ROLE OF CHLORIDE, RHO-KINASE AND STORE-OPERATED CALCIUM CHANNELS IN U46619-INDUCED CONTRACTION OF RAT PULMONARY ARTERIES

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The present study examined the role of chloride conductance, store-operated calcium channels (SOCC) and Rho-kinase in the contraction of rat pulmonary arteries to the thromboxane A_2 mimetic U46619. The effect of the chloride channel blocker NPPB (Pollock *et al.*, 1998), SOCC blocker 2-APB (Putney, 2004) and the Rho-kinase inhibitor Y-27632 (Ishizaki *et al.*, 2000) was examined on the concentration response curve (CRC) to U46619.

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Male Wistar rats (200-250 g) were killed by cervical dislocation. Ring segments 0.20-0.4cm 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. After washing, cumulative concentration response curves to U46619 were examined in the absence or presence of the 2-APB (100µM), NPPB (50µM) or Y-27632 (30µM), which were pre-incubated for 45 minutes. 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.

NPPB, Y-27632 and 2-APB shifted the CRC for U46619 (1nM–3µM)-induced contraction to the right and reduced the maximum response. The response in the presence of the Rho-kinase inhibitor Y-27632 was further reduced by SOCC blockade with 2-APB and was completely inhibited by the addition of the chloride channel blocker NPPB.

Table 1 : The effect of inhibitors on U46619 pEC_{50} and R_{max}

	pEC_{50}	R_{max} (%)	n
Control	7.79 ± 0.05	130 ± 3	4
NPPB	6.52 ± 0.14*	66 ± 2*	4
2-APB	7.41 ± 0.05*	81 ± 2*	4
Y-27632	6.60 ± 0.10*	69 ± 5*	4
2-APB + Y-27632	6.47 ± 0.27*	37 ± 8*	4

*, $P < 0.0001$ compared with control.

In conclusion, the present study suggests that the contractile response of rat pulmonary arteries to U46619 involves activation of Rho-kinase together with Ca^{2+} entry via store-operated channels. An NPPB-sensitive chloride conductance is also important in the contractile response.

Pollock, N.S. *et al* (1998). *Biophy. J.* **75 (4):** 1759-1766.
Putney, J.W. (2001). *Mol. Interventions*. **1:** 84-94.
Ishizaki, T. *et al* (2000). *Mol. Pharmacol*. **57:** 976-983.