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Affinity Constants and β-Adrenoceptor Reserves for Isoprenaline on Cardiac Tissue from Normotensive and Hypertensive Rats

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Abstract

To determine whether there are differences in cardiac β-adrenoceptor responsiveness, isoprenaline affinity constants and fractional β-adrenoceptor occupancy–response relationships for isoprenaline in the early stages of established hypertension, we studied the effects of bromoacetylalprenololmenthane (BAAM) and ([3,5-diamino-6-chloro-*N*-(1[*N*-β-(2-hydroxyl-3-α-naphthoxypropylamino)ethylcarbamoyl]-1-methylethyl)-pyrazine-2-carboxamide (ICI 147 798), slowly reversible β-adrenoceptor antagonists, on the isoprenaline responses of the left ventricular papillary muscle and the left and right atria of 6-month-old Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR).

The papillary muscles, but not the right and left atria, of the SHR were less responsive to isoprenaline than those of the WKY. The isoprenaline pD_2 values (the negative logarithms of the molar concentrations of agonist producing 50% of the maximum response) were 7·72 and 8·00 on the SHR and WKY papillary muscles, respectively. On the WKY papillary muscle the isoprenaline K_A values were $2–3 \times 10^{-6}$ M, which is as expected for isoprenaline at β_1 or β_2-adrenoceptors. Isoprenaline had 100-fold greater affinity on the WKY and SHR left atria than on the papillary muscles; the isoprenaline K_A values were $2–4 \times 10^{-8}$ M. On the WKY papillary muscle and left atrium, isoprenaline had to occupy 3–4% of the β-adrenoceptors to produce a 50% maximum response; on the WKY papillary muscle and left atrium isoprenaline had to occupy 25–35% and 55%, respectively, of the β-adrenoceptors to produce a 90% maximum response.

The SHR papillary muscles and left atrium had smaller β-adrenoceptor reserves for isoprenaline than did the WKY tissues. We were unable to obtain isoprenaline K_A values on the WKY right atrium. The isoprenaline K_A value on the SHR right atrium was $1–4 \times 10^{-8}$ M.

Because the isoprenaline K_A values for the left and right atria are markedly different from those previously reported for isoprenaline at β_1 or β_2-adrenoceptors, we suggest that atypical β-adrenoceptors might be present on the atria of WKY and SHR. We have also demonstrated a lower β-adrenoceptor reserve on SHR papillary muscle and atria in the early stages of established hypertension.

Cardiac β-adrenoceptor responsiveness might be reduced in established hypertension in man and rat. Some functional studies indicate impaired contractility in response to β-adrenoceptor agonists in the papillary muscle or whole heart of spontaneously hypertensive rats (SHR) (Bohm et al 1988; Mertens et al 1992). Although the decrease in ventricular β-adrenoceptor responsiveness becomes pronounced in hypertension-induced heart failure of SHR or man (Brodde & Michel 1992), it is not known whether this reduced ventricular β-adrenoceptor responsiveness is present in the hearts of SHR or man soon after the hypertension has been established. It has been reported that right atrial responsiveness to isoprenaline does not change in the early stages of SHR hypertension (Dyke et al 1989). There are no reports of studies of left atrial β-adrenoceptor responsiveness in hypertension as it progresses to heart failure.

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There is a loss of β-adrenoceptors on the myocardium in advanced hypertension-induced hypertrophy in man (Harding et al 1994). It is not clear whether this loss of β-adrenoceptors is confined to the ventricles or extends to the atria. The stage of the hypertension process in man or rat associated with the decrease in the cardiac β-adrenoceptor numbers is also unknown.

Several characteristics of β-adrenoceptors can be determined from contractility studies using irreversible β-adrenoceptor antagonists to remove a proportion of the β-adrenoceptors. Thus, after studying the effects of an irreversible or slowly reversible antagonist on concentration–response curves to an agonist, at concentrations that have no other actions, K_A values (affinity constants) of the agonist can be determined and the percentage receptor occupancy for each response to the agonist can be calculated.

The first aim of this study was to determine whether there is a difference between the β-adrenoceptor responsiveness of the papillary muscles from the left ventricles and from the right and left atria of Wistar Kyoto rats (WKY) and SHR in the early stages of established hypertension-induced hypertrophy of the SHR left ventricle. SHR hypertension is fully established and maintained from 14–18 weeks onwards (McGuire & Tweitmeyer 1985). We determined the potency of isoprenaline on the papillary muscles, the right and left atria of 6-month-old WKY and SHR. The second part of the study was to determine the K_A values and receptor occupancy–response relationships for isoprenaline on cardiac tissue when the SHR were in the early stages of established hypertension-induced hypertrophy by studying the effects of two very slowly reversible β-adrenoceptor antagonists, bromoacetylalprenololmenthane (BAAM) and ([3,5-diamino-6-chloro-*N*-(1[*N*-β-(2-hydroxyl-3-α-naphthoxypropylamino)ethylcarbamoyl]-1-methylethyl)pyrazine-2-carboxamide (ICI 147 798), on the isoprenaline responses of the left ventricular papillary muscles and atria of 6-month-old WKY and SHR. We have previously used BAAM and ICI 147 798 to determine isoprenaline K_A values (Doggrell & Surman 1995). A preliminary account of the current data has been published elsewhere (Doggrell et al 1995).

Materials and Methods

Drugs

ICI 147 798 ([3,5-diamino-6-chloro-*N*-(1[*N*-β-2-hydroxyl-3-α-naphthoxypropylamino)ethylcarbamoyl]-1-methylethyl)pyrazine-2-carboxamide, donated by ICI, was dissolved in equimolar tartaric acid. BAAM (bromoacetylalprenololmenthane) was from Research Biochemicals and atropine sulphate, guanethidine sulphate and (−)-isoprenaline bitartrate were from Sigma. A solution of BAAM (10^{-2} M) was prepared in absolute ethanol.

Rats

Breeding pairs of Wistar Kyoto rats (WKY) and Okamoto spontaneously hypertensive rats (SHR) were purchased from the Animal Resources Centre, Perth, Western Australia and colonies of these rats were established in the Animal Resources Unit, School of Medicine, University of Auckland. Adult rats were housed three to a cage with free access to standard rat chow and water.

Measurement of body weight and blood pressure

Six-month-old male WKY or SHR were weighed and the systolic blood pressure was measured by use of a tail plethysmograph (11TC Life Sci Model 29). To achieve this the rats were placed in a perspex holding cylinder and left in the dark for 30 min, during which time they routinely went to sleep. The occlusion cuff was placed around the tail which had been warmed to 37 °C under a reading light. The tail cuff was inflated to 250 mm Hg so that the arterial pulse displacements were no longer apparent. The pressure was gradually reduced until the pulse was observed on the chart recorder, and the pulse point was recorded as the tail cuff pressure. Three readings were taken for each rat; these were usually very similar and were averaged.

Contractility experiments

Rats were stunned, exsanguinated and the heart was rapidly removed and placed in Krebs solution saturated with 5% CO_2 in oxygen. Two papillary muscles from the left ventricle or the right and left atria were freed by dissection. All experiments were performed in the presence of modified Krebs solution (composition mM: NaCl, 116; KCl, 5·4; $CaCl_2$, 2·5; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $NaHCO_3$, 22·0; D-glucose, 11·2) at 37 °C which was bubbled with 5% CO_2 in oxygen. Contractile responses were measured isometrically with force-displacement transducers (Grass model FT03.C) and displayed on a polygraph (Grass model 79B). The rate responses of the right atrium were also monitored by means of a tachygraph (Grass model 7P 44B). In each series of experiments the individual values obtained (percentages, slopes, pD_2, K_A) were subject to Student's paired or unpaired *t*-test, as appropriate. Comparison between values from WKY and SHR were made by Student's unpaired *t*-test or by analysis of variance as appropriate.

Differences were considered significant when $P < 0{\cdot}05$. Mean values ± s.e.m. were also determined.

Separate experiments were performed with left ventricle papillary muscles, and right or left atria. The method for studying the effect of irreversible β-blocking on the contractile responses of the electrically driven left atrium is the same as that used previously in our research and has been reported elsewhere (Doggrell 1990). The left atrium was halved. Papillary muscles and atrium halves were mounted longitudinally between two platinum electrodes (approximately 3 cm apart, above and below the tissue). Papillary muscles and the right atrium were mounted under 0·5 g tension and left atrium halves under 1 g tension in 5-mL organ baths containing Krebs solution (with 10^{-5} M guanethidine, to prevent the release of noradrenaline from nerve endings, and 10^{-6} M atropine, to block responses mediated by muscarinic receptors) and left to equilibrate for 75 min. During the equilibration period 500 mL Krebs solution superfused the tissue. The superfusion was then stopped. Stimulation of the papillary muscles or left atrium halves at 4 Hz (5 ms duration, 30 V) and recording of right atrium rate was commenced and, after 6 min, a cumulative challenge with isoprenaline was initiated with further additions of agonist on a 3-min cycle or, if a maximum response to that concentration was not obtained in 3 min, until a maximum response was obtained. The cycle was continued until an overall maximum response was obtained. Stimulation was then stopped and the tissues were then washed by superfusing with 500 mL Krebs solution for 60 min. Some were then treated with BAAM or ICI 147 798 for 30 min while others were treated with vehicle. Tissues were then washed with 500 mL Krebs solution for 60 min. This was followed by a second stimulation or recording of basal rate and challenge to isoprenaline. At the end of the experiment the tissues were removed from the organ baths and the hearts were blotted and weighed.

Assessment of data

For the papillary muscles and left atrium the contractile responses to cardiac stimulation and the combined maximum responses to cardiac stimulation and isoprenaline were expressed as mg tension. Basal right atrium rate and the maximum rate in the presence of isoprenaline were expressed as beats min^{-1}. Also the cardiac force or basal rate responses just before the second challenge with isoprenaline were calculated as a percentage of the response before the first challenge with isoprenaline. If the responses to cardiac stimulation alone or the basal rate were significantly different for treated and untreated tissues the percentage difference was calculated.

The overall responses in the presence of isoprenaline were calculated as a percentage of the overall maximum force or rate response from the first challenge to isoprenaline. If the cardiac stimulation force or basal rate responses for treated and untreated tissues were not significantly different the isoprenaline responses were separated by subtracting the responses just before the addition of isoprenaline from the overall response. The responses to isoprenaline alone were then calculated as a percentage of the maximum force or rate response to isoprenaline during the first challenge. pD_2 values (negative logarithms of the molar concentrations of agonist producing 50% of the maximum response) were obtained from the first challenge to isoprenaline and slopes of concentration–response curves were determined for both isoprenaline challenges. The slope (difference in percentage maximum of the response per unit of logarithm of molar concentration of agonist) and the pD_2 value were computed by regression line analysis over the steepest part of the concentration–response curve.

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 or ICI 147 798. The concentration–response curve of an agonist before and after partial receptor inactivation with the irreversible antagonists BAAM or ICI 147 798 was expressed as the equation:

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

where [A] and [A′] are corresponding equi-effective 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 are determined from plots of the reciprocals of isoprenaline concentration before fractional receptor inactivation (1/[A]) against the reciprocals of the corresponding equi-effective 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 only if the equi-effective concentrations from the linear part of concentration–response curves were used in 'double reciprocal' plots. Consequently we used the equi-effective concentrations from the linear part of the curves and these yielded straight lines in accord with receptor theory. The K_A of isoprenaline was

then calculated from the slope and intercept of the resulting 'double reciprocal' plots by use of the equation:

$$K_A = (\text{slope} - 1)/\text{intercept} \quad (2)$$

Fractional β_1-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_1-adrenoceptor occupancy by isoprenaline:

$$\% \text{ receptor occupancy} = ([A]/K_A + [A]) \times 100 \quad (3)$$

The occupancy–response relationships were constructed by plotting the calculated β_1-adrenoceptor occupancy for isoprenaline, against the corresponding response from the concentration–response curve.

Results

Characteristics of rats and hearts

The WKY (n = 74) and SHR (n = 68) were age-matched; the ages were 170 ± 5 and 168 ± 5 days, respectively ($P > 0.05$). The systolic blood pressures of the SHR, 200 ± 2 mm Hg, were much higher than those of the WKY rats 131 ± 2 mm Hg ($P < 0.0001$). The hearts of the SHR were heavier than those of the WKY; the weights, as a percentage of body weight, were 0·2812 ± 0·0010% and 0·3440 ± 0·0020%, respectively ($P < 0.01$).

Contractile responses of the left papillary muscle

The magnitude of the contractile responses to cardiac stimulation and the maximum response to isoprenaline in the presence of cardiac stimulation were similar for WKY (n = 28) and SHR (n = 23) papillary muscles. For the WKY and SHR muscles the responses to cardiac stimulation were 555 ± 65 and 493 ± 75 mg, respectively ($P > 0.05$) and the isoprenaline maximum responses in the presence of cardiac stimulation were 1206 ± 139 and 1232 ± 175 mg, respectively ($P > 0.05$). The sensitivity of papillary muscle to isoprenaline was less for the SHR (pD_2 7·72 ± 0·06) than for WKY (8·00 ± 0·07) ($P < 0.005$).

Left papillary muscle; effects of BAAM and ICI 147 798

After treatment of the WKY (n = 12) or SHR (n = 13) left papillary muscle with BAAM at 10^{-5} M for 30 min and washing for 60 min, the responses to cardiac stimulation were not altered but the combined submaximum and maximum responses to cardiac stimulation and isoprenaline were reduced (Figure 1). BAAM resulted in non-parallel rightward shifts of the isoprenaline response curves (Figure 1); thus the slopes for untreated and BAAM-treated WKY papillary muscles were 106 ± 13 and 45 ± 5, respectively ($P < 0.001$) and those for untreated and BAAM-treated SHR papillary muscles were 99 ± 11 and 41 ± 4, respectively ($P < 0.0001$). The data from the individual isoprenaline curves from BAAM-treated tissue were used to determine the K_A (affinity constant) values for isoprenaline. The K_A values for isoprenaline were calculated from equation 2 and were not significantly different for the WKY and SHR. The isoprenaline K_A values were $2.79 \pm 0.51 \times 10^{-6}$ and $2.06 \pm 0.29 \times 10^{-6}$ M ($P > 0.05$) for the left ventricular papillary muscles from WKY and SHR, respectively. For each bath concentration of isoprenaline, the receptor occupancy (%) was calculated from equation 3 using individual K_A values. The relationship between receptor occupancy and response for isoprenaline showed that there was a smaller β-adrenoceptor reserve for the 50% response to isoprenaline for SHR left papillary muscle than for that of WKY (Figure 2). Thus isoprenaline produced a half-maximum response by occupying 4 and 8% of the

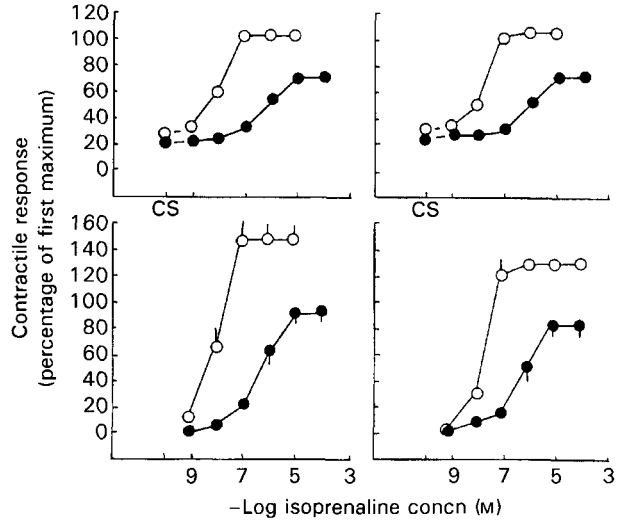

Figure 1. The effects of BAAM on the contractile responses of WKY (left) and SHR (right) left papillary muscle to cardiac stimulation and isoprenaline. Top, responses to cardiac stimulation (CS) and then cardiac stimulation and isoprenaline; bottom, responses to isoprenaline of untreated tissue (○) and of tissue treated with BAAM at 10^{-5} M (●). The responses were calculated as a percentage of the maximum responses during the first, before treatment, challenge and plotted against the logarithm of the molar concentration of isoprenaline. Each value is the mean ± s.e.m. of results from 12 or 13 determinations.

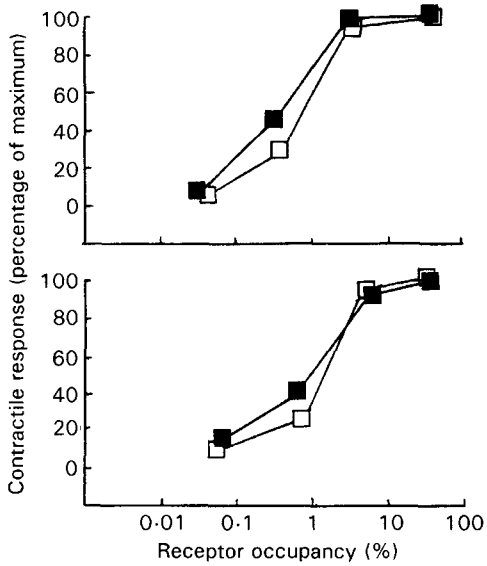

Figure 2. The relationship between receptor occupancy and response to isoprenaline of the left papillary muscle of WKY (■) and SHR (□). Data obtained from the mean K_A value with BAAM (top) and ICI 147 798 (bottom) as the antagonist.

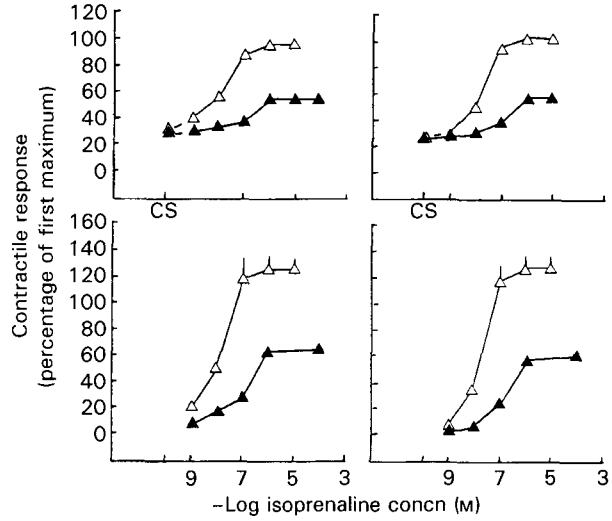

Figure 3. The effects of ICI 147 798 on the contractile responses of WKY (left) and SHR (right) left papillary muscle to cardiac stimulation and isoprenaline. Top, responses to cardiac stimulation (CS) and then cardiac stimulation and isoprenaline; bottom, responses to isoprenaline of untreated tissue (△) and of tissue treated with ICI 147 798 at 10^{-7} M (▲). The responses were calculated as a percentage of the maximum responses during the first, before treatment, challenge and plotted against the logarithm of the molar concentration of isoprenaline. Each value is the mean ± s.e.m. of results from 11–15 determinations.

available β-adrenoceptors on the WKY and SHR left papillary muscles, respectively. Isoprenaline produced a near maximum response (95% maximum) on both WKY and SHR left papillary muscle by occupying between 25–35% of the receptors.

Similar K_A values and receptor occupancy–response relationships were obtained when ICI 147 798 was used as the slowly reversible β-adrenoceptor antagonist. Thus, after treatment of the WKY or SHR left papillary muscle with ICI 147 798 at 10^{-7} M for 30 min and washing for 60 min the responses to cardiac stimulation were not altered but the combined submaximum and maximum responses to cardiac stimulation and isoprenaline were reduced (Figure 3). ICI 147 798 resulted in non-parallel rightward shifts of the isoprenaline response curves (Figure 3); thus the slopes for untreated (n = 15) and ICI 147 798-treated (n = 14) WKY papillary muscles were 91 ± 16 and 47 ± 7, respectively ($P < 0.02$), and those for untreated (n = 11) and ICI 147 798-treated (n = 11) SHR papillary muscles were 92 ± 13 and 44 ± 9, respectively ($P < 0.005$). The isoprenaline K_A values were not significantly different for WKY and SHR papillary muscles nor for the values obtained with BAAM as the β-adrenoceptor antagonist. From the experiments with ICI 147 798 and the muscles from the WKY (n = 12) and SHR (n = 14)s the isoprenaline K_A values were $2.10 \pm 0.49 \times 10^{-6}$ and $2.28 \pm 0.60 \times 10^{-6}$ M, respectively ($P > 0.05$). There was a smaller β-adrenoceptor reserve for the 50% response to isoprenaline on the SHR left papillary muscle than on that from the WKY (Figure 2). Thus isoprenaline produced a half-maximum response by occupying 7 and 14% of the available β-adrenoceptor on WKY and SHR left papillary muscles, respectively. On both the WKY and SHR left papillary muscles, isoprenaline produced a near maximum response by occupying 40–50% of the available β_1-adrenoceptors (Figure 2).

Contractile responses of the right and left atria

For isolated right atria of SHR (n = 42) and WKY (n = 44) the resting SHR heart-rate was lower (330 ± 7 and 297 ± 7 beats min^{-1} ($P < 0.02$) for the WKY and SHR right atria, respectively) but the maximum heart-rate in the presence of isoprenaline was similar (403 ± 6 and 390 ± 5 beats min^{-1} ($P > 0.05$), respectively). The sensitivity to isoprenaline was similar for the WKY and SHR right atria; pD_2 values were 9.16 ± 0.09 and 9.16 ± 0.07, respectively, $P > 0.05$. The force response to cardiac stimulation was greater for SHR (n = 48) than for WKY (n = 52) isolated left atria (148 ± 8 mg and 118 ± 7 ($P < 0.01$), respectively) but the maximum responses in the presence of isoprenaline were similar (211 ± 10 and 195 ± 10 mg($P > 0.05$), respectively). The sensitivity to isoprenaline was similar for the WKY and SHR left atria (pD_2 values

$9{\cdot}28 \pm 0{\cdot}12$ and $9{\cdot}32 \pm 0{\cdot}11$ ($P > 0{\cdot}05$), respectively).

The effects of BAAM and ICI 147 798 on the right atrium

Treatment of the WKY right atrium with BAAM at 3×10^{-6} or 10^{-5} M for 30 min reduced the heart-rate and the submaximum, but not the maximum, response in the presence of isoprenaline, and these data were not suitable for determining isoprenaline K_A values because there were non-selective effects and no depression of the maximum response. In contrast, treatment of the SHR right atrium with BAAM at 3×10^{-6} M for 30 min had no effect on the heart rate and reduced both the submaximum and maximum responses in the presence of isoprenaline. There was a non-parallel rightward shift of the isoprenaline response curve (Figure 4) with the slope being 78 ± 8 for untreated (n = 12) and 49 ± 13 for BAAM-treated (n = 8) tissue ($P < 0{\cdot}05$). The isoprenaline K_A value was $1{\cdot}11 \pm 3{\cdot}01 \times 10^{-8}$ M for the SHR right atrium (n = 8), and isoprenaline had to occupy approximately 5 and 70% of the β-adrenoceptors to induce 50 and 95% of the maximum response (Figure 4).

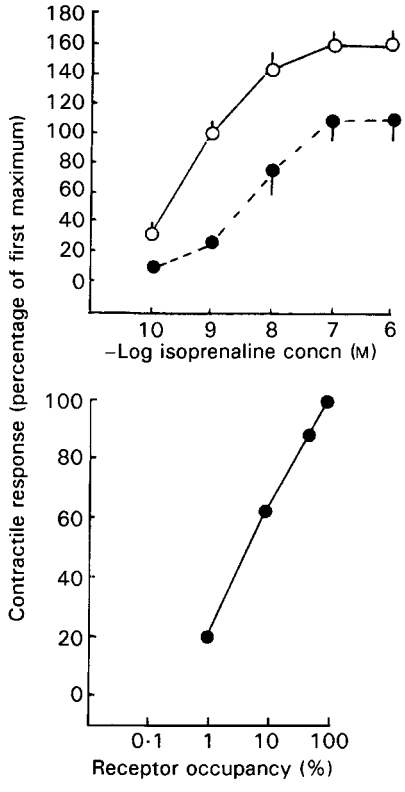

Figure 4. Effect of BAAM on the responses to isoprenaline (top) and receptor occupancy–response relationship (bottom) for the SHR right atrium. Top, responses to isoprenaline from untreated tissue (○; n = 8) and from tissue treated with BAAM at 3×10^{-6} M (●; n = 9). Each value is the mean ± s.e.m. Bottom, data obtained from the mean K_A value with BAAM.

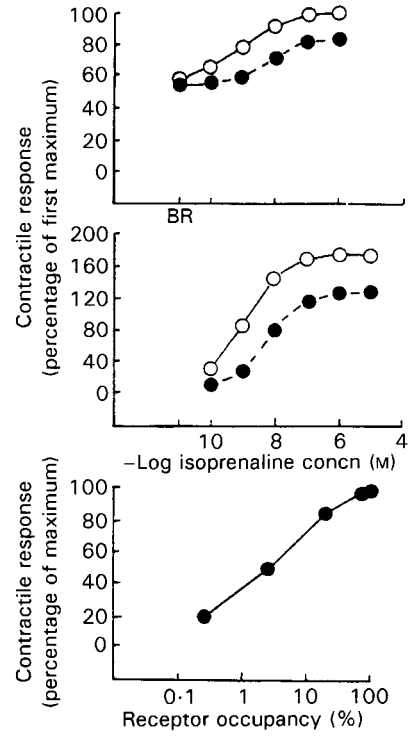

Figure 5. The effect of ICI 147 798 on the contractile responses of the SHR right atrium to cardiac stimulation and isoprenaline, and the receptor occupancy–response relationship. Top, basal heart rate (BR) and then heart rate in the presence of isoprenaline; middle, responses to isoprenaline of untreated tissue (○) and of tissue treated with ICI 147 798 at 10^{-7} M (●). The responses were calculated as a percentage of the maximum responses during the first, before treatment, challenge and plotted against the logarithm of the molar concentration of isoprenaline. Each value is the mean ± s.e.m. of results from 8 or 9 determinations. Bottom, data obtained from the mean K_A value with ICI 147 798.

Treatment of the SHR right atrium with a higher concentration of BAAM (10^{-5} M) caused an additional reduction of the basal heart-rate and these data were not suitable for the determination of K_A values.

Treatment of the WKY right atrium with ICI 147 798 at 3×10^{-9} M for 30 min had no effect on contractions (n = 4, data not shown). Treatment with higher concentrations of ICI 147 798 (10^{-8}, 3×10^{-8} and 10^{-7} M) resulted in reductions of the basal heart rates, and was not suitable for determination of isoprenaline K_A values. In contrast we were able to obtain data suitable for determination of K_A values with ICI 147 798 on the SHR right atrium. Thus treatment with ICI 147 798 at 3×10^{-8} M for 30 min had no effect on the basal heart rate but reduced the maximum response in the presence of isoprenaline (Figure 5). The slopes of the isoprenaline response curves were 80 ± 12 and 55 ± 9 ($P < 0{\cdot}05$), respectively, for untreated tissues (n = 8) and for tissues treated with ICI 147 798 at 3×10^{-8} M (n = 9). The K_A value for the action

of isoprenaline on the SHR right atrium was $3{\cdot}98 \pm 2{\cdot}47 \times 10^{-8}$ M, which was similar to the K_A value obtained using BAAM as the irreversible β-blocker on this tissue. Isoprenaline had to occupy 4% of the β-adrenoceptors to produce a 50% maximum response and 65% of the receptors to cause a near maximum (95%) response (Figure 5); these values were not significantly different from those obtained with BAAM on the SHR right atrium.

The effects of BAAM and ICI 147 798 on the left atrium

Treatment of the WKY left atrium with BAAM at 3×10^{-6} or 10^{-5} M resulted in depression of the basal force without reduction of the maximum response in the presence of isoprenaline. Treatment of the SHR left atrium with BAAM at 3×10^{-6} M had no effect on the basal force or the maximum force in the presence of isoprenaline, whereas BAAM at 10^{-5} M reduced the basal force and the maximum contraction in the presence of isoprenaline. Thus we were unable to obtain isoprenaline K_A values on the WKY or SHR left atria by using BAAM as the irreversible β-blocker. We were able to obtain K_A values on the WKY and SHR left atrium from our ICI 147 798 data. Treatment of the WKY left atrium with ICI 147 798 at 10^{-8} or 10^{-7} M had no effect on the basal force but reduced the maximum response to isoprenaline (Figure 6). The slopes of the isoprenaline curves were 77 ± 7 for untreated tissue ($n = 12$) and 59 ± 4 ($P < 0{\cdot}05$) and 29 ± 9 ($P < 0{\cdot}02$) for tissues treated with ICI 147 798 at 10^{-8} M ($n = 11$) and 10^{-7} M, ($n = 5$), respectively. Data obtained by use of ICI 147 798 at 10^{-8} M ($n = 11$) and 10^{-7} M ($n = 5$) on the WKY left atrium resulted in isoprenaline K_A values of $3.69 \pm 0{\cdot}53 \times 10^{-8}$ M and $3{\cdot}75 \pm 3{\cdot}35 \times 10^{-8}$ M, respectively, values that were not significantly different from each other, or from the values obtained for the SHR right atrium. On the WKY left atrium isoprenaline had to occupy approximately 3 and 55%, respectively, of the β-adrenoceptors to produce a 50 and 95% maximum response (Figure 6).

Treatment of the SHR left atrium with ICI 147 798 at 3×10^{-8} and 10^{-7} M had no effect on the basal force but reduced the isoprenaline maxima (Figure 6). The slopes of the isoprenaline response curves were 66 ± 8 for untreated tissue ($n = 13$) and 47 ± 6 ($P < 0{\cdot}05$) and 14 ± 8 ($P < 0{\cdot}01$) for tissue treated with ICI 147 798 at 3×10^{-8} M ($n = 11$) and 10^{-7} M ($n = 4$), respectively. The marked depression of isoprenaline maximum responses in the presence of ICI 147 798 at 10^{-7} M made these data unsuitable for K_A determination. From the data

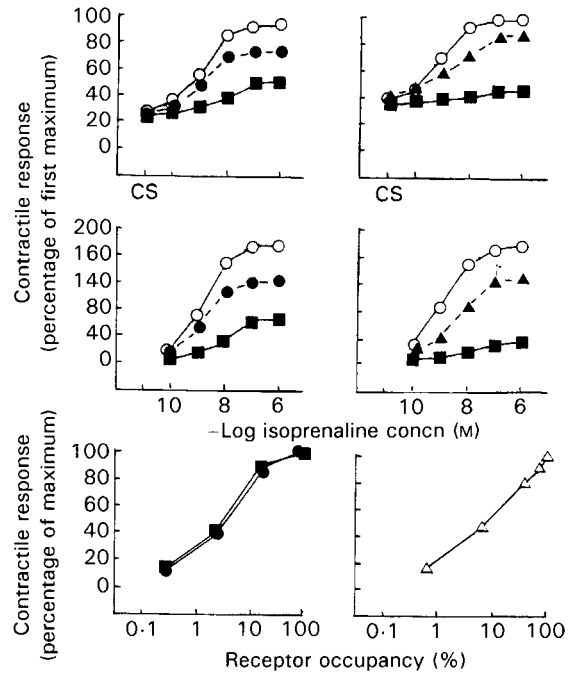

Figure 6. The effects of ICI 147 798 on the contractile responses of WKY (left) and SHR left atrium (right) to isoprenaline, and the receptor occupancy–response relationship. Top, cardiac stimulation response (CS) and then in the presence of isoprenaline; middle, response to isoprenaline of untreated tissue (○) and of tissue treated with ICI 147 798 at 10^{-8} (●), 3×10^{-8} (▲) and 10^{-7} M (■). The responses were calculated as a percentage of the maximum responses during the first, before treatment, challenge and plotted against the logarithm of the molar concentration of isoprenaline. Each value is the mean ± s.e.m. of results from 4–13 determinations. Bottom, data obtained from the mean K_A value with ICI 147 798 at 10^{-8} (●), 3×10^{-8} (△) and 10^{-7} M (■).

obtained with ICI 147 798 at 3×10^{-8} M on the SHR left atrium ($n = 11$), the isoprenaline K_A value was found to be $1{\cdot}65 \pm 2{\cdot}57 \times 10^{-8}$ M, a value not significantly differentfrom that obtained for the WKY left atrium or the SHR right atrium. The receptor reserve for isoprenaline was less on the SHR left atrium than on the WKY left atrium; on the SHR left atrium, isoprenaline had to occupy approximately 9 and 96%, respectively, of the β-adrenoceptors to induce 50% and 95% maximum responses.

Discussion

Hypertrophy is closely associated with hypertension in the SHR (Engelmann et al 1987). In the current study we have demonstrated that the hearts of the 6-month-old SHR are heavier than those of the WKY, indicating that hypertrophy is present.

This study has shown that in the early stages of established hypertension SHR left papillary muscles are slightly less sensitive to isoprenaline than those of the WKY, but that there are no dif-

ferences in the sensitivity of WKY and SHR atria to isoprenaline. Thus the marked decrease in responsiveness to isoprenaline observed in heart failure (Harding et al 1994) must occur either in the later stages of hypertension or in the heart failure. Although the responsiveness of WKY and SHR atria to isoprenaline was not different, there were some differences with hypertension; basal heart-rate was lower and force was greater. To the best of our knowledge this is the first report that left atrium force is greater in hypertension. A previous study (Dyke et al 1989) has suggested that the basal heart-rate is similar for the isolated right atrium of WKY and SHR. The reason for the discrepancy between studies might be that we performed our study in the presence of guanethidine as an extra safeguard to prevent the release of noradrenaline from noradrenergic nerves during stimulation, whereas Dyke et al (1989) did not and consequently could have had a greater leak of noradrenaline in the SHR right atrium than in that from WKY. Another possible explanation for the discrepancy is that we used larger groups (42 and 44) and were able to observe smaller changes than Dyke et al (1989) who used groups of 4–6.

In this study we were able to use concentrations of BAAM and ICI 147 798 without non-specific effects to obtain K_A values on the WKY and SHR papillary muscles and left atria, and SHR right atrium, but not the WKY right atrium. The tissues for which we did determine K_A values had large β-adrenoceptor reserves for near-maximum responses to isoprenaline. On the WKY right atrium large concentrations of the antagonists did not depress the maximum responses to isoprenaline, which suggests that the WKY right atrium has an equal or greater β-adrenoceptor reserve than the other cardiac tissues tested.

There have been few attempts to determine the K_A values of isoprenaline at β_1- or β_2-adrenoceptors using contractility studies with irreversible β-adrenoceptor antagonists, probably because many of the previous antagonists tested have had other actions. We have previously used irreversible antagonists to determine isoprenaline K_A values of approximately 10^{-6} M for isoprenaline at β_1- and β_2-adrenoceptors on cardiovascular tissues, including the left atrium from Wistar rats bred in Auckland (Wistar Auckland rats) (Doggrell 1990; Doggrell & Surman 1995; Doggrell et al 1997). Consistent with these previous findings, we now report K_A values of approximately 10^{-6} M for the action of isoprenaline on the left papillary muscles from WKY and SHR.

The most interesting finding of this study was that the K_A values for the action of isoprenaline on WKY and SHR left atria and SHR right atrium were 100-fold greater than those previously reported at other β_1- or β_2-adrenoceptors, including those of the Wistar Auckland rat left atrium (Doggrell 1990). It has been suggested that differences between SHR and Wistar normotensive rats other than WKY are more likely to be related to hypertension than differences between SHR and WKY because the SHR were derived from WKY, WKY have a high incidence of high blood pressure, and WKY might share with the SHR some of the genes responsible for the hypertension (Louis & Howes 1990). As a greater isoprenaline K_A value is observed for the WKY and SHR left atria but not for the Wistar Auckland rat left atrium, it is possible that this unusual value is related to the presence of hypertension. This indicates that the β-adrenoceptors on the WKY and SHR atrial tissues might be different from the β_1 and β_2-adrenoceptors previously characterized on Wistar rat atria. Previous studies have also highlighted differences between different colonies of normotensive rats (Clineschmidt et al 1970). Other recent studies have suggested that the β-adrenoceptors of the atria of man and rat might be distinct from the β_1, β_2 and β_3-adrenoceptors previously described (Kaumann 1996), and so further characterization of atrial β-adrenoceptors is clearly required. These studies should include radioligand-binding studies and be performed in normotensive and hypertensive animals.

Previous studies have shown that the normotensive rat left atrium and papillary muscles have a large β-adrenoceptor reserve for isoprenaline (Doggrell 1990). The current study confirms this and also demonstrates that the β-adrenoceptor reserve is smaller on the SHR left atrium and papillary muscle in the early stages of established hypertension.

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