Endothelial Cells Freshly Isolated From Small Pulmonary Arteries of the Rat Possess Multiple Distinct K⁺ Current Profiles

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Abstract. This study demonstrates for the first time that endothelial cells freshly isolated from small pulmonary arteries of the rat, based on their electrophysiological profile, possess two distinct populations of cells. Immunohistochemical staining revealed the presence of both anti-Kv1.5 and anti-Kir2.1 immunoreactivity in the endothelium of small pulmonary arteries. Patch-clamp studies demonstrated that 90% of cells studied exhibited an electrophysiological profile that was characterized by a delayed rectifier K⁺ conductance. However, the remaining 10% of cells studied showed the complete absence of a delayed rectifier K⁺ current and were characterized by an inward rectifier K⁺ conductance. Together these results indicate that endothelial cells isolated from rat small pulmonary arteries possess a heterogeneous population of cells that may be distinguished by their markedly different electrophysiological profiles. These different populations of cells may differ in their control of the resting membrane potential of endothelial cells, and thereby altering Ca²⁺ homeostasis and release of vasoactive compounds. These findings may therefore have important implications for understanding the regulation of pulmonary vascular tone.

Key words: Pulmonary artery—Inward rectifier K⁺ channel—Delayed rectifier K⁺ channel—Endothelial cells freshly isolated—Heterogeneous populations.

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Introduction

In the pulmonary circulation, autonomic nerves, gasses, circulating mediators, and hormones can modulate the contractile state of small pulmonary arteries, \( \sim 200-400 \) \( \mu \)m in diameter, ultimately influencing pulmonary vascular tone [6]. Many of these vasoactive neurotransmitters, humoral stimuli such as endothelin-1 (ET-1) [27, 28], and gasses, such as nitric oxide (NO) [3] and a reduction in arterial PO\(_2\) [24, 32, 42], can all modulate pulmonary vascular tone by regulating K\(^+\) channels. Therefore, K\(^+\) channels appear to play a critical role in the control of pulmonary vascular tone by modulating arterial smooth muscle resting membrane potential \( (E_m) \) and its subsequent effects on the intracellular Ca\(^{2+}\) concentration \( ([Ca^{2+}]_i) \) [18]. Thus, K\(^+\) channel inhibition can lead to membrane depolarization, and activation of voltage-gated Ca\(^{2+}\) channels causing a resultant increase in \([Ca^{2+}]_i\) [18], while K\(^+\) channel activation hyperpolarizes PASMC and inhibits the rise in \([Ca^{2+}]_i\) causing relaxation [3]. The importance of K\(^+\) channels within the pulmonary circulation is further exemplified by the findings that altered K\(^+\) channel function may be involved in the pathogenesis of primary pulmonary hypertension [41].

The role of pulmonary arterial smooth muscle in regulating pulmonary vascular tone is well characterized but the influence of the endothelium is less studied. The vascular endothelium exerts a crucial role in controlling blood vessel diameter since it has the capacity to release vasoactive mediators such as ET-1 and NO [22, 40]. In addition, the effects of many principal neurotransmitters (including norepinephrine, serotonin, and ATP), humoral substances, and respiratory gasses are either influenced by or exert their actions via endothelium-dependent mechanisms [1, 15, 26]. The importance of the endothelium is further demonstrated by findings that it is responsible for release of a pulmonary-selective vasoconstrictor under hypoxic conditions [25] that may be pivotal for sustained hypoxic pulmonary vasoconstriction [37, 38].

The release of many vasoactive substances by the endothelium is determined by the transmembrane influx of extracellular Ca\(^{2+}\) [2, 7], the driving force of which is determined by the endothelial cell \( E_m \). K\(^+\) channels appear to be central to the electrogensis of the resting \( E_m \) of endothelial cells [19], although the identity of the K\(^+\) channels involved remain unclear. Recently, it has been shown that freshly isolated endothelial cells from resistance-sized pulmonary arteries possess a unique electrophysiological profile [11]. This study showed that the macroscopic membrane currents were characterized by the presence of a voltage-dependent outwardly rectifying K\(^+\) \( (K_V) \) conductance. Furthermore, \( K_V1.5 \) channel immunoreactivity has also been observed in pulmonary arterial endothelium [5, 11], suggesting the presence of this channel protein. However, reports also suggest that functional inward rectifier K\(^+\) currents also dominate in some pulmonary arterial preparations [16, 20]. Kamouchi et al., [16], using both RT-PCR and functional data from cultured bovine pulmonary arterial endothelial cells (PAEC), suggested that the molecular identity of the inward rectifier K\(^+\) channel \( (K_{IR}) \) within these cells is probably Kir2.1. K\(^+\) channels present in endothelial cells ultimately appear to mediate endothelium-dependent control of pulmonary vascular tone, like their