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Inhibition of mitochondrial complex III attenuates thromboxane-mediated contractions of porcine coronary artery through activation of AMP kinase

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Introduction: Mitochondria may regulate blood vessel tone (1) and we have previously demonstrated that simvastatin-induced relaxation of porcine coronary artery involves inhibition of mitochondrial function, possibly at complex III (2). In this study we determined the effect of the complex III inhibitor antimycin A on coronary artery contractions and the role of AMP kinase.

Methods: Coronary arteries from pigs were mounted in isolated tissue baths. Tissues were incubated with antimycin A (1, 3, and 10 µM) for 2 hrs in the presence or absence of calcium. In other experiments, tissues were incubated with a single concentration of antimycin A (10 µM) with and without AMPK inhibitor dorsomorphin (10 µM). After the incubation period, the thromboxane mimetic U46619 was added cumulatively (1nM-300nM). AMP kinase activity in arteries in the presence of antimycin A was determined by measuring changes in the phosphorylation of the kinase at Thr172 using Western immunoblotting.

Results: Removal of extracellular calcium inhibited the contraction to U46619 (Rmax in the presence of calcium 86.3±7.4% v 12.5±2.7% in the absence of calcium, n=6). Antimycin A at 10µM also inhibited the contraction to U46619 in the presence of calcium (Rmax 44.4 ± 11.7% with antimycin A, n=6) and in the absence of calcium (Rmax 10.5 ± 0.7% with antimycin A, n=7). The inhibitory effect of antimycin A on the U46619 contraction in the presence of calcium was prevented by dorsomorphin. In contrast, in the absence of calcium, dorsomorphin enhanced the inhibitory effect of antimycin A on the U46619-induced contraction, reducing the Rmax to 7.3 ± 0.5%. Western immunoblotting demonstrated an increase in phosphorylation of AMP kinase with 10µM antimycin A, indicating increased activity.

Conclusion: These data demonstrate that the complex III inhibitor antimycin A inhibits U46619-induced contractions in the porcine coronary artery and activates AMP kinase. Inhibition of AMP kinase prevents the inhibitory effect of antimycin A, at least in the presence of calcium, indicating that inhibition of complex III leads to activation of AMP kinase, which inhibits contraction. The fact that this was only seen in the presence of calcium suggests that AMP kinase may act to inhibit calcium channels. The further inhibitory effect of the AMP kinase inhibitior on the U46619-induced contraction in the absence of calcium cannot be explained at this moment in time and requires further experimentation.

References 1-Xi Q et al. (2005). Circ. Res., **97**: 354-362 2-Almukhtar H et al. (2016). Toxicol. Appl. Pharmacol., **305:** 176-185.