Evidence for altered ET_B receptor characteristics during development and progression of ventricular cardiomyocyte hypertrophy

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ETB receptors were detected on SHR cardiomyocytes as early as 8 wk than WKY cells at 12 wk (44). Increased plasma levels of ET-1 occur during onset of LVH; involvement of nonhemodynamic factors has also provided a useful model of human hypertension and LVH (11, 17). These data highlight the importance of longitudinal studies that utilize cells obtained ex vivo from diseased hearts to address the temporal dependence of expression by cardiomyocytes of preproET-1 and ET receptor mRNA and the relative abundance of and responsiveness to each receptor subtype during onset and progression of LVH in vivo.

CONCENTRIC LEFT VENTRICULAR hypertrophy (LVH) occurs after pressure overload; endothelin receptor antagonist characteristics during development and progression of ventricular cardiomyocyte hypertrophy. ETB receptors were not detected on WKY cells until 20 wk. Ventricular cardiomyocytes were isolated from SHRs before onset (8 and 12 wk) and during progression (16, 20, and 24 wk) of LVH and compared with age-matched normotensive Wistar-Kyoto (WKY) rats. PreproET-1 mRNA expression was elevated in SHR (P < 0.05) relative to WKY cardiomyocytes at 20–24 wk. ET binding-site density was twofold greater in SHR than WKY cells at 12 wk (P < 0.05) but normalized at 20 wk. ET_B receptors were detected on SHR cardiomyocytes as early as 8 wk and their affinity increased progressively with age (P < 0.05), whereas ET_A receptors were not detected on WKY cells until 20 wk. ET-1 stimulated protein synthesis with similar maximum responses between strains (21–30%), in contrast with sarafotoxin 6c, which stimulated protein synthesis in SHR (13–20%) but not WKY cells at 12–20 wk. In SHR but not WKY cells, the ET_B receptor-selective ligand A-192621 increased protein synthesis progressively with the development of LVH (15% maximum effect). In conclusion, the presence of ET_A receptors (8–12 wk) coupled with functional responsiveness of SHR cells but not WKY cells to sarafotoxin 6c at 12 wk supports the involvement of ET_A receptors before the onset of cardiomyocyte hypertrophy, whereas altered ET_B receptor characteristics during active hypertrophy (16–24 wk) indicate that ET_B receptor mechanisms may also contribute to disease progression.

spontaneously hypertensive rats; pressure overload; endothelin receptor

hypertension and heart failure and correlate with severity of LVH (15). ET receptor antagonists attenuate LVH in some experimental models in vivo (17, 20). It is unclear whether this occurs as a direct result of ET receptor blockade on cardiomyocytes or represents an indirect effect that is due to reduction in systolic pressure; elevated ET-like immunoreactivity and binding-site density in cardiac tissue indicate that locally derived ET may contribute to ventricular remodeling (3, 6, 35, 45). The actions of ET are mediated by ET_A and ET_B receptors, which are both present in heart (13, 39): ET_A receptors have greater affinity for ET-1 than ET-3, whereas ET_B receptors bind these peptides with equal affinity (2). Sarafotoxin 6c (S6c) is an ET_B receptor-selective agonist; BQ-123 and ABT-627 {2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[N,N-di(n-butyl)amino carbonylmethyl]-pyrrolidine-3-carboxylic acid} are selective antagonists of ET_A receptors, whereas A-192621 [2-(4-propoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(2,5-ethylphenyl)amino carbonylmethyl-pyrrolidine-3-carboxylic acid] is a very potent and highly selective antagonist of ET_B receptors (8, 17). ET-1 elicits a positive inotropic effect on the myocardium; both ET_A and ET_B receptors are implicated in the contractile responses to ET-1 in normal and diseased cardiomyocytes (22).

ET-1, via ET_A and ET_B receptors, initiates increased mass of adult cardiomyocytes in vitro, in which the influence of mechanical loading is eliminated (8). ET_A receptor mRNA is upregulated in hypertrophying neonatal cardiomyocytes (19). ET-3 and mechanical stretch induce expression of preproET-1 mRNA, whereas hypertrophy of neonatal cardiomyocytes by each stimulus in vitro is attenuated by BQ-123 (38, 43). Nonmyocytes provide an additional source of ET-1 and ET-3 within myocardium (41). ET peptides may initiate cardiomyocyte hypertrophy with additional factors taking over a maintenance role because the initial attenuation by BQ-123 of the onset of LVH after aortic banding of adult rats is not sustained (17). These data highlight the importance of longitudinal studies that utilize cells obtained ex vivo from diseased hearts to address the temporal dependence of expression by cardiomyocytes of preproET-1 and ET receptor mRNA and the relative abundance of and responsiveness to each receptor subtype during onset and progression of LVH in vivo.

The spontaneously hypertensive strain of Wistar rat (SHR) provides a useful model of human hypertension and LVH (11, 31). Hypertension develops gradually in SHRs a few weeks after birth; onset of LVH occurs between 10 and 20 wk. Despite severe elevations of systemic arterial pressure, cardiac...
output is maintained initially by moderate LVH. Because alterations in cardiac performance may reflect many influences (intrinsc muscle properties, loading conditions, altered systemic and/or coronary hemodynamics), studies in cardiomyocytes specifically are useful to dissect out adaptations intrinsic to them from those of fibrosis and nonmyocyte proliferation.

We have characterized SHRs comprehensively at the cardiomyocyte level allowing precise application of this model in investigations of pathogenetic mechanisms; hypertension is followed by active hypertrophic growth between 16 and 20 wk. This is evidenced by increased cell mass and width, which subsequently decelerate at 24 wk as stable compensation is attained (4). There are conflicting data regarding whether plasma ET-1 levels are elevated in SHRs (23, 39). Chronic intervention with ET receptor antagonists attenuates hypertension only when overexpression of ET-1 in blood vessel walls is demonstrable (28, 29). Evidence that bosentan causes some regression of LVH without an appreciable reduction in blood pressure indicates that ET-1 may exert a local influence on cardiomyocyte hypertrophy independent of systemic pressor effects (20). For this reason, it is important to examine expression of the peptide and alterations in ET receptors and/or responsiveness within SHR hearts; conflicting evidence has been obtained in this regard depending on the approach used, tissue source, and sampling time (5, 18, 32), and little evidence exists of effects on isolated cardiomyocytes (10).

The aim of this longitudinal study was to investigate whether alterations in the ET receptors are initiated in cardiomyocytes before the onset of LVH in SHRs, and if so, whether these alterations are associated with the development and progression of ventricular cell hypertrophy. Appropriate comparisons were made using cardiomyocytes isolated from age-matched normotensive Wistar-Kyoto (WKY) rats.

METHODS

Experimental model. Male WKY rats and SHRs were obtained from Harlan at 4 wk and maintained until sampling at 8, 12, 16, 20, and 24 wk of age. The study was performed in accordance with Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, published by Her Majesty’s Stationary Office, London. After administration of deep anesthesia to rats using isoflurane (Abbott Laboratories), the hearts were rapidly excised and placed on ice-cold saline, and blood was collected from the chest cavity into ice-cold tubes containing EDTA (2 mmol/l) and aprotinin (500 IU/ml; Sigma Chemical). Immunoreactive ET was extracted from plasma using C18 Sep-Pak cartridges (Waters Associates) and measured by enzyme immunoassay (Abbott Laboratories), the hearts were rapidly excised and placed on ice-cold saline, and blood was collected from the chest cavity into ice-cold tubes containing EDTA (2 mmol/l) and aprotinin (500 IU/ml; Sigma Chemical). Immunoreactive ET was extracted from plasma using C18 Sep-Pak cartridges (Waters Associates) and measured by enzyme immunoassay (Abbott Laboratories).

Cardiomyocyte isolation. Excised hearts were cannulated through the ascending aorta, and ventricular cardiomyocytes were isolated by enzymatic digestion (collagenase, 0.4 mg/ml) using Langendorff perfusion (8). After purification, cells were suspended at 1.5 × 106 viable cardiomyocytes/ml in a creatinine-carnitine-taurine (CCT) medium that consisted of modified glutamine-free medium 199 (M199) supplemented with Earle’s salts (GIBCO), HEPES (15 mM), creatinine (5 mM), l-carnitine (2 mM), taurine (5 mM), ascorbic acid (100 μM), penicillin (100 IU/ml), and streptomycin (100 μg/ml). The medium was also supplemented with cytokine β-D arabinofuranoside (10 μM) to prevent growth of nonmyocytes (Sigma; Ref. 8).

RT-PCR. Total cellular RNA was isolated by a modification of the acid guanidinium thiocyanate-phenol-chloroform method of Chomczynski and Sacchi (7). First-strand cDNA was synthesized from 2 μg of total RNA by reverse transcriptase (Reverse-IT kit; Abgene). Gene-specific primers were based upon those previously reported (37). After initial denaturation at 94°C for 4 min, cycling profiles included specific annealing temperatures and cycle numbers [pre-ET-1: 54°C, 31; endothelin-converting enzyme (ECE): 60°C, 28; ET₄/ET₆ receptors: 55°C, 32; respectively], followed by extension at 72°C for 60 s. PCR products were electrophoresed on 2% agarose gels and stained with ethidium bromide. Gels were visualized under ultraviolet illumination and analyzed using a Gene Genius Gel documentation system with Gene Tools analysis software (Syngene). Band intensity was expressed as the ratio of target mRNA to GAPDH mRNA.

Preparation of sarcolemmas. Viable cardiomyocytes were suspended in a HEPES (20 mmol/l) buffer that contained the protease inhibitors aprotinin (0.8 μmol/l), bacitracin (0.1 mmol/l), benzamidine (0.1 mmol/l), EDTA (5 mmol/l), leupeptin (2 μmol/l), and PMSF (0.1 mmol/l) (all from Sigma), and were homogenized at 9,500 rpm (Ultra-Turax-T25; Janke and Kunkel) for 30 s. Disrupted cells were centrifuged (2,000 rpm for 5 min at 4°C; Mistral MSE 400) to sediment cell nuclei and mitochondrial fractions, and supernatants were then centrifuged three times at 20,000 rpm for 30 min at 4°C. The pellets were stored at −70°C.

Homologous/heterologous competition binding. Sarcolemmas were suspended (20 μg/ml) in a Tris buffer (20 mmol/l, pH 7.4, 37°C) that contained EDTA (5 mmol/l) and protease inhibitors (as above) and incubated (for 2 h at 37°C) with [125I]ET-1 (20 pmol/l; Amersham Pharmacia Biotech) in the absence and presence of ET-1 (0.002–20 nmol/l, American Peptide), ET-3 (0.0001–200 nmol/l; American Peptide), or A-192621 (0.00002–1 μmol/l; Abbott Laboratories). Excess unlabeled ET-1 (200 nmol/l) was used to measure nonspecific binding (NSB; 9.4 ± 0.9%; n = 22), and total binding (TB) was determined in the absence of unlabeled ET peptide. Receptor-bound [125I]ET-1 was separated from unbound after dilution with ice-cold Tris buffer (20 mmol/l) that contained bovine serum albumin (2% wt/vol; Sigma) and bacitracin (0.1 mM/l). Separation occurred under vacuum filtration (Millipore) across glass microfiber filters (25 mm diameter; Whatman) and radioactivity on each filter was counted (Wallac 1410). Specific binding (SB) was calculated as TB − NSB. Data were analyzed by nonlinear regression and fitted to a one- or two-site model (GraphPad Prism), and regression analysis of the data was used to determine a two-site model when P < 0.05.

Protein synthesis. Petri dishes (35 mm diameter) were preincubated for 2 h with fetal calf serum (4% vol/vol) in M199. Aliquots of cell suspension (1 ml) were pipetted gently onto petri dishes, and after 1 h viable cardiomyocytes became attached to the surface of the dish. The dishes were then washed with fresh CCT medium to remove nonattached cells and cell debris, and the attached cells were exposed for 24 h to 1-μCi [3H]phenylalanine (0.1 μCi/ml of culture medium; Amersham Pharmacia Biotech). Incorporation of radioactivity into the acid-insoluble cell fraction was determined under basal conditions and in the presence of ET receptor agonists/antagonists (8). The attached cells were then washed with a 1-mI aliquot of ice-cold PBS before the addition of a 1-m1 aliquot of ice-cold trichloroacetic acid (10% wt/vol). After storage overnight at 4°C, the acid containing the intracellular precursor pool was removed from the dishes, and the attached cells were washed with a 1-m1 aliquot of PBS. The precipitate remaining on the culture dishes was dissolved in a 1-ml aliquot of 1 M NaOH-SDS (0.01% wt/vol) by overnight incubation at 37°C. In these samples, concentration of DNA was determined by a spectrophotometric method in which bis-benzamidine dye was incorporated into DNA and the radioactivity was counted. The ratio of 1-μCi [3H]phenylalanine incorporated into DNA per culture served as a measure of de novo protein synthesis.

Contractile amplitude. Cardiomyocytes were subjected to field stimulation at 0.5 Hz with biphasic pulses of 0.5-ms duration at 60 V under basal conditions and in the presence of ET receptor agonists/antagonists. Cell shortening was assessed by video edge detection (VED 104; Crescent Electronics; Ref. 22), and data were digitized,
recorded, and analyzed using WCP for Windows software (version 1.8) provided by Dr. John Dempster (University of Strathclyde).

Data analysis. Data are expressed as means ± SE, where n denotes the number of rats in which plasma immunoreactive ET-1 was measured or the number of heart cell preparations used to analyze gene expression, contractile amplitude, receptor binding, or protein synthesis. Statistical analyses were performed by ANOVA to detect significant differences for between-group or within-group effects and post hoc comparisons by Bonferroni or an unpaired Student’s t-test as appropriate.

RESULTS

Expression. ET-1 concentration (in pmol/l) was greater (P < 0.05) in plasma of SHRs (3.98 ± 1.3; n = 4) than WKY rats (1.1 ± 0.1; n = 7) at 8 wk; thereafter, values were similar between strains. Plasma concentration did not alter with age in WKY rats (1.1 ± 0.1; n = 7 at 8 wk vs. 0.9 ± 0.1; n = 3 at 24 wk). PreproET-1 mRNA expression was greater in SHR than WKY cardiomyocytes at 20 and 24 wk (P < 0.05); ECE mRNA was not different between SHR and WKY cells at any age (Fig. 1, A and B). Expression of cardiomyocyte ETA and ETB receptor mRNAs increased from 12 to 20 wk in both strains but were not different between strains (Fig. 1, C and D).

Homologous binding. A one-site model was obtained using nonlinear regression of data from each experiment (Fig. 2). In cardiomyocyte membranes of SHRs at 12 wk, ET receptor number was greater (P < 0.05) than that of WKY rats, whereas at 20 wk, receptor number had declined to that of WKY rats. Affinity (Kd) of ET-1 for ET receptors did not decrease significantly between 12 and 20 wk and was similar between strains at each age.

Heterologous binding. A two-site model was obtained via nonlinear regression of data from each experiment using ET-3 to displace [125I]ET-1 binding to cardiomyocyte membranes from SHRs at 12, 16, and 20 wk and from WKY rats at 20 wk (Fig. 3). IC50 values are given in Table 1. The proportion of high-affinity binding sites was 22–25%. The identities of the high- (in pmol/l) and low- (in nmol/l) affinity binding sites for ET-3 were confirmed as the ETA and ETB receptors, respectively, in heterologous competition experiments using the ETB receptor-selective antagonist A-192621 (data not shown). In contrast, a one-site model was obtained using nonlinear regression of data from WKY rats at 12 and 16 wk, which indicated that only the low-affinity binding site was present on these cells. The affinity of ET-3 for the ETB receptor (the pmol/l site) in SHR membranes increased (P < 0.05) with disease progression and was greater (P < 0.05) than that of WKY rats at 20 wk. The affinity of ET-3 for the ETA receptor (the nmol/l site) decreased (P < 0.05) with age in WKY rats.

Hypertrophic function. ET-1 stimulated (P < 0.05) protein synthesis in SHR and WKY cardiomyocytes at 12, 16, and 20 wk (Fig. 4). The response to ET-1 (10−9 mol/l) was not altered with age or strain. S6c (10−7 mol/l) increased (P < 0.05) protein synthesis in SHR but not WKY cardiomyocytes at each age (Fig. 4). A-192621, at a concentration selective for interaction with ETB receptors (10−10 mol/l), did not alter basal protein synthesis in WKY cardiomyocytes at any age but paradoxically displayed agonist activity (P < 0.05) per se in SHR cardiomyocytes at 12 wk and, markedly, at 16 and 20 wk (Fig. 5). In the presence of A-192621 (10−10 mol/l), the response to ET-1 (10−9 mol/l) was attenuated (P < 0.05) in SHR cardiomyocytes at 12 wk but not at 16 and 20 wk nor in WKY cardiomyocytes at any age (Fig. 6). Similarly, the response to S6c in SHR cardiomyocytes was abolished by A-192621 (10−10 mol/l) at 12 wk but not at 16–20 wk.

Contraction. EC50 values (range, 4.6 × 10−11 to 1.8 × 10−10 mol/l) and contraction maxima (range 38.0–50.8% increase from basal) for ET-1 did not alter with age in SHR cardiomyocytes (data not shown). The response to a submaximal concentration of ET-1 (Fig. 7; 10−9 mol/l) was I) not altered between strains, 2) abolished by the ETA receptor-
selective antagonist ABT-627 (10⁻⁹ mol/l), but 3) not altered by the ET₄ receptor-selective antagonist A-192621 (10⁻⁷ mol/l) in WKY or SHR cardiomyocytes (8–24 wk). S6c (10⁻⁷ mol/l) did not affect cell shortening in either strain at any age.

**DISCUSSION**

This study has provided evidence that ET₄ receptors are already present on cardiomyocytes of SHRs before development of LVH but are absent from cardiomyocytes of normotensive WKY rats. Furthermore, the pharmacological characteristics of these receptors become altered during progression of cardiomyocyte hypertrophy in vivo. Taken together, these findings have implications for the role of endogenous endothelin signaling pathways and the contribution of ET₄ receptors specifically during the development of LVH.

Increased plasma levels of ETs are positively correlated with severity of LVH in humans (15). There are conflicting data, however, regarding whether plasma ET-1 levels are elevated in SHRs. Thibault et al. (39) found elevated levels at 18 wk when moderate LVH was evident; others (23, 26), including ourselves, detected no change. In contrast with the work of Kohno
et al. (23), who reported normal levels in prehypertensive SHRs at 6 wk, we observed a transient increase in plasma ET-1 levels at 8 wk subsequent to the onset of hypertension and preceding development of LVH, which suggests a possible association. However, ET-1 has a short half-life (4–7 min; Ref. 30), which may limit the influence of plasma-derived peptide directly on the myocardium. The mixed ET$_{AB}$ antagonist bosentan causes some regression of LVH without reducing blood pressure, which indicates that locally derived ET may exert a direct influence on cardiomyocyte hypertrophy independently of the pressor effects of the peptide (20). Iyer et al. (18) reported increased ET-1 content in hearts of SHRs at 8 but not 4 wk relative to WKY rats; others detected no change at 10–12 wk (5) and 18 wk (39). Increased mechanical stretch and paracrine mediators induce expression of preproET-1 mRNA in neonatal cardiomyocytes in vitro (38, 43). Although hypertension in vivo might be expected initially to increase mechanical stretch of cardiomyocytes, we found no differences in preproET-1 or ECE mRNA expression between strains before onset of LVH. Secretion of ET-1 from cardiac mesothelial cells is enhanced in SHRs at 9 wk (25); nonmyocytes could provide an alternative source of ET-1 to initiate LVH (41).

ET receptor number was greater in cardiomyocyte membranes of SHRs than WKY rats at 12 wk. ET receptor number was also elevated early in development of LVH on aortic banding in rats (3), which supports early recruitment of ET-dependent signaling mechanisms after pressure overload. In contrast, others have reported decreased ET-1 binding-site density in ventricular membranes from SHRs at 10–14 wk (5, 14) compared with WKY rats. At 20 wk, when compensated LVH was present, receptor number declined to values similar to or lower than those of WKY membranes in agreement with the decrease reported by Gu et al. (14) at 25 wk. Crude ventricular membrane preparations may not, however, accurately reflect changes occurring directly in cardiomyocytes because nonmyocytes possess a high density of ET receptors (21).

Cardiomyocyte ET$_A$ and ET$_B$ receptor mRNA expression was similar between SHRs and WKY rats at all ages, which indicates that posttranscriptional mechanisms account for differences between strains in relative abundance of receptor subpopulations. This contrasts with the finding of Kanno et al. (19) that ET$_B$ receptor mRNA expression is upregulated in hypertrophying neonatal cardiomyocytes in vitro and raises questions regarding extrapolation from neonates to adults. Inability to detect ET$_B$ receptors on cardiomyocytes of WKY rats at 12 and 16 wk agrees with the study of Fareh et al. (13), who reported that >90% of ET-1-binding sites on ventricular cardiomyocyte membranes from young adult Sprague-Dawley rats were of the ET$_A$ subtype. Although Thibault et al. (39) reported ratios of 25% ET$_B$ to 75% ET$_A$ in both strains at 18 wk, crude ventricular membranes were used, so cellular localization of each receptor subpopulation cannot be ascertained. Although the total number of ET-1 binding sites present on SHR cardiomyocytes was lower at 20 than at 12 wk, the proportion of each subtype did not change, which indicates that both subpopulations are downregulated with progression from onset to attainment of compensated LVH. Greater ET-1 binding-site density in WKY cardiomyocytes at 20 than at 12 wk.

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**Table 1. IC$_{50}$ values determined from nonlinear regression analysis of heterologous competitive binding**

<table>
<thead>
<tr>
<th></th>
<th>Wistar-Kyoto Rats</th>
<th>Spontaneously Hypertensive Rats</th>
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<tbody>
<tr>
<td>Age, wk</td>
<td>ET$_B$ (Picomolar)</td>
<td>ET$_A$ (Nanomolar)</td>
</tr>
<tr>
<td>8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>None</td>
<td>1.90±0.1</td>
</tr>
<tr>
<td>16</td>
<td>2.72±0.05</td>
<td>11.5±7</td>
</tr>
<tr>
<td>20</td>
<td>123±10</td>
<td>5.32±0.53*</td>
</tr>
</tbody>
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Values are means ± SE; $n = 3$–5 rats. ET, endothelin; ND, not determined. *P < 0.05 between preceding age within strain; †P < 0.05 between strain (equivalent age-matched site).

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**Fig. 4. Effects of ET-1 (10$^{-9}$ mol/l) and sarafotoxin 6c (S6c; 10$^{-7}$ mol/l) on incorporation of L-$\mu$-[14C]phenylalanine into protein in ventricular cardiomyocytes isolated from SHRs and WKY rats at 12, 16, and 20 wk and maintained in culture (24 h). Radioactivity incorporated was corrected for DNA content as an index of cell number. Data are percentage differences from basal level and represent means ± SE of 8 preparations. *P < 0.05 vs. age-matched WKY rats; †P < 0.05 vs. basal response.**

**Fig. 5. Effects of A-192621 (10$^{-10}$ mol/l) on incorporation of L-$\mu$-[14C]phenylalanine into protein in ventricular cardiomyocytes isolated from SHRs and WKY rats at 12, 16, and 20 wk and maintained in culture (24 h). Radioactivity incorporated was corrected for DNA content as an index of cell number. Data are percentage differences from basal numbers and represent means ± SE of 10 preparations. *P < 0.05 vs. response in absence of ligand.**
Fig. 6. Effects of A-192621 (A-192; 10^{-10} mol/l) on incorporation of l-[U-^{14}C]phenylalanine into protein in the presence of ET-1 (10^{-9} mol/l) and sarafotoxin 6c (Sfx; 10^{-7} mol/l), respectively, in ventricular cardiomyocytes isolated from SHRs and WKY rats at 12 (A, B), 16 (C, D), and 20 (E, F) wk and maintained in culture (24 h). Radioactivity incorporated was corrected for DNA content as an index of cell number. Data are percentage differences from basal values and represent means ± SE of 3–6 preparations. *P < 0.05 vs. basal response.

Fig. 7. Effects of ET-1 (10^{-9} mol/l; A), sarafotoxin 6c (Sfx; 10^{-7} mol/l; B), and ET-1 (10^{-9} mol/l) in the presence of ABT-627 (10^{-10} mol/l; C, D) on contractile amplitude of ventricular cardiomyocytes isolated from SHRs and WKY rats at 8–24 wk. Cell shortening is expressed as a percentage (ΔL%) of resting length (L). Data are means ± SE of 3–6 preparations.
could reflect the appearance of ET\(_B\) receptors in this strain with advancing age, which occurs later than in SHRs. This is consistent with the proposition that LVH after pressure overload represents accelerated myocardial aging.

In contrast with the hypertrophic response elicited in WKY cells, which was almost exclusively attributable to ET\(_A\) receptor stimulation, the majority of the response initiated in SHR cardiomyocytes at 12 wk was associated with ET\(_A\) receptor involvement, because S6c elicited a similar response to ET-1 although ET\(_B\) receptors accounted for only \(\sim 25\%\) of ET receptors present. Involvement of both receptor subtypes in initiating hypertrophy of neonatal (17, 38) and adult (8) cardiomyocytes has been demonstrated in vitro, although the proportion of ET\(_B\) receptors present on cardiomyocytes obtained from healthy rats is negligible (<10%; Ref. 13). The number of receptors present does not necessarily imply involvement in, or relate to efficacy of, a particular response; this highlights the importance of combining investigation of binding characteristics with appropriate functional bioassays. Reduced receptor number in SHR cells at 20 wk was not associated with decreased maximum response to ET-1, which indicates the presence of surplus receptors.

The homologous competition data obtained at 12 wk indicate that ET-1 binds with high affinity (pmol/l) to cardiomyocyte ET receptors representing binding predominantly ET\(_A\) receptors in SHR and predominantly so in WKY. It is likely that levels of ET-1 in the vicinity of healthy cardiomyocytes would be significantly less than the \(K_d\) value of 2 \(\times\) \(10^{-10}\) mol/l reported (13, 23, 26). However, the presence of ET\(_B\) receptors on SHR cardiomyocytes would enable picomolar levels of ET-3, which would not interact significantly with ET\(_A\) receptors, to act in concert with ET-1 in initiating LVH; indeed, ET-3 is secreted by nonmyocytes and initiates hypertrophy of neonatal cardiomyocytes in vitro (38, 41, 43).

The affinity of ET\(_B\) receptors present on SHR cells was enhanced with disease progression. Such changes have implications for modulation of cell responsiveness to the growth effects of ET-1 and ET-3 during development of LVH in vivo, because significant stimulation might be achieved via ET\(_B\) receptor-mediated signaling mechanisms to initiate and maintain hypertrophic growth even in the absence of elevated peptide levels. Enhanced affinity might be attributed to post-translational modification of the receptor protein. The antagonist A-192621, which binds selectively to ET\(_B\) receptors (8), paradoxically displayed agonist activity in SHR cells. This observation is also compatible with specific structural changes to the receptor protein resulting in altered intrinsic efficacy of the A-192621-receptor complex. A-192621 displayed partial agonist activity at 12 wk and partially attenuated the response to the full agonists ET-1 and S6c but acquired almost full agonist activity at 16–20 wk such that A-192621 no longer antagonized the responses to ET-1 and S6c. Changes to receptor characteristics mainly occurred between 12 and 16 wk, which corresponds to the onset and early progression of hypertrophic growth of the SHR myocardium (4); additional increases in the affinity of ET-3 for the ET\(_B\) receptor and in the activity of A-19261 were marginal at 20 wk. Structural alterations might be accounted for by oxidation of amino, thiol, diazo, and tyrosyl groups. Evidence is emerging to support a role for oxidative stress and its interaction with ET-1 in development of LVH (16, 34). Oxidative stress can influence binding of aldosterone to mineralocorticoid receptors (33). Lipid peroxidation modifies plasmalemmal proteins (12) and enhances the opening probability of sarcoplasmic reticulum ryanodine receptors (1), and the binding of calmodulin to calcium-release channels in skeletal muscle (46); attenuated dopamine D\(_{1A}\) receptor-effector coupling has been attributed to lipid peroxidation of receptors in the proximal renal tubule of SHRs (42).

The positive contractile effect of ET-1 was exclusively attributed to ET\(_A\) receptor activation in both SHR and WKY cells. It is unclear whether levels of ET-1 present in the vicinity of the cardiomyocytes (23, 26, 39) would enable the peptide to elicit an inotropic response in vivo even in hypertrophying SHR myocardium. In contrast to that of ET\(_B\) receptors, the affinity of ET\(_A\) receptors was not enhanced with disease progression. The maximum response to ET-1 was constant with age in SHR cells and was not different to that of WKY cells, which confirms the finding of Delbridge et al. (10) at 12 wk, despite reduced values for maximal binding capacity in SHR cells at 20 wk compared with 12 wk. These data support the presence of “spare” ET\(_A\) receptors.

In conclusion, the presence of ET\(_B\) receptors before onset of cardiomyocyte hypertrophy coupled with responsiveness to S6c of SHR but not WKY cells support the involvement of ET\(_B\) receptors in initiating cardiomyocyte hypertrophy after pressure overload, whereas altered ET\(_B\) receptor characteristics during active hypertrophic growth indicate that ET\(_B\) receptor-dependent mechanisms may also contribute to disease progression. These findings indicate a more prominent role for ET\(_B\) receptors than previously envisaged in the pathogenesis of LVH and have important implications for the current debate regarding the choice of receptor subtype selective or nonselective endothelin antagonists in therapeutic intervention. Early intervention with ET\(_B\) receptor-selective antagonists may be beneficial in preventing or retarding development of LVH in hypertensive patients, although this benefit might be offset by attenuated ET\(_B\)-mediated vasodilatation and hence exacerbate already elevated blood pressure. Intervention studies with ET\(_B\) receptor-selective antagonists in SHRs and other experimental models are now clearly warranted.

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