Normal regional distribution of membrane current density in rat left ventricle is altered in catecholamine-induced hypertrophy

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Abstract

Objective: To test the hypothesis that changes in the normal regional distribution of potassium and calcium currents contribute to the different regional changes in action potential duration in isoprenaline-induced hypertrophy in rats. Methods: Hypertrophy was elicited in rats by seven daily injections of isoprenaline. Left ventricular myocytes were isolated from basal sub-endocardial, basal mid-myocardial and apical sub-epicardial tissue. Membrane currents were measured using the whole-cell patch-clamp technique at 35±1°C. Results: Cell membrane capacitance was similar in all three groups and was increased by 17% in hypertrophy (P<0.001, t-test). Changes in the calcium-independent transient outward current (I_{to}) density in hypertrophy were different in the three regions (P<0.05, ANOVA). I_{to} was reduced in sub-epicardial (control, 23.4±2.0 pA pF; hypertrophy, 15.8±1.5 pA pF, P<0.01 ANOVA) and in mid-myocardial myocytes (control, 24.0±2.8 pA pF; hypertrophy, 13.8±1.3 pA pF, P<0.01 ANOVA) and was not significantly altered in sub-endocardial myocytes (control, 8.5±0.7 pA pF; hypertrophy, 7.4±1.8 pA pF). Steady-state background current density was reduced in hypertrophy (P<0.05, ANOVA). The regional difference in steady-state background current in control hearts (P<0.05, ANOVA) was altered in hypertrophy. Calcium current (I_{ca}) density was similar in the three regions studied in both control and hypertrophied hearts. I_{ca} was reduced in hypertrophy (P<0.05, ANOVA). Conclusion: The normal regional differences in I_{to} are reduced, in steady-state background current are altered and in I_{ca} are unchanged in catecholamine-induced hypertrophy in the rat left ventricle. These data may in part explain the reduction in the normal regional differences in APD observed in hypertrophy. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Transient outward current; Calcium current; Rat; Hypertrophy; Ventricular myocytes

1. Introduction

The most consistent electrophysiological change in myocardial hypertrophy is prolongation of action potential duration (APD) (for review see [1]). However, we have recently shown that in hypertrophy prolongation of APD is not uniform throughout the left ventricle [2,3]. In a model of pressure overload hypertrophy in guinea-pig, APD at 90% repolarisation (APD_{90}) was prolonged in sub-epicardial myocytes and unchanged in sub-endocardial myocytes [2]. Similarly in catecholamine-induced hypertrophy in rat, APD at 25% repolarisation (APD_{25}) was prolonged in sub-epicardial and unaltered in sub-endocardial myocytes [3]. Moreover, changes in APD at 75% repolarisation (APD_{75}) were different in sub-endocardial and sub-epicardial myocytes, APD_{75} was prolonged in sub-epicardial and was reduced in sub-endocardial myocytes [3].

Prolongation of APD in a number of models of cardiac hypertrophy in rat is thought to be attributable in part to a reduction in the density of the calcium-independent transient outward current (I_{to}) [4–8]. In normal hearts however, I_{to} density is greater in sub-epicardial than in sub-endocardial myocytes in the rat [9], dog [10], cat [11] and man [12,13]. Recent studies have suggested that the
regional variation of $I_{\text{o1}}$ is reduced in failing human hearts compared to nondiseased donor control [12] and in hypertrophy [14]. However, the relative contribution of differential changes in membrane currents to the regional changes in APD in hypertrophy is unknown.

The aim of this study was to test the hypothesis that differential changes in several membrane current systems in catecholamine-induced hypertrophy in sub-endocardial, mid-myocardial and sub-epicardial contribute to the regionally different changes in APD in hypertrophy.

2. Methods

2.1. Model of hypertrophy

Male Wistar rats (weighing 180–200 g) were injected with isoprenaline 1 mg intraperitoneally, daily for 7 days. Weight-matched controls received the same volume (1 ml) of saline. Animals were sacrificed 24 h after the last injection [3,4]. Treatment of animals was in accordance with *Home Office Guidance on the operation of the Animals* (Scientific Procedures) Act 1986, (H.M.S.O).

2.2. Isolation of myocytes

Rats were killed by cervical dislocation. The heart was quickly excised, suspended from a Langendorff column and perfused with a nominally calcium-free Tyrode solution for 2 min, followed by an enzyme solution containing collagenase (Type I, 0.75 mg ml$^{-1}$, Worthington Biochemical, New Jersey, NJ, USA) and protease (derived from *Streptomyces griseus*, 0.025 mg ml$^{-1}$, Sigma, St. Louis, MO, USA) for a further 7 min. The left ventricular free wall was then removed and divided into apical and basal sections. Thin slices of tissue were dissected from the basal endocardial and apical epicardial surfaces, which yielded sub-endocardial and sub-epicardial myocytes respectively (see Fig. 1). A slice of tissue was excised below the basal sub-endocardium and discarded before a third slice of tissue was retrieved, from which mid-myocardial myocytes were isolated. The three tissue samples were then placed in separate flasks containing fresh enzyme solution (with no protease added). Cells were harvested after further 5- and 10-min digestion periods, and were washed twice in Tyrode solution containing 5 mg ml$^{-1}$ bovine serum albumin (Sigma). Myocytes were then resuspended in Dulbecco’s modified Eagle medium supplemented with 2 mg ml$^{-1}$ Ultrasere G (Gibco, Paisley, UK). The myocyte suspension was stored at 20±1°C and cells were used within 12 h of isolation.

2.3. Electrophysiological techniques

Cells were layered onto the glass floor of a perfusion chamber situated on the stage of an inverted microscope (Nikon Diaphot). Myocytes were perfused with a modified Tyrode solution at 35±1°C. Patch pipettes (resistance 2–4 MΩ when filled with solution) were pulled from filamented glass tubing (GC150TF-15 Clark Electromedical Instruments) on a horizontal puller (BB-CH-PC Mecanex, Switzerland). Membrane current was measured using the whole-cell patch-clamp technique (Axopatch 2A, Axon Instruments, USA). Capacity transients were corrected (up to a maximum of 100 pF) and series resistance was routinely compensated by ≈80–90%. All analogue signals were digitised using a 12 bit A/D converter (1401, C.E.D., Cambridge, UK) and digitally stored for off-line analysis.

Calcium-independent outward currents were elicited by 500-ms step depolarisations to test potentials ranging from −40 mV to +70 mV from a holding potential of −70 mV. Membrane current elicited in this way consisted of two components: an early calcium-independent transient outward current component ($I_{\text{o1}}$) and a sustained outward component ($I_{\text{o2}}$) [15]. $I_{\text{o1}}$ was measured as the difference between peak current amplitude and current measured at the end of the step depolarisation.

Assuming a linear current–voltage relationship for the fully activated current, steady-state activation curves were estimated by determination of the relative chord conductance as a function of membrane voltage:

$$G = I/(V_m - V_{\text{rev}})$$

where $G$ is the relative chord conductance, $V_m$ is membrane potential and $V_{\text{rev}}$ is the reversal potential. For each cell a Boltzmann distribution was fitted to the normalised currents:

$$G/G_{\text{max}} = 1/(1 + \exp [(V_m - V_{\text{rev},0.5})/k])$$

where $G_{\text{max}}$ is the maximum chord conductance, $G$ is the relative chord conductance measured at the membrane potential $V_m$, $V_{\text{rev},0.5}$ is the potential at which the conductance is half-maximally activated and $k$ is the slope factor. With the assumption that $I_{\text{o1}}$ is carried by K$^+$ ions $V_{\text{rev},0.5}$ calculated from the Nernst equation was −84 mV. However, this may be more negative than $V_{\text{rev}}$ values calculated experimentally as $I_{\text{o1}}$ has a finite permeability to other ions such as sodium [16–18]. Our method may therefore result in a small, systematic error (<2 mV) in the voltage dependence of activation of $I_{\text{o1}}$; however comparison between the groups will be unaffected.

Steady-state inactivation of $I_{\text{o1}}$ was measured using a standard double-pulse protocol. Step depolarisations to conditioning potentials (ranging from −60 mV to +20 mV) of 500 ms duration were made before a step depolarisation to the test potential (+60 mV). The current elicited at the test potential was normalised to the maximum current measured and plotted as a function of the conditioning voltage. Data from each individual cell were fitted by a Boltzmann function from which the half-inactivation potential ($V_{\text{rev},0.5}$) and slope factor ($k$) were obtained.
Steady-state background current was elicited by step depolarisations to test potentials ranging from −100 to −10 mV from a holding potential of −70 mV for a duration of 300 ms. Steady-state background current was measured at 295 ms.

Calcium current ($I_{Ca}$) was elicited by 500-ms step depolarisations to test potentials ranging from −40 to +80 mV. Although a holding potential of −45 mV was used to inactivate $I_{Na}$, it is possible $I_{Ca}$ may be slightly inactivated at this voltage which will affect the amplitude and steady-state kinetics of the current. 4-Aminopyridine (3 mmol l$^{-1}$) was used to block $I_{Na}$. Peak inward current was measured with respect to the current at the end of the step depolarisation.

2.4. Solutions

Isolation tyrode contained (mmol l$^{-1}$): NaCl 130, KCl 5.4, MgCl$_2$ 1.2, Na$_2$HPO$_4$ 0.4, glucose 10, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) 5, taurine 20; pH 7.2 adjusted with NaOH. For the measurement of K$^+$ currents cells were superfused with a sodium-free salt solution containing (mmol l$^{-1}$): choline CI 140, KCl 5.4, MgCl$_2$ 1.2, glucose 10, HEPES 10, CaCl$_2$ 0.5, CaCl$_2$, 1, atropine 0.01, pH 7.4 with KOH. The pipette solution contained (mmol l$^{-1}$): KCl 140, MgCl$_2$, 1, EGTA 5, Mg$^{2+}$–ATP 5, HEPES 10, pH 7.2 with KOH. Although divalent cations have been shown to shift the voltage dependence of activation of $I_{Ca}$ to more positive voltages, [19] the use of high concentration of organic calcium channel blockers would also have been problematic as these compounds affect both the amplitude and the kinetics of $I_{Ca}$ [20,21].

For the measurement of $I_{Na}$, the superfusion solution contained (mmol l$^{-1}$): NaCl 137, KCl 5.4, MgCl$_2$ 1.2, CaCl$_2$ 1.8, glucose 10, HEPES 5; pH 7.4 with NaOH and the pipette solution contained (mmol l$^{-1}$): KCl 140, MgCl$_2$, 2, MgATP 5, Na$_2$ phosphocreatine 5, HEPES 10; pH 7.4 with KOH. A stock solution of 3 mol l$^{-1}$ 4-aminopyridine (4-AP) was diluted in perfusion Tyrode to give a final concentration of 3 mmol l$^{-1}$.

2.5. Statistics

Data are expressed as mean±S.E.M. and n indicates the number of cells used in each group. Data were collected from approximately equal numbers of cells per region from each animal. Four pairs of animals were used in this study. The Kolmogorov–Smirnov goodness of fit test was used to verify a normal distribution for all parameters. In order to assess the influence of both hypertrophy and region on membrane current characteristics, data from all six groups were initially analysed with two-way analysis of variance (two-way ANOVA) using hypertrophy and region as factors. A significant interaction between the two factors shows that the effects of hypertrophy are different in the different regions and therefore regional differences should be assessed separately in control and hypertrophied hearts. One-way analysis of variance (one-way ANOVA) was then used to assess regional differences in the properties of myocytes isolated from either control or hypertrophied hearts. Appropriate comparisons between the individual groups were then made using either Student–Newman–Keuls post hoc analysis or Student’s unpaired t-test. Current–voltage relationship curves were compared over selected voltage ranges using repeated measures analysis of variance using voltage as the repeated measure. Statistical significance was assessed at the 5% level. Representative records (as shown in Fig. 1) were selected on the criteria that the amplitude of the current density closely resembled the mean amplitude.

3. Results

3.1. Cell membrane capacitance

Cell membrane capacitance was similar in the three regions studied in both control and hypertrophied hearts and was increased by 17% in hypertrophy; (control: sub-endocardial myocytes 124±6 pF (17), mid-myocardial myocytes 113±5 pF (15), sub-epicardial myocytes 122±5 pF (17); hypertrophy: sub-endocardial myocytes 145±8 pF (19), mid-myocardial myocytes 138±6 pF (17), sub-epicardial myocytes 135±6 pF (17), P<0.001, two-way ANOVA).

3.2. Calcium-independent transient outward current

Fig. 1 shows representative families of current records taken from individual sub-endocardial, mid-myocardial and sub-epicardial myocytes isolated from control and hypertrophied hearts. Mean $I_{Na}$–voltage relationship curves are shown in Fig. 2.

In control hearts (Fig. 2A) the $I_{Na}$–voltage relationship is different in the three regions studied (P<0.001, repeated measures ANOVA, voltage range 0 to +70 mV). Mean $I_{Na}$ density (elicited by a step depolarisation to +60 mV) is substantially smaller in sub-endocardial myocytes than in sub-epicardial or mid-myocardial myocytes (P<0.001 one-way ANOVA).

The changes in $I_{Na}$ density associated with hypertrophy are different in the three regions studied (P<0.05, interaction two-way ANOVA). Fig. 2C shows the effect of hypertrophy on the density of $I_{Na}$ (elicited by a step depolarisation to +60 mV) in the three regions studied. Hypertrophy is associated with a significant reduction in the density of $I_{Na}$ in mid-myocardial (by 42%, from control 24.0±2.8 (15) to hypertrophy 13.8±1.3 (17) pA pF$^{-1}$ and in sub-epicardial (by 32%, from control 23.4±2.0 (17) to hypertrophy 15.8±1.5 (17) pA pF$^{-1}$) myocytes. In sub-endocardial myocytes the mean density
Fig. 1. Representative families of transient outward current records taken from individual sub-endocardial, mid-myocardial and sub-epicardial myocytes isolated from control (middle panel) and hypertrophied (bottom panel) hearts. Membrane current was elicited by a step depolarisation to 0, +20, +40 and +60 mV. For the sake of clarity the capacity transient is not shown and only the first 300 ms of the step depolarisation is shown. An exploded view line diagram of the heart (top centre) illustrates the regions from where the three groups of myocytes were isolated.

of $I_{\text{to1}}$ was non-significantly decreased by 13%. (from control $8.5 \pm 0.7$ (17) to hypertrophy $7.4 \pm 1.8$ (19) pA pF$^{-1}$).

In hypertrophied hearts although there are significant regional differences in $I_{\text{to1}}$ density (Table 1) and in the $I_{\text{to1}}$ density–voltage relationship ($P<0.001$, repeated measures ANOVA, voltage range 0 to +70 mV) there is a reduction in the magnitude of these differences (Fig. 2B).

### 3.3. Steady-state activation and inactivation of $I_{\text{to1}}$

Mean steady-state activation and inactivation curves obtained from sub-endocardial, mid-myocardial and sub-epicardial myocytes isolated from both control and hypertrophied hearts are shown in Fig. 3.

Changes in steady-state activation of $I_{\text{to1}}$ associated with hypertrophy were different in the three regions ($P<0.001$, interaction two-way ANOVA). In hypertrophy the steady-state activation curve was shifted to less positive potentials in sub-endocardial myocytes ($P<0.01$, t-test) and unchanged in mid-myocardial and sub-epicardial myocytes (Fig. 3 and Table 1). The slope ($k$) of the steady-state activation curves was similar in the three regions and not altered in hypertrophy.

Steady-state inactivation of $I_{\text{to1}}$ was not altered in hypertrophy ($P=0.4$, two-way ANOVA, Table 1). However, the potential at which half-inactivation occurred was different between the three regions studied in both control and hypertrophied hearts ($P<0.001$, two-way ANOVA). The slope of the inactivation curves were similar in all three regions studied and unchanged in hypertrophy.

### 3.4. Sustained outward current

Mean current–voltage curves for the sustained outward current ($I_{\text{so}}$, measured at 495 ms) obtained from sub-endocardial, mid-myocardial and sub-epicardial myocytes isolated from control and hypertrophied hearts are plotted in Fig. 4. The $I_{\text{so}}$ density–voltage relationship was similar in the three regions studied ($P=0.8$, repeated measures ANOVA, voltage range 0 to +70 mV) and was not altered in hypertrophy ($P=0.9$, repeated measures ANOVA, voltage range 0 to +70 mV). Mean values for $I_{\text{so}}$ density (elicited by a step depolarisation to +60 mV) were similar
myocardial, by 13% in sub-endocardial and by 22% in sub-epicardial myocytes.

Changes in the current density–voltage relationship associated with hypertrophy were different in the three regions studied ($P<0.05$, interaction repeated measures ANOVA, voltage range $-70$ to $-20$ mV). The decrease in outward current was greatest in the mid-myocardial myocytes, thus the pattern of regional differences are altered in hypertrophy (Table 2).

### 3.6. L-type calcium current

Fig. 6 shows representative current records (elicited by a step depolarisation to 0 mV from a holding potential of $-45$ mV) recorded in sub-endocardial, mid-myocardial and sub-epicardial myocytes isolated from control and hypertrophied hearts. There was no significant regional difference in the density of $I_{Ca}$ in either control or hypertrophied hearts, although hypertrophy was associated with a 23% reduction in mean peak $I_{Ca}$ density (Table 2).

Mean $I_{Ca}$ density–voltage curves are plotted in Fig. 7. Comparison of the curves suggest that hypertrophy is associated with a similar decrease in $I_{Ca}$ density in the three regions studied ($P<0.01$, repeated measures ANOVA, voltage range $-40$ to $+40$ mV).

### 4. Discussion

The principal finding of this study is that the differential effects of hypertrophy on APD [3] can be attributed at least in part to a decrease in $I_{to}$ density in sub-epicardial and mid-myocardial myocytes [3]. The effects of hypertrophy are different on current systems within a given region, which implies that channel expression and/or control is altered in a channel and a region-specific fashion.

#### 4.1. Regional differences in $I_{to1}$ in normal rat hearts

In the normal rat heart we found that the density of $I_{to1}$ in sub-epicardial myocytes was similar to that in mid-myocardial and greater than that in sub-endocardial myocytes. These findings agree with earlier studies which showed that $I_{to1}$ density was greater in sub-epicardial than sub-endocardial myocytes in the rat [9,22], cat [11], rabbit [23] and human [12,13], and show a similar rank order of $I_{to1}$ density to that reported in the normal canine heart (i.e. sub-epicardial >mid-myocardial >sub-endocardial myocytes) [10]. The transient outward current is the major determinant of early repolarisation in the rat. The pattern of regional distribution of $I_{to1}$ density reported in this study correlates closely with the previously identified regional differences in APD$_{25}$ in normal rat hearts [3] where APD$_{25}$ is longer in sub-endocardial (11 ms) than in mid-myocardial myocytes (8 ms) and shortest in sub-epicardial myocytes.
Table 1
Regional differences in the characteristics of calcium-independent outward current in myocytes from control and hypertrophied hearts

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypertrophy</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub-endocardial</td>
<td>Mid-myocardial</td>
<td>Sub-epicardial</td>
</tr>
<tr>
<td>Peak (I_{\text{to}})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>17</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>At (+60) mV (pA pF(^{-1}))</td>
<td>8.5±0.7</td>
<td>24.0±2.8</td>
<td>23.4±2.0</td>
</tr>
<tr>
<td>(r^d)</td>
<td>7.4±1.8</td>
<td>13.8±1.3**</td>
<td>15.8±1.5**</td>
</tr>
<tr>
<td>Steady-state activation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>17</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>(V_{1/2}) (mV)</td>
<td>30.3±2.4</td>
<td>30.9±1.3</td>
<td>30.3±1.2</td>
</tr>
<tr>
<td>(k) (mV)</td>
<td>17.9±1.0</td>
<td>20.5±1.1</td>
<td>18.7±0.8</td>
</tr>
<tr>
<td>(r^d)</td>
<td>20.9±2.1</td>
<td>17.8±0.9</td>
<td>18.6±1.2</td>
</tr>
<tr>
<td>Steady-state inactivation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n)</td>
<td>12(^b)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>(V_{1/2}) (mV)</td>
<td>(-27.4±1.4)</td>
<td>(-24.7±0.7)</td>
<td>(-25.6±0.8)</td>
</tr>
<tr>
<td>(k) (mV)</td>
<td>4.3±0.3</td>
<td>4.5±0.3</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td>(r)</td>
<td>4.2±0.5</td>
<td>3.6±0.1</td>
<td>3.7±0.2</td>
</tr>
</tbody>
</table>

\(^a\) Values are mean±S.E.M. \(n\) is the number of myocytes studied, \(V_{1/2}\) is the membrane potential at which half-activation or inactivation occurs, \(k\) is the slope factor of the activation and inactivation curves. \(^{**}\)\(P<0.01\), \(^{***}\)\(P<0.001\) (t-test) control vs. hypertrophy for the same region; ns, not significant.

\(^b\) R, \(P<0.05\) region influences value.

\(^c\) \(r, P<0.05\) hypertrophy and region interact to influence value, two-way ANOVA.

\(^d\) \(r, P<0.05\) one-way ANOVA for the three regions in either control or hypertrophy.

myocytes (5 ms) [3]. Thus regional differences in \(I_{\text{to}}\) density contribute to the regional differences in APD\(_{25}\) in the normal rat ventricle.

4.2. Alteration in the regional differences in \(I_{\text{to}}\) density associated with hypertrophy

A reduction in \(I_{\text{to}}\) density in left ventricular myocytes has been described in whole heart isolates from this model of hypertrophy [4] as well as from other models of hypertrophy [5–8,14,24,25], pacing-induced heart failure [26,27] and cardiomyopathies [28,29]. However, data presented here show that changes in \(I_{\text{to}}\) density associated with catecholamine-induced hypertrophy are not uniform within the left ventricle. The density of \(I_{\text{to}}\) was reduced in sub-epicardial and mid-myocardial myocytes and unchanged in sub-endocardial myocytes, and thus regional differences in \(I_{\text{to}}\) density within the left ventricle (although remaining significant) are reduced in hypertrophy. Although Shimoni et al. [22] found that the reduction in \(I_{\text{to}}\) density was similar in sub-endocardial and sub-epicardial myocytes in hyperthyroid rats, a reduction in the regional variation of \(I_{\text{to}}\) has been shown in failing human hearts compared to undiseased donor control hearts [12] and in hypertrophy [14].

Prolongation of APD in hypertrophy is attributable in part to a reduction in \(I_{\text{to}}\) density [5–8,25,27]. Our data strongly suggest that the reduction in \(I_{\text{to}}\) density observed in sub-epicardial and mid-myocardial myocytes contributes to the prolongation of APD\(_{25}\) and reduction in sensitivity to 4-AP previously reported in these regions in this model of hypertrophy in the rat [3]. Moreover, the lack of any change in \(I_{\text{to}}\) density in sub-endocardial myocytes is consistent with the finding that ADF\(_{25}\) was unaltered in sub-endocardial myocytes in hypertrophy. Thus the dif-

Fig. 3. Steady-state activation (\(G/G_{\text{max}}\)) and inactivation (relative current amplitude) curves for sub-endocardial (○), mid-myocardial (△) and sub-epicardial (□) myocytes isolated from control (open symbols) and hypertrophied (closed symbols) hearts. Data shown are mean±S.E.M. The lines shown for the individual regions were calculated by fitting the mean data points to the appropriate Boltzmann function using the mean values for the voltage at which half-activation or inactivation occurs (\(V_{1/2}\)) and slope of the relationships (\(k\)) shown in Table 1.
to mRNA levels there is a strong similarity between our measures of \( I_{so} \) density and the regional distribution of Kv4.2 mRNA expression in the normal rat left ventricle, in that they are both greatest in the epicardium, least in the endocardium and of intermediate values in the mid-myocardium. Moreover, prolongation of APD and a reduction in \( I_{so} \) density in a post-MI-induced model of left ventricular hypertrophy in rat [32] has been ascribed, in part, to a decrease in Kv4.2 mRNA and protein levels [33]. Thus we may speculate that the differential reduction in \( I_{so} \) in hypertrophy in this study may be due, at least in part, to a more pronounced decrease in the expression of Kv4.2 and/or Kv4.3 in sub-epicardial than sub-endocardial myocytes. Recently Kaab et al. [34] have demonstrated transmural gradients in the expression of Kv4.3 and Kv4.1 mRNA across the left ventricular free wall in man.

4.3. Sustained outward potassium current \( I_{so} \)

Our data suggest that this current does not have a role in the differential changes in APD that occur in hypertrophy, although at present the physiological role of \( I_{so} \) is unclear.

4.4. Steady-state background current \( I_{Ks1} \)

The density of \( I_{Ks1} \) has been reported to be either more outward in sub-endocardial than in sub-epicardial myocytes in cat [35] or similar in these two regions in rat [9,22] and dog [10]. Our data show that the current–voltage relationship for the steady-state background current (over the voltage range −20 to −50 mV) is different in the three regions. Though the regional differences in the steady-state background current are small they may contribute to the heterogeneity of APD in normal rat hearts [3] Fig. 4. Current density–voltage relationship curves of the sustained outward current \( I_{so} \) (measured at 495 ms) for sub-endocardial (○), mid-myocardial (△) and sub-epicardial (□) myocytes isolated from control (open symbols) and hypertrophied (closed symbols) hearts. Data shown are mean±S.E.M.

The mechanism(s) that regulate the differential regional reduction in \( I_{so} \) density are unclear. Within the rat ventricle two genes have been identified that encode channels that are likely to contribute to \( I_{so} \), Kv4.2 and Kv4.3 [30,31]. Interestingly, although Kv4.3 mRNA is equally expressed in endocardial and epicardial tissue [30], there is a gradient of expression of Kv4.2 mRNA across the left ventricular free wall in rat [30,31]. Although protein content and function cannot be correlated directly
channel recordings) and must contain a component of other overlapping currents.

4.5. Calcium current

We found no significant regional difference in $I_{Ca}$ density in sub-endocardial, mid-myocardial and sub-epicardial myocytes isolated from either control or hypertrophied hearts, similar to previous data obtained from normal guinea-pig [2] and cat [42] hearts.

Changes in $I_{Ca}$ associated with hypertrophy are varied (reviewed in [1]) and may be related to the severity of the hypertrophy, with $I_{Ca}$ apparently increasing in mild and decreasing in severe hypertrophy. A decrease in $I_{Ca}$ has been reported in a number of studies of severe hypertrophy in cat [43], Syrian hamster [44], guinea-pig [45] and rat [5, 25]. Although the regional distribution of $I_{Ca}$ density is not altered in hypertrophy, the reduction in $I_{Ca}$ would be expected to shorten APD. Thus it is possible that the effect of reducing $I_{Ca}$ is more pronounced in sub-endocardial myocytes where $I_{Ca}$ density is unchanged and that the decrease in $I_{Ca}$ density in sub-endocardial myocytes contri-

Table 2
Regional differences in membrane currents in myocytes from control and hypertrophied hearts

<table>
<thead>
<tr>
<th>Current Type</th>
<th>Control</th>
<th>Hypertrophy</th>
<th>ANOVA</th>
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<tbody>
<tr>
<td>$I_{so}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>At +60 mV (pA pF⁻¹)</td>
<td>11.9±1.1</td>
<td>12.9±1.0</td>
<td>10.1±0.8</td>
</tr>
<tr>
<td>$I_{Ca}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>At −40 mV (pA pF⁻¹)</td>
<td>1.5±0.2</td>
<td>1.1±0.2</td>
<td>0.9±0.1*</td>
</tr>
<tr>
<td>$I_{st}$</td>
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<td>n</td>
<td>17</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>At 0 mV (pA pF⁻¹)</td>
<td>−7.4±0.4</td>
<td>−8.1±0.9</td>
<td>−7.1±0.6</td>
</tr>
</tbody>
</table>

* Values are mean±S.E.M.; n is the number of myocytes studied. *P<0.05 one-way ANOVA vs. sub-endocardial myocytes in either control or hypertrophied hearts.

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<tr>
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<th>Hypertrophy</th>
<th>ANOVA</th>
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<tr>
<td>n</td>
<td>17</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>At +60 mV (pA pF⁻¹)</td>
<td>11.9±1.1</td>
<td>12.9±1.0</td>
<td>10.1±0.8</td>
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<td>$I_{Ca}$</td>
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<td>20</td>
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<tr>
<td>At −40 mV (pA pF⁻¹)</td>
<td>1.5±0.2</td>
<td>1.1±0.2</td>
<td>0.9±0.1*</td>
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* Values are mean±S.E.M.; n is the number of myocytes studied. *P<0.05 one-way ANOVA vs. sub-endocardial myocytes in either control or hypertrophied hearts.

b R p<0.05 region influences value.

c H, P<0.05 hypertrophy influences value.

d r, P<0.05 one-way ANOVA difference between the three regions in either control or hypertrophied hearts.

Fig. 5. Current density–voltage relationship curves of the steady-state background current for sub-endocardial (○), mid-myocardial (△) and sub-epicardial (□) myocytes isolated from control (open symbols) and hypertrophied (closed symbols) hearts. Data shown are mean±S.E.M.
but to the shortening of APD_{75} observed in these myocytes in hypertrophy.

4.6. Implications for the loss of regional differences in APD in hypertrophy

In isolated rat ventricular myocytes the transient outward current is the major determinant of the rapid early phase of repolarisation (phase 1) [46] and the prominent low plateau phase is attributable to I_{Ca} and I_{NaCa} [47], [3,48] The final repolarisation phase will also be influenced by I_{K1} [49]. Regional differences in repolarisation in the normal heart (APD shorter in epicardium than endocardium) serve to ensure that the sequence of ventricular repolarisation is opposite to that of depolarisation (resulting in a concordant QRS complex and T wave) and will tend to reduce the likelihood of intramural re-entry. These data show that the normal regional differences in I_{to} are reduced in hypertrophy which will contribute to the reduction in the normal regional differences in APD in hypertrophy, and may thus underlie in part T wave inversion observed in this model of hypertrophy [3].

The configuration of ventricular repolarisation in vivo will also be influenced both by cell-to-cell coupling and by mechano-electrical feedback. In normal myocardium when myocytes are well-coupled (via gap junctions which create low-resistance pathways), regional differences in action potential duration of the myocytes will be smaller than measurements taken from isolated cells from the same regions [50]. However, the presence of fibrous tissue and the decrease in lateral myocyte interconnections in hypertrophy [51,52] would be expected to alter the action
potential configuration as a consequence of non-uniform spread of excitation. APD would be relatively longer in directions of slower conduction and relatively shorter at sites where activation spread accelerates [53]. Isoprenaline-induced hypertrophy is known to be associated with micro-infarcts and an increase in fibrosis [54–56] that may cause intra-ventricular conduction defects and alter the normal sequence of repolarisation. Thus in vivo the effects of hypertrophy on the action potential duration of single myocytes may be further modulated by the effects of changes in ventricular conduction consequent on fibrosis and/or ischaemia, and more experiments are needed to explore the contribution of changes in cell-to-cell conduction now that the regional properties of the myocytes have been defined.

Loss of the normal repolarization gradient across the ventricular wall may be expected to favour the establishment of re-entry circuits both within the wall and at the Purkinje–endocardial interface, and the differential effects of fibrosis on conduction velocity and refractory periods will further increase the likelihood that after-depolarizations could result in propagated and sustained ventricular tachycardia. The results of this study are necessary for computer modelling which will be required to evaluate the role of these and other pro-arrhythmic mechanisms in hypertrophied hearts.

Acknowledgements

This work was supported by the British Heart Foundation. Jane Shipsey was a British Heart Foundation Junior Research Fellow.

References

[36] Le Grand B, Hatem SN, Deroubaux E, Couetil J-P, Coraboeuf E.


