Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol14Issue2abst014P.pdf

Downregulation of connexin 43 (Cx43) is associated with pulmonary vascular dysfunction in mice

Pulmonary arterial hypertension (PAH) is a chronic disease which is associated with vasoconstriction and remodelling of pulmonary vasculature. Cellular communication is pivotal in the vasoconstriction and vascular remodelling processes. Connexins are gap junction forming transmembrane proteins and gap junctions permit the exchange or transfer of molecules less than 1kD between neighbouring cells. Recent evidence highlighted that dysregulation in connexin-mediated signalling has a role in the development of PAH (1-2). Therefore, we investigated pulmonary vascular reactivity in isolated intra-lobar pulmonary arteries (IPAs) of connexin 43 heterozygous (Cx43+/-) mice. In addition we also investigated the effects of ^{37,43}Gap 27 (pharmacological blocker of connexins 37 and 43) on pulmonary vascular reactivity. Both male and female mice were used in the experiments as there is a female gender bias in PAH (3).

Male and female Cx43+/- (4) and wild type (WT) littermates (20 to 28 weeks old) were euthanized by intraperitoneal injection of phenobarbitone (60mg/kg). IPAs (~350µm in diameter.) were dissected out and mounted (Krebs-Henseleit solution, 37 ˚C) on wire myographs (Danish Myo Technology). Cumulative contraction concentration-response curves (CRCs) to 5-hydroxytryptamine (5-HT; 1nM-0.3mM) and endothelin-1 (ET-1; 0.1nM-0.1µM) were constructed. For relaxation experiments, methacholine (MCh; 0.1nM-0.3µM) was used after pre-constriction with phenylephrine (PE; 3µM). For gap junction blockade during the relaxation studies, ^{37,43}Gap 27 (100µM) was incubated for 30min after pre-constriction with phenylephrine (PE; 3µM) and relaxation responses were induced by MCh; 0.1nM-0.3µM). Quantitative real-time polymerase chain reaction (RT-PCR) method was used to study Cx43 gene expression. Statistical analyses were done using two-way ANOVA with Bonferroni's multiple comparison post-test or Student's t-test where appropriate. Data were presented as mean±s.e.m.

The potency of ET-1 was greater in the IPAs from male Cx43+/- mice when compared with male WT mice (pEC50: 8.77±0.12, n=5 c/f 8.46±0.08, n=6, $p<0.05$). There was no significant difference in the CRCs to 5-HT between the IPAs derived from male Cx43+/- and WT mice. Moreover, both female Cx43+/- and WT mice showed no significant differences in the CRCs to ET-1 or 5-HT in the IPAs. The maximal relaxation responses (R_{max}) produced by MCh in the IPAs from both male and female Cx43+/- mice were significantly reduced compared to WT mice (R_{max} male mice: 31.5±7.76%,n=5 c/f 56.89±7.01%,n=5, $p<0.05$; (R_{max} female mice: 26.8±7.77%, n=6, c/f 55.34%±8.48%, n=6 $p<0.05$). In the presence of ^{37,43}Gap 27 (100µM), the R_{max} in the IPAs from male and female WT mice were significantly reduced compared to their controls (R_{max} male mice: 22.91±5.56%, n=6 c/f 42.07±4.61%, n=6, $p<0.05$; R_{max} female mice: 42.22±5.24%, n=6, c/f 63.08±4.81%, n=6, $p<0.05$). Both male and female Cx43+/- mice showed significant downregulation of Cx43 gene expression in the main and branch pulmonary arteries (n=6 for each group, $p<0.05$).

In conclusion, reduced Cx43 expression and pharmacological blockade of Cx43 is associated with pulmonary vascular dysfunction.

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