INVOLVEMENT OF NIFEDIPINE-SENSITIVE CALCIUM CHANNEL IN THE U46619-INDUCED RESPONSE IN RAT PULMONARY ARTERIES: INFLUENCE OF THE ENDOTHELIUM

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Endothelium-derived hyperpolarising factors, by increasing the K^+ conductance in smooth muscle, may reduce tone through inactivation of voltage-operated calcium channels (VOCC). The present study investigated the involvement of nifedipine-sensitive VOCC in the contractile response of rat pulmonary arteries to the thromboxane A_2 mimetic U46619 in the presence or absence of the endothelium (ENDO) or in the presence of the K^+ channel blockers (Nelson & Quayle, 1995) tetraethyl ammonium (TEA, non-selective), 4-AP (K_V), charybdotoxin ($BK_{Ca)}$, iberiotoxin and apamin (BK_{Ca} and SK_{Ca}) and PNU37883 (K_{ATP}) (Cui *et al* 2003 *Br.J.Pharmacol* **139:** 122).

Male Wistar rats (200-250 g) were killed by cervical dislocation. Ring segments 0.2-0.4cm in diameter from the 2nd and 3rd pulmonary arterial generations were dissected out and mounted (9.81mN of tension) on a small vessel wire myograph (Danish Myotech, Denmark) for isometric recording in Krebs physiological saline solution gassed with 95/5% O_2/CO_2 at 37°C. Tissues were allowed to equilibrate for 1 hour before the addition of drugs. All tissues were first contracted with 60mM KCl. Channel blockers were pre-incubated for 45mins, and cumulative concentration response curves (CRC) to U46619 constructed. The endothelium was denuded by gentle abrasion of the intimal surface. Results are expressed as a percentage of the potassium chloride-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, pEC50 and Rmax, +endo, 7.54 ± 0.08, 99 ± 4%, n=4; +endo + nifedipine, 7.40 ± 0.09, 103 ± 4%, n=4. Removal of the endothelium caused a leftward shift which was reversed by nifedipine, pEC50 and Rmax, -endo, 8.37 ± 0.04, 117 ± 2%, n=4; -endo + nifedipine, 7.23 ± 0.06, 103 ± 3%, n=4. TEA (1mM), 4-AP (100nM), charybdotoxin (100nM), iberiotoxin and apamin (both 100nM) but not PNU 37883 (10 μM) caused a leftward shift of the U46619 concentration response curve, pEC50 and Rmax, TEA, 8.22 ± 0.05, 132 ± 3%, n=4; 4-AP, 8.33 ± 0.04, 113 ± 2%, n=4; Charybdotoxin, 8.32 ± 0.07, 147 ± 4%, n=4; iberiotoxin + apamin, 8.16 ± 0.08, 112 ± 4%, n=4. Nifedipine reversed the shift by 4-AP and partially reversed the shift by TEA but did not alter the increased sensitivity produced by charybdotoxin or iberiotoxin and apamin, pEC50 and Rmax, TEA + nifedipine, 7.8 ± 0.03, 110 ± 2%, n=4, P,0.0001; charybdotoxin + nifedipine, 7.36 ± 0.07, 99 ± 3%, n=4, P<0.0001.

The present study shows that removal of the endothelium from rat pulmonary arteries significantly increases the tissue sensitivity to U46619. Since the increased sensitivity was prevented by nifedipine this may indicate that an endothelium-derived hyperpolarising factor may prevent the activation of a nifedipine-sensitive calcium channel. That 4-AP produced a similar nifedipine-sensitive increase in sensitivity suggests that the endothelium normally inhibits a K_V channel in the smooth muscle.

Nelson, M.T. & Quayle, J.M. (1995). *Cell Physiol*. **37:** 799-822.