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 directly determine the effects of these QTLs on renal function, we generated congenic strains 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+/+Rf2+/+), double congenic rats to that of ACI.FHH(Rf1+/+Rf2–/–) single congenics and the ACI and FHH parental strains. Albuminuria (UA) and SBP were regularly assessed in rats following unilateral nephrectomy (UNX) at 6–7 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 lower than that of Rf1–/–Rf2–/– congenics. In the single and double congenics, the contribution of Bpfh1 to the increased SBP remains to be established. However, renal susceptibility in the (ACI.FHH(Rf1+/+Rf2+/+)) strain was significantly higher compared to ACI and Rf1+/+ single congenics, and FHH, indicative of an impaired autoregulation in these three strains. These findings indicate 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/Rf2 QTL increases SBP and markedly enhances susceptibility to renal failure following UNX. Whether the increased renal susceptibility is due to impaired autoregulation or remains to be established. However, renal susceptibility in the (Rf1–/–Rf2–/–) double congenic is still below that of the parental FHH rat indicating that the other three Rf/QTLS will have additional effects to further enhance renal susceptibility.

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 insulin resistance. However, the mechanism involved in the renal natriuresis/diuresis in obese Zucker rats remains unknown. The Na+, K+-ATPase (NKA) is thought to be the only mechanism by which the AT2 receptors mediate natriuresis. We used PT suspension isolated from lean and obese Zucker rats. The AT2 agonist CGP42112 produced a concentration-dependent (0.1 nM - 100 nM) inhibition (~33% max. at 100 nM) of the NKA activity in the PTs of obese but not in lean Zucker rats. The presence of the AT2 antagonist PD123319 (1 µM), but not the AT1 antagonist losartan (1 µM), significantly diminished the CGP42112-induced inhibition of the NKA activity in obese rats, suggesting that it is AT2 mediated. The AT2 agonist (10 nM)-induced inhibition of the NKA was completely abolished by ODQ (10 µM), soluble guanylate cyclase inhibitor or L-NAME (100 µM). NOS synthase inhibitor L-NAME, but not PD123319, prevented the inhibition of cGMP/NOS pathway in the AT2-mediated NKA inhibition. Also, we investigated the effect of AT2 receptor activation on cGMP and NO accumulation in the PTs. The AT2 agonist CGP42112 produced a concentration-dependent (0.1 nM - 100 nM) increase in cGMP and NO (measured as nitrite/nitrate) accumulation in the PTs of obese but not lean rats. The maximum stimulation of 5-folds for cGMP and 3.4-folds for NO was observed at 100 nM of the agonist. The AT2 agonist-induced stimulation of NO and cGMP was blocked by PD123319 (1 µM), L-NAME (100 µM), DDD (10 µM), but not by losartan (1 µM). The data demonstrate that the AT2 receptor activation, via the stimulation of NO/cGMP pathway, leads to the inhibition of the tubular NKA activity, which provide a plausible mechanism of the AT2 receptor-mediated natriuresis/diuresis reported earlier in obese Zucker rats. (supported by NIH grant R01-DK61578)

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

Antonella 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; B. Becker, D.M., Berlin, Germany; Maria J Santos, Robson A Santos, Federal Univ of Minas Gerais, Belo Horizonte, Brazil

Acute Angiotensin(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 (A-1–7)] in a congenic 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-splanchnal fluid (CSF) and brain and increased sympathetic and pressor responses to CSF [Na+]i. In the congenic strain, C10SLi16, a segment of Chromosome 10 of the Dahl S rat is replaced with a homologous segment of the Lewis rat. Dahl SLi16 have a blood pressure lower than that of Dahl S rats, indicating the presence of a quantitative trait locus (QTL) in the interval of D10Rat204 and D10Rat9, C10LiT2. The present study was designed to test whether this QTL could be responsible for enhanced responsiveness to CSF Na+ and [Na+]i in response to a high salt diet. At 8–12 wk of age, Dahl S (n=7), Lewis (n=8), and C10SLi16 (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+.

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

Babbette B LaMarca, Lee Grubbs, Jennifer Bain, Cathy 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 this report, 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 progestogen 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 on the modulation of 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) between virgin (V), normal pregnant (NP) rats, and ovariectomized (OVX) rats chronically treated with 17β-estradiol (E) and/or progestogen (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/or P release pellets. Minimotamic 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 vs 101±1 mmHg, V). In contrast, TNF-α had no effect in virgin rats (125±3 mmHg, V vs 125±2 mmHg, NP). Moreover, TNF-α had no effect on blood pressure in progestogen-treated rats (130±4 mmHg, P+TNF vs 129±4 mmHg, P). Estradiol-treated rats (122±7 mmHg, E+TNF vs 107±5 mmHg, E), or estrogen and progestogen-treated rats (124±4 mmHg, E+P+TNF vs 118±3 mmHg, E+P). We conclude that sex steroids have no effect on blood pressure response to TNF-α during pregnancy is not likely due to modulation of the vascular responses to TNF-α by sex steroids.
Use of a Community-Based Study to Define Gene-Gender Interaction in Central Sodium in Wistar Rats

Bing S Huang, Warren J Cheung, Univ of Ottawa Heart Institute, Ottawa, Canada; Hao Wang, Clinic Rock Institute of Montreal, Montreal, Canada; Junhui Tan, Roselyn A White, Fran H Leenen; Univ of Ottawa Heart Institute, Ottawa, Canada

Functional studies indicate that the sympathetic and hypertensive responses to central infusion of Na+-rich crystalloidal-serosal fluid (aCSF) or high salt intake in e.g. Dahl S rats are mediated by brain mineralocorticoid receptors (MR) activation, ouabain-like compounds (O1C) release and then AT1 receptor stimulation. In the present study, we assessed the effects of Na+-rich (0.8 M Na+) aCSF (HNa), on components of the brain RAAS. For this, Wistar rats received via cisterna minipump an intra-cerebroventricular (icv) infusion of aCSF or HNa, combined with either icv infusion of spironolactone, or of antibody Fab fragments (to bind O1C) and its control γ-globulins. After 2 wks of infusions, resting BP and HR were measured in conscious rats. Adrenaline (Ado) and O1C content in the hypothalamus were assessed by RIA and a specific ELISA, and ACE and AT1 binding densities in various brain nuclei using [125I]-labelled 351 A and [125I]-labelled Ang II. Data are mean±SE. *p<0.05, vs aCSF, or HNa. These data indicate that in Wistar rats a chronic increase in CSF [Na+]1 increases brain Ado and, via increasing Ado binding to MR, increases brain O1C. The latter increases AT1 and ACE densities in brain areas involved in cardiovascular regulation.

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

Carmen Hinjosa-Laborde, Jaci A Castaneda, Patricia M de Paula, Steven W Miffin; Univ of Texas Health Science Center, 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 ovarioctomized (OVX) females responded to CIH with an increase in blood pressure similar to males. In OVX 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 6 hours. Control animals breathed room air 24 h/4d. The relationship between renal perfusion pressure (RPP) and sodium excretion and urine flow were determined using ultrasound and normoxemic–female (N), normoxidemic-oVX (OVX), and CIH-oVX rats. Results are mean±SEM, and * 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 sex-enhanced hypertensive response in females, is dependent on the presence of female sex hormones. We speculate that female sex hormones facilitate renal sodium and water excretion during CIH and act to protect against elevations in blood pressure associated with CIH.

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 single nucleotide polymorphisms (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 effects in BP. We ascertained 611 male and 656 female age-matched, unrelated Caucasian-Americans within the 5th percentiles of high and low diastolic BP (DBP) extremes among >5,000 people screened through a primary-care, health maintenance program (Kaiser, Southern California). This approach has >90% power to detect loci contributing >3% BP. Genotyping subjects at 44 SNPs in 30 autosomal and 2 X-linked genes in adrenocortical and renal components that regulate BP revealed 11 SNPs that interacted with sex. Males demonstrated substantially more positive associations than did females, including association of several adrenocortical pathway loci SNPs, including ones in alpha2A- and beta2-adrenergic receptors and monomeric oxime oxidase-A. In females, only PLOC1 n=2255613 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 DBP 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-1 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.

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

Carmine Savoia, Rhyon M Touyz, Dierk Endemann, Gian Piu, Eun A Ko, Carolina DeCicucesso, Ernesto L Schiffrin; 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 and atenolol (123±4/70±5 vs 144±3/80±6; p<0.004). Elastin and collagen content of the media were decreased in both treated groups by 25±1% (p<0.005; 128 vs 144±3 mmHg vs 144±3/80±6; p<0.005) but not after atenolol (10.6±1 vs 9.9±0.9; ns). The stress-strain curve of vessels from atenolol-treated patients was shifted to the left whereas that of vessels from valsartan-treated patients was unchanged. In conclusion, diabetes hypertensive patients good control of BP with valsartan or atenolol was associated with improved vascular remodeling in valsartan-treated patients, but unchanged remodeling and a stiffer wall in vessels from atenolol-treated patients. Addition of an ARB on top of other antihypertensive medication that includes ACE inhibitor thus results in improved resistance artery parameters in diabetic hypertensive patients.

Adventitial Neuronal Somata (ANNIES) from the Rat Mesenteric Branch Arteries Express Palladin, a Novel Actin-associated Protein

Chandra Somasundaram, Richard D Bukoski, Timothy D Coleman, North Carolina Central Univ, Durham, NC, Debra I Diz; Univ of Nebraska, Lincoln, NE, Washington D.C., VA

We recently described a new cell type from peripheral adventitia of rat mesenteric branch arteries (MBA), termed ANNIES (adventitial neuronal somata), that express neural cell adhesion molecule (NCAM), the angiotensin II type I receptor (AT1), endothelin receptors (ETB, ET1), antigens of the peripheral nervous system, and the cadherin cadherin-10. We have investigated the effect of AN (70% body weight) on the characteristics of ANNIES. Using immunocytochemical confocal laser scanning microscopy, we characterized ANNIES as cells with neurite markers residing in vascular wall, we measured palladin expression in adult rat MBA and in ANNIES dissociated from MBA. Cells were isolated and cultured on glass coverslips. Immunocytochemical staining revealed that ANNIES and rat MBA express palladin, a novel actin-associated protein.
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 (ANNEES). 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% ANNEES and 100% of all SMC had positive staining for palladin (n = 3). Palladin was also co-localized with CGRP in multiple foci of rat MBA (n = 3). Immunoblotting from MBA confirmed the presence of palladin as two isoforms of 90–92 and 140 kD (n = 3). This protein pattern is different from other adult tissues such as brain that expresses three isoforms (80–82, 140 and 200 kD) or SMC where only one isoform (80–92 kDa) is expressed. In summary, palladin is present both in SMC and ANNEES, 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 neural cells in the vascular wall may participate in response to injury and vasodilator mechanisms as part of a perivascular sensory neural network.

**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 receptor agonist, 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, 3 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 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 receptor mRNA levels. Immunostaining experiments also demonstrated that GABA 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 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.

**Salt and Oxidative Stress in Essential Hypertension**

Cheryl L Laffer, New York Med College, New York, NY; Rodney J Boltemann, 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) hypertension. Therefore, we explored whether there is a differential effect of salt on OX of SS during a salt-load. We suggest that increased OX in SS during a salt-load. The 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 receptor agonist, 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, 3 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 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 receptor mRNA levels. Immunostaining experiments also demonstrated that GABA 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 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.

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

Chunyu Zeng, Laureano D Asisco, Georgetown Univ Med Cntr, Washington, DC; Ulrich Hopter, 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 Jones; 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 enhanced sodium transport. Disrupting the dopaminergic/D1/D3 receptor subtypes in mouse kidneys reduces ETB receptor expression in the renal proximal tubule. The D3 receptor regulates ETB receptors by physical receptor interaction. In 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 Cuioces, Gianluca E Boari, Francesca Zanci, Marco Miclini, Silvia Piaardi, Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Guido A Tiberio, Stefano M Giuliun, Chair of General Surgery, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Enrico Agostino Rossiei, Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy

Introduction: Endothelial dysfunction in coronary or brachial circulation is associated to a greater incidence of cardiovascular (CV) events. Hypothesis: To investigate the presence of a
Effects of Insulin on Endothelial and Contractile Function of Small Resistance Arteries from Hypertensive and Diabetic Patients

Damiano Rizzoni, Enzo Porteri, Carolina De Ciuceis, Gianluca E Boari, Francesca Zani, Marco Miclci, 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 Paisati, 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 resistance arteries of spontaneously 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 normotensive 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 a isometric myograph. Concentration-response curves (CRC) to NE (from 10–10 to 10–5 M/L) and acetylcholine (from 10–8 to 10–5 M/L) in presence or absence of insulin (715 pMol/L, low dose) and 715 M/Mol (high dose, hd). Results: The results are summarized in the Table (*p<0.05, **p<0.005 vs. basal). A significant reduction in the contractile response to NE was observed in NT after pre-incubation of the vessels with both low and high dose insulin. No reduction was observed in NIDDM and EH+NIDDM, while a significant decrease was obtained in EH with HD insulin. Moreover, a significant difference in reduction in contractile response at maximal dose of NE in presence of insulin was observed in NT compared to EH (p=0.03, NIDDM (p=0.02), and EH and NIDDM (p=0.05) whereas no difference was observed with hd insulin. No difference was observed in the CRC to ACH in NT, EH, or either low or high dose insulin were observed in any group. Conclusions: Insulin at low (physiologic) doses seems to induce a decrease in the reactivity to NE in subcutaneous small resistance arteries of NT subjects, but this effect was lost in EH, NIDDM and EH+NIDDM. This effect does not seem to involve endothelium-dependent mechanisms.

Table

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<th>Basal NE 10–5 mol/L (kPa)</th>
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L16 Changes in Extracellular Matrix in Subcutaneous Small Resistance Arteries of Patients with Primary Aldosteronism

Damiano Rizzoni, Enzo Porteri, Silvia Paisati, Carolina De Ciuceis, Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Luigi Rodella, Rita Rezzani, Chair of Human Anatomy, Univ of Brescia, Brescia, Italy; Gianluca E Boari, Francesca Zani, Marco Miclci, Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Guido A Tiberio, Stefano M Giuliani, Chair of General Surgery, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy; Rosella Bianchi, Chair of Human Anatomy, Univ of Brescia, Brescia, Italy; Enrico Agabiti Rosei; Chair of Internal Medicine, Dept of Med and Surgical Sciences, Univ of Brescia, Brescia, Italy

Introduction: It has been previously demonstrated that aldosteronism may possess a strong proliferative action in vitro and in animal models of genetic or experimental. Hypothesis: To investigate the presence of such a proliferotic 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 lumen ratio (M/L) was measured. The total collagen content within the tunica media was measured (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 (0.09±0.02) and in essential hypertension (0.10±0.03) compared with normotensive 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±8/10) and greater than in normotensive controls (115/80±11/6, P<0.05). Total collagen and type 11 collagen were significantly higher in primary aldosteronism (2.81±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 type and total collagen in respect with the two hypertensive groups (2.33±1.63 and 1.86±0.64, P<0.001). Type I collagen was less in primary aldosteronism (2.91±1.01) than in normotensive controls (2.11±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.

L19 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 angiotension II (AngII)-salt hypertension, and the mechanisms may include actions that are independent of renal injury. Other laboratories have shown separately that activators of the peroxisome proliferator activated receptor-alpha (PPAR-α) decrease blood pressure and IL-6 production. Therefore, this study tested the hypothesis that the deletion of PPAR-α receptors would augment the hypertensive response to chronic AngII infuion. Male PPAR-α knockout (PPAR-α KO) and wild-type (WT) controls, 120Sv (svim), were implanted with biotelemetry devices, and mean arterial pressure (MAP) was measured 18 hours/day throughout the study. Baseline MAP during the control period averaged 121±6 and 114±5 mmHg for PPAR-α KO and WT mice, respectively. AngII (90 ng/min, s.c) caused a rapid increase in MAP in both groups, averaging 144±6 mmHg (PPAR-α KO) and 139±5 mmHg (WT) by day 2. Although blood pressure plateaued at this level in WT mice (143±2 mmHg on day 4), MAP continued to increase in PPAR-α KO mice, averaging 164±6 mmHg on day 4 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-α KO and WT mice, respectively. These data suggest that PPAR-α-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.

L20 Regulation of Cardiac Angiotensin Converting Enzyme 2 (ACE2) and ACE by Aldosterone

A. Ann Tallant, Carlos M Ferrario, Patricia E Gallagher, Wake Forest Univ Sch of Medicine, Winston-Salem, NC

Angiotensin converting enzyme 2 (ACE2) is a new enzyme of the renin-angiotensin-aldosterone system that converts angiotension II (AngII) into angiotension-(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 vasoconstrictor and metabolic 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 its effects on ACE2 in cardiomyocytes. Myocytes were isolated from neonatal rat hearts and pretreated for 24 h in growth medium 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 0.98 ± 0.03 vs 0.95 ± 0.05). The aldosterone 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 gene expression. In conclusion, treatment of 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/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 ACE2/AEC2 by mineralocorticoid antagonists such as spironolactone may participate in their attenuation of the accumulation and structural remodeling of the collagen matrix.

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 the ventricles from WT and Mas-KO mice were compared and the levels of collagen I, III, VI and fibronectin present were determined. Statistical analysis was performed using the Mann Whitney test. We observed that the expression of several matrix proteins was significantly increased in the ventricles of adult KO mice hearts compared to control mice: type I collagen (44.2 ± 4.90 vs 59.09 ± 2.25 in WT mice, P<0.0001), type III collagen (97.33 ± 3.72 vs 48.10 ± 2.91 in WT mice, P<0.01), and fibronectin (78.89 ± 2.908 vs 53.11 ± 2.00 in WT mice, P<0.0001). The expression of type VI collagen was decreased (15.22 ± 1.007 vs 41.58 ± 2.62 in WT mice, P<0.0001). No major differences were detected in the aorta. 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 performance in Mas-KO mice. Supported by: Support Program for Centers of Excellence Program, CNPq/FAPESP.

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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 conduct (aorta-AO) and resistance arteries (mesenteric-arteries-MA) of DOCA-salt rats, and the implication of ET1 receptor in ROS generation. DOCA-salt rats (n=8) received 5 different treatments: apocynin (NAD(P)H oxidase inhibitor, 1.5 mM/L), lipoxygenase (xanthine oxidase inhibitor, 100 mg/kg/day), bosantan (ET receptors antagonist, 100 mg/kg/day), BMS182874 (ET1 antagonist, 40 mg/kg/day), and hydralazine (25 mg/kg/day). Data were compared with untreated (p<0.01). Pretreatment with hydralazine (25 mg/kg/day) prevented increased ROS production in DOCA-salt rats (10 ± 0.19 vs 15 ± 0.00, p<0.05). These data suggests that blockade of the aldosterone-induced increase in ACE2/AEC2 by mineralocorticoid antagonists such as spironolactone may participate in their attenuation of the accumulation and structural remodeling of the collagen matrix.

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catacholamine catalytic enzyme MAOA, renal dopamine D1 receptor (DRD1; 3 SNPs), and two signaling components: SNX13 (RSGPSX1; 45SNPs), and ROCK2 (rho kinase. 4 SNPs). Biallelic SNPs were scored by a two-stage base-extension assay, discriminating the alleles by MALDI mass spectrometry. RESULTS: Microalbumin excretion displayed $\text{H}^{-37.8 \pm 7.43\%}$ $(p=0.0000033)$. Microalbumin and catacholamine excretions paralleled. Genotypes showed associations between microalbumin excretion and polymorphisms at TH (G-62414, p=0.00286), 4/14 SNPs spanning the CHGA locus (A-101814, T-41514, GyuN4Ser), DRD1 (G04A, p=0.02287), SNX13 (p=0.0221), and ROCK2 (p=0.0412). There was evidence of epistasis (gene/gene interactions, or cooperativity) in SOLAR among ROCK2 SNPs and those at TH C-824T, SNX13 C105820T, and CHGA T-4145. CONCLUSIONS: We conclude that urinexcretions of microalbumin and catacholamines are heritable, parallel traits, suggesting adrenergic mediation of early glomerular permeability alterations. Genotypic data suggest that variability in microalbumin excretion is predicted by allelic variations at multiple genes, and is thus a polygenic trait attributable to variation at multiple points in the adrenergic pathway. }

Renal Venous Oxygen Tension After ACE-inhibition Predicts a Functional Renal Artery Stenosis in Two-kidney, One Clip Hypertension

Fredrik Palm, Margarida Mendonca, William J Welsh, Christopher S Wilcox; Georgetown Univ Med Ctr, Washington, DC

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. We evaluated renal venous oxygen tension (RVpO2) and its response to ACE inhibition in the two-kidney, one clip hypertension (2K1C) model. Three weeks after left renal artery stenosis (57%) or sham surgery, baseline RVpO2 and systolic blood pressure (BP) were measured. Baseline RVpO2 was significantly lower (44% lower normalized to sham control) in 2K1C rats compared to sham controls. After 4-week, renal wrap hypertension, baseline RVpO2 from the left clipped kidney was significantly decreased (44% lower normalized to sham control) in 2K1C rats compared to sham controls. However, after enalaprilat, the RVpO2 from the left clipped kidney was significantly increased (44% higher normalized to sham control) in 2K1C rats compared to sham controls.

Salt-resistant Rats 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 supersoxide 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), or the superoxide dismutase mimetic tempol (100 mg/kg). Baseline blood pressure (BP) and heart rate (HR) were recorded. Catecholamine containing neurons from HT (9.9 ± 0.8 pA/pF) and NT (7.8 ± 0.6 pA/pF) were tested for the presence of high calcium current (HVA) and low calcium current (LVA) activity, as estimated by membrane capacitance, was not different comparing neurons from HT and NT. Voltage-gated calcium currents (3.9 ± 2.6 pA/pF; p < 0.0005) were measured by whole cell patch clamp. HVA currents were significantly greater in mice with HF as shown below. We concluded that in HF, myogenic tone enhanced the pressor response to acute stress by increasing 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, Minghua Ye, Northwestern Univ Feinberg Sch of Medicine, Chicago, IL; Hong Xiao, Kenneth E Bernstein, Emory Univ Dept of Pathology, Atlanta, GA; Susan B Gurley, Thomas M Coffman, Duke Univ Med Ctr, Durham, NC; Daniel Batlle, Northwestern Univ Feinberg Sch of Medicine, Chicago, IL

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 ACE2 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/ACE2) and 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 kD 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 kD band was absent and ACE2 activity was barely detectable despite the presence of another 67 kD band detected by our ACE2 antibody. Renal cortex ACE2 activity had no correlation with renal cortex ACE2 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 (46.4 ± 3.4 vs 1.27 ± 0.24 RFU/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/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 renal cortex. Our ACE2 activity assay allows for the detection of differences in tissue specific enzymatic activity in 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

Jasper J Saris, Alexander H Danzer; Dept. Pharmacology, Erasmus Med Ctr, Rotterdam, The Netherlands

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 receptor (Nguyen et al, 2002). Here, we investigated, in neonatal rat cardiomyocytes, 1) (pro)renin 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 (239 ± 65%, at 45 min), but not p42/p44 MAP kinase activation over a 120-min period. Rat microarray gene (n = 4805) transcription profiling of myocardium stimulated with PR (2 µM, 4 hrs, mRNA pool of 5 isolations, dye-swap) detected 259 regulated genes (p < 0.0003). Genes involved in cardiac function and regulation of epithelial sodium transporters in the kidney and aldosterone activity were up-regulated. We conclude that PR may mediate, at least in part, drinking responses in this animal model.

Salt-Induced Water Intake in a Salt-Sensitive Hypertensive Rat Model

Jaiping Li, Donna H Wang; Michigan State Univ, East Lansing, MI

Renal afferent nerves play a key role in maintenance of renal excretory function and sodium and water homeostasis. To determine the role of afferent nerves to the kidneys in the pathogenesis of hypertension, we evaluated the in vivo effects of AT2-R down-regulation on drinking in SD rats induced by s.c. prorenin. A group of 10 Sprague-Dawley rats, each weighing 400 ± 45 g, was divided into the following four groups: 1) SHAM (n = 6, m), though not

C Reactive Protein (CRP), an Intermediate Phenotype for Inflammation: Human Twin Studies Reveal Its Heritability, Association with Blood Pressure and the Metabolic Syndrome, and the Epistatic Influence of Common Polymorphisms at Catechoaminergic/Beta-Adrenergic Pathway Loci

Jennifer Wessels, Guillermo Moratorio, Fangwen Rao, Lian Zhang, Pauline Huang, Manjula Mahata, William Greene, Sriskrinda Khandrika, Brinda K Rana, Brian P Kennedy, Elizabeth O Liddle, Douglas W Smith, Michael G Ziegler, Nicholas J Schork, Geert W Schmid-Schoenbein, Daniel T O’Connor; Univ California at San Diego, San Diego, CA

Rationale. CRP may be an index of inflammation associated with hypertension and cardiovascular disease. Twins allow estimation of the role of heredity in any trait, as heritability estimates range from 0.4 to 0.7. CRP may be an index of inflammation associated with hypertension and the metabolic syndrome. We measured CRP in twins from the Cardiovascular Health Study of the Framingham Heart Study, and evaluated the heritability of CRP using twin correlations. The interaction between genetic and environmental factors was also evaluated using epistatic analysis. Finally, we evaluated whether the genetic architecture of CRP was similar in hypertensive and non-hypertensive individuals. We expected that the genetic architecture of CRP in hypertensive and non-hypertensive individuals would be similar, as we previously observed similar genetic and environmental correlations across these groups.

Methodology: CRP was measured by nephelometry in the Framingham Heart Study. Twin correlations were analyzed using best-estimate models for categorical traits. Heritability estimates were obtained from twin correlations using the animal model (the univariate NL MIXED procedure in SAS). Interaction between genetic and environmental factors was evaluated using epistatic analysis (the UNIVARIATE procedure in SAS). Finally, we evaluated whether the genetic architecture of CRP was similar in hypertensive and non-hypertensive individuals by comparing the correlation between genetic and environmental factors across the groups.

Results: The heritability of CRP was 0.37 in hypertensive individuals and 0.30 in non-hypertensive individuals. The interaction between genetic and environmental factors was statistically significant in both groups, but the magnitude of the interaction was greater in hypertensive individuals. Finally, the heritability of CRP was similar in hypertensive and non-hypertensive individuals, as indicated by the similar correlation between genetic and environmental factors across the groups.

Conclusion: The genetic architecture of CRP is similar in hypertensive and non-hypertensive individuals, and the interaction between genetic and environmental factors is statistically significant in both groups. The magnitude of the interaction is greater in hypertensive individuals, but the heritability of CRP is similar in both groups.
Antigens II Induced Hypertensive Response is Modulated Through Tumor Necrosis Factor-alpha: Role of Nox1, Nox4 and gp91phox

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

Antigens II (ANG II) and Tumor necrosis factor-a (TNF-a) play an important role in the pathogenesis of cardiovascular disease. Recent evidence suggests that both ANG II and TNF-a oxidize oxidative stress and contribute to development of heart disease. In this study, we examined whether ANG II induced hypertensive effect is modulated through cytokines and whether the gp91phox and its homologues, Nox1 and Nox4 are involved in this effect. Method. Wildtype (B6129SF2/J) and TNF-a(-/-) mice were implanted with osmotic minipumps containing ANG II (12 μg/kg/min) or saline for 14 days. In a group of TNF-a(-/-) mice, human recombinant TNF-a 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 the LV was removed for the measurement of gp91phox, Nox1 and Nox4 using real time PCR. Results. The real time PCR values are shown as ΔCT values (GAPDH - the gene of interest) and the fold increase compared to control is 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-a. 2) ANG II-induced hypertensive response is also in part mediated through TNF-a signaling pathways.

BP and GS (% increase in GS/mmHg increase in systolic BP) was lower in the Ang groups (Ang L 0.19 vs. saline 0.6; p < 0.001). Western blot analysis was performed to evaluate iNOS expression. ERM12, P2X4, Akt/PI3K, SHIP-2, and Ang II receptors A1 and A2 expression. Activity of PPAR-y, ERK1/2, Akt/PKB and PDK1 was also analyzed. Rosiglitazone treatment significantly increased nuclear PPAR-y expression and activity in VSMC 2-fold (p < 0.01). Rosiglitazone decreased ERK1/2 peak activity by 58% (p < 0.01) for the 14-day period analyzed. Both of these effects were noted in the Ang II group. Ang II induced SHIP-2 activity was also decreased (p < 0.01) by rosiglitazone. In conclusion, PPAR-y activators PGL2 and rosiglitazone reduced Ang II-induced VSMC growth associated with inhibition of ERK1/2, Akt/PKB. We demonstrate for the first time that SHIP-2 mediates Ang II-induced VSMC growth and that PPAR-y activators may reduce Ang II-induced VSMC growth.

Evidence suggests a relationship between short-term blood pressure (BP) variability and cardiovascular disease. 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. Twenty subjects underwent ambulatory BP monitoring and the other 16 who could not undergo the test assessed their BP at home. The results of the two groups were similar. Evidence suggests a relationship between short-term blood pressure variability and cardiovascular disease. A possible relationship between nocturnal blood pressure variability with coronary artery disease in diabetic nephropathy.
nighttime systolic BP variability (partial R² = 0.490, p < 0.001; and partial R² = 0.470, p < 0.001), and demonstrated that body mass index and Phe were primary determinants of nighttime diastolic BP variability (partial R² = 0.539, p < 0.0005; and partial R² = 0.504, p < 0.005). 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 (10.4 mmHg versus 7.2 mmHg, p < 0.005) variability. In Ang-II hypertensive rats, a dose of a 2 mg/100 g body wt, twice on consecutive days and were sacrificed on the third days. LSN-HO-1-A showed a 38% decrease (p < 0.05) in renal and vascular HO derived CO and bilirubin synthesis compared LXSN. HO showed, but not HO-2 protein levels were decreased in renal and vascular tissues from LSN-HO-1-A rats. Markers of autophagy activation, including the LC3 α-1 protein, were increased in LXSN-HO-1-A compared to LXSN. Isolated mesenteric arteries obtained from LSN-HO-1-A exhibited contraction to PE (10⁻⁶ - 10⁻¹ M), and relaxation and relaxation were markedly increased and decreased, respectively. Vascular smooth muscle protein expression in aortic tissue (ACh 10⁻⁵ M) was increased from 94.8 ± 3.9 to 109 ± 9.1% in heme treated transgenic rats. SOD protein levels decreased in renal and vascular tissues in LSN-HO-1-A relative to LSXSN. Administration of heme caused a significant increase in EC-SOD in both LXSN and LSN-HO-1-A whereas administration of HO inhibitor, to LSXSN 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.

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**A Novel Regulatory Effect of AT1 Receptor-Interacting Molecular In Cardiomyocytes**

Koushi Tamura, Yutaka Tanaka, Yuko Tsurumi, Masashi Sakai, Toyochihiro Shigenaga, Motoko Ozawa, Kosaku Iwatsubo, Tashio Hashimoto, Minoru Kihara, Tomoaki Ishigami, Nobuhiro Hirawa, Yoshituki Toyo, Yokohama City Univ, Yokohama, Japan; Masatsugu Horiuchi, Ehime Univ, Shigenobu, Japan; Satoshi Umemura, Yokohama City Univ, Yokohama, Japan

Activation of cardiac AT1R signaling plays an important role in cardiac hypertrophy. We previously cloned a novel molecule interacting with AT1R, ATRAP (for AT receptor 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 immunofluorescent staining under baseline conditions. ATRAP was markedly co-localized with 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 adenoviral gene transfer significantly decreased the number of AT1R on the surface of cardiomyocytes compared to lacZ control, suggesting an inhibitory effect of ATRAP on the recycling of AT1R. Interestingly, overexpression of ATRAP specifically inhibited Ang II-mediated phosphorylation of p38MAPK (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 transfection (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 furthermore attenuates Ang II-mediated hypertrophic responses in cardiomyocytes, and may suggest a novel strategy to inhibit cardiac hypertrophy.

**LB42**

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

LICY L. YANES, Damian G. Romero, Radul Iliescu, Cezio E. Gomez-Sanchez, Jane F. Rackelhoff; Univ OF MISSISSISSIP Med Ctr, JACKSON, MS

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 females 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%) sodium diet. Mean arterial pressure (MAP) was followed by tail-cuff. Prepro ET-1 and ETB-R mRNA (n=10) and protein (n=6) levels were measured in kidney cortex and medulla 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 ± 1.5 mm Hg; LS vs. HS p < 0.05) and females (from 115 ± 3 to 133 ± 4 mmHg; LS vs. HS p < 0.05). Castration in males slowed the progression of hypertension (from 121 ± 2 to 145 ± 3 mmHg; LS vs. HS p = 0.05) and ovariectomy accentuated the increases in MAP (133 ± 3 to 191 ± 6 mmHg; 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 ± 0.1 to 3.6 ± 0.1). This effect was paralleled by an increased expression of ETA-R mRNA levels in kidneys (2.5 ± 0.5 LS vs. HS p < 0.05). This study reports that chronic reductions in uterine perfusion pressure (RUPP) in pregnant rats result in hypertension and enhanced endothelin production, factors linking placental ischemia and endothelial dysfunction in preeclamptic women. While we have previously reported that chronic reductions in uterine perfusion pressure (RUPP) in pregnant rats result 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 determine 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 1 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 6 and 18 hours after stimulation and used to quantitate endothelin production. Six hours after exposure to RUPP serum (n = 17), cell media endothelin concentration was 18.4 ± 2.7 pg/ml as compared to 9.22 ± 1.3 pg/ml from cells exposed to serum from normal pregnant rats (n = 9). Eighteen hours after exposure to RUPP serum (n = 14), cell media endothelin concentration was 30.5 ± 3.8 pg/ml as compared to 12.8 ± 1.5 pg/ml from cells exposed to normal pregnant rat serum (n = 6). Pretreatment of HUVEC with an AT1 receptor antagonist, Losartan (15μM), markedly attenuated the increased endothelin production observed with serum from RUPP rats. Eighteen hours after exposure to RUPP serum (n = 14), cell media endothelin concentration was 21.3 ± 2.2 pg/ml as compared to 16.4 ± 3.9 pg/ml from cells exposed to normal pregnant rat serum (n = 10). These data indicate that serum from pregnant rats exposed to chronic reductions in uterine perfusion induced endothelin production by endothelial cells, an effect that is, in part, mediated by AT1 receptor activation.

**LB45**

**The Link Between Nitric Oxide System and Aldosterone: Long Term Oral Supplementation of L-arginine Lowers Aldosterone Levels, Improves Arterial Elasticity and Hemodynamic Variables**

Marina Sharogradsky, Hila Gutman, Dov Gavish, Ruth Brendes, Wolfson Med Ctr, Holon, Israel; Reuven Zimlichman, Wolfson Med Ctr, Dept of Medicine and Hypertension and head of the Brunner Cardiovascular Research Institute, Tel Aviv Univ, Holon, Israel

**Background:** Dietary Supplementation with L-arginine enhances activity of the NO system. We aimed to evaluate, in a double blind, placebo controlled randomized study, the effects of long-term oral supplementation of L-arginine on arterial hemodynamic, and humoral and hemodynamic variables. Patients and Methods: We evaluated the effects of 6 month oral supplementation of 6 grams/day of L-arginine as once daily oral additive on arterial elasticity and arterial properties of arteries in 32 patients on placebo and 45 patients with multiple risk factors or end-stage vascular failure. Patients were treated with L-arginine. Large vascular compliance were derived from radial artery waveforms, obtained using a calibrated tonometer (model CR00-2000, HDI Inc, Eagan, MN). Results: Systolic blood pressure did not change in the placebo group, 146.4 ± 25.3 mmHg before and 146.4 ± 20.3 mmHg after 6 months of treatment. In the L-arginine group (Xg) systolic blood pressure increased from 143.2 ± 20.8 to 146.9 ± 20.3 mmHg (p = 0.001). Aortic outflow (α) wave velocity increased from 6.7 ± 1.6 to 6.9 ± 1.2 mmHg (p = 0.007). Large artery elasticity (LAE) decreased in the placebo group from 11.6 ± 4.4/6.4 to 8.0/2.1 ± 8.21 but in the Xg the Xg-L-arginine increased arterial elasticity from 10.03 to 12.76 ± 0.43 mmHg (p = 0.0001). Systemic vascular resistance (SVR) increased slightly in the placebo group from 1739.6 ± 371.98 to 1925.814.089 dynsec cm⁻³ and decreased in the placebo group from 1096.33 ± 330.97 to 1577 ± 222.11 dynsec cm⁻³. Treatment with L-arginine, decreased plasma aldosterone levels by 30% as opposed to no change in the placebo group (p = 0.032). Furthermore, grouping of various risk factors revealed that the L-arginine treatment significantly reduced glucose, cholesterol, triglycerides, LDL, creatine/d, C reactive protein (CRP), GHB, HaCa and fibrinogen levels in blood. L-arginine
treatment also significantly increased insulin index and HDL% 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, hemodynamic and humoral parameters.

Obesity and Insulin Resistance in α-Calcitonin Gene-Related Peptide (α-CGRP) Knockout Mice

Mark C Bowers, Texas A&M Univ, Temple, TX; Kushred A Katki, Deborah B Kuck, Donald J DiPette, Scotti White Health System, Temple, TX; Scott C Sopowit; Texas A&M Univ, Temple, TX

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 ± 3.4 vs 34.2 ± 1.8 g, P<0.01, SD) vs. WT mice. The purpose of this study was to determine if the α-CGRP KO mice have increased insulin resistance in different age groups (10 –12 weeks, ~1 yr old and 2 yrs 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 (1.0 ± 0.2 at 10 –12 weeks; 1.1 ± 0.1 at ~1 yr old, 0.45 ± 0.11 at 2 yrs old). Plasma glucose levels were significantly higher at all study ages in the α-CGRP KO mice compared to their WT counterparts. Plasma insulin levels (ug/L) in the α-CGRP KO mice were 0.64 ± 0.09 (10 –12 wks), 0.78 ± 0.12 (1 yr), and 0.96 ± 0.12 (2 yrs) compared to 0.39 ± 0.08, 0.40 ± 0.06, and 0.59 ± 0.04 for WT mice, respectively. Glycerated 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 wks), 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 neuropetide, may play a significant role in mediating the components associated with the metabolic X syndrome, specifically hypertension, obesity, and insulin resistance.

In Vivo Trafficking Of Distal Na+/Cl- Cotransporter (NCC) in Response to Changes in Dietary NaCl

Monica B Sandberg, USC Keck Sch of Medicine, Los Angeles, CA; Anvid B Maunbach, Dept of Cell Biology, Aarhus, Denmark; Alicia A McDonough; USC Keck Sch of Medicine, Los Angeles, CA

The Na+–Cl cotransporter (NCC) is expressed in the apical membrane of distal convoluted tubule and is responsible for the reabsorption of >5% 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 decreased during high salt diet, and mineralocorticoid blockade. Aim: The aim of this study was to test the hypothesis that plasma measured, 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) or 0.07% NaCl restricted diet (SR) for 1 week. After anesthesia (ketamine/xylazine), kidneys were excised, renal cortex dissected, and analyzed by: 1) immunofluorescence microscopy; 2) electron microscopy with fixation in 1% glutaraldehyde, 1% Na-cacodylate or 2) homogenized, subjected to density gradient centrifugation on sorbitol cacodylate or 2) homogenized, subjected to density gradient centrifugation on sorbitol gradients, fractions collected and analyzed by immuno blot. NCC was detected with anti-ATG-α (D. Ellisme) in EM samples, fractions and total membranes. Results: One week HS (vs. LS) caused a redistribution of NCC from low density membranes containing markers of apical membranes to higher density membranes and decreased NCC total abundance to 0.45 ± 0.11 of LS. 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 endosomes. Conclusion: In conclusion, dietary salt both changes the abundance of NCC and provides trafficking of NCC between functional domains in the plasma membrane and sub apical endosomal pools, a mechanism that would facilitate more rapid regulatory responses of sodium chloride co-transport to dietary salt. Supported by DK34316, AHA pre-doctoral fellowship

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). The hypertension in the RUPP rats was associated with significant elevations in SBP (169 ± 3 vs. 157 ± 2 mmHg) and DBP (100 ± 7 vs. 91 ± 4 mmHg). Adequacy of renal denervation was verified by a greater than 90% decrease in renal norepinephrine content (LBW: 22 ± 4 (BRD) vs. 30 ± 2 (SD) ng/g, P<0.01 vs. control) and 31 ± 6 (BRD) vs. 193 ± 13 (SD) ng/g, P<0.01. In addition, renal norepinephrine content was significantly elevated in intact LBW offspring as compared to intact control offspring (P<0.01, SD LBW vs. SD control, respectively). However, bilateral renal denervation abolished hypertension in LBW offspring (104 ± 1 vs. 92 ± 1 mmHg) and did not alter MAP in control offspring (102 ± 3 mmHg). The hypertension in the RUPP rats was associated with significant elevations in SBP (169 ± 3 vs. 157 ± 2 mmHg) and DBP (100 ± 7 vs. 91 ± 4 mmHg). 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 antagonist resulted in significant decreases in MAP in both NP (181 ± 2 vs 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 agonistic antibodies. Moreover, AT1 receptor activation contributes to the hypertension in response to chronic reductions in uterine perfusion in pregnant rats.

The Genomic Signatures of Hypertension

Onjed Saéda, Johanne Tremblay, Rsch Cntr CHUM, Montreal, Canada; Ettore Merlo, École Polytechnique de Montréal, Montréal, Canada; Daniel Gaudet, Complexe Hospier de la Sagamie, Chicoutimi, Canada; Ulrich Broeckel, Med College of Wisconsin, Milwaukee, WI; Gerard Bouchard, Université du Québec à Chicoutimi, Chicoutimi, Canada; Giulio Antonio, Univ of Sannio, Benevento, Italy; Pierre-Luc Brunelle, Alexandre Garaus, Francis Dossaux, Javier Pintos, Rusch Cntr CHUM, Montreal, Canada; Theodore A Kotchen, Allen W Cowley, Jr., Med College of Wisconsin, Milwaukee, WI; Pavel Harnett; Rusch Cntr CHUM, Montreal, Canada

In a set of 120 hypertensive and dyslipidemic French-Canadian families from the Sagueneay-Lac-St-Jean region of Quebec, Canada, in whom 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 metabolic syndrome were genotyped using Affymetrix GeneChip Human Mapping 50K Array. These were performed for the purpose of identifying those loci 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 31.5 for set of chr.3, chr.11, 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 though being involved in apoptosis (Fatso gene on chr. 16) and triglycerides (KLC3 gene on chr. 16). In addition to higher BP, the subjects with the highest aOR have also increased HDL, 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 though being involved in apoptosis (Fatso gene on chr. 16) and triglycerides (KLC3 gene on chr. 16).
Primary Aldosteronism Contributes to Poorly Controlled Hypertension in Diabetic Subjects

Paul T Cantey, Karen Luster, Arlene Chavez, 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 (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 mmHg ≥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/μl 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 mg/day were considered as having PA. Results: Seventeen subjects were screened for PA by the study protocol. Sixteen patients had a positive screen, and 3 patients have ruled out for PA. The prevalence of PA is 12.9% (95%CI: 6.8-20.1%). 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 suggested to reduce the long-term complications associated with mineralocorticoid excess.

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

Pete D Meliagros, Nancy L Howell, Brandon A Kemp, Shetal H Padia, Robert M Carey; Univ of Virginia Sch of Medicine, Charlottesville, VA

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 cell proliferation and stimulating vasodilation. 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 antagonist, 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/day) 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 mmol/kg/min, each dose for 30 min), or CGP combined with AT1 receptor specific antagonist PD-123319 (PD; 10 μg/kg/min). Mean arterial pressure (MAP) was monitored via the direct carotid method. RESULTS: UNaV was .027 ± .006 μmol/min prior to infusion of CGP. At 20, 40, and 60 mmol/kg/min of CGP, U NaVV increased to .050 ± .010, .045 ± .001*, and .045 ± .003* umol/min, respectively (∗P < .05; ∗∗P < .01). For the control kidney, U NaV was .013 ± .003 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 (P = .023 * .006, .013 * .003, .013 * .005, and .014 * .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

Radu Bizescu, Valeria Cucchiarelli, Icy L Yanes, Jane F Reckelhoff; Univ of Mississippi Med Ctr, Jackson, MS

The presence of androgens is required for the male spontaneously hypertensive rats (SHR) to exhibit high blood pressure (BP) levels than 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. Oxidation potentials measured in the suspensions of the kidneys and the basal production of superoxide anion as well as the activity of the NADPH oxidase (in the presence of excess NADPH) was measured in kidney cortex homogenates by lucigenin chemiluminescence. As an index of NADPH oxidase activation, differential protein expression of the p47phox subunit of the enzyme and 7-aminocoumarin fluorescence versus total cellular homogenate from renal cortical tissue was determined by western blotting. Apocynin treatment decreased BP in intact male rats (155.3 ± 3.8 vs 169.7 ± 1.8 mmHg), an effect paralleled by a reduction of both basal and NADPH-induced superoxide anion production in renal cortex. Gonadectomy lowered BP (153.1 ± 2.4 mmHg) and abolished the hypertensive effect of apocynin (158.1 ± 2.2 mmHg). Gonadectomy also decreased superoxide anion production and NADPH oxidase activity and attenuated the antioxidant effect of apocynin. Activation of NADPH oxidase in renal cortical tissue was inhibited by gonadectomy, as indicated by a 40% reduction in membrane-associated p47phox expression. Our data suggest that androgens stimulate renal cortical superoxide generation by activating NADPH oxidase and contribute to hypertension in SHR.

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

Rhami M Touyz, Kidney Resch Ctrr; OHRI, Ottawa, Canada; Carmine Savoia, Clinical Resch Institute of Montreal, Montreal, Canada; Ying He, Kidney Resch Ctrr; OHRI, Ottawa, Canada; Derek Endemann, Qian Pu, Eun A Ko, Ernesto L DeCruces, Ernesto L Schiffinr; Clinical Resch Institute of Montreal, Montreal, Canada

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 hypoglycemic agents showed no difference in body weight before and after the 3-day salt load were considered as having PA. Results: Seventeen subjects were screened for PA by the study protocol. Sixteen patients had a positive screen, and 3 patients have ruled out for PA. The prevalence of PA is 12.9% (95%CI: 6.8-20.1%). 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 suggested to reduce the long-term complications associated with mineralocorticoid excess.

Late Breaking Presentations

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Chromosome 2p Shows Genome Wide Significant Linkage to Anti-Hypertensive Medication Response in the British Genetics of Hypertension (BRIGHT) Study.

Sandoosh Padmanabhan, BHF Glasgow Cardiovascular Res Ctr, Univ of Glasgow, Glasgow, United Kingdom; Chris Wallace, Patricia B Munroe, Clinical Pharmacology and Bar, and the London Genomic Ctr, William Harvey Resch Institute, Barts and the London Sch of Medicine, London, United Kingdom; Morris Brown, Clinical Pharmacology and the Cambridge Institute of Med Res, Univ of Cambridge, Cambridge, United Kingdom; Nilesh Samani, Dept of Cardiology, Univ of Leicester, Leicester, United Kingdom; David Clayton, Clinical Pharmacology and the Cambridge Institute of Med Res, Univ of Cambridge, Cambridge, United Kingdom; Martin Farrall, Dept of Cardiovascular Medicine, Univ of Oxford, Wellcome Trust Ctr for Human Genetics, Oxford, United Kingdom; John Webster, Medicine and Therapeutics, Aberdeen Royal Infirmary, Aberdeen, United Kingdom; Mark Lathrop, Ctr National de Genotypage, Evry, France; Mark Caulfield, Clinical Pharmacology and Bar, and the London Genomic Ctr, William Harvey Resch Institute, Barts and the London Sch of Medicine, London, United Kingdom; Anna F Dominiczak, John M Connell; BHF Glasgow Cardiovascular Res Ctr, Univ of Glasgow, Glasgow, United Kingdom

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 resistance to the four classes commonly used (ACEI/ARB, B-beta-blockers) or not (CCB, D-duretics). Non-responders had a BP of >140/90 or a BP reduction of <20/15mmHg. 288 sibling pairs (AB/CD) were identified who were non-responders on A/B/C/D therapy only. Of these, 89 pairs (AB) were AB only and 76 pairs (CD) were on C/D only. NPL analysis was performed on 10 cM genome scan in the three groups using MERLIN in combination with Merlin6. Results Significant linkage was observed in the AB group on chromosome 2 (Figures), multipoint LOD 4.84 at 90.68 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 classes of drug response. The focus 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
Shereen J Veerasingham, Univ of Florida, Gainesville, FL; Kathleen Berecek, Univ of Alabama, Birmingham, AL; J M Wyss, Univ of Alabama, Birmingham, AL; Mohan K Raizada; Univ of Florida, Gainesville, FL

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 were to verify increased apelin expression in the SHR RVLM and ii) determine signal transduction mechanisms of apelin that relate to neuropepmodulation. 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 (APJ) 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 phosphoinositide 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 oxidase 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 (AdDp85) was used to determine the sequence of PI3-kinase activation and ROS generation. Expression of the AdDp85 in WKY neurons attenuated apelin 13 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
Srđevi Kolluri, New York Med College, Valhalla, NY; Rita Rezzani, Luigi Rodello, Univ of Brescia, Brescia, Italy; Saadet Turkseven, New York Med College, Valhalla, NY; Barbara Buffoli, Damiano Rizzoni, Enzo Paieria, Univ of Brescia, Brescia, Italy; Nader G Abraham, New York Med College, Valhalla, NY; Attallah Kappas; The Rockefeller Univ, New York, NY

Increased heme oxygenase (HO)-1 activity has been successful in attenuating endothelial cell apoptosis; decreasing oxidative stress and in the correction of vascular impairment (Türkseven et al AJP Heart 2005). We examined the effects of increasing HO-1 protein and activity on superoxide anion level, superoxide dismutase (EC-SOD, Cu/Zn Mn), inductive and endothelial nitric oxide synthase (iNOS and eNOS) levels and, vascular responses to acetylcholine (ACh) in control and spontaneously hypertensive rats (SHR). Renal iNOS protein levels were significantly reduced in SHR compared to normotensive control (Wistar Kyoto, WKY) 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), conferred an increase in EC-SOD but no significant change in CuZn-SOD was seen. Increased HO-1 activity in SHR pretreated with CoPP was associated with a decrease in iNOS and superoxide anion (O2-) but an increase in e-NOS levels. ACh-induced (10-3 to 10-2 M) relaxation in ring segments from mesenteric small arteries (100–250 μm diameter) of untreated hypertensive rats was significantly reduced as compared to untreated WKY controls. This decrease in arterial relaxation was reverted in SHR pretreated with CoPP (p < 0.01) but not with an inhibitor of HO activity, tin mesoporphyrin (SnMPS), nor with CoPP in the presence of NOS inhibitor nitro-L-arginine methyl ester (L-NAME) suggesting that HO-1 induction may prevent hypertension by a NOS-dependent mechanism perhaps by increasing the bioavailability of NO. These observations, along with the experimental hypertension suggest that pharmacological preconditioning of HO-1 attenuates oxidative stress by decreasing O2- and iNOS levels, and increasing e-NOS and EC-SOD.

Evidence for a Secreted and Active Form of ACE2 in Cerebrospinal Fluid
Steven J Newton, E. Ann Tallant, Mark C Chappell, Carlos M Ferrario, Patricia E Gallagher; Wake Forest Univ Sch of Medicine, Winston-Salem, NC

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 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 and adult brain. Since central components, such as Ang II and the precursor protein angiotensinogen, were present in cerebrospinal fluid (CSF), the present study investigated the presence 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 bioactively active in CSF collected from male SD rats (5.47 ± 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 Ang II to Ang-(1–7), as well as Ang I to Ang-(1–8), 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.

Characterization of a 14,15-Epoxyeicosatrienoic Acid Binding Site/Receptor with a New Agostin Ligand 14,15-Epoxyeicosatrienoic Acid Phenylalosulfonamide
Wenzi Yang, Med College of Wisconsin, Milwaukee, WI; Bythie B Holmes, Med College of Wisconsin, Milwaukee, WI; T. Venugopal Raju, J. R Falk, Univ of Texas Southwest Med Sch, Dallas, TX; William B Campbell; Med College of Wisconsin, Milwaukee, WI

Endothelium-derived hyperpolarizing factor (EDHF) is reduced in diseases such as hypertension and diabetes. Epoxyeicosatrienoic acids (ETEs) represent EDHFs in many vascular beds and regulate vascular tone. ETEs activate smooth muscle calcium-activated K+ channels hence cause hyperpolarization and relaxation of coronary arteries. However, whether ETEs act through a membrane receptor is not known. Here, we developed a stable iodinated 14,15-EET agonist, 14,15-EET-phenylalosulfonamide (14,15-EET-PSA), to characterize the putative 14,15-EET receptor. In bovine coronary artery rings precontracted with U46619, 14,15-EET-PSA induced
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-PSA 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-PSA 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-PSA was 33 nM. When 14,15-EET-PSA 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 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 (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 ± 0.25, 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.
Late Breaking Abstracts From the 59th Annual Fall Conference and Scientific Sessions of the Council for High Blood Pressure Research in Association With the Council on Kidney in Cardiovascular Disease

Hypertension. 2005;46:875-887

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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http://hyper.ahajournals.org/content/46/5/875.citation

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