P10. The prostanoid F receptor inotropic effect in rat left ventricle is mediated through enhancing myosin light chain phosphorylation

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Abstract – In rat ventricle, activation of the Gq/11-coupled Prostanoid F receptor (FPR) increases contractility and formation of inositol (1,4,5) trisphosphate (IP3) & diacylglycerol (DAG).

Objectives: 1) Determine if the FPR inotropic effect is mediated by increased phosphorylation of myosin light chain-2 (MLC-2) 2) Elucidate the signaling pathway(s) activated by FPRs to regulate the phosphorylation state of MLC-2.

Methods: Contractility was measured in left ventricular strips from adult male Wistar rats. Strips were also snap frozen and the phosphorylation level of both MLC-2 & myosin phosphatase targeting subunit-2 (MYPT-2) was quantified.

Results: Stimulation of the FPR with fluprostenol increased contractility by ~100% above basal and increased phosphorylation of both MLC-2 (by ~30%) & MYPT-2 (by ~50%). The myosin light chain kinase (MLCK) inhibitor ML-7 and an inhibitor of Ca++/calmodulin (W-7), the primary activator of MLCK, reduced both the FPR inotropic effect & MLC-2 phosphorylation (by ~70 & ~40%, respectively). Inhibition of Rho-associated kinase (ROCK) by Y-27632 reduced the FPR inotropic effect & MLC-2 phosphorylation by ~45% and MYPT-2 phosphorylation by ~70%. ML-7 and Y-27632 together reduced contractility by ~82%. The FPR inotropic effect was modestly reduced by IP3 receptor blockade (2-APB; by ~25%), but not by PKC inhibition.

Conclusions: The FPR inotropic effect is mediated by increasing phosphorylation of MLC-2 through regulation of both MLCK and myosin phosphatase activity. IP3-mediated Ca2+ release may be involved in the FPR inotropic effect but PKC is not. Therefore, FPR signaling mechanism(s) regulating MLC-2 phosphorylation likely extend beyond those currently established for Gq/11-coupled receptors.