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Responsiveness, affinity constants and β-adrenoceptor reserves for isoprenaline on aortae from normo-, pre- and hypertensive rats

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Abstract

The objective of this study was to determine the responsiveness, affinity constants and β-adrenoceptor reserves for isoprenaline on the isolated aorta in the maturation of normotensive and hypertensive rats. The effects of a very slowly reversible antagonist, bromoacetylalprenololmenthane (BAAM), on the relaxant responses of the aortae of 5- and 14-week-old Wistar Kyoto normotensive rats (WKY) and spontaneously hypertensive rats (SHRs) to isoprenaline were determined. Five-week-old SHRs are pre-hypertensive and the aortic rings are less responsive to isoprenaline than age-matched WKY (pD_2 values: WKY, 8.40; SHRs, 8.03). Similar relaxant responses to forskolin were obtained on the aortae of 5- and 14-week-old WKY and SHRs. The K_A value for isoprenaline at the aortic β_2-adrenoceptors of the 5-week-old WKY was 2.1×10^{-7} M, and similar values were obtained on the aortae of 5-week-old SHR and 14-week-old WKY and SHRs. In the maturation of the WKY aortae from 5 to 14 weeks, there was a reduction in the maximum response, a major loss of sensitivity and a loss of β_2-adrenoceptor reserve for isoprenaline. On 5-week-old SHR aorta, the sensitivity to isoprenaline was 2.5-fold lower, and the β_2-adrenoceptor reserve was less than on age-matched WKY. In the development of hypertension on the SHR aorta from 5 to 14 weeks, there was a reduction in the maximum response to isoprenaline. At 14 weeks, the sensitivity and the β_2-adrenoceptor reserve to isoprenaline were similar, but the maximum responses were lower on the SHR than WKY. As there are differences in pre-hypertensive SHR and age-matched WKY aortic responses to isoprenaline, it is no longer valid to consider that the loss of responsiveness to isoprenaline in hypertension is solely owing to the hypertension. There are no changes in affinity, but major changes in the sensitivity, maximum responses and aortic β_2-adrenoceptor reserves to isoprenaline in the maturation of normotensive and pre-hypertensive aortae.

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Introduction

β_2-Adrenoceptor responsiveness declines on arterioles and some veins between young adulthood and old age in humans and rats (Fleisch 1980; Ford et al 1992). On the rat aorta, the relaxant responses to isoprenaline are reduced with age, whereas the responses to forskolin are not (Kazanietz & Enero 1991), and this suggests that the loss of responsiveness is at the level of the β_2-adrenoceptor or G proteins. The responsiveness to isoprenaline decreases on the Wistar rat aorta during maturation from 5 to 10 weeks old (Borkowski et al 1992). There have been no studies of the affinity and receptor reserves for isoprenaline at the β_2-adrenoceptor during maturation.

β_2-Adrenoceptor responsiveness is selectively reduced on veins and arterioles in human borderline hypertension and hypertension (Feldman 1990; Naslund et al 1990; Feldman et al 1993). On arterioles, the losses of β_2-adrenoceptor responsiveness and reserve in established human and rat hypertension are considered by most to be caused by the hypertension (Brodde & Michel 1992). On spontaneously hypertensive rat (SHR) aortae in hypertension, the responsiveness to isoprenaline is reduced, whereas that to forskolin is not (Asano et al 1988), suggesting that the loss of responsiveness is at the level of the β_2-adrenoceptor or G proteins. The lesser responsiveness to isoprenaline in the SHR may not be caused by the hypertension, as the responsiveness is also lesser on the femoral and superior mesenteric arteries of pre-hypertensive SHRs (Fujimoto et al 1987).

Many studies have focussed on G protein function and β_2-adrenoceptor–G protein coupling in the loss of responsiveness to isoprenaline in ageing and hypertension (Fleisch 1980; Asano et al 1988; Gurdal et al 1995; Feldman & Chorazyczewski 1997), with little consideration of changes at the β-adrenoceptor alone. The aim of our study was to determine the responsiveness, affinity and receptor reserves for isoprenaline at the level of the aortic β-adrenoceptor in the maturation of normotensive and hypertensive rats. We isolated the aorta from 5- and 14-week-old Wistar Kyoto normotensive rats (WKY) and SHRs. At 5 and 14 weeks of age, the SHRs in our colony are pre-hypertensive and hypertensive, respectively (Doggrell & Liang 1998; Nand & Doggrell 1999). We investigated the effects of bromoacetylalprenololmenthane (BAAM), a very slowly reversible β-adrenoceptor antagonist (Surman & Doggrell 1993) on the isoprenaline responses. The data obtained from contractility studies with BAAM can be used to determine affinity constants and percentage receptor occupancy–response relationships for agonists at β-adrenoceptors (Doggrell & Surman 1995; Doggrell et al 1998). A preliminary account of this data was presented to the British Pharmacological Society (Doggrell & Chen 1999).

Materials and Methods

Drugs

The drugs used were BAAM and forskolin (Research Biochemicals Inc.), and (−)-isoprenaline bitartrate (Sigma Chemicals Co.). Isoprenaline was dissolved in distilled water; BAAM (10^{-1} M) and forskolin (10^{-2} M) were dissolved in absolute ethanol.

Animals

Breeding pairs of WKY and Okamoto SHRs were purchased from the Animal Resources Centre, Perth, Western Australia, and then colonies of these rats were established in the Animal Resources Unit, School of Medicine, The University of Auckland. Adult rats were housed, three rats per cage, with free access to standard rat chow and water. The Animal Ethical Committee at the University of Auckland approved the experimental procedures.

Measurement of blood pressure

Male 5- and 14-week-old WKY and SHRs were weighed and then their tail cuff pressure (which approximates systolic blood pressure) was measured using a tail plethysmograph (model 29; Life Sciences Inc). Two similar readings of tail cuff pressure in mmHg were obtained for each rat.

General

Rats were stunned and exsanguinated. The thoracic aorta was removed and placed in Krebs solution saturated with 5% carbon dioxide in oxygen. All of the experiments were performed in the presence of a modified Krebs solution (composition in mM: 116 NaCl; 5.4 KCl; 2.5 $CaCl_2$; 1.2 $MgCl_2$; 1.2 NaH_2PO_4; 22.0 $NaHCO_3$; 11.2 D-glucose), which was bubbled with 5% CO_2 in O_2 at 37°C.

Contractility

Each endothelium-intact thoracic aorta ring of approximately 3 mm in length was suspended in a 5-mL organ bath under 1.5 g tension. Contractile responses were measured isometrically with force displacement transducers (Grass model FTO3.C) and displayed on a polygraph (Grass model 79B). Aortae were equilibrated for 30 min during which time 250 mL Krebs superfused the tissues. Aortae were then treated with 10^{-4} M phenoxybenzamine for 40–50 min to block α-adrenoceptors and the extraneuronal uptake process. The tissues were washed with 250 mL Krebs solution over 30 min and then three series of experiments were performed.

In preliminary experiments, the effects of KCl were evaluated. Each aorta was cumulatively challenged with KCl (at 5, 10, 15 mM), which was added to the bath concentration on a 5-min cycle until a maximum response was reached. Studies had shown that a maximal response to individual concentrations of KCl was ob-

tained in 5 min. The tissues were washed for 60 min with Krebs solution and this produced a relaxation to baseline. Some tissues were then challenged for a second time to increasing concentrations of KCl. This study showed that 40–60% of the maximum response to KCl was obtained with 15 mM KCl on the 5- and 14-week-old WKY and SHRs, and that there were no differences between the first and second challenge to KCl. Other tissues were challenged with 15 mM KCl and the contractions were measured every 5 min for 60 min. This study showed that the contractions to 15 mM KCl were maintained over 60 min.

In the second series, the responses to isoprenaline and forskolin were determined. Tissues were contracted by KCl and when a plateau response had been obtained, a cumulative challenge with isoprenaline was made on a 5-min cycle. The tissues were then washed with 500 mL Krebs solution over 60 min. Superfusion was stopped, and tissues were contracted by KCl and challenged with forskolin on a 10-min cycle.

In the main experiments, the effects of BAAM on the isoprenaline responses were determined. A cumulative challenge with isoprenaline was made on KCl-contracted aortae and then the tissues were washed with 500 mL Krebs solution over 60 min. Tissues were treated with BAAM or with the ethanol vehicle for 30 min and then washed with 500 mL Krebs solution over 60 min. The superfusion was stopped, the tissues allowed to stabilize and a second challenge to KCl and isoprenaline was made. Our studies showed that there was a small non-significant loss of responsiveness to isoprenaline in the second compared with the first challenge. In determining the effects of BAAM, the effects of the second challenge to isoprenaline on the tissues untreated with BAAM were used as controls, in order to account for the small loss of responsiveness to isoprenaline.

At the end of each experiment the aortae were removed from the organ baths, blotted, weighed, and the weights calculated as mg mm^{-1}.

Data assessment

The contraction amplitude induced by KCl before the addition of the relaxant agent was measured. Curves of attenuation in response to isoprenaline and forskolin were calculated in two ways. First, they were calculated as percentage attenuation of the KCl contraction. From this, the maximum attenuation was determined, with abolition of contractile activity being an attenuation of 100%. Slopes of these concentration–attenuation response curves (difference in % attenuation of the response/logarithm molar concentration) were computed by regression line analysis over the steepest part of the concentration–response curve. Second, in order to obtain pD_2 values (the negative logarithm of the molar concentration that causes 50% of the maximum attenuation), responses were calculated as a percentage of the maximum attenuation observed with isoprenaline or forskolin. The pD_2 values were determined from regression line analyses over 20–80% maximum response.

The dissociation constant (K_A) of isoprenaline was determined by the method of Furchgott & Bursztyn (1967). Isoprenaline response curves were obtained from untreated tissues and tissues that had been treated for 30 min with BAAM. The following equation describes the relationship that exists between the concentration–response curve of an agonist before and after partial receptor inactivation with the slowly reversible antagonist BAAM:

$$1/[A] = ((1-q)/qK_A)+1/q[A']$$

where [A] and [A′] are corresponding equieffective concentrations of agonist before and after partial irreversible receptor inactivation, respectively, and q is the fraction of active receptors remaining after partial irreversible blockade. K_A values were determined from plots of the reciprocals of isoprenaline concentration before fractional receptor inactivation (1/[A]) against the reciprocals of the corresponding equieffective concentrations of isoprenaline after receptor inactivation (1/[A′]) for individual curves. Furchgott & Bursztyn (1967) demonstrated that more accurate estimates of K_A values were obtained if only the equieffective concentrations from the linear part of concentration–response curves are used in "double reciprocal" plots. Therefore, we used the equieffective concentrations from the linear part of the curves and these yielded straight lines in accordance with receptor theory. The K_A of isoprenaline was then calculated from the slope and intercept of the resulting "double reciprocal" plots by the following equation:

$$K_A = (\text{slope}-1)/\text{intercept}$$

Fractional β_2-adrenoceptor occupancy by isoprenaline was calculated for each bath concentration studied ([A]) using both the individual and mean dissociation constant (K_A) values obtained from the interaction of isoprenaline with postjunctional β_1-adrenoceptors according to the procedure of Ruffolo (1982). Thus, the following relationship between agonist concentration ([A]) and dissociation constant was used to calculate β_2-adrenoceptor occupancy by isoprenaline:

$$\text{Receptor occupancy}(\%) = ([A]/(K_A+[A]))\times 100$$

The occupancy–response relationships were constructed by plotting the calculated β_2-adrenoceptor occupancy for isoprenaline against the corresponding response from the normalized concentration–response control curve.

The individual values (percentages, slope, pD_2 values, K_A values) obtained from same age group rats were compared by Student's unpaired *t*-test. Comparison between multi-groups involved analysis of variance testing followed by *t*-test. $P < 0.05$ was considered statistically significant. Mean values±s.e.m. were also determined.

Results

Rat and aortae characteristics (Table 1)

WKY bodyweight, aorta weight and tail cuff systolic blood pressure increased between 5 and 14 weeks of age. The WKY and SHRs were age- and weight-matched at 5 and 14 weeks. At 5 weeks, the blood pressures and aorta weights of WKY and SHRs were similar. The 14-week-old SHRs had much higher blood pressures than age-matched WKY. SHR aorta weights increased between 5 and 14 weeks, and at 14 weeks were greater than those of age-matched WKY.

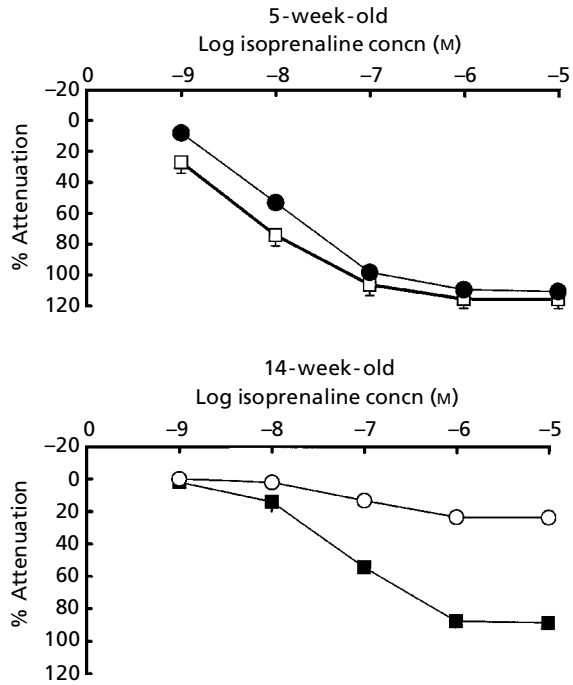

Figure 1 Relaxant effects of isoprenaline on the aortae of 5-week-old WKY (□) and SHR (●) and 14-week-old WKY (■) and SHR (○). Responses are calculated as% attenuation of the KCl contraction and plotted against the negative logarithm of the molar concentration of isoprenaline. Each value is the mean±s.e.m. of 8–14 values. Error bars not shown are within symbol size.

Relaxant responses to isoprenaline and forskolin

Isoprenaline at 10^{-8}–10^{-5} M relaxed the KCl-contracted WKY aortae (Figure 1). In the maturation of the WKY aorta from 5 to 14 weeks, there was a rightward shift of the isoprenaline response curve and a reduction in the maximum responses to isoprenaline (maximal responses: 5-week-old, 115±6%, n = 10; 14-week-old, 88±7%, n = 9; $P < 0.01$). This data, calculated as a percentage of the maximum attenuation to isoprenaline, demonstrated a major loss of sensitivity to isoprenaline on the WKY aorta from 5 to 14 weeks (pD_2 values: 5-week-old, 8.40±0.04, n = 10; 14-week-old, 7.21±0.10, n = 9; $P < 0.01$).

We also studied the responses to isoprenaline on SHR aorta compared with age-matched WKY and showed a lower sensitivity at 5 weeks, and a lower maximum

Table 1 Age, bodyweight, tail cuff blood pressure and thoracic aortae weight.

	Age (days)	Body weight (g)	Blood pressure (mmHg)	Aorta weight (mg mm^{-1})	n
5-week-old					
WKY	37±1	80±3	106±2	0.43±0.02	20
SHR	37±1	81±2	106±2	0.40±0.03	25
14-week-old					
WKY	100±1	264±10#	139±3#	0.69±0.05#	23
SHR	98±1	281±12	202±3*	0.90±0.06*	17

Values are the mean±s.e.m. (n = number of rats). *$P < 0.01$ between age-matched WKY and SHRs. #$P < 0.01$ between 14- and 5-week-old WKY.

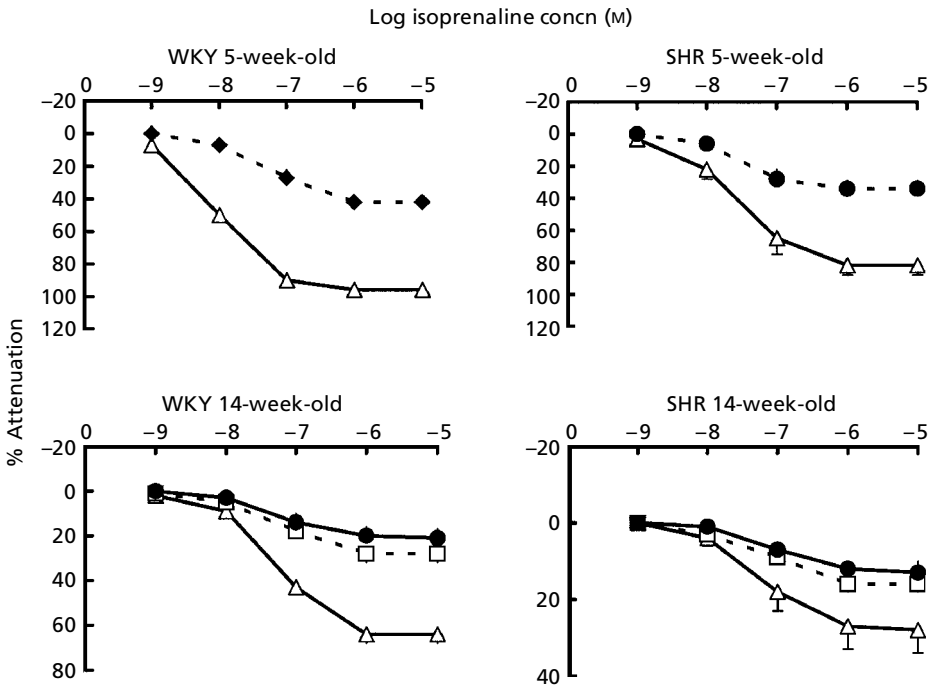

Figure 2 Effects of BAAM on the relaxant responses to isoprenaline on aortae from 5- and 14-week-old WKY and SHRs. Relaxant effects of isoprenaline on the untreated aortae (△) and aortae treated with BAAM at 10^{-7} (□), 3×10^{-7} (●) and 10^{-6} (◆) M. Responses are calculated as% attenuation of the KCl contraction and plotted against the negative logarithm of the molar concentration of isoprenaline. Each value is the mean±s.e.m. of 6–14 values. Error bars not shown are within symbol size.

Table 2 K_A values and percentage receptor occupancy–response relationships.

	K_A values ($\times10^{-7}$) (M)	% Receptor occupancy			n
		20% Response	50% Response	95% Response	
5-week-old					
WKY	2.1±0.7	3±1	9±2	45±8	10
SHR	2.6±0.8	6±1*	22±5*	68±6*	11
14-week-old					
WKY	0.7±0.4	19±2#	43±4#	85±3#	15
SHR	0.9±0.4	20±4	44±6	89±2	9

Values are the mean±s.e.m. (n = number of aortae tested). *$P<0.01$ between 5-week-old WKY and SHRs. #$P<0.01$ between 5- and 14-week-old WKY.

response at 14 weeks (Figure 1). On 5-week-old WKY and SHR aortae, the maximum responses to isoprenaline were similar. On 5-week-old SHR aorta, the sensitivity to isoprenaline was 2.5-fold lower than on age-matched WKY (pD_2 values: WKY, 8.40±0.04, n = 10; SHR, 8.03±0.10, n = 14; $P<0.05$). This hyporesponsiveness to isoprenaline was not apparent on 14-week-old SHR aorta compared with age-matched WKY (pD_2 values: WKY, 7.21±0.01, n = 9; SHR, 7.10±0.11, n = 8; not significantly different).

Forskolin at 10^{-8}–10^{-5} M relaxed the KCl-contracted aortae. The maximum responses and sensitivity to forskolin were not altered by the maturation of the WKY aorta from 5 to 14 weeks (pD_2 values: 5-week-old, 7.98±0.09, n = 9; 14-week-old, 7.74±0.14, n = 8; not significantly different). The aortic responses to forskolin

were also similar on the aortae of 5-week-old WKY and SHR, and were not altered by the onset of hypertension from 5 to 14 weeks in the SHR (pD_2 values: 5-week-old, 7.86±0.08, n = 9; 14-week-old, 7.58±0.15, n = 9; not significantly different).

Effects of BAAM on relaxant responses to isoprenaline

Treatment with BAAM at 1×10^{-7}–1×10^{-6} M for 30 min had no effect on the quiescent aortae of 5- and 14-week old WKY and SHRs (data not shown). This BAAM treatment, followed by washing for 60 min, had no effect on the KCl contractions of the 5- and 14-week-old WKY and SHR aortae (data not shown).

On the 5-week-old WKY aorta, BAAM at 1×10^{-6} M caused non-parallel rightward shifts of the isoprenaline concentration–response curves with a reduction in the maximum responses to isoprenaline (Figure 2). The maximum responses to isoprenaline were 96±3% (n = 8) and 42±4% (n = 14), $P< 0.01$, on the 5-week-old WKY aortae in the untreated and BAAM-treated, respectively. The same treatment with BAAM (1×10^{-6} M) completely abolished the isoprenaline responses on the 5-week-old SHR aorta (data not shown). A lower concentration of BAAM (3×10^{-7} M) was required to cause non-parallel rightward shifts of the concentration–response curves with a reduction in the maximum responses of the 5-week-old SHR aorta to isoprenaline (Figure 2). The maximum responses were 82±6% (n = 7) and 23±6% (n = 9), $P< 0.01$, on the 5-week-old SHR aortae in the untreated and 3×10^{-7} M BAAM-treated, respectively.

On the 14-week-old WKY and SHR aortae, treatment with BAAM at 10^{-7} or 3×10^{-7} M for 30 min caused non-parallel rightward shifts of the isoprenaline concentration–response curves with a reduction in the maximum responses (Figure 2). On the 14-week-old aortae, similar K_A values and β_2-adrenoceptor occupancy–response relationship results were obtained with 10^{-7} and 3×10^{-7} M BAAM, and the data has been combined.

On the WKY aortae, there was no change in affinity for isoprenaline, and a loss in β_2-adrenoceptor reserve between 5 and 14 weeks. The isoprenaline K_A value was 2.1×10^{-7} M on 5-week-old WKY aorta. Isoprenaline had to occupy about 3, 9 and 45% of the β_2-adrenoceptors on the 5-week-old WKY aorta, and this was increased to 19, 43 and 85% at 14 weeks to produce 20, 50 and 95% of the maximum response, respectively (Table 2).

On the 5- and 14-week-old SHR aortae, the isoprenaline affinity constants were similar to age-matched WKY (Table 2). On the 5-week-old SHR, there was a lesser β_2-adrenoceptor reserve for the responses than on age-matched WKY aortae (Table 2). By 14 weeks, there was a similar β_2-adrenoceptor reserve on WKY and SHR aortae (Table 2).

Discussion

We have previously shown that the 5- and 14-week-old SHRs in our colony are pre-hypertensive and hypertensive, respectively (Doggrell & Liang 1998; Nand & Doggrell 1999). The blood pressure recordings in the present study confirmed this. Hypertension induces hypertrophy of blood vessels subject to systemic pressure (Diez 1992). In the present study, there was a weight gain on 14-week-old, but not 5-week-old, SHR aorta. This weight gain may represent hypertrophy or hyperplasia.

The responsiveness of the rat aorta to forskolin is not altered in ageing (Kazanietz & Enero 1991) or hypertension (Asano et al 1988). In addition, our study shows that the responsiveness to forskolin on the aorta is similar in maturation, and at the onset of hypertension. As there are no changes in responsiveness to forskolin, the difference in responses to isoprenaline that we observed in maturation, pre-hypertension and hypertension cannot be owing to any non-selective changes in contractility, and must have occurred at the level of the β_2-adrenoceptor or G protein.

This is the first study to report a major loss of sensitivity, a reduction in maximum, and a loss of β_2-adrenoceptor reserve to isoprenaline in the maturation of WKY aorta from 5 to 14 weeks. A previous study has shown a similar loss of sensitivity and maximum response in the maturation of the Wistar aorta from 5 to 15 weeks (Borkowski et al 1992). There are further losses in sensitivity and maximum responses to β_2-adrenoceptor stimulation with ageing (Fleisch 1980). One implication of this finding is that in the evaluation of drugs that have β_2-adrenoceptor-mediated effects, the animals should be age-matched.

Low concentrations of noradrenaline contract and then higher concentrations stimulate the aortic β_2-adrenoceptors to reverse the contraction (Arribas et al 1994). There is a lesser reversal of the noradrenaline contractions on the 5-week-old SHR than age-matched WKY aorta (Arribas et al 1994), and this suggests a lower responsiveness to β_2-adrenoceptor stimulation on pre-hypertensive SHR aorta. This was confirmed in the present study showing a lower sensitivity to isoprenaline

on pre-hypertensive SHR compared with age-matched WKY aortae.

A previous study showed no difference in the sensitivity to isoprenaline on phenylephrine-contracted aortae of pre-hypertensive SHRs compared with age-matched Wistar rats (Borkowsi et al 1992). A major difference between our study and that of Borkowski et al (1992) is the use of different control rats: WKY and Wistar, respectively. Other differences are the pre-contracting agent and magnitude of contraction. Both the pre-contracting agent and the magnitude of contraction influence the vasodilator potencies of drugs (O'Donnell & Wanstall 1987). We pre-contracted to 50% maximum KCl contraction, and the sensitivity to KCl was similar on 5- and 14-week-old WKY and SHR aortae. Differences in pre-contractions to 40 mM KCl are responsible for apparent differences in relaxations to isoprenaline between WKY and SHR mesenteric resistance arteries (Blankesteijn et al 1996). Borkowski et al (1992) pre-contracted to 80% maximum phenylephrine response, and the sensitivity to phenylephrine was different on 5-week-old Wistar and SHR aortae. This difference in sensitivity to phenylephrine may have affected the relaxant responses to isoprenaline.

We are not the first to report a lower responsiveness to isoprenaline on SHR pre-hypertensive vessels. A lower responsiveness to isoprenaline also occurs on the femoral and superior mesenteric arteries of pre-hypertensive SHRs (Fujimoto et al 1987). The lower responsiveness to isoprenaline of pre-hypertensive SHR vessels cannot be caused by the hypertension, but is associated with the onset of hypertension.

The major aim of the present study was to determine whether there were any differences in the affinity and β_2-adrenoceptor reserves for isoprenaline in maturation, pre-hypertension and hypertension by investigating the effects of BAAM. To be valid, the K_A values derived from contractility studies with very slowly reversible antagonists should be independent of the concentration of antagonist (Kenakin 1987). In the present study, similar results were obtained with two concentrations of BAAM on the 14-week-old aortae, and this validates our use of BAAM to derive K_A values.

Similar K_A values ($1–2 \times 10^{-7}$ M) for isoprenaline were obtained on the 5- and 14-week-old WKY and SHR aortae. These values are similar to those previously reported for β_2-adrenoceptors on the portal veins of WKY and SHR (Doggrell & Surman 1995). This suggests that the binding sites of the β_2-adrenoceptor for isoprenaline on the aorta are not different in maturation, pre-hypertension and hypertension. Radioligand studies have previously demonstrated that the dissociation constants for iodocyanopindolol at the β-adrenoceptors in 13-week-old WKY and SHR femoral and mesenteric arteries are similar (Asano et al 1991).

On the 5-and 14-week-old SHR aortae, a lower concentration of BAAM was required to cause non-parallel rightward shifts of the isoprenaline–response curves than on the 5-week-old WKY aorta. As the K_A values were similar on 5- and 14-week-old aortae, this suggests that there is a lower β_2-adrenoceptor reserve for isoprenaline on 5-week-old SHR and 14-week-old WKY and SHR aortae than on 5-week-old WKY aorta. Our calculations showed a lesser β_2-adrenoceptor reserve for isoprenaline on the aorta of 5-week-old SHR than age-matched WKY aorta. A lesser number of β-adrenoceptors has previously been demonstrated on the pre-hypertensive/borderline hypertensive SHR aorta (Limas & Limas 1979).

There was some loss of receptor reserve on both the WKY and SHR between 5 and 14 weeks resulting in similar β_2-adrenoceptor reserves for isoprenaline being present on 14-week-old WKY and SHR aortae. This result is in agreement with radioligand binding studies of β-adrenoceptor numbers in other blood vessels of similarly aged SHRs. The 13-week-old WKY and SHR femoral and mesenteric arteries have a similar number of β-adrenoceptor binding sites (Asano et al 1991).

Despite there being few studies of the SHR in pre-hypertension, it is widely accepted that the changes in responses mediated by β_2-adrenoceptors in hypertension are caused by the hypertension (Brodde & Michel 1992). Our study shows that there are considerable differences in the isoprenaline/β_2-adrenoceptor responses before hypertension, which are not apparent in early hypertension. These are a lesser sensitivity and a lower β_2-adrenoceptor reserve for isoprenaline on the aorta of the pre-hypertensive SHR compared with age-matched WKY, and these differences are lost in early hypertension. The differences in isoprenaline/β_2-adrenoceptor responses in pre-hypertension may be distinct, or pre-determinants of the differences in early hypertension. The major difference in isoprenaline/β_2-adrenoceptor responses in early hypertension was a reduction in the maximum response in the hypertensive SHR compared with the age-matched WKY.

In conclusion, a major finding of the present study is that there are differences in pre-hypertensive SHR and WKY aortic responses to isoprenaline. Thus, the widely held view that the loss of responsiveness at β_2-adrenoceptors in hypertension is solely owing to the hypertension is no longer valid. Another major finding of the present study is the substantial changes in the sensitivity, maximum responses and aortic β_2-adrenoceptor reserves

to isoprenaline in the maturation of normotensive and pre-hypertensive aortae. Further studies will be required to determine whether this finding is limited to the aorta or observed in other vascular tissue.

References

Arribas, S., Marin, J., Ponte, A., Balfagon, G., Salaices, M. (1994) Norepinephrine-induced relaxations in rat aorta mediated by endothelial beta adrenoceptors. Impairment by ageing and hypertension. *J. Pharmacol. Exp. Ther.* **270**: 520–570

Asano, M., Masuzawa, K., Matsuda, T., Asano, T. (1988) Reduced function of the stimulatory GTP-binding protein in beta adrenoceptor-adenylate cyclase system of femoral arteries from spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.* **246**: 709–718

Asano, M., Masuzawa, K., Matsuda, T., Asano, T. (1991) Decreased responsiveness to beta-adrenoceptor agonists in arterial strips from spontaneously hypertensive rats is not associated with alterations in beta-adrenoceptors. *J. Hypertens.* **9**: 607–613

Blankesteijn, W. M., Raat, N. J., Willems, P. H., Thien, T. (1996) β-Adrenergic relaxation in mesenteric resistance arteries of spontaneously hypertensive and Wistar-Kyoto rats: the role of precontraction and intracellular Ca^{2+}. *J. Cardiovasc. Pharmacol.* **27**: 27–32

Borkowski, K. R., Gros, R., Schneider, H. (1992) Vascular β-adrenoceptor-mediated responses in hypertension and ageing in rats. *J. Auton. Pharmacol.* **12**: 389–401

Brodde, O.-E., Michel, M. C. (1992) Adrenergic receptors and their signal transduction mechanisms in hypertension. *J. Hypertens. Suppl.* **10**: S133–145

Diez, J. (1992) Cardiovascular growth associated with arterial hypertension. *J. Cardiovasc. Pharmacol.* **20** (Suppl. B): S1–S5

Doggrell, S. A., Chen, Y.-Y. (1999) β-Adrenoceptors in the maturation of aortae from normo- and pre-hypertensive rats. *Br. J. Pharmacol.* **128**: 85P

Doggrell, S. A., Liang, L. (1998) The relaxing effect of BDF 9148 on the KCl-contracted aorta from normo- and hyper-tensive rats. *Naunyn Schmiedebergs Arch. Pharmacol.* **357**: 126–132

Doggrell, S. A., Surman, A. J. (1995) Loss of maximum attenuation and receptor reserve for isoprenaline at the β_2-adrenoceptors of the portal veins of hypertensive rats. *J. Hypertens.* **13**: 1023–1029

Doggrell, S. A., Petcu, E. B., Barnett, C. (1998) Affinity constants and β-adrenoceptor reserves for isoprenaline on cardiac tissue from normo- and hypertensive rats. *J. Pharm. Pharmacol.* **50**: 215–223

Feldman, R. D. (1990) Defective venous β-adrenergic response in borderline hypertensive subjects is corrected by a low sodium diet. *J. Clin. Invest.* **85**: 647–652

Feldman, R. D., Chorazyczewski, J. (1997) G-protein function is reduced in hypertension. *Hypertension* **29**: 422–427

Feldman, R. D., Freeman, D. J., Bierbrier, G. S., Anthony, S. E., Brown, J. E. (1993) β-Adrenergic responsiveness is regulated selectively in hypertension. *Clin. Pharmacol. Ther.* **54**: 654–660

Fleisch, J. H. (1980) Age-related changes in the sensitivity of blood vessels to drugs. *Pharmacol. Ther.* **8**: 477–487

Ford, G. A., Hoffman, B. B., Vestal, R. E., Blaschke, T. F. (1992) Age-related changes in adenosine and β-adrenoceptor responsiveness of vascular smooth muscle in man. *Br. J. Clin. Pharmacol.* **33**: 83–87

Furchgott, R., Bursztyn, P. (1967) Comparison of the dissociation constants and of relative efficacies of selective agonists acting on parasympathetic receptors. *Ann. N. Y. Acad. Sci.* **144**: 882–889

Fujimoto, S., Dohi, Y., Aoki, K., Asano, M., Matsuda, T. (1987) Diminished β-adrenoceptor-mediated relaxation of arteries from spontaneously hypertensive rats before and during development of hypertension. *Eur. J. Pharmacol.* **136**: 179–187

Gurdal, H., Friedman, E., Johnson, M. D. (1995) Beta-adrenoceptor-G alpha S coupling decreases with age in rat aorta. *Mol. Pharmacol.* **47**: 722–728

Kazanietz, M. G., Enero, M. A. (1991) Decreased beta-adrenoceptor-mediated vasodilation in aorta from aged rats: possible involvement of a stimulatory GTP-binding protein. *Eur. J. Pharmacol.* **198**: 177–181

Kenakin, T. P. (1987) *Pharmacologic analysis of drug–receptor interaction.* Raven Press, New York

Limas, C. J., Limas, C. (1979) Reduced number of β-adrenergic receptors in hypertensive vessels. *Biochim. Biophys. Acta* **582**: 533–536

Nand, V., Doggrell, S. A. (1999) The effects of BDF 9148 on the action potentials and contractions of the left ventricle from normand hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* **26**: 212–220

Naslund, T., Silberstein, D. J., Merrell, W. J., Nadeau, J. H., Wood, A. J. J. (1990) Low sodium intake corrects abnormality in β-receptor mediated arterial vasodilation in patients with hypertension: correlation with β-receptor function in vitro. *Clin. Pharmacol. Ther.* **48**: 87–95

O'Donnell, S. R., Wanstall, J. C. (1987) Choice and concentration of contractile agent influence responses of rat aorta to vascular relaxant drugs. *J. Pharm. Pharmacol.* **39**: 848–850

Ruffolo, R. R. (1982) Important concepts of receptor theory. *J. Auton. Pharmacol.* **2**: 277–295

Surman, A. J., Doggrell, S. A. (1993) Alprenolol and bromoacetylalprenololmenthane (BAAM) are slowly reversible antagonists at the β_1-adrenoceptors of the rat left atria. *J. Cardiovasc. Pharmacol.* **21**: 35–39