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Effects of mitochondrial complex inhibitors on porcine coronary tone: role of reactive oxygen species

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The electron transport chain is the primary site of reactive oxygen species (ROS) generation within the mitochondria (Turrens, 2003, Gutterman, 2005). Electrons travel down an electrochemical gradient from complex I to complex III and IV, and are finally used to reduce oxygen to water. Although the majority of molecular oxygen is reduced, 1–4% of the oxygen is incompletely reduced to O_2^-, which can result in ROS generation, primarily at complexes I and III. Inhibition of complex I or III blocks the transfer of electrons and, therefore, increases the release of ROS (Chen et al., 2003). The aim of this study was to determine the effects of different mitochondrial complex inhibitors on the isolated porcine coronary artery (PCA) tone and the potential role of ROS in such effects.

Coronary arteries from pigs of both sexes were obtained from a local abattoir and mounted in isolated tissue baths in oxygenated Kreb's- Henseleit buffer solution at 37^{o}C. Tissues were contracted with U46619, a thromboxane A_2 agonist, prior to addition of single concentrations of the mitochondrial complex I inhibitor rotenone (1 to 10µM), the complex III inhibitors antimycin A and myxothiazol (both 1 to 10µM), or the complex II inhibitor 3-NP. The effects of the mitochondrial uncoupler (FCCP, 1 µM) or the ATP synthase inhibitor oligomycin were also assessed. Changes in tone were then measured over 2 hours. The response to antimycin A was also measured in the absence or presence of rotenone (10µM) or FCCP (1µM). Changes in ROS generation in segments of porcine coronary artery were measured in a fluorescence spectrophotometer using the fluorescent dye dichlorofluoroscein. Statistical analysis was carried out using two-way ANOVA followed by a Bonferroni post-hoc test.

Antimycin A elicited a slow, concentration dependent relaxation in porcine coronary arteries compared to vehicle controls (DMSO (0.1% v/v)) with a maximum relaxation of 80.6 ± 30% (n=9) for 10µM antimycin A at 120 mins ($p<0.001$ v vehicle control). A similar relaxation was obtained with myxothiazol (40 ±10 %, $p<0.05$, n=9). Rotenone, 3-NP, FCCP, and oligomycin had no effect on porcine tone over the 2 hour time period. However, FCCP (1µM) caused a 55% inhibition of the maximum relaxation to 10 µM antimycin A ($p<0.001$, n= 6), and rotenone (10µM) delayed the onset of relaxation to antimycin A ($p<0.05$, n= 6). Antimycin A caused a 2-fold increase in fluorescence in intact coronary arteries loaded with dichlorofluoroscein, compared to vehicle control ($p<0.01$, n=6), which is indicative of ROS generation.

These data indicate that inhibiting mitochondrial complex III antimycin produces relaxation of the porcine coronary artery. This relaxation may be due to increased production of reactive oxygen species through disruption of the transfer of electrons through complex III.

Chen, et al. *J Biol Chem,* 278**,** 36027-31.

Gutterman,. 2005. *Circ Res,* 97**,** 302-4.

Turrens, . 2003. *J Physiol*, 552**,** 335-44.