Calcium signaling and the novel anti-proliferative effect of the UTP-sensitive $P2Y_{11}$ receptor in rat cardiac myofibroblasts

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ABSTRACT

During myocardial ischemia and reperfusion both purines and pyrimidines are released into the extracellular milieu, thus creating a signaling wave that propagates to neighboring cells via membrane-bound P2purinoceptors activation. Cardiac fibroblasts (CF) are important players in heart remodeling, electrophysiological changes and hemodynamic alterations following myocardial infarction. Here, we investigated the role UTP on calcium signaling and proliferation of CF cultured from ventricles of adult rats. Co-expression of discoidin domain receptor 2 and α-smooth muscle actin indicate that cultured CF are activated myofibroblasts. Intracellular calcium ($[Ca^{2+}]_i$) signals were monitored in cells loaded with Fluo-4 NW. CF proliferation was evaluated by the MTT assay. UTP and the selective $P2Y_4$ agonist, MRS4062, caused a fast desensitizing $[Ca^{2+}]_i$ rise originated from thapsigargin-sensitive internal stores, which partially declined to a plateau providing the existence of Ca^{2+} in the extracellular fluid. The biphasic $[Ca^{2+}]_i$ response to UTP was attenuated respectively by $P2Y_4$ blockers, like reactive blue-2 and suramin, and by the $P2Y_{11}$ antagonist, NF340. UTP and the $P2Y_2$ receptor agonist MRS2768 increased, whereas the selective $P2Y_{11}$ agonist NF546 decreased, CF growth; MRS4062 was ineffective. Blockage of the $P2Y_{11}$ receptor or its coupling to adenylate cyclase boosted UTP-induced CF proliferation. Confocal microscopy and Western blot analysis confirmed the presence of $P2Y_2$, $P2Y_4$ and $P2Y_{11}$ receptors. Data indicate that besides $P2Y_4$ and $P2Y_2$ receptors which are responsible for UTP-induced $[Ca^{2+}]_i$ transients and growth of CF, respectively, synchronous activation of the previously unrecognized $P2Y_{11}$ receptor may represent an important target for anti-fibrotic intervention in cardiac remodeling.

KEYWORDS: Cardiac fibroblasts; Myofibroblast; UTP; $P2Y_2$ receptor; $P2Y_4$ receptor; $P2Y_{11}$ receptor; Intracellular calcium; Fibroblast cell growth; ATP release; CX43-containing hemichannels; Adenylate cyclase; Cyclic AMP; EPAC