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Endothelial modulation of α_1-adrenoceptor contractile responses in the tail artery of spontaneously hypertensive rats

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1 Vascular contraction induced by phenylephrine was studied in tail artery rings from spontaneously hypertensive (SHR) and Wistar Kyoto rats (WKY) with particular focus on the role of endothelium. The influence of receptor reserve and the density of α_1-adrenoceptors on the possible differences observed were also analysed.

2 Phenylephrine (0.01 - 100 μM) induced concentration-dependent vasoconstrictions. The maximum response (α, $P<0.001$) was greater but the pEC_{50} ($P<0.05$) smaller in rings from SHR than from WKY rats irrespective of the presence or absence of endothelium.

3 Removal of endothelial cells resulted in a decrease of the maximum contraction with no modification in the pEC_{50} in arteries from both WKY and SHR.

4 The density of α_1-adrenoceptors (B_{max}) and the dissociation constant (K_D) were found to be the same for preparations from SHR and WKY rats in [^{3}H]-prazosin binding experiments.

5 The apparent affinity (pK_A) determined by the nested hyperbolic method and the operational model was similar in tail arteries from the two rat strains, irrespective of the presence or absence of endothelium. However, in endothelium-denuded rings, the pK_A value was enhanced when compared with intact rings, in both SHR and WKY rats.

6 In rings from hypertensive rats, the operational parameter maximum possible effect (E_m) was greater and the agonist efficacy (τ) was smaller than in rings from normotensive rats. When the endothelium was removed log τ and E_m diminished in preparations from both rat strains.

7 In summary, the increased maximum responsiveness to phenylephrine in rings from SHR could be due to enhancement in E_m. The log τ values indicate a deterioration in the transduction of the stimulus provided by the agonist in tail arteries from hypertensive animals. This study also suggests that the absence of endothelium modifies the α_1-adrenoceptor-mediated vasocontriction probably by altering the transduction signalling mechanisms. The importance of analysing the degree of endothelium functionality when comparing results from different groups of rats is stated.

Keywords: Tail artery; α_1-adrenoceptors; hypertension; endothelium; [^{3}H]-prazosin binding

Introduction

One of the pathogenic factors of animal hypertension is thought to be an enhanced vascular responsiveness to catecholamines, leading to an elevation of the total peripheral resistance. The sympathetic nervous system controls vascular tone predominantly through α-adrenoceptors. Some, but not all functional studies, describe an increased vascular reactivity following α-adrenoceptor stimulation in different animal models of hypertension (Marín, 1993). Thus, the tail artery but not the aorta from spontaneously hypertensive rats (SHR) shows a greater maximum contractile response to noradrenaline than the age-matched Wistar Kyoto (WKY) normotensive animals (Vila *et al.*, 1993). Some authors speculate that an alteration in α-adrenoceptor density or in postreceptor events may account for the increase in vascular sensitivity to catecholamines (Perry & Webb, 1988). Nevertheless, only a few studies correlate hyperresponsiveness to catecholamines with changes in α_1-adrenoceptor density, α_1-adrenoceptor reserve and/or postreceptor events (Aqel *et al.*, 1987; Li & Triggle, 1993).

It is widely accepted that the endothelium is a modulator of contractile responses induced by α-adrenoceptor agonists in different vascular beds. There is evidence that the endothelium can reduce the contractile reponse to several vasoconstrictors and may play an important role in the mediation of smooth muscle contraction of the small arteries concerned with the regulation of peripheral vascular resistance. The removal of the endothelium enhances the contractile response to several agonists in vascular preparations (Godfraind *et al.*, 1985; Topouzis *et al.*, 1991; Seager *et al.*, 1992; Tabernero & Vila, 1995). A relationship between endothelial modulation of agonist-induced contractile response and receptor reserve (Alosachie & Godfraind, 1988; Topouzis *et al.*, 1991; Tabernero *et al.*, 1996) is proposed in different blood vessels. Thus, responses to a partial agonist are enhanced to a greater extent than responses to a full agonist by the absence of endothelium or the presence of L-NAME (Topouzis *et al.*, 1991; Tabernero *et al.*, 1996).

Essential hypertension seems to be associated with generalized endothelial dysfunction (Moncada & Higgs, 1993; Änggard, 1994; Küng & Lüscher, 1995). There appears to be no debate concerning endothelial dysfunction in large conduit arteries, but the evidence for such a derangement in smaller vessels must now be considered controversial. In hypertensive patients and in different models of hypertension, there exists a reduction of endothelium-dependent relaxations and an enhancement of the agonist-induced contractions which are negatively modulated by the endothelium (Marín, 1993). The loss of endothelial function in hypertension may involve an altered basal and/or stimulated release of nitric oxide as well as an enhanced sensitivity of vascular smooth muscle to contractile factors (Lüscher *et al.*, 1992).

The influence of the endothelium on contractile responses to several agonists in blood vessels from SHR and WKY rats has been analysed (Lüscher *et al.*, 1992; Yokota *et al.*, 1994; Lang *et al.*, 1995). However, most studies of α-adrenoceptor mediated responses in tail arteries and in other blood vessels from

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hypertensive rats, do not consider the integrity of the endothelium (Aqel *et al.*, 1987; Li & Triggle, 1993; Vila *et al.*, 1993; Thorin-Trescases *et al.*, 1994). This fact could explain at least in part, the apparent differences caused by hypertension on α-adrenoceptor-mediated responses noted in different studies.

The objective of our study was to compare the contractile responses following α_1-adrenoceptor stimulation in tail arteries from SHR and WKY rats, taking into account the presence or absence of the endothelium. To explain the possible differences between strains, the α_1-adrenoceptor reserve in rings with and without endothelium, and the α_1-adrenoceptor density were explored. Parameters governing agonism were quantified by means of the nested hyperbolic method (Parker & Waud, 1971; James *et al.*, 1989) and the operational model of agonism (Black & Leff, 1983).

Methods

The experiments were performed on 16–18 weeks old male SHR (354.21 ± 6.25 g) and age-matched normotensive WKY (339.36 ± 4.93 g) rats. The animals were killed by a sharp blow to the head and the tail artery quickly removed, cleaned of adherent tissue and placed in gassed (95% O_2, 5% CO_2) physiological salt solution (PSS) of the following composition (in mM): NaCl 112.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.1, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.1 Yohimbine (0.1 μM) was present throughout the experiment to prevent stimulation of α_2-adrenoceptors (Bao *et al.*, 1993).

Contractile studies

Rings of proximal tail artery (2–3 mm) were set up in 5 ml organ baths containing PSS maintained at 37 ± 0.5°C and continuously gassed with 95% O_2, 5% CO_2. A resting tension of 7.4 mN was applied and changes in tension recorded with a PIODEN (UF-1) isometric transducer attached to a Omniscribe pen recorder. The preparations were left to equilibrate for 30 min and tension was readjusted if necessary. The tissues were contracted 3 or 4 times with KCl (75 mM) every 5 min until the amplitude of contractile response was similar in magnitude. After a 20 min equilibration each ring was contracted with noradrenaline (0.03 μM) and relaxed with acetylcholine (1 μM). Preparations were considered to have an intact endothelium (+E) when they relaxed by more than 60% and to be denuded (−E) when they failed (0%) to relax. Those preparations that relaxed between 0% and 60% were discarded. Rings were washed with PSS and after a further 30 min equilibration, a cumulative agonist concentration-effect (E/[A]) curve to phenylephrine (0.01–100 μM) was constructed. The bath was then washed repeatedly with PSS until preparations reached their initial tension. Afterwards, tissues were exposed for 10 min to the alkylating agent, phenoxybenzamine (0.03 μM), to obtain a partial α_1-adrenoceptor inactivation. The rings were then washed successively every 5 min for 30 min, after which the agonist E/[A] curve was repeated. Control experiments were run in parallel to check the reproducibility over time between two curves performed under the above mentioned conditions.

[^{3}H]-prazosin binding assay

Binding studies were performed by incubation of rat tail artery rings (1.5–2 mg wet weight) under conditions similar to those previously described for the mesenteric artery (Morel & Godfraind, 1991). Briefly, rings of tail artery (one/tube) were incubated in triplicate with [^{3}H]-prazosin (0.05–8 nM) in 0.25 ml (final volume) buffer pH 7.4 (composition in mM: HEPES 8.3, NaCl 130.0, KCl 5.6, $CaCl_2$ 2.0, $MgCl_2$ 0.24 and glucose 11.0) maintained at 37 ± 0.5°C and gassed with a mixture of 95% O_2, 5% CO_2. At the end of the incubation period each ring was washed in buffer solution (5 s), dried on filter paper, weighed and dissolved in 0.1 ml of a mixture of perchloric acid: H_2O_2 (1 : 1). Radioactivity of the tissue was determined by liquid scintillation spectrometry with a counting efficiency of 60%. Non specific binding was defined as that unaffected by 100 μM phentolamine and represented approximately 50–60% of the total binding at a concentration of [^{3}H]-prazosin close to the K_D.

Data analysis

Pragmatic logistic curve fitting Each individual set of E/[A] curve data was fitted to a logistic function of the form:

$$E = \frac{\alpha[A]^m}{[EC_{50}]^m + [A]^m} \quad (1)$$

in which E and [A] are the pharmacological effect and the concentration of agonist, respectively; α, EC_{50} and m are the asymptote, location and slope parameters, respectively. Location parameters were actually estimated as pEC_{50} (i.e. the negative logarithm of the concentration required to cause 50% of the maximal response).

Nested hyperbolic method Data obtained from receptor inactivation experiments were analysed by the nested hyperbolic method (James *et al.*, 1989). This method, which is analytically simpler than the classical method of Furchgott (1966), involves fitting the control E/[A] curve data to equation 1 whilst simultaneously fitting the postinactivation E/[A] curve data to the following equation:

$$E = \frac{\alpha}{\left(\frac{EC_{50}}{qK_A[A']}(K_A + [A'](1-q))\right)^m + 1} \quad (2)$$

where q represents the fractional receptor concentration which remains following inactivation.

Operational model-fitting E/[A] data obtained experimentally were fitted to the operational model of agonism to estimate agonist efficacies and affinities (Black & Leff, 1983; Black *et al.*, 1985):

$$E = \frac{E_m \cdot \tau^n \cdot [A]^n}{(K_A + [A])^n + \tau^n \cdot [A]^n} \quad (3)$$

in which E_m is the maximum possible effect; K_A is the dissociation constant of the agonist from the receptor (estimated as the negative logarithm, that is, pK_A); τ is the ratio $[R_0]/K_E$ where $[R_0]$ is the total functional receptor concentration and K_E defines the value of occupancy [AR], for half E_m; n is the slope parameter for the assumed logistic relation linking [AR] to effect, E. Operationally, τ defines the efficacy of an agonist in a system.

Statistics

Experimental points and results from pragmatic logistic curve fitting are expressed as mean ± s.e.mean. The number of animals for contractile studies and the number of experiments performed in triplicate in binding assays (n) are indicated in the figures and tables. The forces developed in contractile responses are expressed in absolute values (mN).

Experimental data were directly fitted to the mathematical models described above with the 'AR' programme (derivative-free, nonlinear, regression analysis) within the BMDP statistical software package (Dixon, 1990), implemented on a Vax 6610 computer. Each individual experiment was analysed by the three mathematical models and then, in each experimental group, mean ± s.e.mean was calculated for each parameter. It is assumed that estimates of K_A, EC_{50}, and τ are log-normally distributed; therefore, each of these are expressed as a logarithmic value. α, m, E_m and n are assumed to be approximately

normally distributed on the natural scale (Fleming *et al.*, 1972; Leff *et al.*, 1990). Two-way analysis of variance was applied to investigate whether each parameter was affected by the strain and/or the endothelium. Analysis were carried out with the SAS statistical package (Littell *et al.*, 1991) using a general linear model (PROC GLM) which allows for the occurrence of unbalanced designs. In all cases significance was set as a P value of less than 0.05. Ligand binding data were analysed by iterative non linear curve-fitting programmes as described by McPherson (1983).

Drugs and isotopes

(−)-Noradrenaline bitartrate, phenylephrine HCl, acetylcholine HCl and yohimbine HCl were purchased from Sigma Chemical Co; [^{3}H]-prazosin from Amersham International; phenoxybenzamine HCl and phentolamine mesylate from Research Biochemical Incorporated (RBI). All drugs were prepared in physiological salt solution except noradrenaline that was prepared in 23 μM Na_2EDTA and phenoxybenzamine in 0.1 M tartaric acid. All other chemicals used were of analytical grade.

Results

Contractile studies

The contractile responses induced by phenylephrine in tail artery rings, in the presence and absence, of endothelium are shown in Figures 1 and 2, respectively. Phenylephrine contracted in a concentration-related manner the tail artery in both strains of rats. Parameters estimated by pragmatic logistic curve fitting are shown in Table 1. The maximum response (α) achieved by the agonist was greater in SHR than in WKY. In contrast, normotensive animals showed a higher pEC_{50} value when compared to hypertensive rats. Removal of endothelium decreased to a similar extent the maximum contraction by phenylephrine in both strains of animals. Nevertheless, the pEC_{50} was not affected by the absence of endothelial cells. The statistical analysis of these data (Table 3) showed that all the differences observed were significant.

Vasoconstrictor responses to a depolarizing solution of 75 mM KCl were similar in WKY (+E: 6.91±0.52 mN, n=13) and SHR (+E: 8.23±0.82, n=14) rats. The removal of endothelium did not modify the KCl-induced contractile responses.

The effects of phenoxybenzamine on phenylephrine E/[A] curves were examined in the tail arteries from both groups of rats, taking into account the presence (Figure 1) and the absence (Figure 2) of endothelium. In tail artery from WKY, phenoxybenzamine produced a shift to the right of the E/[A] curves to phenylephrine and depressed the maximum response around 50% in rings with or without endothelium. Similarly, the presence of the alkylating agent shifted to the right the E/[A] curves of agonist but produced a greater decrease of maximum contraction in denuded (47%) than in intact (34%) tail artery rings from SHR.

Pharmacological analysis of agonism by irreversible receptor inactivation with phenoxybenzamine was carried out by both the nested hyperbolic method and the operational model of agonism. As described in the Methods section, the experiments were performed with a multiple curve design. In this design, a single piece of tissue provides two curves (control and phenoxybenzamine-exposed) that are analysed simultaneously by the corresponding aforementioned equations. The statistical significances are shown in Table 3. All the E/[A] curves depicted in Figures 1 and 2 were included.

The nested hyperbolic method provided pK_A values that were significantly increased when the endothelium was removed in rings from WKY (+E: 4.33±0.10, n=13; −E: 4.71±0.12, n=12) and from SHR (+E: 4.43±0.13, n=14; −E: 4.73±0.08, n=16). A slight difference between strains (Table 3) in the fractional receptor concentration remaining following inactivation (q%) was observed in both the presence (WKY: 7.18±3.05, n=13; SHR: 14.05±4.14, n=14), and the absence (WKY: 8.10±1.36; n=12; SHR: 14.35±3.22, n=16) of endothelium.

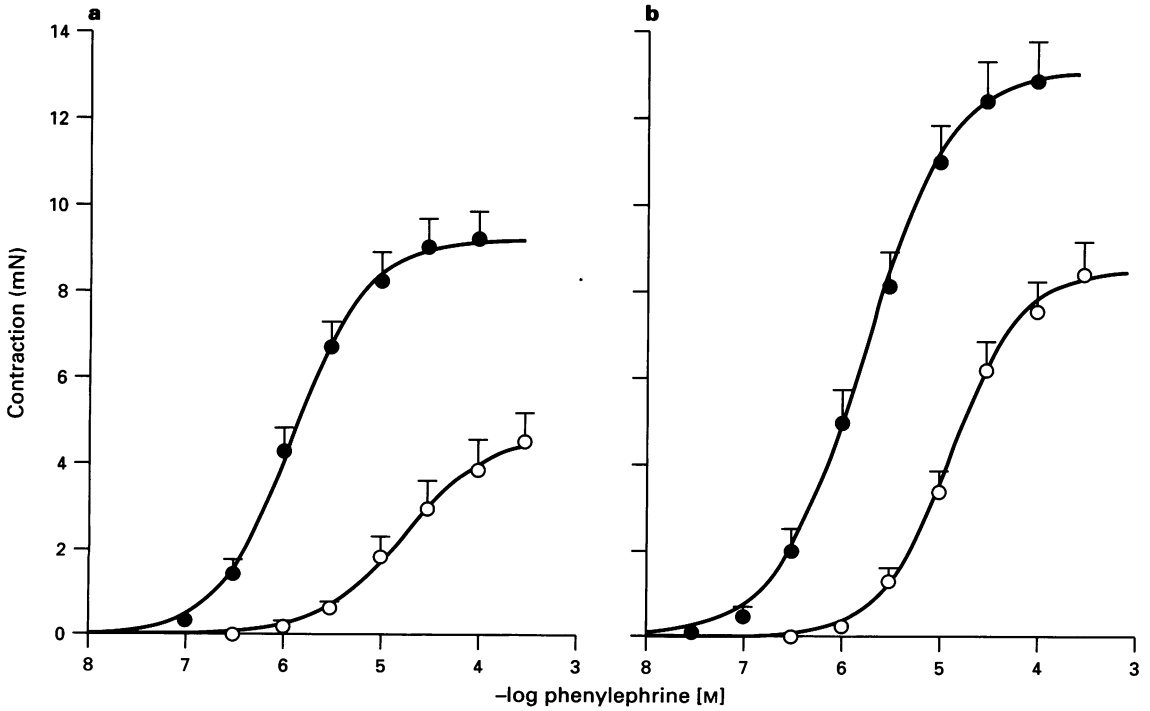

Figure 1 Concentration-response curves for phenylephrine-induced contraction in intact tail artery rings from (a) WKY and (b) SHR obtained before (●) and after (○) a 10 min exposure to 0.03 μM phenoxybenzamine. The lines drawn through the data are the results of pragmatic logistic fitting (see Methods). Each point represents the mean of 12–14 experiments; vertical lines show s.e.mean.

The operational model analyses agonist action by fitting each pair of E/[A] curves, before and after partial inactivation of receptors, to equation 3. In each piece of tissue, E_m, n, and pK_A are restricted to a common value, whereas log τ is allowed to have different values for control and phenoxybenzamine-exposed curves (Leff *et al.*, 1990). Results are summarized in Table 2 and the statistical analysis in Table 3. For the sake of simplicity, only the log τ estimate to the system in the absence of the alkylating agent is shown. The pK_A values obtained by this method of analysis were similar to those obtained by the nested hyperbolic method. The maximum possible effect in the receptor system (E_m) differed significantly between strains, being higher in SHR than in WKY tail artery rings. In addition, the removal of endothelial cells resulted in a similar reduction of E_m in preparations from both strains of rats. In contrast, the efficacy (log τ) was greater in rings from normotensive than from hypertensive animals. The removal of the endothelium decreased, to the same extent, the efficacy in tail arteries from either of the strains of rats studied.

Binding studies

In rings of tail artery from normotensive and hypertensive rats [^{3}H]-prazosin binding was specific, saturable and showed high affinity. Non linear regression analysis of the saturation data was consistent with the presence of a single population of binding sites. No significant differences were observed between the K_D and B_{max} values (Table 4) calculated for tissues from the two species.

Discussion

In this study the influence of the endothelium on α_1-adrenoceptor-mediated contractions in tail artery rings from 16–18 weeks old SHR and age-matched WKY rats was analysed. For this purpose, the parameters governing agonism were analysed by the nested hyperbolic method and the operational model of fitting, which offer the advantage of directly fitting the experimental data (James *et al.*, 1989; Leff *et al.*, 1990). The α_1-adrenoceptor density was also evaluated in tail artery rings from the two rat strains.

Contractile responses induced by phenylephrine in rings from SHR showed higher maximum tension, but lower pEC_{50} values than those obtained in rings from WKY rats, irrespective of the presence or absence of endothelium. These results correlate with those obtained previously with other α_1-adrenoceptor agonists in the same blood vessel (Aqel *et al.*, 1987; Li & Triggle, 1993; Vila *et al.*, 1993). However, it is important to note that the integrity of the endothelium was not checked in some of these studies (Aqel *et al.*, 1987; Vila *et al.*, 1993). In addition, Li & Triggle (1993) assumed that when preparations relaxed by 20% to acetylcholine the endothelium do not influence the α_1-adrenoceptor-mediated response, results that do not agree with observations done in our laboratory (unpublished results) in the rat tail artery.

Aqel *et al.* (1987) observed that the maximum contraction induced by noradrenaline or methoxamine in the tail artery from SHR was enhanced when compared to WKY rats, without differences in the total number of α_1-adrenoceptors. Our binding experiments also revealed the existence of an

Table 1 Parameter estimates for phenylephrine obtained from the pragmatic logistic curve fitting

	α (mN)	pEC_{50}	m	n
Endothelium intact				
WKY	9.14±0.64	5.91±0.06	1.26±0.05	13
SHR	12.74±0.88	5.72±0.07	1.23±0.11	14
Endothelium denuded				
WKY	6.01±0.32	5.95±0.09	1.30±0.08	12
SHR	9.35±0.75	5.70±0.05	1.24±0.05	16

Values are mean±s.e.mean; *n* is the number of experiments.

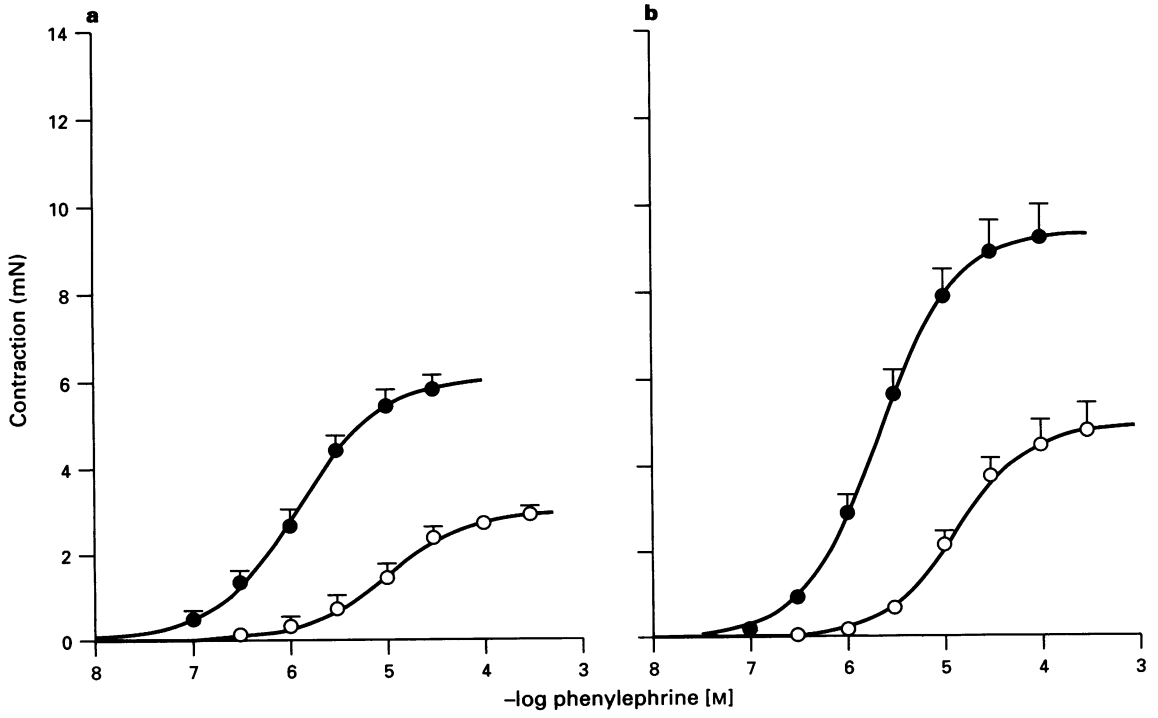

Figure 2 Concentration-response curves for phenylephrine-induced contraction in endothelium denuded tail artery rings from (a) WKY and (b) SHR obtained before (●) and after (○) a 10 min exposure to 0.03 μM phenoxybenzamine. The lines drawn through the data are the results of pragmatic logistic fitting (see Methods). Each point represents the mean of 13–16 experiments, vertical lines show s.e.mean.

Table 2 Parameter estimates from the operational model-fitting to phenylephrine E/[A] curves

	E_m (mN)	pK_A	n	$log\ \tau$	n
Endothelium intact					
WKY	9.43±0.64	4.36±0.09	1.29±0.05	1.55±0.13	13
SHR	13.61±0.94	4.43±0.13	1.32±0.17	1.29±0.15	14
Endothelium denuded					
WKY	6.24±0.35	4.71±0.13	1.34±0.09	1.24±0.09	12
SHR	11.31±1.18	4.75±0.08	1.33±0.06	0.93±0.13	16

Values are mean±s.e.mean; n is the number of experiments.

Table 3 Two-way analysis of variance for parameters estimated by the pragmatic logistic curve fitting, the nested hyperbolic method and the operational model

Pragmatic logistic curve fitting	α	pCE_{50}	m
Strain	$P<0.001$	$P<0.05$	NS
Endothelium	$P<0.001$	NS	NS
Strain*Endothelium	NS	NS	NS
Nested hyperbolic method	q	pK_A	
Strain	$P<0.05$	NS	
Endothelium	NS	$P<0.01$	
Strain*Endothelium	NS	NS	
Operational model fitting	E_m	n	$log\ \tau$
Strain	$P<0.001$	NS	$P<0.05$
Endothelium	$P<0.01$	NS	$P<0.05$
Strain*Endothelium	NS	NS	NS

Table 4 Binding parameters of [^{3}H]-prazosin in rat tail artery rings from SHR and WKY rats

	Saturation binding experiments		
	K_D (nM)	B_{max} (fmol mg^{-1} tissue)	n
WKY	1.56±0.20	14.28±2.74	4
SHR	2.88±0.53	22.13±3.61	5

n is the number of experiments performed in triplicate.

homogeneous population of receptors and a similar B_{max} in both strains of rats. Moreover, when a system such as the tail artery has a great receptor reserve (Tabernero *et al.*, 1996), variations in the maximum contraction to the agonist do not necessarily reflect changes in α_1-adrenoceptor density. It has also been shown that there is an increased contraction in response to noradrenaline in SHR tail artery due to an increase in α_2-adrenoceptors (Hicks *et al.*, 1984; De Moraes *et al.*, 1988). However, here we have used phenylephrine, a selective α_1-adrenoceptor agonist, and performed experiments in the presence of yohimbine to block stimulation of α_2-adrenoceptors. Thus, the differences in maximal contraction observed cannot be attributed to either a difference in the type of receptor or the number of α_1-adrenoceptors.

Contractile responses obtained with 75 mM KCl were also greater, though not significantly so in rings from SHR than from WKY. Potassium-induced vasoconstrictions in tail artery were mediated by smooth muscle depolarization and to the release of endogenous noradrenaline (Fouda *et al.*, 1991; Xiao & Rand, 1991). The slight increase in KCl-induced contraction observed in preparations from SHR may be related to a different stimulation of α_1-adrenoceptors by the noradrenaline released rather than to an alteration in the basic contractile machinery.

The removal of endothelium leads to a decrease of the maximum contractile effect of phenylephrine in tissues from each rat strain. These results are in contrast with previous data showing that the lack of endothelium produces an increase in the contractile responses to different α_1-adrenoceptor agonists in rat aorta (Malta *et al.*, 1986; Alosachie & Godfraind, 1988; Topouzis *et al.*, 1991). The possibility of damage to the smooth muscle cells by mechanical rubbing of the endothelial layer, that could also explain the decrease in the maximum phenylephrine contraction, should be discarded since KCl responses were not modified by this procedure. In addition, the integrity of the vascular smooth muscle cells of some denuded preparations was verified by confocal microscopy (Arribas & Tabernero, results not shown). We have shown that endothelial modulation of α_1-adrenoceptor-mediated responses varies with the blood vessel studied. The removing of endothelial cells led to an increase of the maximum contraction to noradrenaline in aorta but not in tail artery rings from Sprague-Dawley rats (Tabernero & Vila, 1995). Similar results were obtained in the tail artery when the effect of N^G-nitro-L-arginine-methyl ester (L-NAME) was studied on phenylephrine contractile responses (Tabernero *et al.*, 1996). The influence of the endothelium or L-NAME on α_1-adrenoceptor-mediated responses was related to the receptor reserve. Thus, the variability observed between the studies in the modulation of agonist-induced responses by endothelium could be ascribed to either the vessel and/or the strain studied.

When rings of tail artery were exposed to phenoxybenzamine, the maximum effect and the pEC_{50} values for phenylephrine-induced contraction decreased in both groups of animals as a consequence of the reduction of the total number of receptors. Analysis of E/[A] curves before and after exposure of the tail artery to phenoxybenzamine by the nested hyperbolic method demonstrated that there were no differences in the affinity constants (pK_A) for phenylephrine between WKY and SHR, indicating that the affinity of the agonist does not change as a consequence of hypertension. The removal of the endothelium resulted in an unexpected increase in pK_A values. Nevertheless, when the same data were analysed by the operational model of agonism, the affinity constants obtained were in good agreement with those obtained by the nested hyperbolic method. The estimates of apparent affinity for agonists do not depend on the method of calculation used because, as previously demonstrated (Sallés *et al.*, 1994; 1996) both methods gave similar answers. Differences in agonist affinity (K_A) for α_1-adrenoceptors have also been described in rabbit blood vessels (Oriowo *et al.*, 1991).

The log τ value for phenylephrine was greater in WKY than in SHR. In terms of the operational model (Black & Leff, 1983), since the pK_A values did not change between normotensive and hypertensive animals, the increased agonist efficacy observed in WKY could explain the greater pEC_{50} value observed in this strain of rats. Differences observed in log τ values can be attributed to either of its components, namely, [R_0] (receptor population) and/or K_E (transducer constant). Our binding study shows that there is no significant difference in the number of receptors. Thus, these data would indicate that α_1-adrenoceptors from WKY are more efficient than those from SHR in promoting signal transduction after stimulation

by agonists. The removal of endothelial cells increased pK_A values and diminished agonist efficacy in tail artery from both rat strains. The opposite changes induced by the absence of endothelium on the aforementioned parameters could cancel each other, explaining why the pEC_{50} values obtained by pragmatic logistic fitting were not modified.

The theoretical E_m values estimated with the operational model of agonism were close to the corresponding experimental α. Our results indicate that, either in presence or in absence of the endothelium, in the SHR a higher E_m parameter is obtained than in WKY rats. So, the maximum contraction observed in SHR when compared to WKY could be explained by a higher capacity of the system for contraction in hypertensive rats. A higher inositol phosphate formation induced by noradrenaline was previously observed in SHR vs WKY in intact tail artery rings (Vila *et al.*, 1993) that could partially explain the higher capacity for contraction observed in SHR rats. In the absence of the endothelium, the agonist efficacy and the maximum capacity of the system decreased. It is known that vascular endothelium synthesizes and releases multiple factors that regulate a variety of physiological processes. The endothelial factors are linked to several second messenger systems (Sánchez Ferrer & Marín, 1990). We hypothesize that the final contraction mediated by α_1-adrenoceptor agonists is influenced by a cross-talk between the different second messengers that contribute to the contraction. Thus, the changes observed on both agonist affinity and efficacy after removing the endothelium are likely to be due to differences in the transduction signal mechanisms.

In summary, tail artery rings from SHR showed a greater capacity for contraction but a smaller efficacy in the transduction of the stimulus than those from WKY rats, irrespective of the presence or absence of endothelium. Furthermore, the lack of endothelium produced an increase in the affinity but decreased the asymptote (α), E_m and log τ in both strains of rats. Therefore, conflicting results in the literature, when comparing vascular α_1-adrenoceptor-mediated responses from SHR and WKY rats, could be attributed to a different degree of integrity of the endothelial layer. We would like to note that in situations where the functionality of the endothelium is not confirmed, changes due to the existence of hypertension and modifications ascribed to the absence of endothelium could cancel each other.

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