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Evidence for signaling via gap junctions from smooth muscle to endothelial cells in rat mesenteric arteries: possible implication of a second messenger

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Abstract

We investigated heterocellular communication in rat mesenteric arterial strips at the cellular level using confocal microscopy. To visualize Ca^{2+} changes in different cell populations, smooth muscle cells (SMCs) were loaded with Fluo-4 and endothelial cells (ECs) with Fura red. SMC contraction was stimulated using high K^+ solution and Phenylephrine. Depending on vasoconstrictor concentration, intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increased in a subpopulation of ECs 5–11 s after a $[Ca^{2+}]_i$ rise was observed in adjacent SMCs. This time interval suggests chemical coupling between SMCs and ECs via gap junctions. As potential chemical mediators we investigated Ca^{2+} or inositol 1,4,5-trisphosphate (IP_3). First, phospholipase C inhibitor U-73122 was added to prevent IP_3 production in response to the $[Ca^{2+}]_i$ increase in SMCs. In high K^+ solution, all SMCs presented global and synchronous $[Ca^{2+}]_i$ increase, but no $[Ca^{2+}]_i$ variations were detected in ECs. Second, 2-aminoethoxydiphenylborate, an inhibitor of IP_3-induced Ca^{2+} release, reduced the number of flashing ECs by $75 \pm 3\%$ ($n = 6$). The number of flashing ECs was similarly reduced by adding the gap junction uncoupler palmitoleic acid. Thus, our results suggest a heterocellular communication through gap junctions from SMCs to ECs by diffusion, probably of IP_3.

Keywords: Heterocellular communication; Inositol 1,4,5-Trisphosphate; Rat mesenteric artery; Smooth muscle cells; Endothelial cells

1. Introduction

Intercellular communication through gap junctions between smooth muscle cells (SMCs) and endothelial cells (ECs) in the arterial wall plays a major role into the regulation of the arterial diameter. Arterial contraction is due to an increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in SMCs. A number of studies correlated the increase of $[Ca^{2+}]_i$ in ECs to the increase of $[Ca^{2+}]_i$ in SMCs [1–3]. A consequence of the $[Ca^{2+}]_i$ augmentation in ECs is the synthesis of endothelium-derived relaxing factors such as nitric oxide (NO). NO released by ECs induces SMCs relaxation [4,5]. It has also been suggested that electrical communication through gap junctions contributes significantly to the relaxation of rat mesenteric arteries [6]. The mechanism responsible for a $[Ca^{2+}]_i$ increase in ECs following a $[Ca^{2+}]_i$ elevation in SMCs remains unclear and the implication of gap junctions has not been demonstrated yet. Evidence of gap junctional communication between SMCs and ECs essentially concerns electrical coupling [7–9]. However, since ECs are non-excitable cells, electrical communication between SMCs and ECs is not likely to induce an increase of $[Ca^{2+}]_i$ in ECs. Indeed, a depolarization propagating from SMCs to ECs would actually decrease $[Ca^{2+}]_i$ as a result of a decrease of Ca^{2+} influx, reflecting the reduction of the electromotive force [10,11]. Gap junctions can also mediate chem-

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ical coupling; however, this process has been less studied. Diffusion is a slow process compared with electrical spreading, and chemical coupling would imply a measurable time interval between the augmentation of $[Ca^{2+}]_i$ in SMCs and in ECs. This time interval corresponds to the time necessary for molecules to diffuse from one type of cell to the other. However, to the best of our knowledge, such a time delay has not been reported yet and no diffusing molecule has been identified. Two major candidates for chemical communication are Ca^{2+} and inositol 1,4,5-trisphosphate (IP_3). Both are likely to diffuse from SMCs to the ECs, inducing an increase of $[Ca^{2+}]_i$ in ECs. $[Ca^{2+}]_i$ in SMCs can be increased by many processes; among them by stimulation of phospholipase C (PLC) that will release Ca^{2+} form the sarcoplasmic reticulum (SR) or by depolarizing the cells with a high K^+ solution, which opens voltage-gated Ca^{2+} channels (VOCs). In both cases not only Ca^{2+} will increase but also IP_3 [12], since an increase of $[Ca^{2+}]_i$ in SMCs induces IP_3 formation via phospholipase C (PLC) δ. IP_3 would then diffuse to ECs and release Ca^{2+} from the endoplasmic reticulum (ER) via IP_3 receptors Ca^{2+} channels that are sensitive to both, Ca^{2+} and IP_3. PLC δ is the only isoform of the PLC that is activated by Ca^{2+} without the need of extracellular agonists and this PLC has been detected in rat mesenteric arteries [13]. The release of Ca^{2+} from intracellular stores is accompanied by Ca^{2+} influx [14] through Ca^{2+} dependent non-selective cationic channels [15] in the plasma membrane.

The aim of the present study was to assess the chemical coupling between SMCs and ECs via gap junctions. The existence of a diffusion process would imply a time interval between $[Ca^{2+}]_i$ elevations between both cell types. Indeed, we have demonstrated the occurrence of this time lag; we have further tested whether IP_3 was the diffusing molecule by inhibiting either its production or its effect on ECs ER. We first inhibited the phosphatidylinositol-selective PLC with U-73122 in SMCs [16], using as control the inactive analog U-73343 [17]. Second, we used the membrane permeable, selective IP_3 receptor inhibitor 2-Aminoethoxydiphenylborate (2-APB) that inhibits the IP_3 induced Ca^{2+} release in ECs [18–20]. The possible implication of gap junctions in this process was controlled by inhibiting cell-to-cell communication with the gap junction uncoupler palmitoleic acid (PA).

2. Materials and methods

2.1. Preparation of arteries

Male Wistar rats (280 ± 50 g) were anesthetized with Isoflurane and then decapitated in agreement with the Care of Animals (edited by "Académie Suisse des Sciences Médicales" and "Société Helvétique des Sciences Naturelles"). The mesenteric arcade was excised and placed into a control solution containing (in mM) 145 NaCl, 5 KCl, 1 $CaCl_2$, 0.5 $MgSO_4$, 1 Na_2HPO_4, 20 HEPES, 23 Tris base and 10.1 Glucose. A second-order rat mesenteric artery was cleaned from extracellular tissue.

To measure $[Ca^{2+}]_i$ variations in SMCs and ECs simultaneously, the cleaned artery was mounted on two stainless steel cannulas in a custom-built micro-organ cannulation chamber [3]. The proximal cannula was connected to a syringe pump and was continuously perfused with the control solution at a rate of 50 μl/min (Fig. 1A). The artery was then loaded with Ca^{2+} fluorescent dyes as described in Section 2.2. After loading, the artery was decannulated and opened to have a strip (Fig. 1B).

For the experiments with the gap junction uncoupler PA and with the selective IP_3 receptor inhibitor 2-APB, $[Ca^{2+}]_i$ variations were measured in either SMCs or ECs. Thus, before loading with fluorescent dyes, the cleaned arteries were cut longitudinally into strips (see Section 2.3 for fluorescence technique). All experiments with rat mesenteric arterial strips were performed using confocal microscopy (see Section 2.4).

Furthermore, conventional microscopy was used to control the direct effect of high K^+ solution and PE on $[Ca^{2+}]_i$ variations in ECs. This technique consisted of loading a rat mesenteric artery under physiological conditions with the Ca^{2+} fluorescent probe Fura-2 [3,21–23]. The cleaned artery was mounted on two stainless steel cannulas in the micro-organ chamber (Fig. 1A). The proximal cannula was connected to an infusion pump containing the control solution.

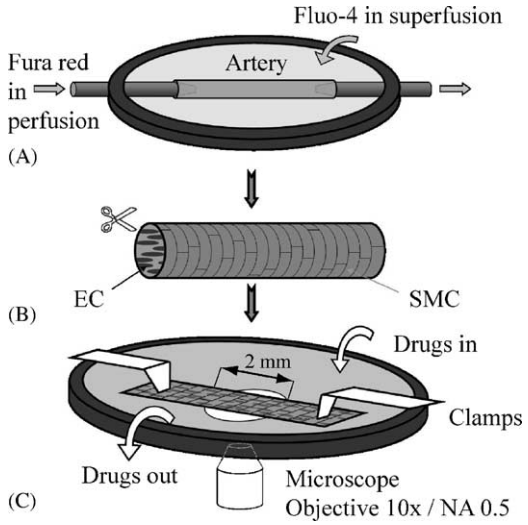

Fig. 1. (A) Rat mesenteric arteries were cannulated at both ends using stainless steel cannulas and perfused at a rate of 50 μl/min with control solution. A 40 μM Fura red AM solution containing 2.2 μl DMSO was added in perfusion for 30 min in order to directly load the ECs. A similar solution of Fluo-4 AM was added for 90 min through the superfusion system to load the SMCs. (B) The loaded artery was then decannulated and gently opened in order to obtain an arterial strip with SMCs and ECs selectively loaded with $[Ca^{2+}]_i$ fluorescent probes. (C) The strip mounted intimal face down in a homebuilt chamber was then ready for fluorescence observations using a confocal microscope. The observation area had a diameter of 2 mm. Gravity led Krebs-Ringer solution and drugs to the chamber perpendicularly to the vessel axis (drugs in). A pump reabsorbed the solutions at the other side (drugs out).