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Influence of adrenodemedullation on β_2- and β_3-adrenoceptors mediating relaxation of oesophageal smooth muscle of spontaneously hypertensive rats

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1 In oesophageal smooth muscle strips from spontaneously hypertensive rats (SHR) of 8-10 and 22-24 weeks of age, respectively, β-adrenoceptor-mediated relaxation was investigated, by use of the β-agonists, (-)-isoprenaline and fenoterol (both in the absence and presence of the β_2-selective antagonist ICI 118,551) and the selective β_3-agonist, BRL 37,344.

2 In preparations from 8-10 week SHR, (-)-isoprenaline- and fenoterol-induced concentration-response curves (CRCs) were hardly antagonized by ICI 118,551 at concentrations up to 1 μM, indicating only a minor contribution of β_2-adrenoceptors. pA_2-values for ICI 118,551 of 5.30 ((-)-isoprenaline as agonist) and 5.46 (fenoterol as agonist), estimated from the shifts at the highest (10-100 μM) antagonist concentrations, are consistent with affinity at a β_3-adrenoceptor, similar to that in Wistar rat oesophageal smooth muscle.

3 In 8-10 week SHR, adrenodemedullated at 4 weeks of age (SHR-ADM4) the potency of fenoterol was markedly increased and CRCs were shallow. In addition, ICI 118,551 (0.1 μM) now produced a clear rightward shift accompanied by a steepening of the CRC. A marked further shift was observed only at 100 μM of the antagonist. The data are compatible with the involvement of both β_2- and β_3-adrenoceptors.

4 In 22-24 week animals, the same differences between SHR and SHR-ADM4 were observed with fenoterol as in 8-10 week animals, though β-adrenoceptor responsiveness was slightly decreased. The potency of ICI 118,551 at β_3-adrenoceptors ($pA_2 = 5.11$) was significantly different from the pA_2 value of 5.46 obtained with the younger animals.

5 Responses to the β_3-adrenoceptor agonist, BRL 37,344, were similar in Wistar rat and SHR preparations. In 8-10 week SHR, a small decrease in the maximal response was observed, which in animals of 22-24 weeks of age was accompanied by a small decrease in the pEC_{50} value as well.

6 The results clearly indicate that β_2-adrenoceptors in SHR oesophageal muscularis mucosae are desensitized, whereas β_3-adrenoceptor-mediated responses are unaffected and similar to the responses observed in the Wistar rat oesophagus. The functional presence of β_2-adrenoceptor-responses in SHR-ADM4 suggests a major role for adrenal-derived adrenaline in the desensitization of the β_2-adrenoceptor-population.

Keywords: β_2- and β_3-adrenoceptors; rat oesophagus; SHR; β-adrenoceptor desensitization; adrenal demedullation

Introduction

Essential hypertension is a disease state in which sympathetic activity, adrenoceptor responsiveness, and in some cases adrenoceptor densities are altered (for a review see Michel *et al.*, 1990). These changes could be important factors in the development and maintenance of the hypertensive state. It has been suggested that an enhanced α-adrenoceptor-mediated vasoconstriction may play a role in the increased peripheral vascular resistance observed in hypertension (Doyle & Fraser, 1961; Mendlowitz, 1973). Furthermore, diminished vasodilatation resulting from a decrease in (vascular) β_2-adrenoceptor responsiveness (Cohen & Berkowitz, 1976; Borkowski & Porter, 1984; Toal & Leenen, 1984) may also contribute to the overall increase in peripheral resistance. Also in other systems, alterations in the number and/or the responsiveness of β-adrenoceptors have been reported in relation to blood pressure elevation (Michel *et al.*, 1993) or cardiac hypertrophy (Böhm *et al.*, 1994).

The spontaneously hypertensive rat (SHR) is widely used to study the factors involved in or contributing to hypertension. Sympathetic activity, as reflected by plasma noradrenaline levels or catecholamine release following sympathetic nerve stimulation, is elevated (Grobecker *et al.*, 1975; Ekas & Lokhandwala, 1981; Hano & Rho, 1989; Remie *et al.*, 1992). This state of increased sympathetic activity may account for the reported alterations in β-adrenoceptor function in various organs (for review see Michel *et al.*, 1990), which, in turn, may contribute to the maintenance of the hypertensive state.

Unlike classical β_1- and β_2-adrenoceptors, β_3-adrenoceptors are less prone to short-term agonist-induced desensitization (Granneman, 1992; Nantel *et al.*, 1993; Liggett *et al.*, 1993), probably due to structural differences within the receptor-molecule itself (Emorine *et al.*, 1991). In contrast, the consequences of long term regulation are more equivocal. Exposure of rats to 4°C for three days, which increases sympathetic nerve activity, reduced adipose tissue β_3-receptor mRNA levels *in vivo*, as did repeated subcutaneous injections of noradrenaline (Granneman & Lahners, 1992). On the other hand, six day noradrenaline infusion in hamster adipose tissue desensitized β_1- and β_2-adrenoceptor-mediated lipolytic responses, but did not alter β_3-adrenoceptor responsiveness (Carpéné *et al.*, 1992), while 6-30 h exposure to isoprenaline in murine 3T3-F442A cells caused an up-regulation of β_3-mRNA (Thomas *et al.*, 1992).

In view of these findings, it was considered of interest to investigate the adaptive effects of chronically elevated catecholamine levels, present in SHR, on the functional re-

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sponses mediated by β_2- and β_3-adrenoceptors which have been shown to co-exist in rat oesophageal muscularis mucosae (De Boer *et al.*, 1993). Oesophageal smooth muscle strips from SHR with developing and established hypertension and SHR adrenodemedullated at 4 weeks of age, were compared in terms of their responses following β-adrenoceptor stimulation.

Methods

Animal care and surgery

Male SHR (45–55 g) underwent surgical bilateral adrenodemedullation under halothane anaesthesia at 4 weeks of age, as described previously (Coppes *et al.*, 1994). After adrenal demedullation the rats had free access to 0.9% (w/v) NaCl in addition to normal drinking water, although they hardly used the saline solution. Tap water and standard laboratory chow were supplied *ad libitum*. SHR that had not been operated upon and Wistar rats were group-housed and given free access to water and standard laboratory chow *ad libitum*. Lights were on from 07 h 00 min to 19 h 00 min.

The animals were killed when they were 8–10 weeks (weighing 240–270 g) or 22–24 weeks old (weighing 400–480 g).

Tissue preparation

Oesophageal smooth muscle strips were prepared as described previously (De Boer *et al.*, 1993). Briefly, male SHR were killed by a blow on the head and exsanguinated. Each oesophagus was rapidly removed and the outer striated muscle layer dissected. Longitudinal strips (5 × 1.5 mm) of the remaining muscularis mucosae were prepared and mounted in 20 ml water-jacketed organ baths at 37°C, filled with Krebs-Henseleit buffer solution, composed of (mM): NaCl 117.5, KCl 5.6, $MgSO_4$ 1.18, $CaCl_2$ 2.52, NaH_2PO_4 1.28, $NaHCO_3$ 25.0, glucose 5.5, gassed with 95% O_2/5% CO_2, pH 7.4, for isotonic recording under 0.2 g load. After equilibration for a period of at least 30 min, tissues showed neither resting tone nor spontaneous activity throughout the experiments.

Concentration-response curves

After two methacholine concentration-response curves (CRCs), the preparations were contracted with methacholine (1 μM or 3 μM), to induce approximately 50% of the maximal contraction. CRCs to (−)-isoprenaline, fenoterol (both in the absence and presence of the selective β_2-antagonist, ICI 118,551, 0.1 to 100 μM), or BRL 37,344 were then constructed as described previously (De Boer *et al.*, 1993). At the end of each CRC, preparations were washed twice to obtain basal tone again.

All experiments were performed in duplicate each day on individual strips from the same animal, providing one data-set for the mean results.

Data analysis

All CRCs were expressed as a percentage of the methacholine-induced contraction and data are presented as means ± s.e.mean of (n) determinations. Schild plots were constructed according to Arunlakshana & Schild (1959) with agonist dose ratios (DRs) obtained from the individual EC_{50} values with and without antagonist. EC_{50} values in the presence of 100 μM ICI 118,551 were corrected for the spontaneous left-ward shift resulting from the depression of the methacholine-induced contraction by this high antagonist-concentration (De Boer *et al.*, 1993). In cases where the Schild plot was biphasic, the slope was calculated from the steep part of the plot only, subtracting the log (DR − 1) values obtained with low antagonist concentrations (Bond & Clarke, 1988; De Boer *et al.*, 1993). A mean pA_2 value was obtained from individual estimates, using the formula $pA_2 = -\log\{[\text{antagonist}]/(DR-1)\}$ after verifying that the slope of the Schild plot did not deviate significantly from unity (MacKay, 1978).

Statistical analyses were performed using Student's two-tailed t test (unpaired, $\alpha < 0.05$).

Drugs

(−)-Isoprenaline hydrochloride was purchased from Sigma (St. Louis, U.S.A.) and acetyl-β-methylcholine (methacholine) chloride form Aldrich (Milwaukee, U.S.A.). BRL 37, 344 (4-[2-[(2-hydroxy-2-(3-chlorophenyl)ethyl)amino]-propyl]-phenoxyacetic acid), ICI 118,551 (erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamino)-butan-2-ol) and (±)-fenoterol hydrobromide were kind gifts from SmithKline Beecham (Epsom, U.K.), Zeneca (Macclesfield, U.K.) and Boehringer Ingelheim (Ingelheim, Germany) respectively. All buffer salts were from Merck (Amsterdam, The Netherlands).

Results

Figure 1 shows the concentration-dependent relaxations to (−)-isoprenaline of preparations from Wistar rats (Figure 1a), and of SHR, 8–10 weeks of age (Figure 1b). The potency of (−)-isoprenaline in inducing relaxation of SHR-oesophageal muscularis mucosae ($pEC_{50} = 7.07 \pm 0.10$ (6)) was significantly ($P < 0.001$) lower than in Wistar rat oesophagus (7.64 ± 0.07 (7)). In the presence of the β_2-selective antagonist, ICI 118,551, at concentrations of 0.1 and 1 μM, only a minor rightward shift of the (−)-isoprenaline-CRC was observed in SHR, in contrast to a clear rightward shift in Wistar rat. Only at a high concentration of 100 μM of the antagonist, were the CRCs of (−)-isoprenaline significantly shifted further. The slopes of the Schild plots obtained at 10 and 100 μM of ICI 118,551 were 1.12 ± 0.05 (SHR) and 1.09 ± 0.09 (Wistar), not different from unity in either case; pA_2-values of 5.30 ± 0.08 (10) and 5.31 ± 0.10 (13), respectively, were calculated.

To investigate the effects of increased sympatho-adrenal activity on the β_2- and β_3-adrenoceptor populations in animals with developing and established hypertension, respectively, we constructed concentration-response curves to fenoterol using SHR of 8–10 and 22–24 weeks of age.

In 8–10 week SHR, as with (−)-isoprenaline, relaxations to fenoterol ($pEC_{50} = 5.81 \pm 0.06$ (8)) were hardly antagonized by ICI 118,551 at concentrations up to 1 μM. Only at the higher concentrations was a pronounced shift to the right observed (Figure 2a). The slope of the corresponding Schild plot (1.08 ± 0.03) was not significantly different from unity, yielding a pA_2-value of 5.46 ± 0.06 (13).

However, when fenoterol-induced relaxations were studied in SHR of the same age which had been adrenodemedullated at 4 weeks of age (SHR-ADM4), a different picture emerged (Figure 2b). Not only had the potency of fenoterol increased dramatically ($pEC_{50} = 7.06 \pm 0.21$ (4)), but also the shape of the CRC had changed from steep to shallow. In addition, with ICI 118,551 at 0.1 μM, a clear rightward shift, accompanied by a steepening of the CRC was observed. A marked further shift was observed only at 10 and 100 μM of the antagonist, represented by the steep part of the Schild plot with a slope of 1.00 ± 0.09 and concomitant pA_2 value of 5.36 ± 0.13 (8).

At 22–24 weeks of age, the potency of fenoterol in inducing relaxation of SHR oesophageal smooth muscle was further decreased to 5.59 ± 0.07 (8) compared to 8–10 week old animals. Again, only with 100 μM ICI 118,551, a pronounced rightward shift of the control-CRC was found (Figure 3a). The slope of the corresponding Schild plot (1.02 ± 0.05) was not significantly different from unity. However, the resulting pA_2-value of 5.11 ± 0.09 (15) was significantly different from the value of 5.46 ± 0.06 (13) for antagonism of fenoterol-induced relaxation in 8–10 weeks old SHR ($P < 0.01$). In contrast, the position of the CRC induced by fenoterol in 22–24 week old SHR, demedullated

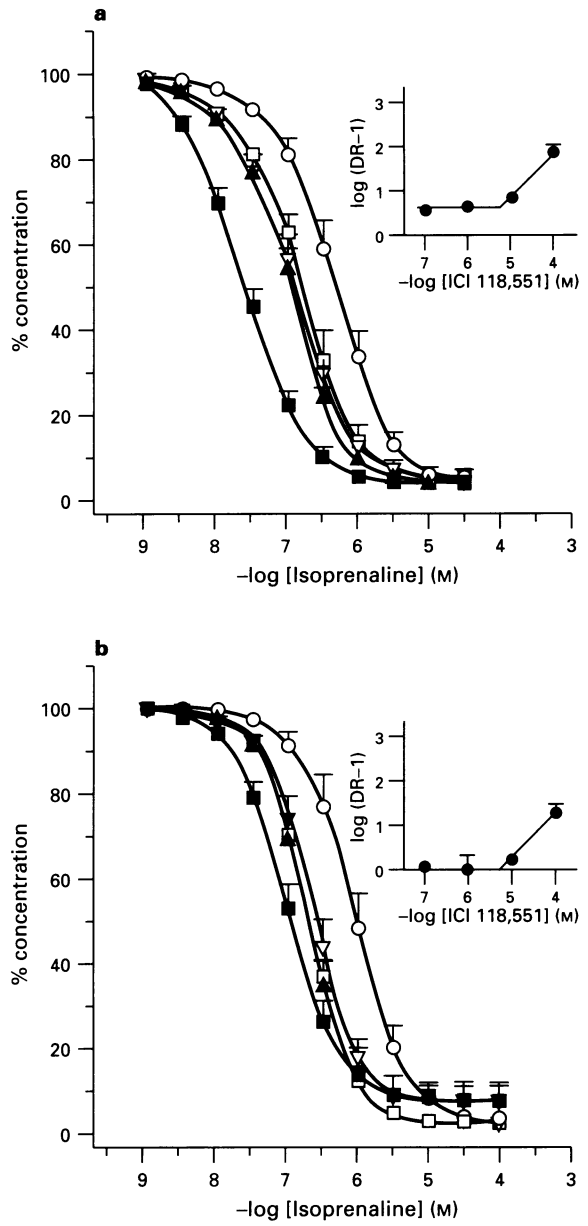

Figure 1 Antagonism of (−)-isoprenaline-induced relaxation of oesophageal muscularis mucosae from 8–10 week old Wistar rats (a) and SHR (b) by ICI 118,551. Control (■), ICI 118,551 100 nM (▲), 1 μM (▽), 10 μM (□), and 100 μM (○). Shown are the mean of five to eight experiments, each performed in duplicate. The inset shows the corresponding Schild plot.

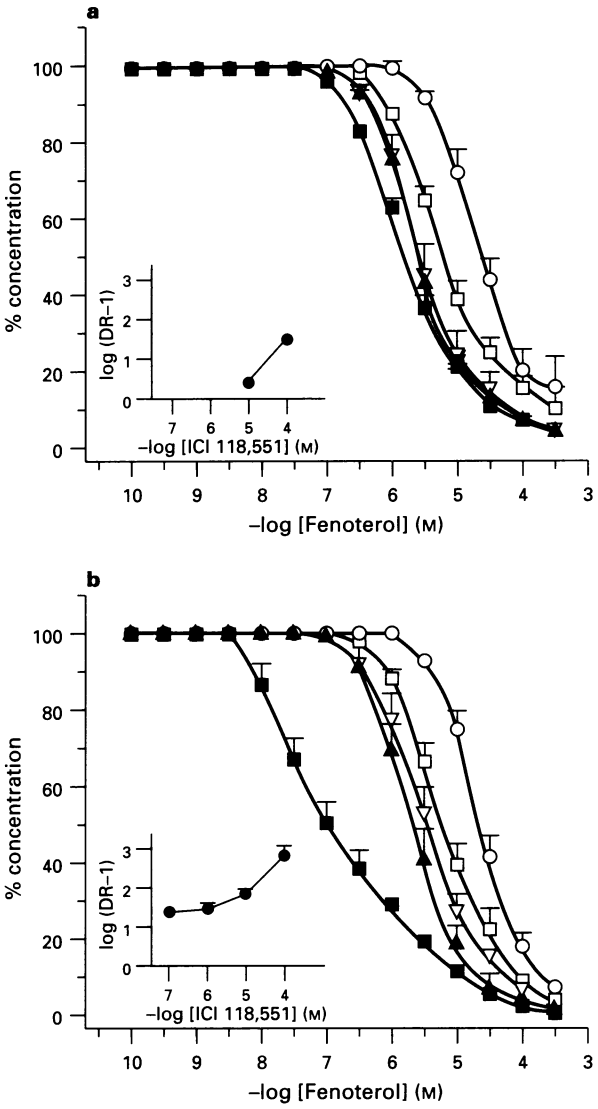

Figure 2 Antagonism by ICI 118,551 of fenoterol-induced relaxation of oesophageal muscularis mucosae from 8–10 week old SHR (a) and 8–10 week old SHR which were adrenodemedullated 4 weeks after birth (b). Control (■), ICI 118,551 100 nM (▲), 1 μM (▽), 10 μM (□), and 100 μM (○). Shown are the mean of four to eight experiments, each performed in duplicate. The inset shows the corresponding Schild plot.

at 4 weeks of age, again was markedly to the left of the CRC in (non-demedullated) SHR of the same age (Figure 3b). Also with ICI 118,551 a picture similar to the findings in the younger SHR-ADM4 animals were obtained, though the final part of the Schild plot had a slope significantly different from unity (0.79±0.05 (8)). Antagonism of the fenoterol-induced relaxations by ICI 118,551 in preparations from the 4 groups of animals is summarized in Figure 4, demonstrating the effects of demedullation and increasing age.

Relaxations to the β_3-adrenoceptor agonist, BRL 37,344 in 8–10 week old SHR were similar to relaxations in age-matched Wistar rats, though E_{max} values were slightly (but significantly, $P<0.01$) lower in SHR (Figure 5). It should be mentioned that the relaxation observed at the highest concentrations of BRL 37344 (10 and 100 μM) is nonspecific and not caused by β_2- (or β_3-) adrenoceptor activation (De Boer *et al.*, 1993). In 22–24 week old SHR, E_{max} values were further decreased accompanied by a small decrease in pEC_{50}-value (8.04±0.16 (5) vs. 8.47±0.02 (4)).

Discussion

The spontaneously hypertensive rat (SHR), originating as a hypertensive mutant of the Wistar-Kyoto strain (Okamoto & Aoki, 1963) is a widely used animal model for the study of hypertension. Hypertension development gradually starts at about 4 weeks of age to stabilize at 16 weeks (Borkowski, 1991).

In the present study, we have investigated the β-adrenoceptor-mediated relaxant responses of oesophageal smooth muscle of SHR at 8–10 weeks (developing hypertension) and 22–24 weeks (established hypertension) of age. Many investigations have focused on the densities and functionality of α- and β-adrenoceptors in multiple organs of SHR, particularly in heart, vascular smooth muscle, and kidney (Michel *et al.*, 1990). Though data on β-adrenoceptor number in hy-

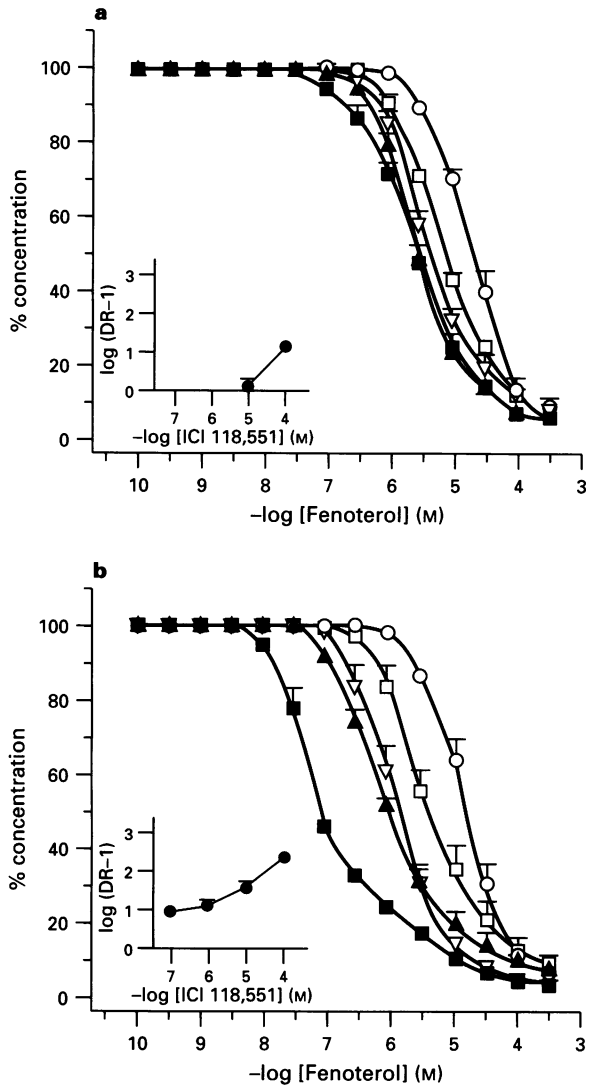

Figure 3 Antagonism by ICI 118,551 of fenoterol-induced relaxation of oesophageal muscularis mucosae from 22–24 week old SHR (a) and 22–24 week old SHR which were adrenodemedullated 4 weeks after birth (b). Control (■), ICI 118,551 100 nM (▲), 1 μM (▽), 10 μM (□), and 100 μM (○). Shown are the mean of four to eight experiments, each performed in duplicate. The inset shows the corresponding Schild plot.

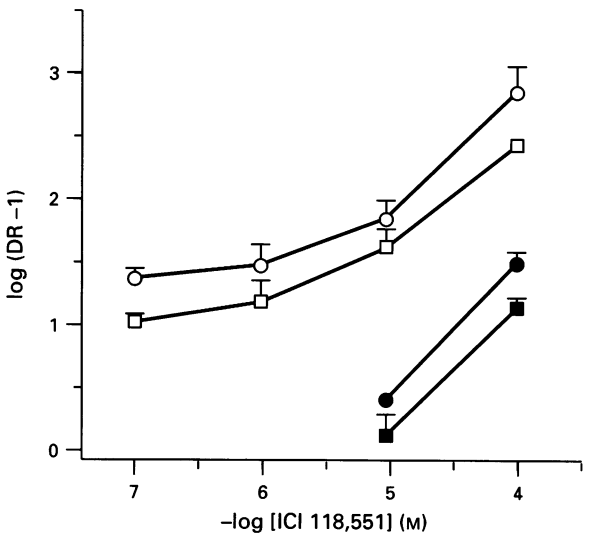

Figure 4 Summary of the Schild plots derived from the antagonism of fenoterol-induced relaxation of oesophageal muscularis mucosae by ICI 118,551 in SHR (●, ■) and SHR, adrenodemedullated at 4 weeks after birth (○, □), at 8–10 weeks (circles) and 22–24 weeks (squares) of age.

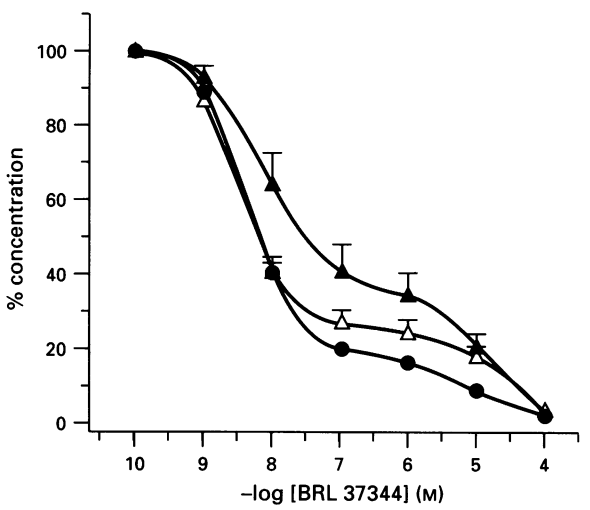

Figure 5 BRL 37,344-induced relaxations of oesophageal muscularis mucosae from 8–10 week old Wistar rat (●), 8–10 week old SHR (△) and 22–24 week old SHR (▲). Shown are the mean of 4–5 (SHR) or 16 (Wistar rat) experiments, each performed in duplicate.

pertensive hearts are rather controversial, the majority of studies on renal β-adrenoceptors report elevations of β-adrenoceptor number (Struyker-Boudier *et al.*, 1986; Michel *et al.*, 1987; 1993), whereas β-adrenoceptor functioning in vascular smooth muscle generally is decreased (Limas & Limas, 1979; Feldman, 1987 and references therein).

We have shown previously that in Wistar rat oesophageal muscularis mucosae, β-adrenoceptor-mediated relaxations involve mainly β_3-, but also β_2-adrenoceptors (De Boer *et al.*, 1993). The contribution of both receptor-subtypes to the (−)-isoprenaline-induced relaxation is shown in the presence of increasing concentrations of the selective β_2-adrenoceptor antagonist, ICI 118,551: a clear shift to the right at the lowest antagonist-concentrations, representing blockade of the β_2-adrenoceptor population, followed by a substantial further shift to the right only at the high concentration of 100 μM, resulting from antagonism of the β_3-adrenoceptors (Figure 1a). In 8–10 week old SHR, however, the initial shift at low concentrations ICI 118,551 (up to 1 μM) is only very minor, indicating that β_2-adrenoceptors are hardly involved in the (−)-isoprenaline-induced relaxation (Figure 1b). Indeed, the low pEC_{50}-value for isoprenaline in SHR (7.07±0.10) is similar to the value of 6.94±0.10 for isoprenaline-induced relaxation in the presence of 1 μM ICI 118,551 (a concentration that occupies virtually all β_2-adrenoceptors) in Wistar rats. However, the clear rightward shift at 100 μM ICI 118,551 demonstrates the functional presence of the β_3-adrenoceptors, both in SHR and Wistar rats. Furthermore, from the similar pA_2-values, it can be concluded that the nature of the β_3-adrenoceptors has not changed during hypertension development. Also with fenoterol as the agonist in 8–10 week old SHR, a similar pA_2-value was found. Again, at most a very minor involvement of a β_2-adrenoceptor was indicated: the control CRC was steep and no clear rightward shift at low antagonist-concentrations occurred. Furthermore, the pEC_{50}-value of 5.81±0.06 was identical to the value of 5.79±0.15 for fenoterol-induced relaxation in the presence of 1 μM ICI 118,551 obtained in the Wistar rat (De Boer *et al.*, 1993).

Thus, it appears that in 8–10 week SHR oesophageal smooth muscle, β_2-adrenoceptors are desensitized, whereas the β_3-adrenoceptor response may have remained unaffected. Since sympathetic activity, as well as plasma concentrations of noradrenaline and adrenaline are elevated in SHR (Grobecker *et al.*, 1975) this may well contribute to the apparent desensitization of the β_2-adrenoceptors. It has been reported that depletion of plasma adrenaline by bilateral adrenal demedullation attenuates the development of hypertension in young SHR (Borkowski & Quinn, 1983; 1985), which could be restored by subcutaneous implantations of adrenaline depots (or other non-catecholamine β_2-adrenoceptor agonists), supporting a prohypertensive effect of adrenaline. More recently, only SHR demedullated at 4–6 weeks of age were found to display an attenuated development of hypertension, whereas demedullation at 7–8 weeks of age, was without effect, suggesting an involvement of the adrenal-derived adrenaline only very early in hypertension development (Borkowski, 1991). Very recently, in patients suffering from phaeochromocytoma, a decrease of surface β_2-adrenoceptor number and response in lymphocytes was reported, which was reversible upon normalization of plasma catecholamine levels after tumour removal (Cases *et al.*, 1995). Furthermore, a strong inverse correlation was found between β_2-adrenoceptor density and the logarithm of plasma adrenaline concentration, but only a weak correlation for noradrenaline, suggesting that β_2-adrenoceptor regulation is mainly dependent on the circulating levels of adrenaline.

Remarkably, adrenodemedullation of SHR at 4 weeks of age completely prevented β_2-adrenoceptor down-regulation in the oesophageal smooth muscle observed at 8–10 weeks of age (Figure 2b). With fenoterol, the typical shallow CRC resulting from the dual stimulation of β_2-adrenoceptors and β_3-adrenoceptors (De Boer *et al.*, 1993) was again obtained, and with ICI 118,551 even at 0.1 μM a profound shift to the right accompanied by a clear steepening of the CRC demonstrates the functional presence of β_2-adrenoceptors. The pEC_{50}-value of fenoterol in the absence of ICI 118,551 was even slightly higher and the rightward shift at the lowest concentration (0.1 μM) of the antagonist significantly greater (DR = 25 vs 6.5) in the SHR-ADM4, compared to Wistar rats. This apparent difference in the contribution of the β_2-adrenoceptor-population may be caused by circulating adrenaline in Wistar rats, which may have led to some desensitization of the β_2-adrenoceptors. In contrast, the pA_2-value of 5.36, calculated from the shifts at antagonist-concentrations above 1 μM indicates no clear alteration in the affinity for the β_3-adrenoceptors. Thus, β_2-adrenoceptors are being desensitized in hypertensive animals, presumably due to elevated plasma levels of adrenaline (80–120 pg ml^{-1} during rest in SHR, 20 pg ml^{-1} or less in Wistar rats; J. Smit, *unpublished observations*) as no desensitization is observed in adrenodemedullated animals. β_3-Adrenoceptor-mediated responses are not altered however, despite the fact that noradrenaline-levels are twice as high in SHR, compared to Wistar rats (Remie *et al.*, 1992) and the noradrenaline-induced relaxation of rat oesophagus smooth muscle is solely mediated by β_3-adrenoceptors (DeBoer *et al.*, 1995). These observations are in line with the findings of Carpéné *et al.* (1992), who found no desensitization of the β_3-adrenoceptor-mediated lipolytic response in hamster adipocytes after long-term infusion of noradrenaline, while β_1- and β_2-receptor-mediated responses were significantly diminished. In contrast, prolonged cold-exposure of rats to enhance sympathetic activity, resulted in a decrease in β_3-receptor mRNA levels (Granneman & Lahners, 1992). However, the relationship between changes in receptor mRNA levels and number and function of β_3-adrenoceptors remains to be elucidated. We found, however, a small, but significant reduction in E_{max}-values of the BRL 37,344-induced relaxations in 8–10 week old SHR compared to Wistar rats, whereas the potency of BRL 37,344 in stimulating the β_3-adrenoceptors in the SHR and Wistar rat was very similar (Figure 5). In 22–24 week old animals with established hypertension, β_2-adrenoceptor responsiveness was further decreased compared to SHR of 8–10 weeks of age. In addition, the β_3-adrenoceptor-mediated response was slightly decreased, witness the smaller rightward shifts of the fenoterol-CRC by ICI 118,551 at all concentrations used. Similar decreases in β-adrenoceptor responsiveness were found in animals of 22–24 weeks, adrenodemedullated at 4 weeks after birth, compared to 8–10 week old SHR-ADM4 (Figure 3 and 4), indicating that these effects do not evolve from prolonged exposure to adrenaline but are merely associated with increasing age. Thus, the smaller DRs in 22–24 week ADM4-animals at low concentrations (0.1–1 μM) of ICI 118,551 (Figure 4), probably involve a decrease in (β_2)-receptor number and/or a change in receptor conformation, in line with previous reports on age-related decreases in β-adrenoceptor responsiveness (Tsujimoto *et al.*, 1986; Deisher *et al.*, 1989; Borkowski *et al.*, 1992), whereas the change in the position of the Schild plot at high (10–100 μM) concentrations of ICI 118,551 rather indicate changes in (β_3-)receptor nature or conformation, as reflected in the (significant) difference in pA_2-values for animals at 22–24 weeks and 8–10 weeks of age (being 5.11 and 5.46, respectively). In accordance with this, relaxations induced by BRL 37,344 also showed a decrease in potency and maximal response (Figure 5).

In conclusion, we have shown that in the oesophageal muscularis mucosae of SHR with developing, as well as established hypertension, β_2-adrenoceptor populations are desensitized. Adrenal demedullation of the animals at 4 weeks of age completely prevented β_2-receptor desensitization, indicating a profound role for adrenaline in desensitizing the β_2-adrenoceptor-mediated responses. In contrast, in 8–10 week old animals, β_3-adrenoceptor-mediated responses were unaffected both in SHR and SHR-ADM4, suggesting a major role for this β-adrenoceptor-subtype, especially under pathophysiological conditons, i.e. when typical (β_2-) adrenoceptor-mediated responses are blunted. In animals of 22–24 weeks of age, a slight decrease in β_3-adrenoceptor responsiveness was observed, both in SHR and in SHR-ADM4, compared to 8–10 week old animals.

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