BRADYKININ B1 AND B2 RECEPTOR AGONISTS SYNERGISTICALLY POTENTIATE INTERLEUKIN-1-INDUCED PROSTAGLANDIN BIOSYNTHESIS IN HUMAN GINGIVAL FIBROBLASTS

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Abstract—The interactions between bradykinin (BK) and interleukin-1 (IL-1) on prostaglandin formation in human gingival fibroblasts have been studied. IL-1α and IL-1β stimulated prostaglandin E₂ (PGE₂) formation in the gingival fibroblasts with IL-1β being the most potent agonist. The effects of both IL-1α and IL-1β on PGE₂ biosynthesis was synergistically potentiated by BK, in a dose-related manner. The synergistic interaction between IL-1β and BK on PGE₂ production was seen both with B1 (des-Arg⁹-BK) and B2 (BK, Lys-BK) BK receptor agonists. No synergistic interaction between BK and IL-1β was seen on arachidonic acid release. These data suggest that BK and IL-1 act in concert to enhance prostanoid formation in inflammatory lesions and that the level of interaction is distal to phospholipase activity.

INTRODUCTION

The nonapeptide bradykinin (BK) is released in inflammatory processes from high-molecular-weight kininogen due to the action of plasma kallikrein (1). BK is best known for its effects on blood vessel dilatation, vessel permeability, and pain, reactions that play an important role in the cardinal symptoms of inflammation (2). Recently, evidence has accumulated that suggests that BK not only stimulates nerves and cells in the vessel wall, but that BK receptors also are present in other cells. Thus, we have reported that BK stimulates prostaglandin
E₂ (PGE₂) formation in human peripheral blood leukocytes (3), in human gingival fibroblasts (4), and in mouse as well as in human osteoblasts (5, 6).

Based upon these observations, and the finding that BK can stimulate bone resorption in vitro via a prostaglandin-dependent mechanism, we have suggested that BK may play an important role in the pathogenesis of bone resorption seen in chronic inflammatory processes (e.g., rheumatoid arthritis, periodontitis, osteomyelitis) (7).

Interleukin-1 (IL-1) is a pleiotropic cytokine produced in inflammatory lesions mainly by macrophages (8). This polypeptide also has been found to stimulate bone resorption (9) and PGE₂ formation in several cells, including fibroblasts (10). In the present investigation, we have studied the interaction between BK and IL-1 on prostanoid formation in human gingival fibroblasts.

MATERIALS AND METHODS

Cell Isolation. Fibroblasts were isolated from explants of human gingiva, obtained from patients 8–12 years of age with no periodontal disease, as previously described (11). The plan to take gingival biopsies was accepted by the Ethical Committee of Karoliska Institute. The cells were cultured in plastic flasks containing α-modified minimum essential medium (α-MEM) with 10% fetal calf serum (FCS). Cells obtained from two different patients (N-17, N-21) were used at passages 17–20 (N-21) and 19–21 (N-17).

Determination of Prostaglandin Biosynthesis. Cells were detached with trypsin–EDTA, seeded in 2-cm² multiwell plastic dishes, and grown to different degrees of subconfluent cell cultures. Then the cell layers were rinsed with Tyrode's solution and preincubated for 30 min in α-MEM without serum. The preincubation media were then discarded and fresh serum-free media without or with different test substances were added. After a 24-h incubation at 37°C in humidified atmosphere and CO₂-air (1:19), the media were withdrawn, acidified to pH 3.5, and stored at −20°C pending analysis. The amount of PGE₂ was determined by radioimmunoassay, using a commercially available kit with [125I]PGE₂ as tracer.

Determination of Arachidonic Acid Release. Cells (2 × 10⁴) were seeded in multiwell dishes (2 cm²) in the presence of 5% FCS and grown to approximately 80% subconfluency. Then the cells were rinsed with serum-free medium and incubated in medium containing [³H]arachidonic acid (1 μCi/ml). After 24 h, the medium was withdrawn and the cells washed three times with serum-free medium. The effect of IL-1β alone, or in combination with BK, on the release of arachidonic acid from cells prelabeled with [³H]arachidonic was then analyzed as previously described (4).

Chemicals. α-MEM and FCS were purchased from Flow Laboratories, Irvine, Scotland; BK, Lys-BK, and des-Arg²-BK from Sigma Chemical Co., St. Louis, Missouri; recombinant human IL-1α and β from Genzyme, Boston, Massachusetts; [³H]arachidonic acid and the radioimmunoassay kit for PGE₂ from Du Pont/New England Nuclear Chemicals, Dreieich, Germany.

Statistics. Statistical analysis were performed with Student's t test for unpaired samples.

RESULTS

IL-1β, at and above 1.5 pg/ml, dose-dependently stimulated PGE₂ formation in human gingival fibroblasts from two different patients (N-17, N-21;