MECHANISMS OF DELAYED PRECONDITIONING WITH A1 ADENOSINE RECEPTOR ACTIVATION IN PORCINE CORONARY SMOOTH MUSCLE CELLS

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This study examined the hypothesis that the activation of A1 adenosine receptor (A1AR) induces delayed cellular protection (DCP) in porcine coronary smooth muscle cells (PCSMC). The following groups of cultured PCSMC, subjected to simulated ischemia (SI) at 20 h were studied: (a) SI: with ischemia alone; (b) A1AR agonist chloro-N6-cyclopentyl adenosine (CCPA: CCPA (1 μM) alone; (c) CCPA + PKC inhibitor chelerythrine chloride (CCL): CCPA and 1 μM CCL; (d) CCPA + iNOS inhibitor S-methylthiourea (SMT): CCPA and 100 nM SMT; (e) CCPA + KATP channel blocker Glibenclamide (Glb): CCPA and 50 μM Glb; (f) CCPA + mitochondrial KATP channel blocker 5-hydroxydecanoate (5-HD): CCPA and 100 μM of 5-HD; (g) CCPA + A1AR antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX): CCPA and 1 μM DPCPX. The release of LDH into the medium as well as the amount of LDH remaining in the cells was used as a marker of cellular injury and cell viability. Up-regulation of A1AR, α-PKC, iNOS and HSP 72i was detected through Western blot analysis. The cellular resistance (%LDH remaining in the cells) acquired by PCSMC due to CCPA (59.42 ± 1.57) was significantly blocked by CCL: 39.30 ± 2.03; SMT: 41.37 ± 1.98; Glb: 47.24 ± 1.31; 5-HD: 47.69 ± 1.40 and DPCPX: 42.92 ± 0.79. CCPA increased the expression of A1AR (1.30 fold), α-PKC (1.20 fold), iNOS (1.50 fold), and HSP 72i (1.70 fold) compared to the controls. CCPA-induced up-regulation of A1AR, α-PKC, iNOS, HSP 72i, and the opening of both mitochondrial and sarcolemmal KATP channels may possibly participate in signaling cascade. Our study suggests that A1AR activation up-regulates iNOS, HSP 72i via α-PKC signaling pathway to activate both mitochondrial and sarcolemmal KATP channels for cellular protection against SI in the cultured PCSMC.

Key words: simulated ischemia, KATP channel, iNOS, HSP, PKC

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