Effect of an increase in coronary perfusion on transmural ventricular repolarization

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Summary

The effect of increased coronary flow on transmural ventricular repolarization was investigated in 6 pentobabital-anaesthetised sheep. The left circumflex coronary artery (LCX) was injected with fresh blood at 10 ml/min in addition to the normal coronary flow. Unipolar electrocardiograms were simultaneously registered from epicardium, mid-myocardium and endocardium with fine plunge needles. Activation-recovery interval (ARI) was measured from the unipolar electrocardiograms and was used as an estimate of ventricular repolarization duration. It was found that intracoronary blood injection (n=3) prolonged ARI in the epicardium, mid-myocardium and endocardium by an average of 34 $\pm$ 16, 28 $\pm$ 18 and 25 $\pm$ 13 msec, respectively ($p<0.01$). Pre-treatment with nitro-L-arginine (n=3), a nitric synthase inhibitor, diminished the flow-induced ARI prolongation across the ventricular wall. In conclusion, an increase in coronary flow lengthens transmural ventricular repolarization duration. These effects appear to be mediated by nitric oxide from the coronary endothelium.

Key words: repolarization, activation-recovery interval, coronary flow, arrhythmia, nitric oxide.

Introduction

An increase in coronary flow, or pressure, is associated with an increase in myocardial oxygen uptake or contractility in the isolated or intact animal heart [Gregg 1963, Feigl 1983]. Increases in coronary flow in the isolated guinea pig heart have also been shown to enhance atrioventricular node conduction [Rubio et al 1985]. In the intact sheep heart, intracoronary injection of blood leads to a flow-dependent T-wave inversion on body surface ECG [Wang 2002], and a prolongation of ventricular repolarization directly measured from the surface of the perfused territories [Li and Wang 2006]. The mechanisms of the flow-induced prolongation in ventricular repolarization are not entirely clear but the synthesis and release of nitric oxide from the coronary endothelium seems play a partial role [Li and Wang 2006].

Several *in vitro* and *in vivo* animal studies have reported regional differences in the duration of action potential and ventricular repolarization between endocardium, mid-myocardium and epicardium [Sicouri C and Antzelevitch 1991, Antzelevitch et al 1999]. A subpopulation of cells, or M cells, shows some unique electrophysiological properties in the mid-myocardium [Sicouri C and Antzelevitch 1991]. The differences between the repolarization duration between M cells and cells from other layers of the myocardium is believed to contribute to the spatial dispersion of ventricular repolarization and hence, the risk of ventricular arrhythmia [Antzelevitch et al 1999]. The primary objectives of the present study are to evaluate the effect of increased coronary flow on ventricular repolarization duration across the ventricular wall.

Materials and Methods

Surgical preparations

The study followed the local ethics guidelines for animal research. The methodologies of intracoronary injection model used in the present study have been previously reported [Wang 2002, Li and Wang 2006]. Briefly, six sheep of both sexes (body weight 25-38 kg)

were anaesthetized with sodium pentobarbital (intravenous infusion at a rate of 3 mg/kg/hr). The animals were intubated and artificially ventilated at a rate of 14-18 strokes/min with room air. The left ventricular pressure was monitored via a 6-french catheter inserted through the left carotid artery into the left ventricle. Body surface ECG (lead II) was continuously monitored during the experiment.

Following a left thoracotomy in the fourth intercostal space, the proximal left circumflex coronary artery (LCX) was cannulated with a thin PE50 polyethylene cannula. Insertion of the intracoronary cannula had no significant effect on coronary flow nor on the body surface or epicardial ECGs. In the presence of normal coronary flow, fresh arterial blood drawn from the left ventricular catheter was injected into LCX with a perfusion pump. The injection rate was 10 ml/min and the duration of each injection was 10 min. Intracoronary injection at this rate on top of the normal coronary flow has been shown to increase the coronary flow by approximately 7 ml/min in [Wang 2002].

Animals were divided into two groups. In group one (n=3) the effect of intracoronary injection on transmural ventricular repolarization was investigated. In group 2 (n=3), the effect of inhibition of nitric oxide synthesis on the injection -induced alterations in ARI was assessed by intravenous administration of nitro-L-arginine (20 mg/kg over 10 min, Aldrich Chemical Co., USA), a nitric oxide synthase inhibitor, before intracoronary blood injection.

Electrocardiogram (ECG) acquisition and analysis

The techniques used for the simultaneous recording of unipolar ECG from epicardium, mid-myocardium and endocardium have been previously described in detail [Wang et al 2002]. In each animal, 4 plunge needles (0.8 mm in diameter) were inserted into the LCX territory at the left anterior-lateral ventricular walls, with a 10 mm distance between the needles. There were 4 unipolar silver electrodes on the shaft of each needle. The distance between electrodes was designed in such a way that the proximal electrode was located in the epicardium (0.5 mm from the epicardial surface), the middle two electrodes were in the

mid-myocardium (4-7 mm from the epicardium) and the distal electrode was in the endocardium (10-11 mm from the epicardium).

These unipolar ECGs were recorded to a portable computer (BriteLite Computer Corp., Carlsbad, CA, USA) through a S11W interface (Engineering Design Team, Inc., Beaverton, OR, USA). Under sinus rhythm (500-700 ms in cardiac cycle length), unipolar ECGs in a 4-second period were sampled at 1000 samples/second, before and at the end of intracoronary perfusion.

Measurement of activation-recovery interval (ARI)

ARI was measured from the unipolar ECGs by a Sun Workstation (SUN Corp., Ichinomiya, Japan) using the S-plus statistical package (Statistical Sciences Inc., Seattle, Washington). The time difference between the most rapid decrease in voltage in the QRS complex (dV/dt_{min}) (activation time) and the most rapid increase (dV/dt_{max}) near the peak of the T wave (repolarization time) was defined as the ARI [8], which was used as an estimate of ventricular repolarization duration [Li and Wang 2006, Wang et al 2002].

Statistical analysis

Data were expressed as means ± SD. In each animal, an average ARI in the same myocardial layers was derived from the 4 epicardial/endocardial or 8 mid-myocardial unipolar ECGs from the 4 plunge needles. Comparisons on average ARI before and after intracoronary perfusion were performed by student t test. $P<0.05$ was considered to be statistically significant.

Results

General findings

In the 3 animals treated with nitro-L-arginine, the left ventricular systolic pressure was increased by 10, 14 and 16 mm Hg respectively, whereas the left ventricular diastolic pressure remained unchanged.

Nitro-L-arginine injection did not affect the ST-T on the body surface or epicardial ECGs. In one animal, the heart rate was temporarily increased by 10 beats/min following drug administration. The heart rate of the other two animals remained unchanged.

Differences in the ARI between the epicardium, midmyocardium and endocardium

At baseline, there was a relatively large variation (3-27 msec) in the ARI recorded from different layers of the myocardium. However, there was no significant difference in the average ARI between the three-myocardial layers in the each of the six animals (Table 1 and 2).

Effect of intracoronary blood perfusion on ARI

In the 3 animals without nitro-L-arginine treatment, the intracoronary blood injection at 10 ml/min prolonged ARI in the three-myocardial layers. The range of the prolongation in the epicardium, midmyocardium and endocardium was 15-68 msec, 6-68 msec and 8-62 msec, respectively.

Because the number of the ARI from each myocardial layer was small in each animal, the ARI from the same myocardial layer of the 3 animals were pooled for analysis. Fig. 1 showed that the mean ARI was prolonged in the three-myocardial layers by intracoronary blood injection.

In each animal, there was no clear evidence to suggest in which myocardial layer the ARI was prolonged the most by the intracoronary blood injection, and the pooled increases in ARI in the same animal showed little difference between the three-myocardial layers (Fig 2).

The pooled ARI increases from the 3 animals were 34 ± 16, 28 ± 18 and 25 ± 13 msec respectively, in the epicardium, mid-myocardium and the endocardium. The increase in the epicardium was greater than that in the mid-myocardium and endocardium ($p<0.01$).

Effect of nitric oxide inhibition on the flow-induced ARI prolongation

In the second group (n=3), the intracoronary blood injection resulted in a similar ARI prolongation to that shown in the Fig 1.

The mean ARI after the pre-treatment of nitro-L-arginine was shown in Table 2. In the first animal, the mean ARI in each of the three-myocardial layers remained unchanged during the intracoronary blood injection. In the other 2 animals, the mean ARI was increased by 2-12 msec, but these changes were not statistically significant ($p>0.05$, Table 2).

Discussion

Measurement of ventricular repolarization across the ventricular wall

Assessment of transmural differences in ventricular repolarization has important clinical implications because the regional differences in, or spatial dispersion of repolarization duration is associated with pathogenesis of life-threatening ventricular arrhythmias, such as Torsade de pointed [Antzelevitch et al 1999]. Suppression of transmural repolarization dispersion with antiarrhythmic drugs or physiological means interrupts the electrophysiological mechanisms of tachycardia, leading to clinical prevention or treatment of these arrhythmias [Antzelevitch et al 1999].

Transmural ventricular repolarization can be measured in a number of ways. In isolated ventricular myocytes, intracellular microelectrode technique is applied to record the action potential duration of different layers of myocardium; this is probably the most accurate measurement of local repolarization [Antzelevitch et al 1999].

In intact animal heart, transmural monophasic action potential (MAP) recording can be used to measure the regional differences of repolarization [Weissenburge et al 1996]. Similar to MAP technique, unipolar electrocardiograms can be registered by a fine needle inserted into the ventricular wall, and ARI can be calculated from these unipolar electrocardiograms [Li and Wang 2006, Wang et al 2002]. This recording technique is able to reasonably measure the changes in transmural repolarization in intact animal heart, and to assess the dispersion in repolarization across the ventricular wall [Antzelevitch et al 1999]. In the present study, although we did not verify the location of M cells with cellular techniques, we did examine the location of needle electrodes after each experiment, ensuring the middle-two electrodes in a plunge needle were indeed located in the middle one-third of the ventricular wall. A number of previous studies on isolated animal hearts have demonstrated that M cells are located in the mid-myocardium of a range of animal species [Antzelevitch et al 1999].

Effect of increased coronary flow on transmural ventricular repolarization

An increase in coronary flow, or coronary shear stress, distends the coronary artery and acts on the intravascular surface of the cells lining the blood vessel walls. This physiological stimulus is transmitted to the adjacent myocardium and this in turn, increases calcium influx [Haneda et al 1989, Kitakaze and Marban 1989]. The increased intracellular calcium not only results in a positive inotropic effect [Kitakaze M and Marban 1989] but also a dromotropic effect on the atrioventricular nodal conduction [Rubio et al 1985].

Our recent studies on pentobarbital-anaesthetized sheep model have demonstrated that when the left circumflex coronary artery is perfused with fresh arterial blood in the presence of normal coronary flow, the T waves on body surface ECG is inverted in a flow-dependent fashion, indicating changes in ventricular repolarization [Wang 2002]. These T wave changes can be diminished by pre-treatment with nitro-L-arginine, a nitric oxide

synthase inhibitor, suggesting that endothelial nitric oxide plays a role in the flow-induced T wave inversion [Wang 2002].

Further studies in the same animal model have clearly demonstrated that an increase in coronary flow prolongs epicardial ARI by up to 33 msec [Li and Wang 2006]. After pre-treatment with nitro-L-arginine, intracoronary perfusion with fresh blood only resulted in only a small increase in ARI, ranging from 3 to 11 msec [Li and Wang 2006]. These results indicate that ventricular repolarization, when measured from the epicardium, is prolonged by an increase in coronary flow through nitric oxide release.

The present study has shown that, when coronary flow is increased, the repolarization duration in the epicardium, mid-myocardium and endocardium is prolonged in a similar fashion. The increase in repolarization duration in the epicardium appears greater than that in the mid-myocardium and endocardium. This may be due to the fact that larger branches of coronary arteries run in the epicardium therefore an increase in coronary flow will impact on the electrophysiology of epicardial myocytes first and most. In addition, the changes in repolarization duration during coronary perfusion appear to be mediated by nitric oxide because after pre-treatment with nitro-L-arginine, the increase in ARI is no longer significant in each of the three animals. These results are consistent with our previous studies where ventricular repolarization is simultaneously measured from more than 30 epicardial sites in each animal [Li and Wang 2006].

A potential limitation of the study is that the change in QT interval on body surface ECG is not measured during the study. Therefore whether an increase in ARI during coronary perfusion is associated with a prolongation in QT interval is unclear. Our previous studies in the same animal model shows that the values of ARI recorded from epicardium are generally shorter than QT intervals on body surface ECG [Wang 2000]. During coronary perfusion, ARI prolongation is associated with an increase in QT interval on the corresponding body surface ECG leads [Wang 2000]. However, perfusion-induced increase in QT dispersion is less than the increase in ARI dispersion [Wang 2000],

indicating that ARI is a more sensitive measure for the evaluation of repolarization inhomogeneity.

In addition**,** number of animals in the study is relatively small but the perfusion-induced ARI changes are consistent between animals. Further studies in a larger number of animals may be required.

Summary

In this study ARI from unipolar electrocardiograms is used to estimate the duration of ventricular repolarization. Under baseline conditions, there is no significant difference in ventricular repolarization duration between epicardium, mid-myocardium and endocardium in the pentobarbital-anaesthetized model. The repolarization duration is significantly prolonged by enhanced coronary perfusion, probably through the release of nitric oxide from coronary endothelium. However, it is unclear as to how does nitric oxide mediate the flow-induced prolongation of transmural repolarization. Further studies in a larger number of animals may be required to clarify the role of nitric oxide in this novel physiological phenomenon.

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Table 1. The pooled activation-recovery intervals (msec) in the three-myocardial layers in each animal.

Animal Number	Endocardium (msec)	Mid-myocardium (msec)	Epicardium (msec)	P value
1	266 ± 9	267 ± 13	271 ± 8	>0.05
2	249 ± 11	244 ± 10	250 ± 13	>0.05
3	268 ± 5	267 ± 8	272 ± 5	>0.05
Average	261 ± 10	259 ± 13	264 ± 11	>0.05

Table 2. The activation-recovery intervals before and during intracoronary blood perfusion in 3 animals pre-treated with both nitro-L-arginine and indomethacin.

Animals	*Positions*	*Baseline*	*Perfusion*	*P*
1				
	EPI	319 ± 13	318 ± 7	>0.05
	MID	320 ± 12	318 ± 13	>0.05
	ENDO	321 ± 6	319 ± 7	>0.05
2				
	EPI	253 ± 14	265 ± 13	>0.05
	MID	250 ± 14	257 ± 14	>0.05
	ENDO	247 ± 14	255 ± 8	>0.05
3				
	EPI	240 ± 9	249 ± 5	>0.05
	MID	239 ± 14	244 ± 14	>0.05
	ENDO	236 ± 10	244 ± 10	>0.05

In each animal, the activation-recovery interval was the mean from 4 different positions in the epicardium (EPI) and endocardium (ENDO), and from 8 different positions in the mid-myocardium (MID).

Figure legends

Fig 1. The pooled activation-recovery intervals from the epicardium (1), mid-myocardium (2) and endocardium (3) before and during intracoronary blood perfusion in three animals without nitro-L-arginine treatment.

Fig 2. The net increases in the activation-recovery intervals (ARI) during the intracoronary blood perfusion in 3 animals without nitro-L-arginine treatment. The increase in ARI in each animal was the mean from the 4 electrodes in the epicardium (EPI) and endocardium (ENDO), and from 8 electrodes in the mid-myocardium (MID).

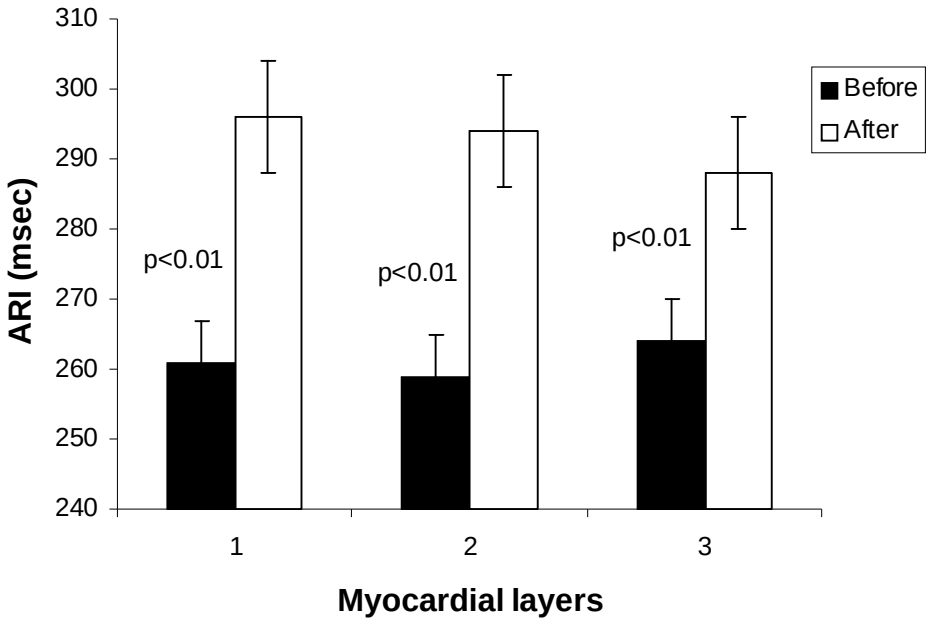
310
300
290
280
270
260
250
240
ARI (msec)
p<0.01
p<0.01
p<0.01
Before
After
1
2
3
Myocardial layers

Fig 1.

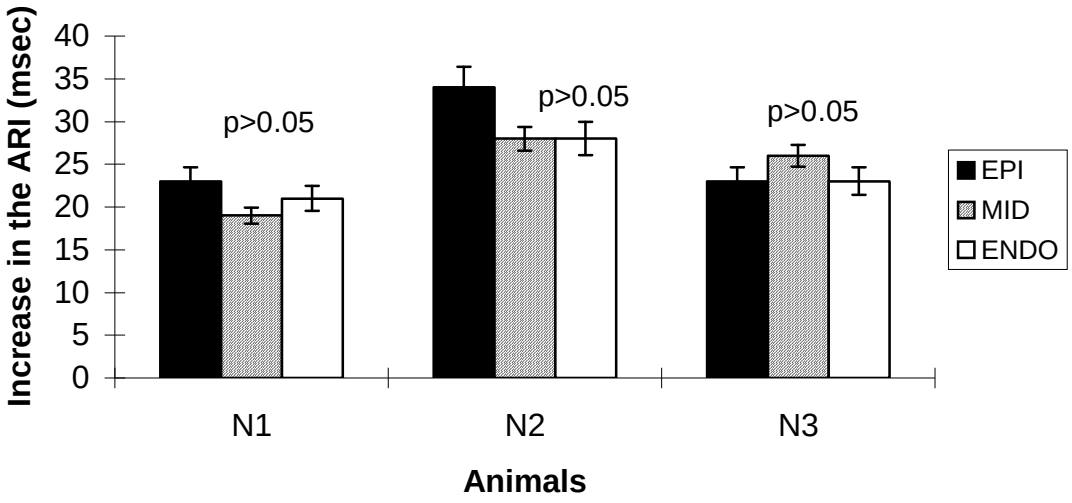
Increase in the ARI (msec)
40
35
30
25
20
15
10
5
0
p>0.05
p>0.05
p>0.05
N1
N2
N3
Animals
EPI
MID
ENDO

Fig 2.