The Effects of the Rf1 and Rf-2/Bpfh1 Region from the FHH Rat on Renal Damage Susceptibility, Blood Pressure and Renal Autoregulation

Abraham P Provoost, Sabine J van Dijk, Patricia A Specht, Ericus MC, Rotterdam, The Netherlands; Jozef Lazar, Howard J Jacob; Med College of Wisconsin, Milwaukee, WI

Susceptibility to renal damage and hypertension of the Fawn Hooded Hypertensive (FHH) rat have been found to be linked to five renal failure QTLs (Rf1 to Rf5) and two blood pressure QTLs (Bpfh1 and -2). The Rf1, Rf2 and Bpfh1 QTLs are in close vicinity on rat chromosome 1. To directly determine the effects of these QTLs, congenic strains were generated carrying the QTLs from FHH on the genomic background of the normotensive renal resistant ACI strain. Here we compare the effect on systolic blood pressure (SBP) and renal damage susceptibility in ACI.FHH(Rf1+/Rf1-Rf2) double congenic rats to that of ACI.FHH(Rf1-Rf2), single congenics and the ACI and FHH parental strains. Albuminuria (UAV) and SBP were regularly assessed in rats following unilateral nephrectomy (UNX) at 5–7 weeks of age. In addition, studies the efficacy of renal blood flow autoregulation was measured in intact two-kidney rats of all four strains. At the end of an 18-week follow-up period UAV and SBP in the Rf1+/Rf2 double congenic rats was significantly higher compared to ACI and Rf1 single congenic rats, but significantly less compared to the double congenic strain carrying the QTLs (Rf1−Rf2) double congenic is still far below that of the parental FHH rat indicating that the other three QTLs impair renal autoregulation was measured in intact two-kidney rats of all four strains. At the end of an 18-week follow-up period UAV and SBP in the Rf1+Rf2 double congenic rats was significantly higher compared to ACI and Rf1 single congenic rats, but significantly less compared to the double congenic strain carrying the QTLs (Rf1−Rf2) double congenic is still far below that of the parental FHH rat indicating that the other three QTLs impair renal autoregulation.

Angiotensin II AT2 Receptor Agonist Inhibits Proximal Tubular Na+, K+ -ATPase via a NO/cGMP Pathway in Obese Zucker Rats

Amer C Hakam, Tahir Hussain; Heart and Kidney Institute, Univ of Houston, Houston, TX

Recently, we have shown that the renal ANG II AT2 receptors are upregulated and are involved in promoting natriuresis/diuresis in obese Zucker rats, a genetic model of obesity associated with cardiac resistance. However, the mechanism involved in the ANG II-mediated natriuresis/diuresis in obese Zucker rats remains unknown. We have recently shown that the sole transfer of the Rf1 QTL from FHH to ACI impairs renal autoregulation, but has only small effects on UAV following UNX. Additional transfer of the Bpfh1/QTL increases SBP and markedly enhances susceptibility to renal damage following UNX. Whether the increased renal susceptibility is due to the transfer of the Bpfh1 region, or to the SBP remains to be established. However, renal susceptibility in the (Rf1+Rf2) double congenic is still far below that of the parental FHH rat indicating that the other three QTLs have will have additional effects to further enhance renal susceptibility.

Decreased Angiotensin II Pressor Response and Increased Hypotensive Effect of Bradykinin In Transgenic Rats Expressing an Angiotensin-(1–7)-producing Fusion Protein

Antonela Murari, Sergio H Santos, Anderson J Ferreira, Federal Univ of Minas Gerais, Belo Horizonte, Brazil; T. L. Reudelhuber, Laboratory of Molecular Biochemistry of Hypertension, Clinical Research Institute of Montreal, Quebec, Canada; M. Bader, MDC, Berlin, Germany; Maria J Santos, Robson A Santos, Federal Univ of Minas Gerais, Belo Horizonte, Brazil

Acute Angiotensin(Ang)-(1–7) administration attenuates the angiotensin II effect and potentiates bradykinin in several preparations. However, the effects of chronic increases in Ang-(1–7) on the cardiovascular effects of angiotensin II and bradykinin are unknown. We have recently engineered a transgenic rat expressing an Ang-(1–7)-producing fusion protein (TGR (A1–7−L3292). Plasma levels of Ang-(1–7) are significantly increased in these animals. In this study we determined the angiotensin II (Ang II) pressor response and the hypotensive effect of bradykinin (BK) in L3292 rats. Female rats were anesthetized with triethanolamine (2.5%, 1ml/100g), and polyethylene catheters were introduced into the femoral artery for mean arterial pressure measurement, in the femoral vein for administration of Ang II or BK saline, and descending aorta (through the carotid artery) for administration of BK or saline. Increasing doses of Ang II (0.25–10 ng, iv); BK (0.25 - 2.0 ug, iv; 5–40 ng, i.a) were administered in TGR L3292 female rats and age-matched female Sprague-Dawley rats (SD). The angiotensin II pressor response was significantly attenuated in L3292 rats in comparison to SD rats (fig 1). In contrast the hypotensive effect produced by intravenous BK was significantly augmented in L3292 rats in comparison to SD rats (fig 2). Similar differences were observed for intra-arterial administration of BK. These data indicate that chronic increases of circulating Ang-(1–7) are associated with significant changes in the vascular reactivity to vasodepressor peptides favoring vasodilatation.

Role of Estrogen and Prognostrogen in Modulating Tumor Necrosis Factor Alpha-induced Increases in Blood Pressure in Pregnant Rats

Babbette B LaMarca, Lee Grubbs, Jennifer Bain, Kathy Cockrell, Michael J Ryan, Joey P Granger; Univ of Mississippi Med Ctr, Jackson, MS

Hypertension during preeclampsia is associated with an increase in plasma levels of tumor necrosis factor alpha (TNF-α), a cytokine known to contribute to endothelial dysfunction. We previously reported that chronic infusion of TNF-α at concentrations mimicking plasma levels in preeclamptic women, increased blood pressure and decreased renal function in pregnant rats. In sharp contrast, TNF-α had no effect in virgin rats, although previous results from in vitro studies suggest that sex steroids may influence the vascular actions of TNF-α, the role of estrogen (E) and/or progesterone in modulating the blood pressure responses to TNF-α during pregnancy are unknown. Therefore, the purpose of this study was to determine the influence of female sex steroids in modulating the blood pressure effects of TNF-α. To achieve this goal, we compared the long-term blood pressure effects of TNF-α (50ng/day for 5 days), in virgin rats, normal pregnant (NP) rats, and ovarietomized (OVX) rats chronically treated with 17B-estradiol (E) and/or progesterone (P). Pellets of E and P were implanted in rats to increase plasma steroids to levels observed during late gestation. Ovariectomized rats received 21 day E and P release pellets. Minimicros pumps containing TNF-α were placed in rats on day 14 of pellet administration. Chronic infusion of TNF-α into normal pregnant rats increased blood pressure by 21 mm Hg (122 ±1 mmHg, NP + TNF vs 101 ± 1 mmHg, NP). In contrast, TNF-α had no effect in virgin rats (125 ±3 mmHg, V + TNF vs 122 ±2 mmHg, V). Moreover, TNF-α had no effect on blood pressure in progestrone-treated rats (130 ±4 mmHg, P + TNF vs 129 ±4 mmHg, P), estrogen-treated rats (122 ±7 mmHg, E + TNF vs 107 ±5 mmHg, E), or estrogen and progesterone treated rats (124 ±4 mmHg, E + P + TNF vs 118 ±3 mmHg, E + P). We conclude that sex steroids blunted the blood pressure response to TNF-α during pregnancy is not likely due to modulation of the vascular responses to TNF-α by sex steroids.

Blunted Development of Salt-sensitive Hypertension Associated with Decreased Responsiveness to Central Na+ in a Congenital Strain of Dahl Salt-sensitive (S) Rats

Bing S Huang, Univ of Ottawa Heart Institute, Ottawa, Canada; Alan Y Deng, Univ of Montreal, Montreal, Canada; Frans H Leenen; Univ of Ottawa Heart Institute, Ottawa, Canada

 Compared to Dahl R, Dahl S rats show on high salt diet increased Na+ entry into the cerebro-spinal fluid (CSF) and brain and increased sympathetic and pressor responses to CSF [Na+] . In the congenic strain, C10SL1c, a segment of Chromosome 10 of the Dahl S rat is replaced with a homologous segment of the Lewis rat. Dahl S rats have a blood pressure lower than that of Dahl S rats, indicating the presence of a quantitative trait locus (QTL) in the interval of D10RatRat204 and D10Rat9, C10LQTL2. The present study was designed to test whether this QTL could be responsible for enhanced responsiveness to CSF Na+ , and [Na+] in CSF in response to a high salt diet. At 2–6 wk of age, Dahl S (n=7), Lewis (n=6), and C10SL1c (n=6) were fed high salt (8% NaCl) for 8–10 days, and the resting mean arterial pressure (MAP) and HR were recorded and CSF withdrawn from the cisterna magna. In another set of conscious rats (n=6–8) on regular diet, MAP, HR and renal sympathetic nerve activity (RSNA) were recorded at rest and in response to intra-cerebroventricular (icv) infusion of artificial CSF (aCSF) and Na-rich aCSF containing 0.2, 0.3 and 0.45 M Na. Late Breaking Presentations

Late Breaking Presentations
Effect of Chronic Intermittent Hypoxia on Renal Pressure-Natriuresis in Female Rats

Carmen Hinjosa-Laborde, Jaci A Castania, Patricia M de Paula, Steven W Miffvin; Univ of Texas Health Science Ctr, San Antonio, TX

We have shown that chronic intermittent hypoxia (CIH) increases blood pressure in male, but not female rats. This protection in females is dependent on female sex hormones because ovarioectomized (OVX) females responded to CIH with an increase in blood pressure similar to males. We have evidence that CIH in males suppresses the pressure-natriuresis/diuresis relationship, and is associated with volume and sodium dysregulation. In this study we investigated the effect of CIH on the renal pressure-natriuresis/diuresis relationship in females and OVX females. OVX surgery was conducted 4 weeks prior to exposure to CIH. Rats were placed in enclosed chambers and exposed to CIH for 7 days. CIH is defined as continuous cycles of 3 minutes of room air (21% O2) and 3 minutes of 10% O2 for 8 hours. Control animals breathed room air 24 hr/day. The relationship between renal perfusion pressure (RPP) and sodium excretion and urine flow were determined under trachea anesthesia in normoxic-female (N), CIH(N), normoxic-OVX (OVX), and CIH-OVX rats. Results are mean ± SEM, * indicates difference compared to respective N group (p < 0.05). Exposure to CIH significantly increased sodium excretion and urine flow in female rats, but had no effect in OVX females. This effect of CIH in females is opposite to previous observations in males. We conclude that the CIH-enhanced sodium excretion is sex-dependent, and this effect is dependent on the presence of female sex hormones. We speculate that female sex hormones facilitate renal sodium flow and water excretion during CIH and act to protect elevations in blood pressure associated with CIH.

Effect of Antihypertensive Treatment on Remodeling of Resistance Arteries in Type 2 Diabetic Hypertensive Patients

Carmine Savioa, Rhiy M Touyz, Dierk Endemann, Qian Pu, Eun A Ko, Carolina DeCucuise, Emesto L Schirrvin, RCM, Montreal, Canada

Normalization of elevated blood pressure (BP) in diabetic hypertensive individuals improves survival and decreases cardiovascular events. We questioned whether remodeling of resistance arteries from hypertensive diabetic patients may improve after one year of tight BP control with addition of either the angiotensin receptor blocker (ARB) valsartan or the beta-blocker (BB) atenolol on top of previous therapy. Twenty-eight hypertensive type 2 diabetic patients (30 to 70 years of age) treated with oral hypoglycemic and antihypertensive agents (that did not include an ARB or a BB) were randomized to double-blind treatment with valsartan (80–160 mg) or atenolol (50–100 mg) once daily, added to previous therapy for one year. Ten normal subjects were studied as a control group. Resistance arteries dissected from gluteal subcutaneous tissues were assessed on a pressurized myograph. After one year of treatment, systolic and diastolic BP were well controlled by valsartan (128 ± 7 mmHg vs 144 ± 3 mmHg, p = 0.002) and atenolol (133 ± 8 mmHg vs 144 ± 3 mmHg, p = 0.006), respectively. Glycemic control was similar in the valsartan and atenolol-treated groups (HbA1c 0.06 ± 0.004 vs 0.06 ± 0.002). Endothelium-dependent and independent relaxation did not change significantly. We observed that L-NAME, L-NMMA, L-arginine, and 1-hydroxyethylxanthine-4-oxide added to the organ bath did not induce further endothelial dysfunction. A significant reduction in the stress–strain relationship was observed in the treated groups between the beginning and end of the study. L-NAME significantly reduced sodium excretion and urine flow in female rats, but had no effect in OVX females. This effect of CIH in females is opposite to previous observations in males. We conclude that the CIH-enhanced sodium excretion is sex-dependent, and this effect is dependent on the presence of female sex hormones. We speculate that female sex hormones facilitate renal sodium flow and water excretion during CIH and act to protect elevations in blood pressure associated with CIH.
from arterial segments of rat MBA by limited collagenase digestion and plated in Ham’s F12 nutrient mixture plus 10% horse serum on a cover glass coated with poly-L-ornithine. The culture medium was replaced with Ham’s F12 nutrient mixture plus N2 supplement after 48 h. This resulted in a mixed population of fibroblasts (20%), a small number (10–20%) of SMC that stained positively for smooth muscle myosin and cells (60%) with sprouted axons that expressed CGRP (ANNIES). The cells and MBA segments were fixed in buffered formalin and co-immunostained with anti-palladin and anti-CGRP using secondary antibodies tagged with alexa fluor 488 and 647, as well as the nuclear stain sytox. Confocal analysis showed that cultured ≥80–90% ANNIES and 100% of all SMC had positive staining for palladin (n = 3). Palladin was also co-localized with CGRP in nucleated structures of rat MBA (n = 3). Immuno blot from MBA confirmed the presence of palladin as two isoforms of 90–92 and 140 kDa (n = 3). This protein pattern is different from other adult tissues such as brain that expresses three isoforms (80–92, 140 and 250 kDa) or SMC where only one isoform (90–92 kDa) is expressed. In summary, palladin is present both in SMC and ANNIES, either in culture or in the rat MBA. This is the first report that cells in the peripheral vasculature with a neuronal phenotype express a marker of active neurite growth. The presence of CGRP-containing neuronal cells in the vascular wall may participate in response to injury and vasodilator mechanisms as part of a perivascular sensory neural network.

**CB11**

**Crosstalk Between the Angiotensin and GABA Systems in NTS Neurons: Contribution to the Long-term Control of Blood Pressure**

Chengwen Sun, Mohan K Raizada, Colin Summers; Univ of Florida, Gainesville, FL

It is established that both the Angiotensin II (Ang II) and gamma-aminobutyric acid (GABA) systems within the brain exert regulatory influences in the control of blood pressure and play an important role in the development and establishment of hypertension. However, there is little information concerning the crosstalk between these two systems in the nucleus tractus solitarius (NTS), a brain area that makes an important contribution to baroreflex integration and blood pressure regulation. In the present study, we examined the effect of Ang II on the neuronal responsiveness to a GABAβ receptor agonist, baclofen, by using a combination of patch clamp and single-cell RT-PCR techniques. Supertreatment of baclofen (10 μM) in control neurons cultured from rat NTS decreased the neuronal firing rate by 39% (from 1.07 ± 0.06 Hz to 0.64 ± 0.05 Hz; n = 6). Pre-treatment of neurons with Ang II (100 nM, 5 hrs) had no effect on the inhibitory action of baclofen (38% inhibition) in AT1 receptor negative neurons. However, in AT1 receptor positive neurons, Ang II (100 nM, 5 hrs) significantly enhanced the inhibitory action of baclofen (63% inhibition). To understand the exaggerated inhibitory response to baclofen following treatment of NTS neurons with Ang II, we examined the effect of Ang II on GABAβ receptor expression in NTS neurons. Real-time PCR data indicate that Ang II treatment (100 nM, 5 hrs) induced a 2-fold increase in GABAβ receptor mRNA levels. Immunostaining experiments also demonstrated that GABAβ receptor expression in NTS neuronal cultures was increased by treatment of neurons with Ang II (100 nM, 5 hrs). Collectively, these experiments indicate that Ang II increases GABAβ receptor expression and consequently enhances the neuronal response to its agonist baclofen (in the NTS). This crosstalk between the Angiotensin and GABA systems may contribute to the central resetting of long-term blood pressure regulation in Ang II-related hypertension.

**CB12**

**Salt and Oxidative Stress in Essential Hypertension**

Cheryl L Laffer, New York Med College, New York, NY; Rodney J Boltemer, Juan Carlos Romero, Mayo Sch of Medicine, Rochester, MN; Fernando Eljovich; New York Med College, New York, NY

The reporting of both benefit and harm of salt-restriction on the outcomes of hypertension has sustained the ongoing controversy on the role of salt in human cardiovascular health. Increased oxidative stress (Ox) due to salt has been documented in experimental salt-sensitive (SS) hypertensive subjects, indicating increased OX in SS during a salt-load. We suggest that this crosstalk between the Angiotensin and GABA systems may contribute to the central resetting of long-term blood pressure regulation in Ang II-related hypertension.

**LB13**

**Loci on Chromosomes 5p and 20q are Linked to Multiple Hypertension Phenotypes in the British Genetics of Hypertension (BRIGHT) Study**

Chris Wallace, Ming-Zhan Xue, Richard Dobson, Carolina Marciano, Johanne Gungadou, Beverly Burke, Abidou Orpinisa, Stephen Newhouse, Janine Pembroke, Bart’s and The London Sch of Medicine and Dentistry, London, United Kingdom; Morris Brown, Univ of Cambridge, Cambridge, United Kingdom; John Connell, Univ of Glasgow, Glasgow, United Kingdom; Niles Samani, Univ of Leicester, Leicester, United Kingdom; Anna Dominiczak, Univ of Glasgow, Glasgow, United Kingdom; G M Latruffi, Cntr National de Genotypage, Evry, France; Tim Birkett, Royal Liver Infirmary, Liverpool, United Kingdom; Martin Farrall, Wellcome Trust Cntr for Human Genetics, Oxford, United Kingdom; Charles Mein, Patricia B Munroe, Bart’s and The London Sch of Medicine and Dentistry, London, United Kingdom; David Clayton, Cambridge Institute for Med Res, Cambridge, United Kingdom; Mark Caulfield, Bart’s and The London Sch of Medicine and Dentistry, London, United Kingdom

Despite strong evidence for genes causing essential hypertension, disentangling the causal variants has proved difficult. Linkage analysis has been used to search for genomic regions that may harbour disease related genes in affected hypertensive family members. By using additional phenotypic data it may be feasible to identify new loci and further redefine signals. The analysis of covariate-defined subsets however, is dependent on researchers’ prior beliefs. Here we report a novel approach to capitalise on additional phenotypic data in linkage studies. The BRIGHT Genetics of Hypertension (BRIGHT) Study has collected biometric and biochemical measurements on 2015 affected sibling pairs recruited from the upper 5% of the blood pressure distribution and has published a 10q M genome scan. We took a unified approach to utilise additional phenotypic variables in the search for genetic signals by testing for dependence between genetic sharing and hypertension covariates within the dataset as a whole. Previous “identity by descent regression” studies have been prohibitively computationally expensive and restricted to studying only a few variables. We have developed a novel and less computationally intensive score test method that remains statistically robust. We found genome-wide significant evidence for linkage of several hypertension phenotypes. The strongest signals were from body mass indexes on chromosome 20 (genomewide p = 0.002) and kidney function measures (creatinine, urea and glomerular filtration rate) on chromosome 5p (genomewide p = 0.008). Correcting for the multiple traits and genetic locations studied, our global genomewide p value is 0.03. The 20q locus coincides with several other obesity-related linkage studies and is particularly interesting as most BRIGHT cases are not obese (median BMI = 28). The locus on chromosome 5p contains several current candidate genes which may harbour kidney function signals, has not been identified in previous hypertension scans. This is the first “identity by descent regression” study in hypertension and demonstrates the value of incorporating additional phenotypic information to define novel loci and new avenues of research.

**LB14**

**Altered Regulation of ETB Endothelin Receptor by D3 Dopamine Receptor in Renal Proximal Tubule Cells of SHRs**

Chunyu Zeng, Laureano D Asico, Georgetown Univ Med Cntr, Washington, DC; Ulrich Hopfer, Case Western Reserve Sch of Medicine, Cleveland, OH; Gilbert M Eisner, Georgetown Univ Med Cntr, Washington, DC; Robin A Felder, Virginia Univ for the Health Sciences, Charlottesville, VA; Pedro A Jose; Georgetown Univ Med Cntr, Washington, DC

The dopaminergic and endothelin systems, by regulating sodium transport in the renal proximal tubule (RPT), play a role in the control of blood pressure. Endothelin B (ETB) receptor deficiency results in pronounced salt-resistant hypertension. Disruption of the dopamine D3 receptor in mice induces hypertension that is associated with a decreased ability to excrete a sodium load. The D3 and ETB receptors are expressed in RPTs, and the D3 receptor may regulate ETB expression. Because D3 receptor regulation and function in RPTs are impaired in spontaneously hypertensive rats (SHRs), we tested the hypothesis that D3 receptor regulation of ETB receptors in RPTs may be impaired in SHRs. D3 and ETB receptors were studied in immortalized RPT cells and brush border membranes from Wistar-Kyoto (WKY) and SHRs, and renal cortical membranes from D3−/− mice using immunoblotting, and immunoprecipitation. In WKY RPT cells, the D3 antagonist, PD128907, increased ETB receptors in a time- and concentration-dependent manner, effects that were blocked by the D3 agonist, USU9194A (10−3 M /24 h). In contrast, in RPT cells from SHRs, PD128907 decreased ETB receptor expression. In spite of similar expression of ETB receptors in WKY and SHRs, basal D3/ETB receptor co-immunoprecipitation was 3 times greater in WKY than in SHRs. The absolute amount of D3/ETB receptor co-immunoprecipitation induced by a D3 agonist was greater in WKY than in SHRs but the percent increases were similar. ETB receptor expression in the renal cortex is decreased in D3 receptor null mice. D3 receptors regulate ETB receptors by physical receptor interaction. D3 receptor regulation of ETB receptors is impaired in RPT cells from SHRs.

**LB15**

**Endothelial Function in Subcutaneous Small Resistance Arteries of Hypertensive Patients is not a Predictor of Cardiovascular Events**

Damiano Rizzoni, Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Enzo Porteri, Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Carolina De Cuioes, Gianluca E Boari, Francesca Zani, Marco Micolini, Silvia Pairedi, Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Guido A Tiberio, Stefano M Giulini, Chair of General Surgery, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Enrico Agabiti Rosei, Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy

Introduction: Endothelial dysfunction or byronal circulatory is associated to a greater incidence of cardiovascular (CV) events. Hypothesis: To investigate the presence of a
Changes in Extracellular Matrix in Subcutaneous Small Resistance Arteries of Patients with Primary Aldosteronism

Damianno Rizzoni, Enzo Porteri, Silvia Paiardi, Francesca Zani, Marco Miclini, Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Guido A Tiberio, Chair of General Surgery, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy.

Introduction: It has been previously demonstrated that aldosteronism may possess a strong profibrotic action in vitro and in animal models of genetic or experimental. Hypothesis: To investigate the presence of such a profibrotic action in the human microcirculation is presently available. Methods: We investigated 13 patients with primary aldosteronism, 7 patients with essential hypertension (EH) and 10 normotensive controls (NT). All subjects were submitted to a biopsy of gluteal or abdominal subcutaneous fat tissue. Small resistance arteries were dissected and mounted on an isometric myograph and the tunica media to internal lumina ratio (M/L) was measured. The total collagen content within the tunica media was detected (Sirius red staining and image analysis) and collagen subtypes were evaluated using polarized light microscopy; under this condition thicker type I collagen fibers appear orange or red, while thinner type III collagen fibers are yellow or green. Results: M/L was significantly increased in primary aldosteronism (0.095 ± 0.02) and in essential hypertension (0.101 ± 0.03) compared with normotensives controls (0.07 ± 0.02, P < 0.05). Clinic blood pressure values were similar in primary aldosteronism (142/89 ± 10/7) and in essential hypertension (151/86 ± 9/10) and greater than in normotensives controls (115/60 ± 11/6, P < 0.05). Total collagen and type III vascular subcollagenous layer was significantly higher in primary aldosteronism (7.81 ± 2.91 and 5.92 ± 1.73, P < 0.01) than in essential hypertension (7.00 ± 2.46 and 5.25 ± 1.64, respectively). Normotensive controls had less total and type III collagen in respect with the two hypertensive groups (2.32 ± 1.63 and 1.80 ± 0.64, P < 0.001). Type I collagen was less in primary aldosteronism (2.1 ± 1.01) than in normotensive controls (2.9 ± 1.03, P < 0.05) with an intermediate result in essential hypertension (2.31 ± 1.06). Conclusions: Our results indicate that in small resistance arteries of patients with primary aldosteronism a pronounced fibrosis may be detected, even more evident that in blood-pressure matched patients with essential hypertension.

Table 1: Changes in Extracellular Matrix in Subcutaneous Small Resistance Arteries of Patients with Primary Aldosteronism

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Changes in Extracellular Matrix in Subcutaneous Small Resistance Arteries of Patients with Primary Aldosteronism

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Introduction: It has been previously demonstrated that aldosteronism may possess a strong profibrotic action in vitro and in animal models of genetic or experimental. Hypothesis: To investigate the presence of such a profibrotic action in the human microcirculation is presently available. Methods: We investigated 13 patients with primary aldosteronism, 7 patients with essential hypertension (EH) and 10 normotensive controls (NT). All subjects were submitted to a biopsy of gluteal or abdominal subcutaneous fat tissue. Small resistance arteries were dissected and mounted on an isometric myograph and the tunica media to internal lumina ratio (M/L) was measured. The total collagen content within the tunica media was detected (Sirius red staining and image analysis) and collagen subtypes were evaluated using polarized light microscopy; under this condition thicker type I collagen fibers appear orange or red, while thinner type III collagen fibers are yellow or green. Results: M/L was significantly increased in primary aldosteronism (0.095 ± 0.02) and in essential hypertension (0.101 ± 0.03) compared with normotensives controls (0.07 ± 0.02, P < 0.05). Clinic blood pressure values were similar in primary aldosteronism (142/89 ± 10/7) and in essential hypertension (151/86 ± 9/10) and greater than in normotensives controls (115/60 ± 11/6, P < 0.05). Total collagen and type III vascular subcollagenous layer was significantly higher in primary aldosteronism (7.81 ± 2.91 and 5.92 ± 1.73, P < 0.01) than in essential hypertension (7.00 ± 2.46 and 5.25 ± 1.64, respectively). Normotensive controls had less total and type III collagen in respect with the two hypertensive groups (2.32 ± 1.63 and 1.80 ± 0.64, P < 0.001). Type I collagen was less in primary aldosteronism (2.1 ± 1.01) than in normotensive controls (2.9 ± 1.03, P < 0.05) with an intermediate result in essential hypertension (2.31 ± 1.06). Conclusions: Our results indicate that in small resistance arteries of patients with primary aldosteronism a pronounced fibrosis may be detected, even more evident that in blood-pressure matched patients with essential hypertension.

Table 1: Changes in Extracellular Matrix in Subcutaneous Small Resistance Arteries of Patients with Primary Aldosteronism

<table>
<thead>
<tr>
<th>Table</th>
<th>Basal NE (10⁻⁵ mol/L)</th>
<th>NE 10⁻⁵ mol/L + insulin id (kPa)</th>
<th>NE 10⁻⁵ mol/L + insulin hd (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT, n=12</td>
<td>11544</td>
<td>7831***</td>
<td>7337a</td>
</tr>
<tr>
<td>EH</td>
<td>8388</td>
<td>7735</td>
<td>6931a</td>
</tr>
<tr>
<td>NIDDM</td>
<td>n=8</td>
<td>8730</td>
<td>8443</td>
</tr>
<tr>
<td>NIDDM + NIDDM</td>
<td>9145</td>
<td>8348</td>
<td>8329</td>
</tr>
</tbody>
</table>
homologous, ACE, which generates Ang II from Ang I. In addition, Ang II and Ang-(1–7) have opposing functions; Ang II is a vasoconstrictor and mediate while Ang-(1–7) causes vasodilation and inhibits cell growth. In previous studies, we showed that Ang-(1–7) inhibits the growth of both cardiomyocytes and cardiac fibroblasts, suggesting that a reduction in formation of the heptapeptide may participate in cardiac hypertrophy and fibrosis. Since aldosterone induces cardiac hypertrophy and fibrosis, we investigated if its effects on ACE2 in cardiac cells. Myocytes were isolated from neonatal rat hearts and pretreated for 24 h in growth media depleted of serum or hormones, to study the transcriptional regulation of ACE2. Treatment of myocytes with aldosterone [5 μM] caused a significant decrease in ACE2 mRNA (relative gene expression of 38.6% ± 13.5 of control, n = 3, p < 0.005). The mineralocorticoid receptor antagonist spironolactone (1 μM) completely blocked the aldosterone-induced down-regulation in ACE2 mRNA (0.65 ± 0.04 relative expression by 100 nM aldosterone versus 1.07 ± 0.02 by aldosterone and 1 μM spironolactone, n = 3, p < 0.05). Spironolactone alone had no effect on ACE2 mRNA expression. In contrast to myocytes with 100 nM aldosterone caused a significant up-regulation of ACE mRNA (1200% ± 170 of control, n = 3, p < 0.05). These results suggest that aldosterone alters the ratio of ACE/AngII in the heart, increasing ACE while concomitantly decreasing AngII. Moreover, aldosterone-induced imbalances in Ang II relative to Ang-(1–7), secondary to changes in expression, favor increased fibrosis and attenuated anti-fibrotic effects. These studies further suggest that blockade of the aldosterone-induced increase in ACE/AngII by mineralocorticoid antagonists such as spironolactone may participate in their attenuation of the accumulation and structural remodeling of the collagen matrix.

**LB21**

Genetic Deletion of the Angiotensin-(1–7) Receptor Mas Markedly Changes Heart Expression of Extracellular Matrix Proteins to a Pro-fibrotic Profile

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The expression of various types of collagen and other matrix proteins has been shown to be altered under various physiological and pathologic conditions. The renin-angiotensin system plays a pivotal role in the biosynthesis of the extracellular matrix within the heart. It has recently been shown that angiotensin-(1–7) is an endogenous ligand for the G protein-coupled Mas receptor. Angiotensin-(1–7) has been reported to modulate a number of important heart functions, as well as affect ECM biosynthesis. However, it has not yet been shown if the Mas receptor is involved in the modulation of extracellular matrix production. In this study we investigated the effects of genetic deletion of the Mas receptor on the expression and distribution pattern of specific matrix proteins in adult and neonatal male mice hearts. Protein quantification in wild-type (WT) and Mas-knockout (Mas-KO) mice was performed using immunofluorescence-labeling techniques and confocal microscopy. Different areas of ventricles from WT and Mas-KO mice were compared and the levels of collagen I, III, IV, and fibrinectin present were determined. Statistical analysis was performed using the Mann Whitney test. We observed that the expression of several matrix proteins were significantly increased in the ventricles of adult KO mice hearts compared to control mice: type I collagen (42.2 ± 4.90 vs 59.00 ± 2.35 in WT mice, P < 0.0001), type III collagen (97.83 ± 3.72 vs 48.01 ± 2.91 in WT mice, P <0.01), and fibronectin (78.89 ± 2.90 vs 53.11 ± 2.00 in WT mice, p < 0.0001). The expression of type VI collagen was decreased (15.22 ± 1.07 vs 41.52 ± 4.50 in WT mice, P < 0.0001). No major differences were detected in the atria. Neonatal KO-Mas mice hearts presented similar patterns as observed in adults. These observations suggest that the Mas receptor is involved in the selective expression of specific extracellular matrix proteins within the ventricles. The profile observed may contribute to the development of cardiac fibrosis observed in the Mas-KO mice. Supported by: Support Program for Centers of Excellence Program, CNpq/FAPENMG.

**LB22**

20-Hydroxyeicosatetraenoic Acid (20-HETE) is Required to Maintain Integrity of Glomerular Protein Permeability Barrier

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Glomerular express cytochrome P450 (CYP450) 4A, the enzyme that synthesizes the eicosanoid 20-HETE. We have further demonstrated that 20-HETE protects the glomerular protein permeability barrier from injury induced by puromycin aminonucleoside (PAN). We hypothesize that 20-HETE not only protects the glomerular permeability barrier from injury, but is required to maintain its functional integrity. We used the CYP450 4A inhibitor 17-ODYA and measured the dose dependence and time course of albumin permeability (P<sub>aw</sub>) response of normal glomeruli in vitro. We then measured P<sub>aw</sub> of glomeruli isolated after in situ renal perfusion with 17-ODYA (100 μM). We determined whether glomeruli isolated from rats pretreated with the CYP450 4A inducer clofibrate were protected against increased P<sub>aw</sub> caused by PAN (5 μM). P<sub>aw</sub> was determined using glomerular volumetric change in response to an oncotic gradient. P<sub>aw</sub> increased within 5 minutes of in vitro inhibition of glomerular CYP450 4A (P<sub>aw</sub> 0.24 ± 0.03 vs 0.84 ± 0.11 in control). This increase was maximal at 15 min (0.10 ± 0.13, p < 0.001 vs control), and was dose-dependent. P<sub>aw</sub> also increased after in situ inhibition of CYP450 4A. Exogenous 20-HETE reversed the increased P<sub>aw</sub> caused by in vitro or in situ treatment with 17-ODYA. Glomeruli isolated from 50 rats treated with clofibrate had increased levels of CYP450 4A and were protected from PAN-induced increase in P<sub>aw</sub> (0.19 ± 0.05 vs n; NO vs control). We conclude that 20-HETE plays a critical role in maintaining the integrity of the glomerular protein permeability barrier. We have shown for the first time that the presence of an eicosanoid is essential for normal barrier function. We postulate that increased glomerular permeability in proteinuric states is due to a relative lack of 20-HETE, and that measures used to treat and/or prevent proteinuria may act to restore or increase glomerular 20-HETE levels.

**LB23**

Xanthine Oxidase and Mitochondrial Enzymes as Potential Sources of Vascular Superoxide Production in DOCA-Salt Rats

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Vascular superoxide (•O2) levels are increased in DOCA-salt rats. The purpose of this study was to investigate the sources of ET-1-induced reactive oxygen species (ROS) production in conduit (aorta-AO) and resistance arteries (mesenteric arteries-MA) of DOCA-salt rats, and the implication of ET<sub>A</sub> receptor in ROS generation. DOCA-salt rats (n = 8) received 5 different treatments: apocynin (NAD(P)H oxidase inhibitor, 1.5 mM/L), allopurinol (xanthine oxidase inhibitor, 100 mg/kg/day), bosantan (ET<sub>A</sub> receptor antagonist, 100 mg/kg/day), BMS182634 (ET<sub>B</sub> antagonist, 40 mg/kg/day), and hydralazine (25 mg/kg/day). Data were compared to uninephrectomized rats (UnNx). After 3 weeks treatment, systolic blood pressure in DOCA-salt rats was reduced by apocynin, BMS, and hydralazine, (P < 0.01). TBARS levels (lipid peroxidation) were increased in DOCA-salt rats (2.8±0.1 μM/L) compared to controls (1.9±0.1 μM/L). BMS (2.0±0.3 μM/L), bosantan (2.2±0.5 μM/L) and hydralazine (1.9±0.4 μM/L) prevented lipid peroxidation increase. Fluorescence confocal microscopy showed reduced •O2 production in MA and AO from bosantan-treated DOCA-salt rats. As well, chemiluminescence analysis of xanthine oxidase activity in MA and AO from DOCA-salt rats compared to controls (413.85 ± 116.51 and 116.10 ± 60.60 ± 1x10<sup>5</sup>cpm/mg dry weight, respectively). All treatments reduced or prevented the increase of xanthine oxidase activity (P < 0.01) in MA whereas bosantan and BMS had no effect in AO. In addition, confocal microscopy showed reduced •O2 when tissues were treated in situ by TFA and CQ (inhibitors of mitochondrial electron transport complexes II and IV). Rottenone (mitochondrial complex I inhibitor) had no effect in MA. Our findings suggest involvement of multiple ROS-generating systems, some of which are ET<sub>B</sub>-sensitive, in the development of hypertension and vascular inflammation in DOCA-salt rats.
Renal Venous Oxygen Tension After ACE-inhibition Predicts a Functional Renal Artery Stenosis in Two-kidney, One Clipping Hypertension

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Clinicians require an immediate, functional test of the significance of renal artery stenosis (RAS) when this is detected incidentally by angiography to provide guidance about the need for angioplasty. We evaluated renal venous oxygen tension (RvO₂) and its response to acute ACE inhibition in the two-kidney, one clipping model (2K1C). Three weeks after left renal artery clipping or sham surgery, right kidneys were studied. Microbubble contrast agent and spatio-temporal image processing were used to define and assess intrarenal and renal venous oxygen metabolism evaluated after administration of vehicle or ACE inhibition (enalaprilat; bolus 0.3 mg/kg bw, infusion 0.3 mg/kg bw/h). An additional clipped group had renal perfusion pressure lowered similarly to the enalaprilat-treated animals by a suprarenal renal artery clipping or sham surgery (sham), rats were inactin-anesthetized and split kidney function studies were performed.

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Removal of Sympathoinhibition by Endothelin A Receptor Blockade Augments the Pressure Response to Environmental Stress in Dahl Salt-resistant Rats

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Endothelin A (ETₐ) receptor blockade enhances the integrated pressor response to acute environmental stress in Dahl salt-resistant (DR) rats, but the mechanism is unknown. We therefore tested the hypothesis that ETₐ receptor antagonism enhances sympathetic nerve activity, as measured by catecholamine release, in a supersensitive dependent manner. Stress was induced by restraint and administration of air jet pulses (3 min) in rats maintained on a normal salt diet before and after 3-day treatment with either the ETₐ receptor antagonist ABT-267 (5 mg/kg) or vehicle. To define the supersensitive baroreceptor afferents, hepatic vein blood flow and pressure in the climbing vena cava were drawn as described involving indwelling catheters, and basal and stress-induced increases in muscle norepinephrine (NE) and epinephrine (Epi) were measured by RIA. Tempol, but not tempol, increased the pressor response with tempol not accompanied by further elevations in plasma catecholamines, suggesting its mechanism is distinct from that of ETₐ receptor antagonism. These data suggest that ETₐ receptor activation suppresses sympathetic nerve activity independent of its ability to promote oxidative stress. We conclude that removal of the sympathoinhibitory effect by ETₐ receptor blockade contributes to augmented pressor response to acute stress in DR rats.
Renal and Heart ACE2 Activity in Models of ACE2 and ACE Ablation and Diabetic Mice

Jan Wysocki, Minjung Chung, Lucas W. Ahlm, David J. Ferrario, Elizabeth O’Gara, Forest W. Stell, Rachel L. Janocko, Kevin S. Skarnulis, Jane A. Shumaker, John L. Hare, Susan B Gurley, William Greiner, and Srikrishna Khandrika

Rationale
ACE2 is the only known and enzymatically active homologue of ACE in the human genome. ACE2 activity may counterbalance the angiotensin II promoting effects of ACE by preventing angiotensin II accumulation in tissues, particularly in the kidney and heart where ACE is predominantly expressed and may exert protective actions. To determine tissue ACE2 activity, we utilized a microplate based fluorometric method using ACE2 specific substrate and specific inhibitors for ACE2. ACE2 activity (RFU/ug protein/hr) was examined in models of ACE and ACE2 gene ablation (ACE2−/−) and ACE2 knock-out mice to investigate ACE2 activity over a wide range of ACE2 and ACE protein expression, respectively.

Methods
Here we investigated, in neonatal rat cardiomyocytes, 1) (P)R receptor expression, and 2) PR gene transcription in cardiomyocytes. Polyclonal antibodies detected membrane bound ACE2 antibody. Renal cortex ACE2 activity had no correlation with renal cortex ACE protein expression (r = −0.02,able role for PR in cardiovascular disease and warrant further research into the local effects of 2nd generation renin inhibitors.

Conclusion
In summary, our findings support a role for PRs in the regulation of prorenin-induced gene transcription in cardiomyocytes. Further research is needed to fully understand the role that PRs play in the regulation of prorenin-induced gene transcription in cardiomyocytes.

Late Breaking Presentations

LB32
Prorenin-Induced Gene Transcription in Cardiomyocytes

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Cardiac accumulation of blood-derived prorenin (PR), the inactive precursor of renin, results in local Ang I generation (Pescott et al., 2002), but could also lead to angiotensin-independent effects through binding to the recently cloned (P)R receptor (Nguyen et al., 2002), Here we present evidence indicating, in neonatal rat cardiomyocytes, 1) (P)R receptor expression, and 2) PR-induced activation of 2nd messenger systems. Polyclonal antibodies detected membrane localized (P)R receptor expression by Western and confocal microscopy. PR induced phosphorylation of PKCdelta (p +39±65%) at 45 min, but no p42 or p44 MAP kinase activation over a 120-min period. Rat microarray gene (n/H11005/H11006) detected 259 regulated genes (p < 0.0003). CRP may be an index of inflammation associated with hypertension and the metabolic syndrome. The pathophysiologic data suggest that variability in CRP secretion is predicted by allelic variations at multiple genes, and is thus a polygenic trait attributable to variation at multiple points in the adenomigratory pathway. Further genetic linkage/association studies in larger numbers of twins and siblings are ongoing to better characterize the role of adenomigratory traits and their genetic polymorphisms in subtle early inflammation.

CRP. Allelic variation at associated SNPs predicted up to 6% of CRP variance. There was number-dependent effects on CRP. TH and ADRB1 haplotype pairs (“diplotypes”) also predicted CRP. Analysis at associated SNPs predicted up to 6% of CRP variance to be evidence of epistasis (non-additive gene * gene interactions, or cooperativity) for particular SNPs at TH and ADRB2. Conclusion. We conclude that CRP is a heritable trait, suggesting adrenomedullary investment of an early inflammatory trait in hypertension and the metabolic syndrome. Studies to date suggest that variability in CRP secretion is predicted by allelic variations at multiple genes, and is thus a polygenic trait attributable to variation at multiple points in the adenomigratory pathway.

LB34
Function and Regulation of Epithelial Sodium Transporters in the Kidney of a Salt-Sensitive Hypertensive Rat Model

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Renal artery nerves play a key role in maintenance of renal excretory function and sodium handling/homeostasis. To evaluate the role of each of the receptors, namely thiazide-sensitive Na+ cotransporter (NCC), Na+ / K+ / 2Cl- cotransporter (NKCC2), and epithelial sodium channel (ENaC) in a salt sensitive hypertensive model induced by sensory nerve denervation, neonatal Wistar rats were treated with capsaicin (Cap, 50 mg/kg, sc) or vehicle on the 1st and 2nd days of life. Male rats were assigned to four groups at 7 wk old with: control plus normal (0.5%), Con-NS (or high (4%), Con-NS) sodium diet, and Cap pretreatment plus a NS (Cap-NS) or HS (Cap-HS) diet. Mean arterial pressure (MAP) was increased in Cap-NS (129 ± 2 mmHg) compared to Cap-NS, Con-NS and Con-HS (104 ± 1 mmHg) (n = 11, p < 0.001). Trichloromethiazide (10 mg/kg, iv) increased urine flow rate (UFR) by about 70% in Cap-HS compared to Cap-NS, Con-HS, and Con-NS (p < 0.01) and decreased MAP in Cap-HS only (p < 0.001). Furosemide (1 mg/kg, iv) increased UFR in Cap-NS and Cap-HS by 200% compared to Con-NS and by 50% compared to Con-HS (p < 0.01), and decreased MAP in Cap-HS only (p < 0.01). Amiloride (1 mg/kg, iv) had no effect on UFR and MAP in any group. Consistently, Western blot showed that NCC expression in the renal cortex was increased in Cap-HS compared to Cap-NS, Con-NS and Con-HS (p < 0.05), and NKCC2 expression in the renal cortex and medulla was increased in Cap-NS compared to Con-NS, ENaC alpha subunit expression in the renal cortex was not different among 4 groups. Release of calcitonin gene-related peptide (pg/100 mg tissues) from freshly isolated renal tissues stimulated by Cap was decreased in Cap-HS (37.0 ± 0.40) and Cap-NS (34.0 ± 0.15) compared to Con-HS (59.5 ± 1.39) and Con-NS (69.6 ± 0.79) (p < 0.001). Thus, NCC and NKCC2 but not ENaC expression were functionally upregulated in the kidney of rats subjected to sensory nerve denervation plus a HS (both NCC and NKCC2) diet, indicating that 1) simultaneous upregulation of NCC and NKCC2 contributes to development of salt-induced hypertension in this model, and 2) sensory neurotransmitters regulate NKCC2 but not NCC and ENaC in the kidney.

LB35
RNA Interference of AT2 Receptor in the Ventral Lateral Septum Decreases Salt-Induced Water Intake

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Studies utilizing pharmacological agents have demonstrated that dopaminergic responses elicited by central administration of dopamine agonists are attenuated in part by CB1 and AT2 receptors (AT2-R). However, the location of these receptors in the brain has not been established. One of the possible areas involved in this response is the ventral lateral septum (VLS). The VLS is involved in the control of drinking behavior, in addition to being an area rich in AT2-R. The objective in this study was to determine the involvement of the VLS AT2-R in the dopaminergic responses elicited by peripheral injection of hypertonic saline. We approached this by injecting dRNAi molecules that elicit specific silencing of the AT2-R. Among the chosen sequences, one dRNAi was able to specifically silence AT2-R specific binding by 60% when compared to a scrambled dRNAi control. After identification of the target sequence, we evaluated the in vivo effects of AT2-R down-regulation on drinking in SD rats induced by s.c. injection of hypertonic saline (2 ml of 2 mol/L). Microinjections of 0.4 micrograms of either AT2-R dRNAi or a control scrambled sequence were administered bilaterally to the VLS. Hypertonic saline-induced water intake was determined 2, 7, 14 and 21 days after injection. Compared with controls (untreated or scrambled dRNAi), we observed a 45 % decrease in saline-induced water intake for the animals treated with AT2 dRNAi, similar to the effects of the AT2-R antagonist PD123319 (1 mg) injected directly into the VLS. The effect of the AT2-R dRNAi was still persistent three weeks after injection. Our results suggest that AT2-R in the VLS mediate, at least in part, drinking responses in this animal model.

Late Breaking Presentations

881
Angiotensin II Induced Hypertensive Response is Modulated Through Tumor Necrosis factor-alpha: Role of Nox1', Nox4 and Gp91phox

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Angiotensin II (ANG II) and Tumor necrosis factor-alpha (TNF-α) play an important role in the pathogenesis of cardiovascular disease. Recent evidence suggests that both ANG II and TNF-α may influence the development of cardiovascular disease. In this study, we evaluated whether ANG II-induced hypertensive response is modulated through cytokines and whether the gp91phox and its homologues, Nox1', Nox4 and Nox4 in involved in this effect. Method: Wildtype (B6129SF2/J) and TNF-α(-/-) mice were implanted with osmotic minipumps containing ANG II (1μg/kg/min) or saline for 14 days. In a group of TNF-α(-/-) mice, human recombinant TNF-α was given at a dose of 10ng/14day for 14 days. Blood pressure was recorded using the tail cuff method. At the end of the study, left ventricular (LV) function was measured using echocardiography. Mice were sacrificed and the LV was removed for the measurement of gp91phox, Nox1' and Nox4 using real time PCR. Results: are tabulated. The real time PCR values are shown as ΔCT values (GAPDH - the gene of interest) and the fold increase compared to control is also shown in parenthesis. Blood pressure increased by day 4 and was sustained for the rest of the study. Baseline, day 8 and 14 values are shown. Conclusions: 1) ANG II-induced hypertensive effect is at least in part mediated through TNF-α. 2) ANG II-induced hypertensive effect is also part mediated through TNF-α. 3) ANG II induces the expression of the oxidase subunits gp91phox, Nox1' and Nox4 in the left ventricle of the mice. 4) Cytokines modulate ANG II induced increase in gp91phox homologues, suggesting a role for these homologues in hypertensive response.

Proliferator Activated Receptor-γ Activators Inhibit Angiotensin II Signaling Pathways in Mesenteric Artery Smooth Muscle Cells

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Peroxisome proliferator-activated receptor (PPAR)-γ activators increase insulin sensitivity and reduce Ang II-induced vascular remodeling. The aim of the present study was to evaluate the effects of PPAR-γ activators on cell growth and Ang II signaling pathways. Vascular smooth muscle cells (VSMC) derived from mesenteric arteries were treated with Ang II (8–10μM) with or without PPAR-γ activators: prostaglandin J2 (PGJ2) and rosiglitazone for 24 hours. PGJ2 (5μM) decreased Ang II-induced protein synthesis by 82% (p<0.001), while both PGJ2 and rosiglitazone (10μM) decreased DNA synthesis induced by Ang II, respectively by 67% and 56% (p<0.001). Western blot analysis was performed to evaluate PPARs isomeric, ERK1,2, PDK, Akt/PKB, SHIP-2, and Ang II receptors A1 and A2 expression. Activity of PPAR-γ, ERK1/2, Akt/PKB and PDK2 was also evaluated. Rosiglitazone treatment significantly increased nuclear PPAR-γ expression and activity in VSMC 2-fold (p<0.01). Rosiglitazone decreased ERK1/2 peak activation by 59% (p<0.01), both Ang II and PDK2 peak activity by 58% (p<0.01), both of which were induced by Ang II. Ang II induced SHIP-2 activity was also decreased (p<0.01) by rosiglitazone. In conclusion, PPAR-γ activators PGJ2 and rosiglitazone reduced Ang II-induced VSMC growth associated with inhibition of ERK1/2, Akt/PKB. We demonstrate for the first time that the PPAR-γ activated SHIP-2 pathway is involved in Ang II-induced vascular remodeling. These results indicate a new pathway by which PPAR-γ activation may inhibit growth effects of Ang II. Downregulation of these pathways by PPAR-γ activators may contribute to regression of vascular remodeling in diabetic and hypertensive patient.

LB38

Roles of Blood Pressure and Absence of Alpha Calcitonin Gene-Related Peptide in Cardiac and Renal Damage Induced by Deoxycorticosterone-Salt Hypertension

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We have previously reported that DOCA-salt hypertension (HTN) (Z1 days), results in enhanced cardiac and renal damage and inflammation in ccr5 KO compared to WT mice, despite an equal increase in MAP in the two groups. Because the KO mice have a ~20 mmHg higher baseline MAP, they consistently have a higher BP than the WT mice during DOCA-salt induced HTN. The aim of this study was to determine the role of the higher BP to the DOCA-salt HTN induced increase in cardiac and renal damage in the absence of ccr5-GFP KO gene. DOCA-salt HTN was induced in telemetry probe implanted 8–10 week old ccr5-GFP KO and WT mice by standard techniques. To equalize the BP to that of the DOCA-salt WT mice, a separate group of DOCA-salt KO mice were given 0.025% hydroxyde (HYD) to drink. Basal MAP was significantly elevated in KO (117±4 mmHg) compared to WT (101±3 mmHg) mice. The DOCA-salt protocol increased the MAP in the KO (to 142±1 mmHg) and WT (to 129±2 mmHg) mice. The MAP of the HYD treated DOCA-salt KO mice was (126±2 mmHg). Using a subjective 0 to 4+ scoring system, cardiac and renal sections from DOCA-salt KO mice exhibited marked histopathologic damage, which was absent in DOCA-salt WT as well as control KO and WT mice. HYD treatment reduced the cardiac (52±0%) and renal (25±8) % damage compared to DOCA-salt KO mice. Plasma C reactive protein (CRP), a marker for inflammation, was significantly elevated (50%) in DOCA-salt KO mice compared to DOCA-salt WT mice and control KO and WT mice. HYD treatment in the DOCA-salt KO mice lowered (~20%) but did not normalize the elevated CRP levels compared to untreated DOCA-salt KO mice. Cardiac hypertrophy and left ventricular strain for each group treatment for 8 month and VCA1-1, VCA1-2, showed a significant but equal elevation of both markers in DOCA-salt WT and DOCA-salt KO mice compared to their controls. HYD treatment had no effect on these elevated levels in the DOCA-salt KO mice. Our data demonstrate that equalization of BP with HYD between the DOCA-salt KO and DOCA-salt WT mice significantly attenuates but does not normalize the increased cardiac and renal damage and plasma CRP levels without an effect on the elevated levels of MCP-1 and VCA1-1, in conclusion, in DOCA-salt hypertensive ccr5-GFP KO mice, both the elevated BP and absence of ccr5-GFP contribute to the cardiac and renal damage and inflammation observed in this setting.

LB39

A Possible Relationship of Nocturnal Blood Pressure Variability with Coronary Artery Disease in Diabetic Nephropathy

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Evidence suggests a relationship between short-term blood pressure (BP) variability and cardiovascular target-organ damage. Although a blunted nocturnal decrease in BP and reduced heart rate variability have been shown to be associated with cardiovascular morbidity in diabetic patients, little information is available on short-term BP variability. In this study, short-term BP variability was assessed in 36 subjects with type 2 diabetes and overt nephropathy who underwent ambulatory BP monitoring and the BP values that corresponded to short-term BP variability were examined. The incidence of coronary artery disease (CAD) was significantly greater in the patients with increased 24-h systolic BP variability (67% versus 11%; p<0.0005), while that of carotid atherosclerosis was not significantly affected (61% versus 50%; not significant). Multiple stepwise regression analysis revealed that mean cholesterol and plasma norepinephrine (p-N) were significant and independent contributors to
nighttime systolic BP variability (partial R² = 0.490, p < 0.001; and partial R² = 0.470, p < 0.001), and that demonstrated body mass index and p-NE were primary determinants of nighttime diastolic BP variability (partial R² = 0.539, p < 0.0005; and partial R² = 0.304, p < 0.05). Diabetic nephropathy patients with CAD had significantly increased daytime systolic (17.8 mmHg versus 13.1 mmHg, p < 0.0005), nighttime systolic (17.4 mmHg versus 10.5 mmHg, p < 0.0001) and nighttime diastolic (-14.2 mmHg versus -7.2 mmHg, p < 0.05) BP variability. Inhibition of Ang II by losartan, a direct blocker of AT1 receptor, administered at a dose of 2 mg/100 g body wt, twice on consecutive days and were scardified on the third days. LSN-HO-1-l showed a 38% decrease (p < 0.05) in renal and vascular hemo derived CO and bilirubin synthesis compared LXS-HO-1, HOB-1, but not HO-2 protein levels were decreased in renal and vascular tissues from LSN-HO-1-l rats. Markers of adaptive vascular VM, p < 0.05.Development of salt sensitive hypertension in male rats slowed the progression of hypertension (from 121 to 130 mmHg, p < 0.05, n = 6) but not that of ERK or JNK in cardiomyocytes. Furthermore, this phenomenon overexpression of ATRAP specifically inhibited Ang II-mediated phosphorylation of p38-MAPK (p < 0.05, n = 6) but not that of ERK or JNK in cardiomyocytes. Interestingly, ATRAP mRNA and protein were endogenously expressed using the yeast two-hybrid strategy. In this study, we tested the hypothesis that cardiomyocytes express ATRAP and that ATRAP modulates Ang II-induced hypertrophic responses in cardiomyocytes. We identified that the ATRAP mRNA and protein were endogenously expressed in cardiomyocytes. There was a partial co-localization of the ATR1 and ATRAP on immunofluorescent staining under baseline condition, suggesting an inhibitory effect of ATRAP on the recycling of the receptor. Interestingly, overexpression of ATRAP significantly inhibited Ang II-mediated phosphorylation of p38-MAPK (p < 0.05, n = 6) but not that of ERK or JNK in cardiomyocytes. Furthermore, this phenomenon was accompanied by inhibition of Ang II-induced activation of c-fos promoter transcription (p < 0.05, n = 6) and amino acid incorporation (p < 0.05, n = 6). These results indicate that ATRAP significantly promotes down-regulation of the ATR1 and further ameliorates Ang II-mediated hypertrophic responses in cardiomyocytes, and may suggest a novel strategy to inhibit cardiomyocyte hypertrophy.

**Sex Hormones and Hypertension in Dahl SS Rat. Role of Renal Endothelin System**

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Growing evidence indicates that the endothelin (ET) system is important in the initiation and maintenance of salt-sensitive hypertension. Sex hormones have been shown to play a role in the development of hypertension in Dahl salt sensitive (SS) rats. The aim of this study was to examine the role of sex hormones in salt induced hypertension in Dahl SS rats and the participation of renal endothelin system in sex hormone modulated hypertension. To achieve this goal, we examined the effects of castration and ovarioctomy in DS rats in low (0.3%) LS and 3 weeks of high (6%) HS sodium diet. Mean arterial pressure (MAP) was followed by telemetry. Prepro ET-1 expression was increased in the number of ET receptors in the surface membrane of ET (50% of lacZ control), suggesting an inhibitory effect of ATRAP on the recycling of the receptor. Interestingly, overexpression of ATRAP specifically inhibited Ang II-mediated phosphorylation of p38-MAPK (p < 0.05, n = 6) but not that of ERK or JNK in cardiomyocytes. Furthermore, this phenomenon was accompanied by inhibition of Ang II-induced activation of c-fos promoter transcription (p < 0.05, n = 6) and amino acid incorporation (p < 0.05, n = 6). These results indicate that ATRAP significantly promotes down-regulation of the ATR1 and further ameliorates Ang II-mediated hypertrophic responses in cardiomyocytes, and may suggest a novel strategy to inhibit cardiomyocyte hypertrophy.

**A Novel Regulatory Effect of AT1 Receptor-Interacting Molecule on Cardiomyocytes**

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Activation of cardiac AT1R signaling plays an important role in cardiac hypertrophy. We previously cloned a novel molecule interacting with AT1R, ATRAP (for AT1R-associated protein), using the yeast two-hybrid strategy. In this study, we tested the hypothesis that cardiomyocytes express ATRAP and that ATRAP modulates Ang II-induced hypertrophic responses in cardiomyocytes. We identified that the ATRAP mRNA and protein were endogenously expressed in cardiomyocytes. There was a partial co-localization of the two proteins in intracellular compartments in stimulated cardiomyocytes, indicating that ATRAP binds to the internalized AT1R and is involved in the intracellular localization of the receptor after Ang II treatment. Overexpression of ATRAP by adenovirus gene transfer significantly decreased the number of AT1R receptors on the surface membrane of cardiomyocytes harvested from 2B-2 control, suggesting an inhibitory effect of ATRAP on the recycling of the receptor. Interestingly, overexpression of ATRAP specifically inhibited Ang II-mediated phosphorylation of p38-MAPK (p < 0.05, n = 6) but not that of ERK or JNK in cardiomyocytes. Furthermore, this phenomenon was accompanied by inhibition of Ang II-induced activation of c-fos promoter transcription (p < 0.05, n = 6) and amino acid incorporation (p < 0.05, n = 6). These results indicate that ATRAP significantly promotes down-regulation of the AT1R and further ameliorates Ang II-mediated hypertrophic responses in cardiomyocytes, and may suggest a novel strategy to inhibit cardiomyocyte hypertrophy.

**Angiotensin Type 1 Receptor Activation Mediates Endothelin Production Induced by Serum From Pregnant Rats Exposed to Chronic Reductions in Uterine Perfusion**

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The initiating event in preeclampsia is thought to be reduced uteroplacental perfusion which leads to widespread dysfunction of the maternal vascular endothelium. Circulating factors such as inflammatory cytokines, VEGF receptor antagonists (fli1), and agonistic autotobodies to the angiotensin II type-1 (AT1) receptor are implicated in the pathogenesis of preeclampsia and endothelial ischemia and endothelial dysfunction in preeclamptic women. While we have previously reported that chronic reductions in uterine perfusion pressure (RUPP) in pregnant rats results in hypertension and enhanced endothelin production, factors linking placental ischemia and endothelial dysfunction in this rat model of preeclampsia remain unclear. The purpose of this study was to investigate the role of AT1 receptor activation on endothelin production induced by serum from pregnant rats exposed to chronic reductions in uterine perfusion. To achieve this goal, human umbilical vein endothelial cells (HUVEC) were exposed for 24 hours to one ml of serum collected from RUPP rats or normal pregnant rats. Exposure media was removed and fresh serum free media was placed on the cells. Cell media was obtained and 18 hours after stimulation and used to quantify endothelin production. Six hours after exposure to RUPP serum (p = 0.017), cell media endothelin concentration was 18.4 ± 2.7 pg/ml as compared to 9.2 ± 1.3 pg/ml from cells exposed to serum from normal pregnant rats (p = 0.01). Exposure media was removed and fresh serum free media was placed on the cells. Cell media was obtained and 18 hours after stimulation and used to quantify endothelin production. Six hours after exposure to RUPP serum (p = 0.017), cell media endothelin concentration was 18.4 ± 2.7 pg/ml as compared to 9.2 ± 1.3 pg/ml from cells exposed to serum from normal pregnant rats (p = 0.01). Pretreatment of HUVEC with an AT1 receptor antagonist, Losartan (15uM), markedly attenuated the increased endothelin production observed with serum from RUPP rats. Eighteen hours after exposure to RUPP serum (p < 0.01), cell media endothelin concentration was 21.3 ± 2.2 pg/ml as compared to 16.4 ± 3.3 pg/ml from cells exposed to serum from normal pregnant rats (p = 0.01). These data indicate that serum from pregnant rats exposed to chronic reductions in uterine perfusion to achieve this goal, human umbilical vein endothelial cells (HUVEC) were exposed for 24 hours to one ml of serum collected from RUPP rats or normal pregnant rats. Exposure media was removed and fresh serum free media was placed on the cells. Cell media was obtained and 18 hours after stimulation and used to quantify endothelin production. Six hours after exposure to RUPP serum (p = 0.017), cell media endothelin concentration was 18.4 ± 2.7 pg/ml as compared to 9.2 ± 1.3 pg/ml from cells exposed to serum from normal pregnant rats (p = 0.01). Pretreatment of HUVEC with an AT1 receptor antagonist, Losartan (15uM), markedly attenuated the increased endothelin production observed with serum from RUPP rats. Eighteen hours after exposure to RUPP serum (p < 0.01), cell media endothelin concentration was 21.3 ± 2.2 pg/ml as compared to 16.4 ± 3.3 pg/ml from cells exposed to serum from normal pregnant rats (p = 0.01). These data indicate that serum from pregnant rats exposed to chronic reductions in uterine perfusion...
treatment also significantly increased insulin index and HDL’s values in blood in these groups of various risk factors. Conclusions: Supplementation of L-Arginine activates the NO system and exerts positive remodeling on the arterial system, improves arterial elasticity, homodynamic and humoral parameters.

Obesity and Insulin Resistance in α-Calcitonin Gene-Related Protein (α-CGRP) Knockout Mice
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Alpha-calcitonin gene-related peptide knockout (α-CGRP KO) mice display a significant increase in basal blood pressure compared to their wild type (WT) counterparts. Initial observations from our laboratory demonstrated that aged (∼2 yr old) α-CGRP KO mice, fed a normal diet, were significantly heavier compared to WT controls (46 ± 1.3 vs 34 ± 1.8 g). Aims: The purpose of this study was to investigate the effects of obesity and insulin resistance in different age groups (10 –12 weeks, ∼1 and 2 yr old) of α-CGRP KO and WT mice. Adiponectin, a protein expressed in adipocytes that attenuates insulin resistance and obesity was significantly lower in the α-CGRP KO compared to WT mice in the 3 different age groups (∼1 yr 0.47 ± 0.05, ∼2 yr 0.46 ± 0.03, and 2 yr 0.45 ± 0.04 mg/ml, respectively). Plasma glucose levels were significantly higher at all study ages in the α-CGRP KO mice compared to their WT counterparts. Plasma insulin levels (u g/l) in the α-CGRP KO mice were 0.61 ± 0.09 (10 –12 weeks), 0.96 ± 0.12 (1 yr), and 0.96 ± 0.12 (2 yrs) compared to 0.39 ± 0.08, 0.40 ± 0.06, and 0.50 ± 0.04 for WT mice, respectively. Glycated albumin, a marker for accelerated glycation of proteins resulting from elevated blood glucose levels, was also significantly higher in the α-CGRP KO mice compared to WT controls at all ages studied (α-CGRP KO mice were 0.55 ± 0.02 mg/ml (10 –12 weeks), 0.9 ± 0.07 mg/ml (1 yr), and 1.3 ± 0.07 mg/ml (2 yrs) compared to 0.30 ± 0.05 mg/ml, 0.34 ± 0.03 mg/ml, and 0.42 ± 0.09 mg/ml for WT mice, respectively). In addition, 60 minutes following an intraperitoneal insulin injection in 1 yr old mice, the α-CGRP KO animals demonstrated a 2.5 fold decrease in glucose uptake compared to WT controls. These data demonstrate that similar to adrenomedullin, another member of the CGRP/calcitonin gene family, α-CGRP, a sensory neuroepithelium, may play a significant role in mediating the components associated with the metabolic X syndrome, specifically hypertension, obesity, and insulin resistance.

In Vivo Tracking Of Distal Na+/Cl Cotransporter (NCC) in Response to Changes in Dietary NaCl
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The Na+/Cl cotransporter (NCC) is expressed in the apical membrane of distal convoluted tubule and is responsible for the reabsorption of 5 – 10% of filtered NaCl. NCC is inhibited by thiazide diuretics and used to treat hypertension. NCC abundance in the kidney is increased during dietary NaCl restriction and by aldosterone and increased during high salt diet, and mineralocorticoid blockade. Aim: The aim of this study was to test the hypothesis that plasma membrane, intracellular distribution of NCC is regulated by changes in dietary salt. Methods: Six week old Sprague Dawley rats were fed a 0.4% NaCl low salt diet (LS) for 3 weeks then a 4% NaCl high salt diet (HS) for 3 weeks. After anesthetization (ketamine/xylazine), kidneys were excised, renal cortex dissected, and analyzed by: a 4% NaCl high salt diet (HS) or 0.07% NaCl salt restricted diet (SR) for 1 week. After membrane to higher density membranes and decreased NCC total abundance to 0.45 ± 0.11 mg/ml. Immunoelectron microscopy revealed that NCC resides almost exclusively in the apical membrane in SR and that HS caused a significant shift from apical membranes to sub apical gradients, fractions collected and analyzed by immunoblot. NCC was detected with anti-TSC membra...
Primary Aldosteronism Contributes to Poorly Controlled Hypertension in Diabetic Subjects

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Background: Data from large clinical trials suggest that most diabetic subjects will require multiple medications for adequate blood pressure (BP) control. Based on these findings, diabetic subjects are rarely screened for secondary forms of hypertension. In non-diabetic subjects primary aldosteronism (PA) is present in a large number (9%-14%) of patients with poorly controlled BP on multiple drugs. Accordingly, we aimed to determine the prevalence of PA in diabetics with poorly controlled hypertension. Methods: Diabetic subjects with a BP ≥140/90 mm Hg on ≥3 anti-hypertensive medications in Diabetes Care Centers were consecutively screened for PA with a plasma aldosterone concentration to plasma renin activity ratio (PAC/PRA). Except for aldosterone inhibitors, patients were continued on their usual BP medications. Subjects with a PAC/PRA <30 ng/dl*umol-1 and a PAC ≥12 ng/dl received a three-day salt suppression testing. Subjects with a PAC ≥8.5 ng/dl or 24-hour urine aldosterone (≥12 mg/24h) or urine sodium (≤10 mmol/L) after the 3-day salt load were considered as having PA. Results: Sixty-two subjects were screened for PA by the study protocol. Seventeen subjects (27.4%) had a positive screen. Eight of the subjects with a positive screen have ruled in for PA as defined by study criteria. The results for 6 others are still pending and 3 patients have ruled out for PA. The prevalence of PA is 12.9% (95%CI: 7.3 - 21.2%). Prior performance of the screening ratio in non-diabetic populations suggests that as many as 85% of those with a positive screen will have PA. Conclusions: Our preliminary study indicates that primary aldosteronism is common in poorly controlled hypertensive diabetic subjects on a multiple drug regimen with PAC/PRA followed by salt suppression testing is indicated to reduce the long-term complications associated with mineralocorticoid excess.

AT2 Receptor-Mediated Natriuresis with CGP-42112a in the Rat

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BACKGROUND: Angiotensin II (Ang II), the major effector peptide of the renin angiotensin system (RAS), acts at two major receptors, AT1 and AT2. Recent studies have shown that AT2 receptors oppose AT1 receptors by decreasing cellular proliferation and stimulating vasodilatation. However, the role of AT2 receptors in the control of renal Na+ excretion is unknown. We tested the hypothesis that selective renal AT1 receptor activation with CGP 42112a (CGP), an AT1 receptor agonist, induces natriuresis in normal Sprague-Dawley rats. METHODS: We employed a 2-kidney rat model (N=6/group) in which CGP was infused directly into the renal interstitial (RI) space of the experimental kidney while the opposite kidney served as control. Systemic AT1 receptors were blocked with an s.c. infusion (osmotic micropump) of candesartan (0.01mg/kg) for 24 hours prior to and during the experiment. Renal Na+ excretion (U NaV) was monitored individually from each kidney in response to cumulative RI infusion of CGP (20, 40, and 60 nmol/kg/min, each dose for 30 min), or CGP combined with AT1 receptor specific antagonist PD-123319 (PD, 10 ug/kg/min). Mean arterial pressure (MAP) was monitored via the direct carotid method. RESULTS: UNaV increased with CGP (0.023 ± 0.006 umol/min prior to infusion of CGP; 0.003 ± 0.003 umol/min, respectively (P<0.05, **P<0.01). For the control kidney, U NaV was 0.13 ± 0.03 umol/kg/min at baseline and did not change with contralateral CGP infusion. MAP was 94 ± 6 mmHg in the control period and did not change in response to CGP. The natriuretic response to CGP was abolished by co-infusion with PD (0.023 ± 0.006, **P<0.01, **P<0.05, and 0.04 ± 0.004 umol/min [P<NS] for each progressive period) and MAP remained unchanged throughout the experiment. CONCLUSIONS: Renal AT1 receptor activation causes natriuresis, opposing the antinatriuretic action of Ang II via AT1 receptors in the rat.

Gonadectomy Attenuates Renal NADPH Oxidase - Dependent Superoxide Generation and Hypertension in Male Spontaneously Hypertensive Rats

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The presence of androgens is required for the male spontaneously hypertensive rats (SHR) to exhibit high blood pressure (BP) levels as females. Renal NADPH oxidase appears to be a major source of oxidative stress, which might impact BP in SHR. We hypothesized that androgens stimulate renal NADPH-dependent superoxide generation which in turn mediates hypertension in male SHR. Intact and castrated male SHR were treated for 1 week with apocynin, an inhibitor of NADPH oxidase which impedes its subunit assembly at the cell membrane. Blood pressure was measured in the conscious animals and the basal production of superoxide by activating NADPH oxidase and contribute to hypertension in SHR.

Late Breaking Presentations

Systemic Inflammation is Increased in Hypertensive Type 2 Diabetic Patients: Improvement after Antihypertensive Therapy

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Experimental and clinical studies suggest that type 2 diabetes is associated with an inflammatory process. Relationships between low-grade inflammation, blood pressure (BP) and diabetes are unclear. We questioned whether hypertensive type 2 diabetic patients have evidence of inflammation and if antihypertensive treatment influences the inflammatory status in these patients. We specifically tested effects of the angiotensin receptor blocker (ARB) valsartan or the beta blocker (BB) atenolol. Hypertensive type 2 diabetic patients (30–70 y, n=28) treated with oral hypoglycemic and antihypertensive agents were randomized to double-blind treatment for one year with valsartan (80–160 mg) or atenolol (50–100 mg) once daily, added to previous therapy. Healthy, age-matched controls (n=12) were also studied. Serum levels of cytokines (IL-6, IL-18, chemokines (MCP-1), adhesion molecules (soluble ICAM (sICAM) and soluble E-selectin (sE-selectin)) were measured by ELISA before and one year after treatment. One year after therapy, BP was similarly controlled by valsartan and atenolol (123±27/4±2 vs 144±34/8±2 mmHg, p<0.005; 128±37/5±2 vs 144±28/3±2 mmHg, p<0.005, respectively). Glycemic control was identical in the two groups. Serum levels of IL-6, IL-18, sICAM and s-selectin were increased (IL-2–4-fold) in patients before treatment compared with controls (p<0.05). IL-6 and IL-18 levels were reduced by valsartan (5-fold, p<0.01) and atenolol (2-fold, p<0.05) compared with pretreatment levels. Whereas valsartan significantly reduced sICAM and MCP-1 concentrations (p<0.05), atenolol effects were only minor. When treatment groups were combined, levels of all proinflammatory mediators were significantly reduced (p<0.05) and comparable to levels in controls. Our findings indicate that 1) proinflammatory mediators are increased in hypertensive type 2 diabetic patients, 2) BP reduction is associated with improved inflammatory status and 3) valsartan and atenolol have differential anti-inflammatory actions. In conclusion, antihypertensive treatment, particularly with valsartan, ameliorates inflammatory processes in diabetic hypertensive patients. Such effects may contribute, in part, to cardiovascular protection by these agents.

ENaC and AS1Cl Proteins are Required for Vascular Smooth Muscle Cell Wound Healing

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In response to vascular injury, vascular smooth muscle cells (VSMCs) migrate from the media toward the intimal injury site, and participate in wound healing. Although injury-induced release of numerous substances may initiate VSMC migration into the intima, evidence also suggests mechanosensitive processes are required. Previously, we demonstrated blockage of putative mechanosensitive Degenerin/Epithelial Na\(^+\) Channel (DEG/ENaC) proteins, using amiloride and benzamil, inhibits VSMC migration. However, it is unknown which specific DEG/ENaC members are required for migration. Therefore, the aim of this study is to determine if DEG/ENaC proteins, α2, β2 or γ ENaC, or Acid Sensing Ion channel 1 (ASIC1) are required for VSMC migration. To address our aim, we evaluated VSMC migration, upon a "wound healing assay", in which confluent VSMC monolayers are “wounded” by scratching with a pipette tip and “healing” is quantified as re-invasion of initial wound area after 24 hours. Disruption of DEG/ENaC levels, using dominant-negative and siRNA approaches, inhibits healing compared to controls (EDP and RSIO as shown in Table 1). To determine if hepatocyte growth factor (HGF), an enhancer of VSMC wound healing, might act by stimulating DEG/ENaC expression, we evaluate HGF-stimulated (20 ng/ml) healing before and after DEG/ENaC blockade with benzamil (1 5–11)- Benzamil (n=6/group) healing before and after DEG/ENaC blockade with benzamil (1 μM). As shown in Table 2, benzamil abolishes HGF-stimulated healing. Our findings suggest DEG/ENaC molecules are required for wound healing and HGF stimulates healing by activating DEG/ENaC channels. In conclusion, our results support a novel role for DEG/ENaC molecules in VSMC migration associated with wound healing.
Chromosome 2p Shows Genome Wide Significant Linkage to Anti-Hypertensive Medication Response in the British Genetics of Hypertension (BRIGHT) Study.

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Introduction
Numerous genome-wide linkage studies have found evidence for loci influencing blood pressure and hypertension status on almost all chromosomes. We hypothesised that drug response could be used to stringently define subsets with reduced genetic and etiologic heterogeneity and thus enhance gene finding. Material and methods: The study population was 2142 severely hypertensive Caucasian ASP (BRIGHT). Antihypertensive therapy was classified into two groups - those that inhibit RAS (A-ACEI/ARB, B-beta-blockers) or not (C-CCB, D-diuretics). Non-responders had a treatment BP change of < -20mmHg.

RESULTS: Significant linkage was observed in the AB group on chromosome 2 (multipoint LOD 4.84 at 9.60 Kosambi cM). Suggestive linkage was also observed for the CD group on chromosome 10 (LOD 2.83 at 125.96 cM) and the combined ABCD group on chromosome 2 in the same region as the AB only group (LOD 1.61 at 90.68 cM). Conclusions: This is the first study to identify significant genome wide linkage by partitioning different determinants of hypertension based on drug response. The locus on chromosome 2p in a subset of Caucasian hypertensives unresponsive to AB drugs coincides with a linked region identified in African American hypertensives. This suggests that the region may contain a gene for the salt-sensitive form of hypertension and/or a pharmacogenomic locus affecting drug response.

Increased Apelin Expression in the SHR Rostral Ventrolateral Medulla

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The rostral ventrolateral medulla (RVLM) is the major source of excitatory input to sympathetic preganglionic neurons and alterations in its function have been implicated in hypertension. Gene expression profiling of the SHR RVLM was utilized to identify potential genetic determinants of hypertension. Of 20,000 transcripts represented on the microarray (Agilent G4130A), 107 transcripts were differentially expressed by >50%. One gene that was upregulated in the RVLM of SHR was apelin. Thus, our aim in the present study was to verify increased apelin expression in the SHR RVLM and ii) determine signal transduction mechanisms of apelin that relate to neuremodulation. Real-time RT-PCR demonstrated a 1.5-fold increase in mRNA abundance of the precursor peptide for apelin, preproapelin, in the RVLM of SHR compared to WKY rats. However, the transcript for its receptor (AP) did not vary significantly between the strains. Primary neuronal cultures derived from the hypothalamus and brainstem areas were incubated with 100nM Apelin 13 for 5 min and its effect on phosphoinositoide 3-kinase (PI3-kinase) and reactive oxygen species (ROS), two key modulators of neuronal activity, was assessed. Apelin 13 increased PI3-kinase activity ~ 2-fold in WKY neurons. In addition, it caused a 30% increase in NADPH oxide derived ROS. In SHR neurons, apelin 13 resulted in a ~ 2.5-fold greater stimulation of PI3-kinase activity than in WKY neurons. A dominant negative mutant for the p85 regulatory subunit of PI3-kinase cloned in an adenoviral vector (Ad2p85) was used to determine the sequence of PI3-kinase activation and ROS generation. Expression of the Ad2p85 in WKY neurons attenuated apelin stimulated ROS generation by ~75%. This demonstrates that PI3-kinase activation induced by apelin occurs upstream of ROS generation. These observations indicate that increased apelin expression in the SHR RVLM and its enhanced signaling may contribute to hypertension in light of the fact that PI3-kinase activity and ROS generation in this area have been implicated in hypertension. Supported by NIH grants HL33610 and HL76312.

The Mechanism of Heme Oxygenase-1 Reversal of Vascular Impairment in the Spontaneously Hypertensive Rat Involves an Increase in Superoxide Dismutase

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Increased heme oxygenase (HO)-1 activity has been successful in attenuating endothelial cell apoptosis; decreasing oxidative stress and in the correction of vascular impairment (Turkseven et al AJP Heart 2005). We examined the effects of increasing HO-1 protein and activity on superoxide anion levels, superoxide dismutase (EC-SOD, Cu/Zn Mn), and HO-1 activity in SHR rats (p < 0.05). Steal HO activity in SHR rats was significantly reduced as compared to age-matched WKY controls, p < 0.0001. Up regulation of HO-1 by intermittent administration of the inducer of HO-1 protein and activity, cobalt protoporphyrin (CoPp), increased an increase in HO activity, tin mesoporphyrin (SnMP), or with CoPp in the presence of NOS inhibitor nitro-L-arginine methyl ester (L-NAME) suggesting that HO-1 induction may prevent hypertension by inhibiting the bioavailability of NO. These observations in experimental hypertension suggest that pharmacological preconditioning of HO-1 attenuates oxidative stress by decreasing O2- levels and, increasing eNOS and levels, and increasing eNOS and EC-SOD.

Evidence for a Secreted and Active Form of ACE2 in Cerebrospinal Fluid

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Angiotensin converting enzyme 2 (ACE2) is a homolog of ACE that preferentially converts the potent vasoconstrictor and growth stimulator angiotensin II (Ang II) to Ang-(1–7), a peptide with vasodilator and anti-proliferative properties. We previously reported ACE2 mRNA and protein in distinct brain regions from both neonatal and adult Sprague-Dawley rats as well as primary cultures of rat astrocytes isolated from the medulla oblongata and cerebellum of neonatal rat brain. Since central components, such as Ang II and the precursor protein angiotensinogen, were detected in cerebrospinal fluid (CSF), the present study investigated the expression of ACE2 in the CSF. Using a specific antibody, we detected a prominent 72 kDa immunoreactive ACE2 in CSF from male Sprague-Dawley rats. We found that the ACE2 was biologically active in CSF collected from male SD rats (S.7 ± 0.47 fmol/µL/min, n = 4) and that enzymatic activity was abolished by a specific ACE2 inhibitor. No difference in ACE2 activity was observed in the CSF from male or female Sprague-Dawley rats [8.12 ± 0.19 fmol/µL/min (male)] vs. 4.83 ± 0.67 (female); n = 4], suggesting that gender does not play an important role in the regulation of ACE2 secretion into the CSF. Additional studies revealed immunoreactive staining for ACE2 in both astrocytes and neurons from brain cell preparations co-stained with cell-specific antibodies. Similar to the CSF results, the conditioned media from cultured astrocytes showed a single, immunoreactive band at approximately 72 kDa, strongly suggesting the presence of a secreted form of ACE2. This secreted enzyme from astrocytes exhibited dose- and time-dependent kinetics when assayed using a fluorescent substrate and converted the endogenous substrate Ang II to Ang-(1–7), as well as Ang I to Ang-(1–9), although the latter reaction was less robust. In summary, the current study is the first to demonstrate the endogenous presence of an enzymatically active form of ACE2 in the CSF. We conclude that astrocytes may constitute a novel paracrine system that maintains the balance of extracellular Ang II and Ang-(1–7) in the brain in part through the secretion of ACE2 into the CSF and interstitial space.
concentration-dependent relaxations, with maximal relaxation of 94.1 ± 5.4% and ED50 of 10^(-6) mol/L. It was equipotent with 14,15-EET. The relaxations to 14,15-EET-PISA were inhibited by the K⁺ channel inhibitor iberiotoxin (100 nmol/L; max relaxation 39.3 ± 12.8%); the 14,15-EET antagonist 14,15-EEZE-SI (10 μmol/L; max relaxation 23.5 ± 9.1%) and abolished by increasing extracellular K⁺ to 20 mmol/L (max relaxation 7.6 ± 12.8%). Relaxations to 14,15-EET were similarly inhibited by these treatments. 14,15-EET-PISA binding to human U937 cell membranes was time- and concentration-dependent. The specific binding reached equilibrium by 15 min at 4°C and remained unchanged at 30 min. With 50 μg of protein, the estimated KD of 14,15-EET-PISA was 33 nM. When 14,15-EET-P125ISA was incubated with myocardial or coronary arterial membranes, a 48kD protein was detected on SDS-PAGE gels. The radiolabeling of the 48kD protein was displaced by unlabeled EETs in a concentration-dependent manner (0.02–200 μmol/L). The order of potency was 11,12–14,15–5,6–8,9-EET. These data suggest that 14,15-EET may exert its effect through a membrane receptor.

Intrarenal Oxidative Stress and Augmented Angiotensinogen (AGT) are Precedent to Diabetic Nephropathy in Zucker Diabetic Fatty Obese Rats

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The Zucker diabetic fatty (ZDF) obese rats (ZDFO) are a model of type 2 diabetes and metabolic syndrome based on impaired glucose tolerance caused by the inherited insulin-resistance gene. ZDFO exhibit progressive nephropathy; however, the mechanisms have remained unclear. A recent study indicates that a temporary blockade of the renin-angiotensin (Ang) system (RAS) during the prediabetic stage attenuates renal injury in another model of type 2 diabetes, suggesting the activated renal RAS in type 2 diabetes. The present study was performed to examine the possible involvement of AGT in diabetic nephropathy of ZDFO. Genetic pairs of male ZDFO and ZDF lean rats (ZDFL) (N=6 each) were maintained on a diet containing 16.7% fat from 12 to 17 weeks of age. ZDFO showed an increased body mass compared to ZDFL (378 ± 18 vs 288 ± 7 g at 17 weeks). Fasting blood glucose levels were also significantly higher in ZDFO compared with ZDFL (148 ± 36 vs 47 ± 5 mg/dl). Urinary levels of 8-isoprostane were significantly increased in ZDFO compared with ZDFL (38.5 ± 4.1 vs 15.7 ± 3.4 ng/day). Systolic BP (SBP) was progressively increased in ZDFO from 120 ± 1 to 137 ± 1 mmHg during this period. In contrast, SBP did not increase in ZDFL. Kidney AGT protein levels were significantly increased in ZDFO compared with ZDFL (1.83 ± 0.34 vs 1.00 ± 0.25, relative ratio). Kidney Ang II contents tended to increase in ZDFO compared with ZDFL (348 ± 100 vs 279 ± 44 pg/g); however, the change was not statistically significant. Expression of Ang II type 1 receptor mRNA was similar between these 2 groups (0.95 ± 0.26 vs 1.00 ± 0.17, relative ratio). Previous papers reported that ZDFO show renal injury at around 25 weeks of age. However, at 17 weeks of age, measured indices of renal damage in the present study (glomerular sclerosis, macrophage infiltration, interstitial expansion, and renal arterial hypertrophy) were not significantly different between these 2 groups. We have previously shown that reactive oxygen species (ROS)-associated AGT enhancement plays an important role in renal damage of genetic salt-sensitive hypertension and the present data suggest that elevated ROS and ROS-induced intrarenal AGT augmentation are present prior to the development of diabetic nephropathy in ZDFO.
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