Histamine \( H_1 \) Receptor-Mediated \( \text{Ca}^{2+} \) Signaling in Cultured Bovine Corneal Endothelial Cells

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The corneal endothelium pumps ions and water from the stroma to the aqueous humor, maintaining corneal transparency. This report investigates the possibility that cultured corneal endothelial cells express neurohormonal \( \text{Ca}^{2+} \) signaling pathways employed by other epithelia to regulate transport or other cellular functions. Agonist-stimulated changes in intracellular calcium ([\( \text{Ca}^{2+} \)]) in single bovine corneal endothelial cells (BCEC) derived from confluent cultures were measured by microspectrofluorimetry using the \( \text{Ca}^{2+} \)-sensitive probe, fura 2. Mean resting [\( \text{Ca}^{2+} \)] in BCEC was 46 ± 2 nM (n = 124). The muscarinic cholinergic agonist, carbachol, did not mobilize \( \text{Ca}^{2+} \), whereas histamine induced a rapid increase in [\( \text{Ca}^{2+} \)] to initial peak levels of 549 ± 22 nM (n = 46) at maximally stimulating doses. The initial rise in [\( \text{Ca}^{2+} \)] in response to histamine was dose dependent, with a minimum effective dose of 50 nM, \( EC_{50} = 0.84 \mu\text{mol/L} \), and a maximum effective dose of 10 \mu\text{mol/L}. [\( \text{Ca}^{2+} \)], decreased from the initial peak, but then stabilized to form an agonist-dependent sustained elevation or abruptly fell back to baseline to begin oscillatory fluctuations. The initial peak was insensitive to removal of extracellular calcium (\( \text{Ca}^{2+} \)), whereas subsequent elevations in [\( \text{Ca}^{2+} \)] or sustained [\( \text{Ca}^{2+} \)] oscillations required \( \text{Ca}^{2+} \). The amplitude of the oscillations in [\( \text{Ca}^{2+} \)] increased with an increase in [histamine]. However, frequency was independent of [histamine] (mean = 0.62 spikes min\(^{-1}\) ± 0.06, n = 33). Histamine-induced \( \text{Ca}^{2+} \) mobilization was inhibited by the \( H_1 \) receptor antagonist triprolidine, but was unaffected by ranitidine (\( H_2 \) antagonist) or thioperamide (\( H_3 \) antagonist). These results indicate that histamine \( H_1 \) receptors are expressed in BCEC cultures and that histamine elicits a dose-dependent release of intracellular calcium and promotes extracellular \( \text{Ca}^{2+} \) entry in these cells. Invest Ophthalmol Vis Sci 33:3041-3049, 1992

Corneal transparency depends on a corneal endothelial pump mechanism that compensates for passive stromal hydration, subsequent thickening, and consequent opacification. This is accomplished by coupling water flow to the aqueous humor with transendothelial ion flow, resulting in maintenance of corneal deturgescence and transparency.\(^{1,2}\) Electrophysiologic and ion flux studies have established that the corneal endothelium transports \( \text{HCO}_3^- \), \( \text{Na}^+ \) and \( \text{H}_2\text{O} \) to the aqueous,\(^{1,3}\) and models for endothelial electrolyte and fluid transport have been proposed.\(^{4,5}\) An important aspect of corneal endothelial cell physiology that has been infrequently explored, however, is whether the endothelial cells have the capacity to express neurohormonal-second messenger signaling pathways that are employed by many other epithelia to regulate electrolyte transport or other cellular functions.

In recent years, cultured corneal endothelial cells have provided a valuable model system for studying endothelial function. A bovine corneal endothelial cell (BCEC) culture system, developed in our laboratory, has been used by us to explore the deposition and organization of the basement membrane,\(^{6}\) and has been used by others to examine mechanisms of ion and water transport as well as intracellular pH regulation.\(^{3,5,7,8}\) Additional laboratories have used other cultured BCEC preparations to study wound healing and growth factor expression,\(^{9}\) arachidonic acid metabolism,\(^{10}\) ion transport,\(^{11,12}\) and muscarinic receptor stimulation.\(^{13}\) As part of their study of arachidonic acid metabolism, Gerritsen et al\(^{10}\) showed that inflammatory mediators such as histamine and bradykinin, as well as the \( \text{Ca}^{2+} \) ionophore A23187, stimulated release of prostaglandin \( E_2 \) from cultured BCEC. These results suggest that elevations in intracellular \( \text{Ca}^{2+} \) ([\( \text{Ca}^{2+} \)]) may mediate histamine-induced prostaglandin synthesis and release.

To further characterize functional properties of the cultured endothelial experimental system and to ex-
explore pathways involved in neurohormonal signaling, we examined the capacity of BCEC to respond to several Ca²⁺-mobilizing neurohormones using microspectrofluorimetric analysis of single cells loaded with the Ca²⁺ probe, fura 2. Whereas stimulation with the muscarinic agonist, carbachol, did not elicit a Ca²⁺ response in these cells, exposure to histamine induced a dose-dependent mobilization of calcium from internal stores and enhanced entry of extracellular calcium. This agonist-induced Ca²⁺ response was selectively blocked by H₁ receptor antagonists. In addition, the Ca²⁺ probe, fura 2, whereas stimulation with the Ca²⁺-mobilizing neurohormones using microspectrofluorimetric analysis of single cells loaded with the Ca²⁺ probe, fura 2. Whereas stimulation with the muscarinic agonist, carbachol, did not elicit a Ca²⁺ response in these cells, exposure to histamine induced a dose-dependent mobilization of calcium from internal stores and enhanced entry of extracellular calcium. This agonist-induced Ca²⁺ response was selectively blocked by H₁ receptor antagonists. In addition, the Ca²⁺ probe, fura 2.

Materials and Methods

Materials

Culture medium—Dulbecco’s modified minimal essential medium (DMEM), with 4.5 g glucose/L—and salt solutions—Earle’s balanced salt solution (EBSS), buffered to pH 7.3 with 20 mmol/l HEPES—were obtained from Gibco (Grand Island, NY). Iron supplemented calf serum was purchased from HyClone (Logan, UT), and crystalline trypsin was from CalBiochem (San Diego, CA). Chemicals were purchased from the following sources: fura 2, pentapotassium salt, and acetoxyethyl ester (fura 2/AM), were from Molecular Probes (Eugene, OR); histamine dihydrochloride, triprolidine hydrochloride, ranitidine hydrochloride, and all other chemicals were from Sigma Chemical (St. Louis, MO). Thioperamide was a gift from Dr. John DelValle. Fura 2/AM was prepared as stock solution (1 mmol/l in dimethyl sulfoxide), fresh for each experiment.

Cell Culture

BCEC cultures were prepared as described by MacCallum et al.⁵ Briefly, adult bovine eyes were obtained from a local abattoir, and extracellular tissue was removed from the globes by dissection. The cornea was excised with an attached 1–2 mm-wide scleral ring and placed, endothelium side up, in a plastic cup with the same shape as the cornea. The corneal endothelial surface was rinsed and subsequently incubated at 37°C for 5–6 min in Ca²⁺-, Mg²⁺-free EBSS that contained 0.6 mmol/l EDTA, and 0.05% trypsin. Endothelial cells were dislodged from Descemet’s membrane by gentle scraping using a spatula with a tapered silicone surgical rubber tip curved to conform to the contour of the cornea. Dislodged cells were aspirated from the eyes with a pipette and added to 5 ml of DMEM containing 10% calf serum and 50 μg/ml gentamicin sulfate (subsequently referred to as “basal media”). The cells were gently centrifuged (600 × g, 2 min) and the pellet was resuspended in basal media. Cells from one eye (∼0.25 × 10⁶) were added in 5 ml of media to a 25 cm² culture flask, gassed with 95% air/5% CO₂, and incubated at 37°C. Cultures were fed 3 times/week and typically were confluent in 5–7 days. Confluent endothelial cultures (approximately 2.0 × 10⁶ cells/flask) were subcultivated using a 3–5 min incubation in Ca²⁺-, Mg²⁺-free EBSS containing 0.6 mmol/l EDTA, and 0.05% trypsin. After centrifugation, the cells were resuspended in an amount of basal media sufficient to initiate four or five subcultures. The data presented in this report were obtained from experiments using cells from the first four passages.

Dye Loading and Measurement of [Ca²⁺]i

Suspensions of cultured cells were harvested by centrifugation and resuspended (approximately 0.5 × 10⁶ cells ml⁻¹) in air-saturated Kreb’s Ringer-Henseleit solution (KRH) buffered with HEPES containing (in mmol/l) 145 NaCl, 4.0 KCl, 1.7 CaCl₂, 1.0 KH₂PO₄, 1.2 MgSO₄, 6 NaHCO₃, 10 HEPES, and 5 glucose and adjusted to pH 7.4 with NaOH. After preincubation for 15 min at 37°C in a gently oscillating water bath, the cells were incubated with 1 μmol/l fura 2/AM in KRH at 37°C for 30 min. To remove extracellular fura 2/AM, the suspension was diluted with 2 volumes of KRH and centrifuged, and the cells were resuspended in the original volume of KRH. The cells were kept at room temperature until they were used.

Measurement of [Ca²⁺]i in fura 2-loaded corneal endothelial cells was performed following procedures previously described for single cell preparations.¹⁴,¹⁵ Briefly, corneal endothelial cells were allowed to attach naturally to the bottom of a chamber (100 μl) that allowed continuous superfusion (1 ml min⁻¹) at 37°C of various solutions. A flow-rate-to-chamber-volume ratio of 10:1 assured a rapid exchange of applied solutions. All experiments were carried out in KRH. Medium designated as Ca²⁺-free was prepared by omission of CaCl₂ and addition of 1 mmol/l EGTA. Fluorescence signals from single cells were monitored using a 40× (numeric aperature = 1.3) objective on a Nikon Diaphot inverted microscope connected to a Spex Fluorolog spectrofluorimeter system (SPEX Industries, Inc., Edison, NJ) to generate dual excitation wavelengths of 340 and 380 nm and to collect and analyze emitted photon counts after passage through a 510 ± 20 nm barrier filter. After correction for autofluorescence (< 10% of absolute photon counts), fluorescence intensity ratios (340/380 nm) were converted to [Ca²⁺]i, as described in detail previously.¹⁴
Statistical Analysis

Data obtained under identical experimental conditions were pooled, and averaged, and are presented in the text as means with standard errors. Differences between mean values in dose-response experiments were tested by one-way analysis of variance (ANOVA). Results were considered significant at \( P < 0.05 \). All traces shown represent at least three separate determinations.

Results

Elevation of \([\text{Ca}^{2+}]_i\) in BCEC in Response to Histamine Stimulation

The mean resting \([\text{Ca}^{2+}]_i\) in single BCEC was 45.8 ± 2.2 nM (n = 124). Responses to histamine were observable as early as the second day of cell culture. Representative measurements of \([\text{Ca}^{2+}]_i\) after exposure of cultured BCEC to supramaximal stimulating doses of histamine (100 \( \mu \text{mol/l} \)) are shown in Figure 1. Histamine induced a rapid increase (10- to 12-fold) in \([\text{Ca}^{2+}]_i\), to peak levels of 564 ± 31 nM (n = 23). As shown in Figure 1A, the signal subsequently declined over the 1 min period of histamine application and returned to baseline after agonist removal. Subsequent restimulation with histamine gave responses similar in amplitude and duration to that of the initial response, ruling out significant receptor desensitization (Figure 1A). When agonist was applied for longer times, \([\text{Ca}^{2+}]_i\), typically decreased from peak values during the first 2–3 min, but then stabilized to form an agonist-dependent sustained elevation (Fig. 1B) or abruptly fell back to baseline to begin oscillatory fluctuations (Fig. 1C).

Dependence of the \([\text{Ca}^{2+}]_i\) Signal Induced by Histamine on Extracellular \( \text{Ca}^{2+} \)

Figure 2A shows that an increase in \([\text{Ca}^{2+}]_i\), still occurred upon stimulation with histamine (100 \( \mu \text{mol/l} \)) in the absence of extracellular \( \text{Ca}^{2+} \) (no added \( \text{Ca}^{2+} \) plus 1 mmol/l EGTA), although the \( \text{Ca}^{2+} \) rise returned to baseline within 1 min, despite the continuous presence of agonist. The transient \( \text{Ca}^{2+} \) signal seen in the absence of medium \( \text{Ca}^{2+} \) resembled in form and amplitude that observed for the initial phase of the histamine response measured in the presence of extracellular \( \text{Ca}^{2+} \) (Fig. 2B). The initial response induced by histamine in the absence of \( \text{Ca}^{2+} \), however, was generally of slightly lower amplitude and took more time (≈2 sec) to reach peak amplitude, suggesting that \( \text{Ca}^{2+} \) entry may contribute somewhat to the initial rapid rise of \([\text{Ca}^{2+}]_i\), induced by histamine.

As indicated in Figure 2B, in contrast to the initial rise in \([\text{Ca}^{2+}]_i\), elicited by histamine, the sustained elevation in \([\text{Ca}^{2+}]_i\), was eliminated in the absence of extracellular \( \text{Ca}^{2+} \). Similarly, removal of extracellular \( \text{Ca}^{2+} \) during the sustained phase of a histamine-induced increase in \([\text{Ca}^{2+}]_i\), resulted in a rapid decline in \([\text{Ca}^{2+}]_i\), to prestimulatory levels (Fig. 2C). This effect was reversed upon readdition of extracellular \( \text{Ca}^{2+} \).
Fig. 2. The effect of extracellular calcium (Ca\textsuperscript{2+}) on histamine-stimulated changes in [Ca\textsuperscript{2+}]\textsubscript{i}. (A) Effect of removal of Ca\textsuperscript{2+} before histamine stimulation. In contrast to the pattern of Ca\textsuperscript{2+} mobilization in the presence of Ca\textsuperscript{2+} (left trace), the response in the absence of Ca\textsuperscript{2+} (right trace) is transient and [Ca\textsuperscript{2+}]\textsubscript{i} rapidly returns to prestimulatory levels despite the continued presence of agonist. (B) The same trace with the two histamine-induced responses superimposed on one another for comparison. Note the initial peak in the absence of Ca\textsuperscript{2+} is of slightly lower amplitude and rises at a slower rate. (C) Effect of removal and replacement of Ca\textsuperscript{2+} during the sustained phase of histamine-stimulated increase in [Ca\textsuperscript{2+}]\textsubscript{i}. (D) Effect of removal and replacement of Ca\textsuperscript{2+} during histamine-induced oscillations in [Ca\textsuperscript{2+}]\textsubscript{i}. After removal of Ca\textsuperscript{2+}, oscillations decline in frequency and then cease until medium Ca\textsuperscript{2+} is replenished.

(Fig. 2C). The sustained elevation in [Ca\textsuperscript{2+}]\textsubscript{i} also was reversibly sensitive to the Ca\textsuperscript{2+} channel blocker, Ni\textsuperscript{2+} \textsuperscript{11} (data not shown). If Ca\textsuperscript{2+} was removed during histamine-induced Ca\textsuperscript{2+} oscillations, a decrease in frequency, and an eventual cessation of spiking, was observed (Fig. 2D). Readdition of Ca\textsuperscript{2+} restored the oscillations to their approximate initial frequency. These results suggest a prominent role for extracellular Ca\textsuperscript{2+} entry in the maintenance of sustained elevations and oscillations of [Ca\textsuperscript{2+}]\textsubscript{i}. In contrast, the initial rise in [Ca\textsuperscript{2+}]\textsubscript{i} as well as initial oscillations in [Ca\textsuperscript{2+}]\textsubscript{i}, are, for the most part, independent of extracellular calcium and must be the result of release from an internal Ca\textsuperscript{2+} store.

Sensitivity and Dose Dependence of Single Cell Ca\textsuperscript{2+} Responses to Histamine

Figure 3 summarizes the Ca\textsuperscript{2+} response data for the individual cells tested in this study. Not all cells responded to agonist with a sustained [Ca\textsuperscript{2+}]\textsubscript{i} rise or oscillations. The likelihood of a response was related to the histamine concentration (Fig. 3A). For example, of the 14 cells tested at [histamine] = 100 nM, only four (28.5%) responded (all showing oscillations). At 10 nM, no cells responded, whereas at histamine ≥5 μmol/l, nearly all of the cells mobilized Ca\textsuperscript{2+}.

Because of this cell-to-cell variability in sensitivity to histamine at low concentration, only [Ca\textsuperscript{2+}]\textsubscript{i} from those cells that responded to agonist were included in the calculation of the mean responses to histamine doses. The dose response to histamine showed that the amplitude of the initial release of Ca\textsuperscript{2+} from the intracellular pool increased with a minimum effective dose of 50 nM histamine, a maximal response at 10 μmol/l, and an EC\textsubscript{50} of 0.84 μmol/l (Fig. 3B). At 10 μmol/l histamine elicited a mean initial rise in [Ca\textsuperscript{2+}]\textsubscript{i}, to 541 ± 54 nM (n = 12) and an average response (during the first 2 min) of 229 ± 25 nM (n = 11). The rise time of the initial response (measured as the time from the beginning of the rise in [Ca\textsuperscript{2+}], to its peak) was inversely related to [histamine] (ANOVA, P < 0.0001). For example, at 100 nM, the mean rise time was 19.8 ± 3.6 sec, whereas at 10 μmol/l it was 7.1 ± 0.8 sec. Among cells showing a sustained elevation in [Ca\textsuperscript{2+}]\textsubscript{i} (eg, Fig. 1B), the magnitude of the elevation above baseline was 110.8 ± 6.5 nM (n = 22) and was independent of dose. This suggests that the
Histamine-Induced Oscillations in $[\text{Ca}^{2+}]_i$

As shown in Figure 1C, histamine generated oscillations in $[\text{Ca}^{2+}]$, in some BCEC. The oscillations persisted in the presence of agonist up to 30 min (the longest time measured) and ceased upon its removal (Fig. 1C) or after the application of receptor antagonist. Although some cells displayed $\text{Ca}^{2+}$ oscillations at all histamine tested, the proportion of responding cells that demonstrated oscillations decreased with increasing agonist concentration. For example, almost all of the cells that responded to [histamine] $\leq 500$ nM showed oscillations in $[\text{Ca}^{2+}]$, whereas at [histamine] $\geq 5$ $\mu$M, sustained elevations in $[\text{Ca}^{2+}]$, predominated and only a minority of cells showed oscillations. This pattern was seen not only when cells individually challenged with different histamine doses were compared, but also when a single cell was subjected to sequential elevations in agonist concentration (Fig. 4).

Representative traces of $[\text{Ca}^{2+}]$, oscillations in single BCEC, elicited at several agonist concentrations, are illustrated in Figure 5. Inspection of these traces indicates that as the dose of histamine increased, the amplitude of the initial rise in $[\text{Ca}^{2+}]$, increased, as did the amplitude of the subsequent $\text{Ca}^{2+}$ spikes. In contrast, the frequency of oscillations appeared to be independent of agonist concentration. Figure 6 summarizes the effect of histamine on the amplitude and frequency of $[\text{Ca}^{2+}]$, oscillations. Histamine elicited a dose-dependent (ANOVA, $P = 0.009$) elevation in spike amplitude with an EC$_{50}$ of 2.04 $\mu$mol/l (Fig. 6), whereas the frequency (mean = 0.62 ± 0.06 spikes/min, n = 33) was independent of histamine concentration (ANOVA, $P > 0.8$). These results suggest amplitude, rather than frequency modulation of the $[\text{Ca}^{2+}]$, signal in oscillatory cells.

Pharmacology of Histamine-Induced $[\text{Ca}^{2+}]$, Signaling

Histamine receptor subtype was investigated by treating single BCEC with specific receptor subtype antagonists prior to and during histamine stimulation. Triprolidine, an H$_1$ receptor antagonist, was able to reversibly inhibit the $[\text{Ca}^{2+}]$, signal during a sustained elevation (Fig. 7A) or during oscillations (Fig. 7B). Triprolidine also was able to prevent an agonist-induced response when applied prior to histamine treatment (Fig. 7C). This inhibition was reversible as an agonist response was attained upon restimulation 5 min after washout of the antagonist. In contrast, ranitidine, an H$_2$ receptor antagonist, had no effect on the histamine-induced elevation in $[\text{Ca}^{2+}]$, even at...
Fig. 5. Representative traces of oscillations in \([Ca^{2+}]_j\) in single cultured bovine corneal endothelial cell treated with various concentrations of histamine. Note that the amplitude of the initial rise in \([Ca^{2+}]_j\), as well as the amplitudes of the individual spikes, increased with \([histamine]\), whereas the frequency was independent of dose.

Lack of \(Ca^{2+}\) Response During Exposure of BCEC to Carbachol

It has been reported that BCEC express muscarinic acetylcholine receptors.\(^{10}\) As shown in Figure 8, cells that were clearly capable of mobilizing \(Ca^{2+}\), as judged by their responsiveness to histamine, were insensitive in this regard to the muscarinic agonist, carbachol. BCEC did not mobilize \(Ca^{2+}\) when treated with up to 0.5 mmol/l carbachol.

Discussion

The results of this study indicate for the first time that histamine \(H_3\) receptors are expressed in cultured BCEC, and that their activation by histamine causes mobilization of \([Ca^{2+}]_j\). The linkage of histamine activation with \(Ca^{2+}\) mobilization suggests mediation of the response in these cells by the \(H_3\) receptor subtype, as has been demonstrated in several other cell types.\(^{16-18}\) This prediction was confirmed in the present study by showing that the \(Ca^{2+}\) response was blocked by \(H_3\)-specific antagonists, whereas exposure to \(H_2\) and \(H_2\)-specific antagonists did not inhibit the response (Fig. 7).

As judged by dependence on the presence of extracellular \(Ca^{2+}\), the histamine-induced rise in \([Ca^{2+}]_j\) exhibited two distinct phases: an initial release of \(Ca^{2+}\) from intracellular stores followed by increased extracellular \(Ca^{2+}\) influx (Fig. 2). This biphasic response is similar to that seen with various \(Ca^{2+}\)-mobilizing agents in a variety of cell types, including parietal cells,\(^{19}\) pancreatic acinar cells,\(^{20}\) mast cells,\(^{21}\) avian salt gland secretory cells,\(^{14}\) and vascular endothelial cells.\(^{22}\) It has been established that agonist-induced mobilization of intracellularly stored calcium arises from production of inositol phosphates, particularly inositol \([1,4,5]\) trisphosphate \((I[1,4,5]P_3)\), which elicits the release of stored \(Ca^{2+}\) in a wide variety of cell types\(^{23}\) and may ultimately contribute to enhanced \(Ca^{2+}\) entry at the plasma membrane.\(^{21}\) As with other \(Ca^{2+}\)-mobilizing receptors,\(^{23,24}\) activation of \(H_3\) receptors in human skin fibroblasts\(^{17}\) and HeLa cells\(^{18}\)
Fig. 7. The effect of receptor antagonists on histamine-stimulated changes in [Ca\(^{2+}\)]. (A) Effect of application and removal of 10 \(\mu\)mol/l triprolidine (a specific H1 receptor antagonist) during a histamine-induced sustained elevation in [Ca\(^{2+}\)]. (B) Effect of 10 nM triprolidine on histamine-induced [Ca\(^{2+}\)] oscillations. (C) Application of triprolidine (50 nM) prior to histamine abolishes the [Ca\(^{2+}\)] response. Note the reversibility of the inhibition by triprolidine. (D) Effect of ranitidine (a specific H2 receptor antagonist) on histamine-induced changes in [Ca\(^{2+}\)]. Pretreatment of an endothelial cell with 10 \(\mu\)mol/l ranitidine for 1 min prior to histamine stimulation had no effect on the response.

is coupled to stimulation of phospholipase C and consequent hydrolysis of plasma membrane phosphatidylinositol bisphosphate (PIP\(_2\)) to diacylglycerol and I(1,4,5)P\(_3\). Therefore, the results presented here regarding agonist-induced Ca\(^{2+}\) mobilization suggest that this proximal part of the Ca\(^{2+}\) signaling second messenger pathway is present in corneal endothelial cells as well, although generation of I(1,4,5)P\(_3\) remains to be confirmed by biochemical assay.

Whereas a variety of neurohormonal receptors are linked to Ca\(^{2+}\) mobilization, such receptors have not been identified previously in corneal endothelial preparations. Recent reports indicate that corneal endothelial cells express muscarinic cholinergic receptors, which in many cell types are coupled to phosphoinositide turnover and Ca\(^{2+}\) mobilization via activation of M3 receptors. However, repeated attempts to demonstrate Ca\(^{2+}\) mobilization with muscarinic agonists in single BCEC, including cells previously or subsequently stimulated with histamine to verify the presence of the requisite Ca\(^{2+}\) signaling pathway, have been unsuccessful (Fig. 8). This suggests that another muscarinic subtype, which presumably is not coupled to phospholipase C activation and I(1,4,5)P\(_3\) generation, is expressed in BCEC.

Histamine has been shown to mobilize Ca\(^{2+}\) in cultured human nonpigmented ciliary epithelium, cultured bovine lens epithelium, and cultured human vascular endothelium. The dose response of BCEC to histamine (EC\(_{50}\) = 0.84 \(\mu\)mol/l, maximal effective dose 10 \(\mu\)mol/l) observed in our study is very similar to that reported by Lee et al (EC\(_{50}\) = 0.7 \(\mu\)mol/l; maximal effective dose 10 \(\mu\)mol/l) for cultured nonpigmented ciliary epithelium (NPCE). The amplitude of the initial response in BCEC was much greater than that reported for NPCE, although this difference may reflect differences in tissue source or methodology.

Oscillations in [Ca\(^{2+}\)], often were a prominent feature of the response to histamine in BCEC, particularly at low agonist concentration. Agonist-induced [Ca\(^{2+}\)], oscillations have been described in a number of cells. Oscillations in BCEC were modulated by [histamine] through the amplitude of [Ca\(^{2+}\)] spikes, rather than their frequency (Fig. 6). In this respect, oscillatory behavior in BCEC is similar to that observed in lacrimal and parotid acinar cells, but differs significantly from that of vascular endothelial cells, hepatocytes, and salt gland cells, in which frequency of oscillations depends on agonist concentration. As with other cells in which agonist-induced
oscillations in \([\text{Ca}^{2+}]_o\) have been described,\(^{27}\) \(\text{Ca}^{2+}\) spikes in BCEC are derived from periodic release of \(\text{Ca}^{2+}\) from intracellular stores, because oscillations can persist when \(\text{Ca}^{2+}\) is removed from the extracellular medium (Fig. 2D). However, although the amplitude of the subsequent spikes initially was unaltered, their frequency declined, and after a few spikes, they ceased altogether. Presumably, cellular \(\text{Ca}^{2+}\) was gradually lost to the EGTA-containing medium, causing a gradual run-down of oscillations, because readdition of \(\text{Ca}^{2+}_o\) restored the oscillations toward their original frequency. Thus, \(\text{Ca}^{2+}_o\) is not directly required for the oscillatory response, but ultimately is necessary for maintaining a source of \(\text{Ca}^{2+}\) for refilling intracellular pools. In addition, the maintenance of the amplitude of spikes in the absence of extracellular \(\text{Ca}^{2+}\) suggests that the intracellular pools may have to be completely refilled before the following oscillatory spike can be triggered.\(^{27}\) The transient reduction in frequency of spikes in the absence of \(\text{Ca}^{2+}\) entry suggests a longer refilling time, presumably from the recycling of previously released \(\text{Ca}^{2+}\) and transfer from other intracellular pools.

Agonist-induced \(\text{Ca}^{2+}\) mobilization affects a wide variety of cellular processes (directly or through protein kinases), including ion and fluid transport,\(^{30,31}\) exocrine secretion,\(^{19}\) and cell growth and proliferation.\(^{17,18}\) In rabbit corneal endothelial cells, an elevation in \([\text{Ca}^{2+}]_i\), activated a nonselective cation channel.\(^{31}\) In nonpigmented ciliary epithelial cells, histamine induced \(\text{Ca}^{2+}\) mobilization was hypothesized to play a potential role in the formation or secretion of aqueous humor.\(^{25}\) Similarly, histamine-induced \(\text{Ca}^{2+}\) signaling in the corneal endothelium may affect ion and fluid transport or may play a role in inflammation or wound repair.

Although histamine has been shown to be present in the aqueous humor,\(^{32,33}\) a physiologic role for histamine-stimulated \(\text{Ca}^{2+}\) mobilization in the corneal endothelium remains to be clearly established. However, histamine and the \(\text{Ca}^{2+}\) ionophore A23187 have been shown to stimulate the release of prostaglandin \(E_2\) (PGE\(_2\)) from cultured BCEC.\(^{10}\) That an increase in \([\text{Ca}^{2+}]_i\), alone caused secretion of prostaglandin strongly suggests that histamine-induced PGE\(_2\) release is mediated by an increase in \([\text{Ca}^{2+}]_i\). Experiments by Jumblatt and colleagues\(^{34}\) demonstrated the importance of PGE\(_2\) in maintaining polygonal morphology and epithelial polarity in cultured rabbit corneal endothelial cells. In addition, early mediators of the inflammatory response, such as histamine, may be involved in the inflammatory cascade in ocular tissues through the \(\text{Ca}^{2+}\)-mediated release of eicosanoids. Responses to experimentally induced inflammation or injury in the eye include release of eicosanoids into the aqueous.\(^{35,36}\)

A few preliminary attempts to elicit \(\text{Ca}^{2+}\) mobilization in corneal endothelial cells harvested by trypsinization or by corneal scraping of adult bovine eyes have been unsuccessful. It is possible that sensitivity of BCEC to histamine (at the receptor or the second messenger level) is compromised during the time between slaughter of the cattle and preparation of endothelial cell samples (ie, 4–6 hr). For example, Bito and Salvador discovered that the aqueous humor of many bovine eyes was completely devoid of glucose 2–3 hr post-mortem upon arrival at the laboratory.\(^{37}\) The observation that BCEC cultures express \(H_1\) histamine receptor activity raises the additional possibility that receptors may be up-regulated in vivo under appropriate physiologic or pathologic conditions. Recently, we observed histamine-induced \(\text{Ca}^{2+}\) mobilization in cultured human infant corneal endothelial cells (data not shown). The pattern of response was very similar to that reported here for BCEC, indicating that this mode of cellular signaling is not restricted to one species and may be relevant to human corneal endothelial function.

**Key words:** calcium, cell culture, corneal endothelium, histamine, oscillations

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**References**


