselective β-adrenoceptor antagonist, did not modify the tone induced by $PGF_{2\alpha}$, but elicited relaxation in rat pulmonary arteries precontracted with phenylephrine. Bupranolol also exerted a relaxant effect in phenylephrine-precontracted rat aorta (Brahmadevara et al. 2003). The relaxant response to bupranolol in phenylephrine-precontracted pulmonary arteries was consistent with an antagonist effect on α_1-adrenoceptors, as its potency to induce relaxation (active concentration range: 0.3 to 30 μM) was very close to its potency to antagonize the effect of phenylephrine (pA_2: 6.38). The lack of bupranolol effect on $PGF_{2\alpha}$ -precontracted arteries argues against the α_1-adrenoceptor agonist property of this compound. This indicates that bupranolol acts as a competitive antagonist on α_1-adrenoceptor in rat pulmonary artery. However, unlike CGP 12177, bupranolol displays no α_1-adrenoceptor agonist activity.

Aryloxypropanolamines were compared with the phenylethanolamines BRL 37344 and CL 316243, two β_3-adrenoceptor agonists that exhibit low affinity (pK_i: 3.75 for BRL 37344 and <3 for CL 316243) for α_1-adrenoceptors (Leblais et al. 2004a). In contrast to aryloxypropanolamines, the effects of BRL 37344 and CL 316243 were independent of the precontractile agent used. CL 316243 had no contractile or relaxant effect in precontracted pulmonary arteries, suggesting a lack of α_1-adrenoceptor agonist or antagonist activity with this compound. The lack of antagonist effect of CL 316243 on α_1-adrenoceptors is further supported by its inability to antagonize the effect of phenylephrine. In both phenylephrine- and $PGF_{2\alpha}$ -precontracted pulmonary arteries, a high concentration (≥30 μM) of BRL 37344 induced a slight relaxation. Compared with other cells and tissues (Granneman

et al. 1991; Hoey et al. 1996), including vascular ones (MacDonald et al. 1999; Tamaoki et al. 1998), the weak potency of BRL 37344 observed here is not consistent with a β_3-adrenoceptor-mediated effect. The relaxant response to high concentrations of BRL 37344, which was independent of the precontractile agent used, may partly result from its β_2-adrenoceptor agonist property (Gauthier et al. 1999; Gibbs and Summers 2001; Shen et al. 1996). The potential antagonist effect of BRL 37344 against α_1-adrenoceptors was further evaluated by its ability to displace the CRC to phenylephrine. In these experiments, BRL 37344 exerted an α_1-adrenoceptor antagonist effect, but with a much weaker potency (pK_b: 4.45) than the aryloxypropanolamines. It should be noticed that the profile of responses to BRL 37344 and CL 316243 does not support the existence of a relaxant role of β_3-adrenoceptors in rat intralobar pulmonary artery. This deserves further investigation using other β_3-adrenoceptor ligands.

It is concluded from this study and our previous one (Leblais et al. 2004a) that there is a close correlation between the binding affinity for α_1-adrenoceptors of the β_3-adrenoceptor ligands studied here and their antagonist potency for α_1-adrenoceptors (in both cases, SR 59230A> bupranolol>CGP 12177>BRL 37344≫CL 316243). The key finding of the present study is that among the aryloxypropanolamines studied, bupranolol and SR 59230A, unlike CGP 12177, do not display partial α_1-adrenoceptor agonist properties in rat pulmonary artery, even though SR 59230A elicited an α-adrenoceptor-independent contraction. Among the phenylethanolamines studied, BRL 37344 at high concentrations, but not CL 316243, also exerts an antagonist effect on α_1-adrenoceptors in this artery. The α-adrenoceptor properties of some of these β_3-adrenoceptor ligands should be considered when interpreting their effects, especially on vascular tone.

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References

Arunlakshana O, Schild HO (1959) Some quantitative uses of drug antagonists. Br J Pharmacol 14:48–58

Brahmadevara N, Shaw AM, MacDonald A (2003) Evidence against beta 3-adrenoceptors or low affinity state of beta 1-adrenoceptors mediating relaxation in rat isolated aorta. Br J Pharmacol 138:99–106

Brahmadevara N, Shaw AM, MacDonald A (2004) Alpha1-adrenoceptor antagonist properties of CGP 12177A and other beta-adrenoceptor ligands: evidence against beta(3)- or atypical beta-adrenoceptors in rat aorta. Br J Pharmacol 142:781–787

Gauthier C, Tavernier G, Trochu JN, Leblais V, Laurent K, Langin D, Escande D, Le Marec H (1999) Interspecies differences in the cardiac negative inotropic effects of beta(3)-adrenoceptor agonists. J Pharmacol Exp Ther 290:687–693

Gibbs ME, Summers RJ (2001) Enhancement of memory consolidation in chicks by beta(3)-adrenoceptor agonists. Eur J Pharmacol 413:235–240

Granneman JG, Lahners KN, Chaudhry A (1991) Molecular cloning and expression of the rat beta 3-adrenergic receptor. Mol Pharmacol 40:895–899

Hoey A, Jackson C, Pegg G, Sillence M (1996) Atypical responses of rat ileum to pindolol, cyanopindolol and iodocyanopindolol. Br J Pharmacol 117:712–716

Leblais V, Pourageaud F, Ivorra MD, Guibert C, Marthan R, Muller B (2004a) Role of alpha-adrenergic receptors in the effect of the beta-adrenergic receptor ligands, CGP 12177, bupranolol, and SR 59230A, on the contraction of rat intrapulmonary artery. J Pharmacol Exp Ther 309:137–145

Leblais V, Pourageaud F, Ivorra MD, Marthan R, Muller B (2004b) The aryloxypropanolamines CGP 12177, bupranolol and SR 59230A, but not the phenylethanolamines CL 316243 and BRL 37344 exhibit alpha1-adrenergic receptor ligand properties. Fund Clin Pharmacol 18:23–126

MacDonald A, McLean M, MacAulay L, Shaw AM (1999) Effects of propranolol and L-NAME on beta-adrenoceptor-mediated relaxation in rat carotid artery. J Auton Pharmacol 19:145–149

Shen YT, Cervoni P, Claus T, Vatner SF (1996) Differences in beta 3-adrenergic receptor cardiovascular regulation in conscious primates, rats and dogs. J Pharmacol Exp Ther 278:1435–1443

Tamaoki J, Tagaya E, Isono K, Nagai A (1998) Atypical adrenoceptor-mediated relaxation of canine pulmonary artery through a cAMP-dependent pathway. Biochem Biophys Res Commun 248:722–727