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Enhanced noradrenergic transmission in the spontaneously hypertensive rat anococcygeus muscle

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1 There is a long-known hyper-responsiveness of vascular adrenergic transmission in the spontaneously hypertensive rat (SHR) that is uncovered specifically in the presence of cocaine and attributed to blockade of the neuronal monoamine transporter. We have now used the rat anococcygeus muscle to investigate whether this phenomenon is generic to sympathetic transmission to smooth muscle rather than a purely vascular phenomenon. We sought the origin of the effect by successively blocking the buffering effects of the neuronal monoamine transporter, prejunctional α_2-adrenoceptors and NO from nitrergic nerves with desipramine (0.1 μM), rauwolscine (0.01 μM) and L-N^G-nitro-arginine (100 μM).

2 In the presence of desipramine, contractile responses to electrical field stimulation but not to noradrenaline (1 nM–100 μM) were greater in SHR than in Wistar–Kyoto (WKY). Neither inhibition of prejunctional α_2-adrenoceptors nor the blockade of neuronal nitric oxide synthase (nNOS) accounted for the differential enhancement of response in SHR. The enhanced effectiveness of motor neurotransmission in SHR becomes most apparent when all known major buffering mechanisms are removed.

3 When nitrergic responses were isolated pharmacologically (phentolamine 1 μM and guanethidine 30 μM; tone raised with carbachol 50 μM), they were not different between SHR and WKY.

4 Western blots showed that both nNOS and tyrosine hydroxylase are expressed to a similar extent in anococcygeus muscle from SHR and WKY, suggesting similar adrenergic and nitrergic innervations in the two strains. This suggests that enhanced motor transmission is due to increased transmitter release per varicosity rather than there being normal transmission from a greater number of sites.

5 We conclude that there is a generic enhancement of sympathetic transmission in SHR rather than this being a vascular phenomenon.
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Abbreviations: ANOVA, analysis of variance; EFS, electrical field stimulation; L-NOARG, L-N^G-nitro-arginine; NO, nitric oxide; NOS, nitric oxide synthase; PSS, physiological salt solution; SHR, spontaneously hypertensive rats; TTX, tetrodotoxin; WKY, Wistar–Kyoto

Introduction

The spontaneously hypertensive rat (SHR) is used as a pathophysiological animal model for human hypertension. It provides an opportunity to study the secondary consequences of hypertension *per se* but the cause of its hypertension, and thus whether it shares this with human essential hypertension, remains controversial.

An attractive possibility is the concept of an over effective sympathetic or under effective parasympathetic nervous system. In mesenteric and caudal arteries from SHR, compared with normotensive controls, cocaine, a blocker of the neuronal monoamine transporter, produced a greater potentiation of the vasoconstrictor response to exogenous noradrenaline or stimulation of the intramural nerves (Whall *et al*., 1980; Webb & Vanhoutte, 1981; Stephens *et al*., 1991). This took on greater significance because Aalkjaer *et al*. (1987) showed a similar effect of cocaine *versus* noradrenaline in the subcutaneous resistance arteries of essential hypertensives, compared with normotensive controls, although this was not reported in relation to nerve-mediated responses.

No mechanistic explanation of this 'enhanced cocaine shift' has been made. A few studies suggested that blood vessels from SHR have a greater adrenergic innervation than Wistar–Kyoto (WKY) rats (Zsoter *et al*., 1982; Cassis *et al*., 1985). If so, when the uptake mechanisms are blocked, the total amount of noradrenaline released by electrical field stimulation (EFS) might be higher in SHR than in WKY rats, and so the contractile responses to released noradrenaline would increase. Until now this has been the most plausible explanation.

The possibility that the key primary defect lies in sympathetic transmission rather than in a specifically vascular effect was investigated by studying the responses of nonvascular tissues of SHR. Altered responsiveness was found in smooth

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muscle such as stomach (Altman *et al*., 1977) and vas deferens (Docherty & Warnock, 1986; Katzuragi *et al*., 1991; Vivas *et al*., 1997). However, there were also negative observations, two of which utilised noradrenaline's contractile response in the anococcygeus muscle of SHR (Laher & Triggle, 1984a) or Dahl hypertensive rats (Laher & Triggle, 1984b; Lee *et al*., 1987). Crucially, perhaps, for the future development of this concept, Laher & Triggle (1984a) could demonstrate the 'enhanced cocaine shift' in tail artery, confirming Webb & Vanhoutte (1981) but they found no such phenomenon in two other blood vessels (aorta and portal vein) or in anococcygeus. This lent support to the idea that the phenomenon was specific to blood vessels and may have diminished further interest in generalisation of the defect. However, these studies did not study neurotransmission.

We have now pursued this issue again in anococcygeus because preliminary experiments showed quite clearly that, following blockade of the neuronal monoamine transporter, the responses to nerve stimulation are larger in SHR than in normotensive WKY controls. If this is true then at least part of the pathophysiology of the SHR is likely to be a general defect of the sympathetic nervous system rather than vascular-specific.

To do this, we had to take other factors into consideration that might buffer adrenergic nerve responses, viz. α_2-adrenoceptor-mediated effects and nitrergic influences.

α_2-Adrenoceptors: In rat mesenteric arteries, differences have been postulated between SHR and WKY rats in the population of postjunctional but not prejunctional α_2-adrenoceptors (Feres *et al*., 1998). In rat anococcygeus, there is evidence for prejunctional but not postjunctional α_2-adrenoceptors (Leighton *et al*., 1979; McGrath, 1984). Therefore, this seems an unlikely explanation but must be tested. Potential differences in α_2-adrenoceptors between SHR and WKY and their influence on responses to nerve activation were examined using rauwolscine, a selective α_2-adrenoceptor antagonist previously characterised on rat anococcygeus (Leighton *et al*., 1979; McGrath, 1984).

Nitrergic mechanisms: Changes have been postulated in SHR; the activity of nonendothelial derived NO, presumed to be released from nitrergic nerves, and NADPH diaphorase staining, indicative of nitrergic innervation, have been reported as diminished in SHR mesenteric bed and superior mesenteric ganglion, respectively (Rabelo *et al*., 2001). These results suggest a diminished role of NO from nitrergic nerves in tissues from SHR. Rat anococcygeus has a dual nitrergic and adrenergic innervation (*via* α_1-adrenoceptors) providing reciprocal, mutually opposing, inhibitory and motor responses when activated simultaneously by EFS (Gillespie & McGrath, 1973; McGrath, 1984; Gillespie *et al*., 1989; Li & Rand, 1989a, b; Gibson *et al*., 1990; Gillespie & Sheng, 1990; Song *et al*., 1993). The noradrenergic and 'nitrergic' nerves have been estimated at 60 and 40% of the total innervation, respectively (for a review, see Gibson & McFadzean, 2001). Thus, the buffering effect of nitrergic responses *per se* as well as their potential modification in the SHR have to be considered.

The expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of catecholamines, and neuronal NO synthase (nNOS), the enzyme that converts L-arginine to NO, were evaluated as index of noradrenergic (Klimek *et al*., 2001) and nitrergic (Takahashi *et al*., 2000; Mbaku *et al*., 2003) innervation.

Methods

The experiments were performed on 6-month-old male SHR and age-matched WKY rats supplied by the Autonomous University of Madrid (Spain). The animals were killed by decapitation and exsanguination. All the protocols used were approved by the Animals Ethics Committee of the Autonomous University of Barcelona and comply with the current laws of Spain. The anococcygeus muscle was dissected as described by Gillespie (1972) and set up in 20 ml organ baths containing 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, maintained at 37°C and continuously gassed with 95% O_2 and 5% CO_2. In some experiments, desipramine (DMI, 0.1 μM) was added to the PSS to inhibit the neuronal uptake of noradrenaline. A resting force of 4.90 mN was placed on the tissue and changes in force were recorded with a PIODEN (UF-1) isometric transducer attached to an Omniscribe pen recorder. The preparations were left to equilibrate for 45 min with frequent washes. Force was readjusted if necessary. Following equilibration, the preparation was contracted three or four times with KCl (60 mM) every 5 min until the contractile responses were of similar magnitude. The tissue was then washed and, when the initial force was achieved, was left to equilibrate for a further 30 min period before starting the experiments.

Responses to noradrenaline

A cumulative concentration–response curve to noradrenaline (1 nM–100 μM) was constructed in the anococcygeus muscle from SHR and WKY rats. To test for the possibility that contractile responses to noradrenaline could be influenced by the neuronal uptake mechanism, in another set of experiments concentration response curves to noradrenaline were repeated but DMI (0.1 μM) was added to the PSS.

Responses to nerve stimulation

To study the EFS-induced responses, the anococcygeus muscle was suspended between a pair of platinum ring electrodes. For contractile responses, square wave pulses of 0.1 ms and supramaximal voltage at 0.25–10 Hz were applied for 10 s every 4 min using a Grass stimulator. For relaxation responses, EFS was applied at supramaximal voltage, 1 ms duration at 0.25–5 Hz for 10 s every 3 min, according to the protocol established by Gillespie (1972). Under these conditions, the inhibitory responses were abolished by tetrodotoxin (TTX, 1 μM) as previously reported (Gillespie, 1972).

First, a frequency–response curve for contraction was performed in parallel on anococcygeus muscles from SHR and WKY rats. To test for the influence of neuronal uptake of catecholamines on contraction induced by EFS, in another set of experiments a frequency–response curve was carried out in both strains but DMI (0.1 μM) was present in the PSS.

To distinguish between the three major factors that can potentiate contractile responses to EFS, pairs of frequency–response curves were constructed pre- and post-drugs as follows: rauwolscine in the presence of DMI; L-NOARG in the presence of rauwolscine plus DMI; DMI in the presence of rauwolscine and L-NOARG. The concentrations of the drugs

involved were: DMI (0.1 μM), rauwolscine (0.01 μM); L-NOARG (100 μM). Time control experiments showed that pairs of curves were similar whether they were carried out in the absence or the presence of DMI.

To observe the nitrergic relaxations to EFS, it is necessary to raise muscle tone and eliminate the contraction due to sympathetic nerve stimulation. The sympatholytic drugs phentolamine (1 μM) and guanethidine (30 μM) were added for 40 and 30 min, respectively, then the tone was raised to a submaximal plateau by carbachol (50 μM). A frequency–response curve was then constructed. After the highest frequency of stimulation used, the tissue was left to rest for 15 min and L-NOARG (30 and 100 μM) was added on top of the carbachol-induced tone. A second frequency–response curve was then performed.

Western blotting

After being homogenized in lysis buffer, tissue samples (40 μg protein) were electrophoretically separated on a 7.5% SDS–PAGE and transferred onto polyvinylidene difluoride membranes. Prestained SDS–PAGE standards of broad range (Bio-Rad Laboratories) were used as molecular mass standards. Western immunoblot was performed with monoclonal antibodies against nNOS (1 : 2000; Transduction Laboratories) and TH (1 : 1000; Chemicon International). Afterwards, membranes were incubated with the second antibody (1 : 2000 horseradish peroxidase-conjugated anti-mouse IgG antibody; Transduction Laboratories). Inmunocomplexes were detected by enhanced chemiluminescence (ECL, Amersham International) and evaluated by densitometry (Biorad). Prestained protein markers (BioRad Laboratories) were used for molecular mass determinations. Signals on the inmunoblot were quantified using A BioRad GS700 and the software Molecular Analyst 1.5 (BioRad). The same membrane was used to determine α-actin expression, and the content of the latter was used to correct nNOS and TH expression in each sample, using a monoclonal antibody anti α-actin (1 : 50,000, Sigma Aldrich).

All experiments were performed in parallel using anococcygeus muscles from SHR and WKY rats.

Statistics

The forces developed in contractile responses were calculated as absolute value (mN). Nitrergic responses were expressed as relaxations (mN) from the tone existing at the onset of nerve stimulation. The experimental points are expressed as mean $\pm$ s.e.m. The number of animals (n) is indicated in the legend of the figures.

The statistical significance for the pEC_{50} (the negative logarithm of the concentration required to cause 50% of the maximum response) and for the quantified enzyme expression was assessed by the two-tailed Student's t-test for unpaired observations. The dependency of contractile response on strain or treatment and frequency or concentration was assessed by a two-way analysis of variance (ANOVA) within the framework of the general linear model approach (Litell *et al*., 1991). Statistical significance was set as a P-value of less than 0.05. Statistical analyses were carried out with the SAS/STAT. (SAS Institute Inc., Cary, NC, U.S.A.) statistical package.

Drugs

(−)-Noradrenaline bitartrate and desipramine HCl were purchased from Sigma Chemical Co., St Louis, MO, U.S.A.; rauwolscine HCl from Research Biochemical Incorporated (RBI) Natick, MD, U.S.A.; L-N^G-nitro-arginine from Carl Biochem, La folla, CA, U.S.A.; SDS and acrylamide from BioRad Laboratories, Hercules, CA, U.S.A. Rauwolscine and DMI were prepared in distilled water, aliquoted and frozen at −20°C. Dilutions were made with PSS. Noradrenaline was prepared daily as stock solution (100 μM) in 0.1 mM ascorbic acid and diluted in PSS. All other chemicals used were of analytical grade and supplied by Merck, KGaA, Darmstadt, Germany.

Results

In either the absence (Figure 1a) or the presence (Figure 1b) of DMI noradrenaline contracted the anococcygeus muscle in a concentration-related manner in both strains of rats. The maximum contraction (E_{max}) to noradrenaline, the contractile response to KCl and the midrange sensitivity (pEC_{50}) were similar between SHR and WKY rats when they were compared in the presence or in the absence of DMI (Table 1). DMI increased the pEC_{50} ($P<0.001$) in both SHR and WKY (Table 1), producing a similar leftward shift; in SHR, 1.29 log units; in WKY, 1.16 log units.

EFS contracted the muscle in a frequency-related manner in both strains in either the absence (Figure 1c) or the presence (Figure 1d) of DMI. Frequency–response curves were similar between strains in the absence of DMI (Figure 1c). After DMI, SHR tissue contraction was potentiated but WKY was not: SHR showed significantly greater responses than WKY. (Figure 1d).

To analyse the influence of prejunctional α_2-adrenoceptors on the observed differences between strains after neuronal uptake inhibition, the influence of rauwolscine was studied on contractions induced by EFS. In either the absence or the presence of DMI (0.1 μM) to block the neuronal uptake mechanism, responses to EFS were not significantly enhanced by rauwolscine (0.01 μM) in WKY rats ($n=7–8$; data not shown). In contrast, in SHR ($n=7–8$), the frequency–response curve was significantly enhanced by the α_2-adrenoceptor antagonist: the effect was significant but small ($P<0.05$; two-way, treatment and frequency, ANOVA with repeated measures in both factors; data not shown). To exclude α_2-adrenoceptors as responsible for the observed difference between strains, the EFS-induced contractions in the two strains were compared in the presence of rauwolscine and DMI. Responses to EFS remained greater ($P<0.05$) in SHR than in WKY by two-way (strain and frequency) ANOVA with repeated measures in the frequency factor (data not shown).

Since field stimulation activates nitrergic nerves, the influence of blocking neuronal uptake was studied in conditions suppressing the nitrergic response, that is, inhibition of nNOS by L-NOARG (100 μM). SHR ($n=7$) remained significantly larger ($P<0.05$; two-way, strain and frequency, ANOVA with repeated measures in the frequency factor) than WKY ($n=8$) after inhibition of nitrergic responses by L-NOARG and α_2-adrenoceptors by rauwolscine (data not shown).

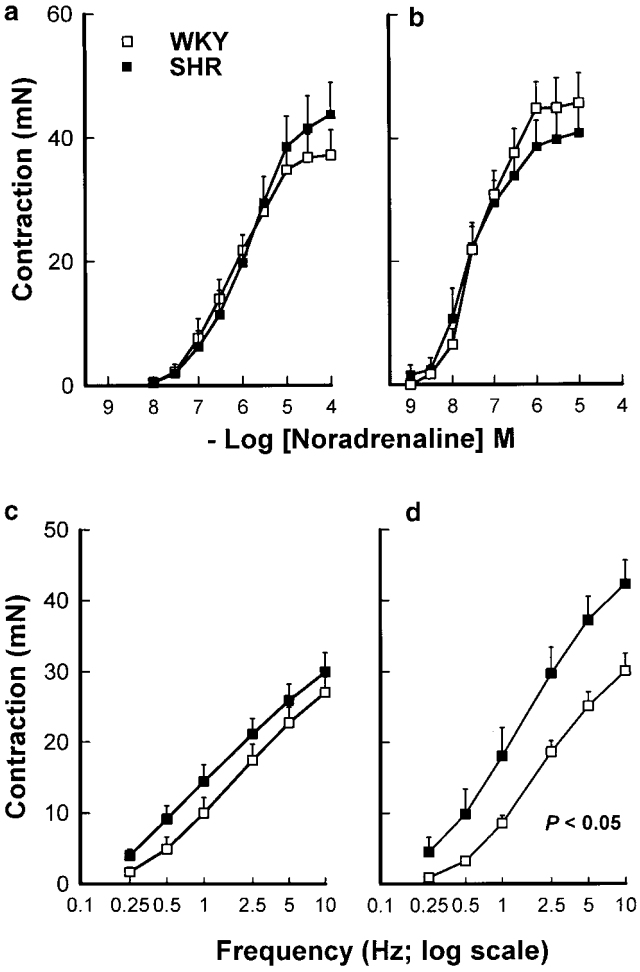

Figure 1 Comparison of contractions to noradrenaline and EFS between strains. Contractile responses to (a,b) noradrenaline and to (c,d) EFS (supramaximal V, 0.1 ms for 10 s, 0.1–10 Hz) in the anococcygeus muscle from SHR and WKY rats in the absence (a,c) and in the presence (b,d) of DMI (0.1 μM). Each point represents the mean$\pm$s.e.m. of six to eight rats. (a,b) SHR and WKY are not significantly different; two-way (strain, concentration) ANOVA with repeated measures on concentration factor. (c,d) SHR and WKY differ significantly ($P<0.05$) by two-way (strain and frequency) ANOVA with repeated measures on frequency factor in the presence (d) but not in the absence (c) of DMI.

Table 1 Influence of desipramine (0.1 μM) on the sensitivity, maximal contraction to noradrenaline and contraction to 60 mM KCl on anococcygeus muscle from normotensive and hypertensive rats

	WKY		*SHR*	
	Control	*Desipramine*	*Control*	*Desipramine*
pEC_{50}	6.31$\pm$0.21	7.47$\pm$0.13^{+++}	5.98$\pm$0.16	7.27$\pm$0.09^{+++}
E_{max} (mN)	37.2$\pm$4.06	43.70$\pm$5.80	40.87$\pm$4.50	45.75$\pm$4.77
60 mM KCl (mN)	39.7$\pm$3.0	43.80$\pm$3.90	44.10$\pm$5.90	44.33$\pm$5.80

$^{+++}P<$ 0.001 control *versus* uptake blockers by unpaired Student's *t*-test; $n=7$.

Having established that the combination of blockade of nNOS and blockade of α_2-adrenoceptors had a uniform effect between strains, the effect of DMI was assessed under these conditions. DMI now dramatically increased responses in SHR

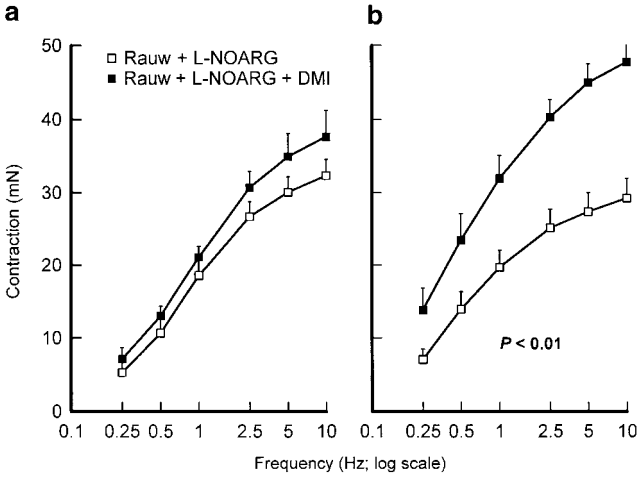

Figure 2 Effects of DMI in the presence of rauwolscine (Rauw) and L-NOARG. Influence of desipramine (DMI, 01 μM) on the contractile responses induced by EFS (supramaximal V, 0.1 ms for 10 s, 0.1–10 Hz) in the anococcygeus muscle from (a) WKY and (b) SHR anococcygeus muscle. Rauwolscine (0.01 μM) and L-NOARG (100 μM) were present in the PSS throughout the experiment. Each point represents the mean$\pm$s.e.m. The number of animals used was WKY = 6–7 and SHR = 7–9. In SHR, but not in WKY, the frequency-response differ significantly ($P<0.01$) by two-way (treatment and frequency) ANOVA with repeated measures in the frequency factor.

(Figure 2b), but had a small and statistically nonsignificant effect on WKY (Figure 2a). This also shows that any theoretical decrease of nitrergic responsiveness in SHR would not explain the larger contractile responses in SHR than in WKY after monoamine transporter and α_2-adrenoceptor blockade.

To make a further analysis of a possible strain difference in the operation of nitrergic nerves, relaxant responses to EFS were studied in conditions where all sympathetic nerve influence was excluded. In the presence of adrenergic blockade, carbachol induced a contraction that was larger ($P<0.001$) in SHR (41.6$\pm$2.82 mN; $n=6$) than in WKY (25.9$\pm$1.9 mN; $n=6$). EFS elicited a relaxation of the carbachol-induced tone in a frequency-related manner in both strains of rat (Figure 3). When expressed in absolute terms, the relaxation induced by EFS was similar between the strains (Figure 3). This confirms that there is no significant decrease of nitrergic responses in the SHR compared to WKY. L-NOARG abolished the frequency-response curves in both strains (data not shown).

Analysis of Western blot data revealed that neither nNOS nor TH expression differed between strains. The TH signal was seen as a single band of approximately 59 KDa (Figure 4a). The positive control used was a homogenate of substantia nigra that was previously seen to express TH (Soriano *et al.*, 1997). The nNOS signal was seen as a single band of approximately 155 kDa. The positive control was rat pituitary (Transduction laboratories). Basal expressions of nNOS and TH were similar in both strains (Figure 4b).

Discussion

In SHR, the contractile responses of anococcygeus to EFS were enhanced to a greater extent than in WKY by removal of

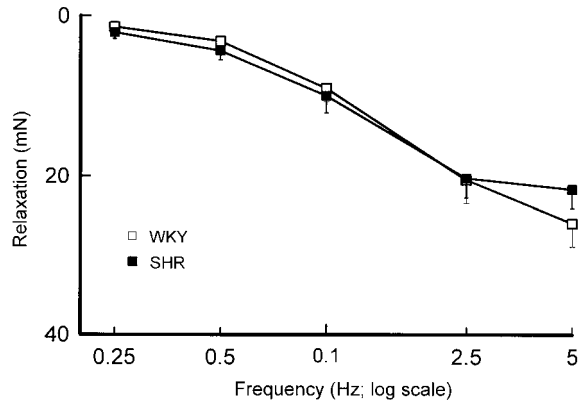

Figure 3 Comparison of relaxation to EFS between strains (NANC/nitrergic nerves). The results were calculated as relaxation (mN) from tone existing at the onset of EFS. Each point represents the mean ± s.e.m. of five to eight experiments. SHR and WKY curves do not differ significantly by two-way (frequency and strain) ANOVA with repeated measures on frequency factor.

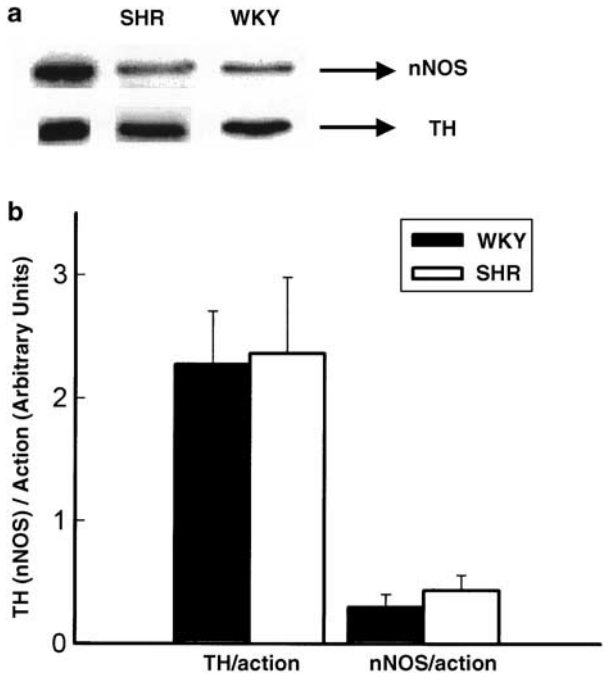

Figure 4 Comparison between strains of protein markers for adrenergic and nitrergic nerves. (a) Representative Western blot for nNOS and TH protein expression in anococcygeus muscle from SHR and WKY rats. The control line is the positive control (nNOS: rat pituitary; TH: rat substantia nigra). Blots were representative of six animals. (b) Densitometry analysis of the Western blot nNOS and TH protein expression. The results are expressed as the ratio between expression of nNOS or TH and the expression of α-actin.

the major buffering influences on sympathetic neurotransmission, resulting in larger responses in SHR. However, there was no parallel effect on responses to noradrenaline. This is similar to the effect seen after cocaine in SHR caudal artery (Webb & Vanhoutte, 1981; Cassis *et al*., 1985), and persisted after the additional blockade of prejunctional α_2-adrenoceptors and nNOS. The increased adrenergic response in SHR was not accompanied by a change in the nitrergic inhibitory responses induced by EFS, so this is not part of the explanation.

In the present study, in the absence of blocking drugs, no difference was observed between SHR and WKY rats in the contractile responses induced by noradrenaline, and by EFS in the anococcygeus muscle. This confirms the previously observed lack of difference between hypertensive and normotensive animals in the rat anococcygeus's responses to noradrenaline with or without cocaine (Laher & Triggle, 1984a, b). So a difference is revealed by DMI for the nerves,but not the postjunctional response to exogenous noradrenaline. This suggests a greater release of transmitter from SHR.

It is also possible that the 'enhanced cocaine shift' is a feature of cocaine rather than a class effect attributable to blockade of the neuronal monoamine transporter. Controversy surrounds whether DMI or cocaine should be the agent of choice for blocking the neuronal monoamine transporter in functional experiments, that is, DMI is unsuitable because it is an α_1-adrenoceptor antagonist (Williamson & Broadley, 1989; Byg *et al*., 1994) or cocaine is unsuitable because it has a general depressant effect on membrane processes due to its well-known local anaesthetic effect (Lew & Angus, 1983). In our two laboratories, we have used both agents in studies of anococcygeus over a long period (e.g. Gillespie & McGrath, 1974; McGrath, 1984; Vila *et al*., 1992; Hoyo *et al*., 1997; 2002) and found that either could potentiate responses to adrenergic nerve stimulation and noradrenaline. One disadvantage of cocaine is that at optimal concentration for blockade of the transporter, it causes a noradrenaline-mediated contraction of the tissue due to release of neuronal noradrenaline and/or potentiation of spontaneously released noradrenaline (Gillespie & McGrath, 1974); this is particularly noticeable after suppression of NO (E. Vila, unpublished observations). For this reason, we used DMI at a concentration with trivial α_1-adrenoceptor antagonism but effective transporter blockade in rat anococcygeus (pA_2 value of 6.6 ± 0.34, $n = 11$, against the transporter-resistant agonist methoxamine, data not shown; IC_{50} value for blockade of noradrenaline uptake, 40 nM, Doggrell & Woodruff, 1977). This approach produced a clear difference between SHR and WKY in the size of motor nerve-mediated but not noradrenaline-mediated contractions.

The influence of α_2-adrenoceptors was found to be consistent with their normal operation in the two strains. When the neuronal uptake mechanism had been inhibited, rauwolscine enhanced the EFS-induced responses to a greater extent in SHR than in WKY rats, which is what would be expected if transmitter release and hence perineuronal noradrenaline concentration is greater in the SHR. This excludes the participation of prejunctional α_2-adrenoceptors in the greater contraction exhibited by SHR in the presence of DMI.

We found no evidence of a change in nitrergic influence between the strains. Functional responses were similar in the two strains both when isolated and when their influence was assessed by the effect of its removal. The expression of nNOS, as an indirect index of nitrergic innervation, was similar in both strains supporting a similar degree of nitrergic innervation.

The results obtained for expression of TH or nNOS do not support the general hypothesis that a greater noradrenergic or lesser nitrergic innervation is responsible for the differences between SHR and WKY rats. Indeed the tightness of the data suggests that this is a reliable way of estimating innervation in this preparation.

The outstanding feature of this study is that in SHR, when the major known buffers of sympathetic neurotransmission are blocked, the response of smooth muscle to motor nerve stimulation is enhanced. Since the smooth muscle sensitivity to exogenous noradrenaline is not enhanced, this suggests either that more transmitter(s) is released or that in SHR some other termination mechanisms are less effective. Since this exactly parallels the SHR caudal artery it suggests a genetic fault in the sympathetic nerves that produces similar consequences in the two tissues. This is supported by several studies in vas deferens that show essentially that nerve-mediated responses are larger in SHR. A substantial part of transmission in the vas is purinergic, and this component has been shown to be enhanced (Docherty & Warnock, 1986; Guitart *et al*., 2002) providing further support for a fault in the transmitter release process. In anococcygeus, responses to EFS have an NPY component (Hoyo *et al*., 2002), so it cannot be assumed that the mechanism here is purely 'adrenergic'.

Since the eponymous characteristic of the SHR is a feature associated with peripheral resistance, and hence by implication with control of arterial vascular smooth muscle, it is logical to seek an explanation there. There is no question that in SHR the smooth muscle in several tissues is aberrant (Altman *et al*., 1977; Docherty & Warnock, 1986; Muir & Wardle, 1989; Katzuragi *et al*., 1991; Marin, 1993; Vila *et al*., 1993; Tabernero *et al*., 1996; Vivas *et al*., 1997). It may be that the combination of a sympathetic transmission defect and variable excitability of smooth muscle has produced complicated experimental data that has frustrated the attainment of a simple consensus.

Overall, since the extent of the sympathetic innervation is unchanged, this suggests that enhanced motor transmission is due to increased transmitter release per varicosity rather than there being normal transmission from a greater number of sites. This should be the target of further investigation.

Regarding the role of sympathetic nerves in the genesis of hypertension, recent developments present alternative mechanisms. An abnormality in SHR in the mechanisms regulating the concentration of noradrenaline at the adventitial/medial biophase could produce other relevant paracrine actions of noradrenaline apart from smooth muscle contraction, particularly involving longer-term effects such as cell growth or cytokinesis. It has recently become clear that noradrenaline has such effects on both adventitial fibroblasts and vascular smooth muscle cells (Faber *et al*., 2001; Eramis *et al*., 2002; McGrath *et al*., 2002; Zhang *et al*., 2002). In their study of SHR caudal artery, Cassis *et al*. (1985) noted not only that the medial smooth muscle layer was thickened, as is characteristic of hypertension, but that at the adventitial-medial border there were smooth muscle-like cells with an abnormal orientation. It has recently been shown that changes in the elastin structure of the internal elastic lamina in resistance vessels from SHR have a significant role in hypertensive remodelling. It may be that the relevance to hypertension of aberrant sympathetic neurotransmission in SHR is related to trophic effects of neurotransmission rather than acute effects on smooth muscle tone.

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