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Title: Role and Therapeutic Potential of Myoregulin in Cardiovascular Disease

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Introduction:
Cardiovascular disease continues to be a major public health problem. It affects approximately 1 in 20 adults in New Zealand and remains the country's leading cause of death; responsible for 33% of deaths annually. As the population ages, and with greater life expectancy, these numbers are expected to increase substantially, necessitating the discovery of new biological markers to aid earlier diagnosis, treatment and prognosis.

A collection of potential markers was recently discovered within regions of DNA, previously considered non-coding 'junk DNA', but now shown to produce functional proteins. The Christchurch Heart Institute is currently investigating one such candidate, a 46 amino acid micropeptide called myoregulin which is proposed to play a key role in controlling muscle calcium balance. This has been demonstrated in knockout studies, improving muscle performance and enhancing calcium handling. Calcium is the principal driver of both contraction and relaxation, therefore myoregulin may be a crucial regulator of heart function; but as yet this is unknown.

Aim:
The aim of this project was to investigate the role of a novel peptide myoregulin on heart function in both healthy and damaged hearts, using an *ex vivo* isolated rat heart model.

Impact:
This project will build on the novel discovery of myoregulin and provide important information on its contribution to heart function. Myoregulin's therapeutic potential will also be assessed, possibly directing the development of therapies which may improve health outcomes and survivability in patients with cardiovascular disease.

Method:
Using an *ex vivo* isolated rat heart model; a method already established in the Christchurch Heart Institute, synthetic myoregulin was administered directly into rat hearts. Male Sprague-Dawley rats weighing 350-400g obtained from the Christchurch Animal Research Facility, University of Otago, Christchurch, were used for the experiments. Rats were anaesthetised by Sodium Pentobarbital and the hearts were rapidly excised and mounted on a specialized rig called a Langendorff apparatus. The hearts were allowed to stabilise for 30 minutes before the experimental protocol commenced. Hearts were randomly assigned to either a control group, administered only perfusion buffer (Krebs-Henseleit solution) or to the myoregulin treatment group. For the treatment group, synthetic myoregulin was diluted in the perfusion buffer and administered directly into the heart via a perfusion line using a syringe pump. For the first set of experiments, incrementing doses of myoregulin (0.3nM to 10nM) were delivered to 'healthy hearts' to determine the dosage required for subsequent experiments. In the next set of experiments, hearts underwent myocardial infarction by 35 minutes of total coronary flow occlusion, followed by a 60 minute reperfusion (ischaemia/reperfusion). Myoregulin was perfused for 30 minutes either prior to ischaemia or starting at the time of reperfusion.

Measures of cardiac function including contractility, left ventricular pressures and coronary flow were made throughout the experiment.

Results:
From the dose/response experiments conducted on the healthy hearts, a dose of 3nM myoregulin was chosen for the ischaemia/reperfusion experiments.

In total, 20 Ischaemia-Reperfusion experiments were performed. A number of assessment criteria were applied to determine if hearts were adequate for analysis; this included the heart beating between 308-310bpm and having the developed pressure above 40mmHg. Of the 20 hearts, 6 did not meet these criteria, leaving 14 for the final analysis in the respective groups; control (n=6), myoregulin before ischaemia (n=4), and after ischaemia (n=4).

No statistically significant results were identified between the control and treatment groups for any measures of cardiac function. However, there was a 15% difference in left ventricular pressure between hearts receiving myoregulin before ischaemia and control hearts, with this pressure having declined more in control hearts.

Conclusion:
This is the first study to assess the functional role of myoregulin in both healthy and damaged hearts. Our preliminary data suggests that when myoregulin is used to precondition the hearts before ischaemia it is reducing heart function in the reperfusion phase. However, it has no effect when administered at the time of reperfusion. Due to the small sample size however, these results require follow up with a larger sample number. Furthermore, only one concentration of myoregulin was used in this study, yet a range of different concentrations could also be investigated.