Abstract

Effects of phenytoin in human gingival fibroblasts

-Augmentation of PHT on the calcium responses

mediated by physiologically active substances-

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【Introduction】

Antiepileptic agent phenytoin (PHT) is known to inhibit voltage-gated Na^+ channel and reduces neuronal excitability by decreasing Na^+ influx. PHT is also known to induce gingival overgrowth, which is likely caused by the proliferation of human gingival fibroblasts (HGF) and the unbalance of collagen metabolism. It is suggested that the exacerbation of Drug-induced gingival enlargement (DIGE) is induced by environmental factors such as gingival inflammation. Bioactive substances produced by inflammatory responses regulate various cellular functions through increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). It is also known that PHT induces $[Ca^{2+}]_i$ elevation in HGF. These findings suggest that PHT causes gingival overgrowth by interacting with these bioactive substances. The purpose of this study was to clarify the mechanism of $[Ca^{2+}]_i$ elevation by PHT in HGF using live cell imaging with Ca^{2+} indicators fura-2 and to investigate the effect of PHT on the Ca^{2+} responses by inflammatory bioactive substances. The results of this study demonstrate that PHT enhances the weak Ca^{2+} response by ATP and Histamine, and this finding suggests the interaction of PHT and inflammatory bioactive substances may cause exacerbations of DIGE.

【Materials & Methods】

HGF（Cell Line Service）were cultured in Dulbecco's Modified Eagle Medium including 10% Fetal bovine serum. HGF were seeded on recording chambers and incubated with fura-2/AM (2 μM) in Hanks' balanced salt solution buffered with HEPES (HBSS-H) for 20 min at room temperature. Fura-2-loaded cells were washed with HBSS-H and allowed to rest for at least 30 min prior to the Ca^{2+} measurement. To monitor fura-2 fluorescence, cells were alternately excited at 345 and 380 nm, and emission signals were recorded at 500-530 nm using an inverted fluorescence microscope TE-2000 (Nikon) coupled with EM-CCD camera and controlled with AQUACOSMOS imaging software (Hamamatsu-photonics). These experiments were performed at room temperature.

【Results & Discussion】

The applications of 100 μM PHT elevated $[Ca^{2+}]_i$ in HGF. This PHT-induced $[Ca^{2+}]_i$ elevation was also observed in the absence of extracellular Ca^{2+}. PHT also enhanced Ca^{2+} elevation after the treatment with thapsigargin (ThG), an inhibitor of endoplasmic reticulum Ca^{2+}pump. Since ThG is known to deplete the intracellular Ca^{2+} stores, the involvement of Ca^{2+} release from intracellular stores in the PHT-induced Ca^{2+} elevations were excluded. Together with these results inhibitory effects of PHT on the excretion of cytosolic Ca^{2+} were suggested.

To investigate the effects of PHT on Na^+/Ca^{2+} exchanger (NCX), one of the intracellular Ca^{2+}

excretion mechanisms, PHT-induced Ca^{2+} elevation in HGF was examined in the presence and absence of extracellular Na^{+}. PHT induced $[Ca^{2+}]_i$ elevation was strongly attenuated in the absence of extracellular Na^{+}. In addition, removal of extracellular Na^{+} increased $[Ca^{2+}]_i$ by the Ca^{2+} entry through the reverse mode of NCX, and this $[Ca^{2+}]_i$ elevation was strongly attenuated by PHT. These results provide strong evidence for the inhibitory effect of PHT on NCX.

These results provide an assumption that the suppression of the excretion of intracellular Ca^{2+} by PHT may enhance Ca^{2+} response with various stimuli, and thus effects of PHT on the Ca^{2+} response by ATP and Histamine were examined. Ca^{2+} responses with 1 μM ATP and 10 μM were diminished at the second application of same concentrations of ATP by the receptor desensitization. However, the second application of ATP was potentiated in the presence of PHT. In particular, cells that were unresponsive to 1 μM ATP showed clear Ca^{2+} responses at the second application in the presence of PHT. Ca^{2+} responses with 3 μM Histamine were also potentiated by PHT. These results suggest that the administration of PHT enhances the Ca^{2+} responses by weak physiological stimulations.

【Conclusion】

The present study elucidated that PHT increases $[Ca^{2+}]_i$ due to the reduction of Ca^{2+} excretion by the suppression of NCX in HGF. Due to the inhibitory effect on the Ca^{2+} excretion, PHT enhanced the Ca^{2+} response of HGF by low concentrations of ATP and Histamine. These results raise the possibility that PHT enhances Ca^{2+} responses by mechanical stimulations and inflammation. These interactions between PHT and bioactive substances may cause the exacerbation of DIGE.