Abstract #486 | Oral

# Unravelling the intracellular signaling pathways in CGRP-induced vasorelaxation in rat coronary arteries

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**Introduction** Calcitonin gene-related peptide (CGRP) may play an important role in modulating coronary microvasculature resistance and is known to be cardioprotective. Widely believed to act via the Gαs-coupled cAMP/PKA signalling pathway, there is evidence that endothelial cells (ECs) and Gβγ can play a role in CGRP-induced vasorelaxation [1]. Despite its clinical importance, the precise underlying pathway is yet to be elucidated in the coronary microvasculature.

**Methods** Coronary septal arteries were isolated from male Wistar rats and mounted in a wire myograph. Cumulative CGRP (1 pM – 100 nM) or isoprenaline (0.1 nM – 10 μM) concentration-response curves (CRCs) were conducted from developed myogenic tone (>0.5 mN/mm). ECs were removed with a hair and confirmed by <10% relaxation to 1 μM acetylcholine. For EC $Ca^{2+}$ imaging, septal arteries were cannulated onto two glass micropipettes, pressurised to 80 mmHg at 37 °C, and left to develop myogenic tone. ECs were selectively loaded with the $Ca^{2+}$ indicator Oregon Green BAPTA-1 (10 μM) and $Ca^{2+}$ activity was recorded using confocal microscopy. Data were analysed using parametric; two-way ANOVA with Sidak's multiple comparisons, or non-parametric tests; Mann-Whitney with multiple comparisons followed with Bonferroni-Dunn correction. $p < 0.05$ was considered statistically significant.

**Results** The $EC_{50}$ of CGRP in rat septal arteries was 50 pM (n=6). The nitric oxide synthase inhibitor, L-NAME (100 μM), caused a ~30-fold right-shift of the CGRP CRC ($EC_{50}$ 1.4 nM), which was significantly different to the control responses at 0.1 and 1 nM CGRP (n=6). Denuding also resulted in a ~30-fold right-shift of the CGRP CRC ($EC_{50}$ 1.6 nM), with significant difference to control at 10 pM – 1 nM CGRP (n=14). The Gβγ-subunit inhibitor, gallein (100 μM), significantly attenuated vasorelaxation to CGRP ($EC_{50}$ 50 nM, n=5). The $EC_{50}$ of isoprenaline was 25 nM, but there was no significant difference to control in the presence of L-NAME or gallein (n=5). No global increase in EC intracellular $Ca^{2+}$ was observed following application of CGRP (10 nM) for 2 mins, but it was consistently observed in response to acetylcholine (100 nM).

**Conclusion** These data indicate that the most potent CGRP-induced vasorelaxation in rat septal arteries is EC-dependent. This finding is clinically relevant because EC dysfunction in the coronary microvasculature underlies several pathological conditions, such as coronary microvascular dysfunction (CMVD), and would result in the reduced bioavailability of nitric oxide. Interestingly, it appears that CGRP-induced nitric oxide release relies on a $Ca^{2+}$-independent pathway. Furthermore, these data suggest that both the EC-dependent and -independent components to CGRP vasorelaxation occur via a Gβγ-mediated signalling pathway in rat coronary arteries. This is a novel and therapeutically important finding.

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**References**

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