ATP release and P2 purinergic activation on human fibroblasts from subcutaneous tissue in response to inflammatory mediators

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BACKGROUND. Pain related to the musculoskeletal system is increasingly common and connective tissue seems to be involved on its pathogenesis [1]. Bradykinin (BK) and histamine (Hist) belong to the endogenous chemical substances mediating pain upon cell damage or inflammatory processes [2]. Cell signalling also occurs through nucleotides released via both lytic and non-lytic mechanisms from various cell types upon mechanical or chemical irritation. In this study, we tested whether inflammatory mediators, such as BK and Hist, influence the release of ATP and, thereby, P2 purincoceptors activation on fibroblasts of the human subcutaneous connective tissue.

METHODS. Fibroblasts were obtained from subcutaneous connective tissue of human organ donors (58±17 years old, n=7) with no clinical history of connective tissue diseases (approved by the Ethics Committee). The release of ATP was evaluated using the luciferin/luciferase bioluminescence assay [3]. In some of the experiments, ATP release was inferred from destaining of cells loaded with quinacrine (an ATP-binding intracellular fluorescent dye) by confocal microscopy [4]. Intracellular $[Ca^{2+}]_i$ oscillations were monitored using a microplate reader and by confocal microscopy, by loading the cells with the fluorescent Ca^{2+} dye, Fluo-4NW [5].

RESULTS. Incubation of cultured human fibroblasts with BK (30 μM) and Hist (100 μM) caused a sustained ride of extracellular ATP. Likewise, application of both agents decreased fluorescence of intracellular ATP containing granules loaded with quinacrine. These findings paralleled the accumulation of intracellular $[Ca^{2+}]_i$ measured in cells loaded with Fluo-4NW by confocal microscopy. Intracellular $[Ca^{2+}]_i$ accumulation and quinacrine destaining due to BK and Hist were partially prevented by mefloquine (MFQ, 3 μM), a blocker of connexin-containing hemichannels, and by brefeldin A (BFA, 20 μM), which blocks vesicular trafficking and secretion. Moreover, the non-selective P2 purinoceptor antagonist, PPADS (300 μM), also attenuated $[Ca^{2+}]_i$ oscillations produced by BK and Hist.

CONCLUSIONS. Data suggest that inflammatory mediators, like BK and Hist, trigger intracellular $[Ca^{2+}]_i$ accumulation and ATP release from cultured human fibroblasts. The mechanism(s) by which BK and Hist operate ATP release might involve mefloquine-sensitive connexin hemichannels and vesicle exocytosis. Results also indicate that ATP released endogenously may contribute amplify the inflammatory response on fibroblasts of the human subcutaneous connective tissue via P2 purinoceptors activation.

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