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Responsiveness, affinity constants and receptor reserves for serotonin on aortae of aged normotensive and hypertensive rats

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Abstract

We have previously shown that the potency and affinity constants (K_A values) for serotonin (5-HT) are greater, and the 5-HT_{2A}-receptor reserve is lesser, on the aorta of 6-month-old spontaneously hypertensive rats (SHRs) compared with age-matched Wistar Kyoto normotensive (WKY) rats. The present study was undertaken to investigate whether these parameters are altered on the aorta with ageing and as hypertension progresses to heart failure. The effects of phenoxybenzamine on the serotonergic responses of the aortae of 24-month-old WKY rats and SHRs were determined. On WKY rat aorta, ageing from 6 to 24 months was associated with an increase in sensitivity and affinity for serotonin, and a loss of 5-HT_{2A}-receptor reserve. On SHR aorta, ageing from 6 to 24 months was also associated with an increase in sensitivity and affinity for serotonin, but a loss of 5-HT_{2A}-receptor reserve. The sensitivity to serotonin was greater on the 24-month-old SHR aorta (pD_2 6.53) than age-matched WKY rat aorta (pD_2 5.89). On the aorta of the 24-month-old WKY rats, the K_A value for serotonin was 4.5×10^{-6} M, and the receptor occupancies required for 20 and 50% maximum responses were 12 and 29%, respectively. There was a similar affinity, but greater receptor reserves, for serotonin on the aorta of age-matched SHRs. In summary, we have shown changes in sensitivity, affinity and 5-HT_{2A}-receptor reserves for serotonin on the aorta with ageing and in hypertension/heart failure.

Introduction

There have been few studies to determine whether the responsiveness of blood vessels to serotonin (5-HT) changes with ageing. One recent study suggests that the sensitivity of the rat basilar artery to serotonin does not change between 3 and 21 months (Arribas et al 1997). It is not known whether the sensitivity to serotonin changes on other blood vessels with age.

Several studies have demonstrated enhanced 5-HT_{2A}-receptor mediated responsiveness in the early stages of rat models of genetic hypertension (Webb & Vanhoutte 1985). For instance, we have shown that there is a 3-fold greater sensitivity to serotonin on the aorta of 6-month-old spontaneously hypertensive rats (SHRs) than on the aorta of age-matched Wistar Kyoto normotensive (WKY) rats (Doggrell 1995). It is not known whether this increased sensitivity to serotonin on the aorta is maintained as hypertension progresses to heart failure in the SHR.

Changes at the level of receptors may be distinguished by studying the effects of irreversible blockers on the contractile responses to agonists (Kenakin 1987). Following such studies, the affinity constant ($K_A$value) and fractional occu-

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pancy–response relationships may be determined (Kenakin 1987). Our previous study of the effect of phenoxybenzamine on the contractile responses to serotonin showed that there was a greater affinity, but a lesser 5-HT_{2A}-receptor reserve, on 6-month-old SHRs than age-matched WKY rat aortae (Doggrell 1995). It is not known whether these differences are still apparent between the aortae of aged WKY rats and SHRs.

The aim of this study was to determine whether the sensitivity, affinity and the fractional occupancy–response relationships for serotonin were altered by ageing in normotensive and hypertensive rat aortae. We studied the effects of phenoxybenzamine, an irreversible 5-HT_{2A}-receptor antagonist, on the serotonergic response curves of aortae of 24-month-old WKY rats and SHRs. This is the same method as we previously used on the aortae of 6-month-old WKY rats and SHRs (Doggrell 1995), which allows us to compare the two studies.

Materials and Methods

Drugs

The drugs used were phenoxybenzamine HCl (RBI, Natick, USA) dissolved at 10^{-1} M in absolute alcohol containing 10^{-2} M HCl, and serotonin creatinine sulphate (Sigma Chemical Co., St Louis, MO) at 3×10^{-2} M dissolved in distilled water.

Animals

Breeding pairs of WKY rats 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 to a cage with free access to standard rat chow and water.

Aged rats were stunned and exsanguinated. The thoracic aorta was removed and placed in Krebs solution saturated with 5% CO_2 in O_2. All of the experiments were performed in the presence of a modified Krebs solution (composition in 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) bubbled with 5% CO_2 in O_2 at 37°C.

Contractility experiments

Each endothelium-intact thoracic aorta ring, 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 60 min during which 500 mL Krebs solution superfused the tissues. Each tissue was then cumulatively challenged with serotonin. Exposure to each concentration of serotonin was continued for a minimum of 3 min or, if a maximum response was not obtained in 3 min, until a maximum response was obtained. Tissues were allowed to recover in a wash of 500 mL Krebs solution over 60 min. Three of the rings were then treated with different concentrations of phenoxybenzamine for 30 min and one tissue was left untreated. All rings were then washed with 500 mL drug-free Krebs solution over 60 min. The tissues were then challenged with serotonin for a second time. At the end of each experiment the aortae were removed from the organ baths and the length was measured. The tissues were blotted, weighed and the weights calculated as mg mm^{-1}.

Assessment of data

Responses to each concentration of serotonin, in the absence or presence of phenoxybenzamine, were measured and calculated as a percentage of the maximum obtained during the first challenge. pD_2 values (the negative logarithm of the molar concentration that causes 50% of the maximum response) were determined from regression line analyses over 20–80% of the maximum response in the first challenge.

The affinity constant (K_A) of serotonin was determined by the method of Furchgott & Kaumann (1967). Serotonin response curves were obtained from untreated tissues and tissues that had been treated for 30 min with phenoxybenzamine. The following equation describes the relationship between the concentration–response curve of an agonist before and after partial receptor inactivation with the irreversible antagonist phenoxybenzamine:

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

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 serotonin concentration before fractional receptor inactivation (1/[A]) against the reciprocals of the corresponding equieffective concentrations of serotonin after receptor inactivation (1/[A′])

for individual curves. Furchgott & Kaumann (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 accord with receptor theory. The K_A of serotonin 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 α_1-adrenoceptor occupancy by serotonin was calculated for each bath concentration studied ([A]) using both the individual and mean dissociation constant (K_A) values obtained from the interaction of serotonin with 5-HT_{2A}-receptors according to the procedure of Ruffolo (1982). Thus, the following relationship between agonist concentration ([A]) and dissociation constant was used to calculate 5-HT_{2A}-receptor occupancy by noradrenaline or adrenaline:

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

The occupancy–response relationships were constructed by plotting the calculated 5-HT_{2A}-adrenoceptor occupancy for serotonin against the corresponding response from the normalized concentration–response control curve.

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

Results

The WKY rats were slightly older than the SHRs, but not significantly so. The ages of the WKY rats and SHRs were 752 ± 40 days (n = 9) and 703 ± 21 days (n = 10), respectively. The bodyweights of the SHRs were lighter, but the aortae rings were heavier than those of the WKY rats (bodyweight: 424 ± 21 g (n = 9) and 377 ± 15 g (n = 10) ($P < 0.05$); aortae weight: 0.97 ± 0.06 mg mm^{-1} (n = 9) and 1.44 ± 0.17 mg mm^{-1} (n = 10) ($P < 0.01$), for WKY rats and SHRs, respectively).

Contractile responses to serotonin

Serotonin at $10^{-7} - 10^{-5}$ M contracted the aortae of 24-month-old WKY rats and SHRs. The sensitivity to serotonin was greater on the aged SHR aorta than on WKY rat aorta (Figure 1). The pD_2values were 5.89 ± 0.05 (n = 9) and 6.50 ± 0.07 (n = 10) ($P < 0.01$) on 24-month-old WKY rat and SHR aortae, respectively.

On the aged WKY rat aorta, phenoxybenzamine treatment at 10^{-10} M for 30 min had no effect, but at 10^{-8} M it abolished the serotonergic responses (data not shown). Phenoxybenzamine treatment at 5×10^{-10} and 10^{-9} M caused non-parallel rightward shifts of the aged WKY rat aorta serotonergic responses with a reduction in maximum response (Figure 2). The K_A values for serotonin were independent of phenoxybenzamine concentration, and were $2.4 \times 10^{-6} \pm 0.8$ M (n = 9) and $5.8 \times 10^{-6} \pm 1.0$ M (n = 9) with phenoxybenzamine at 5×10^{-10} and 10^{-9} M, respectively, and the combined mean value was 4.5×10^{-6} M. Serotonin produced 20, 50, 95 and 100% maximum responses by occupying 12 ± 2, 29 ± 4, 63 ± 4 and 71 ± 4% (n = 9), respectively, of the aged WKY rat aorta 5-HT_{2A}-receptors.

There was a similar affinity, but a smaller 5-HT_{2A}-receptor reserve, for serotonin on the aged SHR compared with the WKY rat aortae. On the 24-month-old SHR aorta, phenoxybenzamine treatment at 10^{-9} M for 30 min had no effect, but at 10^{-7} M it abolished the serotonergic responses (data not shown). Phenoxybenzamine treatment at 5×10^{-9} and 1×10^{-8} M caused non-parallel rightward shifts of the aged SHR aorta serotonin response curves with a reduction in maximum response (Figure 2). The K_A values for serotonin on the 24-month-old SHR were independent of phenoxy-

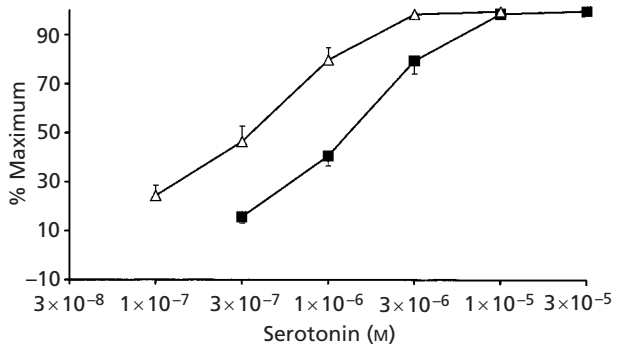

Figure 1 Contractile responses to serotonin on the aortae of 24-month-old WKY rats (■) and SHRs (△). Responses are calculated as % maximum response and plotted against the molar concentration of serotonin. Each value is the mean ± s.e.m. from 9 (WKY) or 10 (SHR) aortae.

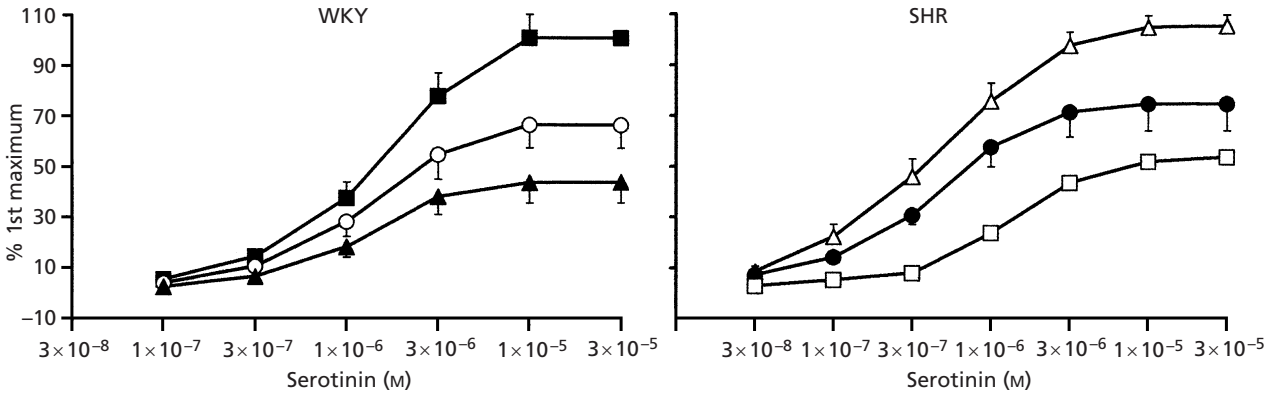

Figure 2 Effect of phenoxybenzamine treatment on the serotonergic responses of 24-month-old WKY rats and SHRs. WKY rats: mean ± s.e.m. from 9 untreated aortae (■) and 9 aortae treated with phenoxybenzamine at 5×10^{-10} M (○) or 10^{-9} M (▲). SHR: mean ± s.e.m. from 10 untreated aortae (△) and 10 aortae treated with phenoxybenzamine at 5×10^{-9} M (●) and 1×10^{-8} M (□). Responses were calculated as % maximum response of the first response (untreated) and plotted against the −log of the concentration of serotonin.

benzamine concentration, and were $1.7 \times 10^{-6} \pm 0.71$ M (n = 10) and $7.73 \times 10^{-6} \pm 3.01$ M (n = 10) with phenoxybenzamine at 5×10^{-9} and 1×10^{-8} M, respectively. Serotonin produced 20, 50, 95 and 100% maximum responses by occupying 5 ± 1, 17 ± 3, 56 ± 5 and 71 ± 4% (n = 10), respectively, of the aged SHR aorta 5-HT_{2A}-receptors. The receptor reserve for 20 and 50% maximum responses to serotonin was significantly greater on the SHR than the WKY rat aortae ($P < 0.05$). The 5-HT_{2A}-receptor reserves for near maximal or maximal responses to serotonin were similar on the aortae of aged WKY rats and SHRs (Figure 2).

Discussion

In this study, the 24-month-old SHR aortae were heavier than those of age-matched WKY rats, indicating that there was hypertension-associated hypertrophy of the aorta. Aged SHRs are a non-invasive and realistic model of hypertension-induced heart failure (Doggrell & Brown 1998). Of the many markers tested by Bing et al (1995), the most consistent marker of the SHR in failure was right ventricular hypertrophy. Bing et al (1995) have divided their 18–24-month-old SHRs into two groups: SHR-F (failing), which have right ventricular hypertrophy, and SHR-NF (non-failing), which do not have right ventricular hypertrophy. Using right ventricular hypertrophy as a marker of heart failure, all the SHRs in our colony have heart failure at > 18 months (Doggrell et al 1999; Nand & Doggrell 2000). Thus, it is likely that the SHR aortae used in the present study were from rats with heart failure. Serotonin may be inactivated by uptake into tryptaminergic or noradrenergic nerves. In most studies with serotonin, it is important to inhibit the neuronal uptake process (e.g. with cocaine) in order to work under equilibrium conditions. The rat thoracic aorta is an unusual blood vessel in that there is no evidence of noradrenergic or tryptaminergic innervation. The rat thoracic aorta content of noradrenaline and serotonin is negligible and the responses to noradrenaline and serotonin are not altered by the neuronal uptake inhibitor cocaine, or by reserpine or 6-hydroxydopamine (Maling et al 1971). The thoracic aortae of 6- and 24-month-old WKY rats and SHRs are unresponsive to nerve stimulation (Doggrell 1995; S. A. Doggrell, unpublished observations). Therefore, on the rat thoracic aorta, it is unnecessary to inhibit the neuronal uptake process when studying the effects of serotonin.

To be valid, the K_A values derived for contractility studies with irreversible antagonists should be independent of the concentration of the antagonist (Kenakin 1987). This requirement is satisfied by the present study, in which the K_A values for serotonin were independent of the concentration of phenoxybenzamine used.

Serotonin acts solely at 5-HT_{2A}-receptors to contract the rat aorta. Spiperone (Cohen et al 1982) and ketanserin, which are selective 5-HT_{2A}-receptor antagonists, but not the selective α_1- and α_2-adrenoceptor antagonists, prazosin and idazoxan (Doggrell 1987), are potent inhibitors of the rat aorta serotonergic response. However, the rat aorta 5-HT_{2A}-receptors may be atypical as the affinity constants (K_A) for serotonin are 10–100-fold greater (Doggrell 1992, 1995, and this study) than those previously reported for serotonin at 5-HT_{2A}-receptors. K_A values for serotonin in the order of $1–8 \times 10^{-7}$ M have been obtained at the 5-HT_{2A}-receptors

of the rabbit aorta (Clancy & Maayani 1985; Leff et al 1987), calf coronary and pulmonary artery (Frenken & Kaumann 1987) and rat kidney vasculature (Bond et al 1989). This suggests that there may be subtypes of 5-HT_{2A}-receptors, with those of the rat aorta being of one subtype and those of the rabbit aorta, calf coronary and pulmonary artery and rat kidney vasculature being of another subtype.

Ageing of the rat basilar artery from 3 to 21 months is not associated with a change in sensitivity to serotonin (Arribas et al 1997). However this is not true for all vessels. Ageing of the WKY rat aorta from 6 to 24 months was associated with an increase in sensitivity and affinity to serotonin (Doggrell 1995, and this study). Ageing of the WKY rat aorta was also associated with a loss in the receptor reserve for serotonin.

Ageing of the SHR aorta from 6 to 24 months is also associated with an increase in sensitivity to serotonin (Doggrell 1995, and this study). On the SHR aorta, the affinity for serotonin is similar at 6 and 24 months. On the SHR aorta there is a gain in receptor reserve for serotonin between 6 and 24 months.

Several studies have shown that the sensitivity of the SHR aorta in early hypertension is greater than on the normotensive aorta (Ahlund et al 1977; Turla & Webb 1990; Doggrell 1995). This study illustrates that there are major changes in sensitivity, affinity and receptor reserve to serotonin in the ageing of the normotensive and hypertensive aortae. Between 6 and 24 months, the sensitivity to serotonin on both the WKY rat and SHR aorta increases, and at both time points there is a greater sensitivity on the SHR than WKY rat aorta. At 6 months, there is a greater affinity for serotonin on the SHR than WKY rat aorta. Between 6 and 24 months, the affinity for serotonin increases on the WKY rat aorta, but not the SHR aorta, and at 24 months, there is no difference in the affinity of serotonin on WKY rat and SHR aortae. At 6 months, there is a lesser 5-HT_{2A}-receptor reserve for serotonergic responses on the SHR than on the WKY rat aorta. Between 6 and 24 months, the receptor reserve for serotonin decreases on the WKY rat aorta, but increases on the SHR aorta. At 24 months, there is a greater 5-HT_{2A}-receptor reserve on the SHR aorta than the age-matched WKY rat aorta.

It is not known whether similar alterations in 5-HT_{2A}-receptors and their functional responses occur in the ageing of human blood vessels. If similar differences were apparent in the ageing of human blood vessels, there could be clinical implications. First, as most pre-clinical pharmacological testing uses tissues from young animals, the pathophysiological role of serotonin may not have been assessed correctly. Second, changes in the dose of 5-HT_{2A}-receptor antagonists (e.g. ketanserin in hypertension) may be required with age.

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