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Histamine-induced inward currents in cultured endothelial cells from human umbilical vein

[1]P. Bregestovski, A. Bakhramov, S. Danilov, A. Moldobaeva & *K. Takeda

Institute of Experimental Cardiology, U.S.S.R. Cardiology Research Center, 3 Cherepkovskaya Street 15A, Moscow 121552, U.S.S.R. and *Université Louis Pasteur, Laboratoire de Pharmacologie Cellulaire et Moléculaire – CNRS UA600, B.P. 10, 67048 Strasbourg Cedex, France

1 The membrane response to applied histamine of cultured endothelial cells from human umbilical vein was studied by use of whole cell and single channel patch clamp techniques. A value of -27 ± 1.4 mV was found for the resting potential under whole cell current clamp. No voltage-gated currents were seen at either the macroscopic or single channel levels.

2 At holding potentials of -20 to -40 mV, histamine evoked slow rising, long lasting whole cell inward currents. The inward current was associated with depolarization and decreased input resistance. The calcium ionophore A23187 provoked similar whole cell inward currents.

3 Single channel currents were observed in cell-attached and inside-out patches for both histamine and A23187. The single channel conductance was about 20 pS with a mean open time of 5 ms and a reversal potential of 0 mV in symmetrical potassium solutions. Internal sodium blocked outward going currents.

4 For cell-attached patches, histamine-dependent channel activity required external calcium and was also seen when histamine was present in the bath but not the pipette. Recording from inside-out patches revealed that decreases in 'internal' calcium resulted in the disappearance of channel activity.

5 The histamine-dependent inward current appears to involve calcium-dependent activation of cationic channels.

Introduction

Endothelial cells possess plasma membrane receptors for a variety of hormones and neurotransmitters (Richardson & Beaulnes, 1971; Buonassisi & Venter, 1976; Makarski, 1981). The physiological roles of these receptors and their controlling mechanisms have attracted much recent interest. For example, certain vasoactive agonists stimulate the production and release of prostacyclin (Weksler *et al.*, 1977), platelet-activating factor (Camussi *et al.*, 1983) or endothelium-derived relaxing factor (EDRF; Furchgott, 1983). The endothelium is also important in low density lipoprotein regulation (Henriksen *et al.*, 1982) and in its interactions with the coagulation cascade. In contrast, only a few reports on the electrophysiological characterization of these various receptors in endothelial cells from intact tissue or in culture have appeared.

Our interest in the electrophysiological study of the actions of histamine in endothelial cells stems from the classical observation that histamine and other inflammatory agents provoke an increase in vascular permeability (Majno & Palade, 1961). This phenomenon has been ascribed to a contraction of endothelial cells and/or dilatation of interendothelial junctions (Majno *et al.*, 1969; Meyrick & Brigham, 1984) and can be demonstrated in cultured human umbilical vein endothelial cells, where an H_1-receptor type has been implicated (e.g. Killackey *et al.*, 1986; Rotrosen & Gallin, 1986). In the presence of phosphodiesterase inhibitors, agonist-dependent increases in endothelial cell cyclic nucleotides have been reported (Buonassisi & Venter, 1976; Makarski, 1981). Histamine acting on endothelial H_1-receptors caused a 2–3 times rise in guanosine 3′:5′-cyclic monophosphate (cyclic GMP) levels (Buonassisi & Venter, 1976; Brotherton, 1986) and is thus apparently not linked to adenylate cyclase. Histamine has also been shown to elevate phosphoinositide metabolism in human umbilical vein endothelial cells (Resink *et al.*, 1987; Lo & Fan,

[1] Author for correspondence.

1987). Calcium influx (D'Amore & Shepro, 1977) and intracellular calcium concentration were increased in the presence of histamine (Rotrosen & Gallin, 1986; Hamilton & Sims, 1987). Depolarization of endothelial cells was reported by Northover (1975a; 1980) following application of histamine. However, no relationship between rises in internal calcium and changes in endothelial cell membrane permeability has been established. In view of these properties and the known agonist action of histamine in liberating EDRF both from endothelial cells in culture and from intact tissue in an external calcium-dependent manner, it was of interest to test the hypothesis that histamine receptor-operated channels are responsible for calcium influx which is necessary for EDRF release. Whole cell and single channel patch clamp techniques were used to study the response to histamine in cultured endothelial cells from human umbilical vein. A preliminary description of some of these results has appeared elsewhere (Bregestovski & Bakhramov, 1987; Bakhramov *et al.*, 1988).

Methods

Cell culture

Endothelial cells were isolated from human umbilical veins and cultured as described elsewhere in detail (Allikmets & Danilov, 1986; Resink *et al.*, 1987). Cells from passages 1 to 7 were harvested by use of trypsin-EDTA and seeded on gelatin-coated glass cover slips in 35 mm plastic Petri dishes. Experiments were carried out on cells 2 to 7 days after seeding.

Electrophysiology

Standard whole cell and single channel current recording techniques were used. Experimental methods, data recording on FM tape (Bruel & Kjaer, Denmark) and offline computer (Labtam, Australia) analysis procedures are similar to those previously described elsewhere in detail (Bregestovski *et al.*, 1986; Takeda *et al.*, 1987). Pipettes for whole cell recording had resistances of 3–7 MΩ. For electrical coupling experiments, double whole cell patch clamp recordings were made as described by Neyton & Trautmann (1985), except that the cell pairs used were from confluent cultures. Because quantitative description of junctional resistance is possible only for isolated pairs of cells (Neyton & Trautmann, 1985), approximate coupling coefficients were estimated simply as the ratio of current amplitudes i_2/i_1 (from cells 2 and 1 respectively) for voltage steps applied in cell 1. Pipettes having resistances of 5–10 MΩ were fire polished and also coated with Sylgard (Dow Corning, USA) for single channel recording. The normal external Na solution contained (in mM): NaCl 140, KCl 2.8, $CaCl_2$ 1, $MgCl_2$ 2 and HEPES 10. The pH was adjusted to 7.4 with NaOH. High external K solution was made by substituting KCl for NaCl and adjusting the pH with KOH. For whole cell recording, the normal intracellular high K pipette solution contained (in mM): KCl 140, $MgCl_2$ 2, $CaCl_2$ 0.6, EGTA 1.1 (calculated pCa 7), HEPES 10, pH 7.4 with KOH. Agonists were bath applied. Solutions with other pCa values were made by using appropriate ratios of Ca/EGTA buffers. Input resistance was calculated from plateau current responses to either depolarizing or hyperpolarizing voltage steps of 10 mV in amplitude. Experiments were carried out at room temperature (20–23°C).

Drugs and other chemicals were obtained from Sigma (USA).

Where appropriate, values given in the text are expressed as the mean ± s.e.mean.

Results

Properties of unstimulated cells

The resting potential of human umbilical vein endothelial cells measured under current clamp after passing from the cell-attached mode to the whole cell recording configuration, with normal external Na bath and internal K pipette solutions was relatively low: -27 ± 1.4 mV ($n = 163$). The measured input resistance of the cells depended on whether or not the cells were in contact with each other: cells that were isolated (as judged visually at 400× with an inverted microscope) had an input resistance of 5.13 ± 2.4 GΩ ($n = 20$). Confluent cells in obvious contact with surrounding cells had an input resistance of 87 ± 10.7 MΩ ($n = 72$).

When either depolarizing or hyperpolarizing test pulses were imposed from a holding potential of −40 mV, the corresponding whole cell currents were essentially linear for a large range of potentials (−150 to 100 mV). At the single channel level, no currents were observed either for rapid voltage jumps or for maintained changes in potential. Thus, cultured endothelial cells from human umbilical veins have no detectable voltage-gated ionic currents.

Histamine-induced inward whole cell current

The action of bath-applied histamine (1–10 μM) was studied by whole cell recording with normal external Na bath and internal K pipette solutions. The cells were maintained at holding potentials of either −20

or −40 mV and the resting potential was measured periodically under current clamp. In about 9% of the cells at confluence tested, histamine produced an inward current ($n = 15$) as illustrated in Figure 1a. This response was associated with a decrease in membrane resistance, as judged by the increased current amplitude to 10 mV voltage steps (Figure 1a). The resting input resistance was 112 ± 27.8 MΩ and after 10 μM histamine, 67 ± 14.2 MΩ. In current clamp, a peak depolarization of 12 ± 5.3 mV was seen after histamine (10 μM). The maximal observed inward current was 274 ± 106 pA at a holding potential of −20 mV. In Figure 1b, another example of the inward current evoked by histamine in an isolated cell is given and the corresponding I–V relationship is shown in Figure 1c (the I–V curve of the histamine response was determined by subtraction of the control I–V in absence of histamine from that in presence of histamine).

Ionic current activation is influenced by calcium

It is possible that the histamine-dependent ionic channel activation depends on an increase in intracellular Ca concentration. We tested this idea using the Ca ionophore A23187. Whole cell I–V relations were obtained for the inward current induced by 5 μM histamine (after subtraction of currents in the absence of histamine; Figure 2a) with normal Na external bath and high K internal pipette solutions. Bath application of A23187 (1 μM) also provoked an inward whole cell current response, with I–V relations similar to those for histamine (Figure 2a). The A23187-evoked inward current resulted in cell depolarization with a decrease in membrane resistance. The interpolated reversal potentials for histamine and A23187 were about −5 mV. Responses to A23187 were only observed in those cells which responded to histamine.

The microscopic basis for these whole cell currents was examined using cell-attached recording of single channel activity associated with bath application of either histamine or A23187 (insets, Figure 2b). The single channel currents evoked by histamine and A23187 had similar slope conductances of about 20 pS (over the linear part of the I–V curves; Figure 2b) and reversal potentials with normal Na external bath and pipette solutions. The average open time for the histamine-induced channels was about 5 ms for a patch hyperpolarization of 30 mV (not shown). In 2 patches studied, external Ca was necessary for the activation of histamine-associated single channel activity (Figure 2c). Cell-attached recording was first established in low Ca (0.5 μM) control Na external solution, with no observed channel activity. In low Ca solution, bath-application of 10 μM histamine did not provoke channel activity, but after addition of

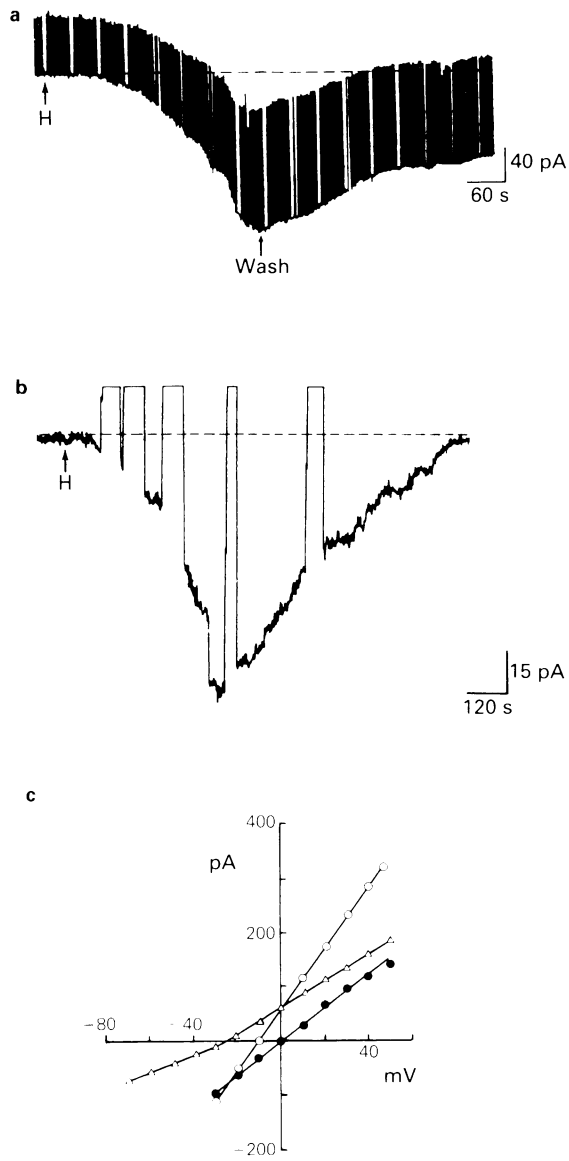

Figure 1 Whole cell current responses of voltage clamped cultured endothelial cells from human umbilical vein to bath-applied 10 μM histamine (arrows). Current jumps in response to 10 mV depolarizing steps were used to calculate input resistance. Broken lines indicate steady current levels necessary to maintain holding potentials of −20 mV. Normal Na external bath and K internal pipette solutions. (a, b) Inward current responses with decrease in membrane input resistance in 2 cells. The breaks in the trace in (b) (truncated) correspond to times at which I–V relations were determined. (c) I–V curves in the absence (△) and presence (○) of histamine; the histamine-induced current was calculated by subtraction (●).

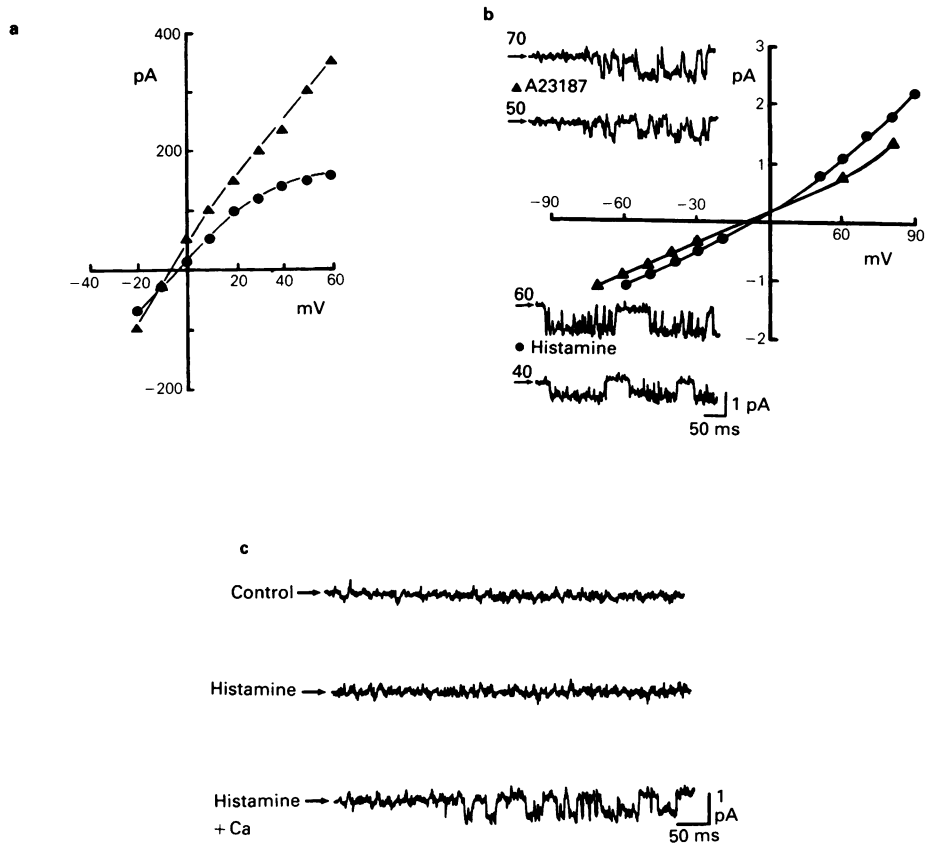

Figure 2 (a) Whole cell current-voltage relations for histamine- (●, 5 μM) and A23187- (▲, 1 μM) induced currents after subtraction of currents in the absence of drugs. Normal Na bath and K pipette solutions. Interpolated reversal potentials were −4 and −7 mV for histamine and A23187 respectively. Holding potential −20 mV. (b) I–V curves for single channels (inset) recorded in the cell-attached mode with normal Na external bath and pipette solutions for histamine (●, 10 μM) and A23187 (▲, 1 μM). Imposed pipette potentials are indicated in the insets. (c) External Ca is required for channel activity. Upper trace, control cell-attached recording in the absence of histamine. Middle trace, no channel activity was seen after 10 μM histamine bath application. Lower trace, appearance of single channels when external bath Ca was increased to 1 mM with histamine 10 μM. Note that the pipette contained low Ca (0.5 μM), Na external solution with no histamine. Pipette potential 40 mV. Arrows indicate closed levels.

Ca to a final concentration of 1 mM, histamine provoked channel opening (Figure 2c). Note that the pipette contained low Ca (0.5 μM), external Na solution and no added histamine.

Direct activation of channel activity was seen in 2 inside-out patches when the pCa of the solution bathing the internal surface of the patch was decreased from 7 to 6 (Figure 3a). Single channel currents after histamine application (10 μM) were first recorded in the cell-attached mode with normal Na external bath and high K pipette solutions. This channel activity continued after formation of an inside-out patch with a high K bathing solution having pCa 6. When the pCa was increased to 7 by bath perfusion, channel activity ceased (top trace, inset Figure 3a). Subsequent perfusion of internal solution with a pCa of 6 resulted in channel opening (bottom trace, inset Figure 3a). The I–V characteristics of these channels are illustrated in Figure 3a and the slope conductance of the channels was about 25 pS for the linear portion of the curve with a reversal potential of 0 mV. The reversal potential

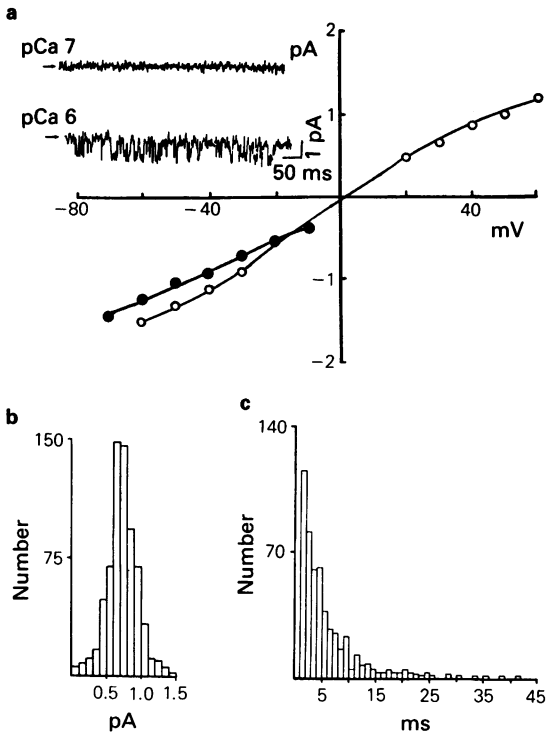

Figure 3 Internal Ca activation of single channels recorded from an inside-out patch. Channel activity was first observed in the cell-attached mode after 10 μM histamine addition to the normal Na bath solution, with a high K pipette solution. Following formation of an inside-out patch, a high K bathing solution with pCa 7 or 6 was introduced (see text). (a) I–V relations for channels recorded inside-out in symmetrical K (○) and after K was replaced by Na (●) in the bathing solution (pCa 6). Note the absence of outward going currents with internal Na (●). Insets show that an internal pCa of 6 was required for channel activity. Patch potential −40 mV; arrows indicate closed levels. (b) Channel amplitude histogram for the patch in (a) (symmetrical K, pCa 6, patch potential −40 mV). Peak amplitude, 0.7 pA. (c) Channel open time histogram for the patch in (b). A single exponential with time constant of 5.2 ms fits the data. Events briefer than 1 ms are not included.

and single channel conductance of the channels were influenced by changes in K concentration (not shown). When the internal K in the bathing solution was replaced by Na, only inward currents were observed (Figure 3a), suggesting a block of outward K current by internal Na. This behaviour was seen for 6 inside-out patches. Typical channel amplitude and open time histograms are shown for a patch potential of −40 mV (Figure 3b,c), with a peak amplitude of about 0.7 pA and a fitted single exponential time constant of 5.2 ms. The average open time was 5.0 ± 0.4 ms (n = 3).

Electrical coupling between cells

A possible explanation for histamine-dependent increases in vascular permeability is that histamine causes endothelial cell gap junction uncoupling (Meyrick & Brigham, 1984). Double whole cell patch clamp recordings were made as illustrated in Figure 4. Cell-attached and subsequently whole cell recording were established on adjacent cells using 2 patch pipettes. Both cells were held at −20 mV and 10 mV steps were given in one cell (C1, top trace Figure 4). The resulting currents in the neighbouring cell C2 are a reflection of the junctional resistance due to electrical coupling (see Neyton & Trautmann, 1985). For 24 different pairs of cells, 4 were uncoupled and the 20 others had approximate junctional coupling coefficients ranging from 0.1 to 0.8. When 10 μM histamine was bath applied, inward current was observed in both cells (Figure 4), without a marked change in junctional resistance (n = 3). In 9 other cell pairs, a marked increase in junctional resistance (corresponding to a 2–3 fold decrease in coupling

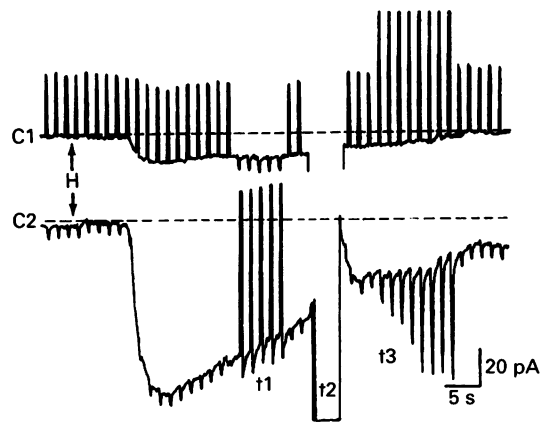

Figure 4 Double patch clamp whole cell recording from two neighbouring cells. Both cells were held at −20 mV. Arrow indicates time of histamine (10 μM) bath application. In cell 1 (C1, upper trace), the upward current deflections are produced by 10 mV depolarizing steps. Electrical coupling between the 2 cells is demonstrated by the downward current deflections in cell 2 (C2, lower trace). Both cells responded to histamine with an inward current. At t1, the 10 mV depolarizing pulses were applied in C2. At t2, large hyperpolarizing pulses were applied in C1 (both responses are truncated). At t3, increasing depolarizing steps of a larger amplitude were applied in C1 (truncated). Dotted lines indicate 0 current level.

coefficient) was seen after about 5 min of histamine application (not shown), as if the cells became uncoupled.

Discussion

There are two points of interest concerning the resting behaviour of human umbilical vein endothelial cells. Firstly, the measured membrane potential was lower than that recently found by use of similar techniques in cultured bovine arterial endothelial cells (Takeda *et al.*, 1987; Colden-Stanfield *et al.*, 1987; Adams *et al.*, 1987; Johns *et al.*, 1987). However, similar low values of resting potentials have been reported in confluent endothelial cell cultures (Venter *et al.*, 1975; Richter & Halle, 1983) and in intact tissue (Northover, 1980), where gap junctions and electrical coupling between cells can be expected to be present (Simionescu *et al.*, 1975; Venter *et al.*, 1975). The observed differences in input resistance probably also result from confluent cells being electrically coupled. Secondly, no voltage-activated ionic currents were observed here, thus ruling out voltage-gated Ca current as the basis for the external Ca dependence of EDRF release. This is in agreement with the results obtained in bovine endothelial cells, but differs in that a hyperpolarization-activated, inward rectifier K current was observed in the bovine cells (Takeda *et al.*, 1987; Colden-Stanfield *et al.*, 1987; Adams *et al.*, 1987; Johns *et al.*, 1987). The reasons for these discrepancies are not clear, but may be species-dependent. Alternatively, it may be that the embryonic nature of the umbilical vein underlies differences in cellular behaviour compared to adult tissue or that differences exist between endothelial cells of arterial and venous origin.

It has been suggested (Northover, 1975a) that external Ca-dependent, histamine-induced depolarization of endothelial cells is associated with morphological and histochemical changes of the endothelium (Northover, 1975b) seen in intact guinea-pig aorta. The inward current evoked by histamine in human endothelial cells (present work) may generate this depolarization. Evidence that Ca was implicated in the activation of the inward current comes from whole cell experiments with the Ca ionophore A23187 (Figure 2a). Furthermore, at the single channel level (Figure 2b), Ca was shown to be important for this response. Firstly, external Ca was necessary to record histamine-dependent channel activity in the cell-attached mode. Secondly, increasing the concentration of Ca bathing the cytoplasmic surface of inside-out patches resulted in channel activation (Figure 3).

The physiological source of Ca necessary for channel activity remains poorly defined, as we have not yet made extended investigations on the ionic selectivity of the inward current. Since the reversal potential is near 0 mV in normal solutions (Figure 3a), it appears that the channel displays poor selectivity for cations and thus Ca may permeate. A similar, presumably small permeability for Ca via nonspecific cation channels activated by stretch (Lansman *et al.*, 1987) and thrombin or bradykinin (Adams *et al.*, 1987; Johns *et al.*, 1987) has been recently reported in other cultured endothelial cells. On the other hand, the blocking action of internal Na for outward currents (Figure 3a) suggests that these channels display properties closer to those of other K-selective channels. Alternatively, another yet undetected, histamine-activated conductance pathway for Ca, perhaps linked to phosphoinositol metabolism may exist. Together, these then might form the basis for the external Ca dependence of histamine-induced EDRF release, in agreement with previous studies where rises in internal endothelial cell Ca were measured following histamine (D'Amore & Shepro, 1977; Rotrosen & Gallin, 1986; Hamilton & Sims, 1987). A role for second messenger signalling seems likely as bath-applied histamine evoked channel activity in cell-attached patches without histamine in the pipette. Histamine might also induce a release of Ca from some intracellular store subsequent to a rise in inositol trisphosphate levels (Resink *et al.*, 1987; Lo & Fan, 1987).

The double patch clamp experiments demonstrate directly the presence of electrical coupling between confluent endothelial cells in culture. This coupling might be important in propagating histamine or other agonist-associated currents (Venter *et al.*, 1975). The apparent uncoupling actions of histamine may arise subsequent to an elevation of internal Ca concentration, and may underly the previously reported histamine-induced increases in vascular permeability.

A variety of transmitter-like actions have been found for histamine in different cell types, some associated with hyperpolarization, some with depolarization, with or without changes in membrane conductance. Perhaps the best characterized electrophysiological actions of histamine are in molluscan neurones, where a slow hyperpolarization due to a K conductance increase has been demonstrated convincingly (Ascher & Chesnoy-Marchais, 1982; McCaman & Weinreich, 1982). In ileal smooth muscle from guinea-pig, histamine and carbachol were shown to activate ion channels permeable to Na, K and probably Ca, causing depolarization and contraction (Bolton *et al.*, 1981). In contrast, histamine-induced depolarization and increased action potential discharge in guinea-pig myenteric neurones were associated with an increased input

resistance (Nemeth *et al.*, 1984). Similar effects of histamine on rat hippocampal pyramidal cells have been reported (Segal, 1980), and additionally, a decrease in Ca-activated K current (Haas & Konnerth, 1983).

Recently, voltage clamp studies of the actions of bradykinin and thrombin on cultured vascular endothelial cells have appeared (Colden-Stanfield *et al.*, 1987; Adams *et al.*, 1987; Johns *et al.*, 1987). Bradykinin appeared to induce an internal Ca-dependent increase in presumably outward K current at depolarized potentials (Colden-Stanfield *et al.*, 1987). On the other hand, both bradykinin and thrombin were reported (Adams *et al.*, 1987; Johns *et al.*, 1987) to cause increases in inward current and it was shown that Ca was permeant. These results lend support to the notion that the various hormone and agonist receptors present on vascular endothelium play important roles in the regulation of local circulation.

The histamine-induced current observed here in cultured endothelial cells from human umbilical vein may be of influence in several of the known cellular actions of histamine. Should the currents be associated with changes in intracellular Ca concentration, they might modulate phenomena as widespread as increased vascular permeability due to endothelial cell contraction and/or gap junction uncoupling, external Ca-dependent release of EDRF, increase in cyclic GMP levels, and more speculatively, perhaps also stimulation of prostacyclin and platelet activating factor synthesis.

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