432g The Role of Aquaporins-1 in H2o Transport across the Endothelium of the Aorta

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Atherosclerosis is the leading cause of illness and deaths in the United States and the Western world. It involves the accumulation of extracellular lipoproteins in the inner layers of medium and large arteries that develops into lesions that can lead to strokes and heart attacks. This accumulation is often associated with high plasma low-density lipoprotein (LDL) concentrations and its transendothelial transport is the focus of numerous studies. The earliest events of lesion formation involve the passage of plasma proteins such as low-density lipoprotein (LDL) cholesterol across the vascular endothelium into the subendothelial intima. Permeability studies using HRP, EBA or 125I-LDL have shown that the transport of solute across the wall of the artery occurs mainly by pressure-driven convection through rare localized endothelial leaks. Many of these leaks have been associated with the junctions of cells in turnover, although a portion of the transport may also occur via intra-cytoplasmic vesicles. It has therefore become necessary to investigate the details of the water flow that drive the convective transport through the walls of these arteries. Note that the higher the driving pressure (as in chronic hypertension), the greater the expected transendothelial convective plasma proteins transport. The main parameter that characterizes the filtration water flow across tissue is the hydraulic conductivity Lp. It is defined via Starling's 1896 Law of fluid filtration, conceived for microvessels but since used on larger vessels as well, which states that the rate Jv of fluid flow across these walls is proportional to the hydrostatic pressure difference DP minus the osmotic pressure difference DP, or $Jv = LpS (\Delta P - \Delta \Pi)$. Tedgui & Lever 1984, Baldwin &Wilson 1992 measured Lp of rabbit aorta, and we have previously reported Lp measurements of rat aorta and other vessels, taken on each vessel both with intact and subsequently with denuded endothelium. These data allow us to calculate the Lp of the endothelium of these vessels. It is natural to ask whether this Lp value is intrinsic to the endothelium and immutable, or whether it depends on the conditions to which the endothelium has been exposed. Traditionally, one assumes the former, i.e., that the membrane simply reacts passively to hydrostatic and osmotic pressure gradients imposed on it by allowing water to pass through, e.g., the interendothelial junctions. Here we focus on the molecular factors that determine the measured Lp in an attempt to delve further into this question. During the last decade, Nobel laureate Peter Agre and coworkers have identified a family of ubiquitous water channel plasma membrane proteins called aquaporins. Aquaporins play the central role in many biological water transport processes, e.g., in the kidney, yet maintain a very high specificity for water, preventing even the passage of individual protons. In this paper we use immunohistochemistry with polyclonal antibodies to AQP1 and confocal microscopy to show the existence and distribution of aquaporin-1 molecules in the membrane of Sprague-Dawley rat aortic endothelial cells. We use this direct evidence to corroborate our studies using the known aquaporin blocker HgCl2, which reacts with the Cys189 residue situated close to the narrowing of the aquaporin-1 molecule's water pore. These studies show a depression of up 30% in the Lp of excised rat aorta upon the administration of HgCl2 over the pressure range of 60-140 mmHg. The presence of aquaporins in these membranes and their apparent strong contribution to endothelial Lp may suggest an active role in regulating a substantial fraction of the vessel's endothelial hydraulic conductivity and, by implication, its transendothelial convective transport. To begin to investigate this possibility, we perform Western blot analysis to quantify the amount of aquaporin-1 on rat aortic endothelial cells, and to see how this number varies between normotensive and chronically (spontaneously) hypertensive Wistar Kyoto rats. We shall report on these results and whether they indicate active control in response to chronic transmural pressures.