Wall stress-induced arrhythmias in the working rat heart as left ventricular hypertrophy regresses during captopril treatment

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Abstract

Objectives: (1) To determine whether regression of left ventricular hypertrophy (LVH) leads to a reduction in wall stress induced arrhythmia. (2) To determine the relationship between the time course of LVH regression and changes in arrhythmias in the spontaneously hypertensive rat heart.

Methods: 67 male spontaneously hypertensive rats (SHR) and 67 normotensive Wistar Kyoto rats (WKY) rats were studied at 100 days of age. 39 of each were treated with the ACE inhibitor captopril 2 mg/ml in drinking water, and the remaining 28 were controls. At 0, 2, 4, 8 and 16 weeks hearts were removed and perfused in the working heart mode. Control afterload was 80 mm Hg and perfusate K+ was 2.4 mM. Step increases in afterload (20, 40 and 80 mm Hg rises; 20 s duration; 2 min between each) were applied in random order to increase ventricular wall stress and induce arrhythmias. Results: Total number of ventricular premature beats (VPBs) elicited by each afterload step were counted. The ratio of left ventricular weight to body weight in the SHR (an index of LVH) showed a rapid and marked decline with captopril treatment (2.65 ± 0.07 mg/g after 2 weeks treatment compared to 3.38 ± 0.08 before treatment; P < 0.01), indicating that captopril produced rapid regression of LVH. In contrast, the number of wall stress-induced arrhythmias in SHR did not show a significant decline over the 16 week treatment period. However, when the effect of regression of LVH on wall thickness was taken into account, and compensation was made for differences in wall stress applied, there did appear to be a slow reduction in arrhythmias in SHR. This decline in VPBs was significant after 16 weeks treatment for 40 and 80 mm Hg rises in afterload (P < 0.05).

Conclusions: Treatment with captopril produced a rapid regression of LVH in the SHR. In contrast, arrhythmias declined more slowly over the 16 week period. There did not appear to be a direct relationship between the degree of regression of LVH and wall stress-induced arrhythmias in this model.

Keywords: Rat, spontaneously hypertensive; Hypertrophy; Regression; Arrhythmias; Wall stress; ACE inhibitors

1. Introduction

Hypertension is among the most common causes of death in developed countries. It contributes to the development of left ventricular hypertrophy (LVH). LVH is associated with an increased number of ventricular arrhythmias and is a powerful predisposing factor for sudden cardiac death (SCD). In the Framingham study it was reported that males with LVH were six times more likely to die suddenly compared to those without LVH. Although infrequent coronary occlusion was the cause of SCD, hypertensive patients have been estimated to have 15-20% of about 10,000 deaths from coronary heart disease (WHO report on sudden cardiac death, 1985).

Hypertension is usually treated pharmacologically. Some but not all pharmacological treatments of hypertension have been shown to produce regression of LVH. Those having the largest effects include angiotensin converting...
enzyme (ACE) inhibitors, calcium (Ca) channel blockers and centrally acting anti-adrenergic agents [5]. So far, however, there is little information about the effect of these treatments on the incidence of arrhythmia, or on the time course over which arrhythmias might decline during treatment. Some small clinical studies on hypertensives with LVH have reported a reduction in both LVH and ventricular arrhythmias after 6 months treatment with either isradipine (a Ca channel blocker) or the ACE inhibitor, captopril [6,7]. Previous animal studies have shown that LVH can be made to regress with captopril [8–10], and one study has shown less arrhythmias after 11 months treatment with enalapril [11].

The spontaneously hypertensive rat (SHR) is widely used as a model of human essential hypertension and LVH [12,13]. Furthermore, it has been shown using the working heart preparation that the SHR develops more wall stress-induced arrhythmia (both simple and complex) when compared to normotensive control hearts [14,15]. The purpose of this study was to assess the time course of LVH regression with captopril treatment, and to compare this to the time course over which any change in arrhythmia might occur.

We have chosen to examine wall-stress induced arrhythmias in the working heart model since these arrhythmias might be of importance clinically. Hypertensive patients are known to have more labile arterial blood pressure [16,17], and it has been shown that sudden increases in ventricular wall stress brought about by sudden increases in blood pressure are arrhythmogenic in man [18,19]. The mechanism by which increases in wall stress produce arrhythmias is not completely understood. It is well established that myocardial stretch modulates the electrophysiological properties of the heart (‘mechanoelectrical feedback’) [20] and it has also been shown that ventricular dilatation shortens the effective refractory period in a regionally non-uniform manner that might serve as a substrate for re-entrant circuits [21].

We treated SHR animals (and their accepted normotensive control – Wistar-Kyoto rat, WKY) with captopril for 16 weeks to produce regression of LVH. Our results show that LVH regressed with a rapid time course. In contrast, the number of arrhythmias declined slowly over the 16 weeks of treatment. These data indicate that there might not be a close relationship between the extent of LVH and the occurrence of arrhythmias.

2. Methods

Experiments were performed in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, published by HMSO, London. The isolated working heart model [22] including the modifications of Taegtmeier et al. [23] was used to study arrhythmias. Step increases in afterload produce increases in pressure when measured by the transducer distal to the aorta (see Fig. 1A, site A; e.g. [15]). However it was necessary also to characterise the relationship between step increases of afterload and the change in left ventricular pressure, since any change in ventricular wall stress is dependent directly on the level of intraventricular pressure. A series of preliminary experiments was therefore performed to investigate the relationship between the pressure measured distal to the aorta (point A) and pressure within the left ventricle (point B, Fig. 1).

2.1. Regression of hypertrophy experiments

Male SHR and WKY rats were purchased from Charles River Breeding Laboratories (Margate, UK). The animals were kept in house at the School of Medical Sciences, Bristol. They were kept in an environment with a 12 h light/dark cycle and were fed on a standard diet and allowed to drink freely.

67 SHR and 67 WKY male rats were divided into two groups: (1) a treatment group (given captopril 2 mg/ml drinking water and allowed to drink freely) and (2) an untreated group (given tap water only). Captopril was purchased from Sigma. Treated animals were killed at 2, 4, 8 and 16 weeks. Untreated control rats were killed at 0, 8 and 16 weeks. Each group contained between 7 and 11 animals.

2.1.1. Anaesthesia

All animals were anaesthetised with halothane prior to removal of the heart. A gas mixture of 95% O₂ + 5% CO₂ was passed through a conventional human halothane regulator and into a glass chamber into which the animal was placed for induction of anaesthesia. The use of the O₂/CO₂ mixture ensured that the animal was well oxygenated prior to removal of the heart. Once stable anaesthesia was achieved, the animal was removed from the chamber and placed on an operating surface. The animal’s muzzle was placed in the mouth of a 50 ml syringe into which a gentle flow of anaesthetic/gas mixture was passed in order to maintain anaesthesia. The regulator allowed the gaseous concentration of halothane to be varied accurately between 0.5% and 4%. For induction of anaesthesia a level of 4% was used, when the animal was unconscious it was reduced to between 2 and 2.5%. Whilst measuring aortic pressure from the abdominal aorta the halothane concentration was reduced to 1% for 2–4 min to attenuate its depressant effect on arterial pressure. Aortic blood pressure was measured by making a longitudinal abdominal incision exposing the small bowel which was lifted up and retracted out side the abdominal cavity on the right thus exposing the aorta. The peritoneum covering the aorta was gently rubbed from the aortic surface. A 25 gauge needle attached to a fine bore tube (i.d. 0.4 mm, o.d. 0.8 mm) was inserted into the abdominal aorta and blood pressure was recorded using a Piezo crystal transducer. The pressure trace was recorded onto a Gould chart recorder.
2.2. Preparation of hearts for perfusion

Under stable anaesthesia, animals were given heparin 500 U into a femoral vein 2 min prior to removal of the heart. The upper abdomen was opened with a transverse incision and the chest opened with two lateral incisions. The heart was excised ensuring adequate lengths of aorta and the pulmonary veins. The heart was placed immediately in Tyrode's solution chilled to 4°C and then mounted by the aorta onto a Langendorff perfusion apparatus. The time taken from removal of heart to establishment of Langendorff perfusion was usually less than 60 s. Once

![Diagram of working heart apparatus](image)

**Fig. 1.** (A) Schematic diagram of the working heart apparatus. Tyrode's solution is fed into the oxygenation flask and then into the left atrium. The left ventricle pumps fluid out into the compression chamber and pressure is measured at the transducer. The afterload against which the heart pumps is set by adjusting the height of the fluid column in the outflow line. Afterload can be changed suddenly by turning the 3 way tap so that fluid is diverted to a second fluid column set at a different height. (B) Right panel: sample traces showing the differences in pressure recorded at site A distal to the aorta and site B within the left ventricle. (C) and (D) show the effect of 10 mm Hg step incremental rises in afterload (0–90 mm Hg) on the mean systolic and diastolic pressures recorded at sites A and B shown in (A). (E) shows the relationship between the 10 mm Hg pressure rises induced at the pressure transducer (site A (A)) and the increases in pressure recorded within the left ventricle (site B (A)).
Langendorff perfusion had been established, the pulmonary artery was identified and a small incision made into it to ensure free coronary drainage from the right atrium. The pulmonary veins were then identified and cannulated thus establishing a conduit allowing Tyrode's solution to pass into the left atrium. When a tight seal with no leaks had been established Langendorff perfusion was discontinued and anterograde perfusion of the left atrium at a constant preload was commenced.

At the end of the experiment the heart was blotted dry, the atria and right ventricular free wall were dissected away, and the left ventricle weighed.

2.2.1. Experimental solutions

Solutions were made fresh each day. The stock solution was a bicarbonate buffered Tyrode's solution containing (mM): NaCl 114; NaHCO₃ 25; NaH₂P0₄ 1.0; CaCl₂ 2.6; MgCl₂ 1.0 and glucose 11.1. KCl was added to give a concentration of 2.4 or 6 mM. Tyrode's solution circulating in the working heart apparatus was continuously gassed with 95% O₂ and 5% CO₂ and maintained at 37°C.

2.3. Baseline cardiac function tests

All hearts were initially perfused with Tyrode's solution containing 6.0 mM K⁺ and allowed to stabilise for a 15 min period prior to the start of the experiment. The preload was set to a level of 20 cm water for both WKY and SHR hearts. The afterload systolic pressure was set by adjusting the height of the column of fluid from the aortic out-flow to achieve a systolic pressure of 80 mm Hg, the pulse pressure was varied by adjusting the amount of air in the compression chamber to give a pulse pressure between 20 and 40 mm Hg (see Fig. 1). Aortic flow was measured using a flow meter in the aortic flow line (Fig. 1). Coronary flow was collected from the perfusion chamber overflow cannula. Maximum systolic pressure generated whilst briefly pacing at 300 beats/min during aortic cross-clamping (Pmax) was used as an index of contractility. This method of assessing cardiac function approximates to the peak isovolumic pressure since aortic ejection is prevented and is an accepted index of contractility [24–27]. Pmax, aortic flow and coronary flow were measured at the end of the 15 min stabilisation period at the start of the experiment whilst perfused with Tyrode's solution containing 6 mM K⁺.

The perfusate was then changed to one containing K⁺ 2.4 mM for the remainder of the experiment since this has been shown in previous studies to increase wall stress-induced arrhythmias [28]. After a further 15 min stabilisation period and thus an increase in left ventricular wall stress. By turning the 3 way tap (see Fig. 1A), the aortic outflow could be switched from the fixed baseline afterload to a pre-set higher afterload producing 20, 40 and 80 mm Hg rises in systolic pressure measured at site A distal to the aorta. Hearts were allowed a stabilisation period of 2 min between afterload rises. The order of rise was applied randomly and three runs were performed for each level of afterload.

2.5. Pressure measurement experiments

Preliminary experiments measuring pressure at the site of the pressure transducer in the outflow circuit (site A, Fig. 1) and from within the left ventricle (site B, Fig. 1) following step increases in afterload (10 90 mm Hg, 10 mm Hg increments) were performed using a perfusate containing K⁺ 6.0 mM. Hearts from eight Wistar rats were used. These pressures were measured by inserting a fine bore polythene tube (i.d. 0.5 mm, o.d. 0.9 mm) connected to a 23 gauge needle into the two sites.

2.6. Data acquisition and analysis

The electrocardiogram (ECG) was recorded from electrodes attached to the metal aortic and left atrial perfusion cannulae and a third metal cannula draining the perfusion chamber. The height of the cannula within the perfusion chamber allowed the chamber to be partially flooded to a level submerging the apex of the heart, thus establishing an electrical contact with the apex.

The ECG and aortic pressure were recorded continuously on a chart recorder (Gould 8000 series). Pressure was monitored distal to the aorta (site A, Fig. 1) using a Gould diaphragm pressure transducer (model P23 ID) placed in the aortic outflow line. Data was digitised using a CED 1401 analogue to digital interface (Cambridge Electronic Design) and an Instrutech CRC-VR-100B digital recorder (Instrutech, USA) and recorded on a video recorder (Panasonic NV-SD30B) for later analysis.

Arrhythmias were analysed in accordance with the Lambeth convention [29]. Ventricular premature beats (VPBs) were defined as QRS complexes morphologically different from those occurring during the normal sinus rhythm. Ventricular tachycardia (VT) was defined as four or more consecutive VPBs (in this study a series of four or more VPBs had to be of identical morphology and coupling interval to be accepted as VT). Ventricular fibrillation (VF) was defined as a signal for which individual QRS deflections could no longer be distinguished from one another (implying morphological instability) and for which the rate could no longer be measured.

The number of VPBs independent of VT, and the number of VPBs involved in VT were counted for each 20 s increase in afterload.
2.7. Statistical analysis

For each heart, the average value for arrhythmias for the 3 runs performed for each afterload level was taken. Results were expressed as mean ± standard error of mean (S.E.M.). Statistical comparisons were made using 3 way ANOVA, if significant ($P < 0.05$), pair-wise comparisons were made using Student's $t$ test unless otherwise stated. Analysis was performed using Excel and SPSS statistical software packages.

3. Results

3.1. Measurement of pressure at different sites

Pressure rises of between 10 and 90 mm Hg (in 10 mm Hg increments) measured at site A (Fig. 1A) were applied in random order by switching the 3 way tap. Pressure was also measured directly from within the ventricle (site B). Fig. 1B shows sample pressure traces recorded from the two positions. Pressure measured at site A had a 'saw tooth' waveform with systolic and diastolic pressures at the basal set levels (80 systolic, 40 diastolic). Pressure measured within the ventricle (site B) had a higher systolic than measured at position A, and a diastolic near zero.

The mean systolic and diastolic pressures recorded in each position, for a given step increase of afterload, are displayed in Fig. 1C–D ($n = 8$). It can be seen that for each increase in afterload step there is a progressive increase in systolic pressure distal to the aorta (site A) and within the left ventricle (B). There was little change in ventricular diastolic pressure with increases in afterload. In Fig. 1E we plotted the relationship between the step increases in pressure recorded at the transducer in its normal position (site A), with each rise in afterload, and the corresponding step increases in pressure within the left ventricle (site B). There was an approximately linear relationship between these two variables (Pearson's correlation coefficient $r = 0.967$, 95% confidence intervals 0.863–0.992). The implication of this strong correlation is that a given increase in aortic afterload produces a similar and graded increase in systolic pressure within the left ventricle, and therefore a graded increase in ventricular wall stress.

![Figure 1A](image1a.png)

![Figure 1B](image1b.png)

![Figure 1C](image1c.png)

![Figure 1D](image1d.png)

![Figure 1E](image1e.png)

Fig. 2. The effect of captopril treatment on: (A) systolic pressure; (B) left ventricular weight/body weight; (C) maximum pressure generated whilst paced at 300 beats/min during aortic cross-clamping; (D) coronary flow per gram heart weight. The left panel of each shows WKY and the right panel SHR. Untreated groups are represented by open symbols and treated groups by closed symbols. * = $P < 0.05$ compared to untreated control; ** = $P < 0.01$ compared to untreated control.
Table 1
Mean body weights and LV weights of animals (mean ± s.e.m.)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Untreated</th>
<th>Treated</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Week</td>
<td>Week</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>WKY</td>
<td>Body weight (g)</td>
<td>314 ± 6</td>
</tr>
<tr>
<td></td>
<td>LV weight (mg)</td>
<td>954 ± 38</td>
</tr>
<tr>
<td>SHR</td>
<td>Body weight (g)</td>
<td>310 ± 7</td>
</tr>
<tr>
<td></td>
<td>LV weight (mg)</td>
<td>1047 ± 28</td>
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</tbody>
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3.2. Effect of captopril treatment on arterial blood pressure, LVH and cardiac function

Fig. 2A shows the peak systolic arterial pressure in WKY and SHR, for control and captopril treated animals, during the 16 week period of the study. Peak systolic pressure was significantly higher in untreated SHR compared to untreated WKY at 0, 8 and 16 weeks (open symbols, Fig. 2A; \( P < 0.01 \)). In both SHR and WKY, treatment led to a significant fall in peak systolic arterial pressure (closed symbols, Fig. 2A). This fall occurred rapidly over the first 4 weeks of treatment then tended to stabilise at a plateau level.

Table 1 shows body and heart weights for each group of animals. Left ventricular weight as a ratio of body weight (LB/BW, Fig. 2B) was calculated as an index of LVH. This ratio was significantly greater in untreated SHR compared to untreated WKY at 0, 8 and 16 weeks (open symbols; \( P < 0.05 \), \( P < 0.05 \) and \( P < 0.01 \) respectively). With treatment there was a rapid fall in LV/BW ratio in
SHR which was significant as early as 2 weeks ($P < 0.01$) with a further reduction over the 16 week period. This shows that captopril caused a rapid regression of LVH in the SHR. In WKY there was also a fall in LV/BW ratio with treatment ($P < 0.05$ at 2 weeks). These data show that even in the absence of LVH, treatment with captopril produces a reduction in LV mass. Interestingly, the LV/BW ratio in both SHR and WKY fell to a similar plateau level with treatment.

3.3. Baseline cardiac functions

There was no significant difference in heart rate between untreated SHR and untreated WKY (194 ± 10 vs. 182 ± 13) and treatment had no significant effect on heart rate. Similarly there was no significant difference in cardiac output (aortic flow + coronary flow) for untreated SHR or WKY, and again treatment had no significant effect.

Contractility (Fig. 2C), as assessed by $P_{\text{max}}$, was significantly greater in untreated SHR compared to untreated WKY (open symbols; $P < 0.01$). Ageing had a significant affect on this difference which became greater as the animals aged. With treatment there was a fall in $P_{\text{max}}$ for both SHR and WKY compared to untreated SHR and WKY. For SHR this was significant at 4 ($P < 0.05$), 8 and 16 weeks ($P < 0.01$). Therefore contractility fell in the SHR as LVH regressed during captopril treatment.

Coronary flow (expressed as ml·min$^{-1}$·g$^{-1}$ total heart weight; Fig. 2D) in untreated WKY was significantly greater than in untreated SHR ($P < 0.01$, open symbols; Fig. 2D). Captopril treatment led to an increase in coronary flow in SHR ($P < 0.01$ at 2, 4 and 16 weeks) and WKY ($P < 0.01$ at 16 weeks).

3.4. The effect of captopril treatment on ventricular ectopics elicited by step increases in afterload

Fig. 3 shows four representative recordings made from hearts in each group. The lower trace of each pair shows the pressure recorded distal to the aorta (Fig. 1, site A). The trace starts with a baseline pressure of 80/40, the 3 way tap was then turned to produce a 40 mm Hg rise in afterload. This afterload increase was maintained for a period of 20 s. The upper trace shows the simultaneous ECG recording. At the start, the heart was in sinus rhythm, when the afterload was increased ventricular premature beats (VPBs) were induced and appeared as complexes which were premature and of irregular morphology. The two panels on the left in Fig. 3 display traces from an untreated WKY (upper) and untreated SHR (lower). It can be seen from these traces that untreated SHR displayed more arrhythmias for the same afterload step than the WKY (as has been shown previously [28]). The two right panels show the effect on arrhythmias after 8 weeks of captopril treatment to cause regression of LVH. It is clear for the SHR (lower right panel) that it still developed arrhythmias with the 40 mm Hg afterload step, and the number of arrhythmias was similar to the untreated SHR heart (lower left panel). Similarly for the WKY heart after

![Fig. 4](image-url)
8 weeks of captopril (upper right panel), this also developed a similar number of arrhythmias compared to the untreated WKY heart.

The mean number of VPBs elicited by each level of afterload step was counted, and the results are displayed in Fig. 4. The top panels show the number of arrhythmias elicited in WKY hearts over the 16 week period of the study, for both untreated (open symbols) and captopril treated animals (solid symbols). The number of arrhythmias elicited by 20, 40 and 80 mm Hg afterload steps are shown in the respective panels. The lower panels show the number of arrhythmias in SHR hearts.

There appeared to be a small decline in VPBs in untreated SHR for 20 and 80 mm Hg rises in afterload over the 16 week period. However, these apparent changes in arrhythmias with ageing over the 16 week period in this study were not statistically significant. When untreated SHR were compared to untreated WKY there were significantly more arrhythmias in SHR ($P < 0.05$ for 20, and $P < 0.01$ for 40 and 80 mm Hg rises in afterload), consistent with the findings of Evans et al. [15] who demonstrated increased arrhythmias in SHR compared to WKY in the working heart model.

The primary objective of this study was to ascertain the effect of captopril treatment on arrhythmias. Fig. 4 suggests that for SHR, for each level of afterload there may be a slow progressive decline in the number of VPBs with captopril treatment over the 16 week treatment period. This difference, however, was not significant for treated SHR compared to untreated SHR. For WKY there also appeared to be less VPBs in treated animals, this difference was only significant for treated WKY compared to untreated WKY at 16 weeks, and for 80 mm Hg rises in afterload. Since we showed in SHR that captopril treatment produced a rapid regression of LVH (Fig. 2B), and since these data indicate only a minimal, non-significant change in arrhythmias, it appears that regression of LVH is not simply associated with a reduction in wall stress-induced arrhythmias.

3.5. Relation of arrhythmias to wall stress

Taking the results at face value, they appear to indicate that for SHR, as LVH regressed during captopril treatment, there was no significant change in the number of wall stress-induced arrhythmias. Thus, the early and rapid reduction in LVH did not appear to be associated with an early change in the arrhythmic behaviour of the heart.

One possible difficulty in interpreting these results is that arrhythmias were elicited by standard step increases of afterload. The increase in wall stress produced in each heart, however, will be related not just to the resulting step increase in intraventricular pressure, but also to the thickness of the ventricular wall. As LVH regressed, the LV wall will have become thinner and thus a given increase of intraventricular pressure will produce a larger increase in wall stress. Therefore, whether the heart becomes intrinsically more or less arrhythmic as LVH regresses should be more closely related to the number of arrhythmias elicited by a given rise in wall stress, rather than the rise of intraventricular pressure (i.e. afterload) imposed on the heart. Because of this, we have performed an additional analysis of these data to express arrhythmias as a function of wall stress in each group of animals.

3.6. Relation of ventricular wall thickness to LV mass

Since the left ventricle was removed and weighed for each group of animals treated with captopril, and since the density of heart tissue is 1.05 [30] this allowed us to calculate the volume of LV tissue in each heart. Modelling the LV either as a sphere or as an ellipsoid, the volume of LV tissue is then proportional to $h^3$, where $h$ is the wall thickness of the LV [30], this makes the assumption that volume of the LV cavity remained constant as LV mass regressed, the main differences being due to changes in wall thickness. This appears to be the case for two reasons. First, Litwin et al. [31] found in rats with concentric LV hypertrophy that LV cavity size remained normal as LVH developed, and that treatment with an ACE inhibitor did not produce a change in LV cavity dimension (except when the heart went into failure). Second, we performed measurements of LV cavity size in animals after 16 weeks captopril treatment. Hearts from four animals in each group were taken and a thin transverse section was cut midway along the axis of the left ventricle. The diameter of the intraventricular cavity was then measured using a dissecting microscope (untreated WKY: 4.0 ± 0.6 mm; treated WKY: 3.75 ± 0.3 mm; untreated SHR: 4.3 ± 0.3 and treated SHR: 3.8 ± 0.3). There was no significant difference in cavity size between untreated WKY and SHR. In addition, treatment of either species with captopril had no effect on cavity size. The primary effect was a thinning of the LV wall.

Therefore LV mass = 1.05 volume LV tissue $\propto h^3$.

It follows that $h \propto \sqrt[3]{\text{LV mass}}$

3.7. Calculation of wall stress

Wall stress is strictly defined as wall tension per unit cross sectional area of LV muscle. Using the Laplace Law, wall stress ($S$) for a sphere or ellipsoid can be expressed as

$$S = \frac{Pa}{2h}$$

where $a =$ internal radius of ventricle and $P =$ pressure within the left ventricle [30]. Since the internal radius ($a$) remained constant,

$$S \propto \frac{P}{h}$$
Fig. 5. Mean number of VPBs per unit wall stress applied plotted for WKY (top panel) and SHR (bottom panel) for each rise in afterload. Untreated groups are represented by open symbols and treated groups by solid symbols. Wall stress applied was estimated from the formula:

\[ S = \frac{P}{\sqrt{LV \text{ mass}}} \]

(see Section 3). When making this compensation for differences in wall stress applied due to changes in wall thickness the decline in VPBs with treatment in SHR is significant at 16 weeks for 40 and 80 mmHg rises in afterload. Despite making this compensation for differences in wall stress applied there remains a disparity between the time course of changes in arrhythmias in SHR which decline slowly over the 16 week treatment period and regression of LVH (Fig. 2B) which occurs rapidly with treatment.

Fig. 6. The top panel shows the mean number of complexes involved in episodes of VT during 20 s rises for 20, 40 and 80 mm Hg rises in afterload in WKY. The bottom panel shows those recorded in SHR. Untreated groups (open symbols) are shown at 0, 8 and 16 weeks, treated groups (closed symbols) at 2, 4, 8 and 16 weeks. Treatment significantly increased VT in WKY at 16 weeks for 40 mm Hg rises in afterload and at 8 and 16 weeks for 80 mmHg rises in afterload. There was no significant change in VT for SHR over the 16 treatment period.
thus

\[ S \propto \frac{P}{\sqrt{\text{LV mass}}} \]

Therefore in Fig. 5 we have plotted the mean number of VPBs per unit of wall stress (from the formula:

\[ S \propto \frac{P}{\sqrt{\text{LV mass}}} \].

Values for \( P \) used were the peak intraventricular pressures attained for each of the afterload rises in the preliminary pressure experiments (159, 173 and 206 mm Hg for 20, 40 and 80 mm Hg rises in afterload above the control level). It is important to note that this intraventricular pressure data was obtained from untreated WKY hearts. In these calculations we make the assumption that afterload will be the major determinant of intraventricular pressure, and that intraventricular pressure attained for each afterload increase will be independent of the type of heart (WKY or SHR, treated or untreated). It is, however, possible that a difference in compliance between WKY and SHR might lead to a small difference in wall stress attained for given afterload steps. Having compensated for differences in wall stress due to differences in wall thickness, we observed in Fig. 5 that for SHR, the decline in arrhythmias was now significant at 16 weeks for 40 and 80 mm Hg rises at 16 weeks. Despite making compensation for these differences in wall stress, however, there remained a dissociation between the time course of LVH regression (highly significant after just 2 weeks treatment) and the slow decline in arrhythmias (reaching significance at 16 weeks for 40 and 80 mm Hg rises in afterload).

3.8. Complex ventricular arrhythmias

Fig. 6 shows the mean number of complexes involved in episodes of VT during a 20 s increase in afterload. The top panel shows the mean number of VT complexes in WKY for 20, 40 and 80 mm Hg rises in afterload and the bottom panel shows those in SHR. Whilst captopril treatment had no significant effect on VT in SHR this did not appear to be the case for WKY, which showed a significant increase in VT at 16 weeks treatment for 40 mm Hg rises in afterload and at 8 and 16 weeks treatment for 80 mm Hg rises in afterload (\( P < 0.05 \)).

Ventricular fibrillation (VF), when it occurred, was frequently terminal and when hearts died during experiments it was always due to terminal VF. The percentage of hearts dying from terminal VF in each group is shown in Fig. 7. Comparisons were made using the Mantel-Haenszel nested \( \chi^2 \) test. More untreated SHR died than untreated WKY (\( P < 0.05 \)). Whilst there was a trend towards an increased mortality in the early stages of captopril treatment for both WKY and SHR these trends were statistically non-significant. These data indicate that regression of LVH was not associated with a change in the incidence of VF.

4. Discussion

This study confirms that treatment of SHR with captopril produces regression of LVH. This regression occurred rapidly – the LV/BW ratio became significantly smaller as early as 2 weeks into the treatment period, and the LV/BW ratio reached a baseline level after 4 weeks treatment. In contrast, the number of VPBs declined slowly with treatment. This was only significant when numbers of VPBs were expressed per unit of wall stress, and also was only significant following 16 weeks treatment, for 40 and 80 mm Hg rises in afterload. Complex arrhythmias (VT and VF) did not show any significant decline over the same time period. From these results it would appear that the relationship between LVH regression and changes in arrhythmias is not a direct one.

4.1. Step increases of afterload, intraventricular pressure and ventricular wall stress

Preliminary experiments performed to assess the changes in intraventricular pressure produced by increases in afterload showed that an increase in afterload (applied distal to the aorta) produced a similar increase in peak intraventricular pressure. Moreover, the changes of intraventricular pressure were graded with the increase in afterload. Although we did not measure wall stress directly, according to Laplace’s law, wall stress is directly proportional to intraventricular pressure. Therefore, we can assume that the step increases in afterload we applied to each heart will have produced graded increases in wall stress in each heart.
4.2. Effect of long-term captopril treatment on LV mass and cardiac function

4.2.1. LV mass

There was a clear and rapid fall in LV mass in both WKY and SHR during captopril treatment. This had a similar time course to the change of arterial pressure. LV mass became significantly smaller after 2 weeks treatment, and reached a baseline level after 4 weeks. One possible cause of the decline in LV mass in both SHR and WKY might be the fall of arterial pressure, allowing the heart to adapt to the reduced afterload by reducing muscle mass. A second possibility for the fall of LV mass might be the known growth inhibiting effects of ACE inhibitors on the local cardiac renin-angiotensin system [32].

4.2.2. $P_{\text{max}}$

As an index of contractility we measured $P_{\text{max}}$. This also fell in both groups of animals with captopril treatment, consistent with the reduction in LV mass that occurred simultaneously.

4.2.3. Coronary flow

Interestingly, captopril treatment produced an increase in coronary flow in both groups of animals. In untreated SHR coronary flow was lower than in untreated WKY, but this increased rapidly after 2 weeks captopril treatment to approximately the same level as WKY. The fact that coronary flow increased rapidly whilst arrhythmias remained unchanged during the first 4 weeks of treatment would appear to indicate that any possible ischaemia associated with a reduced coronary flow in SHR was not an important factor causing the arrhythmias recorded in this study.

4.3. Effect of captopril treatment on wall stress-induced arrhythmias

When results were taken at face value they appeared to indicate that for SHR animals, as LVH regressed during captopril treatment, there was only a small and non-significant change in the number of wall stress-induced arrhythmias. Thus, the early and rapid reduction in LVH did not appear to be associated with an early change in the arrhythmic behaviour of the heart. Only when compensation was made for differences in wall stress did a significant reduction in arrhythmias emerge in treated SHR. This reduction was only significant for 40 and 80 mm Hg rises after 16 weeks treatment. Despite compensating for these differences in wall stress however there remained a dissociation between the time course of LVH regression (highly significant after just 2 weeks treatment) and the slow decline in arrhythmias (reaching significance at 16 weeks).

4.4. Relation to previous studies

A number of previous studies have investigated the relationship between LVH and arrhythmias, and the general conclusion has been that regression of LVH is associated with reduced arrhythmia. Two small clinical studies [6,7], one using captopril and the other using the Ca channel blocker isradipine, found that after 6 months treatment LVH had regressed and this was accompanied by a reduction in spontaneous arrhythmias. Pahor et al. [11] showed that treatment of SHR with enalapril for 11 months reduced both spontaneous ventricular arrhythmias and electrically stimulated arrhythmias. Kohya et al. [33] treated SHR animals with captopril and found regression of LVH and a reduction of ischaemia-induced arrhythmias. A number of other studies have reported that regression of LVH with ACE inhibitors is associated with a reduction of reperfusion arrhythmias (e.g. [34-36]). Our results are consistent with these studies, since we also found that VPBs declined slowly with treatment when differences in wall stress were accounted for. In contrast, however, we found no decline in complex arrhythmias over the 16 week treatment period despite regression of LVH. It is possible that the aetiology of simple VPBs and more complex arrhythmias is different. Rials et al. [37] showed that regression of cardiac hypertrophy leads to normalisation of the prolonged action potential. We have also observed that the prolonged action potential in rat cardiac hypertrophy normalises following 8 weeks treatment with captopril (G. Dalton, unpublished observations). These changes in electrophysiology might account for the reduced number of VPBs seen. Some studies (e.g. [38,39]) have shown that regression of myocyte hypertrophy is associated with a relative increase in the ratio of collagen to muscle. We have preliminary evidence that this appears to be the case with the treatment protocol used in this study. The increased collagen to muscle ratio might lead to the development of re-entry circuits that could account for the persistence in complex arrhythmias found in this study.

In summary, we have shown that treatment with captopril produces a rapid regression of LV mass in SHR. VPBs also appeared to decline slowly with treatment. However, there was a dissociation between the time course of changes in VPBs, which appear to decline slowly with treatment, and the time course of LV mass regression which occurred much more rapidly. The cause of this temporal discrepancy remains to be identified. Future study directed at the time course of changes in muscle fibre and connective tissue with treatment, and the effect LVH regression on the electrophysiological properties of myocytes, might help to further elucidate the mechanisms involved.

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