

FINAL PROJECT REPORT

Name:

John J Evans

Project Title:

Why do males die younger? Sex steroids and vasoactive peptides.

Please copy the "Outcome(s)" statement, entered on your application form, in the space below.

Vascular disease is an extremely important health concern but there has been limited progress on delineating the mechanism(s) of the clear contribution of sex steroids [41, 42]. When the reasons that steroid HRT and OCs cause vascular pathologies is better understood, steroidal molecules with favourable vascular activity can be developed. Endothelial cells which line the blood vessels are of primary importance because they are in immediate contact with and respond to circulating hormones. The hormonal network may explain some treatment failures and unexpected side effects. We propose that gender-related and individual responses of vasoactive peptides to steroids may be profiled and ultimately incorporated in prophylaxis and designer treatments.

Will your work contribute to this outcome(s) in the manner you envisaged? If not, what has changed?

The differential effects of female and male sex hormones on vasoactive peptides that were observed, together with data from other workers, can be expected to allow the eventual construction of peptide response profiles for the two sexes.

Please copy the "Specific Objective(s)" statement, entered on your application form, in the space below.

To determine the effect:

1. on the percent of endothelial cells that secretes adrenomedullin of long (12h) exposure to oestradiol;
2. on the percent of endothelial cells that secretes adrenomedullin-2 and on the expression of adrenomedullin-2 mRNA of exposure to oestradiol, testosterone and angiotensin-II;
3. on the percent of endothelial cells that secretes endothelin-1 and on the expression of endothelin-1 mRNA of exposure to oestradiol, testosterone and angiotensin-II;
4. on the expression of adrenomedullin, adrenomedullin-2 and endothelin-1 mRNAs in rat vascular tissues of in vivo administration of testosterone and oestradiol.

Briefly describe how successful you were in achieving the stated objective(s). If the objective(s) was not achieved, explain why that is the case and describe what you did manage to achieve.

1. Determining mRNA expression was found to be a robust method for answering the relevant questions. We revealed that oestradiol increased adrenomedullin mRNA expression at 12 hours ($127 \pm 11\%$, $p < 0.05$; $n=7$). Arterial cells treated with oestradiol had higher expression levels than cells treated with testosterone ($p < 0.001$).

2. Incubation of arterial cells for 3 hours with testosterone elicited an increase in adrenomedullin-2 expression (to $235\% \pm 38\%$, $p < 0.01$, $n=9$) but not at 12 hours ($85 \pm 11\%$, $n=9$) relative to control incubations. No significant change in adrenomedullin-2 mRNA was observed for oestradiol ($135\% \pm 36\%$ and $85 \pm 12\%$ ($n=8$)) or dexamethasone at 3 hours or 12 hours respectively. Angiotensin-II did not alter adrenomedullin-2 mRNA expression at 3 hours but decreased IMD mRNA expression at 12 hours ($73 \pm 6\%$, $p < 0.001$).

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3. The number of cells secreting endothelin-1 increased in a concentration-dependant manner when exposed to testosterone ($p < 0.05$). A significant increase was observed with 3500 nM ($p=0.05$, $n=4$) testosterone relative to control cells incubated without testosterone. This dose response was linear. ($R^2=0.94$). Oestradiol did not significantly alter the number of cells secreting endothelin-1 but reduced the number of cells secreting endothelin-1 stimulated with angiotensin-II. ($P < 0.01$).

After 3 hours treatment testosterone (350 nM) increased the level of endothelin-1 mRNA $120 \pm 7\%$ ($P < 0.05$, $n=9$). Neither angiotensin II ($1 \mu\text{M}$) $113 \pm 8\%$ ($n=8$), oestradiol (37nM) $101 \pm 4\%$ ($n=8$), nor dexamethasone $106 \pm 8\%$ ($n=3$) significantly altered endothelin-1 mRNA levels at 3 hours.

After 12 hours treatment testosterone and angiotensin II decreased endothelin-1 mRNA levels by $72 \pm 9\%$ ($P < 0.05$, $n=7$) and $66 \pm 10\%$ ($P < 0.05$, $n=7$) respectively. Neither dexamethasone $94 \pm 8\%$ ($n=4$) nor oestradiol significantly altered endothelin-1 mRNA levels after 12 hours incubation ($100 \pm 9\%$, $n=7$).

4. Aortic cells of the luminal surface of the aorta of rats, which contains endothelial cells, were examined. The effects of exogenous steroids would not provide useful additional information in the context of other observations. The endothelial cell layer had detectable levels of adrenomedullin mRNA but not adrenomedullin-2 mRNA. Cells from deeper within the aortic wall also exhibited measurable adrenomedullin mRNA but not adrenomedullin-2 mRNA. These differential expressions of adrenomedullin and adrenomedullin-2 fits with our hypothesis these two peptides have distinct roles in vascular modulation.

These results are included in a series of conference presentations and international publications (Pearson *et al.* 2006; Pearson *et al.* 2008; Pearson *et al.* 2009).

Pearson LJ, Rait C, Nicholls MG, Yandle TG & Evans JJ 2006 Regulation of adrenomedullin release from human endothelial cells by sex steroids and angiotensin-II. *Journal of Endocrinology* **191** 179-188.

Pearson LJ, Yandle TG, Nicholls MG & Evans JJ 2008 Regulation of endothelin-1 release from human endothelial cells by sex steroids and angiotensin-II. *Peptides* **29** 1057-1061.

Pearson LJ, Yandle TG, Nicholls MG & Evans JJ 2009 Intermedin (Adrenomedullin-2): a Potential Protective Role in Human Aortic Endothelial Cells. *Cellular and Physiological Biochemistry* **23** 97-108.

Please confirm delivery of the outputs listed on your application form. If these outputs were not to be delivered, please explain why.

Outputs of several conference presentations and international peer-reviewed manuscripts were achieved.