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, Erasmus 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 Bpfh2). The Rf1, Rf2 and Bpfh1 QTLs are in close vicinity on rat chromosome 1. To determine how the effects of these QTLs can contribute to the increased SBP we compared congenic strains generated carrying the QTLs from FHH on the genomic background of the normotensive 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/Rf1-Rf2). The other three QTLs have been found to be linked to five renal failure QTLs (Rf3 to Rf5) and two blood pressure QTLs (Bpfh1/Rf1 and Bpfh1/Rf2) from FHH on the genomic background of the normotensive ACI strain. Here we compare the effect on SBP and renal damage susceptibility in ACI.FHH(Rf3/Rf3-Rf4), Rf4 double congenic rats to that of ACI.FHH(Rf3/Rf3-Rf4) and ACI.FHH(Rf3/Rf3-Rf4-Rf5), triple congenic rats.

To establish whether renal susceptibility in the (Rf1 × ACI)F1 progeny is significantly higher compared to ACI, FHH and ACI × FHH congenic rats, we measured susceptibility to renal damage following UNX. Whether the increased renal susceptibility is due to a higher SBP or the presence of a different strain background will be established. The results indicate that the renal susceptibility to renal damage in congenic rats is significantly higher than that of parental FHH rats indicating that the other three QTL regions are not only linked to the congenic region.

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

Carmen Hinjosa-Laborde, Jaci A Castania, Patricia M de Paula, Steven W Mintoff; 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 ovarietomized (OVX) females responded to CIH with an increase in blood pressure similar to males. In OVX females, CIH resulted in a fall in plasma sodium concentration and a reduced sodium excretion, suggesting CIH may affect renal 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. CIH 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 h/ day.

The relationship between renal perfusion pressure (RPP) and sodium excretion and urine flow were determined under trachea anesthetized in normoxemic (N)- and hyperoxic (HO)-exposed female (F) and OVX females. CIH-OVX rats. Results are mean ± SEM, and Student t test was used to determine significance of the difference.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sodium Excretion (µEq/min/kg)</th>
<th>Urine Flow (µL/min)</th>
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<tr>
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<td>0.08±0.02</td>
<td>0.29±0.14</td>
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<tr>
<td>CIH-F(n=10)</td>
<td>0.63±0.18*</td>
<td>2.25±0.54*</td>
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<td>N-OVX(n=6)</td>
<td>0.17±0.10</td>
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<td>CIH-OVX(n=6)</td>
<td>0.14±0.09</td>
<td>0.36±0.23</td>
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</table>

RPP = 90

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

Carmina Savioa, Rhy M Tousy, Dierk Endemann, Gian Pu, Eun A Ko, Carolina DeCuidece, Ernesto L Schiffrin; IRCM, 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 an ACE inhibitor 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. One after another of treatment, systolic and diastolic BP were well controlled by valsartan (123±27/74±17 mmHg vs 144±3/84±22 mmHg, p<0.005) and atenolol (123±27/74±17 mmHg vs 144±3/84±22 mmHg, p<0.005, respectively). Glycemic control was similar in the valsartan and atenolol-treated groups (HbA1c 0.06±0.04 vs 0.06±0.02). Endothelium-dependent and independent relaxation did not change significantly with the treatments. L-NAME, L-arginine, and L-NMMA significantly reduced acetylcholine-induced dilation equally in all groups. Resistance artery media-to-lumen ratio was reduced after treatment with valsartan (8.9±6 vs 7.9±0.5%, p<0.005) but not under atenolol (10.6±1 vs 9.8±0.9%, p=0.3). The strain-area curve of vessels from atenolol-treated patients was shifted to the left whereas that of vessels from valsartan-treated patients was unchanged. In conclusion, in diabetic hypertensive patients good control of BP with valsartan or atenolol was associated with improved vascular remodeling in valsartan-treated patients. Addition of an ARB on top of other antihypertensive medication that includes ACE inhibitors thus results in improved resistance artery parameters in diabetic hypertensive patients.

Use of a Community-Based Study to Define Gene-Gender Interaction in Blood Pressure

Brinda K Rana, Paul A Insel, Nicholas J Schork, Daniel T O‘Connor; Univ of California, San Diego, La Jolla, CA

Genetic association studies of blood pressure (BP) and essential hypertension (EH) have primarily focused on identifying monogenic dysmorphisms (SNPs) that influence ethnic or inter-individual differences within a population but have largely ignored a role for gender. In the current study, we utilized a unique community-based approach to test for gender-gene interactions in blood pressure phenotypes. In this unique study, we included 1,790 African-Americans within the 5th percentiles of high and low diastolic BP (DBP) extremes among Southern California. This approach has >90% power to detect loci contributing >3% BP variance. Genotyping subjects at 44 SNPs in 30 autosomal and 2 X-linked genes in adrenergic and renal components that regulate BP revealed 11 SNPs that interacted with sex. Males displayed substantially more positive associations than did females, including association of several adrenergic pathway loci SNPs, including ones in alpha2A- and beta2-adrenergic receptors and monooxygenase oxiad-A. In females, only PLOC1 n22356/3 was associated with elevated DBP; a common haplotype of 2 PLOC1 SNPs showed even stronger association. We also observed opposite effects of genetic variants on BP in males vs. females: elevated DBP in males carrying two (but not fewer) copies of the ADRA2A haplotype but lower DBP in females who carried 1–2 copies. An angiotensin-2 haplotype also showed gender differences. These findings reveal the utility of a community-based approach to assess genetic contribution of SNPs in candidate genes that contribute to BP, including the identification of gender-specific differences in genes that may underlie etiology and clinical features of EH.
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 GABA (ANIES). The cells and MBA segments were fixed in buffered formalin and co-immunostained with anti-palladin and anti-CD34 using secondary antibodies tagged with Alexa fluor 488 and 647, as well as the nuclear stain 4,6-diamidino-2-phenylindole (DAPI). Confocal analysis showed that cultured 80–90% ANIES and 100% of all SMC had positive staining for palladin (n = 3). Palladin was also co-immunostained with GABA in immuno-stained images of rat MBA (n = 3). Immunoblot 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 200 kDa) or SMC where only one isoform (90–92 kDa) is expressed. In summary, palladin is present both in SMC and ANIES, 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 CD34-containing neural cells in the vascular wall may participate in response to injury and vasodilator mechanisms as part of a perivascular sensory neural network.

**LB11**

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 interactions 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 GABA_4 receptor antagonist, baclofen, by using a combination of patch clamp and single-cell RT-PCR techniques. Suppression 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 exacerbated inhibitory response to baclofen following treatment of NTS neurons with Ang II, we examined the effect of Ang II on GABA_A receptor expression in NTS neurons. Real-time PCR data indicate that Ang II treatment (100 nM, 5 hrs) induced a 2-fold increase in GABA_A receptor mRNA levels. Immunostaining experiments also demonstrated that GABA_A 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 GABA_A 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.

**LB12**

Salt and Oxidative Stress in Essential Hypertension

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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) hypertension. Therefore, we explored whether there is a differential effect of salt on OX of SS (n = 14) vs salt-resistant (SR, n = 13) hypertensive subjects, indicating increased OX in SS during a salt-load. We suggest that controversial results of epidemiological studies of the effect of salt on cardiovascular disease may be due to the pitfalls of studying phenotypically heterogeneous hypertensive subjects. Clarifying this issue may require assessment of salt-sensitivity of blood pressure in the studied population.

**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 Gungadoo, Beverly Burke, Abidun Opara, 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; M G Lathrop, Ctr for National of Genotypage, Edinburgh, United Kingdom; Sven-Ingvar Holm, Royal Infirmary, Birmingham, United Kingdom; Martin Farrall, Wellcome Trust Ctr 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 refine existing signals. The analysis of covariate-defined subsets however, is dependent on researchers’ prior beliefs which might bias further research. Here we report a novel approach to capitalise on additional phenotypic data in linkage studies. The BRIGHT Genes 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 10cM 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 index and measures on chromosome 20 (genomewide p < 0.002) and kidney function measures (creatinine clearance and glomerular filtration rate) on chromosome 5p (genomewide p < 0.008). Correcting for the multiple traits and genetic locations studied, our overall 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 = 27). The locus on chromosome 5p contains several candidate genes which may influence kidney function, but 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

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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 (ETB) receptor deficiency results in self-sustaining hypotension. Disruption of D3 dopamine receptor (D3) 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 receptor (PB2136807), increased ETB receptors in a time- and concentration-dependent manner, effects that were blocked by the D3 antagonist, U99194A (10−4 M/24 h). In contrast, in RPT cells from SHRs, PB2136807 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 extent 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. The 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

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Introduction: Endothelial dysfunction or cytoplasmic circularization is associated to a greater incidence of cardiovascular (CV) events. Hypothesis: To investigate the presence of a
prognostic role of endothelial dysfunction in human small subcutaneous arteries. Methods: Ninety subjects (normotensive, essential or secondary hyperten-

sives, type 2 diabetics) were included in our study. All subjects underwent a biopsy of gluteal or abdominal subcutaneous fat tissue. Small resistance arteries were dissected and mounted on an isometric myograph. Endothelium-dependent and independent vasodilatation were assessed by concentration-

response curves (from 10−9 to 10−5 mol/L) to acetylcholine (ACH) and sodium nitroprusside (SNP), respectively, in vessels precontracted with norepinephrine 10−6 mol/L. The subjects were re-evaluated (by clinical visit or telephonic interview) after an average follow-up time of 5.6 years (2.6–10.7). Results: Twenty-nine subjects had a documented fatal or non-fatal CV event (3.67% events % per year). CV events were observed in 5 normotensive subjects (10%), in 14 hypertensive patients (39%), in 2 patients with phaeochromocytoma (20%), in 3 patients with primary aldosteronism (27%), in 4 patients with renovascular hypertension (40%), and in 5 normotensive diabetic patients (38%). A lack of vasodilatation of small arteries was similar in subjects with or without CV events (see table). No correlation to SNP was similar in the two groups (max vasodilatation: −72.0% (20.6% vs. −76.4% (17.8%). Analogous results were obtained subvibidng patients in the different subgroups (essential or secondary hypertension, etc.). Conclusions: Our results suggest that endothelial dysfunction in the microcirculation does not predict CV events. It means that a prognostic role for endothelial dysfunction in such a vascular district may be detected only in low-medium risk patients, or in other vascular districts prone to atherosclerosis.

Table

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<tr>
<td>CV events</td>
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<td>-20.8 ± 15.6</td>
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<td>-60.5 ± 18.9</td>
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<td>No CV events</td>
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Effects of Insulin on Endothelial and Contractile Function of Small Resistance Arteries from Hypertensive and Diabetic Patients

Damiano Rizzoni, Enzo Porteri, Carolina De Cucchi, Gianluca E Boari, 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; Stefano M Giuliani, Chair of General Surgery, Dept. of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Silvia Pasadri, Enrico Agabiti Rosei; Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy

Introduction: We have previously demonstrated that high-dose insulin may induce an increase in the reactivity to norepinephrine (NE) in mesenteric small subcutaneous arteries of hypertensive rats. Hypothesis: To evaluate the effect of low- and high-dose insulin on the dose-response curves to NE and acetylcholine (ACH) in subcutaneous small resistance arteries of hypertensive and diabetic patients. Methods: Twelve normoensensitive subjects (NT), 11 patients with essential hypertension (EH), 8 patients with non-insulin-dependent diabetes mellitus (NIDDM), and 8 patients with both EH and NIDDM (EH-NIDDM) were included in the study. Subcutaneous small resistance arteries were dissected and mounted on an isometric myograph. Concentration-response curves (CRC) to NE (from 10−9 to 10−5 mol/L) and acetylcholine (from 10−8 to 10−6 mol/L) were performed in presence or absence of insulin 715 pmol/L (insulin ld) and 715 mol/L (high dose, hd). Results: The results are summarized in the Table (*p<0.05, ***p<0.001 vs. basal). A significant reduction in the contractile response to NE was observed in NT after pre-incubation of the vessels with both ld and hd insulin. No reduction was observed in NIDDM and EH-NIDDM. A significant decrease was observed in EH with hd insulin. Moreover, a significant difference in reduction in contractile response at maximal dose of NE in presence of insulin ld was observed in NT compared to EH (p=0.03, NIDDM (p=0.02), and EH-NIDDM (p=0.05) whereas no difference was observed with hd insulin. No difference was observed in CRC to ACH before or after pre-contraction with either ld or hd insulin were observed.

Table

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<th>Table</th>
<th>Basal NE 10−5 mol/L (kPa)</th>
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LB18

Relationships Between Plasma Asymmetric Dimethylarginine and Vascular cNOS Activity and Endothelial Function in ADPKD

Dan Wang, Georgetown Univ, Washington, DC; Svend Strandgaard, Herlev Hosp, Copenhagen, Denmark; Julie Raggio, Georgetown Univ, Washington, DC; Anne Leone, 30Noxon Bioanalyses Inc., Okland, CA; Christopher S Wilcox; Georgetown Univ, Washington, DC

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase (NOS). Our previous studies have shown that endothelial dysfunction, mediated by down-regulation of NOS and a decreased nitrite oxide (NO) is present in autopsies of patients with renal insufficiency. ADMA. Methods: The relationship between ADMA levels and NOS activity in ADPKD was assessed. The results show that ADMA and NOS activity in ADPKD is unclear. We hypothesized that plasma ADMA concentration decreases NO2/NO3 contributes to the endothelial dysfunction of ADPKD. PON2, symmetrical dimethylarginine (PON2) and L-arginine (P arg) were determined in relation to plasma nitrate levels (NO2/NO3) and NO2/NO3 activity. The results show that PON2 and PON2 concentrations in ADPKD patients were significantly higher than in controls (0.64±0.16 vs 0.41±0.07 mol/L in PON2, 0.69±0.06 vs 0.40±0.06 mol/L in NO2/NO3, all with p<0.002). P arg concentrations were significantly different between the two groups (19.6±13.1 mol/L in PON2, 5.9±3.6 and 42 ± 20 vs 33 ± 19 vs 43 ± 39 mol/mg protein in NO2/NO3 in CN, all with p<0.005). PON2 was inversely correlated vs NO2/NO3 (r=0.59, p=0.001) and NO2/NO3 were determined by relevant analysis kts. EDR was determined in isolated small subcutaneous resistance arteries using Mulvany-Halpern myograph. The results show that PON2 and PON2 concentrations in ADPKD patients were significantly higher than those in control group (0.64±0.16 vs 0.41±0.07 mol/L in PON2, 0.69±0.06 vs 0.40±0.06 mol/L in NO2/NO3, all with p<0.002). P arg concentrations were significantly different between the two groups. PON2-NOS, VNO2 and EDR were similar lower in ADPKD patients than in controls (55.7±7 vs 73±13 mol/L in PON2, 14/16 vs 33/49 ± 33 ± 4 pmol/mg protein in NO2/NO3, 85/36; 51±15% in EDR, all with p<0.05). PON2 was inversely correlated with NO2/NO3 (r=0.59, p=0.001) and NO2/NO3 were determined by relevant analysis kts. In conclusion, in patients with ADPKD, ADMA is significantly increased before renal failure, and may contribute to the development of endothelial dysfunction and cardiovascular disease.

LB19

Chronic AngII Infusion Causes Greater Hypertension and Increased IL-6 in Mice with Knockout of Peroxisome Proliferator Activated Receptor-alpha

Dexter L Lee, Jennifer S Pollock, Michael W Brands; Med College of Georgia, Augusta, GA

Previous results from our laboratory suggest that interleukin-6 (IL-6) plays a major role in mediating angiotensin II (AngII)-salt hypertension, and the mechanism may include actions that are independent of renal injury. Other laboratories have shown separately that activators of the peroxisome proliferator activated receptor-alpha (PPAR-alpha) decrease blood pressure and IL-6 production. Therefore, this study tested the hypothesis that the deletion of PPAR-alpha receptors would augment the hypertensive response to chronic AngII infusion. Male PPAR-alpha knockout (PPAR-alpha KO) mice and their wild-type (WT) controls, 129/SvEvj mice, were implanted with blood pressure telemetry devices, and mean arterial pressure (MAP) was measured 14 hours/day throughout the study. Baseline MAP during the control period averaged 121±6 mmHg and 114±5 mmHg for PPAR-alpha KO and WT mice, respectively. AngII (90 ng/min, 5x) caused a rapid increase in MAP in both groups, averaging 144±6 mmHg (PPAR-alpha KO) and 139±5 mmHg (WT) by day 2. Although blood pressure plateaued at this level in WT mice (143±6 mmHg) by day 4, MAP continued to increase in the PPAR-alpha KO mice, averaging 164±6 mmHg on day 7 and 173±5 mmHg on day 7 of AngII. IL-6 measurements in plasma samples taken on day 7 were 109±30 and 44±20 pg/mL for PPAR-alpha KO and WT mice, respectively. These data suggest that PPAR-alpha dependent mechanisms play a major role in limiting the hypertensive response to chronic increases in AngII, and that suppression of AngII stimulation of IL-6 may mediate that effect.

LB20

Regulation of Cardiac Angiotensin Converting Enzyme 2 (ACE2) and ACE by Aldosterone

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Angiotensin converting enzyme 2 (ACE2) is a new enzyme of the renin-angiotensin-aldosterone system that converts angiotensin II (AngII) into angiotensin-(1–7) (Ang-(1–7)), in contrast to its
homologous, ACE, which generates Ang II from Ang I. In addition, Ang II and Ang-(1–7) have opposing functions; Ang II is a vasococontractor and mitigates 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 the effects of aldosterone on cell cycle. 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 38.8±1.35 of control, n=3, p<0.05). The mineralocorticoid receptor antagonist spironolactone (1 μM) completely blocked the aldosterone-induced down-regulation in ACE2 mRNA (0.65±0.04 relative gene 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 or Ang-(1–7) expression. In contrast, treatment of myocytes with 100 nM aldosterone caused a significant up-regulation of ACE mRNA (1200% of control, n=3, p<0.05). These results suggest that aldosterone alters the ratio of ACE/AEC2 in the heart, increasing ACE while concomitantly decreasing ACE2. 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/AEC2 by mineralocorticoid antagonists such as spironolactone may participate in their attenuation of the accumulation and structural remodeling of the collagen matrix.

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

Emile C Vie, Karim Benkirane, Rhiann M Touyz, Ernesto L Schiffrin; Clinical Research Institute of Montreal, Montreal, Canada

Vascular superoxide (O₂⁻) levels are increased in DOCA-salt rats. The purpose of this study was to investigate the sources of the endothelial reactive oxygen species (ROS) production in conduit (aorta-AO) and resistance arteries (mesenteric arteries-MA) of DOCA-salt rats, and the implication of ETA receptor in ROS generation. DOCA-salt rats (n=8) received 5 different treatments: apocynin (NAD(P)H oxidase inhibitor, 1.5 mM/L), lipoallopurose (xanthine oxidase inhibitor, 100 mg/kg/day), bosenian (ET receptor antagonist, 100 mg/kg/day), BMS182874 (ET, antagonist, 40 mg/kg/day), and hydralazine (25 mg/kg/day). Data were compared to unphrectomized rats (Unknl). 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), bosentan (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 bosenian-treated DOCA-salt rats. As well, chemiluminescence analysis showed increased oxidative damage in MA and AO from DOCA-salt rats compared to controls (413.8±0.85 vs 116±0.51 and 118±0.10 vs 60±1 x 10¹⁰ cm²/g dry weight, respectively). All treatments reduced or prevented the increase of xanthine oxidase activity (P<0.01) in MA whereas bosenian and BMS had no effect in AO. In addition, confocal microscopy showed reduced O₂⁻ when tissues were treated in situ with TFA and COOP inhibitors (of mitochondrial electron transport complexes II and V). Rotenone (mitochondrial complex I inhibitor) had no effect in MA. Our findings suggest involvement of multiple ROS-generating systems, some of which are ET, sensitive, in the development of hypertension and vascular inflammation in DOCA-salt rats.
Salt-resistant Rats

Hypertension Augments the Pressor Response to Environmental Stress in Dahl Salt-resistant Rats

Gerard D’Angelo, Jennifer S Pollock, David M Pollock; Med College of Georgia, Augusta, GA

Endothelin A (ET1) 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 ET1 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 ET1 receptor antagonist ABT-267 (5 mg/kg/day) or vehicle. Both the superfused aorta minus m.im. and the drinking water were drawn as described involving indwelling catheters, and basal and stress-induced increases in microsphere norepinephrine (NE) and epinephrine (Epi) were measured by RIA. Tempol, but not ABT-267, significantly reduced plasma TBARS (11.7±0.6 vs. 9.5±0.2 nmol/ml, untreated vs. tempol). In telemetry-instrumented animals, both ABT-267 (8.1±1.7 vs. 36.3±6.2 nmol/ml, x 3 min, untreated vs. ABT-267, p<0.05) and tempol (17.2±2.3 vs. 27.6±2.0 mmHg x 3 min, untreated vs. tempol, p<0.05) augmented the total pressor response (area under the curve) to air jet stress. ABT-267 caused significant increases in basal (156±7 vs. 285±31 pg/ml, untreated vs. ABT-267) and stress-mediated increases (201±22 vs. 419±36 pg/ml, untreated vs. ABT-267) of NE. Epi was unaffected by ABT-267. Conversely, tempol had no effect on baseline NE and Epi, and on the stress-mediated increase in NE; the stress-induced rise in Epi was attenuated by tempol (705±107 vs. 460±72 pg/ml, untreated vs. tempol, p<0.05). In summary, ABT-267 and tempol potentiated the pressor response to acute stress. Unlike ABT-267, the increased pressor response with tempol was not accompanied by further elevations in plasma catecholamines, suggesting its mechanism is distinct from that of ET1 receptor antagonism. These data suggest that ET1 receptor activation suppresses sympathetic nerve activity independent of its ability to promote oxidative stress. We conclude that removal of the sympathomimetic effect by ET1 receptor blockade contributes to augmented pressor response to acute stress in DR rats.

Changes in Ca2+ Influx Via Voltage Gated Calcium Channels in Nucleus of the Solitary Tract Neurons From Renal Wrap Hypertensive Rats

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The nucleus of the solitary tract (NTS) is the central site of termination of baroreceptor afferents. We hypothesize that changes occur in voltage-gated calcium channels (VGCCs) within NTS neurons as a consequence of hypertension. Whole cell patch clamp recordings were obtained from adult normotensive (NT, 109±2 mmHg, n=63) and 4-week, renal wrap hypertensive (HT, 158±6 mmHg n=24) rats. In some experiments the tracer DiA was applied to the aortic nerve to visualize NTS neurons receiving baroreceptor synaptic contacts. Cell size, as estimated by membrane capacitance, was not different comparing neurons from HT (7.9±1.0 pF, n=7) and NT (7.2±1.6 pF, n=28) rats. This was also observed in NTS neurons receiving arterial baroreceptor inputs as determined by the presence of soma DiA labeled apoppositions. Membrane capacitance was not different comparing DiA labeled NTS neurons from HT (9.0±1.6 pF, n=9) and NT (9.0±1.8 pF, n=7) (p=0.8) rats. Ba2+ currents (500ms, -80mV pre-potential, 500ms voltage steps in 5mV increments to -50mV peaked by 1.7±0.3 ms) were significantly larger in cells from HT (36.9±3.6 pA/pF at -80mV and 43.7±4.0 pA/pF at -100mV, n=20) compared to NT (26.6±2.6 pA/pF at -80mV; 32.3±3.8 pA/pF at -100mV, n=28) (p=0.02 for Vh -60mV and for Vh-100mV). Peak LVA currents were not different between HT (8.9±3.8 pA/pF) and NT (7.8±2.9 pA/pF) NTS neurons from HT rats (p=0.7). These findings demonstrate that 4 weeks of renal wrap hypertension can increase Ca2+ influx through HVA VGCCs in NTS neurons receiving arterial baroreceptor inputs.

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

Gerard D’Angelo, Jennifer S Pollock, David M Pollock; Med College of Georgia, Augusta, GA

Endothelin A (ET1) 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 ET1 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 ET1 receptor antagonist ABT-267 (5 mg/kg/day) or vehicle. Both the superfused aorta minus m.im. and the drinking water were drawn as described involving indwelling catheters, and basal and stress-induced increases in microsphere norepinephrine (NE) and epinephrine (Epi) were measured by RIA. Tempol, but not ABT-267, significantly reduced plasma TBARS (11.7±0.6 vs. 9.5±0.2 nmol/ml, untreated vs. tempol). In telemetry-instrumented animals, both ABT-267 (8.1±1.7 vs. 36.3±6.2 nmol/ml, x 3 min, untreated vs. ABT-267, p<0.05) and tempol (17.2±2.3 vs. 27.6±2.0 mmHg x 3 min, untreated vs. tempol, p<0.05) augmented the total pressor response (area under the curve) to air jet stress. ABT-267 caused significant increases in basal (156±7 vs. 285±31 pg/ml, untreated vs. ABT-267) and stress-mediated increases (201±22 vs. 419±36 pg/ml, untreated vs. ABT-267) of NE. Epi was unaffected by ABT-267. Conversely, tempol had no effect on baseline NE and Epi, and on the stress-mediated increase in NE; the stress-induced rise in Epi was attenuated by tempol (705±107 vs. 460±72 pg/ml, untreated vs. tempol, p<0.05). In summary, ABT-267 and tempol potentiated the pressor response to acute stress. Unlike ABT-267, the increased pressor response with tempol was not accompanied by further elevations in plasma catecholamines, suggesting its mechanism is distinct from that of ET1 receptor antagonism. These data suggest that ET1 receptor activation suppresses sympathetic nerve activity independent of its ability to promote oxidative stress. We conclude that removal of the sympathomimetic effect by ET1 receptor blockade contributes to augmented pressor response to acute stress in DR rats.

Renal Venous Oxygen Tension After ACE-inhibition Predicts a Functional Renal Artery Stenosis in Two-kidney, One Clipped 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 intervention. The purpose of this study was to determine whether renal venous oxygen tension (RVpO2) and its response to acute ACE inhibition predict the functional significance of RAS.

Using a rat model of renovascular hypertension, we evaluated RVpO2 and its response to acute ACE inhibition following aortic clipping or sham surgery (sham). Rats were inactin-anesthetized and split kidney function assessed in vivo using RVpO2 electrodes inserted into one RV. The contralateral kidney served as a control. rats were randomized to receive vehicle or captopril (0.3 mg/kg bw, infusion 0.3 mg/kg bw/h). An additional clipped group had tempol (10 mg/kg bw, iv) added to captopril. 2K1C rats had elevated blood pressure (161±8 vs. sham 109±7 mmHg; p<0.05), right kidney hypertrophy (1.6±0.1 g; p<0.05), and renal perfusion pressure lowered similarly to the captopril-treated animals by a suprarenal aortic clamp (enalaprilat; bolus 0.3 mg/kg bw, infusion 0.3 mg/kg bw/h). An additional clipped group had tempol (10 mg/kg bw, iv) added to captopril. 2K1C rats had elevated blood pressure (161±8 vs. sham 109±7 mmHg; p<0.05), right kidney hypertrophy (1.6±0.1 g; p<0.05), and renal perfusion pressure lowered similarly to the enalaprilat-treated animals by a suprarenal aortic clamp (enalaprilat; bolus 0.3 mg/kg bw, infusion 0.3 mg/kg bw/h). An additional clipped group had tempol (10 mg/kg bw, iv) added to captopril. 2K1C rats had elevated blood pressure (161±8 vs. sham 109±7 mmHg; p<0.05), right kidney hypertrophy (1.6±0.1 g; p<0.05), and renal perfusion pressure lowered similarly to the enalaprilat-treated animals by a suprarenal aortic clamp (enalaprilat; bolus 0.3 mg/kg bw, infusion 0.3 mg/kg bw/h). An additional clipped group had tempol (10 mg/kg bw, iv) added to captopril.
Renal and Heart ACE2 Activity in Models of ACE2 and ACE Ablation and Diabetic Mice

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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 (ACE-ACE2, ACE-ACE2 knock-out mice) to investigate ACE2 activity over a wide range of ACE2 and ACE protein expression, respectively. ACE2 activity had a strong positive correlation with renal cortex ACE2 protein expression (90 kDa band recognized by our non-commercial ACE2 antibody) in both knockout models and their respective wild-type littermates (r=0.94, p<0.01). In the ACE2 knockout, the 90 kDa band was absent and ACE2 activity was barely detectable despite the presence of another 67 kDa band detected by our ACE2 antibody. Renal cortex ACE2 activity had no correlation with renal cortex ACE protein expression (r=0.02, p=NS, renal cortex) demonstrating the specificity of our assay for ACE2. In 24-week-old db/db mice, ACE2 activity in renal cortex was about twenty-fold higher than in the heart (20.4 ± 3.4 vs 1.27 ± 0.24 RFU/ug protein/hr, respectively). In renal cortex of 8 weeks old db/db mice, ACE2 activity was increased as compared to db/m controls (db/db 46.7 ± 4.4 vs db/m 22.0 ± 4.7 RFU/ug protein/hr, p=0.01), which is in concordance with the higher levels of ACE2 protein in the renal cortex of the db/db model of type 2 diabetes. We conclude that ACE2 enzymatic activity reflects the relative abundance of the ACE2-immunoreactive protein in the renal cortex and the db/db model of type 2 diabetes and may be useful in other pathophysiological states such as hypertension, where ACE2 activity measurements may provide mechanistic insight.

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 (pro)renin (PR) receptor (Nguyen et al., 2002). Here we show that, in neonatal rat cardiomyocytes, 1) PR receptor expression, and 2) PR-induced activation of 2nd messenger systems. Polyclonal antibodies detected membrane localized (PR) receptor expression by Western and confocal microscopy. PR increased p38 MAP kinase activity was barely detectable despite the presence of another 67 kDa band detected by our ACE2 antibody. Renal cortex ACE2 activity had no correlation with renal cortex ACE protein expression (r=0.02, p=NS, renal cortex) demonstrating the specificity of our assay for ACE2. In 24-week-old db/db mice, ACE2 activity in renal cortex was about twenty-fold higher than in the heart (20.4 ± 3.4 vs 1.27 ± 0.24 RFU/ug protein/hr, respectively). In renal cortex of 8 weeks old db/db mice, ACE2 activity was increased as compared to db/m controls (db/db 46.7 ± 4.4 vs db/m 22.0 ± 4.7 RFU/ug protein/hr, p=0.01), which is in concordance with the higher levels of ACE2 protein in the renal cortex of the db/db model of type 2 diabetes. We conclude that ACE2 enzymatic activity reflects the relative abundance of the ACE2-immunoreactive protein in the renal cortex and the db/db model of type 2 diabetes and may be useful in other pathophysiological states such as hypertension, where ACE2 activity measurements may provide mechanistic insight.

Salt-Induced Water Intake

Central de Venezuela. Instituto de Medicina Experimental. Escuela de Medicina Luis Razetti, Caracas, Venezuela; Carlos Diaz-Freire, Mohan K Raizada, Colin Summerson, Univ of Florida, Gainesville, FL

Studies utilizing pharmacological agents have demonstrated that dipsogenic responses elicited by blood, or as hyperosmotic water deprivation are mediated by perifornical lateral septum (VLS) 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 of being an area rich in AT2-R. For this reason, we studied the role of early inflammation for influence by adrenergic pathway polymorphisms. Our approach involved down-regulation of AT2-R expression via RNA interference. For this, we characterized a series of dsRNA molecules that elicited specific silencing of the AT2-R. Among the chosen sequences, one dsRNA was able to significantly decrease AT2-R specific binding by 60% when compared to a scrambled dsRNA 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 0.5 micrograms of either AT2-R dsRNA or a control of 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 dsRNA), we observed a 45 % decrease in salines-induced water intake for the animals treated with AT2 dsRNA, similar to the effects of the AT2-R antagonist PD123319 (1 mg) injected directly into the VLS. This effect of the AT2-R dsRNA 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
Angiotensin II Induced Hypertensive Response is Modulated Through Tumor Necrosis Factor-alpha: Role of Nrx1, Nrx4 and G9p1phox

Julie Hufnagel, Anuradha Guggilam, Masudul Haque, Inder Sehgal, Joseph Francis; Louisiana State Univ, Baton Rouge, LA

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 induce hypertensive stress and contribute to development of heart disease. In this study, we examined whether Ang II induced hypertensive effect is modulated through cytokinins and whether the g9p1phox and its homologues, Nrx1 and Nrx4 are involved in this effect. Method: Wildtype (B6129SF2/Jand TNF-/-/-) mice were implanted with osmotic minipumps containing Ang II (1µg/kg/min) or saline for 14 days. In a group of TNF-/-/- mice, human recombiant TNF-α was given at a dose of 10ng/day 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 LV was removed for the measurement of g9p1phox, Nrx1 and Nrx4 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 parentheses. 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 hypertrophic response is also part mediated through TNF-α. 3) Ang II induces the expression of the cytokine subunits g9p1phox, Nrx1 and Nrx4 in the left ventricle of the mice. 4) Cytokinins modulate Ang II induced increase in g9p1phox homologues, suggesting a role for these homologues in hypertensive response.

TABLE

<table>
<thead>
<tr>
<th>Group</th>
<th>LV IVS/D (mm)</th>
<th>LV function</th>
<th>Blood Pressure</th>
<th>Blood Pressure</th>
<th>Nrx1</th>
<th>g9p1phox Nrx4 Nrx4 (mRNA)</th>
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<td>Wildtype</td>
<td>0.40 ± 0.03</td>
<td>29.5</td>
<td>119.67 121</td>
<td>116.7 -9.2</td>
<td>0.39 ± 0.2</td>
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<td>+Saline</td>
<td>-</td>
<td>7.1</td>
<td>0.9 ± 0.7</td>
<td>7.4 ± 0.4</td>
<td>0.02 ± 0.3</td>
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<td>+Ang II</td>
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<td>0.04</td>
<td>2.3</td>
<td>±1.18</td>
<td>5.8 ± 0.1</td>
<td>±0.4 ± 0.2</td>
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<tr>
<td>II (n=6)</td>
<td>-</td>
<td>(34)</td>
<td>(5)</td>
<td>(37)</td>
<td>(44)</td>
<td>(29)</td>
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<tr>
<td>TNF-α</td>
<td>-</td>
<td>±0.4</td>
<td>±2</td>
<td>±4.7</td>
<td>±12.8</td>
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<td>+ANG II</td>
<td>-</td>
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<td>±12.8</td>
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Angiotensin II (Ang II) is postulated to cause tailing through both BP-dependent as well as direct BP independent pathogenetic mechanisms based on the demonstration of such potential patho-physiological effects through these pathways. Ang II magnifies the deleterious effects of hypertension (HTN) on target tissues. However, for such interpretations in vivo models is primarily based on isolated and intermittent tail cuff BP measurements. Direct comparisons of the quantitative relationships between BP and renal damage in the presence and absence of Ang II have not been performed using the more accurate direct and chronic radiotelemetric BP monitoring. These relationships were examined in the normotensive 3/4 M/R by surgical excision model in which HTN was superimposed ~2 weeks after RMR either by the substitution of 1% NaCl as drinking fluid or by continuous SC infusion of Ang IV via Alzet pumps in doses of 125ng=2175ng=3215ng and 2300ng=500ng=100ng kg=day. After 3 weeks, proteinuria (mp24hrs) was measured and % glomerulosclerosis (GS) including ischemic, quantitated in a blinded manner in perfusion fixed remnant kidneys. Due to the lack of significant differences for any of the parameters between the Ang 125 and 175 and the Ang 225 and 300 groups, the results for these two groups are combined and presented as low and high dose Ang (Ang L and Ang H, respectively). Results: mean ± SEM.

TABLE

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Systolic BP (mmHg)</th>
<th>Proteinuria</th>
<th>% GS</th>
</tr>
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<tbody>
<tr>
<td>1% NaCl (n=23)</td>
<td>165 ± 3</td>
<td>90 ± 10</td>
<td>17 ± 2.3</td>
</tr>
<tr>
<td>Ang I (L=25)</td>
<td>142 ± 7*</td>
<td>70 ± 29</td>
<td>17 ± 2.3</td>
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<tr>
<td>Ang II (H=15)</td>
<td>166 ± 6*</td>
<td>24 ± 24</td>
<td>17 ± 2.3</td>
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* p < 0.05 compared to other groups Linear regression analysis showed good correlations between the average systolic BP during the 3 wks and % GS in all groups combined (p<0.001). However, the slope of the relationship between GP and % GS increased in systolic model was lower than that in Ang II groups (1 mHg=10% vs 3 mHg=1% of K15 vs 1% NaCl=0.12). These data not only fail to demonstrate additional BP-independent deleterious effects of Ang II on GS but suggest that risk GS may be seen with a given BP with the lower doses of Ang, possibly due to preglomerular vascular resistance.
activating cardia 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 AT1R and ATRAP on immunofluorescence under basal condition, and a substantial ATRAP 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 on the surface of cardiomyocytes (35.9% in lacZ control), suggesting an inhibitory effect of ATRAP on the recycling of AT1R. 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 attenuates Ang II-mediated hypertrophic responses in cardiomyocytes, and may suggest a novel strategy to inhibit cardiac hypertrophy hyper trophy.

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 (DS) rats. The aim of this study was to examine the role of salt sensitivity in DS in relation to estrous cycle and the participation of renal endothelin system in sex hormone modulated hypertension. To achieve this goal, we examined the effects of castration and ovariectomy 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. The number of ET receptors on the surface of cardiomyocytes was measured in kidney cortex and media by real-time RT-PCR. Placement of DS rats on HS for 3 weeks caused a progressive increase in MAP in males (from 121 ± 2 to 168 ± 2 mm Hg; LS vs. HS p < 0.05) and females (from 115 ± 3 to 133 ± 4 mm Hg; LS vs. HS p < 0.05). Castration in males slowed the progression of hypertension (from 121 ± 2 to 145 ± 3 mm Hg; LS vs. HS p < 0.05) and ovariectomy accelerated the increases in MAP (133 ± 3 to 191 ± 6 mm Hg; LS vs. HS p < 0.05). Development of salt sensitive hypertension in male DS rats was associated with a marked increase of prepro-ET-1 expression in kidney cortex (from 1.7 ± 0.01 to 3.8 ± 0.01), suggesting the occurrence of ET overexpression in ET signaling pathways, although, to a lesser extent than males. Expression of ET receptors was not altered by salt or by ovariectomy in females. Sexual hormones are important modulators of blood pressure in DS rats. The protective effect of estradiol on the pressor response to HS in females is not mediated by changes in renal endothelin system. Testosterone plays a major role in the development of salt sensitive hypertension in DS rats perhaps via disregulation in renal endothelin system.

Lack of Heme Oxgenase -1 in Transgenic Rats Increases Vascular Reactivity and Impairs Endothelial Function

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The heme-heme oxygenase (HO) system has been implicated in the regulation of extracellular superoxide dismutase (EC-SOD), inducible nitric oxide synthase (iNOS), and superoxide anion (O2-) formation. This study examined the consequences of HO-1 suppression, using retroviral mediated HO-1 gene in antisense orientation, on EC-SOD, iNOS and acetylcholine-induced relaxation in mesenteric small arteries (100–200 μm diameter). Five-day-old Sprague-Dawley rats received an intra-luminal ventricular injection of approximately 5×105 cfu/ml of retroviruses containing rat HO-1 in antisense orientation (L5SH-RO-K0-1-ΔS, or control retrovirus (L5SH). Three days later, the two groups were weight-matched. The preconstriction was achieved at a dose of 2 mg/100 g body wt, twice on consecutive days and were sacrificed on the third days. LSN-HO-1-AS showed a 38% decrease (p < 0.05) in renal and vascular HO derived CO and bilirubin synthesis compared LXS. HO-1, but not HO-2 protein levels were decreased in renal and vascular tissues from LSN-HO-1-AS rats. Moreover, arterial VACAM expression, were increased in LSN-HO-1-AS compared to LXS. Isolated mesenteric arteries obtained from LSN-HO-1-AS exhibited contraction to FE (10-10 M) and relaxation to Ach (10-10 to 10-4 M) contractions and relaxation were markedly increased and decreased, respectively. Vasorelaxation was increased by Ach. HO-1 mRNA level was increased from 9.4 ± 3.9 to 10.4 ± 9.1% in heme treated transgenic rats vessels. SOD protein levels decreased in renal and vascular tissues in LSN-HO-1-AS relative to LXS. Administration of HO enzyme caused a significant increase in EC-SOD in both LXS and LSN-HO-1-AS whereas administration of HO inhibitor, to LXS prevented HO-1 mediated increase in EC-SOD suggesting that the HO-1-derived products, exhibit the ability to upregulate EC-SOD. Collectively, these results implicate HO-1 and its catalytic products as a regulatory modulators of the antioxidant system. Such function may underlie, at least in part, the antioxidant properties of HO-1 overexpression and may contribute to its role in protecting endothelial function and preventing increases in vascular reactivity and blood pressure.
Obesity and Insulin Resistance in α-Calcitonin Gene-Related Peptide (CGRP) Knockout Mice

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α-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 (48.1 ± 1.3 vs 34.2 ± 1.8 g). In the present study, 8–10-wk-old α-CGRP KO mice were compared to their WT counterparts, with obesity (calculated from body weight/height 2) being significantly higher in the α-CGRP KO mice compared to WT controls (44.0 ± 0.5 mg/ml, 0.30 ± 0.07 mg/ml vs 0.42 ± 0.08 mg/ml, respectively). Compared to WT mice, α-CGRP KO mice demonstrated a significant increase in basal blood pressure (120/80 vs 110/60 mm Hg). In conclusion, dietary salt both changes the abundance of NCC and receptor activation plays a role in mediating RUPP-induced hypertension. AT1R-αA were detected by the chromatic response to AT1 receptor-mediated stimulation of cultured neonatal rat cardiomyocytes coupled with receptor-specific antagonists. Mean arterial pressure (MAP) was significantly higher in RUPP rats (137 ± 1 mmHg) than in normal pregnant (NP) rats (107 ± 1 mmHg). The hypertension in the RUPP rats was associated with significant elevations in angiotensin II levels and a significant activation of AT1 receptor (RUPP: 5.4 ± 1.6 vs NP, 0.6 ± 0.3 units). To determine the importance of AT1 receptor activation in the hypertension in this rat model of preeclampsia, we infused AT1 receptor antagonist, L-188809, for 5 days (days 14 to 19 of gestation) in NP and RUPP rats. Chronic administration of the AT1 receptor antagonist resulted in significant decreases in MAP in both NP (115 ± 2 to 101 ± 2 mm Hg) and RUPP rats (122 ± 2 vs 90 ± 2 mm Hg). However, the decrease in MAP was significantly greater in the RUPP rats (Δ 32 mm Hg) vs NP rats (Δ 20 mm Hg). In summary, hypertension in RUPP rats is associated with significant elevations in AT1 receptor agonist antibodies. Moreover, AT1 receptor activation contributes to the hypertension in response to chronic reductions in uterine perfusion in pregnant rats.

Bilateral Renal Denervation Prevents Development of Hypertension in a Model of Fetal Programming Induced by Placental Insufficiency in the Rat

Norma Ojeda, W. R Johnson, Terry M Dwyer, Barbara T Alexander; Univ of Mississippi Med Ctr, Jackson, MS

Low birth weight (LBW) is a risk factor for development of hypertension in humans. We previously reported that reduced uterine perfusion initiated at day 14 of gestation in the pregnant rat results in LBW offspring predisposed to the development of hypertension. In addition, we have shown that bilateral renal denervation (BRD) abolishes hypertension in adult LBW offspring suggesting that the renal nerves play an important role in the maintenance of hypertension in this model of LBW. The purpose of this study was to determine the importance of the renal nerves in the development of LBW-induced hypertension. Bilateral renal denervation (BRD) or sham denervation (SD) was initiated at 4 weeks of age. Mean arterial pressure (MAP) was determined 2 weeks later at 6 weeks of age in conscious chronically instrumented animals. At 6 weeks of age MAP was significantly increased in intact LBW offspring as compared to intact control offspring (114±3 vs 103±2 mmHg, P<0.01, SD LBW vs. SD control, respectively). However, bilateral renal denervation abolished hypertension in LBW offspring (104±1 mmHg, P<0.01 vs. LBW SD), but did not alter MAP in control offspring (102±3 mmHg). Adequacy of renal denervation was verified by a greater than 90% reduction in renal nephrinepine content (LBW: 22.7±4 BRD: 301±22 mg/g, P<0.01 and control: 31±6 BRD: vs 193±13 SD mg/g, P<0.01). In addition, renin nephrinepine content was significantly elevated in intact LBW offspring as compared to intact control offspring (P<0.01, SD LBW vs. SD control). Thus, the renal nerves may participate in the etiology of hypertension in LBW offspring induced by placental insufficiency in the rat.

The Genomic Signatures of Hypertension

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In a set of 120 hypertensive and dyslipidemic French-Canadian families from the Saguenay-Lac-St-Jean region of Quebec, Canada, in which we have recently described 46 loci significantly linked to hypertension (HT) and its metabolic components, we searched for the genomic signatures (specific haplotype sets) of HT. After performing a rigorous phenotyping with battery of >50 anthropometric, metabolic and humoral traits, 274 individuals (168 hypertensive) from 25 families presenting HT with extremes of high and low density of hypertensive status were genotyped using Affymetrix GeneChip Human Mapping 50K Array. These were performed at a genome-wide significance for haplotypes with hypertension status and specifically those with highest predictive value concerning HT. Among the initial set of 438 haplotypes associated with HT, we identified four haplotypes on chromosomes 3, 11, 16 and 18 with the highest predictive value. The presence of the individual haplotypes conferred a sex, age and family-adjusted odds ratio (aOR) of HT of 2.0–2.6, however, combination of any 3 of the haplotypes increases the aOR (up to 3.1 for set of 3, 16 and 18). The degree of risk of HT increased with age, was independent of sex, and was seen in most pedigrees analyzed. In addition to higher BP (SBP 146±19 vs. 118±10 mmHg, P<0.01), the subjects with the highest aOR have also increased total and LDL cholesterol, triglycerides and extracellular/intracellular water volume compared to subjects with none of these haplotypes. Only the chr. 11 and 16 haplotypes localize to known genes, none of which previously associated with HT through being involved in apoptosis (Fas gene on chr. 16) and protein folding (zinc finger C3L3 on chr.11). At the depth of 12 layers/generations, the ancestors of families whose members carry any of the 4 haplotypes showed a higher transmission to the current generation in contrast to families in which at least one patient carries all 4 haplotypes (80% vs. 62%) with overall ancestral separability by 42%. In conclusion, using a genome-wide SNP typing approach in an extensively annotated population in terms of genealogy, genetic makeup and phenotype, we have identified genomic signatures providing strong predictive value of hypertension status.

Agnostic Autoantibodies to the AT1 Receptor in a Rat Model of Preeclampsia Induced by Chronic Reductions in Uterine Perfusion Pressure (RUPP)

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Recent studies have found that the IgG fraction from preeclamptic women contains an angiotensin-1 receptor autoantibody (AT1R-αA) that antagonizes the angiotensin II type I (AT1) receptor. Therefore, we have suggested that these antibodies could contribute to the pathogenesis of preeclamp- sia. While chronic reductions in uterine perfusion pressure (RUPP) in pregnant rats result in a hypertensive state that closely resembles preeclampsia in women, the role of AT1 receptor autoantibodies and AT1 receptor activation in contributing to the hypertension in this rat model of preeclampsia is unknown. The purpose of this study was to determine whether pregnant rats with chronic RUPP develop antibodies against AT1 receptors and whether AT1 receptor activation plays a role in mediating RUPP-induced hypertension. AT1R-αA were detected by the chromatic response to AT1 receptor-mediated stimulation of cultured neonatal rat cardiomyocytes coupled with receptor-specific antagonists. Mean arterial pressure (MAP) was significantly higher in RUPP rats (137 ± 1 mmHg) than in normal pregnant (NP) rats (107 ± 1 mmHg). The hypertension in the RUPP rats was associated with significant elevations in angiotensin II levels and a significant activation of AT1 receptor (RUPP: 5.4 ± 1.6 vs NP, 0.6 ± 0.3 units). To determine the importance of AT1 receptor activation in the hypertension in this rat model of preeclampsia, we infused AT1 receptor antagonist, L-188809, for 5 days (days 14 to 19 of gestation) in NP and RUPP rats. Chronic administration of the AT1 receptor antagonist resulted in significant decreases in MAP in both NP (115 ± 2 to 101 ± 2 mm Hg) and RUPP rats (122 ± 2 vs 90 ± 2 mm Hg). However, the decrease in MAP was significantly greater in the RUPP rats (Δ 32 mm Hg) vs NP rats (Δ 20 mm Hg). In summary, hypertension in RUPP rats is associated with significant elevations in AT1 receptor agonist antibodies. Moreover, AT1 receptor activation contributes to the hypertension in response to chronic reductions in uterine perfusion in pregnant rats.
Primary Aldosteronism Contributes to Poorly Controlled Hypertension in Diabetic Subjects

Paul T Cantey, Karen Luster, Arlene Chapman, Guillermo Umpierrez; Emory Sch of Medicine, Atlanta, GA

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 (8%-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 mmHg or ≥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/mmHg and a PAC ≥12 ng/dl received a three-day salt suppression testing. Subjects with a PAC ≥85 ng/dl or 24-hour urine aldosterone ≥2 mcg/dl or serum uric acid ≥10 mg/dl 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. In 3 patients have ruled out for PA. The prevalence of PA is 12% (75.0% in males, 4.6–21.2%). 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 subjects with type 2 diabetes. Screening of 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.

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 yrs, n=28) treated with oral hydralazine 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 and soluble E-selection) were measured by ELISA before and one year after treatment. One year after therapy, BP was similarly controlled by valsartan and atenolol (123±27/4t vs 144±33/4t mmHg, p<0.005; 128±37/5t vs 144±28/3t mmHg, p<0.005, respectively). Glycemic control was identical in the two groups. Serum levels of IL-6, IL-18, sICAM and sE-selectin were increased (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<0.01) 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.
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 is a phenotype which may be used to strongly define a genetic region generated by genetic 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, diuretics). Non-responders had a treatment BP >140/90 or a BP reduction of <20mmHg. 258 sibling pairs (ABCD) were identified who were non-responders on A/B/CD therapy only. Of them 89 pairs (AB) were on AB only, and 76 pairs (CD) were on C/D only. NPL analysis was performed on a 10 cM genome scan in the three groups using MERLIN in combination with MLSix. Results Significant linkage was observed in the AB group on chromosome 2 (Figure, multipoint LOD 4.84 at 90.68 Kosambi cM). Suggestive linkage was also observed for the C 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 pathways of hypertension based on drug response. The locus on chromosome 2p in a subset of Caucasian hypertensives unresolved 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 pharmacogenetic locus affecting drug response.

Increased Apelin Expression in the SHR Rstral Ventrolateral Medulla

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The rostral ventrolateral medulla (RVLM) is the major source of excitatory input to sympathetic neurons. A dominant negative mutant for the p85 regulatory subunit of PI3-kinase cloned in an MLSix. performed on a 10 cM genome scan in the three groups using MERLIN in combination with MLSix.

Results

- Increased apelin expression in 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%.
- 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 aims in the present study were to:
  - Verify increased apelin expression in the SHR RVLM and ii) determine signal transduction mechanisms of apelin that relate to neuronomodulation.
- Real-time RT-PCR demonstrated an increase in mRNA abundance of the receptor precursor for apelin, preproapelin, in the RVLM of SHR compared with WKY rats. However, the transcript for its receptor (APJ) did not vary significantly in SHR compared to WKY.
- The rostral ventrolateral medulla (RVLM) is the major source of excitatory input to sympathetic neurons. A dominant negative mutant for the p85 regulatory subunit of PI3-kinase cloned in an MLSix. performed on a 10 cM genome scan in the three groups using MERLIN in combination with MLSix.

Conclusions

- This is the first study to identify significant genome wide linkage by partitioning different pathways of hypertension based on drug response. The locus on chromosome 2p in a subset of Caucasian hypertensives unresolved 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 pharmacogenetic locus affecting drug response.

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

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Angiotensin converting enzyme 2 (ACE2) is a homologue of ACE that preferentially converts the potent vasoconstrictor and growth stimulator angiotensin II (Ang II) to Ang-(1–7), a peptide with vasodilatory 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 angiotensinogen, were absent in cerebrospinal fluid, 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 (54.7 ± 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 apparent 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 angiotensinogen to Ang-(1–7), a peptide with vasodilatory and anti-proliferative properties.

Characterization of a 14,15-Epoxyeicosatrienoic Acid Binding Site/Receptor with a New Agonist Ligand 14,15-EET-PISA

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Endothelium-derived hyperpolarizing factor (EDHF) is reduced in diseases such as hypertension and diabetes. Epoxyeicosatrienoic acids (EETs) represent EDHFs in many vascular beds and regulate vascular tone. EETs activate smooth muscle calcium-activated K+ channels cause hyperpolarization and relaxation of coronary arteries. However, whether EETs act through a membrane receptor is not known. Here, we developed a stable iodinated 14,15-EET agonist, 14,15-EET-phenylsulfonyl fluoride (14,15-EET-PSF), to characterize the putative 14,15-EET receptor. In bovine coronary artery rings precontracted with U46619, 14,15-EET-PSF induced expression in the SHR RVLM and its enhanced signaling may contribute to hypertension in light of the fact that P3K-kinase activity and ROS generation in this area have been implicated in hypertension. Supported by NIH grants HL33610 and HL76312.
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 ± 12.9%); 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-P125ISA 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-P125ISA 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.

**LB62**

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

Yuki Suzaki, Hiroyuki Kobori, Yuri Ozawa, L. G Navar; Tulane Univ Health Sciences Cntr, New Orleans, LA

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 286 ± 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 (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.