THE INVOLVEMENT OF VOLTAGE OPERATED CALCIUM CHANNELS (VOCC) IN THE CONTRACTIONS MEDIATED BY U46619 & 5-HT IN BOVINE PULMONARY ARTERIES.

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The contractile response to the thromboxane A_2 mimetic U46619 but not 5HT in bovine pulmonary arteries (BPA) is sensitive to the chloride channel blockers 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) and 9-anthracene carboxylic acid (9-AC) (Alapati *et al.,* 2004*).* Since an increase in the chloride conductance of the plasma membrane may produce an inward depolarizing current (Chipperfield *et al.,* 2000) this study investigated the involvement of voltage operated calcium channels (VOCC) in contractile responses to U46619 and 5-HT.

Bovine lungs were obtained fresh from the local abattoir. Ring segments (0.3-0.5cm in diameter, dissected from the 3rd and 4th arterial generations) were mounted in 10 ml organ baths suspended between stainless steel hooks in Krebs-Henseleit buffer (37°C) under a tension of 2 g and gassed with a mixture of O_2:CO_2 95%/5% v/v. Tissues were allowed to equilibrate for 1 hour before the addition of drugs. All tissues were first contracted with to 60 mM KCl. After washing, cumulative concentration response curves (CRCs) were constructed to U46619 or to 5HT in the absence or presence of one of the following VOCC blockers; nifedipine, verapamil, mibefradil (1-10μM) or ω conotoxin MVIIC (1μM), which were pre-incubated for 45 minutes before the addition of U46619 or 5HT. Results are expressed as a percentage of the potassium chloride- (60 mM) 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 concentration response curve to U46619 (100pM-10μM) was unaffected by any of the VOCC blockers.

The concentration response curve to 5-HT (1nM – 300μM) was shifted to the right and the maximum response (R_{max}.) reduced by verapamil and mibefradil (both 10μM) but not nifedipine (10μM) or ω conotoxin MVIIC. The inhibition by the combination of verapamil and mibefradil was additive, pEC_{50} values: control, 5.47 ± 0.07, n=9; verapamil 5.03 ± 0.05, n=6 P< 0.001; mibefradil, 5.1 ± 0.08, n=6, P< 0.001; verapamil + mibifradil, 4.8 ± 0.05, n=6, P< 0.001; R_{max}., control, 195 ± 7% n=9; verapamil, 140 ± 4.7% n=6 P< 0.001; mibefradil, 121 ± 6.5% n=6, P< 0.001; verapamil + mibifradil, 52 ±1.8% n=6, P< 0.001.

In BPA the U46619-induced contraction, which is sensitive to the chloride channel blockers, does not appear to involve any of the VOCC. The contraction to 5HT, which was insensitive to chloride channel blockers, appears to involve the activation of the low voltage activated L- (verapamil sensitive) and T- (mibefradil sensitive) type VOCC. This suggests that the inhibitory effect of the chloride channel blockers NPPB and 9-AC on U46619-induced contraction of the BPA is not consistent with blockade of an inward depolarizing current.

Chipperfield A.R. *et al.,* (2000) *Progress in Biophy. & Mol. Bio.* **74** 175–221.
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