Short communication

Permeation properties of a non-selective cation channel in human vascular endothelial cells

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SUMMARY

Endothelial cells obtained from human umbilical cord have been studied by the patch clamp method. An ion channel is described that is activated by μM concentrations of histamine and shows a slow run-down in cell-attached patches. After excision, channel activity quickly runs down to zero open probability. In symmetrical potassium concentrations (140 μM K in the bath and the pipette), the single channel conductance is 28 ± 2 pS and the reversal potential is 0.3 ± 0.8 mV (mean ± SEM, n=4). With 140 mM Na in the pipette, the conductance is 26 ± 2 pS. A reversal potential of -1.5 ± 0.9 mV (n=7) was measured. With 60 mM Ca and 70 mM Na in the pipette, 140 mM K in the bath, the reversal potential was -11 ± 3 mV, the single channel conductance 16 ± 3 pS (n=5). The single channel conductance in 110 mM Ca (pipette) and 140 mM K (bath) is 8 ± 2 pS and the reversal potential is -10 ± 6 mV (n=3). From analysis of the reversal potentials, a permeation ratio of K:Na:Ca = 1:0.9:0.2 was calculated. This ligand-gated non-selective cation channel in human endothelial cells is Ca permeable and could induce a sustained agonist-mediated Ca influx.

Keywords: endothelium - histamin - non-selective cation channel - Ca permeability.

INTRODUCTION

Endothelial cells play a crucial role in the control of blood circulation. Humoral agents, for example histamine, bradykinin, thrombin, acetylcholine, and ATP can induce relaxation of vascular smooth muscle in the presence of endothelium due to release of endothelium-derived relaxation factor (EDRF). In umbilical-vein endothelial cells, histamine induces an intracellular Ca transient probably by IP3 and IP4 (Pollock et al. 1988). A sustained increase in intracellular Ca induced by histamine and other vasoactive hormones seems to depend exclusively on extracellular Ca and is mediated by a Ca influx (Jacob et al. 1988, Schilling et al. 1988, Danthuluri et al. 1988). A possible candidate for histamine induced influx of extracellular Ca into endothelial cells is a non-selective cation channel (Bregestovski et al. 1988). In this paper, a histamine-induced channel in human endothelial cells from umbilical vein is described that is permeable for calcium ions.

METHODS

Endothelial cells were isolated from the human umbilical vein by a method described by Jaffe et al. (1973) and cultured in Parker medium. Cells were identified by their "cobblestone" morphology, and by the presence of von Willebrand factor shown by immunofluorescence. Only cells from subcultures 1 to 3 were used. The cells were characterized morphologically and immunochemically. Ion channels were measured in the cell-attached or cell-free (inside-out) configuration by a standard patch-clamp device (Hamill et al. 1981). Records contained 512 or 1024 samples. Currents were sampled with 1 kHz and filtered with a 4-pole Bessel filter set at 1 kHz. Bath solutions contained (mM): A. 140 KCl, 1 MgCl2, 5 Hapes, titrated with KOH to pH 7.2, 100 μM CaCl2 were added to the bath solution. Pipette solutions contained (mM): A. 140 NaCl, 1 CaCl2, 1 MgCl2, 10 Hapes, pH 7.4 with NaOH; B. 140 KCl, 1 CaCl2, 1 MgCl2, 10 Hapes, pH 7.4 with KOH; C. 60 CaCl2, 70 NaCl, 1 MgCl2, 10 Hapes, pH 7.4 with NaOH; D. 110 CaCl2, 10 Hapes, pH 7.4 with Ca(OH)2. Histamine (VEB Jenapharm, GDR) was applied in a concentration of 5 μM into the bath. Current-voltage relationships measured from amplitude histograms or linear voltage ramps were best approximated by a Levenberg-Marquard least square fitting routine according to the respective equations given by Lewis and Stevens (1979), and Weber and Siemen (1989). Pooled data are given in mean ± SEM.

RESULTS AND DISCUSSION

Application of histamine into the bath induced, in cell-attached patches, openings of an ion channel (figure 1). Channel openings can be measured in the presence of chloride or aspartate as the anion. In 5 cells (cell-attached patches, K asp in the bath solution, NaCl in the pipette) a single channel conductance of 28 pS was recorded at 0 mV was measured. No shift occurred after excision. The same result was obtained in two cell-free patches in the presence of aspartate as anion in the bath solution. It is therefore concluded that the channel conducts cations. Channel activity arises about 25 seconds after application of histamine and is characterized by a high open state probability. At -40 mV the amplitude of channel openings in the presence of asymmetrical solutions (solution A in the pipette) is -1 pA.
After excision, the open probability of the channels runs down rapidly, the survival time is between several seconds and 5 minutes as a maximum (in the presence of 100 μM GTPyS in the bath solution, figure 1). Therefore, in excised patches with free access to either side of the membrane, current-voltage (IV) relationships were mostly obtained by use of linear voltage ramps (figure 2). IV curves were described by a two barrier (6b)-one binding site (6s) model (Lewis and Stevens 1979, Weber and Siemen 1989). For sake of simplicity, both barriers (6b) were regarded to be identical in height. This model (see also figure 2) predicts for a single species on both sides of the membrane the single channel conductance by

\[ g = \frac{g_{\text{max}}}{1 + K_\text{X}/X} \]  

(1)

where g is the single channel conductance at the membrane potential 0 mV for the cation X at the concentration X, K_X the concentration of halfmaximal saturation of the site. g_{\text{max}} can be directly obtained by

\[ g_{\text{max}} = \frac{v e_0 F}{R T} \exp\left(-\frac{G_\text{a} - G_\text{s}}{R T}\right) \]  

(2)

where v is the molecular vibration frequency (v = kT/h, k: Boltzmann’s constant, h: Planck’s constant), e_0 is the elementary charge, F, R, T have their usual meaning, G_a and G_s are the respective barriers and the single well). For asymmetrical solutions, g was obtained from linear regression. In the presence of 140 mM KCl at both sides of the membrane in cell-free patches and also in cell-attached patches with 140 mM KCl in the pipette and in the bath, a single channel conductance of \( 28 \pm 2 \) was measured (4 patches). In asymmetrical solutions (140 mM NaCl in the pipette, 140 mM KCl in the bath), a single channel conductance of \( 26 \pm 2 \) was obtained (7 patches). Figure 2 left shows IV curves obtained from linear voltage ramps and the respective fits by the energy barrier model.

\[ g_{\text{b,Na}} = 24.3 \text{ kJ/M}, \quad G_{\text{s,Na}} = -8.2 \text{ kJ/M}, \quad \text{electrical distance } 0.49. \]

Right: instantaneous current-voltage relationships with 110 Ca in the pipette and 140 K in the bath. The records were taken immediately after excision. The smooth curve has been calculated by the energy barrier profile seen at the bottom. The reversal potential obtained is -17.5 mV. The single channel conductance is approximately 9 pS. Data obtained from best fits are:

\[ G_{\text{b,Ca}} = 29.5 \text{ kJ/M}, \quad G_{\text{s,Ca}} = -9.5 \text{ kJ/M}, \quad d = 0.5. \]

A similar conductance (25 pS) was obtained in excised patches with symmetrical Na solutions. This non-selective cation channel share a similar conductance as known for a class of non-selective cation channels in other tissues (see Patridge and Swandulla 1988 for a review). By use of 110 mM Ca in the patch pipette without any other cation, inward currents could be measured (figure 2, right). This results is different from similar experiments in brown adipocytes (Weber and Siemen 1989). In these cells a permeation ratio \( P_{\text{Ca}}/P_{\text{Na}} = 0.02 \) was found that is approximately 10 times less than in this study. A single channel conductance of \( 8 \pm 2 \) pS was measured from 3 patches. Table 1 summarizes the results obtained from measurements of the