Transmural changes in size, contractile and electrical properties of SHR left ventricular myocytes during compensated hypertrophy

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Abstract

Objective: Some hypertrophic stimuli provoke responses from myocytes that vary across the thickness of the left ventricular wall. The Spontaneously Hypertensive Rat (SHR) is a well-established genetic model of hypertension and whole heart hypertrophy. Details of transmural responses to hypertension in the SHR are few, but are needed if the properties of this model are to be fully understood. We therefore tested the hypothesis that left ventricular myocytes of the SHR do not respond uniformly to their hypertensive environment.

Methods: The volume, contraction and action potentials of enzymically isolated sub-epicardial (EPI) mid-myocardial (MID) and sub-endocardial (ENDO) myocytes from the left ventricle of 20-week-old SHR and normotensive Wistar--Kyoto (WKY) control rats were compared.

Results: Compared to WKY, as a single population, SHR myocytes displayed concentric hypertrophy (larger volumes with smaller length:width) increased t-tubule spacing, larger and prolonged cell shortening and intracellular calcium ([Ca$^{2+}$]$_i$) transients and longer action potentials. However, these responses differed across the left ventricular wall. MID myocytes showed significantly less hypertrophy than EPI and ENDO myocytes. EPI myocytes showed the largest (and significant) increases in cell shortening, [Ca$^{2+}$]$_i$ transients and action potential duration, whilst MID myocytes showed the smallest (and non-significant) changes in these parameters. Real time reverse transcription polymerase chain reaction analysis on cardiac tissue suggest that increased expression of mRNA for fibronectin-1 and protein kinase C$_q$ are involved in the hypertrophic response of the whole heart.

Conclusions: Our findings show that in the SHR, the effect of hypertension upon the morphology, mechanical activity and electrical activity of left ventricular myocytes is dependent upon their transmural location. Therefore, in addition to the overall compensating response to hypertension, i.e. increased contractility, there are likely to be regionally specific alterations in mechano-electric interactions that may influence the properties of this important model, e.g. its predisposition to arrhythmia whilst still in a compensated state.

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Keywords: Calcium; Contractile function; Hypertension; Hypertrophy; Myocytes

1. Introduction

The Spontaneously Hypertensive Rat (SHR) is a well-established genetic model of hypertension and whole heart hypertrophy when compared to its normotensive equivalent, the Wistar--Kyoto (WKY). The compensated state of the SHR model has been reported as early as 3 months but is more often studied closer to 6 months, while failure is reported at 18–24 months [1,2]. Hypertrophy has been demonstrated in SHR single myocytes (e.g. [3,4,5,2]) and whilst still in the compensated stage there is an increase in myocyte contraction, intracellular Ca$^{2+}$ ([Ca$^{2+}$]$_i$) transients and action potential duration (APD) [4].

However, these studies were performed on mixed populations of ventricular myocytes. It is known that there are differences in the in vivo environment (e.g. in transmural stress and strain [6,7]) and in the basic structural, electrical and mechanical properties of myocytes across the wall of the left ventricle [8,9,10,11]. More importantly, it has been shown that responses to certain hypertrophic stimuli are dependent upon myocyte origin. For example, constriction-induced pressure overload caused greater hypertrophy in the sub-endocardial (ENDO) than sub-epicardial (EPI) myocytes [12], isopren-
aline-induced hypertrophy caused selective lengthening of EPI APD [13] and exercise caused greater hypertrophy [11] and increased myofilament Ca$^{2+}$ sensitivity [14] in ENDO compared to EPI myocytes. The influence of myocyte regional origin on the response to hypertension in the SHR is little understood, even though any effects will be important in understanding the response of the whole organ to hypertension (e.g. its pre-disposition to arrhythmia whilst still in a compensated state [15]). Given that some hypertrophic stimuli have been shown to produce a transmurally varied response, we tested the hypothesis that myocytes from different regions of the SHR left ventricle do not respond uniformly to the hypertensive environment.

2. Methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23 revised 1996). Data are presented from 27 WKY and 39 SHR animals.

2.1. Measurement of systolic blood pressure

Systolic blood pressure was measured in 20-week-old SHR and WKY rats by the tail-cuff method using a sphygmomanometer (Infrasonde, Model D4000) set to 240 mm Hg, at a tail surface temperature between 27 and 29 °C.

2.2. Cardiac myocyte isolation

Twenty-week-old male SHR or WKY rats were killed and hearts removed. The hearts were perfused at 37 °C on a Langendorff apparatus at a constant flow rate of ~8 ml/min. for a period of ~4 min with isolation solution containing (in mmol/l): NaCl, 130; KCl, 5.4; MgCl$_2$, 1.4; NaH$_2$PO$_4$, 0.4; HEPES, 5; Glucose 10; Taurine, 20; Creatine, 10, plus 750 µmol/l CaCl$_2$; pH 7.3 at room temperature. CaCl$_2$ was replaced with 100 µM EGTA for a further 4 min. Finally, the heart was exposed to isolation solution (containing 0.75 mg/ml collagenase (Worthington, USA) and 0.075 mg/ml protease (Sigma) and 50 µmol/l CaCl$_2$) for 7–8 min. Thin strips of left ventricular free wall, EPI and ENDO tissue were excised with fine scissors. The remaining free wall tissue was then trimmed on both surfaces to provide a MID region spatially distinct from the EPI and ENDO regions. Further digestion was carried out in separate conical flasks.

2.3. Isolation of total RNA

EPI, MID and ENDO tissue samples (40–50 mg) were excised from the left ventricles of three 20-week-old SHR and three WKY hearts, snap-frozen in liquid nitrogen and cut into 25 µm sections on a cryostat. Samples of single myocytes were isolated from each strain and region as described above. All samples were stored at −80 °C until RNA extraction. Total RNA was extracted from all samples using a modified Qiagen mini-kit protocol for heart and skeletal muscle [16]. Frozen samples were homogenised (rotor-stator homogeniser, Ultra Turrax T8, IKA) in warm lysis (RLT) buffer (45 °C) for 2 min, diluted with DEPC-treated water, Proteinase K (600 mAU/ml ~ 20 mg/ml; Qiagen) added and the sample vortexed and incubated in a water bath for 1 h at 45 °C. Samples were centrifuged at 13000 rpm for 5 min, room temperature and the supernatant retained. 2 ml of RLT buffer was added before ethanol precipitation and binding of the RNA to the Qiagen mini-column. DNase I treatment was carried out to prevent contamination from co-extracted DNA (RNase free kit, Qiagen). RNA samples were separated by electrophoresis through 0.6% formaldehyde/1% agarose gels, visualised by EtBr fluorescence and the concentration of RNA adjusted to 1 µg/µl. The resulting samples were reverse transcribed using random hexamers with the Superscript II First Strand cDNA synthesis Kit (Life Technologies, Rockville, MD, USA) and the cDNA diluted 1:20 in TE and stored in 1 µl aliquots at −20 °C until required.

2.4. Real-time reverse transcriptase polymerase chain reaction (RT-PCR)

Real-time RT-PCR was performed using a Roche® LightCycler (Roche Applied Systems, Indianapolis, IN, USA). Primers were designed to specifically amplify protein kinase C epsilon (PKCe) or fibronectin-1 and amplification was performed according to Fast Starts SYBR® Green I Master Kit instructions (Roche Diagnostics, Mannheim, Germany). Primers are given in Table 1. One-microliter aliquots of the cDNAs were combined with 9 µl of master mix in the LightCycler capillaries. Magnesium and primer concentrations were adjusted for each amplified cDNA fragment as determined by a series of test runs for each primer pair. The thermocycling program consisted of: 10 min activation at 95 °C followed by 35–40 cycles of PCR, 95 °C for 15 s, 49–55 °C, depending on primers, for 3 s, 72 °C for 20 s. Melting curves were generated by 0.1 °C/s gradients between 60 and 95 °C with continuous fluores-

<table>
<thead>
<tr>
<th>Gene of interest</th>
<th>Protein kinase Cε</th>
<th>Fibronectin-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genbank accession number</td>
<td>E02148</td>
<td>NM019143</td>
</tr>
<tr>
<td>Forward primer sequence (5’–3’)</td>
<td>aagcataactacactgtagttgagtactgttcggtattc</td>
<td>ggttagctgctagagta</td>
</tr>
<tr>
<td>Reverse primer sequence (5’–3’)</td>
<td>attgtagttgagggtctccc</td>
<td>agttagccccgggctt</td>
</tr>
<tr>
<td>Predicted size of Amplicon (bp)</td>
<td>190</td>
<td>329</td>
</tr>
</tbody>
</table>

Primers were designed against the published GeneBank sequences for the rat homologues of the genes stated. Sequence for PKCe was obtained from GeneBank, Patent: JP1990000433, Takeda Chem.
cence acquisition. In each case, product identity was demonstrated by the presence of a single peak on derivative melting curve plots and a single band of the appropriate size on gel electrophoresis. DNA sequencing provided final confirmation of PCR product identity. For all transcripts, three independent measurements were performed for each cDNA sample and their values averaged and related to the values for 18S cDNA to correct for variations in input total cDNA. Levels of 18S have been observed to remain stable across a variety of tissues, whereas other commonly used 'housekeepers' such as β-actin are reported to be increased in response to aortic-banding in rat myocardium [17]. Six of the experimental cDNA samples were chosen as reference standards and were measured in each run. Relative quantification was performed with help of the “fit point” method using the pre-installed software program of the LightCycler. For each run, ratios relative to each of the reference standards were determined based on the respective delta CTs and an average efficiency (determined graphically, S.D. 2–3%). These ratios were then averaged over all three runs and related to the average content of 18S cDNA in each sample. The obtained “mRNA values” are therefore relative values based on total RNA content.

2.5. Confocal microscopy, measurement of t-tubules and myocyte volume

Myocytes were loaded with the lipophilic fluorescent indicator di-8-ANEPPS (5 μmol/l for 2 min at 22–24 °C, see [18,19]) to label the sarcolemma and t-tubule system. Labeled cells did not manifest signs of toxicity or changes in shape when compared to unlabelled cells. ANEPPS was not used in any studies in which functional measurements were made. Cells were imaged by Laser Scanning Confocal Microscopy (LSCM), excitation light had a wavelength of 514 nm and emitted light recorded at a wavelength of 600 nm. Cell depth (D) was measured on-line by scanning through the z-axis of the cell using optical sections down to 1 μm in thickness. The top and bottom limits of the cell were points at which 50% of the cell disappeared from the focal plane and cell depth was taken as the difference between these two limits. Maximum cell length (L) and width (W) were also measured from this image. Myocyte L, W and D were used to calculate cell volume based on [20] who found rat ventricular myocytes were elongated ellipses, whose volumes were equal to 54% of a block defined by cell length × width × depth. T-tubule spacing was measured by taking a fluorescence intensity profile across the length of the cell. Regular peaks in the intensity plot indicated the position of t-tubules, allowing them to be counted and the mean distance between t-tubules calculated.

2.6. Measurement of contractility and [Ca^{2+}]i

Cells were placed in an experimental chamber on the stage of an inverted microscope and superfused with solution at 37 °C containing (in mmol/l): NaCl, 136.9; KCl, 5.4; NaH2PO4, 0.37; MgCl2, 0.57; HEPES, 5; Glucose, 5.6 and CaCl2 1; pH to 7.4 at 37 °C with NaOH (all chemicals from Sigma, UK). Cells were field stimulated to contract at a rate of 1 Hz. Cell shortening, expressed as a percentage of resting cell length, was our index of contractility. This was measured using a video-edge motion detector system sampling at 200 Hz. The video image was also used to measure the width of these cells. The mean W:D (2.79 see Results) from all confocally imaged cells was used in the calculation of volume in cells viewed under conventional microscopy.

Myocytes were loaded with the fluorescent Ca^{2+} indicator Fura-2 AM (acetoxyethyl form, 3 μmol/l, for 10–12 min at 22–24 °C). The ratio of fluorescent emission at 510 nm in response to alternate excitation at 340 and 380 nm (340/380 ratio) was our index of [Ca^{2+}]i. Because we were primarily interested in comparative rather than absolute levels of [Ca^{2+}]i, the 340:380 ratio was not calibrated for [Ca^{2+}]i. Brooksby et al. [4] previously showed that the intracellular characteristics of Fura 2, including organelle loading, were not different in the WKY and SHR.

2.7. Measurement of action potentials

Action potentials were measured using the perforated patch clamp technique. Micropipettes had a resistance of 2–5 MΩ when filled with solution containing: (in mmol/l) KCl, 10; NaCl, 10; MgCl2•6H2O, 1; CaCl2, 1; K+ glutamate, 110; HEPES, 5; pH 7.2 at room temperature (with KOH). Amphotericin B at a concentration of 400 μg/ml was included to perforate the membrane patch. Pipettes were first dipped in Amphotericin free solution before back-filling. Access resistance and cell capacitance were measured and compensated up to 75% and 100 pF (the maximum available to the Axon 2B amplifier), respectively. A liquid junction potential of + 10 mV between the pipette (K+-glutamate based) and intracellular solution was measured and corrected for using the method of Neher [21]. Action potentials were triggered by application of 2 ms current pulses of just supra-threshold amplitude at a frequency of 1 Hz at 37 °C. Resting membrane potential and action potential duration at 25% (early) 50% (mid) and 75% (late) repolarisation were measured (APD_{25}, APD_{50} and APD_{75}, respectively).

2.8. Statistics

All data is expressed as mean ± S.E.M. Statistical analysis followed that of Shipsey et al. [13] and Bryant et al. [22] on similar data sets. Two-way Analysis of Variance was used to detect an effect of strain (S) (SHR vs. WKY), region (R) (EPI vs. MID vs. ENDO) and interaction (I) (of S and R). When significant differences (of P < 0.05) were
identified, pairwise comparisons corrected for multiple comparisons were performed.

### 3. Results

#### 3.1. Blood pressure and whole heart size

To confirm that the 20-week-old SHR animals used in this study had developed hypertension and cardiac hypertrophy (compared to WKY), blood pressure and heart size were measured in a sample of animals. SHR animals had significantly elevated systolic blood pressure, whole heart/body weight ratios and left ventricular/body weight ratios compared to WKY animals (Table 2). These findings indicate hypertension and cardiac hypertrophy.

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**Table 2**

Whole animal and cardiac parameters in WKY and SHR, BW (body weight), SBP (systolic blood pressure), HW (blotted whole heart weight), LVW (blotted left ventricular weight)

<table>
<thead>
<tr>
<th>Strain</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (animals)</td>
<td>10–14</td>
<td>14–16</td>
</tr>
<tr>
<td>BW (g)</td>
<td>348.9 ± 2.50</td>
<td>414.3 ± 4.65*</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>112.4 ± 3.4</td>
<td>169 ± 3.7*</td>
</tr>
<tr>
<td>HW (g)</td>
<td>1.34 ± 0.02</td>
<td>1.67 ± 0.02*</td>
</tr>
<tr>
<td>LVW (g)</td>
<td>1.07 ± 0.02</td>
<td>1.55 ± 0.03*</td>
</tr>
<tr>
<td>HW/BW ratio (mg/g)</td>
<td>3.84 ± 0.05</td>
<td>4.03 ± 0.05*</td>
</tr>
<tr>
<td>LVW/BW ratio (mg/g)</td>
<td>3.06 ± 0.06</td>
<td>3.73 ± 0.06*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. SHR displayed elevated systolic blood pressure and whole heart (HW/BW) and left ventricular (LVW/BW) hypertrophy when compared to WKY rats. * Indicates significant difference from WKY.

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#### 3.2. Altered expression of mRNA in SHR

Cardiac hypertrophy in SHR is likely to be the result of increased protein expression driven by the increased expression of mRNAs of modulatory genes. Real time RT-PCR was used to measure the relative expression of mRNA for candidate genes, protein kinase Cε and fibronectin-1 (see Discussion). In contrast to samples from tissue, the RNA isolated from myocyte preparations varied in quality (28S:18S ratios between 1.6 and 1, gel band pattern below 18S was somewhat variable), probably due to varying amounts of non-viable cells. PCR data are only presented for the tissue samples. Levels of expression of mRNA for fibronectin-1 and PKCε, were significantly increased in the

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![Confocal image of a myocyte showing the cell boundary and t-tubules as vertical striations. B inset in A at increased magnification.](image)

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Fig. 1. Relative expression levels (normalized to 18S RNA) of Fibronectin (A) and PKCε (B) in SHR and WKY ventricular samples (n = 9, samples from three hearts). Transmural expression of Fibronectin (C) and PKCε (D) mRNA in SHR and WKY tissue (n = 3 samples for each region) isolated from sub-epicardial (EPI), mid-myocardial (MID) and sub-endocardial (ENDO) regions. Values are expressed as mean ± S.E.M. (*P < 0.05, two-way ANOVA, effect of strain). Expression of both mRNAs was greater in SHR than WKY.
SHR (Fig. 1A,B). Differences in the regional levels of expression were not significant (Fig. 1C,D).

3.3. Myocyte morphology

Cells were labeled with di-8 ANEPPS (see Methods and Fig. 2) to allow us to calculate spacing of t-tubules (an important site for ion channels and exchangers associated with excitation–contraction coupling, E–C coupling) and cell W:D (to enable estimation of cell volume from cell L and W alone, when using conventional microscopy). The mean t-tubule spacing of SHR myocytes (1.85 ± 0.013 μm, \(n=108\)) was significantly longer than WKY myocytes (1.81 ± 0.012 μm, \(n=143\)) (\(P<0.05\), two-way ANOVA, effect of strain), there were no regional differences in t-tubule spacing within strains. There were no significant differences in the cell W:D between any of the six groups of myocytes. Therefore, the overall mean cell W:D was used in the calculation of myocyte volume (2.79 ± 0.14, \(n=360\) myocytes) (see Methods).

Consistent with whole heart data (Table 2), SHR myocytes were hypertrophied compared to WKY myocytes, i.e. they had a significantly larger volume (Fig. 3) as a result of a significantly larger L and W (Table 3). Furthermore, this hypertrophy was concentric due to the significantly smaller L:W in SHR myocytes (\(P<0.05\), two-way ANOVA, effect of strain, Table 2). However, the hypertrophy was not uniform across the SHR left ventricle. Significant increases in cell L, W and volume were only seen in SHR EPI and ENDO regions. Significant increases in cell L, W and volume were only seen in SHR EPI and ENDO regions.

Table 3

<table>
<thead>
<tr>
<th>Strain</th>
<th>WKY</th>
<th>SHR</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td>EPI</td>
<td>MID</td>
<td>ENDO</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>n (cells)</td>
<td>82</td>
<td>85</td>
<td>76</td>
</tr>
<tr>
<td>Cell length (μM)</td>
<td>144.1 ± 2.5*</td>
<td>142.3 ± 2.4*</td>
<td>133.0 ± 2.6</td>
</tr>
<tr>
<td>Cell width (μM)</td>
<td>31.9 ± 0.8</td>
<td>33.3 ± 0.8</td>
<td>33.0 ± 0.9</td>
</tr>
<tr>
<td>Length/width</td>
<td>4.80 ± 0.16*</td>
<td>4.49 ± 0.13</td>
<td>4.27 ± 0.15</td>
</tr>
</tbody>
</table>

Sub-epicardial (EPI), mid-myocardial (MID) and sub-endocardial (ENDO) myocytes (n = number of myocytes) studied from 30 to 42 animals. Values are expressed as mean ± S.E.M. Data was analysed using two-way ANOVA followed by t-tests corrected for multiple comparisons. S denotes significant (\(P<0.05\)) influence of strain (WKY against SHR) and R, of region (EPI, MID, ENDO). SHR myocytes displayed concentric hypertrophy (i.e. greater length and width but smaller L/W ratios) compared to WKY myocytes. This effect was more pronounced in EPI and ENDO myocytes than MID myocytes.

* Denotes significant difference between SHR and WKY for a given region.

* Significant difference from ENDO within a given strain.

* Significant difference from EPI within a given strain.
endo but not in MID (* in Table 3, Fig. 3). Thus, the SHR displays transmurally dependent, concentric myocyte hypertrophy when compared to the WKY.

3.4. Cell contractility and [Ca^{2+}]_i transients

We observed significantly larger and longer contractions (Fig. 4A) and [Ca^{2+}]_i transients (Fig. 4B) in SHR myocytes (P<0.05, two-way ANOVA, effect of strain). When these data were analysed with respect to myocyte regional origin, we observed a significant increase in the magnitude of change between SHR and WKY EPI cells (shortening and [Ca^{2+}]_i) and END0 cells ([Ca^{2+}]_i), but no differences in MID cells (Figs. 5A and 6A). However, the time to peak (Fig. 5B) and time from peak to half resting level (Fig. 5C) of the contraction were significantly prolonged in all regions

Fig. 5. Cell shortening in SHR (open bars) and WKY (filled bars) myocytes. (A) Amplitude of shortening expressed as % of resting cell length, (B) time from stimulation to peak shortening. (C) Time from peak shortening to half relaxation. Data are mean ± S.E.M. for numbers of myocytes indicated from sub-epicardial (EPI) mid-myocardial (MID) and sub-endocardial (END0) regions of the left ventricle. All three parameters were significantly greater in SHR than WKY (two-way ANOVA, effect of strain). Time courses were prolonged in all SHR regions (*, B, C) but regional differences in the amplitude of shortening (*, A) between SHR and WKY only reached significance in the EPI regions.

Fig. 6. Intracellular calcium transients in SHR (open bars) and WKY (filled bars) myocytes. (A) Amplitude of transients. (B) Time from stimulation to peak transient. (C) Time from peak transient to half resting value. Data are mean ± S.E.M. for numbers of myocytes indicated from sub-epicardial (EPI) mid-myocardial (MID) and sub-endocardial (END0) regions of the left ventricle. All 3 parameters were significantly greater in SHR than WKY (two-way ANOVA, effect of strain). Decay times were prolonged in all SHR regions (*, C) but transient amplitudes and times to peak were only increased in the EPI and END0 regions (*, A, B) and not in the MID region. († indicates significant differences within strain).
of the SHR vs. WKY. Similar observations were seen for the time course of the \([\text{Ca}^{2+}]_i\) (Fig. 6B,C) except for MID time to peak. Consistent with data in Fig. 5C, the time constant of a single exponential fit to the decaying \([\text{Ca}^{2+}]_i\) transient was significantly prolonged in SHR myocytes (SHR, 162 ± 6 ms, WKY, 101 ± 2 ms, \(P<0.05\), two-way ANOVA, effect of strain).

3.5. Action potential duration

There was no significant effect of strain or region on resting membrane potential (SHR, −68.0 mV ± 0.4, \(n=37\), WKY, −66.8 ± 0.8 mV, \(n=25\) myocytes) However, APD was significantly prolonged in SHR compared to WKY myocytes at 25%, 50% and 75% of repolarisation (Fig. 7, \(P<0.05\) two-way ANOVA, effect of strain). Although mean APD was longer in each SHR region than its WKY equivalent, this only reached statistical significance for the EPI region (Fig. 8).

4. Discussion

4.1. Whole animal data

Before studying the characteristics of SHR myocytes, it is important to demonstrate that hypertension and cardiac hypertrophy have developed in the whole animal at the time of experimentation. The level of hypertension displayed in our SHR animals is similar to that previously reported (e.g. [3,4,2]). Whole heart hypertrophy, as indicated by a significantly greater HW:BW, (22% at 20 weeks) is consistent with previous observations (34% at 6 months [2]).

4.2. mRNA expression

PKC\(\varepsilon\) is a second messenger that is activated by many upstream triggers (see [23]). Because it is a signal transduction ‘node’ we chose it’s mRNA as an indicator for hypertension-induced long-term changes in gene expression. The identification of the hypertrophic triggers per se (e.g. brain or atrial naturetic peptides, angiotensin II, endothelin-1) or possible targets was not the purpose of this study. PKC\(\varepsilon\) mRNA was reported to be increased in a model

Fig. 8. Action potential duration (APD) at (A) 25% repolarisation, (B) 50% repolarisation and (C) 75% repolarisation. Data are mean ± S.E.M. for number of myocytes indicated. Overall APD was significantly longer in SHR (open bars) than WKY (closed bars) (two-way ANOVA, effect of strain), but SHR APDs were only significantly longer than WKY in the EPI region (*) († indicates significant differences within strain).
of compensated concentric hypertrophy in mice [23]. Our findings in SHR are consistent with those in mice. However, levels of transmural expression could not be linked to transmural changes in myocyte volume. This may be because the expression from tissue samples was a combined signal from myocytes and other cell types (e.g., fibroblasts, vascular tissue) that are also affected by hypertension. The theory that we have a mixed signal is supported by the increased expression of fibronectin-1 from SHR tissue samples (consistent with [24]) and our limited data from myocyte preparations (not shown), where fibronectin mRNA was several fold lower in the myocytes compared to heart tissue but PKCε was at similar levels.

Other potential targets for interaction of transmural region and physiological/pathological interventions are the myofibrillar proteins, for example, there is an increase in expression of β- myosin heavy chain in ENDO compared to EPI and in response to pressure overload (e.g., [25]).

4.3. Myocyte hypertrophy

SHR myocytes are hypertrophied compared to WKY myocytes [3,4,2,5]. We have shown that at 20 weeks the L:W was significantly smaller in SHR myocytes, this observation taken with the increased volume of SHR myocytes is evidence for concentric hypertrophy, consistent with the findings in intact SHR hearts [5] and other hypertensive models (e.g., [26,27,23]). This is a compensatory response of the myocytes to normalize increased wall stress arising from the elevated blood pressure [28].

Hypertrophy may be graded across the left ventricle because of a graded stimuli, e.g., in wall stress [29]. A previous study did not detect transmural differences in SHR myocyte volume, but only compared the inner and outer halves of the ventricle at 12 weeks [30]. By contrast, we found that hypertrophy at 20 weeks is not uniform across the left ventricular wall, significant changes in myocyte L, W and volume were only seen in the EPI and ENDO regions and not in the MID region.

Calculation of myocyte volume based on linear dimensions (length, width and when available, depth) together with a geometric correction factor (assuming an oblong, elliptical or cylindrical cross-section) is common (see review by Gerdes and Capasso [27]). But, when comparing volumes, an error would be introduced if hypertrophy altered the cross-sectional geometry. We used a factor derived from rendered volumes of optically sectioned adult rat myocytes [20] that was not reported changed following pressure-induced hypertrophy by aortic banding [31].

4.4. Contraction, [Ca2+]i and APD

An increase in myocyte cross-sectional area (due to increased W and D), would increase their ability to generate tension, and this may be a major part of the SHR’s compensatory adaptation to hypertension. In addition, as a mixed ventricular myocyte population, SHR cells had larger contractions, [Ca2+]i transients and APDs (in agreement with [3,4]). Although we found a trend for these effects in all three regions, they were only consistently statistically significant in the EPI region and did not reach significance at all in the MID region.

Brooksby et al. [4] suggested the increased contraction and [Ca2+]i transients in the SHR resulted from the prolonged APD, as differences between strains were abolished under voltage clamp. However, Shorofsky et al. [2] did see larger SHR [Ca2+]i transients under voltage clamp and proposed altered coupling between sarcolemmal Ca2+ entry and Ca2+ release from the sarcoplasmic reticulum. L-type Ca2+ channel density was not increased in SHR in either study, levels of phospholamban, SERCA2a and ryanodine receptor are reported unchanged, but Ca2+ spark amplitude was larger in SHR [2]. Transmural differences in the contribution of SERCA and Na–Ca exchange to the [Ca2+]i transient are unknown. Our observation that the transmural gradient in APD did not result in a similar gradient in [Ca2+]i transient profile in either strain argues against the APD prolongation in the SHR being the sole cause of the differences between WKY and SHR.

The resting membrane potentials in this report, and the lack of effect of strain [4] and region [13,11] on resting membrane potentials, are similar to previous findings. The selective lengthening of the SHR, EPI, APD is particularly interesting as a similar effect has been reported in hypertrophy induced by isoprenaline [13] and by exercise [11], thus lengthening of EPI, APD may be a common response in hypertrophic models of the rat. A likely candidate for the lengthening of the EPI APD is a reduced density of the transient outward K+ current, Ito ([13,22], but see [32]). Altered [Ca2+]i characteristics may also influence the transmural changes in electrical activity via effects upon Ca2+ activated currents and stress–strain (stretch) relationships. The pattern of APD75 (where ENDO>MID=EPI) seen in the WKY is less pronounced in the SHR, this may have implications for the susceptibility of the model to arrhythmia ([13], and see below).

4.5. T-tubule spacing

Evidence suggests that there is an increase in the spacing of t-tubules in failing ventricular myocytes [33], which could be detrimental to E–C coupling. Although we found a significantly larger t-tubule spacing in compensated SHR myocytes, this does not appear to compromise E–C coupling given the enhanced [Ca2+]i transients we see and the unaltered density of Ica reported by Brooksby et al. [4] and Shorofsky et al. [2].

4.6. Transmural responses to hypertension

Transmurally varied responses in some hypertrophic models have been previously reported but not in the SHR (see...
Introduction), and yet the SHR is the most commonly used model of human hypertension. In our study, in the compensatory state, the MID cells were least responsive, interestingly, in humans with pressure overload-induced concentric hypertrophy, the midwall is affected differently in that endocardial fractional shortening is maintained or increased, velocity of shortening increased and the stress to shortening relationship is maintained while midwall fractional shortening and velocity of shortening is decreased and the stress to shortening relationship depressed [34,35,36]. Changes in wall stress and strain may be triggers for the changes seen in the SHR [6]. Fibre orientation in the MID region differs from EPI and ENDO [37] and it has been shown that local wall stress is sensitive to fibre orientation [38].

The complexity of the heart has led to extensive computer modeling in order to understand the transmural interactions of electrical and mechanical activity. Models now acknowledge the importance of inhomogeneities and incorporate transmural mechanical inhomogeneity (e.g. [7]) and their mechano-electric correlates (e.g. [39]). Our findings indicate that if pathology is to be accurately simulated (a major goal of such modeling), a uniform response to interventions like hypertension cannot be assumed. Our data contributes important information to specific modeling of the SHR [40].

Our findings have another important implication for the understanding of the SHR heart. We have shown that the lengthening of the APD in the SHR is not uniform, thus the profile of depolarization and repolarization between EPI and ENDO via MID regions of the ventricle will be altered. The transmural gradient in APD is thought to be important in the sequenced depolarization (ENDO to EPI) and repolarization (EPI to ENDO) of the ventricle (e.g. [10,13], but see [41]). Changes in this gradient can alter refractoriness and predispose to arrhythmia. If the gradient is reduced, re-entry arrhythmias caused by EPI activation of the ENDO can occur. It is known that the compensated SHR heart is more susceptible to arrhythmic stimuli than the WKY heart (e.g. [15]).

In summary, our findings demonstrate that the response to hypertension is not uniform across the SHR left ventricular wall and indicate that spatial as well as temporal factors [42] are important in fully describing and understanding the myocardial response to hypertension.

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