91

Autocrine Role of ATP in Masking Mechanosensitivity of ENaC in Cell-Attached Recordings. He-Ping Ma, Department of Medicine, Division of Nephrology and NRTC, University of Alabama at Birmingham, Birmingham, AL 35294

The mechanosensitivity of ENaC is a matter of controversy. ENaC in lipid bilayers or expressed in Xenopus oocytes can be activated by membrane stretch (*Biophys. J.* 72:1182, 1997 and *Am. J. Physiol.* 275:C1182, 1998). However, activation of ENaC in the rat cortical collecting tubule by mechanical perturbation is shown to be inconsistent (*J. Gen. Physiol.* 107:35, 1996). Since my previous studies have shown that ATP inhibit ENaC in A6 distal nephron cells (*J. Am. Soc. Nephrol.* 7:1284, 1996), an autocrine mechanism of ATP is hypothesized to be simultaneously activated by membrane tension and play a role in masking mechanosensitivity of ENaC. To test this hypothesis, cell-attached recordings were established in A6 cells. Consistent with the results from the rat cortical collecting tubule, ENaC activation by negative pressure was seen in only 3 out of 9 cell-attached patches. The mean P_o (open probability) was not significantly changed ($P > 0.5$). However, negative pressure stimulated ENaC activity in all 7 cell-attached patches after trapping ATP in the patch pipettes with 10 U/ml hexokinase plus 5 mM glucose; the mean P_o was increased, from 0.38 ± 0.19 to 0.67 ± 0.28 ($P < 0.01$). Furthermore, low mean P_o of ENaC (0.09 ± 0.08, $n = 5$) was recorded when patch pipettes contained 200 μM ATP. These data together suggest that ENaC is mechanosensitive, but ATP acts as a "mask" to its mechanosensitivity via an autocrine mechanism.

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92

INTERLEUKIN-1 INCREASES VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) GENE EXPRESSION IN ALVEOLAR EPITHELIAL CELLS

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VEGF is a secreted endothelial mitogen and potent endothelial permeability factor that is highly expressed in lung, particularly within respiratory epithelial cells. Acute lung injury (ALI) is a disorder characterized by increased endothelial permeability in the lung. We postulated that alveolar epithelial VEGF gene expression is increased in ALI in response to the elevation in proinflammatory cytokines characteristic of ALI. We evaluated VEGF gene expression in human A549 alveolar epithelial cells in vitro after stimulation for 6 hr with IL-1, TNF, and TGF beta under normoxic conditions as a test of this hypothesis. Total RNA was isolated, size fractionated on agarose gels, and VEGF expression was quantitated by densitometric analysis of bands on Northern blots. Results were analyzed by paired t-test comparing mean density of VEGF bands normalized to beta-actin of 3 paired experiments. We found that IL-1 increased VEGF RNA expression by a factor of 1.6 over vehicle control (p less than .05). We conclude that at least one proinflammatory cytokine increases VEGF expression in alveolar epithelium. Increased VEGF release by alveolar epithelium may promote endothelial leak in ALI. Supported by: NIH K08 HL035454-03 (JM)

93

IMAGING DOCUMENTATION OF ARRESTED LYMPHATIC DEVELOPMENT IN ANGIOPOIETIN-2 KNOCKOUT MICE. C. Martin, C. Suri, M. Wilte, C. Wilte, G. Yancopoulos. Dept. of Surgery, U. of AZ, Tucson, AZ and Regeneron Pharmaceuticals, Inc., Tarrytown, NY.

The mammalian lymphatic system (LS) arises from distinct, anatomically separate primary lymph sacs, which only later link up to form continuous collecting trunks transporting lymph through interposed lymph nodes back to the central venous system. Controversy persists, however, about the embryonic origin of lymphatic endothelium, whether budding from preexisting veins (centrifugal theory) or de novo from tissue mesenchyme (centripetal theory). Whereas VEGF and angiopoietin families of growth factors and their receptor ligands are clearly implicated in blood vessel formation ("hemangiogenesis"), molecular control of "lymphangiogenesis" remains poorly understood. Recently, chylous ascites (triglyceride rich intestinal lymph free in the peritoneal cavity) accompanied by intestinal lymphangiectasia has been observed in newborn angiopoietin-2 knockout (KO) mice (Suri and Yancopoulos, unpublished observation). To dynamically image LS abnormalities, Evans blue dye (EBD) was injected intradermally under ketamine anesthesia in the dorsum of both hindpaws of wild type (+/+) or heterozygous (+/-) and angiopoietin-2 KO (-/-) neonatal mice (~2.3-4.6 g). Under a dissecting microscope, EBD-stained streamers and lymph nodes were examined from the hindlimbs to the cisterna chyli and thoracic duct. In 8/8 +/+ or +/-, no pleural or peritoneal fluid was detected, and EBD-stained lymphatics and lymph nodes were progressively visualized. In contrast, in 7/7 -/-, milky peritoneal and/or pleural fluid was found, and no EBD-stained hindlimb or abdominal lymphatic collectors or nodes could be identified. Thus, angiopoietin-2 appears to play an important role in murine LS development and may be involved in analogous human structural/functional anomalies of the peripheral and visceral LS associated with fetal demise and serious birth defects.

94

SYSTEMIC VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF-1) INCREASES RAT INTESTINAL TRANSCAPILLARY (LYMPHATIC) WATER AND PROTEIN FLUX BY ENHANCING FILTERING SURFACE AREA. C. Martin, M. Wilte, M. Bemas, D. Way, F. Travis and C. Wilte. Dept. of Surgery, U. of AZ, Tucson, AZ 85724.

VEGF-1 ($VEGF_{165}$), a dimeric glycoprotein angiogenic cytokine, was originally described as Vascular Permeability Factor (VPF) for its potent enhancement of local capillary fluid leakage. To elucidate further the underlying mechanism and relative participation of enhanced permeability (P) vs. increased filtering surface area (S) in VEGF's elevation of the PS product, we followed transcapillary water (Jv) and protein (Jp) fluxes by serially determining lymph flow and total protein (TP) concentration (refractometry) in anesthetized Sprague-Dawley rats with indwelling intestinal lymphatic cannulae. Before and up to 120 min after a single IV injection of 100 μg recombinant human VEGF-1 or control (saline or vehicle) and then after acute superior mesenteric vein (SMV) constriction (hypertension), lymph was serially collected in 10 min volumes (Jv in ml/10 min). Jp (mg/10 min) was calculated as Jv x TP (mg/ml). RESULTS (Mean±SE): Table [% Baseline (=100% at 0 min)]:

Group	(n)	Flux	30 min	60 min	90 min
VEGF	(5)	Jv	462 ± 79.0%	181 ± 26.3%	120 ± 20.8%
		Jp	582 ± 86.8	212 ± 35.8	141 ± 18.6
Control	(7)	Jv	77.9 ± 14.6	56.9 ± 12.2	40.9 ± 17.6
		Jp	97.6 ± 20.6	68.1 ± 22.7	56.2 ± 20.9

VEGF-1 markedly increased Jv by 30 min ($p<0.001$), after which Jv steadily declined to baseline. As lymph TP was unchanged and protein fractions unaltered, Jp showed a similar response ($p<0.001$). Intestinal capillary integrity was maintained as evidenced by prompt lymph protein dilution or "washdown" (increased Jv with unchanged Jp) during SMV hypertension. Thus, VEGF, like cytokines IL-2 and TNF, exerts its microcirculatory action primarily by expanding capillary filtering surface area (S), thereby raising the PS product and equivalently enhancing transcapillary movement of both water and protein.

95

IN VITRO INHIBITION OF GLIAL CELLS BY ADCON-L. M Moveddat, DL Way, AJ Hamilton, (SPON: MH Witte)

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Glioma is the most common major subgroup of primary nervous system tumors. Frequent dissemination through the central nervous system is a common feature of gliomas. Anti-invasive therapy would allow effective local therapies, such as surgery and irradiation, to provide local control while limiting the infiltration of the glioma cells beyond the reach of these modalities. We have investigated the role of ADCON-L, a resorbable anti-adhesion barrier gel, in inhibiting glioma invasiveness by examining it's effects on glial cell migration. Rat C6 glioma cells were obtained and propagated in monolayer cultures. C6 cells were allowed to adhere to the bottom half of tissue culture plates and were allowed to migrate to the top half of the plates with or without an ADCON-L coating. Images were captured at 48, 96 and 144 hours. Our results indicate that in the presence of ADCON-L, C6 cells freely migrate to the ADCON-L coating interface, and not beyond. In the absence of ADCON-L (control), C6 cells migrate without resistance to the top of the tissue culture plates. These results highlight the inhibitory role of ADCON-L on glial cell migration and suggest that it may serve as a therapy in future strategies at blocking invasion by gliomas.

96

DIFFERENCES BETWEEN ALVEOLAR MACROPHAGES AND CELL LINES IN ACTIVATION OF MITOGEN-ACTIVATED PROTEIN KINASES (MAPKs) STIMULATED BY LIPO-POLYSACCHARIDE (LPS). T. Meighan and K.M.K. Rao. PPRB/HELD/NIOSH, Morgantown, WV 26505.

We have shown previously that the lung surfactant inhibits LPS-stimulated nitric oxide (NO) production in alveolar macrophages (Miles et al., Am.J.Physiol. 276:L186, 1999). But lung surfactant had no effect on NO production in two macrophage cell lines RAW 264.7 and J-774. Stimulation of primary macrophages and cell lines by LPS has been shown to activate MAPKs. To determine if differences in MAPK activation may explain the difference in response to lung surfactant between primary macrophages and the cell lines, we compared the activation of MAPKs, p38, extracellular-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK/SAPK), in rat alveolar macrophages and three macrophage cell lines, RAW 264.7, J-774, and NR8383. In alveolar macrophages there was basal phosphorylation of p38 and ERK which did not significantly increase on LPS stimulation. There was no detectable JNK phosphorylation in unstimulated or LPS-stimulated alveolar macrophages. In contrast, in all three cell lines LPS induced a significant increase in phosphorylation of p38. Differences in activation of MAP kinases may determine the response to lung surfactant.