U46619-induced Contractile Responses in Rat Pulmonary Arteries: Influence of the Endothelium

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Thromboxane A_2 (TXA_2) is a pulmonary vasoconstrictor that has been implicated in the development of pulmonary hypertension (Roy and Courier, 2006). The present studyinvestigated the involvement of the inositol 1,4,5-trisphosphate (IP_3) receptor chloride, voltage-operated calcium (VOCC) and store-operated calcium channels (SOCC) in the contractile response of rat pulmonary arteries to the TXA_2 mimetic, U46619. Concentration response curves (CRC) were constructed for U46619 in the presence or absence of the chloride channel blocker, niflumic acid (NFA), the VOCC blocker, nifedipine, the IP_3 receptor antagonist 2-APB and the SOCC blocker, SKF-96365. To investigate the influence of the endothelium on the contractile mechanisms, experiments were performed in endothelium-intact and denuded arteries.

Male Wistar rats (200-250 g) were killed by cervical dislocation. Ring segments 0.2-0.4cm in diameter were mounted on a small vessel wire myograph with a resting tension of 9.81mN in Krebs-Henseleit physiological salt solution at 37°C and gassed with 95/5% O_2/CO_2. Changes in isometric tension were recorded using Powerlab data collection and Chart 5 software. Tissues were allowed to equilibrate for 1 hour and then contracted with 60mM KCl then washed. Tissues were incubated with drugs for 45mins, and CRCs to U46619 were then constructed. The endothelium was denuded by gentle abrasion of the intimal surface. Results are expressed as a percentage of the KCl-induced contraction and are the means ± S.E.M. Statistical analysis was carried out using Student's t-test and $p<0.05$ is considered significant.

The U46619 (1nM-3μM) CRC was unaffected by nifedipine (1μM), 2-APB (30μM) or NFA (30μM) in endothelium-intact tissue, pEC_{50} and R_{max}, ENDO +, 7.78 ± 0.03, 128 ± 2%, n=5 ($p<0.05$). Removal increased the sensitivity to U46619 and this curve was shifted to the right and the maximum response reduced by 2-APB or nifedipine and markedly reduced by the combination of both 2-APB and nifedipine, pEC_{50} and R_{max}, ENDO -, 8.24 ± 0.05, 123 ± 3%, n=5; 2-APB, 7.9 ± 0.09, 103 ± 4%, n=5 ($p<0.05$); nifedipine, 7.2 ± 0.06, 103 ± 3%, n=5($p<0.05$); 2-APB + nifedipine, 6.8 ± 0.1, 56 ± 4%, n=5 ($p<0.05$). NFA shifted the CRC for U46619 in denuded vessels to the right and reduced the maximum response. Addition of nifedipine to NFA caused no greater inhibition, however addition of 2-APB did, pEC_{50} and R_{max}, ENDO -, 8.24 ± 0.05, 123 ± 3%, n=5; NFA, 7.2 ± 0.06, 103 ± 4%, n=5 ($p<0.05$); nifedipine + NFA 7.2 ± 0.05, 103 ± 3%, n=5 ($p<0.05$); 2-APB + NFA, 6.8 ± 0.08, 5 ± 2%, n=5 ($p<0.05$). The response in endothelium-denuded vessels was unaffected by SKF-96365 (10μM), however a rightward shift and a reduced maximum in was seen in the endothelium-intact vessels, pEC_{50} and R_{max}, ENDO +, 7.8 ± 0.05, 112 ± 2%, n=5; SKF-96365, 7.5 ± 0.08, 71 ± 3, n=5 ($p<0.05$).

This study suggests that in endothelium-denuded, but not endothelium-intact arteries, the U46619-induced response involves Ca^{2+} influx through a nifedipine-sensitive VOCC and a 2-APB-sensitive mechanism possibly IP_3-mediated Ca^{2+} release. An NFA-sensitive chloride conductance appears to be important in the activation of this VOCC. The sensitivity of the endothelium-intact response to SKF-96365 may indicate the involvement of Ca^{2+} entry through SOC channels.