

## A Novel Sheep Model to Study the Effect of High Rate Intracoronary Perfusion on Cardiac Electrophysiology

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**Abstract.** An increase in coronary flow is known to enhance myocardial metabolism and contractility (the Gregg effect) but the effect on cardiac electrophysiology is unclear. In 5 pentobarbital-anesthetised open-chest sheep, left circumflex coronary artery was perfused with fresh arterial blood at 6 and 10 ml/min respectively in the presence of normal coronary flow. The perfusion was repeated in these animals after treatment with nitro-L-arginine, a nitric oxide synthase inhibitor. The high rate intracoronary perfusion caused a flow-dependent T wave inversion on body surface ECG in all animals ( $p < 0.01$ ). Pre-treatment with nitro-L-arginine abolished T wave inversion during 6 ml/min perfusion, and diminished the T inversion during 10 ml/min perfusion. Conclusion: An increase in coronary flow alters ventricular repolarisation through nitric oxide release from coronary endothelium.

**Key words:** Cardiac electrophysiology — Ventricular repolarisation — Coronary flow — Nitric oxide

### Introduction

The Gregg (1963) effect refers to a physiological phenomenon that abrupt or gradual increase in coronary perfusion pressure for several minutes enhances oxygen uptake and contractility of the left ventricular myocardium. The flow-dependent inotropic effect has been confirmed by several other investigators in the isolated or intact animal heart, e.g. Feigl (1983). A recent study in the isolated guinea pig heart showed that, intracoronary perfusion at a rate between 6 and 25 ml/min enhances atrioventricular node conduction without affecting the conduction within ventricular myocardium (Rubio et al. 1995). The effect of increased coronary flow on ventricular repolarisation has not been reported.

The signalling mechanism between coronary flow increase and changes in cardiac metabolism and function is not well understood. Previous studies have shown that an increase in vascular flow stimulates endothelial synthesis and release of

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nitric oxide (Karwatowska-Prokopczuk et al. 1989; Stewart et al. 1994). However, whether nitric oxide has a role in the Gregg effect remains in question. The primary purpose of the preliminary study was to investigate the influence of high rate intracoronary perfusion on ventricular repolarisation, and the potential role of nitric oxide in the flow-induced alteration of ventricular repolarisation.

## Materials and Methods

The study followed the guidelines of the National Health and Medical Research Council of Australia. Five sheep of both sexes (body weight 28–35 kg) were anaesthetised with sodium pentobarbital (30 mg/kg b.w. i.v. followed by slow infusion at 3 mg/kg b.w./hour). They were intubated and artificially ventilated at a rate of 16–18 strokes/min with room air. The left ventricular pressure was monitored *via* a 6F catheter inserted through the left carotid artery into the left ventricle. Body surface ECG (6 limb leads) was continuously monitored during the experiment, and the T waves on the ECG were used to represent ventricular repolarisation.

Following a left thoracotomy in the fourth intercostal space, the proximal left circumflex coronary artery (LCX) was cannulated with a thin PE50 polyethylene cannula. In the presence of normal coronary flow, fresh arterial blood drawn from the ventricular catheter was injected into LCX with a large syringe preheated to 37°C. In each animal, two perfusions at a rate of 6 and 10 ml/min were performed respectively. The duration of each perfusion was 10 min, with an equilibration period between perfusions of at least 60 min. The pericardial temperature was monitored with a thermometer throughout experiment. Coronary flow was monitored by an electromagnetic flow meter placed distally in the LCX.

Nitro-L-arginine (NOLA, 20 mg/kg b.w.), a nitric oxide synthase inhibitor, was intravenously administered over 10 min in these animals after the two intracoronary perfusions were completed. Intracoronary perfusion at 6 and 10 ml/min was repeated 20 min after NOLA administration.

The amplitude of ST segment shift and inverted T waves was manually measured on the body surface ECG lead II. ST shift was measured from the baseline to the J point of the ST segment. The amplitude of T waves was measured from the baseline to the top of the T waves. The parameters used further in this study were averages of three cardiac cycles.

Data were expressed as means  $\pm$  SD. Comparisons of heart rate, ventricular pressure, ST segment and T wave changes between protocols were performed by a single factor ANOVA test.  $p < 0.05$  was considered to be statistically significant.

## Results

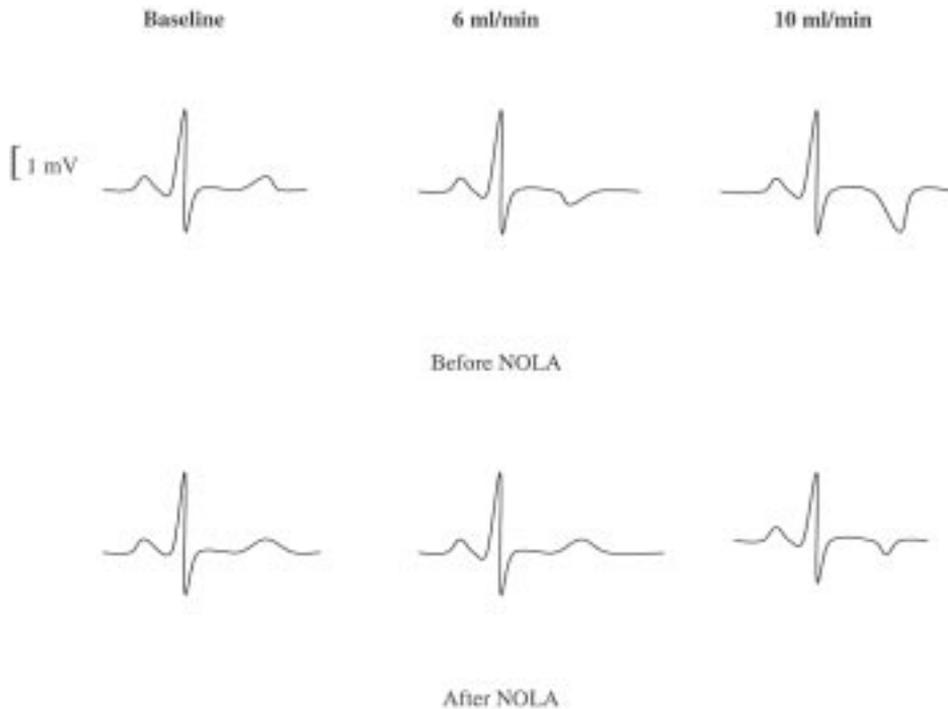
The baseline coronary flow of the five animals was  $29 \pm 2$  ml/min, which was increased by an average of 4 ml/min during 6 ml/min perfusion, and 7 ml/min during 10 ml/min perfusion.

Intracoronary perfusion at 6 and 10 ml/min did not affect heart rate or ventricular systolic pressure (Table 1). The ST segment on body surface ECG also remained unchanged (Fig. 1). The pericardial temperature remained constant at approximately 37°C during each perfusion.

**Table 1.** Changes in heart rate (HR), left ventricular systolic pressure (LVSP) and T wave amplitude before and after NOLA

Parameters	Before IP	IP 6 ml/min	IP 10 ml/min
HR (bpm)	105 ± 6	108 ± 8	106 ± 10
LVSP (mm Hg)	109 ± 10	111 ± 10	108 ± 12
T amplitude before NOLA (mV)	0.28 ± 0.06	-0.21 ± 0.04	-0.29 ± 0.05*
T amplitude after NOLA (mV)	0.27 ± 0.07	0.25 ± 0.05	-0.12 ± 0.02**

\*,  $p = 0.03$  compared with that during 6 ml/min perfusion; \*\*,  $p < 0.01$  compared with that at 10 ml/min perfusion before NOLA; -, Inverted T waves; IP, Intracoronary perfusion.



**Figure 1.** T wave changes on body surface ECG lead II during intracoronary perfusion at 6 and 10 ml/min in a sheep before and after the nitro-L-arginine (NOLA) treatment.

Intracoronary perfusion elicited a significant T wave inversion in all animals (Fig. 1). The T wave changes were observed on leads II, III and aVF, which were consistent with the territory supplied by the LCX in this sheep model. There was a significant increase in the degree of T wave inversion when the perfusion rate was increased from 6 to 10 ml/min ( $p < 0.05$ , Table 1). T wave inversion occurred at 2–4 seconds from the onset of perfusion, and returned to the baseline 3–5 min after the cessation of perfusion.

The same intracoronary perfusion protocols were repeated twice in each animal, resulting in identical changes in T waves.

Administration of NOLA *per se* had no significant effect on baseline ventricular pressure, HR or ST-T ( $p > 0.05$ ). Pre-treatment with NOLA did not affect the baseline coronary flow in the five animals ( $29 \pm 2$  vs.  $28 \pm 3$  ml/min,  $p > 0.05$ ).

Following NOLA administration, intracoronary perfusions resulted in a similar degree of increase in coronary flow to that before NOLA treatment. However, intracoronary perfusion at 6 ml/min failed to depress T waves, whereas perfusion at 10 ml/min elicited T wave inversion with a much less degree than that before NOLA treatment (Table 1, Fig. 1).

## Discussion

To the best of our knowledge, there have been no reported animal experiments investigating the effect of increased coronary perfusion on ventricular repolarisation. The results from this study show that an increase in coronary flow affects ventricular repolarisation, which is manifested as T wave inversion on body surface ECG. Endothelial nitric oxide appears to be an important mediator of this novel phenomenon.

Although ischemia is a primary cause of T wave depression or inversion, it does not seem to be the cause of the T wave changes in this sheep model in which coronary blood flow is increased. The flow-dependent nature of T wave changes is also against ischemia being the source of T inversion observed in these animals.

Baseline endothelial nitric oxide does not appear to play a critical role in regulating coronary flow in this model. Pre-treatment with NOLA did not cause any significant reduction in baseline coronary flow; neither did it affect the flow increase during intracoronary perfusion at 6 or 10 ml/min. However, inhibition of nitric oxide production largely diminished the action of intracoronary perfusion on ventricular repolarization.

There is emerging evidence to support the important role of nitric oxide in regulating ventricular repolarisation. Fei et al. (1997) showed that in denervated dogs, pericardial administration of L-arginine, a nitric oxide precursor, reduced the shortening of ventricular effective refractory period induced by sympathetic nerve stimulation. Simultaneous administration of N<sup>G</sup>-monomethyl-L-arginine, a nitric oxide synthase inhibitor, abolished the inhibitory effect of L-arginine. Goulielmos et al. (1995) showed that inhibition of nitric oxide synthesis exacerbated the reduction in action potential duration during ischemia, while administration of nitric oxide

diminished the reduction of action potential duration. The interaction pathway between nitric oxide and ventricular repolarization remains unclear.

In summary, the preliminary study in this open chest sheep model has shown, for the first time, that an increase in coronary flow results in a significant change in ventricular repolarisation in a flow-dependent manner. Endothelial release of nitric oxide is the most likely signalling mechanism between coronary flow and myocytes. These findings are of fundamental importance for our understanding of the relationships between coronary endothelial function and ventricular repolarisation.

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