Spatial heterogeneity of myocardial perfusion predicts local potassium channel expression and action potential duration

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Received 19 September 2006; revised 1 November 2007; accepted 4 November 2007; online publish-ahead-of-print 9 November 2007

Time for primary review: 26 days

Aims In the heart, there is not only a transmural gradient of left ventricular perfusion and action potential duration (APD), but also spatial heterogeneity within each myocardial layer, where local blood flow and energy turnover vary more than three-fold between individual regions. We analysed at high spatial resolution whether a corresponding heterogeneity also extends to ion channel gene expression and APD.

Methods and results In the open-chest beagle dog, left ventricular 300 \( \mu \)L samples of very low or high flow were identified by radioactive microspheres and expression levels determined by quantitative PCR. The distribution of epicardial APD was assessed by mapping local activation repolarization intervals (ARIs) and QT interval (QT). ERG, the potassium channel mediating \( I_{Kr} \), and KChIP2, the interacting protein modulating \( I_{to} \), were increased in Low flow (3.3- and 2.5-fold, \( P < 0.001 \) and \( P < 0.05 \), respectively; \( n = 6 \) hearts, 30–31 samples each) as compared with High flow areas. This suggested enhanced repolarizing currents in Low flow areas, and in consequence, mathematical model analysis predicted a shorter local APD upon enhanced ERG and \( I_{Kr} \). Epicardial mapping revealed a patchy, temporally stable APD pattern (\( n = 11 \)), a small apico-basal gradient and an APD prolongation induced by the ERG blocker dofetilide predominantly in areas of short basal ARI or QT, respectively (\( n = 9 \)). In addition, in Short QT areas, ERG expression was three-fold increased (\( P < 0.05 \), \( n = 4 \)).

Conclusion The spatial pattern of perfusion is matched by the novel patterns of \( K^+ \) channel expression and APD. Whenever this newly recognized intramural dispersion of APD increases, it may contribute to arrhythmogenesis.

KEYWORDS
Ion channels; Gene expression; Action potentials; Coronary circulation; Regional blood flow; Mapping; Microspheres; Computer modelling

1. Introduction
In the past decade, it is becoming increasingly evident that the left ventricular myocardium cannot be simply regarded as a homogeneous structure, which serves as a single, contractile function. It is rather a complex organ, rich in its cellular diversity, which displays substantial spatial heterogeneity of function, metabolism as well as gene and protein expression. In the left ventricular myocardium, a transmural gradient of perfusion and electrophysiological properties, e.g. action potential duration (APD), is well established. Under physiological conditions, subendocardial blood flow exceeds subepicardial perfusion by about 1.25:1, reflecting greater oxygen requirements and systolic wall stress in the deeper myocardial layers.

This transmural flow gradient is paralleled by a gradient of APD, where the longer APD in the subendocardium results in later recovery from depolarization compared with the subepicardium. In most species, there is a marked gradient of the transient outward current (\( I_{to} \)) from the subepicardium to the subendocardium. This \( I_{to} \) gradient is based on an expression gradient of the \( K^+ \) channel subunits Kv4.3 and KChIP2, the Kv channel-interacting protein. It contributes to the prominent phase 1 repolarization and the shorter action potential in the subepicardium (for review:). In the canine heart, a transmural gradient of the rapid component of the delayed rectifier \( K^+ \) current (\( I_{Kr} \)) mediated by the two-fold enhanced ERG (KCNH2 or LQT2) expression in the subepicardium was recently reported as well. In addition to these transmural gradients, there is also a gradient in APD from apex to base, consistent with the late activation and early repolarization of the myocardial base.

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Beyond these transmural gradients of flow and functional properties, within each myocardial layer there is also a spatial heterogeneity: initial studies, focusing on myocardial blood flow, demonstrated a fractal pattern in various species\textsuperscript{10,11} that proved to be temporally stable for weeks.\textsuperscript{12} For example at a myocardial resolution of 200 $\mu$m in sheep or baboons, a spatial dispersion of blood flow of 20% was observed, where local perfusion between individual myocardial tissue samples differed more than three-fold. Subsequent studies elucidated the metabolic and molecular basis of this spatial heterogeneity. Low flow areas, i.e. areas receiving less than 60% of the average left ventricular perfusion under physiological conditions, are not hypoxic.\textsuperscript{13} High flow areas, i.e. areas receiving $>$140% of the mean blood flow, display enhanced fatty acid uptake\textsuperscript{14} and energy turnover.\textsuperscript{15} In fact, the local flow pattern reflects the local energy turnover and demand.\textsuperscript{13,15} In spite of a similar morphology, Low and High flow areas differ also in their local proteome, which favours fatty acid oxidation in high flow regions.\textsuperscript{12} Although the above data translate into a coherent spatial pattern of flow, metabolism and gene and protein expression, both the underlying mechanism and functional correlates remain elusive.

Since the transmural gradient of myocardial perfusion was paralleled by a subendo-/subepicardial gradient of APD based on differential expression of potassium channels, we wondered whether the spatial heterogeneity of flow, metabolism and gene expression observed within each myocardial layer extended also to ion channel expression and APD. Specifically, we compared Low, Mean and High flow samples and tested the hypothesis that in Low flow areas, i.e. areas receiving less than 60% of the average left ventricular perfusion under physiological conditions, are not hypoxic.\textsuperscript{13} High flow areas, i.e. areas receiving $>$140% of the mean blood flow, display enhanced fatty acid uptake\textsuperscript{14} and energy turnover.\textsuperscript{15} In fact, the local flow pattern reflects the local energy turnover and demand.\textsuperscript{13,15} In spite of a similar morphology, Low and High flow areas differ also in their local proteome, which favours fatty acid oxidation in high flow regions.\textsuperscript{12} Although the above data translate into a coherent spatial pattern of flow, metabolism and gene and protein expression, both the underlying mechanism and functional correlates remain elusive.

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Having established significant differences in potassium channel expression, in the second series ($n = 11$) the spatial distribution of APD and the role of potassium channel expression was assessed by epicardial mapping. In most experiments of this series ($n = 9$), the effect of the ERG-blocker dofetilide (0.1 mg/kg) on QT interval (QT) and blood flow was assessed. In a subset of experiments ($n = 5$), the cardiac cycle length was varied by electrical stimulation in the range from 400 to 550 ms before and after dofetilide to assess its effect on QT and ARI independent of its effect on heart rate.

To identify areas of short and long QT under basal conditions, in four of these hearts epicardial mapping of the anterior free wall was repeated five times in 15 min intervals prior to pacing and dofetilide and the time-averaged QT mean for each electrode determined. Selected areas were marked with an intramyocardial injection of 25 $\mu$L trypan blue at the end of the experiment. After withdrawal of the electrode sock, 3 mg subepicardial biopsies were obtained using a 16G biopsy needle (Temno Evolution, Allegiance) with a 4 mm biopsy range. This tissue was immediately stored in RNA-later (Qiagen) for further preparation. Thereafter, the left ventricular free wall was excised and treated as described above.

### 2.3 Epicardial mapping of local surface electrograms

A nylon mesh sock equipped with 128 electrodes (J. Davis, Utah) equally spaced at 6–8 mm distance on the epicardial surface was employed. Following pericardiotomy, the sock was gently pulled across the complete left and right ventricular surface. The junction of left anterior descending and circumflex artery served as anatomical landmark for a consistent placement. Once a steady-state was attained, unipolar electrograms, measured against a common chest reference electrode, were acquired at a sampling rate of 1 kHz by a 16-channel A/D-converter (ADInstruments). The electrodes were divided into eight groups and within 3 min all traces were obtained while heart rate and blood pressure remained stable. The traces were manually analysed for RR, QT and also ARI as a measure of local APD.\textsuperscript{16} While QT was defined as the period between the initial deflection of the activation complex (QRS correlate) and the end of the T wave, while ARI is the delay between the moment of minimum dV/dt during the activation complex and the moment of maximum dV/dt during the T wave\textsuperscript{17} (see also Figure 3A). The latter is independent of the polarity of the T wave.\textsuperscript{17} QT and ARI were corrected for heart rate by Van de Water’s formula [e.g. QTc = QT – 0.087(RR – 1000)].\textsuperscript{18} Preliminary experiments had indicated that for each individual electrode, the variability of the measured QTc and ARIc, respectively, was only about $\pm 3$ and $\pm 2$% and independent of the local time-averaged QTc or ARIc, which differed by more than 60 and 40 ms, respectively. As a result, an individual electrode displaying e.g. a short QTc in one measurement showed a relatively short QTc in all registrations throughout the experiment. To further enhance the reliability, for local biopsies and selective flow determinations, areas of Short and Long QTc were identified on the basis of average values from five consecutive measurements.

### 2.4 RNA preparation, reverse transcription, and quantitative real-time PCR analysis

Total RNA was isolated from 31 Low, 53 Mean, and 30 High flow samples and in another set of experiments from biopsy tissue...
using the RNeasy Fibrous Tissue Mini Kit and the RNeasy Micro Kit, respectively (Qiagen). First-strand cDNA was synthesized using Superscript II RT (Invitrogen) in the presence of 1 µg of total RNA and oligo (dT)12–18 primers.

Gene expression of K,A3, KChIP2, K,LQT1, minK, ERG, Kir2.1, Na,1,5, and Ca,1,2 was determined by quantitative real-time PCR. For gene amplification, cDNA was amplified using SYBR Green PCR Core Reagents (Applied Biosystems) and primers as listed in Table 1.

The comparative CT method was used for relative quantification, relating the number of cycles of the target genes to β-actin at CT 0.5 and at CT 1.0. Because cycle efficiency was 100% in the range studied, relative amount was determined by using the formula 2^−ΔCT where ΔCT describes the difference between β-actin and the six genes.

### 2.5 Model analysis of ion channel expression and APD

To evaluate the relevance of different ion channel expression levels observed in Low, Mean, and High flow areas for APD, a mathematical model of canine ventricular cardiomyocyte electrophysiology was employed. This model had been translated to run within the Cellular Open Resource (COR) environment. Consistent with experimental observations, all simulations were carried out at a cycle length of 550 ms and only steady-state data were used for further analysis. The standard parameter set of the model was taken to present mean flow areas. To simulate Low and High flow areas, the maximal channel conductances were modulated in proportion to average flow and were designated Low and High flow areas, respectively (see below).

### 2.6 Statistics

All data are given as mean ± standard deviation except where indicated. Regional differences within the heart between Low and High flow samples were compared using Student’s t-test for paired data, taking separate samples from the same flow or QT domain as replicate measurements. One-way ANOVA followed by Bonferroni’s correction for multiple comparisons was applied where appropriate. Results were considered significant at P < 0.05.

### 3. Results

In the 17 anaesthetized dogs studied, basal heart rate, mean aortic pressure, and myocardial blood flow were 82 ± 25 bpm, 79 ± 11 mmHg, and 0.91 ± 0.42 mL/min/g, the subendo-/subepicardial perfusion ratio being 1.21 ± 0.12.

When analysed at the spatial resolution of 300 µL (sample mass 60 mg dry weight), there was substantial flow heterogeneity within each myocardial layer. The coefficients of variation (CV) for subepicardial, midmyo- and subendocardial flow ranged from 0.29 to 0.36, the overall CV being 0.32 ± 0.08. In each dog, about one-tenth of samples received less than 60% or more than 140% of the overall average flow and were designated Low and High flow areas, respectively. The flow difference between Low and High flow areas exceeded the small transmural gradient by far.

#### 3.1 Ion channel expression in Low and High flow areas

When comparing Low, Mean, and High flow samples from all transmural layers, a significant inverse correlation between ERG, but also KChIP2 expression in the individual samples and their flow could be observed (Figure 1A). While ERG mediates I,Ko, KChIP2 is the K, channel-interacting protein modulating K, and thus I,K, which is dependent on K,LQT1 and minK. Blocking ERG by dofetilide was simulated by a 90% decrease of maximal ERG conductance.

### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence 5’ → 3’</th>
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<tr>
<td>β-Actin</td>
<td>AATCATACGGGTACCTACAGAGA CATACGGTTCCTACAGGTA</td>
</tr>
<tr>
<td>β-Actin</td>
<td>GGGCAAAACGCTCTCCAAACC TCTGATCCCTTGGACCTGCA</td>
</tr>
<tr>
<td>KvLQT1</td>
<td>ACCACAACACTGTCACCAAC TCTGATCTCTGCGTGGAG</td>
</tr>
<tr>
<td>Cav1.2</td>
<td>AF394940, U17869, AF465484 TCTCCCTATGGTCAKRTCGCAG</td>
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</tbody>
</table>

### Table 2

<table>
<thead>
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<th>Gene</th>
<th>Ion current</th>
<th>LF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>K,A3</td>
<td>I,Ko</td>
<td>126 ± 53</td>
<td>84 ± 17</td>
</tr>
<tr>
<td>KChIP2</td>
<td>I,Ko</td>
<td>161 ± 80*</td>
<td>65 ± 11*</td>
</tr>
<tr>
<td>K,LQT1</td>
<td>I,Ks</td>
<td>67 ± 50</td>
<td>123 ± 42</td>
</tr>
<tr>
<td>minK</td>
<td>I,Ks</td>
<td>91 ± 25</td>
<td>99 ± 24</td>
</tr>
<tr>
<td>ERG</td>
<td>I,Kr</td>
<td>192 ± 54**</td>
<td>58 ± 13**</td>
</tr>
<tr>
<td>Kir2.1</td>
<td>I,K1</td>
<td>106 ± 50</td>
<td>107 ± 19</td>
</tr>
<tr>
<td>Na,1.5</td>
<td>I,Na</td>
<td>137 ± 57</td>
<td>153 ± 42</td>
</tr>
<tr>
<td>Ca,1.2</td>
<td>I,Ca</td>
<td>149 ± 41</td>
<td>92 ± 42</td>
</tr>
</tbody>
</table>

*P < 0.05 and **P < 0.001 for LF and HF vs. MF, n = 5–6 hearts, 15-30 samples each.
domains (Figure 1B). Thus, the correlation seen was indeed related to flow.

There was no flow-related difference in mRNA expression of KvLQT1 and minK, mediating, respectively, modulating $I_{Ks,i}$ in Kir2.1, the inward rectifier $K^+$-channel, nor in Nav1.5, the voltage-gated $Na^+$-channel or Cav1.2, the $\alpha$-subunit of the voltage-gated $Ca^{2+}$-channel (Table 2).

When comparing subepi- and subendocardial Mean flow samples, there was no difference in $K_4.3$, $K_{LQT1}$, minK, ERG, and $Ca_{1.2}$, the respective ratios ranging from 0.75 to 1.37. Only KChIP2 tended to display a 2.86-fold greater expression in the subepicardium that however did not reach the level of statistical significance.

3.2 Model analysis of ion channel expression and action potential duration

To predict the effects of enhanced potassium channel expression on APD in Low flow areas, a mathematical model of the isolated canine ventricular myocyte was employed. At a cycle length of 550 ms chosen according to experimental data, the model calculated the AP and the APD at 90% of repolarization ($APD_{90}$) (Figure 2A).

Increasing selectively the maximal conductance for $I_{Kr}$ according to the enhanced ERG expression in Low flow areas resulted in a $>10$ ms shortening of the predicted $APD_{90}$. Reducing the $I_{Kr}$ conductance consistent with the lower ERG expression in High flow areas prolonged $APD_{90}$ to a smaller extent (Figure 2B, $I_{Kr}$). Similarly, enhancing the maximal conductance of $I_{to}$ according to the expression data shortened $APD_{90}$ while reducing $I_{to}$ had hardly any effect (Figure 2B, $I_{to}$). The non-significant differential expression of $KvLQT1$ and minK governing $I_{to}$ had no discernible effect on $APD_{90}$ (data not shown). When modulating both the maximal conductance for $I_{to}$, $I_{Kr}$, and $I_{Ks}$ taking all the expression data from Table 2 for Low and High flow areas into account, the effect on the predicted action potentials was much greater than upon modulation of a single channel conductance only (Figures 2A and B, $I_{to}$, $I_{Kr}$, and $I_{Ks}$). The model also predicted that blocking ERG by 90% (dofetilde) would result in an increase in $APD_{90}$ predominantly in areas characterized by enhanced ERG expression, i.e. in Low flow areas (Figure 2B, $I_{to}$, $I_{Kr}$, and $I_{Ks}$ + dofetilde).

3.3 Epicardial mapping to assess spatial differences in local electrograms

We next explored whether the predicted heterogeneity of APD (Figure 2A) translated into a spatial heterogeneity in
the intact beating heart. To this end, 128 local cardiac electrograms (Figure 3A) were obtained per heart, enabling the reliable detection of local QT intervals and ARIs. Figure 3B demonstrates a representative map of the anterior cardiac QT distribution. Consistent with the local differences in potassium channel expression, a spatial pattern of epicardial QT intervals was observed, with differences between the shortest and longest left ventricular QTc of 51 ± 15 ms. There was also a similar pattern of local ARI, the average difference between shortest and longest ARIC being 55 ± 17 ms. These patterns were temporally stable for at least 60 min (data not shown). Qualitatively similar maps were obtained in 11 experiments, the average QTc in a total of 1315 traces being 320 ± 48 ms and the average ARIC being 247 ± 31 ms. Within a given heart, the average coefficient of variation was 3.7 and 5.6%, respectively. There was no difference between the left and right ventricular traces in terms of mean QTc (320 ± 48 vs. 317 ± 48 ms) and ARIC (249 ± 32 vs. 237 ± 28 ms); LV and RV traces also did not differ in variability within each ventricle (QTc CV 3.7 vs. 3.5%, and ARIC CV 5 vs. 4.1%). As expected, QTc was about 7 ms and ARIC 14 ms shorter in the basal compared with the apical region (P < 0.05 each).

### 3.4 ERG channel blockade, local APD prolongation and perfusion

To test the model prediction of a preferential APD prolongation by ERG blockade in areas of high ERG expression (Figure 2B, dofetilide), the effects of dofetilide on the local QTc and ARIC were measured in the heart *in vivo*. Dofetilide increased mean QTc from 311 by 34 ms (+10 ± 6%) and mean ARIC from 243 by 31 ms (+12 ± 7%). The QT prolongation was significantly greater in areas of short basal QTc and smaller in areas of long basal QTc (Figure 4A). In consequence, when correlating the increase in local QTc to its basal value in each heart (n = 9), the average slope was −0.68 ± 0.17 at an average r² of 0.32. When allocating the basal values of each heart to nine percentile classes and calculating the dofetilide-induced increase, there was a significantly greater increase in the two lowest and a much smaller increase in the two highest QTc classes compared

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Spatial heterogeneity of local unipolar electrograms as observed by epicardial mapping. (A) Unipolar electrogram from a single electrode site. (B) Representative anterior surface map of local QT durations (grey to black colour code: 287 to 349 ms). LV and RV: left and right ventricular surface; LAD: left anterior descending artery.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Greater APD prolongation by dofetilide in areas of short basal APD as assessed by QTc and ARIC. (A) Basal QTc values from nine hearts were grouped in percentile classes. The average increase for each class induced by dofetilide was determined. **P < 0.01 vs. mean ΔQTc. (B) Using the same approach, the increase in ARIC was assessed. ***P < 0.001 vs. ARIC class shown.**
with the overall mean (Figure 4A). In fact, in three hearts in areas of long basal QTc dofetilide induced hardly any rise in QTc (≤3%). When relating the dofetilide-induced rise in local ARic to basal ARic, the data were qualitatively the same: dofetilide caused a significantly greater ARi prolongation in the shortest basal ARic class than in three out of four long ARi classes (Figure 4B).

To test whether a shorter APD in low flow areas might impair the local Ca$^{2+}$-transient and energy demand and thus directly contribute to the lower flow, in most of the experiments ($n = 7$) the effects of APD prolongation by dofetilide on local flow were determined. While dofetilide increased both QTc and ARic substantially in Short QTc, respectively, Short ARic and thus presumably Low flow areas (Figure 4), there was only a modest elevation of myocardial perfusion in Low flow regions. When correlating flow before and after application of dofetilide, both the positive offset and the slope being $<1$ reflected a preferential rise in perfusion in Low flow areas ($P < 0.001$, Figure 5), while the spatial heterogeneity of perfusion was not affected by dofetilide.

### 3.5 ERG expression in Short and Long QTc areas

To directly demonstrate enhanced ERG and KChIP2 expression in short QTc regions, a total of 20 Short and Long local QTc ($<97$ vs. $>103$% of the mean) areas were identified by averaging of repeated QTc measurements, which reduced the local SEM to 1.3%. Analysis of biopsies from Short QTc areas indicated a modest increase in KChIP2 and revealed a threefold higher ERG expression (Figure 6), consistent with a substantial role of Kr in APD shortening. When relating local ERG expression to ARic in those Short and Long QTc areas, there was a significant negative correlation, the slope being $-9.8$, i.e. a doubling of ERG expression upon a 10% shorter basal ARic.

### 4. Discussion

While across the left ventricular myocardial wall, transmural gradients have long been established, the current study adds a novel dimension to the spatial heterogeneity in the heart. At high spatial resolution, there is not only a fractal patchwork of perfusion within each myocardial layer,11 but there is also substantial variability in potassium channel expression, notably in ERG and KChIP2, which mediate, respectively, govern $I_{Kr}$ and $I_{Ks}$. In fact, Low flow areas, receiving $<60\%$ of the average left ventricular blood flow under physiological conditions, display an up to 3.5-fold higher expression of ERG and KChIP2 resulting locally in a shorter action potential. This may contribute to a reduced energy demand in Low flow regions.13 The spatial dispersion of APD seen on the epicardial surface could well increase under pathophysiological conditions such as myocardial hypertrophy and subsequently form the arrhythmogenic substrate e.g. of drug-induced torsades de points.21

#### 4.1 Spatial heterogeneity of ion channel expression and function

Previous low resolution studies focused on transmural gradients, e.g. the expression gradient of the $K^+$ channel subunits Kv4.3 and KChIP2 governing $I_{Ks}$.5,6 In the present study, a three-fold higher KChIP2 expression in the subepicardium might have been significant when restricting our subendocardial and subepicardial samples to a small rim. A prominent subepicardial ERG expression had been reported in the ferret and the canine heart.4,22 However, in the present and other canine studies there was no transmural difference in $I_{Kr}$21 and ERG expression (Figure 1B). Another traditional focus was the apico-basal gradient. In previous9 and in the present study, a longer apical APD was seen, suggesting enhanced inward or decreased outward currents. However, there was no apico-basal ERG gradient in the canine and human heart24 and no evidence for decreased apical $I_{Kr}$ or $I_{Ks}$.

These previous studies could not assess the spatial pattern of ERG and KChIP2 expression now revealed, since they either integrated across large tissue volumes or randomly picked few tissue samples, most probably from Mean flow...
areas. Only the present high-resolution approach, i.e. systematically dividing subendo-, mid-, and subepicardium of the left ventricular wall and selecting 300 μL samples according to local perfusion made it possible to detect the several-fold differences in ERG and KChIP2 expression between Low and High flow samples. Model analysis indicated substantial consequences for the local APD, consistent with the spatial heterogeneity of the local ARI and QT demonstrated by epicardial mapping (Figures 3–4). The specific ERG blocker doxetilide predominantly prolonged ARI and QT in short basal ARI and QT areas, respectively (Figure 4), which can be attributed to an enhanced local ERG expression. ARI is an established index of the APD at the site of the electrode.23 However, the close correlation of QT prolongation and basal QT compared with the looser dependence of ARI prolongation on basal ARI (cf. Figure 4A and B) and the predictive power of basal QT for the level of ERG expression (Figure 4) may indicate that local QT is also a measure of APD in the vicinity of the electrode.

The spatial heterogeneity of ERG and KChIP2 is consistent with the non-uniform, patchy ERG distribution seen in the ferret heart.22 Since flow-related differences in ERG and KChIP2 were present also in the subendocardium (Figure 1), a contribution of M cells to these differences is highly unlikely.23

4.2 Spatial heterogeneity in the heart and its potential basis

Although the myocardium is still viewed by many as a very homogeneous tissue, recent research has firmly established spatial patterns of gene and protein expression,12 flow10,11 metabolism,25 energy turnover,15 and demand13 even within the left ventricular free wall, revealing a rather complex and nevertheless coherent structure. The current study adds potassium channel gene expression and APD as an additional dimension to this already impressive list.

On the transmural level, under physiological conditions, there is a higher subendocardial blood flow2 associated with enhanced energy turnover. The transmural flow gradient is paralleled by a gradient of APD induced by transmural differences in K⁺ channel expression (Kv4.3 and KChIP2) and 

\[ \text{APD} = \text{Kv4.3 and KChIP2} \]

To what extent this APD gradient contributes to gradients of the Ca²⁺ transient and energy demand remains to be seen.

When studying the myocardium at higher resolution, within each myocardial layer a substantial spatial heterogeneity is revealed (for review:1). Local blood flow differs more than three-fold between Low and High flow areas. The fractal pattern of myocardial perfusion correlates with local metabolism, gene, and protein expression, and the current study extends these observations to local potassium channel expression and APD. The spatial heterogeneity of perfusion reflects the local energy turnover and demand.13,15 Due to the inherently higher energy demand, High flow areas are more likely to suffer from stop-flow ischaemia and reperfusion.26 It is most likely that the quantitative differences between local myocardial tissue areas will increase at even higher spatial resolution, and therefore may represent only the ‘tip of the iceberg’. However, current technology for flow measurements27 does not allow to enhance the spatial resolution while still correlating precisely local perfusion and gene or protein expression in one tissue sample.

Surprisingly, the fundamental basis of this spatial heterogeneity is not well understood. In Low flow areas in the present study, the shortened APD was expected to induce a lower energy demand. However, since ERG blockade increased APD (Figure 4), but only slightly enhanced local perfusion in Low flow (and thus short APD) regions (Figure 5), the observed differences in ERG and APD can only explain a minor fraction of the variability of perfusion. To what extent the local differences in KChIP2 expression and \( I_{\text{K}} \) govern local Ca²⁺ transients, energy demand and flow remains to be seen. A further important factor may be local differences in myocardial fiber strain and work,28 which consecutively result in local variations of flow, gene, and protein expression.12 However, the relevant mechano-sensitive feedback transduction mechanisms have not been elucidated so far. Also the regulatory mechanisms in the ventricular expression of ion channels are incompletely understood and the transcription factors governing transmural or intramural ion channel expression are only beginning to emerge. In the mouse heart, e.g. a transmural expression gradient of a transcription factor governs the gradient for Kv4.2 and \( I_{\text{K}} \).29 However, since Kv4.2 is not expressed in larger species, this finding cannot explain the heterogeneity seen in the present study.

4.3 Possible implications and outlook

The current study indicates a spatial heterogeneity of APD at least in the epicardium, induced by a newly recognized spatial pattern of ion channel expression clearly present in each transmural layer. Although in the past only transmural and apico-basal expression gradients were studied, we have now demonstrated a local pattern of ERG and KChIP2 expression that is related to local flow and present in both the subendo-, midmyo-, and subepicardial layer. This pattern results in a dispersion of ventricular APD and contributes to the pattern of ventricular repolarization as well.30 Enhanced spatial heterogeneity may be of considerable importance under pathophysiological conditions, e.g. during heart failure. Proarrhythmic properties of drugs inducing QT prolongation are frequently studied in the AV-block dog.31 Since in this model doxetilide is well known to induce torsades de pointes, it is tempting to speculate that the spatial heterogeneity of flow, ion channel expression, and APD is increased, possibly resulting in a critical level of dispersion of repolarization. This could also be true in heart failure or myocardial hypertrophy, creating the substrate for functional reentry responsible for the development of life-threatening arrhythmias.32

Acknowledgements

We are grateful to Jürgen Schrader, MD, for continuous advice and support and to Penny Noble and Alan Garny, Oxford, for providing the CellML translation of the Fox model and the COR environment. We appreciated helpful discussions with Dieter Hafner, Department of Pharmacology, Düsseldorf and skillful technical assistance by Gerald Große, Artyom Verner, and Daniela Haubs.

Conflict of interest: none declared.
Funding
This study was supported by the Deutsche Forschungsgemeinschaft (SFB-612-A4 and De 487/7-1). Dofetilide was provided by Pfizer (Karlsruhe, Germany) free of charge.

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