33

NOX1 IS REQUIRED FOR SMOOTH MUSCLE CELL ACTIVATION OF MATRIX METALLOPROTEINASE 9 AND CELL MIGRATION IN RESPONSE TO THROMBIN. J.K. Dammanahalli, R.C. Bhalla, B. Banfi, F. Miller, University of Iowa, Iowa City, IA.

Smooth muscle cell (SMC) degradation of the extracellular matrix and migration to the intima are fundamental processes in the vascular response to injury. NADPH oxidase–derived reactive oxygen species (ROS) are involved in development of vascular disease; however, the specific contribution of Nox1 and Nox4, the primary catalytic subunits of NADPH oxidase in SMC, is poorly understood. We hypothesized that Nox1-derived ROS mediate thrombin-dependent activation of matrix metalloproteinase 9 (MMP-9) and migration of SMCs. Studies were performed in SMCs cultured from the aorta of Nox1 null and littermate control mice. Thrombin (2 U/mL) increased superoxide levels in control SMCs, as measured by dihydroethidium, and this response was inhibited by the flavoenzyme inhibitor diphenylene iodonium (DPI, 10 μM). In contrast, thrombin failed to increase ROS in Nox1 null SMCs. Previous studies have identified Src and mitogen-activated protein kinases as key redox-dependent regulatory proteins in thrombin-stimulated responses. Five minutes following thrombin stimulation, both Src and ERK1/2 phosphorylation were significantly decreased in Nox1 null SMCs compared with normal SMCs, measured by densitometry of Western blots. In addition, in response to thrombin, epidermal growth factor receptor (EGFR) phosphorylation was reduced in Nox1 null VSMCs. Conditioned media was collected 24 hours after cells were treated with thrombin and MMP-9 activity measured by gelatin zymography. Thrombin increased MMP-9 more than twofold in control cells; however, thrombin failed to increase MMP-9 activity in Nox1 null cells. Using a wound-scratch assay, the number and distance of cells migrating into the injured area were markedly reduced in SMCs deficient in Nox1. In conclusion, the Nox1 subunit of NADPH oxidase is required by SMCs for thrombin-dependent activation of MMP-9 and cell migration. In addition, Nox1 generation of ROS participates in phosphorylation of Src and of ERK1/2. These findings suggest that Nox1 may play an important role in the pathogenesis of vascular disease.

34

ALTERATIONS AT THE NEUROEFFECTOR JUNCTION OF PERIARTERIAL AND PERIVENOUS NERVES IN A SALT-DEPENDENT MODEL OF HYPERTENSION. S.L. Demel, J.J. Galligan, Michigan State University, East Lansing, MI.

About 33% of American adults have hypertension. However, we can only identify and therefore target an underlying cause in 5 to 10% of cases. Although suboptimal blood pressure may be caused by many contributing factors, sympathetic nervous system activity is increased in human and animal models of hypertension. Norepinephrine and ATP are coreleased from sympathetic nerve endings onto arteries and veins, maintaining vascular tone and controlling blood pressure. Importantly, periarterial and perivenous nerves originate from different neurons in prevertebral ganglia, and these nerve fibers have neurochemical and anatomic differences. There are also differences in the adrenergic and purinergic receptor subtypes found on arterial and venous smooth muscle cells. Studies in our laboratory and others have shown that in animal models of hypertension, increased neurotransmitter release and increased sensitivity of smooth muscle cells in veins precede changes in arteries. In the present work, immunohistochemistry, contractility studies, and electrophysiologic techniques were used to study these differences between arteries and veins. To explore changes occurring at the neuroeffector junction in hypertension, we used the deoxycorticosterone acetate (DOCA)-salt model in rats. Mesenteric arteries and veins maintained in vitro were used. Perivascular nerves were stimulated electrically. These studies provide insight into (a) differential regulation of resistance arteries and capacitance veins, (b) the neurotransmitters involved in regulating vascular tone, and (c) alterations in the neuroeffector junction in salt-dependent hypertension. Data from frequency-response curves (0.2–30 Hz) confirmed that veins are more sensitive to nerve stimulation than arteries ($p < .05$), but there were no differences in responses between DOCA-salt and sham arteries or veins ($n = 4$–9, $p > .05$). Electophysiologic studies showed that the amplitude of excitatory junction potentials (EJPs) mediated by ATP were not different in sham and DOCA arterial smooth muscle cells. Clonidine (an α_2-adrenergic receptor agonist that stimulates prejunctional autoreceptors) caused a concentration-dependent inhibition of EJPs in sham and DOCA-salt arteries, but EJPs in DOCA-salt arteries were less sensitive to inhibition by clonidine ($p < .05$). A better understanding of neurotransmitters released at the neuroeffector junction and changes that occur in a salt-dependent model of high blood pressure may provide novel pharmaceutical targets for hypertension in the future.

35

***BACILLUS ANTHRACIS racE1* AND *racE2* ENCODE FUNCTIONAL GLUTAMATE RACEMASES WITH DISTINCT PROPERTIES.** D. Dodd, J.G. Reese, C.R. Louer, J.D. Ballard, M.A. Spies, S.R. Blanke, University of Illinois, Urbana-Champaign, Urbana, IL; Oklahoma City, OK.

Bacillus anthracis synthesizes two complex structures, a peptidoglycan cell wall and poly-γ-D-glutamic acid (PDGA) capsule, which require an accessible pool of D-glutamate. The mechanisms, however, underlying the establishment of accessible pools of D-glutamate for *B. anthracis* are poorly understood. *B. anthracis* harbors two genes, *racE1* and *racE2*, which are each predicted to encode a glutamate racemase capable of converting L-glutamate to D-glutamate. However, the respective roles, if any, of RacE1 or RacE2 in catalyzing the racemization of D-glutamate to L-glutamate have not been investigated. The objective of this study was to compare the in vitro properties of the *racE1* and *racE2* gene products, with the explicit purpose of establishing whether either or both of these proteins have the capacity to catalyze the conversion of L-glutamate to D-glutamate. *racE1* or *racE2* were cloned from *B. anthracis* Sterne 7702 and expressed as recombinant proteins in *Escherichia coli.* Each protein was purified to homogeneity. We developed an assay based on circular dichroism for directly monitoring glutamate racemase activity. Both RacE1 and RacE2 exhibited detectable glutamate racemase activity. Characterization of both enzymes revealed similar pH profiles and a lack of dependency for various metal cofactors. However, the catalytic efficiency (k_{cat}/K_M) of RacE2 was twice that of RacE1. In addition, RacE2 exists in solution as a dimer, whereas RacE1 exists primarily as a monomer. RacE1 forms a higher-ordered complex in the presence of L-glutamate, whereas the quaternary structure of RacE2 is largely independent of substrate. Collectively, these data indicate that although both *racE1* and *racE2* encode proteins that catalyze the racemization of L-glutamate to D-glutamate in vitro, differences in the properties of these two enzymes suggest that these two enzymes may have distinct cellular roles.

36

RENIN-ANGIOTENSIN SYSTEM ACTIVATION IS ASSOCIATED WITH IMPAIRED MICROVASCULAR RESPONSES AND ORGAN DYSFUNCTION IN HUMAN SEPSIS. K. Doerschug, A. Delsing, A. Ashare, University of Iowa, Iowa City, IA.

Severe sepsis is a systemic response to infection that results in organ dysfunction. Microvascular dysregulation characterized by hyporesponsive and heterogeneous blood flow is implicated in the pathogenesis of organ failure. Although the renin-angiotensin system (RAS) has known effects on the microvasculature and is activated in sepsis, the relationships between RAS and organ injury in human sepsis remain unclear. We hypothesized that systemic RAS mediators are associated with microvascular hyporesponsiveness and organ dysfunction in human sepsis. We studied 30 severe sepsis subjects, and healthy volunteers served as controls. Septic subjects were studied 24 hours after the initial recognition of organ dysfunction; 12 subjects were enrolled such that an initial study was also performed 8 hours after organ dysfunction. Plasma was analyzed for plasma renin activity (PRA) and angiotensin II (Ang II) concentration. Organ failure was assessed quantitatively with the Sequential Organ Failure Assessment (SOFA) score. Using near-infrared spectroscopy, we measured the rate of increase in the oxygen saturation of microvascular hemoglobin in thenar muscles after 5 minutes of forearm ischemia. In so doing, we assessed bulk arteriolar and capillary hemoglobin influx to the tissue during reactive hyperemia. At 8 hours, Ang II was markedly elevated and significantly correlated with the extent of organ failure in septic subjects. After 24 hours of resuscitation to achieve clinically defined end points of preload and arterial pressure, Ang II remained elevated in septic subjects. There was a strong linear relationship between PRA and Ang II in the systemic circulation. Notably, both PRA and Ang II significantly and negatively correlated with the rate of microvascular reoxygenation during reactive hyperemia in septic subjects. We conclude that RAS is activated in severe sepsis, and systemic RAS mediators correlate with measures of microvascular dysregulation and organ failure.

37

AN ACUTE LUNG INJURY–ASSOCIATED CORTACTIN POLYMORPHISM ALTERS PULMONARY ENDOTHELIAL CELL BARRIER FUNCTION. S. Dudek, S. Camp, S. Kunznetsov, S. Ma, J. Garcia, University of Chicago, Chicago, IL.

Introduction: Acute lung injury (ALI) syndromes are highly morbid consequences of systemic inflammatory conditions such as sepsis. Inflammation-induced disruption during ALI of the endothelial cell (EC) barrier that lines the pulmonary vasculature results in leakage of fluid, protein, and cells into the airspaces of the lung, resulting in respiratory failure. We have previously described (Dudek et al. J Biol Chem 2004;279:24692–700) a critical role for the actin-binding protein cortactin in mediating EC cytoskeletal rearrangements that regulate in vitro barrier function. **Methods/Results:** We now report immunofluorescence and coimmunoprecipitation data demonstrating increased association of cortactin with the junctional proteins β-catenin, focal adhesion kinase, and vinculin during the recovery phase following thrombin-induced permeability. Thus, cortactin interacts at critical cell-cell and cell-matrix junctional sites as the EC monolayer recovers after inflammation-induced disruption. As a translational approach building upon these in vitro observations, single-nucleotide polymorphism (SNP) discovery of the cortactin gene was performed using direct fluorescence-based resequencing. Cortactin SNP discovery in 36 patients, subdivided into those with sepsis-induced ALI, sepsis alone and healthy controls, identified 26 SNPs within the human cortactin gene, including a single novel coding variant. This SNP at amino acid position 484 results in a serine to asparagine change (Ser484Asn), which was enriched in patients with ALI and sepsis compared with controls. Since this S484N site is in close proximity to a critical p60src-targeted tyrosine residue (Y486), a major regulatory site of cortactin function, we generated wild-type and S484N mutant constructs for overexpression in human pulmonary ECs. These studies revealed greatly increased phosphorylation of cortactin at Y486 in the S484N mutant under baseline and EC barrier-altering conditions. In addition, overexpression of S484N in ECs inhibited the potent barrier-promoting effects of sphingosine 1-phosphate as well as delayed barrier recovery after the barrier disruption by thrombin. **Summary:** Cortactin plays a critical role in mediating cytoskeletal and junctional protein rearrangements that regulate EC barrier function. Given that phosphorylation of Y486 modulates cortactin function, the S484N cortactin SNP may increase ALI susceptibility via this mechanism.

38

KRUPPEL-LIKE FACTOR 15 IS A NOVEL REGULATOR OF CARDIOMYOCYTE HYPERTROPHY. S. Fisch, S. Gray, S. Heymans, S. Haldar, B. Wang, Y. Zhu, R. Liao, Y.M. Pinto, M.K. Jain, Cleveland, OH; Maastricht, the Netherlands; Brigham and Women's Hospital, Boston, MA.

Cardiac hypertrophy is a common response to injury and hemodynamic stress and an important harbinger of heart failure and death. Herein we identify Kruppel-like factor 15 (KLF15) as a novel inhibitor of cardiac hypertrophy. Myocardial expression of KLF15 is reduced in rodent models of hypertrophy and in biopsy samples from patients with pressure overload induced by chronic valvular aortic stenosis. Overexpression of KLF15 in neonatal rat ventricular cardiomyocytes inhibits cell size, protein synthesis, and hypertrophic gene expression. KLF15-null mice are viable but, in response to pressure overload, develop an eccentric form of cardiac hypertrophy characterized by increased heart weight, exaggerated expression of hypertrophic genes, left ventricular cavity dilatation with increased myocyte size, and reduced left ventricular systolic function. Mechanistically, a combination of promoter analyses and gel-shift studies suggests that KLF15 can inhibit GATA4 and MEF2 function. These studies identify KLF15 as part of a heretofore unrecognized pathway regulating the cardiac response to hemodynamic stress.

39

MANIC DISTRACTIBILITY AND FRONTOSUBCORTICAL PROCESSING. D.E. Fleck, J.C. Eliassen, M. Durling, J. Adams, M. Lamy, K. Williams, S.M. Strakowski, University of Cincinnati, Cincinnati, OH; Notre Dame, IN.

The aim of this research was to examine state-related neurocognitive changes to advance understanding of the neurophysiology and treatment of bipolar disorder. Specifically, we examined the temporal (time limited) brain dynamics of sustained attention in relation to mood state. The central hypothesis was that the dorsolateral prefrontal cortex is "recruited" to maintain sustained attention in bipolar disorder owing to subcortical brain abnormalities. Sustained attention decrements were predicted when distractibility was increased by mania, which interferes with limited channel capacity (executive) control processes of prefrontal cortex. To test this hypothesis, we examined differences in the temporal dynamics of prefrontal-striatal-thalamic brain circuit activation during a continuous performance test (CPT) using high-field (4 Tesla) functional magnetic resonance imaging (fMRI). We examined chronometric changes during the simultaneous acquisition of fMRI and CPT data in three separate groups of manic bipolar, euthymic bipolar, and demographically similar healthy comparison subjects. Preliminary data suggest that manic distractibility has a mediating influence between the neurophysiologic consequences of bipolar disorder and information processing efficiency. Clinically, the results indicate that neural reorganization may allow patients with bipolar disorder to learn or enhance compensatory strategies in support of efficient information processing and better functional recovery. Although neuropsychological and anecdotal evidence suggests that this is indeed the case, to our knowledge, these are the first corroborating neuroimaging data pertaining to the spatial and temporal dynamics of neural reorganization in bipolar diorder.

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