Biochemical Pharmacology, Vol. 52, pp. 1015–1023, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00417-0

Histamine H_1 Receptor-Induced Ca^{2+} Mobilization and Prostaglandin E_2 Release in Human Gingival Fibroblasts

POSSIBLE ROLE OF RECEPTOR-OPERATED CA^{2+} INFLUX

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ABSTRACT. Stimulation of human gingival fibroblasts with histamine elicited an increase in the intracellular concentration of free calcium ($[Ca^{2+}]_i$) and the formation of inositol 1,4,5-trisphosphate ($InsP_3$) in a concentration- and time-dependent manner. The histamine-induced increase in $[Ca^{2+}]_i$ was attenuated completely by chlorpheniramine, an H_1 antagonist, but not by cimetidine, an H_2 antagonist. The histamine-induced Ca^{2+} response consisted of an initial transient peak response and a subsequent sustained increase. The transient phase can be largely attributed to Ca^{2+} release from intracellular $InsP_3$-sensitive stores since the increased $[Ca^{2+}]_i$ effect of histamine completely disappeared after depletion of intracellular Ca^{2+} stores with thapsigargin in the absence of extracellular Ca^{2+}. The sustained phase was due to Ca^{2+} influx which was attenuated in the absence of extracellular Ca^{2+}. The Ca^{2+} influx required the continuous binding of histamine to the receptor, since chlorpheniramine attenuated the increase in $[Ca^{2+}]_i$ observed when extracellular Ca^{2+} was re-applied to the cells after stimulation with histamine in the absence of extracellular Ca^{2+}. Pretreatment with the Ca^{2+} channel blocker SK&F96365 inhibited the Ca^{2+} influx component, suggesting that histamine stimulates Ca^{2+} influx through an H_1 receptor-operated Ca^{2+} channel. Histamine also evoked a concentration- and time-dependent release of prostaglandin E_2 (PGE_2). The histamine-evoked PGE_2 release was reduced markedly by exclusion of extracellular Ca^{2+} or pretreatment with SK&F96365 or an H_1 antagonist. These results indicate that histamine stimulates both the intracellular Ca^{2+} release from $InsP_3$-sensitive stores and the H_1 receptor-operated Ca^{2+} influx from extracellular sites. The increased $[Ca^{2+}]_i$ due to the Ca^{2+} influx causes PGE_2 release in human gingival fibroblasts. BIOCHEM PHARMACOL 52;7:1015–1023, 1996.

KEY WORDS. human gingival fibroblasts; intracellular free calcium concentration; histamine; H_1 receptor; Ca^{2+} influx; prostaglandin E_2

The inflammatory process involves complex regulation by various factors such as chemical mediators, cytokines, growth factors and hormones. Histamine, the most extensively studied chemical mediator of inflammation, is distributed widely in mammalian tissues. It is present in abundance in mast cells and basophilic leukocytes [1], where it is thought to be bound as a heparin–protein complex. Interaction of antigen and IgE at the cell surface induces the release of histamine from this complex [2]. Histamine appears to act as a growth factor and a chemoattractant in the inflammatory process and in allergic reactions [3, 4].

Periodontitis is an inflammatory disease in the oral region. This inflammatory reaction represents the response of the host plaque microorganisms and their products: the immunological response to the plaque microbiota results in tissue destruction and bone loss [5]. In this inflammation process, histamine appears to play an important role; endotoxins from various Gram-negative organisms induce the synthesis of histamine by mediating the activation of the enzyme histidine decarboxylase [6]. On the other hand, bacterial endotoxins and enzymes produced in dental plaque can cause mast cell degranulation, which results in the release of histamine [7]. Furthermore, endotoxin is capable of sensitizing the host of histamine [8]. When the number of mast cells were counted in the gingival tissue, normal gingiva contained more mast cells than moderately inflamed tissue, whereas severely inflamed tissue contained the least number [9, 10], implying that the distribution of mast cells in human gingiva appears to be correlated inversely with the degree of inflammation in the tissue. In addition, PMNL†

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† *Abbreviations:* PMNL, polymorphonuclear leukocytes; $[Ca^{2+}]_i$, intracellular concentration of free calcium; PGE_2, prostaglandin E_2; $InsP_3$, inositol 1,4,5-trisphosphate; PLC, phospholipase C; α-MEM, α-minimal essential medium; and KRH, Krebs–Ringer–Hepes.

Received 17 March 1995; accepted 19 April 1996.

in gingival crevicular fluid and gingival tissues may cause the degranulation of mast cells through the action of lysosomes from PMNL during the inflammatory response [7]. Therefore, it is most likely that mast cell degranulation causes the increase in histamine content in local regions.

Most chemical mediators induce an inflammation response in conjunction with bioactive substances such as prostanoids; PGE_2 is one candidate. If may play crucial roles in inflammation since extracellular stimuli invoke the synthesis of PGE_2 through cell surface signaling in fibroblasts [11], neutrophils [12], endothelial cells [13], and monocytes [14] at inflammatory sites. Prostaglandins can regulate bone formation positively and negatively [15], being potent stimulators of bone resorption in organ cultures [16] and inhibitors of resorption by isolated osteoclasts [17]. Furthermore, various factors such as interleukin-1 [18], bradykinin [19], and epidermal growth factor [20] regulate PGE_2 production and stimulate bone resorption through PGE_2 function(s). Therefore, PGE_2 is considered to be a crucial factor with an involvement in bone resorption in periodontal disease. The levels of PGE_2 in gingival crevicular fluid from patients with periodonititis are elevated significantly compared with patients with gingivitis [21], suggesting that PGE_2 is a crucial factor in periodontal destruction and bone resorption. While periodontal sites are selected on the basis of clinical and radiographic evidence of bone resorption, some sites display low PGE_2 levels while others have high PGE_2 levels, suggesting the presence of both inactive and active sites. There is, however, little information available on the cells in periodontal tissues that produce prostanoids and on the mechanism of their biosynthesis and release. In this study, we have focused on investigating the histamine-mediated signaling pathway and PGE_2 release in periodontal tissue cells to increase our understanding of PGE_2-related periodontal destruction during the inflammatory process.

Histamine-mediated responses are elicited via cell surface receptors categorized as H_1, H_2, and H_3 receptors. These three subtypes of histamine receptors differ in their sensitivity to antagonists and mediate different actions. H_1 receptor activation leads to a rapid breakdown of inositol phospholipids [22], whereas the H_2 receptor is coupled to the cyclic AMP-generating system [23]. The more recently introduced H_3 receptor, present in the nervous system and in mast cells, is an autoreceptor modulating the release of endogenous histamine [24]. Many tissues and cell lines have cell surface receptors containing histamine H_1 receptors that couple to PLC via a regulatory GTP-binding protein. Upon activation, PLC hydrolyses the plasma-membrane phospholipid, phosphatidylinositol 4,5-bisphosphate, generating two second messengers, $InsP_3$ and 1,2-diacylglycerol [25]. $InsP_3$ triggers the release of Ca^{2+} from intracellular stores, inducing an elevation in $[Ca^{2+}]_i$, whereas 1,2-diacylglycerol activates cytosolic protein kinase C [26–28].

In this study, we demonstrate that histamine H_1 receptor-operated Ca^{2+} influx induced PGE_2 release in human gingival fibroblasts.

MATERIALS AND METHODS

Materials

α-MEM, penicillin, streptomycin, and trypsin-EDTA were purchased from Gibco (Grand Island, NY, U.S.A.); fetal bovine serum was from Irvine Scientific (Santa Ana, CA, U.S.A.), and fura-2/AM from Dojindo Laboratories (Kumamoto, Japan); histamine, thapsigargin, and chlorpheniramine were obtained from Wako Pure Chemical Ltd. (Osaka, Japan); the PGE_2 [^{125}I] assay system and the D-*myo*-$InsP_3$ [^{3}H] assay system were obtained from Amersham Life Science (Arlington Heights, IL, U.S.A.). SK&F96365 was a gift from Smith, Kline and French (Hert, U.K.).

Cell Culture

Human gingival fibroblasts were obtained from explants of healthy human gingival connective tissue according to the method of Somerman *et al.* [29]. For cell culture, α-MEM supplemented with 10% fetal bovine serum and antibiotics (100 U/mL of penicillin and 100 μg/mL of streptomycin) was used. When the cells surrounding the tissue explants were confluent, they were subcultured with 0.05% trypsin and 0.53 mM EDTA in Hanks' balanced salt solution and then transferred to a tissue culture flask (75 cm^2) [30].

Measurement of Intracellular Free Calcium

Confluent cells were preincubated with 2 μM fura-2/AM in α-MEM for 30 min at 37°. We used trypsin–EDTA solution to detach the fura-2-loaded cells from the tissue culture flask, washed them twice, and resuspended them in KRH solution: 120 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 0.96 mM NaH_2PO_4, 0.2% glucose, 0.1% BSA, 1 mM $CaCl_2$ (Ca^{2+}-containing solution) or 1 mM EGTA (Ca^{2+}-free solution), and 20 mM HEPES (pH 7.4). The fluorescence of fura-2-loaded cells was measured with a CAF-110 spectrophotometer (Nihon Bunkou, Tokyo, Japan) with excitation at 340 and 380 nm and emission at 500 nm. The determination of $[Ca^{2+}]_i$ was by calculation from the ratio of fluorescence intensities [30–32].

Protein Binding Assay for $InsP_3$

For the measurement of $InsP_3$, the confluent cells were detached and suspended with KRH solution containing 1 mM Ca^{2+}. The cells were incubated in the buffer at 37° for 5 min before stimulation with histamine. Stimulation was stopped by adding 15% trichloroacetic acid at the indicated times and standing the samples on ice for 30 min. After neutralization, the samples were centrifuged for 15 min at 2000 *g*. Then the supernatant samples were put through $InsP_3$ assay using a commercially available $InsP_3$ protein-binding assay kit (Amersham). The amount of $InsP_3$ was determined from the calibration curve by using the binding protein specific for $InsP_3$ and [^{3}H]$InsP_3$ [30, 31].

Radioimmunoassay for PGE_2

The confluent cells were detached from the tissue culture flask as described above and suspended in KRH solution. To equilibrate the cell suspension, it was incubated for 5 min and then stimulated with histamine at 37°. After stimulation, the cell suspension was chilled rapidly and separated immediately by centrifugation at 4°. The amount of PGE_2 released in the supernatant was determined by a PGE_2 radioimmunoassay with a commercially available kit using ^{125}I-PGE_2 as a tracer (Amersham) [30, 31].

Statistical Analysis

The statistical significance of differences between the control and the experimental groups was determined by Student's *t*-test.

RESULTS

Histamine-Induced Ca^{2+} Mobilization

Figure 1 summarizes the effect of 100 μM histamine on $[Ca^{2+}]_i$ in fura-2-loaded human gingival fibroblasts in the presence and absence of extracellular Ca^{2+}. Histamine induced a rapid increase in $[Ca^{2+}]_i$ (transient phase) that soon declined to a level that was subsequently maintained (sustained phase). When the cells were stimulated with 100 μM histamine in the presence of extracellular Ca^{2+}, the basal $[Ca^{2+}]_i$ level (61.1 ± 4.2 nM, N = 9) rapidly increased to a transient peak (383.8 ± 8.7 nM) and then subsequently declined to a sustained level (91.7 ± 10.1 nM) higher than the basal level. In the Ca^{2+}-free solution containing 1 mM EGTA, the histamine-induced transient peak $[Ca^{2+}]_i$ was reduced to 222.3 ± 17.7 nM (N = 5) and followed by a return to the basal level (27.7 ± 2.3 nM). There was no sustained increase in the absence of extracellular Ca^{2+}. In both cases, we attribute most of the initial rise in $[Ca^{2+}]_i$ to Ca^{2+} released from intracellular stores, whereas the sustained increase of $[Ca^{2+}]_i$ (the plateau phase in Fig. 1) would be due to Ca^{2+} influx from extracellular Ca^{2+} sources. Histamine in concentrations ranging from 100 nM to 1 mM evoked increases in $[Ca^{2+}]_i$ in the presence and absence of extracellular Ca^{2+}. Figure 2 shows the initial peak of $[Ca^{2+}]_i$ induced by different concentrations of histamine. The greatest change in $[Ca^{2+}]_i$ was induced by stimulation with 100 μM histamine.

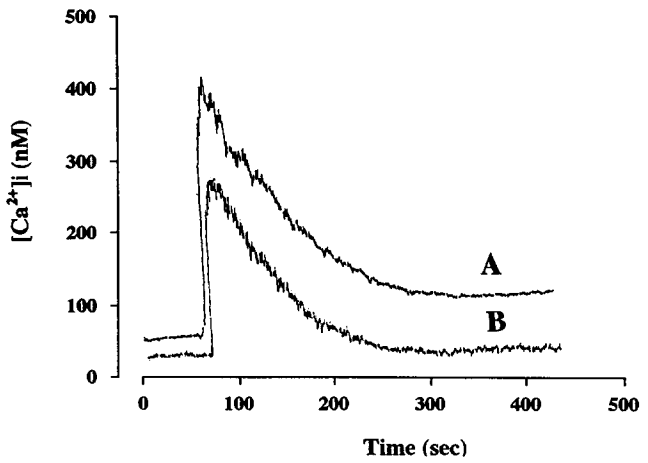

FIG. 1. Effect of histamine on $[Ca^{2+}]_i$ in human gingival fibroblasts. Fura-2-loaded cells were stimulated with 100 μM histamine in the presence (A) or absence (B) of external Ca^{2+}. Similar results were obtained in five other experiments.

Histamine-Stimulated Accumulation of $InsP_3$

To determine whether histamine activates $InsP_3$ formation that results in Ca^{2+} release from intracellular stores in human gingival fibroblasts, we studied the effect of histamine on $InsP_3$ formation. The addition of 100 μM histamine to cells incubated in KRH solution containing 1 mM Ca^{2+} evoked a rapid increase in $InsP_3$ to a peak of about three times the basal level, within 10 sec of stimulation. The level then declined rapidly to almost basal levels (Fig. 3). The concentrations of histamine applied, which ranged from 100 nM to 1 mM, induced an $InsP_3$ formation response that was concentration dependent; the maximal effective concentrations was 100 μM (Fig. 4).

Effect of Thapsigargin on Histamine-Induced Increase in $[Ca^{2+}]_i$

Thapsigargin, a phorbol ester, is an inhibitor of Ca^{2+}-ATPase, which can deplete $InsP_3$-sensitive Ca^{2+} stores [33,

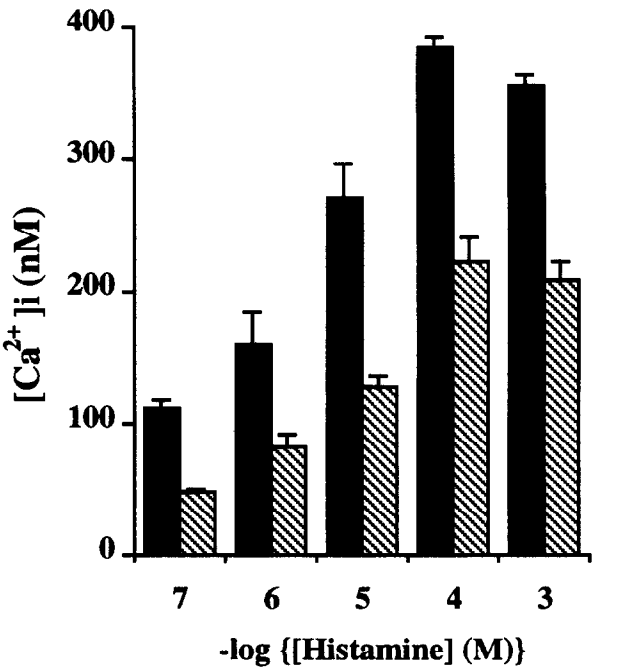

FIG. 2. Transient peak levels in $[Ca^{2+}]_i$ induced by different concentrations of histamine in human gingival fibroblasts. Fura-2-loaded cells were stimulated with the indicated concentrations of histamine in KRH solution containing 1 mM Ca^{2+} (closed columns) or 1 mM EGTA (hatched columns). $[Ca^{2+}]_i$ values are means ± SEM for four independent experiments.

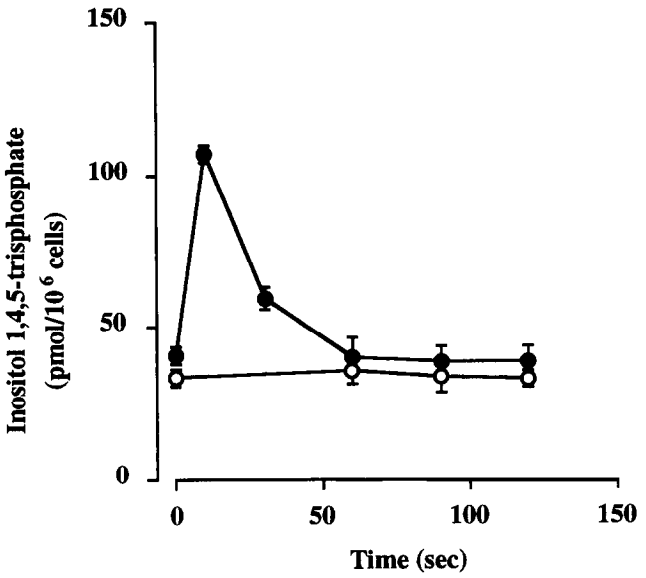

FIG. 3. Histamine-stimulated $InsP_3$ formation in human gingival fibroblasts stimulated with 100 μM histamine (closed circles) or a vehicle (open circles) for the periods shown. $InsP_3$ levels were measured by a protein-binding assay. Values are means ± SEM for three independent experiments.

34]. To demonstrate the relationship between histamine-induced increase in $[Ca^{2+}]_i$ and $InsP_3$ formation, we next studied the effect of thapsigargin on Ca^{2+} mobilization in human gingival fibroblasts.

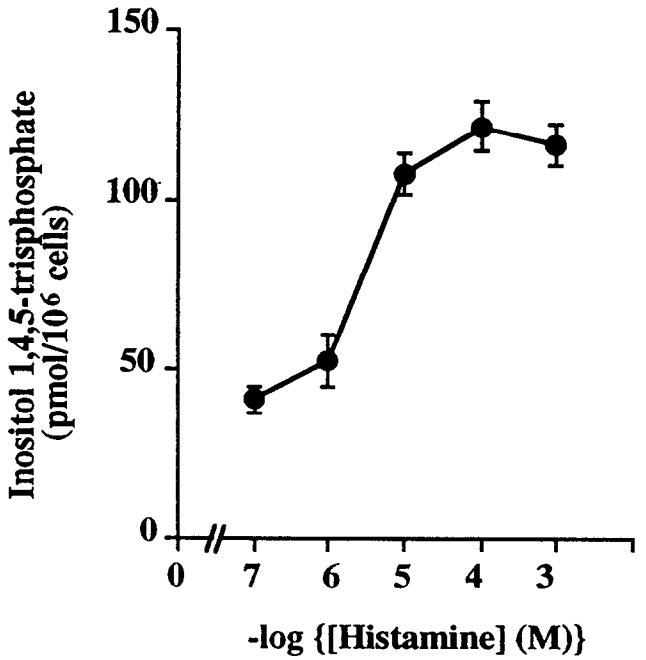

FIG. 4. Concentration-dependent effect of histamine on $InsP_3$ formation in human gingival fibroblasts. The cells were stimulated with histamine at the concentrations indicated for 10 sec in KRH solution containing 1 mM Ca^{2+}. $InsP_3$ levels were measured by a protein-binding assay. Values are means ± SEM for three independent experiments.

Treatment of fura-2-loaded cells in the presence of extracellular Ca^{2+} with 1 μM thapsigargin caused a moderate increase in $[Ca^{2+}]_i$, which declined slowly thereafter (data not shown). When 100 μM histamine was added to the cells during the sustained phase induced by 1 μM thapsigargin, the histamine caused a brief transient increase in $[Ca^{2+}]_i$, which then returned to the sustained level achieved by thapsigargin alone (Fig. 5A). In the absence of extracellular Ca^{2+}, thapsigargin also induced a moderate increase in $[Ca^{2+}]_i$ which slowly returned to the basal level, indicating the depletion of intracellular Ca^{2+} stores by thapsigargin. The addition of 100 μM histamine after the depletion of the intracellular stores failed to induce an increase in $[Ca^{2+}]_i$ (Fig. 5B).

Histamine-Stimulated Ca^{2+} Influx

As already described, the histamine-induced increase in $[Ca^{2+}]_i$ appeared to consist of intracellular Ca^{2+} release and Ca^{2+} influx from extracellular sources. We next investigated the effect of histamine on receptor-operated Ca^{2+} influx. In the absence of extracellular Ca^{2+}, the re-application of extracellular Ca^{2+} (3 mM $CaCl_2$) to the Ca^{2+}-free medium after the histamine-induced transient rise in $[Ca^{2+}]_i$ caused an increase in $[Ca^{2+}]_i$. This re-applied extracellular Ca^{2+} induced a moderate increase in $[Ca^{2+}]_i$ which slowly decreased but which maintained a level higher than the initial basal level (Fig. 6A). The increase in $[Ca^{2+}]_i$ induced by re-application of Ca^{2+} did not occur in the cells pretreated without histamine (Fig. 6B). In cells pretreated with 50 μM SK&F96365, a receptor-operated Ca^{2+} channel blocker [35], the histamine-induced Ca^{2+} influx was reduced, as shown in Fig. 6C. However, SK&F96365 by itself had no effect on $[Ca^{2+}]_i$.

Effect of Histamine Receptors Antagonists on Histamine-Induced Ca^{2+} Mobilization

A breakdown of inositol phospholipids and Ca^{2+} mobilization are generally induced by H_1 receptor activation [27]. We examined the effects of H_1 and H_2 antagonists on histamine-induced Ca^{2+} mobilization in the presence of extracellular Ca^{2+}. Pretreatment of cells with 10 μM chlorpheniramine (an H_1 antagonist) for 3 min completely blocked the histamine-induced increase in $[Ca^{2+}]_i$ (Fig. 7A), implying that the histamine-induced Ca^{2+} signaling is coupled to the H_1 receptor, whereas pretreatment with 100 μM cimetidine, an H_2 antagonist, had no effect on the histamine-induced increase in $[Ca^{2+}]_i$ (Fig. 7B). H_1 and H_2 antagonists had no effect on the basal $[Ca^{2+}]_i$ by themselves. Application of 10 μM chlorpheniramine to histamine-stimulated cells at the sustained phase in the presence of extracellular Ca^{2+} caused the $[Ca^{2+}]_i$ to return immediately to the basal level (Fig. 8A). The addition of 10 μM chlorpheniramine inhibited the rise in $[Ca^{2+}]_i$ observed when Ca^{2+} was re-applied to cells stimulated with histamine in the absence of extracellular Ca^{2+} (Fig. 8B). These

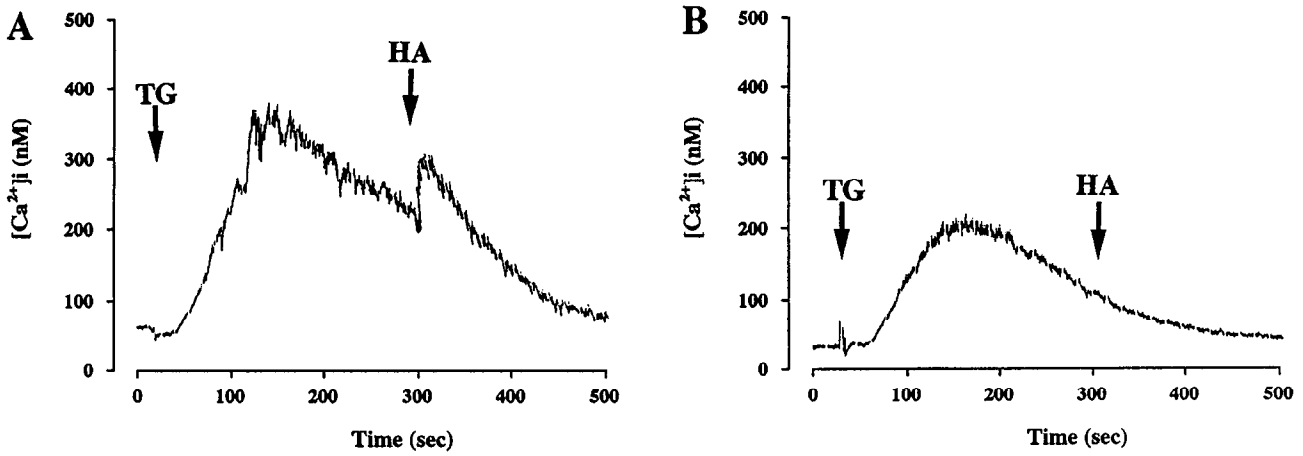

FIG. 5. Effect of histamine on $[Ca^{2+}]_i$ in human gingival fibroblasts pretreated with thapsigargin. Fura-2-loaded cells were stimulated with 100 μM histamine (HA) after pretreatment with 1 μM thapsigargin (TG) in the presence (A) and absence (B) of extracellular Ca^{2+}. These results are representative of three independent experiments.

results suggest that the activation of the H_1 receptor stimulates a receptor-operated Ca^{2+} influx from extracellular sites, possibly through a Ca^{2+} channel(s).

Histamine-Stimulated PGE_2 Release

Histamine is known to be a chemical mediator of inflammation; PGE_2 is considered to be an important clinical indicator in periodontal disease [21]. To understand the physiological role of histamine in human gingival fibroblasts, we examined the effect of histamine on PGE_2 release. As shown in Fig. 9, 100 μM histamine markedly stimulated the release of PGE_2, in proportion to time, for a minimum of 10 min. The basal and histamine-stimulated levels at 10 min were 343.3 ± 25.0 and 1630.7 ± 127.8 $pg/10^5$ cells, respectively. When human gingival fibroblasts were incubated with 100 nM to 1 mM histamine for 10 min in the presence of extracellular Ca^{2+}, PGE_2 release was stimulated in a concentration-dependent manner, as shown in Fig. 10. The optimal histamine concentration for maximal PGE_2 release was 100 μM.

Histamine-Induced PGE_2 Release Coupled to H_1 Receptor Activation and Ca^{2+} Influx

Figure 11 shows the effects of H_1 and H_2 antagonists on histamine-induced PGE_2 release. The pretreatment with 10 μM chlorpheniramine, an H_1 antagonist, reduced histamine-induced PGE_2 release by 78.7%, whereas 100 μM cimetidine, an H_2 antagonist, reduced the histamine-induced PGE_2 release only slightly. These antagonists had no effect on PGE_2 release by themselves. These results indicate that the histamine-induced PGE_2 release is coupled mainly to H_1 receptor-operated Ca^{2+} influx in human gingival fibroblasts.

Figure 11 summarizes the effect of Ca^{2+} influx on PGE_2 release in human gingival fibroblasts. When the extracellular Ca^{2+} was chelated with EGTA, the PGE_2 release induced by 100 μM histamine was reduced significantly (dotted column). The presence of 50 μM SK&F96365 reduced the histamine-induced PGE_2 release by 68.7%, even in the presence of extracellular Ca^{2+}. This receptor-operated Ca^{2+} channel blocker had no effect on the basal PGE_2 release by itself. Taken together, these observations suggest that histamine-induced PGE_2 release is coupled to H_1 receptor-operated Ca^{2+} influx in human gingival fibroblasts.

DISCUSSION

This study has demonstrated that histamine induced Ca^{2+} mobilization, $InsP_3$ formation, and PGE_2 release in human gingival fibroblasts. Histamine-induced Ca^{2+} mobilization consisted of transient and sustained increases in $[Ca^{2+}]_i$. In the absence of extracellular Ca^{2+}, histamine could induce only the transient increase in $[Ca^{2+}]_i$, but not the sustained increase. This suggests that the transient increase in $[Ca^{2+}]_i$ is due at least partly to Ca^{2+} release from the intracellular stores, whereas the sustained phase is maintained by an influx from extracellular sources. The peak of the transient increase in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+} was lower than that in the presence of extracellular Ca^{2+}, which suggests that both intracellular release and influx of Ca^{2+} contribute to it.

We have shown here that $InsP_3$ was increased within 10 sec after stimulation with histamine. The time courses of the two events (the $InsP_3$ and $[Ca^{2+}]_i$ increases) were almost parallel. On the other hand, histamine failed to induce the increase in $[Ca^{2+}]_i$ after depletion of releasable Ca^{2+} stores with thapsigargin. These results are consistent with the idea that the release of intracellular Ca^{2+} from $InsP_3$-sensitive stores elicits the increase in $[Ca^{2+}]_i$ in cells stimulated with histamine and that the Ca^{2+} release is triggered by $InsP_3$.

Histamine also induced a Ca^{2+} influx in human gingival

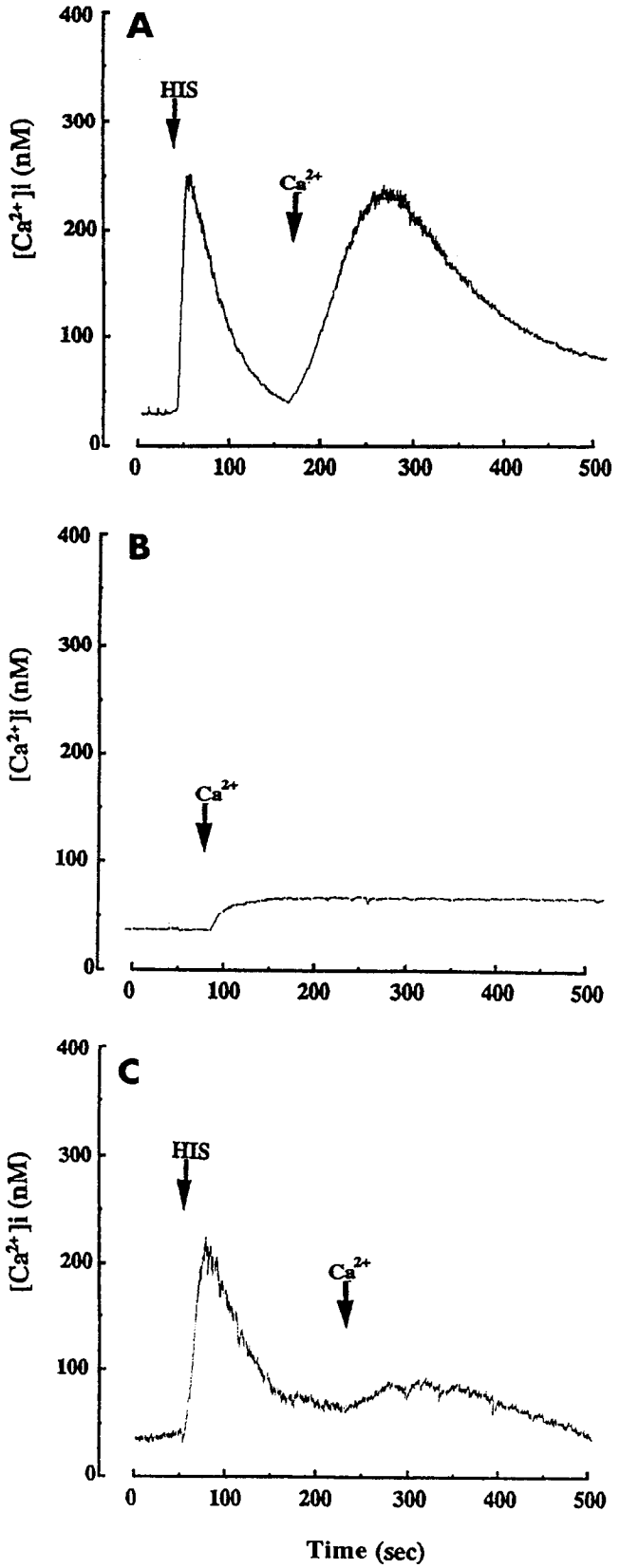

FIG. 6. Effect of SK&F96365 on the histamine receptor-operated Ca^{2+} influx in the absence of extracellular Ca^{2+} in human gingival fibroblasts. (A) Fura-2-loaded cells were stimulated initially with 100 μM histamine (HIS), after which 3 mM $CaCl_2$ (Ca^{2+}) was re-applied, as shown. (B) Without stimulation with 100 μM histamine, 3 mM $CaCl_2$ was added at the time indicated. (C) Same protocol as in panel A, but the cells were pretreated with 50 μM SK&F96365 for 10 min; histamine (100 μM) and $CaCl_2$ (3 mM) were added at the times indicated by the arrows. Similar results were obtained in two other experiments.

fibroblasts. In the absence of extracellular Ca^{2+}, re-application of Ca^{2+} elicited an increase in $[Ca^{2+}]_i$ only in the cells stimulated with histamine. On the other hand, the histamine-induced sustained increase in $[Ca^{2+}]_i$ was attenuated by exclusion of extracellular Ca^{2+}. These observations indicate that the increase in $[Ca^{2+}]_i$ is due to Ca^{2+} influx from extracellular sites through the Ca^{2+} channel in the plasma membrane. The histamine-induced Ca^{2+} influx was inhibited completely by pretreatment with an H_1 antagonist, chlorpheniramine. During the declining phase in histamine-induced Ca^{2+} influx, the addition of chlorpheniramine rapidly reduced $[Ca^{2+}]_i$ to the basal level (data not shown). Furthermore, the histamine-induced Ca^{2+} influx was blocked by pretreatment with SK&F96365, an inhibitor of receptor-operated Ca^{2+} influx [35]. These results suggest that the histamine-induced Ca^{2+} influx is dependent on the activation of histamine H_1 receptors and requires continued H_1 receptor occupancy by histamine. In many non-excitable cells, the mechanism of agonist-stimulated extracellular Ca^{2+} entry is not due to a voltage-dependent Ca^{2+} channel [36]. In human gingival fibroblasts, the Ca^{2+} influx induced by histamine is not caused by voltage-dependent Ca^{2+} channel activation, because high potassium had no effect on Ca^{2+} mobilization, indicating that voltage-dependent Ca^{2+} channels do not contribute significantly to the Ca^{2+} influx in the cells (data not shown). Current experimental data suggest a few possible mechanisms for receptor-operated Ca^{2+} entry, e.g. (1) receptor-operated Ca^{2+} channels, in which receptor activation and channel opening are intimately interconnected, perhaps via a G protein; (2) second messenger-operated Ca^{2+} channels, gated by $InsP_3$, inositol 1,3,4,5-tetrakisphosphate, cyclic GMP, or Ca^{2+} itself; and (3) capacitative Ca^{2+} entry, in which the activation of Ca^{2+} entry is linked to the emptying of the initial $InsP_3$-sensitive Ca^{2+}stores [37]. Further studies are needed to clarify the mechanisms of histamine-induced Ca^{2+} entry.

Stimulation of the histamine receptor triggered PGE_2 release from human gingival fibroblasts. However, histamine failed to induce PGE_2 release in the absence of extracellular Ca^{2+}. Even in the absence of extracellular Ca^{2+}, histamine stimulated $InsP_3$ formation (data not shown) and the subsequent increase in $[Ca^{2+}]_i$ (Fig. 1), suggesting that the $InsP_3$ formation and the increase in intracellular Ca^{2+} release are insufficient for the histamine-induced PGE_2 release. We have demonstrated previously that thapsigargin

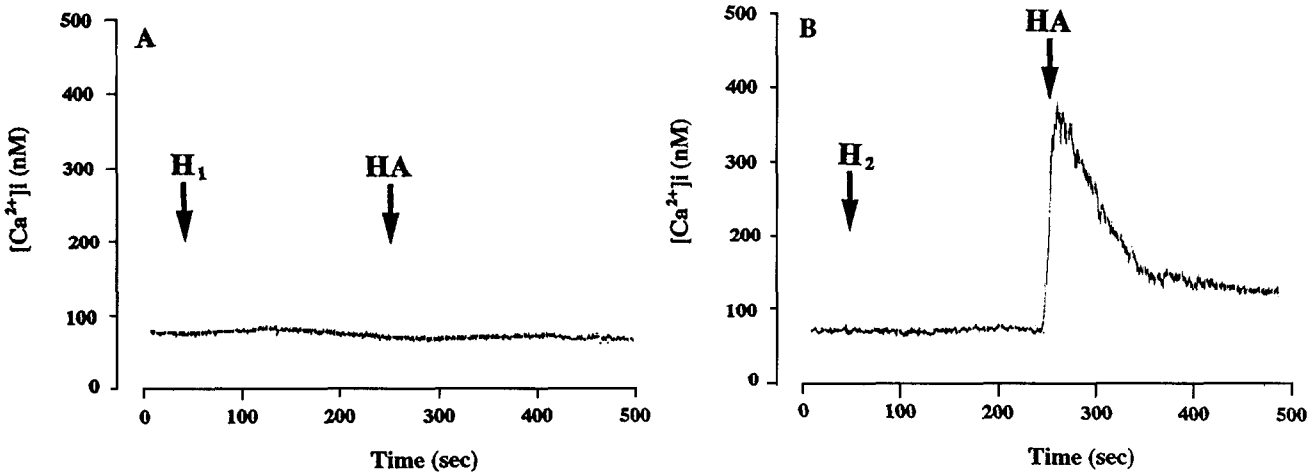

FIG. 7. Effects of H_1 and H_2 antagonists on histamine-induced increase in $[Ca^{2+}]_i$. (A) Fura-2-loaded cells were stimulated with 100 μM histamine (HA) after pretreatment with 10 μM chlorpheniramine (an H_1 receptor antagonist; H_1). (B) Fura-2-loaded cells were stimulated with 100 μM histamine (HA) after pretreatment with 100 μM cimetidine (an H_2 antagonist; H_2). Similar results were obtained in five other experiments.

does not induce PGE_2 release in the absence of extracellular Ca^{2+} and that A23187 (a Ca^{2+} ionophore) mimics the effect of histamine on PGE_2 release in the presence of extracellular Ca^{2+} in human gingival fibroblasts [30]. Furthermore, we have shown that SK&F96365, an inhibitor of Ca^{2+} entry, blocks the histamine-induced PGE_2 release. These findings imply that the histamine-induced PGE_2 release is dependent mostly on an influx of Ca^{2+}.

Chlorpheniramine (an H_1 antagonist) at 10 μM completely blocked Ca^{2+} mobilization and significantly reduced PGE_2 release in response to histamine. Cimetidine (an H_2 antagonist) slightly reduced PGE_2 release, but had no effect on histamine-induced increase in $[Ca^{2+}]_i$. Since H_2 receptors are coupled to the cyclic AMP-signaling pathway [23], these results suggest that the histamine-induced PGE_2 release is coupled not only to Ca^{2+} influx but also to another mechanism such as a cyclic AMP-dependent process.

PGE_2 may play an important role in the inflammation process by responding to the pain and the swelling in the inflamed tissues and in rheumatoid arthritis. The prosta-

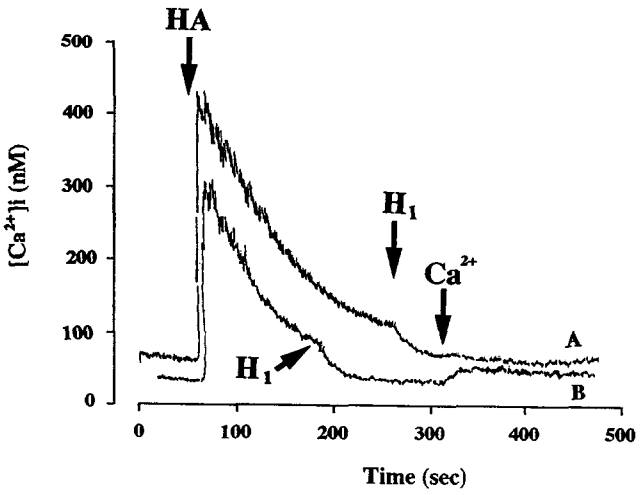

FIG. 8. Effect of an H_1 receptor antagonist on histamine-induced Ca^{2+} influx. (A) Fura-2-loaded cells were stimulated initially with 100 μM histamine (HA) in the presence of extracellular Ca^{2+}, after which 10 μM chlorpheniramine (H_1) was re-applied. (B) Fura-2-loaded cells were stimulated initially with 100 μM histamine in the absence of extracellular Ca^{2+}, after which 10 μM chlorpheniramine (H_1) and 3 mM $CaCl_2$ (Ca^{2+}) were applied at arrows, respectively. These results are representative of three independent experiments.

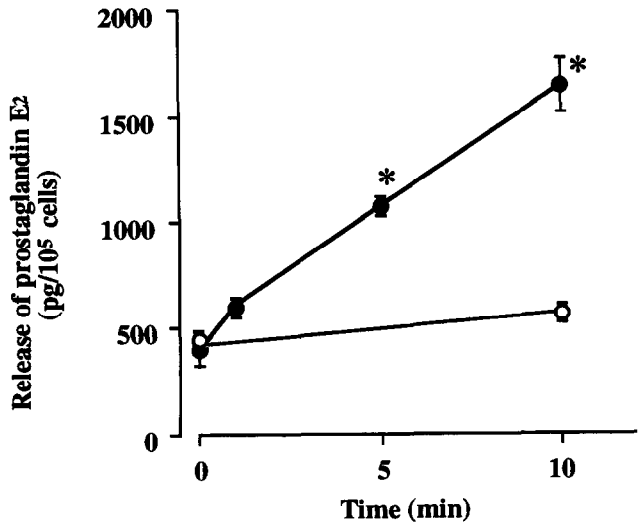

FIG. 9. Time-course of histamine-stimulated PGE_2 release. The cells were stimulated with 100 μM histamine (closed circles) or a vehicle (control; open circles) for indicated times in the presence of extracellular Ca^{2+}. The amount of PGE_2 released into the medium was determined by radioimmunoassay. Values are means ± SEM for three independent experiments. Asterisks indicate significant differences between control (open circles) and treated (closed circles) samples: *$P < 0.01$.

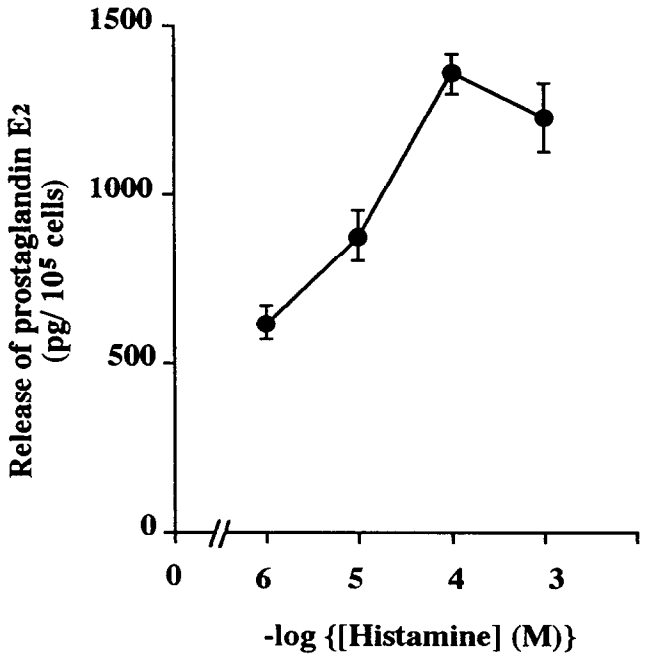

FIG. 10. Concentration-dependent effect of histamine on PGE_2 release in human gingival fibroblasts. The cells were stimulated with the concentrations of histamine indicated for 10 min in the presence of extracellular Ca^{2+}. The amount of PGE_2 released in the medium was detected by radioimmunoassay. Values are means ± SEM for three independent experiments.

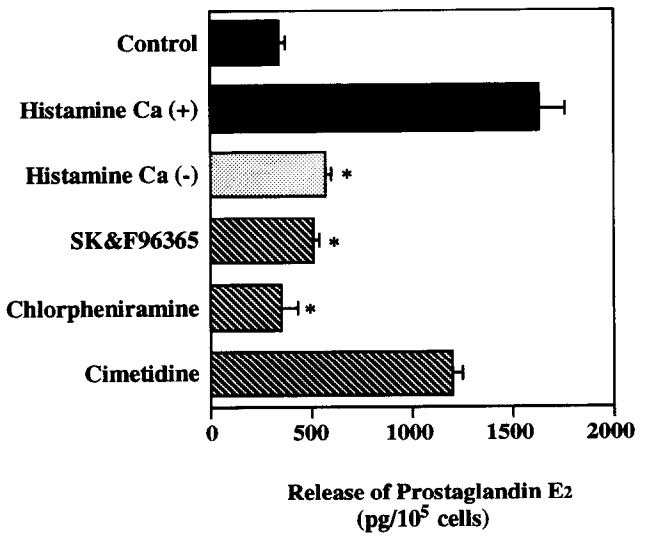

FIG. 11. Effect of extracellular Ca^{2+}, a receptor-operated Ca^{2+} channel blocker, and H_1 and H_2 antagonists on the histamine-induced PGE_2 release in human gingival fibroblasts. The cells were suspended in a Ca^{2+}-containing (closed and hatched columns) or a Ca^{2+}-free (dotted column) KRH solution, and preincubated with a vehicle (control), 10 μM chlorpheniramine, 100 μM cimetidine, or 50 μM SK&F96365 at 37°C for 10 min. After these pretreatments, the cells were stimulated with 100 μM histamine for 10 min except for the control. The amount of PGE_2 released was determined by radioimmunoassay. Values are means ± SD for three independent experiments. Asterisks mark significant differences from PGE_2 levels in cells stimulated by histamine in the presence of extracellular Ca^{2+}: *$P < 0.01$.

glandins, including PGE_2, are complex regulators of bone metabolism. The inflammatory mediator bradykinin induces bone resorption *in vitro* [19]. The action of bradykinin on bone resorption is due to the functions of PGE_2. Evidence for this view includes the findings that prostaglandins stimulate bone resorption *in vitro* and that bradykinin-induced bone resorption is blocked by agents that inhibit prostaglandin synthesis, i.e. indomethacin and hydrocortisone. Furthermore, bradykinin can induce the release of PGE_2 even in human gingival fibroblasts and periodontal ligament cells [30, 31]. Notably, the bradykinin-induced PGE_2 release in these cells is dependent on Ca^{2+} influx. In the present study, we demonstrated that histamine stimulates PGE_2 release, which is dependent on Ca^{2+} influx in human gingival fibroblasts. Taken together, our observations suggest that histamine may have a crucial role in regulating the inflammatory process of periodontal disease through PGE_2 release, which is dependent on H_1 receptor-operated Ca^{2+} influx in human gingival fibroblasts.

The authors thank Dr. Y. Marunaka for helpful advice and critical reading of the manuscript. This paper was prepared with the assistance of the Medical Publication Section of The Hospital of Sick Children. This work was supported by a Grant-in-Aid for Scientific Research (No. 04771546) from the Ministry of Education, Science and Culture of Japan (N. Niisato), a Nihon University Research Grant in 1994, and MPG Corporation research funds.

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