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Impairment of the low-affinity state β_1-adrenoceptor-induced relaxation in spontaneously hypertensive rats

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1 In hypertension, a decrease of the vascular β-adrenergic relaxation has been described. However, the specific involvement of each β-adrenoceptor (β-AR) subtype, in particular the low-affinity state of β_1-AR, has not yet been evaluated. We investigated whether the low-affinity state of β_1-AR-induced relaxation was impaired in Spontaneously Hypertensive Rats (SHR).

2 The relaxant responses to CGP 12177 and cyanopindolol, low-affinity state β_1-AR agonists (with β_1-/β_2-AR antagonistic and partial β_3-AR agonistic properties) were evaluated on thoracic aortic rings isolated from 12-weeks-old Wistar Kyoto rats (WKY) and SHR.

3 In WKY, CGP 12177 and cyanopindolol produced an endothelium and nitric oxide (NO)-independent relaxation. CGP 12177-induced endothelium-independent relaxation was not modified either by β_1-, β_2-AR (nadolol) or β_3-AR (L-748337 or SR 59230A) antagonists but was significantly reduced by high concentrations of CGP 20712A ($P<0.05$). This relaxation was also reduced by adenylyl cyclase inhibitors, SQ 22536 or MDL 12330A.

4 In SHR, CGP 12177 produced mainly an endothelium and NO-dependent relaxation. This effect was not modified by nadolol, but was strongly reduced by β_3-AR blockade. Endothelium-independent relaxation to CGP 12177 was not altered by adenylyl cyclase inhibition, but was amplified in preparations from pertussis toxin-pretreated SHR.

5 The immunohistochemical analysis revealed an upregulation of β_3-AR in the endothelial layer of SHR aorta, whereas the β_3-AR-induced relaxation was not modified.

6 In conclusion, we demonstrated an impaired low-affinity state of the β_1-AR-induced relaxation and an upregulation of the β_3-AR in hypertension. Some clinical implications of those findings are discussed.

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Abbreviations: β-AR, β-adrenoceptor; cAMP, adenosine-3′,5′-monophosphate; CCRC, cumulative concentration–response curve; L-NMMA, N^G-monomethyl-L-arginine monoacetate; NECA, 5′-(*N*-ethylcarbamidoadenosine); NO, nitric oxide; PE, phenylephrine; PTX, pertussis toxin; rβ_3-AR Ab, rat β_3-AR antibody; SBP, systolic blood pressure; SHR, spontaneously hypertensive rat; vWf, von Willebrand factor; WKY, Wistar Kyoto

Introduction

Vascular tone is generated *via* a net balance between vasoconstriction and vasorelaxation established through the effects of numerous neurotransmitters and hormones (epinephrine, norepinephrine, acetylcholine, angiotensin II, nitric oxide (NO), etc.). Thus, hypertension results mainly from an imbalance between vasoconstrictive and vasodilatory mechanisms, partly due to pre- and postsynaptic sympathetic dysfunctions (De Champlain, 2001). Several studies reported a decreased β-adrenoceptors (β-ARs)-mediated relaxation of isolated vascular preparations from Spontaneously Hypertensive Rats (SHR) (Fujimoto *et al*., 1987; Arribas *et al*., 1994; Gros *et al*., 2000). The main identified defects included β-AR downregulation, alteration of G protein levels and impairment of β-AR/G protein/effectors coupling (Werstiuk & Lee, 2000). However, some studies described that β-AR-mediated relaxation of thoracic aorta and mesenteric arteries was unchanged (Borkowski *et al*., 1992; Boonen *et al*., 1993) or enhanced (Carvalho *et al*., 1987) comparatively to what was observed in Wistar Kyoto (WKY) rats. Several explanations may exist for those controversial findings regarding β-AR-induced relaxation in arterial hypertension. Thus, the β-AR-mediated relaxation was dependent on the following: (1) the stage of hypertension (Borkowski *et al*., 1992), (2) the agent used for preconstriction (Konishi & Su, 1983; Carvalho *et al*., 1987) and (3) the precontraction level (Konishi & Su, 1983; Carvalho *et al*., 1987). It is important to note that in all those studies, the β-AR relaxant responses have been investigated by using

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isoprenaline, a nonselective β-AR agonist, and the contribution of each β-AR subtype in the β-AR-mediated relaxation in SHR was not determined.

At the present time, at least three populations of β-AR subtypes have been shown to be involved in the β-adrenergic response in blood vessels (Guimaraes & Moura, 2001). Stimulation of β_1- and β_2-ARs produces a vasorelaxation. In some vascular beds, in addition to β_1- and β_2-ARs, the functional expression of a third β-AR subtype, β_3, has been described in several *in vivo* and *in vitro* studies using β_1-/β_2-AR antagonists and β_3-AR agonists (Tavernier *et al*., 1992; Oriowo, 1994; Hom *et al*., 2001). In rat thoracic aorta, β_3-AR stimulation produced an endothelium and NO-dependent relaxation (Trochu *et al*., 1999; Rautureau *et al*., 2002). Furthermore, vascular relaxation induced by BRL 37344 (a β_3-AR agonist), cyanopindolol or CGP 12177 suggested the presence of an atypical β-AR in vessels (Shafiei & Mahmoudian, 1999; Brawley *et al*., 2000a, b). Such an atypical β-AR has been previously described in heart (Kaumann *et al*., 2001) and adipose tissue (Granneman, 2001) and firstly qualified as 'putative' β_4-AR. Recently, β_4-AR has been demonstrated to actually correspond to a low-affinity state of the β_1-AR (Granneman, 2001). It was activated by cyanopindolol and CGP 12177, which also possesses β_1-/β_2-AR antagonistic and partial β_3-AR agonistic properties.

To date, no available data has been reported on the role of the vascular low-affinity state of the β_1-AR in experimental hypertension. So, the present investigation was carried out in order to evaluate the effect of the low-affinity state of the β_1-AR in the model of SHR by analysing the relaxing responses to CGP 12177.

Methods

Animals

Male SHR at 12 weeks of age and age-matched WKY rats were purchased from CER Janvier (Laval, France). They were housed with standard rat chow and water was provided *ad libitum*.

Systolic blood pressure (SBP) of conscious rats was determined by the tail-cuff method (Bioseb, Chaville, France). The measurements, performed from 16:00 to 18:00 hours by the same investigator, were repeated at least 10 times and the mean values were determined for each animal. SBP was 117.3 ± 2.8 mmHg in WKY ($n = 73$) and 182.8 ± 3.7 mmHg in SHR ($n = 50$; $P < 0.05$ vs WKY).

Preparation of aortic rings

Rats were anaesthetized with pentobarbital (30 mg kg^{-1} i.p.) and exsanguinated. Intact and endothelium-denuded aortic rings were prepared as previously described (Trochu *et al*., 1999). Each aortic ring was suspended on stainless steel wires in a 5 ml organ bath containing Krebs solution maintained at 37°C (Trochu *et al*., 1999). Then, the preparations were progressively stretched in order to get a resting tension of 1 g and allowed to equilibrate for 1 h. Isometric tension was recorded by a force displacement transducer (Electromike, Electrocorporation, Sarasota, U.S.A.) on a potentiometric recorder (Bryans-BS 272, U.S.A.).

Vascular relaxation measurements

Functional endothelium was checked by application of 1 μM acetylcholine resulting in the presence of at least 60% relaxation of precontracted rings with 0.3 μM phenylephrine (PE), an α_1-AR agonist. After washout, aortic rings were contracted with PE at concentrations ranging from 0.1 to 1 μM in order to obtain similar values of the sustained tension for each ring studied (0.9 ± 0.05 g). Then, cumulative concentration–response curves (CCRC) to relaxant agents were conducted after reaching the steady-state amplitude of PE contraction. Some rings were incubated during 30 min in Krebs containing nadolol (β_1-/β_2-AR antagonist), L-748337 (Candelore *et al*., 1999) or SR 59230A (two β_3-AR antagonists), N^G-monomethyl-L-arginine monoacetate (L-NMMA, an NO synthase inhibitor), SQ 22536 or MDL 12330A (two adenylyl cyclase inhibitors) before CCRC establishment. CCRC to CGP 12177 were also constructed in aortic rings isolated from rats pretreated with pertussis toxin (PTX, an inhibitor of $G_{i/0}$ proteins) (10 μg kg^{-1} once daily during 3 days).

Immunohistochemistry analysis

The method used was already described by Rautureau *et al*. (2002). After freezing in 2-methyl butane solution (−50°C), 10 μm sections of thoracic aorta were cut on cryostat microtome and stored at −20°C until use. The presence of the endothelial layer of the preparations was tested by incubation with a von Willebrand factor antibody (vWf Ab; DAKO, Trappes). The rat β_3-AR antibody (rβ_3-AR Ab) (Santa Cruz Biotechnology Inc., U.S.A.) is an affinity-purified goat polyclonal antibody raised against a peptide mapping at the carboxy terminus of the β_3-AR of mouse origin. This antibody reacts with β_3-ARs of mouse and rat origin, without crossreactive signal with β_1- and β_2-ARs. After the secondary hybridization with an anti-goat IgG peroxidase conjugated (Sigma, France), the antibody complexes were revealed for sensitive detection of the enzymatic activity with peroxidase substrate kit AEC (SK4200 – Vector, France). Controls for specificity of the rβ_3-AR antiserum involved the incubation of the primary antiserum overnight at 4°C with a five excess by weight of blocking peptide, the sequence used for the immunization procedure without the immunogenic carrier.

Statistics

Results were expressed as the means ± s.e.m. of n experiments, which refers to the number of rats. Relaxation was expressed as percentage relaxation of contraction induced by PE. Comparison of the different CCRC was performed by a two-way ANOVA with repeated measures. Maximal effect (E_{max}) obtained for the highest concentration of agonist was compared using Student's t-test. A level of $P < 0.05$ was considered statistically significant.

Drugs

Phenylephrine hydrochloride (HCl), acetylcholine chloride, nadolol, 5′-(*N*-ethylcarbamidoadenosine (NECA), CGP 20712A ((±)-2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluoromethyl)-1*H*-imidazol-2-yl]phenoxy]propyl]amino]ethoxy]-benzamide methanesulphonate), (−) cyanopindolol and SR

59230A (3-(2-ethylphenoxy)-1-[(1*S*)1,2,34-tetrahydronapht-1-ylaminol]-(2*S*)-2-propanol oxalate) were obtained from Sigma-Aldrich (France). N^G-monomethyl-L-arginine monoacetate (L-NMMA), SQ 22536, MDL 12330A and PTX were purchased from Calbiochem (France). CGP 12177 (4-[3-t-butylamino-2-hydroxypropoxy] benzimidazol-2-one) was purchased from RBI (France). SR 58611A [(*RS*)-*N*-[(25)-7-ethoxycarbonylmethoxy-1,2,3,4-tetrahydronapht-2-yl]-(2*R*)-2-(3-chlorophenyl)-2 hydroethanamine hydrochloride] was a generous gift from Sanofi Synthelabo Recherche (France) and L-748337 from Merck (U.S.A.). 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 L-748337, MDL 12330A, NECA and cyanopindolol that were dissolved in dimethylsulphoxide (DMSO, Sigma-Aldrich). The final concentration of the solvents in the organ bath was less than 0.1% v v^{-1} and has been tested to induce *per se* no effect on the tissue responses.

Results

Effects of CGP 12177 in WKY aorta

In intact aortic rings from WKY, CGP 12177 (0.1–30 μM) produced a concentration-dependent relaxation ($E_{max} = 55.8 \pm 2.3\%$; $n = 9$). This relaxation was unaffected by endothelium removal ($E_{max} = 59.4 \pm 3.3\%$; $n = 8$), or after pretreatment with 100 μM L-NMMA (Figure 1a). Similar results were obtained with cyanopindolol (10 nM to 3 μM; Figure 1b). In order to determine the β-AR subtype involved in the CGP 12177-induced relaxation, CCRC in endothelium-denuded rings were constructed in the presence of 10 μM nadolol, or 3 μM L-748337 or 1 μM SR 59230A. In all these conditions, the CGP 12177-induced effects were not significantly modified (Figure 2). Conversely, the CGP 12177-mediated relaxation was antagonized by a high concentration (10 μM) of CGP 20712A, a β_1-AR antagonist (Figure 2).

In order to determine a putative involvement of the adenylyl cyclase/adenosine-3′,5′-monophosphate (cAMP) pathway, CCRC to CGP 12177 were constructed in endothelium-denuded rings pretreated by adenylyl cyclase inhibitors, SQ 22536 or MDL 12330A (Satake *et al*., 1996; Hourani *et al*., 2001). These compounds were first tested by performing CCRC to NECA, a nonselective adenosine A_2 receptor agonist, which produces a vasorelaxation through the activation of $A_{2A,B}$ receptors linked to adenylyl cyclases *via* G_s proteins (Fahim *et al*., 2001). In denuded aortic rings, CCRC to NECA ($E_{max} = 91.7 \pm 0.6\%$; $n = 6$) was significantly inhibited in the presence of 200 μM SQ 22536 ($E_{max} = 58.2 \pm 6.3$; $n = 6$) or 30 μM MDL 12330A ($E_{max} = 38.4 \pm 2.78$; $n = 7$). Then, the effect of CGP 12177 was tested and was significantly inhibited in the presence of either 200 μM SQ 22536 ($E_{max} = 23.8 \pm 5.9$, $n = 6$; $P < 0.05$ vs CGP 12177 alone) or 30 μM MDL 12330A ($E_{max} = 25.5 \pm 3.8$, $n = 7$; $P < 0.05$ vs CGP 12177 alone) (Figure 3). Finally, in another set of experiments, we evaluated the CGP 12177-mediated response in aortic rings isolated from rats pretreated with PTX. G_i proteins inhibitory effect of PTX was previously confirmed by establishing CCRC to UK 14304, a selective α_2-AR agonist (Rautureau *et al*., 2002). The endothelium-independent effect of CGP 12177 was not modified by PTX pretreatment ($E_{max} = 66.9 \pm 7.3\%$; $n = 6$) compared to control rats.

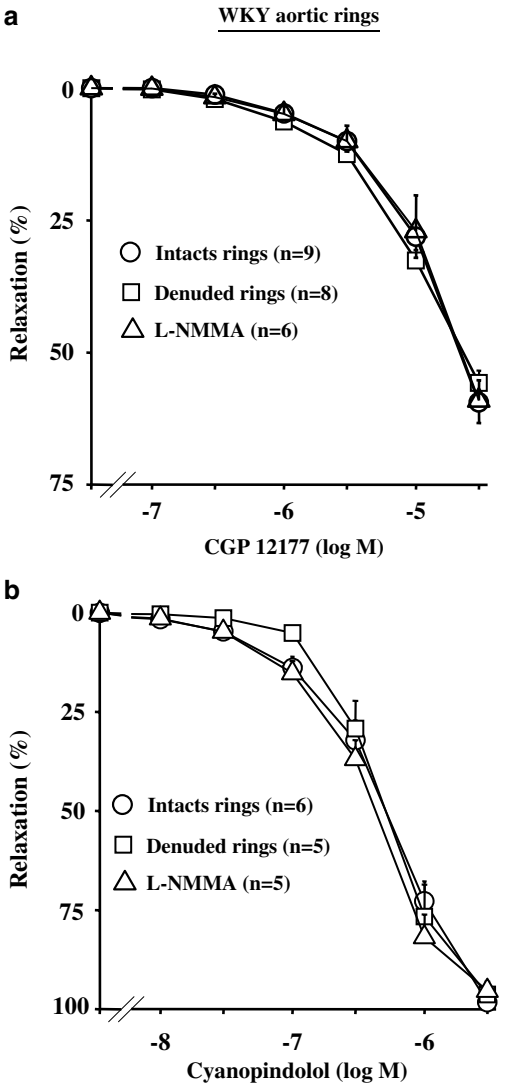

Figure 1 Concentration–response curves to CGP 12177 (a) and cyanopindolol (b) in WKY rats. Curves were performed in intact, denuded rings or in intact rings pretreated with 100 μM L-NMMA for 30 min.

Alteration of the CGP 12177-induced vasorelaxation in SHR aorta

In intact aortic rings from SHR, CGP 12177 produced a concentration-dependent relaxation ($E_{max} = 57.6 \pm 2.5\%$, $n = 12$) (Figure 4). This relaxation was greatly inhibited by endothelium removal or after pretreatment with 100 μM L-NMMA (Figure 4). Interestingly, endothelium-dependent relaxation of CGP 12177 was not modified in the presence of 10 μM nadolol, but was significantly inhibited in the presence of 3 μM L-748337 ($E_{max} = 25.9 \pm 3.3\%$; $n = 7$; $P < 0.05$ vs CGP 12,177 alone) (Figure 5).

Endothelium-independent relaxation to CGP 12177 was not altered in the presence of SQ 22536 (Figure 6) but was amplified by PTX pretreatment ($E_{max} = 36.9 \pm 3.4\%$; $n = 5$; $P < 0.05$ vs rings without PTX by ANOVA) (Figure 6).

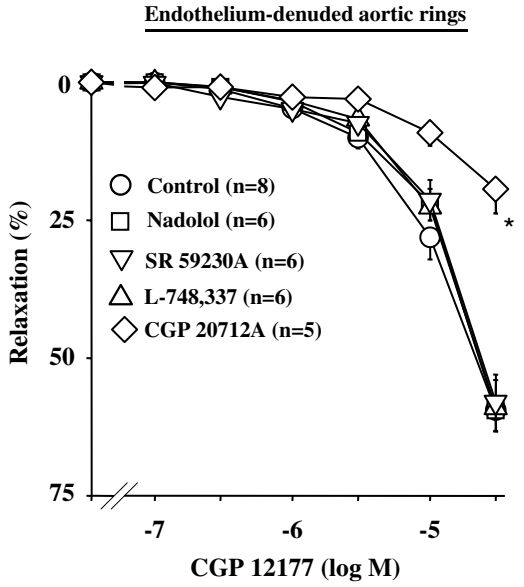

Figure 2 Concentration–response curves to CGP 12177 in denuded aortic rings from WKY rats. Curves were performed in the absence or presence of 10 μM nadolol, 3 μM L-748337, 1 μM SR 59230A or 10 μM GCP 20712A. *$P<0.05$ vs CGP 12177 alone (the curve in the presence of nadolol is mostly obscured under the control one).

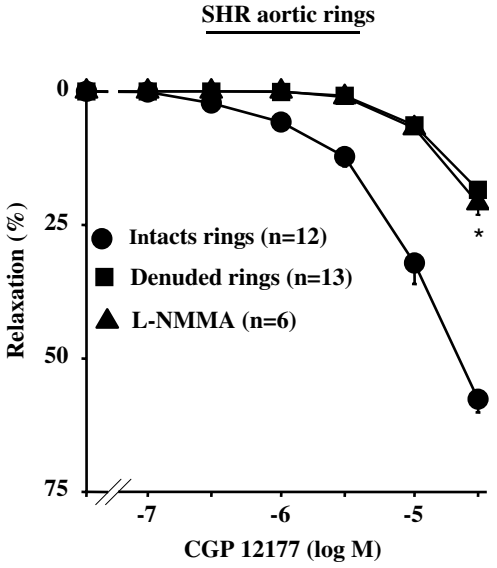

Figure 4 Concentration–response curves to CGP 12177 in SHR rats. Curves were performed in intact, denuded rings or in intact rings pretreated with 100 μM L-NMMA. *$P<0.05$ vs CGP 12177 alone.

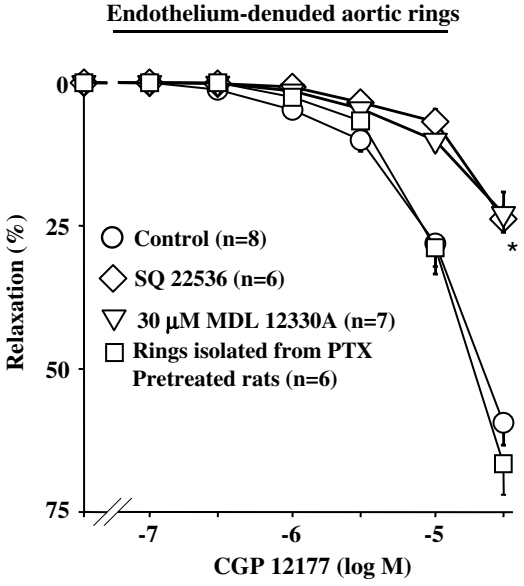

Figure 3 Concentration–response curves to CGP 12177 in denuded aortic rings from WKY rats. Curves were performed in the absence or presence of 200 μM SQ 22536, 30 μM MDL 12330A or after pretreatment of rats with 10 $\mu g\,kg^{-1}$ PTX during 3 days. *$P<0.05$ vs CGP 12177 alone.

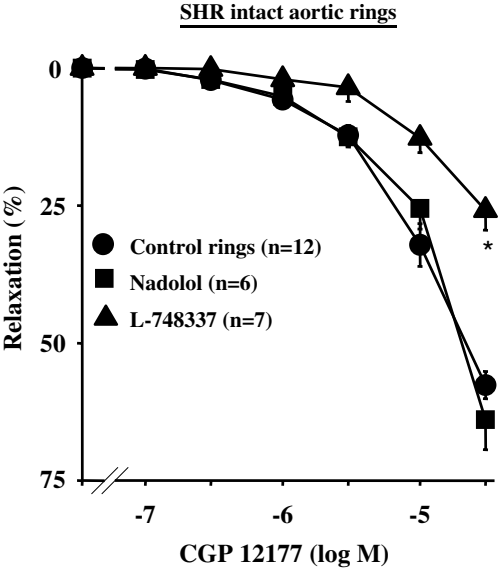

Figure 5 Concentration–response curves to CGP 12177 in intact aortic rings from SHR. Curves were performed in the absence or in the presence of 3 μM L-748337 or 10 μM nadolol. *$P<0.05$ vs CGP 12177 alone.

Modification of β_3-AR expression without alteration of the functional response

As the endothelium-dependent relaxation to CGP 12177 was blunted by a β_3-AR antagonist in SHR, we evaluated the expression level of β_3-AR in both strains of WKY rats and SHR by immunohistochemical analysis. The pattern of rat β_3-AR immunoreactivity was compared with the vWf expression profile (Figure 7a). vWf was used as a marker of the endothelial layer. The rat β_3-AR antibody (rβ_3-AR Ab) highly stained cells from the endothelial layer in a similar distribution and form, to those revealed with the vWf antiserum. Furthermore, the preabsorption of rβ_3-AR antiserum with the synthetic peptide, used for the procedure of immunization, totally abolished the staining observed in the endothelial layer. The same rβ_3-AR Ab staining experiment was performed in WKY rats and SHR aorta ($n=5$, Figure 7b). In both rat

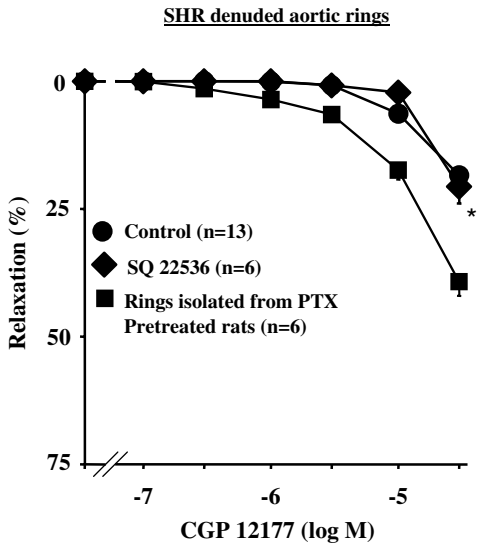

Figure 6 Concentration–response curves to CGP 12177 in denuded aortic rings from SHR. Curves were performed in the absence or in the presence of 200 μM SQ 22536 or after pretreatment of rats with 10 μg kg^{-1} PTX during 3 days. *$P<0.05$ vs CGP 12177 alone.

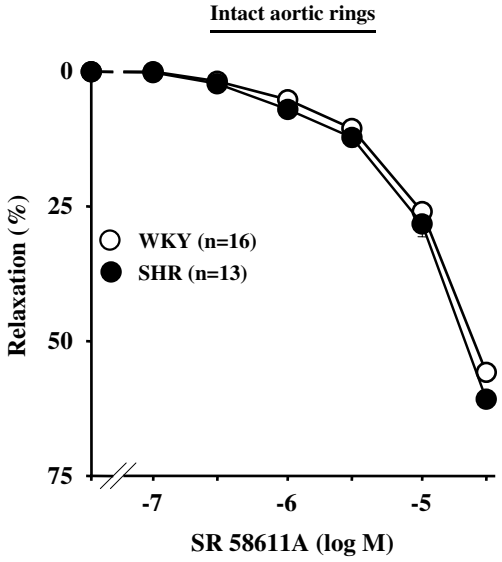

Figure 8 Concentration–response curves to SR 58611A in intact aortic rings from WKY rats or SHR.

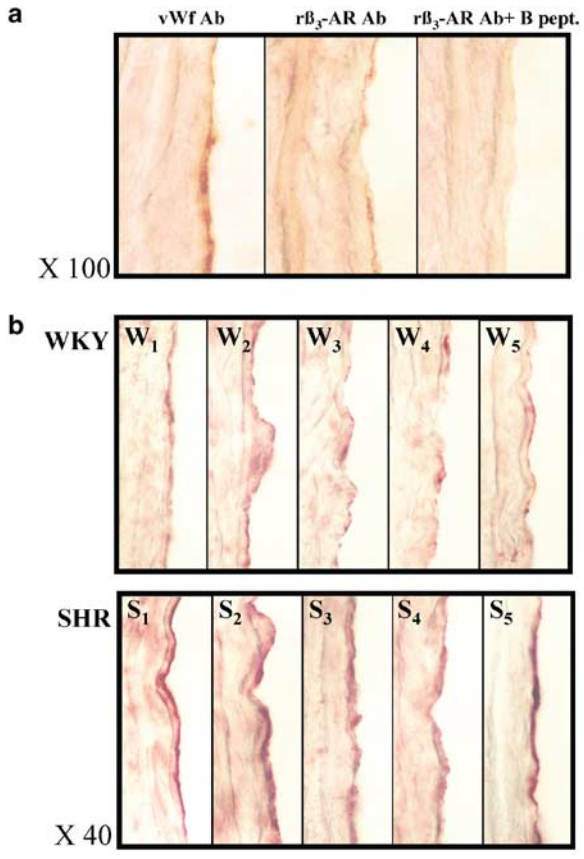

Figure 7 β_3-AR expression in WKY and SHR aorta. Panel a: adjacent 10 μm thick sections were incubated with either von Willebrand factor antibody (vWf Ab) or rat β_3-AR antibody (rβ_3-AR Ab) or after preabsorption of rβ_3-AR Ab with the blocking peptide (rβ_3-AR Ab + B pept.). Panel b: the same rβ_3-AR Ab staining experiment was performed in WKY (W1–W5) and SHR (S1–S5) aorta. vWf and rβ_3-AR antibodies were revealed with peroxidase-conjugated second serum.

strains, the rβ_3-AR Ab revealed a staining for the endothelial layer. A light and discontinuous signal was observed in the endothelial layer of WKY aorta. Conversely, a strong, large and continuous labelling was stained in the endothelial layer of SHR aorta. Furthermore, a light and diffuse signal was observed with the same distribution and intensity in smooth muscle layers of both strains.

On the basis of the results showing an upregulation of β_3-AR, we determined whether β_3-AR-induced effect was potentiated in SHR rats. Then, CCRC to SR 58611A, a preferential β_3-AR agonist, was performed. In SHR intact aortic rings, SR 58611A (0.1–30 μM) induced a concentration-dependent relaxation ($E_{max} = 60.8 \pm 1.6\%$; $n = 13$), which was similar to that obtained in WKY rats ($E_{max} = 55.8 \pm 1.6\%$; $n = 16$) (Figure 8).

Discussion

In the present study, CGP 12177, a low-affinity state β_1-AR agonist (with β_1-/β_2-AR antagonistic and partial β_3-AR agonistic properties) produced an endothelium-independent relaxation of aortic rings isolated from normotensive WKY rats. This finding corroborates results obtained in Wistar rat aorta, where the relaxation to CGP 12177 was found to be mainly endothelium-independent (Brawley *et al*., 2000b). Furthermore, we have shown that the relaxant effect of CGP 12177 was not modified by pretreatment with L-NMMA, an NO synthase inhibitor, suggesting that an endothelial NO pathway was not involved. Thus, present results showed that CGP 12177 activated a receptor located in vascular smooth muscle cells.

CGP 12177 has been demonstrated to activate an atypical β-AR in adipose tissue (Granneman, 2001), in heart (Kaumann *et al*., 2001) and in blood vessels such as aorta (Brawley *et al*., 2000b) and carotid artery (Oriowo, 1994; MacDonald *et al*., 1999). In the present work, the endothelium-independent effect of CGP 12177 was not modified after β_1-/β_2-AR or β_3-AR

blockade, but was inhibited by CGP 20712A, a low-affinity state β_1-AR antagonist at high concentration. Altogether, those results suggested that CGP 12177 acted through the interaction with a β-AR pharmacologically distinct from the well-characterized β_1-, β_2- and β_3-AR subtypes. Using hamster cells stably expressing either human β_1-AR, human β_2-AR or rat β_1-AR, Pak & Fishman (1996) have already found that CGP 12177 behaved as an agonist of β_1-AR in a lower affinity, guanine nucleotide-sensitive state precoupled to G_s proteins. Only the high-affinity uncoupled receptors recognized CGP 12177 as an antagonist. Recent evaluation of recombinant β-AR subtypes, as well as the characterization of the β-adrenergic response of several β-AR-deficient mice, have established the identity of the atypical β-AR as a novel state of the β_1-AR (Kaumann *et al*., 2001) and CGP 12177 produced its agonistic effects by interacting with the lower affinity state of the β_1-AR.

It should be noted that in rat aorta, Brahmadevara *et al*. (2003) proposed that the CGP 12177-mediated relaxation resulted from an α_1-AR antagonism, but not from the activation of the low-affinity state of β_1-AR. However, in the present study, in endothelium-denuded aortic rings of WKY, CGP 12177-induced relaxation was significantly decreased in the presence of adenylyl cyclase inhibitors, suggesting that a cAMP pathway was partly involved in the CGP 12177-mediated relaxation rather than an effect on α_1-AR. The role of cAMP in the response to CGP 12177 has also been reported in rat aorta (Brawley *et al*., 2000b), in rat isolated atria (Kaumann & Lynham, 1997), in guinea-pig taenia caecum (Koike *et al*., 1995) and in mouse adipose tissue (Konkar *et al*., 2000).

Conversely to normotensive rats, the removal of endothelium in aortic rings from SHR greatly reduced the relaxant effect of CGP 12177. Furthermore, adenylyl cyclase inhibitors did not modify the remaining endothelium-independent relaxation to CGP 12177 in SHR aorta. An increase of expression and/or activity of G_i proteins has been shown to occur in hypertension and could contribute to the alteration of the cAMP pathway (Anand-Srivastava, 1996). Interestingly, in our study, the inhibition of $G_{i/0}$ proteins by PTX partly restored the CGP 12177-induced endothelium-independent relaxation. Two hypotheses might explain this effect: (i) the inhibition of G_i proteins restored the adenylyl cyclase activity and/or (ii) the low-affinity state of β_1-AR could be linked to G_i proteins in SHR. Concerning the latter, it has already been shown in a rat model of heart failure, where there was an increase of G_i proteins, that the cardiostimulatory effect of CGP 12177 was lost. However, this cardiostimulatory effect was restored by blockade of G_i proteins with PTX treatment, suggesting that the low-affinity state of β_1-AR was linked to G_i proteins in the rat's failing heart (Kompa & Summers, 1999). Thus, the increased expression and/or activity of G_i proteins could be a mechanism to protect the cardiovascular system against a sustained activation of the sympathetic nervous system, during the hypertensive state. Nevertheless, this compensation could be deleterious by impairing the relaxation induced by activation of receptors linked positively to adenylyl cyclases. It is noticeable that only partial restoration of the low-affinity state of β_1-AR-induced relaxation was achieved by PTX pretreatment. This observation indicates that G_i proteins are not exclusively involved in the impairment of relaxation and other mechanisms could participate in this alteration such as a decrease of the low-affinity state β_1-AR density or an enhanced β-AR kinase activity.

In the present work, the relaxant effect to CGP 12177 was significantly inhibited by a β_3-AR antagonist in SHR, suggesting that CGP 12177 could activate β_3-AR in hypertensive rats. By contrast with a full agonist, a partial agonist, such as CGP 12177, can induce an effect only in a tissue where the receptor density and/or the coupling efficiency are high. Therefore, the unveiled β_3-AR effect in SHR aorta should be related to a high density and/or a high coupling efficiency of β_3-AR. In this respect, the immunohistochemical analysis has revealed the presence of an upregulation of β_3-AR in the endothelial layer of SHR aorta. However, experiments performed with a full β_3-AR agonist, SR 58611A, demonstrated similar relaxant responses in WKY and SHR, indicating that β_3-AR-mediated responses were not enhanced in hypertensive rats. In the same way, in rat brown adipose tissue, the increase in the blood flow in response to a β_3-AR agonist, BRL 26830A, was found to be unaltered in SHR compared to WKY (Iwase *et al*., 2000). As β_3-AR-mediated relaxation is mainly NO-dependent (Trochu *et al*., 1999), the decrease of the NO bioavailability observed in SHR aorta (Kerr *et al*., 1999) might explain why the functional response to SR 58611A was not enhanced.

In conclusion, our results showed an impaired relaxation of aortic rings, induced by the stimulation of the low-affinity state of the β_1-AR by CGP 12177 in 12-week-old SHR. We also demonstrated, for the first time, an upregulation of the β_3-AR expression in an animal model of hypertension. However, this upregulation was not associated with an increase in the β_3-adrenergic-induced vasorelaxation. As the aorta is a conductance vessel, the results obtained in the present study could not be extended to the resistance vessels. Then, additional experiments will be needed to investigate the role of the low-affinity state of β_1-AR in the resistance vessels in hypertension.

In clinics, decreased vascular resistance by vasodilating β-blockers, known as third-generation drugs, are associated with β_3-AR agonism (nebivolol, bucindolol), β_2-AR activation (celiprolol), α-AR blockade (bucindolol), antioxidant activity (carvedilol), potassium channel opening properties (tilisolol) or NO synthesis (Strosberg, 1997; Gosgnach *et al*., 2001; de Groot *et al*., 2003; Toda, 2003). The relaxant effect demonstrated in our work through the activation of the low-affinity state of β_1-AR could represent another possible mechanism underlying vasorelaxant actions of vasodilating β-blockers. In this respect, carvedilol or alprenolol have already been described to activate the human low-affinity state β_1-AR in CHO cells (Baker *et al*., 2003). This activation might include intracellular changes at the short term of secondary messenger and/or at long term of gene transcription. However, it remains to be established in clinical studies whether the beneficial effects of these β-AR antagonists can be partly attributed to the activation of low-affinity state of β_1-AR in blood vessels.

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