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Effects of connexin mimetic peptide Gap27 on mouse pulmonary artery relaxation responses to methacholine and isoprenaline

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Introduction: Abnormal cellular communication within the pulmonary artery is thought to contribute to the constriction and remodelling associated with pulmonary arterial hypertension (PAH). Connexins are transmembrane proteins which can form membrane channels known as hemichannels. Hemichannels can align to form gap junctions and allow the exchange of small signalling molecules between adjacent cells. Recent evidence suggests dysregulation of connexin signalling plays a role in the development of PAH (1). The aim of this study was to assess the role of connexins in mouse intra-lobar pulmonary artery (IPA) relaxation using Gap27, a selective connexin mimetic peptide blocker of connexin 37 and 43. Relaxations in response to classical nitric oxide dependent (methacholine) and cAMP dependent (isoprenaline) pathways were assessed.

Methods: Male mice (4-6 months old) were euthanized by administration of pentobarbitone (60 mg/kg, i.p.). IPAs (~250µm i.d) were dissected free and mounted (Krebs-Heseleit solution, 37 °C) on wire myographs and pre-constricted with phenylephrine (3µM). Cumulative concentration response curves to vasorelaxant drugs methacholine (0.1nM-300µM) and isoprenaline (1nM-300µM) were constructed in the presence/absence of Gap27 (100µM). In order to assess the contribution of nitric oxide to IPA relaxations L-NAME (100µM) was used. Statistical analysis was performed by two-way ANOVA with Bonferroni's multiple comparison test or Student's t-test as appropriate. Data are presented as mean ± s.e.m.

Results: Methacholine mediated a partial relaxation of IPAs (Rmax: 42.1±4.6%, n=6). This was significantly inhibited in the presence of Gap27 (Rmax: 22.9±5.6%, n=6, $P<0.05$). Methacholine-induced relaxation was completely ablated in the presence of L-NAME (Rmax: 38.1±.6.6% c.f. 5.1±1.8%, n=4, $P<0.01$), verifying dependence of this pathway on nitric oxide. Isoprenaline induced a full relaxation of IPAs (Rmax: 92.0±1.2%, n=5) which was slightly but significantly inhibited by Gap27 (Rmax: 80.8±4.2%, n=5, $P<0.05$).

Conclusion: Our results suggest a role for gap junctions in both methacholine (nitric oxide dependent) and isoprenaline (cAMP dependent) vasorelaxation.

References:

1. Dempsie *et al.,* (2015) *Biochem Soc Trans* **43:**524-9