Bradykinin activation of fibroblasts of the rat subcutaneous tissue triggers the release of ATP and P2 purinoceptors activation

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BACKGROUND. Pain related to musculoskeletal system is increasingly common and purines, acting through distinct signaling pathways, are described to be involved in nociception (Schleip & Klingler, 2008). Considering that fibroblasts from subcutaneous connective tissue can release adenine or uridine nucleotides, triggering intracellular Ca^{2+} waves in response to stressful conditions, these cells may play a key role in the propagation/amplification of signals to primary afferent neurons (Furuya *et al.,* 2005). Thus, we decided to investigate the involvement of purinoceptors on bradykinin (BK)-induced activation of fibroblasts of the rat subcutaneous tissue.

METHODS. Experiments were performed in the first subculture of fibroblasts isolated from rat subcutaneous connective tissue. Intracellular ATP release was inferred from destaining of cells loaded with quinacrine (an ATP-binding intracellular fluorescent dye) by confocal microscopy (Orriss *et al.*, 2009). Intracellular Ca^{2+} oscillations were monitored using a microplate reader after loading the cells with the fluorescent Ca^{2+} dye, Fluo-4NW. The expression of P2 purinoceptors on cultured fibroblasts of the rat subcutaneous tissues was evaluated by immunofluorescence confocal microscopy.

RESULTS. Exposure to BK (30 μM) significantly enhanced fluorescence destaining of cultured fibroblasts loaded with quinacrine, suggesting that fibroblasts release intracellular ATP when stimulated with BK. In the presence of brefeldin A, which is a potent inhibitor of vesicular trafficking and secretion, BK (30 μM) was unable to increase intracellular Ca^{2+} accumulation. Moreover, we observed a decrease of the BK-induced Ca^{2+} response in the presence of the Cx36 and Cx50 inhibitor, mefloquine, and of the selective pannexin-1 inhibitor, ^{10}Panx. The intracellular Ca^{2+} accumulation induced by BK was also partially attenuated by apyrase, an enzyme that hydrolyses ATP and ADP directly to AMP. Interestingly, the ecto-NTPDase inhibitor, POM-1, which prevents sequential dephosphorylation of ATP to ADP leading to ATP accumulation and ADP depletion, also prevented BK-induced intracellular Ca^{2+} increase. These results point towards the participation of ADP generated from the extracellular catabolism of released ATP on BK-induced fibroblast Ca^{2+} rises. Fibroblast Ca^{2+} oscillations caused by BK were partially attenuated in the presence of the selective $P2Y_1$ receptor antagonist, MRS 2179, but not upon the selective blockade of $P2Y_{12}$ and $P2Y_{13}$ receptors, respectively with AR-C66096 and MRS 2211. The expression of $P2Y_1$ receptors on cultured fibroblasts from rat the subcutaneous tissue was confirmed by immunofluorescence confocal microscopy.

CONCLUSIONS. Data suggest that BK-evoked responses may be partially mediated by endogenous released adenine nucleotides via a mechanism involving hemichannels and vesicle exocytosis. Extracellular hydrolysis of released ATP into ADP may facilitate the inflammatory responses of BK through the activation of $P2Y_1$ receptors. Understanding how fibroblasts of the subcutaneous tissue trigger the release of signal amplification molecules, such as adenine nucleotides, which may ultimately contribute to activate neighboring cells, including sensory neurons, enlightens the hypothesis that fibroblast may be a novel target for therapeutic intervention in chronic painful conditions.

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