J. Membrane Biol. 173, 107–116 (2000)

The Journal of
Membrane
Biology

Histamine Increases $[Ca^{2+}]_{in}$ and Activates Ca-K and Nonselective Cation Currents in Cultured Human Capillary Endothelial Cells

F. Jow, R. Numann

Cardiovascular Diseases, Wyeth-Ayerst Research, CN-8000, Princeton, NJ 08543, USA

Received: 16 June 1999/Revised: 22 September 1999

Abstract. We characterized the effects of histamine on intracellular Ca^{2+} and activation of ionic currents in human capillary endothelial cells. Histamine produced both a transient and sustained increase in intracellular Ca^{2+}. The transient response was mediated largely through intracellular Ca^{2+} release and the sustained response was due to extracellular Ca^{2+} entry. The increase in intracellular Ca^{2+} by histamine was not affected by the H2 blocker cimetidine. But was entirely blocked by the H1 antagonist diphenhydramine showing that the histamine response in these cells is mediated through the H1 receptor. A transient ionic current is coactivated with the histamine-induced increase in intracellular Ca^{2+} and this current has several properties of a nonselective, Ca^{2+} permeable, cation channel (NSC). The magnitude of the NSC current does not strictly correlate with intracellular Ca^{2+} levels. A Ca^{2+}-activated K^+ current (BKCA) is activated by the increase in intracellular Ca^{2+} and this current is blocked by the selective BKCA blocker iberiotoxin.

Key words: Endothelial cell — Histamine — Nonselective cation channel — BKCA — Ca signaling — Patch clamp

Introduction

Histamine was one of the first substances identified as a mediator of inflammation and has been effectively targeted with antagonists to help moderate allergic and acute inflammatory responses (Schachter, 1973). One of the primary tissues affected by histamine is the endothelium of vessels where histamine produces a marked increase in vascular permeability. Histamine is one of many agonists that also mediate increases in intracellular Ca^{2+} and release of the potent vasodilator, NO from endothelial cells. Ca^{2+} imaging data and simultaneous monitoring of NO have shown that Ca^{2+} entry and not simply an increase in intracellular Ca^{2+} is required for histamine induced stimulation of NO (Lantoine et al., 1998). Others have seen some NO release associated with the initial Ca^{2+} release but much less for subsequent challenges unless Ca^{2+} entry occurs, presumably to refill internal stores (Kishi et al., 1996). Regardless, it appears that histamine and associated Ca^{2+} release and entry play a critical role in modulating the release of NO from endothelial cells.

Ionic currents activated by histamine would be expected to modulate NO release by altering the driving force for Ca^{2+} entry. It has been reported that depolarization of endothelial cells with high K^+ diminishes agonist induced entry of Ca^{2+} and release of NO (Lantoine et al., 1998). Patch-clamp studies of isolated endothelial cells report on a number of ionic currents activated by histamine although the channel type and temporal pattern varies between the individual studies (Nilius, 1997, 1998). To date, a nonselective cation channel with presumed or demonstrated permeability to Ca^{2+} and monovalent cations, an outward K^+ current, a chloride current and an inward rectifying K^+ current have all been seen, although not all from any given study (Bregestovski et al., 1988; Hosoki & Iijima, 1994; Groschner, Graier & Kukovetz, 1994; Nilius, Schwarz & Groogmans, 1993*a*). In addition, there has been one report in which intracellular Ca^{2+} and ionic currents were recorded simultaneously (Nilius et al., 1993*b*).

We report here on the probable identity of ionic currents and intracellular Ca^{2+} response of human microvessel endothelial cells exposed to histamine. The identity and temporal pattern of the ionic currents activated along with the associated changes in intracellular Ca^{2+} appear to be a unique mix from those already re-

Correspondence to: F. Jow

ported and include a novel Ca^{2+} dependent, inactivating nonselective cation current that is highly permeant to Ca^{2+}.

Materials and Methods

Cell Culture

Human capillary endothelial cells (HCEC) cells were obtained from Clonetics Corporation and grown in endothelial cell growth media (EGM™, Clonetics, Kirkland, WA). The composition of this medium was: modified DMEM/F-12 (1:1), 15 mM HEPES buffer, 10% FBS, 50 mg/ml heparin, 50 mg/ml αFGF (bovine pituitary origin). Cells were maintained at low density by passaging on day 5–6 but the culture media was allowed to become conditioned by only replacing it after passaging. The cells were used from passages 2–6.

Electrophysiological Measurements

Human endothelial cells were cultured on 35 mm tissue culture dishes in media and serum as described. They had a flattened (0.1–0.5 μm thick) cobblestone appearance that precluded reproducible whole-cell patch recording. To produce a thicker cell profile, cells were trypsinized and replated and the isolated cells were recorded 10–30 min later. Comparison of flattened endothelial cells with their freshly replated counterparts revealed no differences in either resting membrane potential or ion currents. Endothelial cells responded to bradykinin with an increase in outward current (185% at +100 mV, $n = 4$), suggesting that the trypsin had little deleterious effect on surface receptors. Cells were recorded in whole-cell voltage clamp with the patch-clamp technique (Hamill et al., 1981). Patch electrodes were pulled from borosilicate glass (Sutter Inst. O.D. 1.0 mm, I.D. 0.5 mm.) and had resistances from 3–6 MOhms. The input resistance of cells at −60 mV measured in excess of 1 Gigaohm. Seal resistance was typically 20 Gigaohms or greater. Resting membrane potentials of these isolated cells averaged −60.6 + 0.5 mV, $n = 83$. All recordings were done at room temperature. An EPC9 amplifier with the acquisition program Pulse-PulseFit from HEKA (Lambrecht, Germany) was used for recording, data acquisition and analysis. Series resistance compensation was used and currents were filtered at 3 kHz. Liquid junction potentials were measured, <6 mV, and corrected for. The standard pipette solution for whole-cell recordings was (in mmol/l): 120 K-aspartate, 20 KCl, 0.05 EGTA, 5 HEPES (pH 7.2 with KOH), 5 Mg-ATP, 5 Phosphocreatine-Na_2, 1 $MgCl_2$. In dual recordings 0.05 mM Fura2 acid was added in the standard pipette solution. The standard bath recording solution was modified Hanks' Balance salt solution (HBS) that consisted of (in mmol/l) 140 NaCl, 5.4 KCl, 5 HEPES (pH 7.2 with NaOH), 15 Dextrose, 1.9 $CaCl_2$, 0.8 $MgSO_4$, 0.4 KH_2PO_4, 0.3 Na_2HPO_4. Sodium and chloride substitution experiments used a NaCl-free bath solution in which NMDG-$MeSO_3$ was substituted for NaCl in the standard bath solution. Iberiotoxin (IbTx) was purchased from Peptide Institute, Fura2 acid from Molecular Probe, diphenhydramine and cimetidine from RBI, histamine and all salts from Sigma. All compounds and solutions were added to the HCEC by superfusion at the rate of 1 ml/min to a 35 mm tissue culture dish with a stainless steel insert which reduced the volume to 0.5 ml. A solid bar in the figures shows the duration of application.

Permeability ratios were calculated using the GHK (Goldman-Hodgkin-Katz) modified constant field equation (Lewis, 1979). To estimate P_K/P_{Na} the following equation was used:

$$E_{rev} = \frac{RT}{F} ln\left(\frac{[Na^+]_o + (P_K/P_{Na})[K^+]_o}{[Na^+]_i + (P_K/P_{Na})[K^+]_i}\right)$$

To estimate P_{Ca}/P_{Na} the following equation was used:

$$E_{rev} = \frac{RT}{F} ln\left(\frac{[Na^+]_o + (P_K/P_{Na})[K^+]_o + 4P'_{Ca}[Ca^{2+}]_o}{[Na^+]_i + (P_K/P_{Na})[K^+]_i + 4P'_{Ca}[Ca^{2+}]_i e^{E_{rev}F/RT}}\right)$$

Where $P'_{Ca} = P_{Ca}/P_{Na}\,(1 + e^{E_{rev}F/RT})$, E_{rev} refers to reversal potential, R is the gas constant, T is the absolute temperature and F is Faraday's constant.

Photometry

Intracellular Ca^{2+} was monitored simultaneously with patch-clamp recordings in some of the experiments. A T.I.L.L. Photonics (Germany) photometer adapted for the EPC-9 (Applied Scientific Instrumentation) was used to monitor the fura ratio. Calcium concentration was calculated from min/max ratio, and K_d values entered into the Pulse Fura program and verified against a calcium calibration curve generated with Molecular Probes calibration kit F-6774. Calcium concentrations reported with this technique represented the mean of four individual measurements taken during the voltage ramp or pulse. The population intracellular Ca^{2+} data in Fig. 1*B* was obtained from cells loaded with Fura2AM and imaged with a fluorescence microscope using the Intracellular Imaging software package (Intracellular Imaging, Cincinnati, OH). This system was also calibrated with Molecular Probes calibration kit F-6774.

Results

Histamine Produces an Increase in Intracellular Ca^{2+} in HCEC and this Response is Mediated Through the H1 Receptor

In fura2-loaded cells, histamine at 10 μM produces a transient increase in intracellular Ca^{2+} that slowly decays over 15 min back to a plateau level of sustained, elevated Ca^{2+} (Fig. 1*A*). This increase in intracellular Ca^{2+} is mediated through the H1 receptor as shown in Fig. 1*B* that shows the average intracellular Ca^{2+} response of 19 cells to histamine. An initial control response was elicited by 10 μM histamine. Following washout, histamine was co-applied with the specific H2 antagonist cimetidine (10 μM) which failed to block the response. Again following washout, the specific H1 antagonist diphenhydramine (10 μM) was perfused along with the histamine. This H1 antagonist completely blocked the histamine mediated increase in intracellular Ca^{2+} and was partially reversible upon washout (Fig. 1*B*, end of trace).

The Initial Histamine Mediated Increase in Intracellular Ca^{2+} Occurs Through Intracellular Ca^{2+} Release

Figure 2*A* shows the effect of histamine on a fura2-loaded HCEC in the absence of extracellular

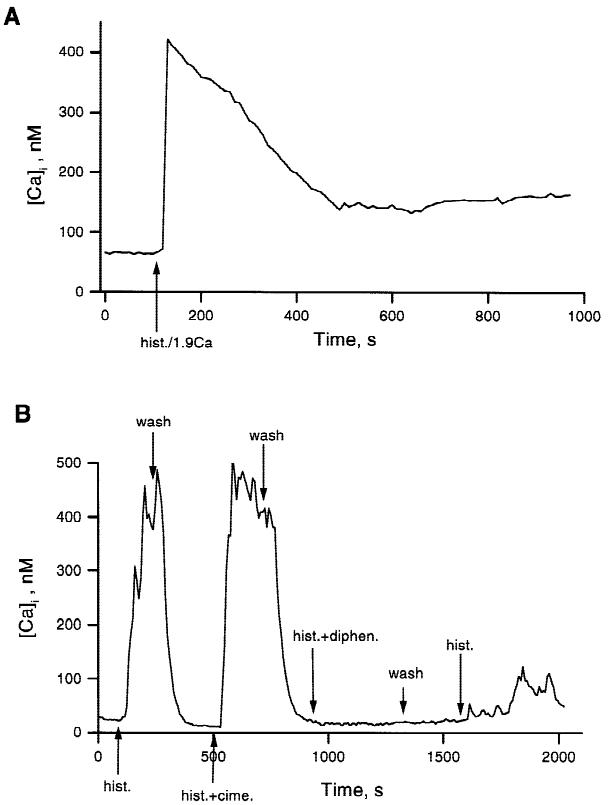

Fig. 1. The intracellular Ca^{2+} response of HCEC to histamine is mediated through H1 receptors and is composed of a transient and sustained increase in $[Ca^{2+}]_i$. Panel *A* shows the intracellular Ca^{2+} response of an endothelial cell exposed to 10 μM histamine in 1.9 mM external Ca^{2+}. Panel *B* shows increases in $[Ca^{2+}]_i$ in response to 10 μM histamine, wash, 10 μM histamine + 10 μM cimetidine, wash, 10 μM histamine + 10 μM diphenhydramine, wash, and 10 μM histamine showing partial recovery from diphenhydramine block.

Ca^{2+}. Histamine (10 μM) elicits an oscillatory increase in intracellular Ca^{2+} which rides upon a gradually declining plateau. In 5 additional cells, in Ca^{2+}-free external solution, histamine (10–40 μM) rapidly caused an increase in $[Ca^{2+}]_i$ from the basal level of 30 to 300 nM which was followed by oscillations. The oscillating spike gets larger and larger with time as the plateau level of Ca^{2+} falls until after 15 min the $[Ca^{2+}]_i$ declines back to the basal level and the $[Ca^{2+}]_i$ stops oscillating. The average spike interval is 2–3 min. The increase in intracellular calcium and its' oscillation in the absence of external calcium indicates that histamine triggers intracellular calcium release. The $[Ca^{2+}]_i$ oscillation phenomenon was seen in 43% ($n = 14$) of the cells recorded in zero external Ca^{2+}. The remainder of the cells responded to histamine with a transient increase in $[Ca^{2+}]_i$ that then returned to baseline. Interestingly under conditions of normal external Ca^{2+} the intracellular Ca^{2+} levels were never observed to oscillate with 10 μM histamine ($n = 19$) (*see* discussion).

Figure 2*B* shows peak inward and outward current from another HCEC exposed to 40 μM histamine in the absence of external Ca^{2+}. The peak inward and outward currents from voltage ramps (−120 to +100 mV) show a similar oscillatory behavior to that of intracellular Ca^{2+}. The inward and outward currents increase 3- to 4-fold in 40 μM histamine and oscillate for about 15 min before returning to basal level ($n = 6$). Buffering intracellular Ca^{2+} with 10 mM EGTA blocked the increase in intracellular Ca^{2+} and the activation of current by 40 μM histamine in all cells tested ($n = 3$; 1.9 mM external Ca^{2+}).

Simultaneous Recording of Ca^{2+} and Ionic Current from HCEC During Application of Histamine

To further investigate histamine's effect on $[Ca^{2+}]_i$ and membrane currents, HCEC were loaded with 50 μM Fura2 acid through the pipette for simultaneous recording of the Ca^{2+} signal and ionic currents. In the absence of external Ca^{2+}, 10 μM histamine induced a transient $[Ca^{2+}]_i$ increase that decayed to below the $[Ca^{2+}]_i$ seen in 1.9 mM external Ca^{2+} prior to the application of histamine (Fig. 3*A*). Readdition of 1.9 mM external Ca^{2+} induced a sustained Ca^{2+} increase similar to that seen in Fig. 1*A*. The transient Ca^{2+} increase in the absence of external Ca^{2+} indicates that histamine triggered intracellular Ca^{2+} release from the cell. The increase in intracellular Ca^{2+} seen with the subsequent addition of 1.9 mM Ca^{2+} to the external solution corresponds to histamine-induced Ca^{2+} influx. The increase in intracellular Ca^{2+} due to intracellular release, activated a transient current (lasting 7 min) that was inward at −120 mV and outward at +100 mV (Fig. 3*B* and *C*). The current amplitude for both inward and outward current increased by 12-fold over background levels in this cell. In 15 cells the average increase was 8.4 ± 1.7-fold. The inset in Fig. 3*B* shows raw current ramps obtained at the times indicated by 1 and 2. The transient current at the peak of its response (time 2) has a linear current voltage relationship that reverses close to zero mV. The inset in Fig. 3*C* shows raw current ramps at the times indicated by 3 and 4 corresponding to the current before and after addition of 1.9 mM external Ca^{2+}. The current ramp at time point 4 shows a noisy, sustained current that is outwardly rectifying and reverses around −60 mV. A transient intracellular calcium wave was elicited simultaneously with the transient current in all 14 cells tested with 40 μM histamine in calcium free external solution. In 4 cells where 1.9 mM calcium was added following the decay of the transient histamine response; a plateau phase of elevated intracellular calcium occurred and was associated with a sustained outwardly rectifying current.

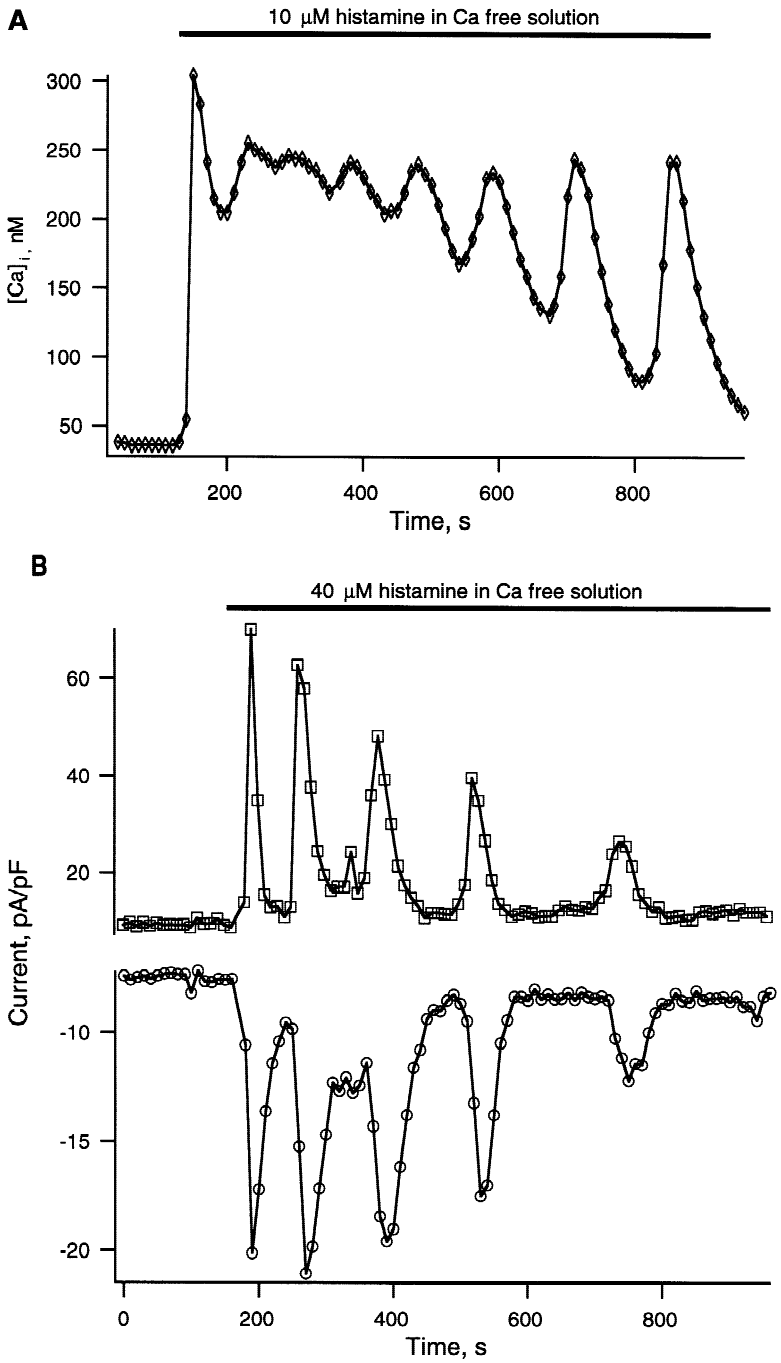

Fig. 2. Oscillations in intracellular Ca^{2+} and ionic currents in response to histamine in Ca^{2+}-free external solution. Figure 2*A* shows oscillatory increase in $[Ca^{2+}]_i$ from a HCEC exposed to 10 μM histamine in Ca^{2+}-free external solution. Figure 2*B* shows oscillations in outward (top) and inward (bottom) ionic current from another cell exposed to 40 μM histamine in Ca^{2+}-free external solution. This cell was recorded in whole cell voltage clamp with a holding potential of −60 mV and stimulated with voltage ramp pulses (−120 to +100 mV; 1 sec duration) at 10 sec interval. Peak inward current at −120 mV and peak outward current at +100 mV was plotted *vs.* time.

Histamine in NMDG-$MeSO_3$ External Solution Induced Ca^{2+}-activated K^+ Membrane Current

To investigate the outwardly rectifying current, activated by histamine, in isolation; NaCl in the external solution was replaced with NMDG-$MeSO_3$ and the pipette solution contained Kaspartate. We have previously found that NMDG blocks the nonselective cation channel (NSC) in these HCEC. In this recording condition with the nonselective cation current blocked, the primary permeant ion will be K^+ flowing through K^+ channels. 40 μM histamine in Ca^{2+}-free external solution was seen to activate a transient (10 min) outwardly rectifying current. Addition of 1.9 mM Ca^{2+} to the external solution activated a larger nondecaying outwardly rectifying current that could be blocked by 100 nM iberiotoxin, a specific blocker of Ca^{2+} activated K^+ current (BKCA) (Fig. 4*A*). The zero current potential obtained from the current voltage ramps of histamine activated current is −62 mV which is close to the K^+ equilibrium potential (Fig. 4*B* 2,

3). Numbers 1, 2, and 3 labeled current ramps correspond to voltage ramps given at the times indicated on Fig. 4*A*. In 4 cells the histamine induced BKCA current was 3.5 ± 0.7-fold above the background level but the absolute amplitude ranged from 5.2 pA/pF to 115 pA/pF reflecting the variability of BKCA channel expression in these cells. In 24% ($n = 33$) of HCEC we saw no evidence for a BKCA current.

Histamine Activates an Ohmic, Nonselective Cation Current (NSC) in the Presence of Iberiotoxin

Histamine (40 μM), in Ca^{2+}-free external solution and with 100 nM iberiotoxin, activates a linear transient current that spontaneously decays over 400 seconds (Fig. 5*A*). Addition of external 1.9 mM Ca^{2+} activates a larger linear transient current (Fig. 5*A*, time 3). The current voltage ramps at the times indicated with 1, 2, and 3 are plotted in Fig. 5*B*. In zero external Ca^{2+}, in the absence of histamine, the current voltage ramp reverses at −52 mV. After histamine application the ramp shifts positive to +7 mV due to activation of the NSC (time 2). Switching to 1.9 mM external Ca^{2+} in the continued presence of histamine further shifts the current voltage ramp positive to a reversal potential of +12.5 mV (time 3). Population data give a reversal potential of 8.7 ± 1.8 mV ($n = 4$) for the histamine response in the absence of Ca^{2+} and this is shifted to 11.7 ± 1.4 mV ($n = 4$) after addition of 1.9 mM Ca^{2+}, presumably due to the additional inward Ca^{2+} flux. The equilibrium potential for Cl^- under these experimental conditions is approximately −48 mV and the BKCA current was blocked by 100 nM iberiotoxin. This current is therefore not a Cl^- current but rather a nonselective cationic current (NSC). In the presence of Ca^{2+}-free external solution with 20 mM TEA or 100 nM Iberiotoxin to block the BKCA current, the average reversal potential of the cells is −3.5 ± 1.7 mV ($n = 13$) and the permeability ratio for P_K/P_{Na} is calculated as 1.1. The permeability ratio P_{Ca}/P_{Na} is calculated for this histamine activated NSC by using the difference reversal potential of the cell in Ca^{2+} free external solution and 1.9 mM Ca^{2+} external solution in the presence of 10 to 40 μM histamine and 100 nM Iberiotoxin. The average P_{Ca}/P_{Na} from 5 different cells is 2 ± 0.2.

Figure 6 summarizes the ionic currents activated by histamine in these HCEC. The cell was initially perfused with 40 μM histamine in the absence of external Ca^{2+} and oscillatory currents were activated which slowly decayed over time. Switching to an external solution containing 1.9 mM Ca^{2+} elicited a large ohmic current which decayed with time (Fig. 6*B*, time 2, inward current). After the decay of the ohmic current a substantial sustained outward current remained which was blocked by iberiotoxin (Fig. 6*A*, time 3). Thus in this cell, and 14 others, histamine coactivates what appears to be a nonselective

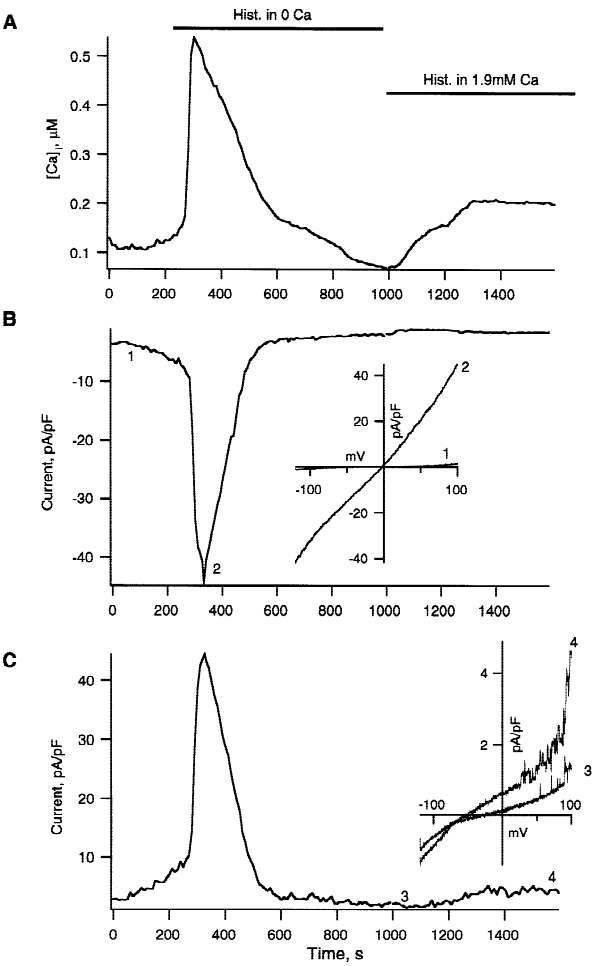

Fig. 3. Simultaneous $[Ca^{2+}]_i$ and current recording of a HCEC activated by 40 μM histamine. Simultaneous whole cell recording of $[Ca^{2+}]_i$ with Fura2 and current from whole cell voltage clamp with a holding potential of −60 mV and 1 sec voltage ramps every 10 sec from −120 to +100 mV. (*A*) $[Ca^{2+}]_i$ stimulated by 40 μM histamine for a HCEC in the absence and presence of external Ca^{2+} (1.9 mM). (*B*) Inward current from the same cell activated by 40 μM histamine taken for the current at −120 mV from the 1 sec voltage ramp. The inset shows current ramps of the cell before (1) and after (2) application of 40 μM histamine and demonstrates that the histamine-activated current reverses near 0 mV at the peak of the response. (*C*) The outward current from the same cell taken for the current at +100 mV from a 1 sec voltage ramp in the presence of 40 μM histamine. The inset shows current ramps from the cell before (3) and after (4) 1.9 mM Ca^{2+} was added to the external solution with histamine. Addition of external Ca^{2+} induced a large noisy outward current in the continued presence of histamine.

cation current (NSC) and a BKCA current. The nonselective cation current appears to require intracellular Ca^{2+} for its activation but then decays with a time course that is independent of the intracellular Ca^{2+} level. This is further illustrated in Fig. 7. In this cell intracellular Ca^{2+} and ionic currents were recorded simultaneously. Application of 40 μM histamine in Ca^{2+} containing external solution produced a large increase in intracellular Ca^{2+}

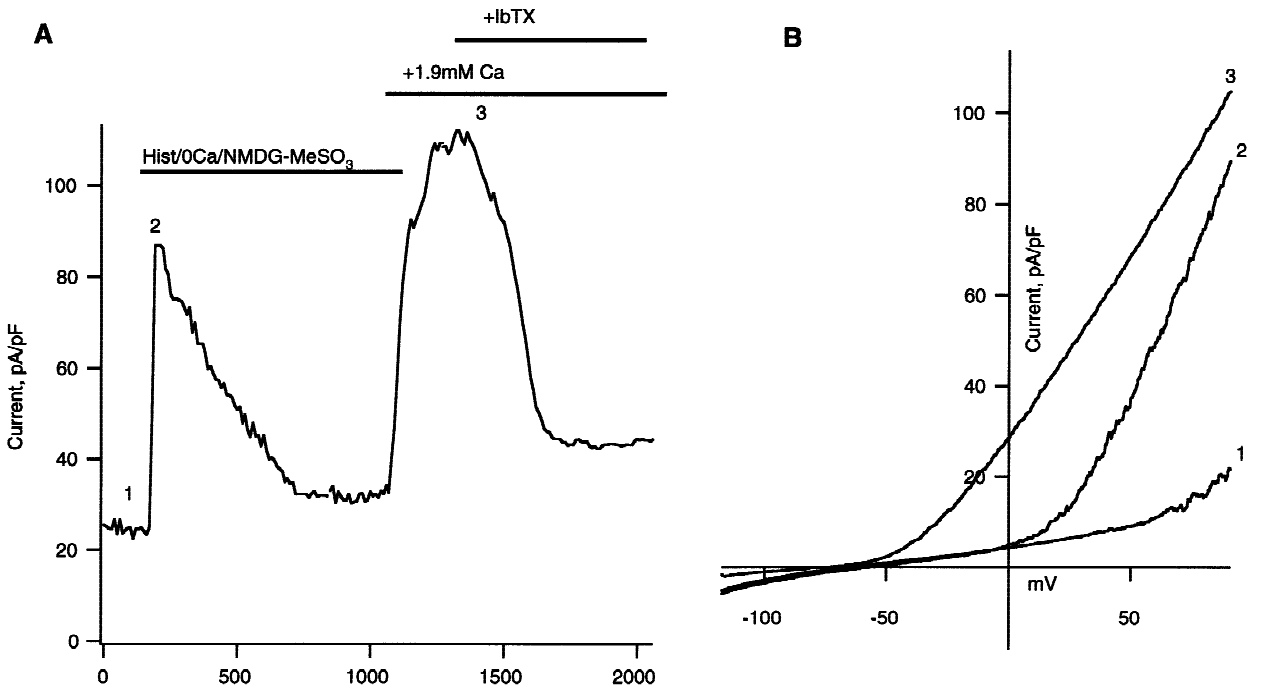

Fig. 4. The outwardly rectifying K^+ current activated by 40 μM histamine in NMDG-$MeSO_3$ external solution is blocked by 100 nM iberiotoxin. (*A*) Peak outward current from a 1 sec voltage ramp to +100 mV in response to 40 μM histamine in nominally free external Ca^{2+} and NMDG-$MeSO_3$, time point 3 shows data after addition of 1.9 mM external Ca^{2+}, and block with 100 nM iberiotoxin (IbTX). (*B*) Current ramps for times indicated by 1, 2 and 3. The ramps for times 2 and 3 reverse near −60 mV in the presence of external NMDG-$MeSO_3$ which is near the reversal potential for K^+.

and a transient current which reversed near +14 mV. There was little evidence for a Ca^{2+} activated K^+ current in this selected cell. The NSC current was transient in nature and decayed back to baseline after 300 sec, although intracellular Ca^{2+} remained elevated for an additional 400 sec. This suggests that the decay of the transient NSC current is not strictly tied to the fall in intracellular Ca^{2+}.

Discussion

Human capillary endothelial cells respond to exogenously applied histamine with a large transient release in intracellular Ca^{2+} followed by a phase of sustained Ca^{2+} entry. Simultaneous with this is activation of a nonselective cation current that reverses at 8.7 ± 1.8 mV in zero external Ca^{2+} and 11.7 ± 1.4 mV ($n = 4$) in 1.9 mM Ca^{2+} suggesting that it may also be permeant to Ca^{2+}. This current decays over a time course of 5 min even with elevated levels of intracellular Ca^{2+} and unmasks the presence of a Ca^{2+}-activated outward current that is blocked by iberiotoxin.

We show that the histamine receptor mediating the changes in intracellular Ca^{2+} in these human capillary endothelial cells is the type I receptor (Fig. 1*B*). This is in accord with previous studies on human endothelial cells that find type I to be the predominant form of the histamine receptor mediating vascular permeability via cell retraction, vasorelaxation, hydrolysis of phosphatidylinositol (4,5)-bisphosphate, and increases in intracellular Ca^{2+} (Furchgott et al., 1984; Matsuki & Ohhashi, 1990; Flavahan & Vanhoutte, 1990; Crawford, MacCallum & Ernst, 1992, 1993; Nijmi, Noso & Yamamoto, 1992).

Histamine-evoked repetitive spikes of cytoplasmic calcium in human endothelial cells have been reported by Jacob et al. (1988). They observed a dose-dependent relationship between histamine and the intracellular Ca^{2+} oscillations with low concentrations (0.1–3 μM) producing repetitive $[Ca^{2+}]_i$ spikes and higher concentrations (3–100 μM) eliciting an initial spike followed by a maintained plateau of elevated $[Ca^{2+}]_i$. They also present data from cells in nominally free Ca^{2+} and the oscillations they describe are very similar to the ones in this study and occur over a similar concentration range of 1–10 μM. Oscillations in intracellular Ca^{2+} from other cell types have been reported for IP_3-mediated Ca^{2+} release and entry (Tsien & Tsien, 1990; Berridge, 1997). The temporal pattern of these oscillations in intracellular Ca^{2+} have recently been tied to activation of specific gene transcription pathways (Dolmetsch, Xu & Lewis, 1998; Li et al., 1998).

The data with 1.9 mM external Ca^{2+} and Ca^{2+}-free external solution (Fig. 3) demonstrate that the histamine mediated increase in intracellular Ca^{2+} occurs by both

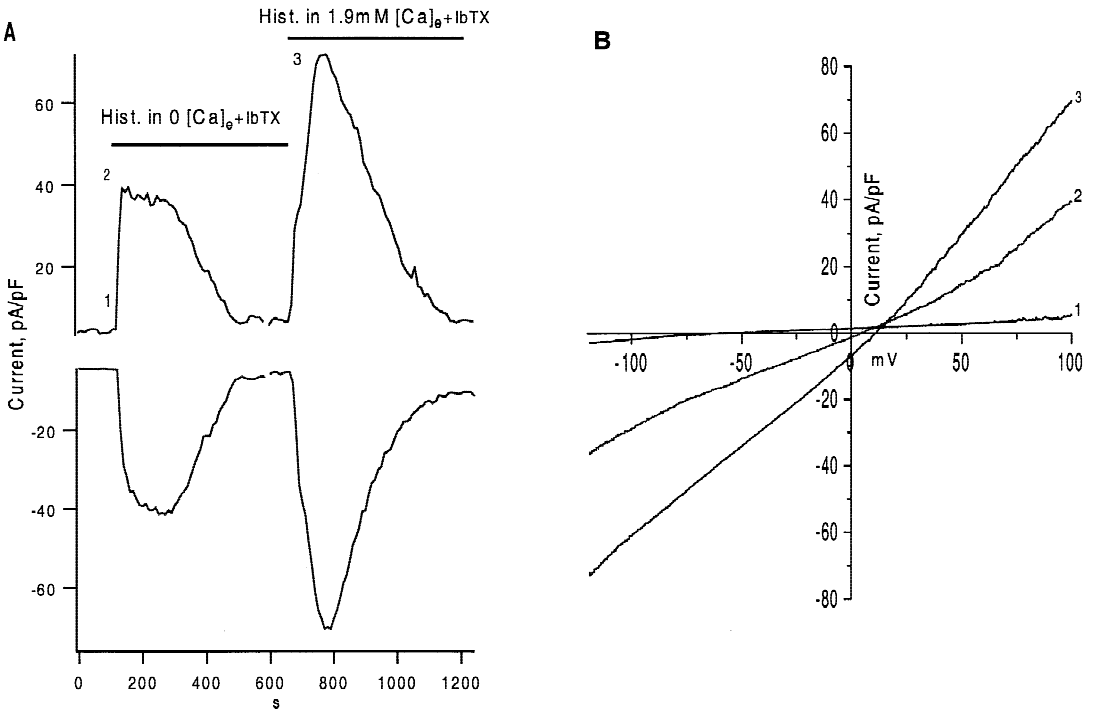

Fig. 5. The transient, ohmic ionic current activated by histamine reverses near 0 mV suggesting that it is a nonselective cation current. (*A*) Outward current (top) was taken from the peak current at +100 mV and inward current (bottom) from the peak current at −120 mV for 1 sec voltage ramps from −120 to +100 mV. Cell continually in the presence of 100 nM iberiotoxin. Time 1 and 2 before and after 40 μM histamine in external solution containing no added Ca^{2+}. Time 3 follows addition of 1.9 mM external Ca^{2+}. (*B*) Current ramp data for times 1, 2 and 3. The ramp for time 1 reversed at −52 mV, time 2 at 7 mV, and the current ramp following addition of 1.9 external Ca for time point 3 reversed at 12.5 mV; suggesting a permeability to Ca^{2+} ions.

intracellular release and Ca^{2+} entry. The initial fast transient increase in intracellular Ca^{2+} appears to primarily be due to intracellular Ca^{2+} release whereas the sustained plateau level of elevated Ca^{2+} reflects Ca^{2+} influx. This same pattern of Ca^{2+} release and entry has been reported by Hallam, Jacob and Merritt (1988) and Nilius et al. (1993*b*), for human umbilical vein and Hosoki et al. (1994) for human aortic endothelial cells, respectively.

The ionic currents activated or modulated by histamine in human endothelial cells have also been explored by other groups. Nilius et al. (1993*a*) have described a histamine-dependent block of the inward rectifier current in human umbilical vein endothelial cells. We see little evidence for histamine block of inward rectifier current (Fig. 4*B*, traces 1, 2). The reason for this difference is not known although their study was with human umbilical vein and they were studying a subpopulation of endothelial cells that were selected because they lacked the normal histamine-activated nonselective cation current.

Bregestovski et al. (1988) describe the activation of what appears to be a nonselective cation current by 10 μM histamine in human umbilical vein endothelial cells. Unlike the nonselective cation current we describe, their current activates very slowly with bath application of histamine (10 μM) requiring six min to reach a peak and then gradually decays back to baseline 10 minutes later. Their current reversed near 0 mV as did ours. They did not report the presence of a histamine-induced Ca^{2+}-activated K^+ current in their cells. This current is not routinely observed in endothelial cells and it's presence seems to be dependent upon tissue type and culturing conditions (Jow & Numann, 1999).

Groschner et al. (1994) describe the activation of three separate ionic currents by 10 μM histamine in human umbilical vein endothelial cells. Immediately following application of histamine they describe a large outward current that reverses close to the K^+ equilibrium potential and is blocked by the K^+ channel blocker tetrabutylammonium at 10 μM. After a significant delay of 20–60 sec a slowly developing inward current that was permeable to Ca^{2+} was observed. In parallel with this current they also observed activation of a Cl^- current. It seems probable that the Ca^{2+}-permeable inward current reflects in part, activation of a nonselective cation current similar to that seen by Bregestovski et al. (1988). The K^+ current may correspond to a Ca^{2+}-activated K^+ current similar to that we have described but this is somewhat speculative due to the nonspecific nature of TPA block of K^+ channels.

Nilius (1990) has described a nonselective cation channel in human umbilical vein endothelial cells that slowly runs down in the cell attached conformation fol-

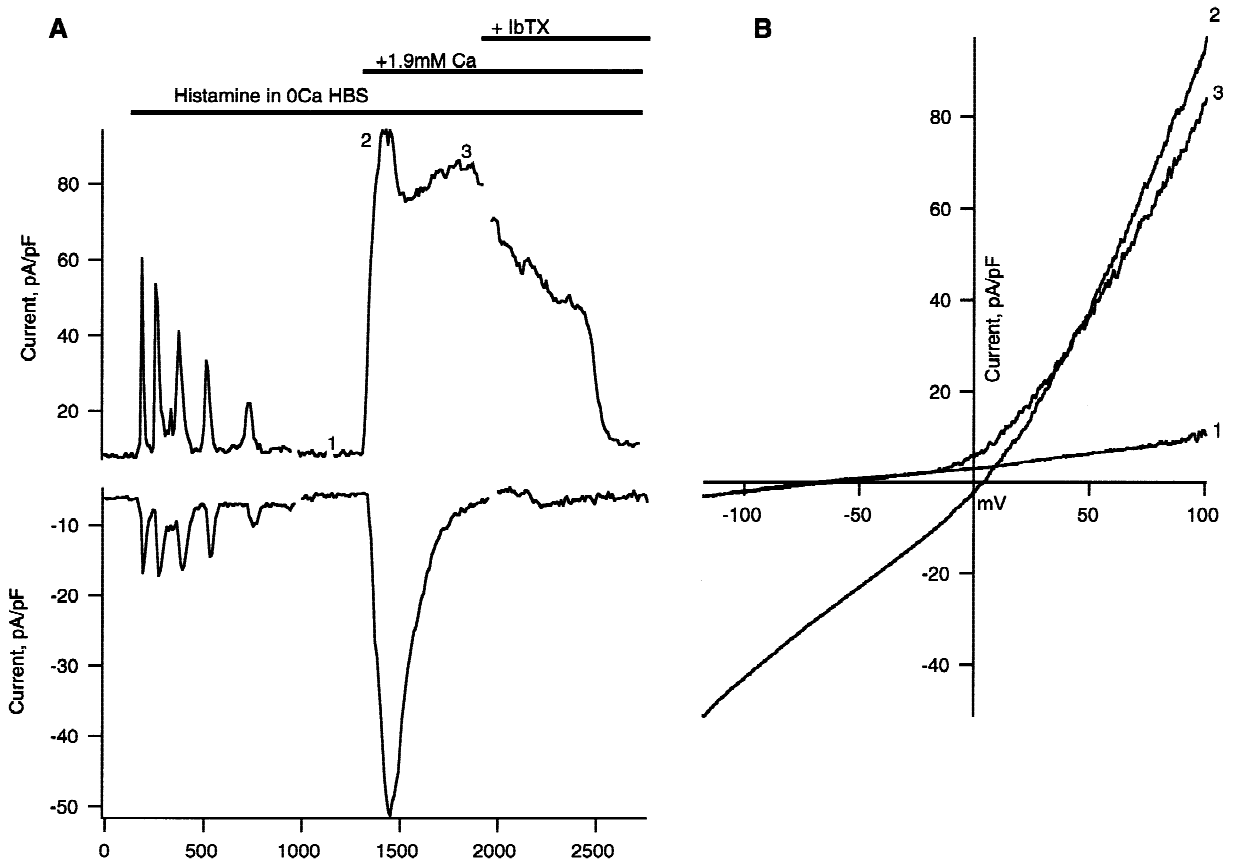

Fig. 6. Histamine, in normal external solutions, activates both the transient nonselective cation channel and the Ca^{2+}-activated K^+ channel. (*A*) Whole cell voltage clamp of a HCEC with a holding potential of −60 mV and stimulated with voltage ramp pulses (−120 to +100 mV; 1 sec duration) at 10 sec interval. Peak inward current at −120 mV and outward current at +100 mV were plotted *vs.* time. (*B*) Current ramps of the same HCEC in 10 μM histamine in Ca^{2+}-free external solution (1), after addition of 1.9 mM Ca^{2+} to the external solution (2), and after application of 100 nM IbTX (3). The current ramp for time point 1 reversed at −52 mV, time point 2 at 8 mV, and time point 3 at −58 mV, reflecting the predominance of nonselective cation current or Ca^{2+}-activated K^+ current active at those times.

lowing histamine activation. This channel was further explored in the whole cell configuration by recording ionic currents and Ca^{2+} transients from human umbilical vein endothelial cells using simultaneous patch clamp and $[Ca]_i$ recording (Nilius et al., 1993*b*). Histamine activated a transient and sustained increase in intracellular Ca^{2+} followed by activation of an ionic current that reversed near 0 mV. The Ca^{2+} transient is different than ours as it appears to activate more slowly to a peak response and then decays to a sustained plateau level in the presence of external Ca^{2+}. The nonselective cation current appears to activated more slowly than the increase in intracellular Ca^{2+}. The current reaches a peak and then slowly decays to roughly half the peak amplitude over the next 10 minutes. In contrast the peak of the nonselective cation current in these HCEC corresponds in time to the peak of the intracellular Ca^{2+} response. The inward current carried by the nonselective cation current in our study then decays completely back to baseline levels within 5 minutes in the continued presence of histamine. Their nonselective cation current reversed at 7.6 mV in 1.5 mM external Ca^{2+} and +13.0 mV in nominally free external Ca^{2+} (Nilius et al., 1993*b*). In contrast the nonselective current in these HCEC reversed at +11.7 mV in 1.9 mM external Ca^{2+} and +8.7 mV in nominally free external Ca^{2+}. No Ca^{2+}-activated K^+ current was activated by histamine in their study.

Manabe, Takano and Noma (1995) have reported on a nonselective cation current in guinea-pig endocardial endothelial cells exposed to histamine. The current in their study was also Ca^{2+} permeant but unlike most other studies it showed a pronounced inward rectification. However, Yamamoto et al. (1992) have seen a similar NSC from intrapulmonary artery endothelial cells that exhibits inward rectification presumably due to a voltage-dependent block by Mg^{2+}. We saw no evidence for rectification of the nonselective cation current in these HCEC even in the presence of internal and external Mg^{2+}.

Ca^{2+} is twofold more permeant than Na^+ through the

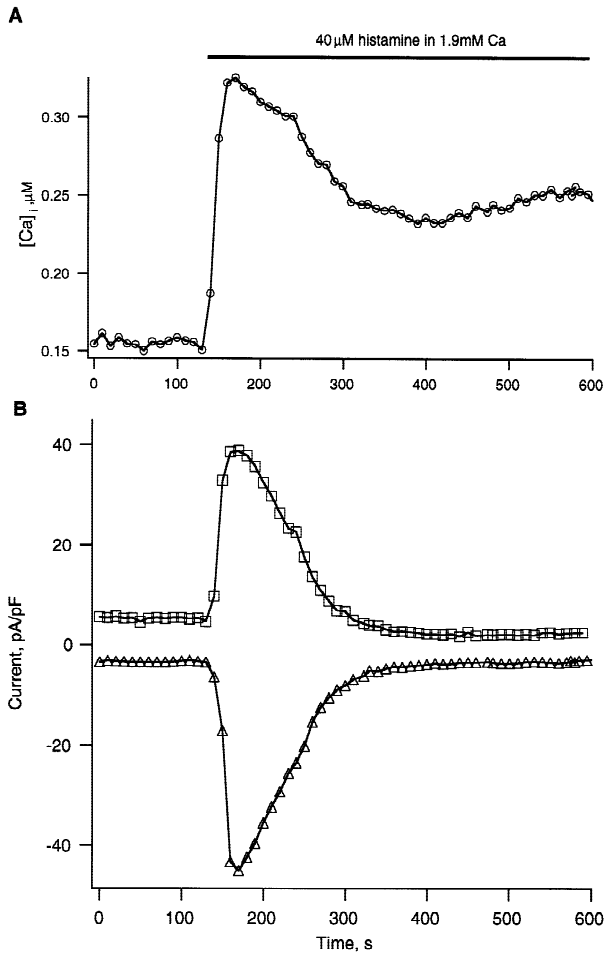

Fig. 7. The transient nature of the nonselective cationic current activated by histamine (panel *B*) is maintained even with elevated levels of $[Ca^{2+}]_i$. Simultaneous recording of $[Ca^{2+}]_i$ and ionic current in a HCEC with little or no Ca^{2+} activated K^+ current. (*A*) 40 μM histamine in 1.9 mM Ca^{2+} produced a transient and sustained level of elevated $[Ca^{2+}]_i$ that persists after the nonselective cationic current has decayed back to baseline. This suggests that the decay of the nonselective ionic current is not entirely mediated by $[Ca^{2+}]_i$.

NSC in these HCEC in contrast to the NSC from human Umbilical Vein Endothelial cells (P_{Ca}/P_{Na} = 0.11, Kamouchi et al., 1999), the NSC in human vascular endothelial cells (P_{Ca}/P_{Na} = 0.2, Nilius, 1990) and the basal NSC in guinea-pig endocardial endothelial cells (P_{Ca}/P_K = 0.57, Manabe et al., 1995). However the histamine operated NSC in rat intrapulmonary artery endothelial cells (P_{Ca}/P_{Na} = 15.7, Yamamoto et al., 1992) and the substance P activated NSC in porcine aortic endothelial cells (P_{Ca}/P_{Na} = 6 (Lansman, Hallam & Rink, 1987)), were both more Ca^{2+} permeant than our NSC.

In summary, none of the previous studies match with the histamine response we observe in these HCEC particularly with respect to the ion currents that are activated. The transient nature of the nonselective cation current seen in our study appears to be unique. The receptor-mediated activation of the NCS is not entirely regulated by intracellular Ca^{2+} levels but does require the initial increase in intracellular Ca^{2+} as it is blocked when 10 mM EGTA is included in the pipette (n = 3). This has also been recently reported by Kamouchi et al. (1999) for a human umbilical vein endothelial cell line, although the NSC in their study had properties different from ours. The coactivation of the nonselective cation channel and the BKCA channel maintain a driving force for Ca^{2+} entry and facilitate increases in intracellular Ca^{2+} (Bregestovski et al., 1988). However, the decay of the nonselective channel even in the maintained presence of histamine will serve to limit the amount of Ca^{2+} influx possibly preventing cell damage.

References

Berridge, M.J. 1997. Elementary and global aspects of calcium signaling. *J. Phys.* **499:**291–306

Bregestovski, P., Bakhramov, A., Danilov, S., Moldobaeva, A., Takeda, K. 1988. Histamine-induced inward currents in cultured endothelial cells from human umbilical vein. *Br. J. Pharmacol.* **95:**429–436

Crawford, K.M., MacCallum, D.K., Ernst, S.A. 1992. Histamine H1 receptor-mediated Ca^{2+} signaling in cultured bovine corneal endothelial cells. *Invest. Ophthalmol. Vis. Sci.* **33:**3041–3049

Crawford, K.M., MacCallum, D.K., Ernst, S.A. 1993. Agonist-induced Ca^{2+} mobilization in cultured bovine and human corneal endothelial cells. *Curr. Eye Res.* **12:**303–311

Dolmetsch, R.E., Xu, K., Lewis, R.S. 1998. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* **392:**933–936

Flavahan, N.A., Vanhoutte, P.M. 1990. G-protein and endothelial responses. *Blood Vessels* **27:**218–229

Furchgott, R.F., Cherry, P.D., Zawadzki, J.V., Jothianandan, D. 1984. Endothelial cells as mediators of vasodilation of arteries. *J. Card. Pharm.* **6:**S336–343

Groschner, K., Graier, W.F., Kukovetz, W.R. 1994. Histamine induces K^+, Ca^{2+}, and Cl^- currents in human vascular endothelial cells. *Circ. Res.* **75:**304–314

Hallam, T., Jacob, R., Merritt, J.E. 1988. Evidence that agonists stimulate bivalent-cation influx into human endothelial cells. *Biochem. J.* **255:**179–184

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Hosoki, E., Iijima, T. 1994. Chloride-sensitive Ca^{2+} entry by histamine and ATP in human aortic endothelial cells. *Eur. J. Pharmacol.* **266:**213–218

Jacob, R., Merritt, J.E., Hallam, T.J., Rink, T.J. 1988. Repetitive spikes in cytoplasmic calcium evoked by histamine in human endothelial cells. *Nature* **335:**40–45

Jow, F., Numann, R. 1999. Induction of Ca^{2+}-activated K^+ current and transient outward current in human capillary endothelial cells. *J. Membrane Biol.* **167:**53–64

Kamouchi, M., Mamin, A., Droogmans, G., Nilius, B. 1999. Nonselective cation channels in endothelial cells derived from human umbilical vein. *J. Membrane Biol.* **169:**29–38

Kishi, F., Nakaya, Y., Takahashi, A., Miyoshi, H., Nomura, M., Saito,

K. 1996. Intracellular and extracellular Ca^{2+} regulate histamine-induced release of nitric oxide in vascular endothelial cells as shown with sensitive and selective nitric oxide electrodes. *Pharmacol. Res.* **33:**123–126

Lansman, J.B., Hallam, T.J., Rink, T.J. 1987. Single stretch-activated ion channels in vascular endothelial cells as mechanotransducers? *Nature* **325:**811–813

Lantoine, F., Iouzalen, L., Devynck, M.A., Millanvoye-Van Brussel, E., David-Dufilho, M. 1998. Nitric oxide production in human endothelial cells stimulated by histamine requires Ca^{2+} influx. *Biochem. J.* **330:**695–699

Lewis, C.A. 1979. Ion-concentration dependence of the reversal potential and the single channel conductance of ion channels at the frog neuromuscular junction. *J. Phys.* **286:**417–445

Li, W., Llopis, J., Whitney, M., Zlokarnik, G., Tsien, R.Y. 1998. Cell-permeant caged $InsP_3$ ester shows that Ca^{2+} spike frequency can optimize gene expression. *Nature* **392:**936–941

Manabe, K., Takano, M., Noma, A. 1995. Nonselective cation current of guinea-pig endocardial endothelial cells. *J. Phys.* **487:**407–419

Matsuki, T., Ohhashi, T. 1990. Endothelium and mechanical responses of isolated monkey pulmonary veins to histamine. *Am. J. Physiol.* **259:**H1032–1037

Nijmi, N., Noso, N., Yamamoto, S. 1992. The effect of histamine on cultured endothelial cells. A study of the mechanism of increased vascular permeability. *Eur. J. Pharmacol.* **221:**325–331

Nilius, B. 1990. Permeation properties of a nonselective cation channel in human vascular endothelial cells. *Pfluegers Arch.* **416:**609–611

Nilius, B., Schwarz, G., Droogmans, G. 1993*a*. Modulation by histamine of an inwardly rectifying potassium channel in human endothelial cells. *J. Physiol.* **472:**359–371

Nilius, B., Schwartz, G., Oike, M., Droogmans, G. 1993*b*. Histamine-activated, nonselective cation currents and Ca^{2+} transients in endothelial cells from human umbilical vein. *Pfluegers Arch.* **424:**285–293

Nilius, B. 1997. Ion channels in vascular endothelium. *Annu. Rev. Physiol.* **59:**145–170

Nilius, B. 1998. Signal transduction in vascular endothelium: The role of intracellular calcium and ion channels. *Verh K. Acad. Geneeskd Belg.* **60:**215–250

Schachter, M. 1973. Histamine and antihistamines Vol. 1 International Encyclopedia of Pharmacology and Therapeutics Section 74, Pergamon, Oxford

Tsien, R.W., Tsien, R.Y. 1990. Calcium channels, stores, and oscillations. *Annu. Rev. Cell Biol.* **6:**715–760

Yamamoto, Y., Chen, G., Miwa, K., Suzuki, H. 1992. Permeability and Mg^{2+} blockade of histamine-operated cation channel in endothelial cells of rat intrapulmonary artery. *J. Phys.* **450:**395–408