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Large conductance Ca^{2+}-activated K^+ channels modulate endothelial cell outward currents and nitric oxide release in the intact rat superior mesenteric artery

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ABSTRACT

Endothelial cells (EC) control vascular smooth muscle cell (VSMC) tone by release of paracrine factors. VSMC may also influence the EC layer, and therefore, the present study hypothesized that the opening of large-conductance Ca^{2+} activated K^+ (BK_{Ca}) channels may indirectly modulate EC hyperpolarization and nitric oxide (NO) release via myoendothelial gap junctions (MEGJ). To address this hypothesis '*in situ*' EC ion current recordings, isolated VSMC patch clamp recordings, and simultaneous measurements of NO concentration and relaxation were conducted using segments of the rat superior mesenteric artery. In arteries constricted by α_1-adrenoceptor activation, ACh (1 μM) evoked EC outward currents, vasorelaxation, and NO release. In contrast to preincubation with iberiotoxin (IbTx, 100 nM) application of IbTx after ACh decreased EC outward currents, NO release and vasorelaxation. Furthermore, in phenylephrine (Phe)-contracted arteries treated with a gap junction uncoupler, cabenoxolone (CBX), IbTx failed to decrease ACh-evoked EC outward currents. In addition, CBX decreased EC outward currents, time constant of the capacitative transients, input capacitance, and increased input resistance. In isolated VSMC CBX did not affect BK_{Ca} currents. Immunohistochemistry revealed only BK_{Ca} channel positive staining in the VSMC layer. Therefore, the present results suggest that BK_{Ca} channels are expressed in the VSMC, and that Phe by activation of VSMC BK_{Ca} channels modulates ACh-evoked EC outward currents, NO release and vasorelaxation via MEGJ in rat superior mesenteric artery.

1. Introduction

In the vessel wall VSMC tone is controlled by endothelium-derived factors including NO and vasoactive prostanoids [1]. Another pathway is associated with the hyperpolarization of the EC and transmitted to the VSMC either by an endothelium-derived hyperpolarizing factor (EDHF) or electronic conduction through myoendothelial gap junctions (MEGJ) [2]. MEGJ also allow the passage of current and small signalling molecules, such as Ca^{2+} and IP_3 [3]. The possibility that VSMC may also influence the EC has been less considered. Vasoconstrictors acting on VSMC of rat aorta evoke depolarization of the endothelial membrane potential [4], and later, the existence of electrotonic conduction from VSMC to EC of large pig coronary arteries was demonstrated [5]. In addition there is also evidence for Ca^{2+} signalling following elevations in VSMC Ca^{2+} by agonists such as Phe. The consequent secondary rise in endothelial Ca^{2+} can enhance the production of both NO and EDHF [6]. Therefore, these studies provide evidence supporting that the VSMC layer may control the EC layer. The importance of the radial communication process between EC and VSMC appears to be heavily dependent on the size of the vessel, being more numerous in small arteries, however MEGJ are also present in the rat superior mesenteric artery [7].

BK_{Ca} channels are considered as smooth muscle key elements, where activation of the channels leads to smooth muscle membrane hyperpolarization and relaxation [8]. In addition, BK_{Ca} channels in the microcirculation play an important negative feedback role during active agonist- and stretch-induced vasoconstriction [9]. However, whether VSMC BK_{Ca} channels can influence EC membrane potential and the release of NO based on the MEGJ-mediated interaction between VSMC and EC as discussed above is unclear.

The hypothesis of the present study was that VSMC BK_{Ca} channels may indirectly modulate EC outward currents, NO release and vessel relaxation via transfer of current through MEGJ. To address

Abbreviations: CBX, carbenoxolone; C_{in}, input capacitance; EC, endothelial cells; MEGJ, myoendothelial gap junctions; PSS, physiological salt solution; R_{in}, input resistance; VSMC, vascular smooth muscle cells.

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this hypothesis, simultaneous recordings of '*in situ*' EC outward currents and vessel tension, as well as simultaneous measurements of NO release and vessel tension were performed. Finally, these findings were further supported by recordings of membrane currents in isolated VSMC and BK_{Ca} immunolabelling in rat superior mesenteric arterial segments.

2. Methods

2.1. Rat mesenteric arteries

All experiments were performed in rat superior mesenteric artery of adult male Wistar-Kyoto rats (12 weeks old). Animals were sacrificed according to the guidelines of the European Union and by following protocols approved by the Local Animal Experimentation Ethics Committee. The mesenteric vascular bed was isolated and transferred into physiological saline solution (PSS).

2.2. Myograph experiments

Arterial segments were dissected and mounted in a microvascular myograph 310-A (DMT A/S, Aarhus, Denmark). The vessel segments were equilibrated in PSS at 37 °C, bubbled with a mixture of 5% CO_2 in 95% O_2, resulting a pH of 7.4. Next, the relationship between resting wall tension and internal circumference L_{100} was calculated [10]. The arteries were set to an internal circumference L_1, as determined by the equation, $L_1 = 0.9 \times L_{100}$, and for the *in situ* patch clamp arteries were set to $L_1 = 0.25 \times L_{100}$ to allow access to the patch pipette. The contractile ability of the vessels was tested by exposing them with 124 mM K^+-rich PSS (KPSS).

2.3. Simultaneous measurements of EC ionic currents and force

As previously described macroscopic ionic currents were recorded '*in situ*' from EC [11] with the whole-cell configuration of the patch-clamp technique using an Axon Multiclamp 700 A amplifier (Molecular Devices, CA. USA). Patch pipettes had resistances of 4-8 MΩ. Stimulation protocols consisted of depolarizing steps from −60 to +140 mV over 400 ms in 20 mV increments from a holding potential (V_h) of −60 mV elicited with a frequency of 0.1 Hz. Data were sampled at 2 kHz, and were digitized by a 16-bit A/D converter and analyzed using 'PowerLab Scope v3.6.4' software package.

2.4. Immunohistochemistry of BK_{Ca} protein

Immunohistochemical processing to evaluate the BK_{Ca} protein distribution was performed as previously described [12]. Incubation with a primary antibody which previously has shown selectivity in Slo1 knockout mice brain for the BK_{Ca} channel protein (1:100, Neuro Mab, clone L6/60, Briggs Hall, CA, USA) and eNOS protein (1:100, Abcam plc, cat# ab 5589 rb, Cambridge, UK) was

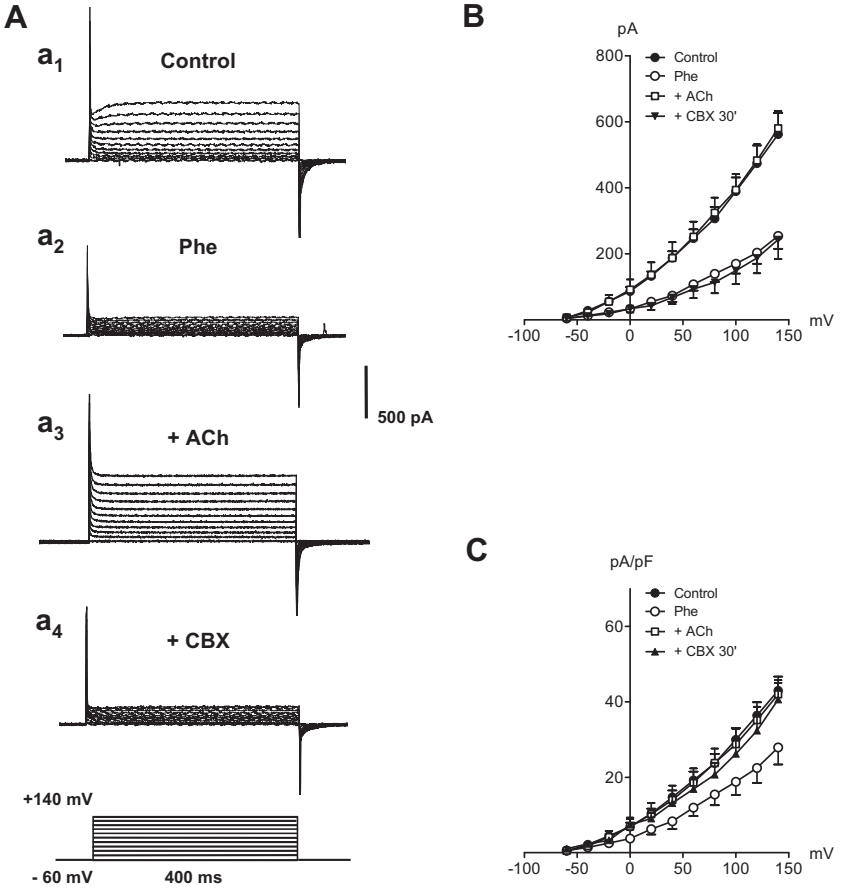

Fig. 1. CBX decreases '*in situ*' ACh-evoked EC outward currents. (A) Whole cell recording of currents in response to the stimulation protocol in control conditions (a_1), after addition of 0.5 μM Phe (a_2), after 1 μM ACh (+ ACh) (a_3) and after 50 min of 100 μM CBX (+ CBX) (a_4). (B) Current-voltage (I/V) relationship (pA) for control vs. Phe, for Phe vs. + ACh, and + ACh vs. + CBX were significantly different ($P < 0.01$, $P < 0.001$ and $P < 0.001$, respectively, $n = 5$, repeated-measures ANOVA). *C*. I/V graph (pA/pF) for control vs. Phe, for Phe vs. ACh was significantly different ($P < 0.05$, $P < 0.01$) but for ACh vs. CBX was similar ($P > 0.05$, $n = 5$; repeated-measures ANOVA). Each point represents the mean ± S.E.M.