THE ROLE OF CHLORIDE IN THE ACTIVATION OF STORE-OPERATED CALCIUM CHANNELS BY CPA AND U46619 IN BOVINE PULMONARY ARTERIES.

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Depleting the calcium content of the sarcoplasmic reticulum (SR), using SERCA inhibitors, such as cyclopiazonic acid (CPA) can, through an unknown signalling pathway, induce calcium influx and tone via activation of capacitative calcium entry or store-operated channels (SOC) (Parehk *et al.,* 1997). Chloride channels are present on the membrane of intracellular organelles, including the SR, and movement of chloride through the membrane of calcium storage organelles has been suggested to be important in the uptake and release of calcium (Pollock *et al.,* 1998; Kitamura & Yamazaki, 2001). This study investigated the effect of the SOC blockers 2-aminoethoxydiphenyl borate (2-APB) and SKF96365 (Putney, 2001) and the chloride channel blockers 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), and niflumic acid on the contractile response of bovine pulmonary arteries (BPA) to CPA and the thromboxane A_2 mimetic U46619.

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, the contractile responses to CPA (10 μM) or cumulative concentration response curves to U46619 were examined in the absence or presence of the SOC blockers 2-APB (100μM) or SKF96365 (100 μM) or the chloride channel blockers NPPB (50 μM) or niflumic acid (30 μM), which were pre-incubated for 45 minutes before the addition of U46619. 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.

CPA (10μM) induced a contraction (70 ± 4 % KCl), which was insensitive to niflumic acid but abolished by 2-APB, SKF 96365 and NPPB (n = 4-6). The concentration response curve to U46619 (100pM – 1 μM) was unaffected by niflumic acid but was shifted to the right and the maximum response (R_{max}.) reduced by 2-APB, SKF96365 and NPPB, pEC_{50} values; control, 7.63 ± 0.1, n = 6; 2-APB 7 ± 0.03, P<0.001, n=6; SKF96365 7.3 ± 0.1, P<0.001, n=6; NPPB, 6.5 0.03, P<0.001 n=6. R_{max}., % KCL response, control, 169 ± 11; 2APB, 106 ± 4.5 n=6; SKF96365, 116 ± 3.5 n=6, NPPB, 91 ± 2 , n=6.

This study suggests that in BPA the contractile response to the CPA is mediated by calcium influx through SOC and that SOC are also involved in mediating the contractile response to U46619. Since the concentration response curve to U46619 was sensitive to NPPB but not niflumic acid and since NPPB but not niflumic acid abolished the contraction to CPA, this may suggest that a NPPB-sensitive chloride conductance is associated with activation of the SOC in this tissue.

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