学 位 論 文 の 要 旨

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学 位 論 文 名 **Spontaneous Oscillation and Mechanically Induced Calcium Waves in Chondrocytes**

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論 文 内 容 の 要 旨

INTRODUCTION

Articular cartilage lacks nerves and blood vessels and the abundant cartilage extracellular matrix separates the chondrocytes from each other. Participation of gap junctions in the propagation of a Ca^{2+} wave between neighboring cells has been proposed, but because chondrocytes in articular cartilage are isolated and lack direct cell-to-cell contact, communication through gap junctions is probably not the major pathway in vivo. Instead of gap junctions, chondrocytes in articular cartilage possibly communicate using paracrine factors including adenosine 5'-triphosphate (ATP). The intracellular Ca^{2+}concentration could have been visualized and measured with the Ca^{2+}-sensitive dye, fura-2 AM recently. To investigate this further, we studied the characteristics of spontaneous Ca^{2+} oscillation in cartilage slices and the propagation of Ca^{2+} waves in primary cultured chondrocytes.

MATERIALS AND METHODS

Articular cartilage was obtained from 6-week-old Japanese white rabbits (1.1–1.3 kg). In the case of measurements using cartilage preparations, a thin cartilage layer was taken by slicing the articular surface. This was then placed in a medium for primary culture and incubated for 1–2 hours prior to use in experience. Articular chondrocytes were isolated and cultured. Chondrocytes were inoculated on circular glass cover slips placed in 35-mm tissue culture dishes. The cells were then incubated for 3 days at 37℃ in a humidified atmosphere of 5% CO_2/95% air. The intracellular Ca^{2+}concentration was measured with fura-2 AM. Sliced articular cartilage or primary cultured chondrocytes were incubated in the presence of 2–20 mM fura-2 AM. Fura-2 fluorescence ratio of single cells was obtained by microfluorometry, wherein fura-2 was excited

by dual wavelengths of 340 and 380 nm, after which the absolute values of the intracellular Ca^{2+} concentration were calculated. Concentrations of extracellular ATP were estimated using a luciferin–luciferase assay and a photon counter.

RESULTS

Individual cells in a cartilage slice were loaded with fura-2, to observe spontaneous oscillation of the intracellular Ca^{2+} concentration in articular cartilage chondrocytes. In cartilage, spontaneous Ca^{2+} oscillation in a cell did not propagate to adjacent cells. Mechanically-induced Ca^{2+} wave did not appear. In contrast, lightly touching chondrocytes in a nearly confluent culture with a fine glass rod stimulated intracellular Ca^{2+} change. A Ca^{2+} wave appeared from the center of the mechanically-stimulated site and spread to surrounding cells. Even when there was no direct cell-to-cell attachment, the Ca^{2+} wave originating from mechanically-stimulated cell was propagated to other cells over a distance. The application of a uridine 5'-triphosphate (UTP) induced a transient increase in intracellular Ca^{2+} and release of ATP in cultured chondrocytes. A P2 receptor antagonist (suramin) and blockers of Cl^- channels, niflumic acid and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), reduced the UTP-induced ATP release.

DISCUSSION

The phenomena such as spontaneous Ca^{2+} oscillations and propagation of Ca^{2+} waves in response to mechanical stimulation suggested that there was intercellular communication mediated by the Ca^{2+} signaling. Our results indicate that there are different mechanisms to work, and probably different roles, in the spontaneous Ca^{2+} oscillation and mechanically-induced Ca^{2+} wave. Extracellular triphosphonucleotides, such as ATP may be one of the paracrine factors because chondrocytes express P2 receptor and can release ATP to the extracellular space. However, direct intercellular communication through the gap junction is implausible in chondrocytes *in vivo*. In sparsely cultured chondrocytes, we observed propagation of Ca^{2+} wave initiated from a mechanically-stimulated single cell to distantly located cells. The Ca^{2+} wave probably expands through gap junctions in confluent culture or clusters of chondrocytes, but in sparsely cultured chondrocytes lacking direct contact between cells, it is possible that ATP works as a paracrine factor to mediate the Ca^{2+} wave. The sequential application of UTP and ATP induced transient Ca^{2+} increases in chondrocytes. This indicated that chondrocytes possess P2 receptor subtypes sensitive to both ATP and UTP, such as $P2Y_2$. The presence of P2 type receptors in chondrocytes and the suppression of UTP induced ATP release by the P2 receptor-blocker suramin suggested the involvement of P2Y receptors. Moreover, inhibitions of UTP induced ATP release with Cl^- channel-inhibitors, niflumic acid and DIDS, suggested involvement of Cl^- channels in release of ATP to the extracellular space. Because ATP-conductive anion channels are found in a variety of cell types, ATP might also be released

via the Cl^- channel in articular chondrocytes.

CONCLUSION

Our results suggested that mechanical stimulation and activation of P2 receptor subtypes of chondrocytes induced the release of ATP to the extracellular space by a process that involves Cl^- channels. Released ATP elicited an increase in the intracellular Ca^{2+} concentration in neighboring cells and triggered further release of ATP to propagate the Ca^{2+} wave. This process might occur in intercellular communication in articular cartilage *in vivo*. The abundant extracellular matrix might decrease the intercellular communication mediated by the Ca^{2+} signaling.