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 (BpF1 and -2). The Rf1, Rf2 and BpF1 QTLs are in close vicinity on rat chromosome 1. To determine the effects of these QTLs congenic strains were generated carrying the QTLs from FHH on the genomic background of the normotensive renal resistant ADI strain. Here we compare the effect on systolic blood pressure (SBP) and renal damage susceptibility in ACI, FHH/Rf1 (Rf1 – Rf2) double congenic rats to that of ACI, FHH/Rf1 (Rf1 – Rf2) and ACI, FHH parental strains. Albuminuria and SBP were regularly assessed in rats following unilateral nephrectomy (UNX) at 6–7 weeks of age. In addition studies the efficacy of renal flow autoregulation was measured in intact two-kidney rats of all four strains. At the end of an 18-week follow-up period UV and SBP in the Rf1 + Rf2 double congenic rats was significantly higher compared to ACI and Rf1 single congenic rats, but significantly less compared to SBP in the single and double congenic rats, i.e. the contribution of BpF1/Rf2 region, amounted to 20–25 mm Hg. When compared with the ACI rat, the renal autoregulatory index in the 100–150 mm Hg arterial pressure range was shown to be increased to a similar extend in ACI, FHH/Rf1, ACI, FHH/Rf1 (Rf1 – Rf2) 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 UV following UNX. Additional transfer of the BpF1/Rf2 QTL increases SBP and markedly enhances susceptibility to renal damage following UNX. Whether the increased renal susceptibility is due to the increased SBP remains to be established. However, renal susceptibility in the (Rf1 + Rf2) double congenic is still far below that of the parental FHH rat indicating that the other three Rf-QTLs will have additional effects to further enhance renal susceptibility.

Effect of Bradykinin In Transgenic Rats Expressing an Angiotensin II AT2 Receptor Agonist Inhibits Proximal Tubular Na+ Reabsorption

Angiotensin II AT2 receptor agonist inhibits proximal tubular Na+, K+-ATPase via a NO/cGMP pathway in obese Zucker rats

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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 salt sensitivity. However, the mechanism involved in these findings has not been described. We have recently found that the enhanced blood pressure response to TNF-α during pregnancy are unknown. Therefore, the purpose of this study was to determine the influence of sex steroids in modulating the blood pressure responses to 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 ovario-cystectomized (Ovx) rats chronically treated with 17-β estradiol (E) and/or progesterone (P). Pellets of E and P were implanted in rats to increase plasma estradiol to levels observed during late gestation. Ovario-cystectomized rats received 121 day E and/or P release pellets. Minipumps 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 mm Hg, NP + TNF vs 101±1 mm Hg, NP). In contrast, TNF-α had no effect in virgin rats (125±3 mm Hg, V vs 125±2 mm Hg, V). Moreover, TNF-α had no effect on blood pressure in progesterone-treated rats (130±4 mm Hg, P + TNF vs 129±4 mm Hg, P), estrogen-treated rats (122±7 mm Hg, E + TNF vs 107±5 mm Hg, E), or estrogen and progesterone-treated rats (124±4 mm Hg, P + E + TNF vs 118±3 mm Hg, P + E). We conclude that normal blood pressure response to TNF-α during pregnancy is not likely due to modulation of the vascular responses to TNF-α by sex steroids.
Effect of Chronic Intermittent Hypoxia on Renal Pressure-Natriuresis in Female Rats

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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. While 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. CIH surgery was conducted 4 weeks prior to exposure to CIH. Rats were placed in enclosed chambers and exposed to CIH for 7 days. CIH is defined as continuous cycles of 3 minutes of room air (21% O2) and 3 minutes of 10% O2 for 6 hours. Control animals breathed room air 24 h/4. The relationship between renal perfusion pressure (RPP) and sodium excretion and urine flow were determined under tract anesthesia in normoxic–female (N-OVX), OVX, CIH-OVX, and OLV-infused 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 exposure to CIH enhances sodium excretion in females, and this effect is dependent on the presence of female sex hormones. We speculate that female sex hormones facilitate renal sodium reabsorption and water excretion in CIH and act to protect elevations in blood pressure associated with CIH.

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

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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 treatment (123 ± 10/74 ± 9 mmHg) vs 144 ± 3/84 ± 10 mmHg; p < 0.005; 123 ± 7/44 ± 5 mmHg vs 144 ± 3/84 ± 10 mmHg; p < 0.005, respectively). Glycemic control was identical in the valsartan and atenolol-treated groups (HbA1c 0.06 ± 0.04 vs 0.06 ± 0.002). Endothelium-dependent and independent relaxation did not differ between the treated groups. L-NAME significantly reduced acetylcholine-induced dilation equally in all groups. Resistance artery media-to-lumen ratio was reduced after treatment with valsartan (9.8 ± 0.6 vs 7.9 ± 0.5%, p < 0.005) but not under 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, in diabetic 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 inhibitors thus results in improved resistance artery parameters in diabetic hypertensive patients.
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 CD56. The cells and MBA segments were fixed in buffered formalin and co-immunostained with anti-palladin and anti-CD56 using secondary antibodies tagged with Alexa fluor 488 and 647, as well as the nuclear stain sytox. Confocal analysis showed that cultured 80–90% ANIES and 100% of all SMC had positive staining for palladin (n = 3). Palladin and also co-localized with CD56 in microvascular structures of rat MBA (n = 3). Immunoblot from MBA confirmed the presence of palladin as two isoforms of 90–92 and 140 kDa (n = 3). This protein pattern is different from other adult tissues such as brain that expresses three isoforms (80–92, 140 and 200 kDa) or SMC where only one isoform (80–92 kDa) is expressed. In summary, palladin is present both in SMC and ANIES, either in culture or in the rat MBA. This is the first report that cells in the peripheral vasculature with a neuronal phenotype express a marker of active neurite growth. The presence of CD56-containing neural cells in the vascular wall may participate in response to injury and vasoconstrictor mechanisms as part of a perivascular sensory neural network.

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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 solitaries (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. Supplementation of baclofen (10 μM) in control neurons cultured from rat NTS decreased the neuronal firing rate by 39% (from 10.07 ± 0.06 Hz to 6.4 ± 0.05 Hz; n = 6). Pre-treatment of neurons with Ang II (100 nM, 5 hrs) had no effect on the inhibitory action of baclofen (38% inhibition) in AT1 receptor negative neurons. However, in AT1 receptor positive neurons, Ang II (100 nM, 5 hrs) significantly enhanced the inhibitory action of baclofen (63% inhibition). To understand the exacerbated inhibitory response to baclofen following treatment of NTS neurons with Ang II, we examined the effect of Ang II on GABA 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.

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The reporting of both benefit and harm of salt restriction on the outcomes of hypertension has sustained the ongoing controversy on the role of salt in human cardiovascular health. Increased oxidative stress (OX) due to salt has been documented in experimental salt-sensitive (SS) hypertensive rats. However, whether there is a differential effect of salt on OX of SS and salt-resistant (SR) hypertensive rats, we tested the hypothesis that D3 receptor regulation of ETB receptors in SS hypertensive rats (SHRs) but the percent increases were similar. ETB receptor expression in the renal cortex is similar expression of ETB receptors in WKY and SHRs, basal D3/ETB receptor co-immunoprecipitation was 3 times greater in WKY than in SHRs. The absolute amount of D3/ETB immunoprecipitation was 3 times greater in WKY than in SHRs. The absolute amount of D3/ETB receptor co-immunoprecipitation induced by the D3 antagonist, 99199A (10 M/24 hr). In contrast, in RPT cells from SHRs, P128987 decreased ETB receptor expression. In spite of similar expression of ETB receptors in WKY and SHRs, basal D3 ETB receptor co-immunoprecipitation was 3 times greater in WKY than in SHRs. The absolute amount of D3/ETB receptor co-immunoprecipitation induced by the D3 antagonist was greater in WKY than in SHRs but the percent increases were similar. ETB receptor expression in the renal cortex is decreased in D3 receptor null mice. D3 receptors regulate ETB receptors by physical receptor interaction. The role of D3 receptor expression in D3 receptor regulation of ETB receptors in RPT cells from SHRs.

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Despite strong evidence for genes causing essential hypertension, disentangling the causal variants has proved difficult. Linkage analysis has been used to search for genomic regions that may harbour disease related genes in affected hypertensive family members. By using additional phenotypic data it may be feasible to identify new loci and further refine existing signals. The analysis of covariance-defined subsets however, is dependent on researchers’ prior beliefs and introduces a multiple testing burden. Here we report a novel approach to capitalise on additional phenotypic data in linkage studies. The British Genetics of Hypertension (BRIGHT) Study has collected biometric and biochemical measurements on 2015 affected sibling pairs recruited from the upper 5% of the blood pressure distribution and has published a 100M genome scan. We took a unified approach to utilise additional phenotypic variables in the search for genetic signals by testing for dependence between genetic sharing and hypertension covariates within the dataset as a whole. Previous “identity by descent regression” studies have been prohibitively computationally expensive and restricted to studying only a few variants. We have developed a novel and less computationally intensive score test method that tests consistently robust. We found genome-wide significant evidence for linkage of several hypertension phenotypes. The strongest signals were from body mass measurements on chromosome 20q (genomewide p = 0.002) and kidney function measures (creatinine and glomerular filtration rate) on chromosome 5p (genomewide p = 0.008). Correcting for the multiple traits and genetic locations studied, our global genomewide p value is 0.03. The 20q locus coincides with several other obesity-related linkage studies and is particularly interesting as most BRIGHT cases are not obese (median BMI =27). The locus on chromosome 5p contains several candidate genes which may influence kidney function and has not been identified in previous hypertension scans. This is the first “identity by descent regression” study in hypertension and demonstrates the value of incorporating additional phenotypic information to define novel loci and new avenues of research.

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The dopaminergic and endothelin systems, by regulating sodium transport in the renal proximal tubule (RPT), play a role in the control of blood pressure. Endothelin B (ETB) receptor deficiency results in increased sensitive hypertension and a decrease in glomerular filtration rate (GFR). Disruption of D3 dopamine receptor gene in mice induces hypertension that is associated with a decreased ability to excrete a sodium load. The D3 and ETB receptors are expressed in RPT, and the D3 receptor may regulate ETB expression. Because D3 receptor regulation and function in RPTs are impaired in spontaneously hypertensive rats (SHRs), we tested the hypothesis that D3 receptor regulation of ETB receptors in RPTs may be impaired in SHRs, D3 and ETB receptors were studied in immortalized RPT cells and brush border membranes from Wistar-Kyoto (WKY) and SHRs, and renal cortical membranes from D3−− mice using immunoblotting, and immunoprecipitation. In WKY RPT cells, the D3 ETB, P128987, increased ETB receptors in a time- and concentration-dependent manner, effects that were blocked by the D3 antagonist, 99199A (10 M/24 hr). In contrast, in RPT cells from SHRs, P128987 decreased ETB receptor expression. In spite of similar expression of ETB receptors in WKY and SHRs, basal D3 ETB receptor co-immunoprecipitation was 3 times greater in WKY than in SHRs. The absolute amount of D3 ETB receptor co-immunoprecipitation induced by the D3 antagonist was greater in WKY than in SHRs but the percent increases were similar. ETB receptor expression in the renal cortex is decreased in D3 receptor null mice. D3 receptors regulate ETB receptors by physical receptor interaction. The role of D3 receptor expression in D3 receptor regulation of ETB receptors in RPT cells from SHRs.

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Introduction: Endothelial dysfunction in cytoplasmic or branchial 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

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Introduction: We have previously demonstrated that high-dose insulin may induce an increase in the reactivity to nor epinephrine (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^{-8} to 10^{-5} Mol/L) and acetylcholine (from 10^{-9} to 10^{-5} Mol/L) were performed in presence of or absence of insulin 715 pmol/l (low dose) and 715 nMOL/l (high dose, hd). Results: The results are summarized in the Table (*p<0.05, **p<0.001 vs. basal). A significant reduction in the contractile response to NE was observed in NT after pre-incubation of the vessels with both hd and hd insulin. No reduction was observed in NIDDM and in 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 hd insulin was observed in NT compared to EH (p<0.03, NIDDM (p<0.02), and EH + NIDDM (p<0.05) whereas no difference was observed with hd insulin. No difference between the CRC to ACH in NGF- efferent or either efferent or afferent nerve were observed in any group. Conclusions: Insulin at (physiologic) doses seem 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>Table</th>
<th>Ach 10^{-8} mol/L</th>
<th>Ach 10^{-7} mol/L</th>
<th>Ach 10^{-6} mol/L</th>
<th>Ach 10^{-5} mol/L</th>
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<tr>
<td>CV events -12.4 ± 14.8</td>
<td>20.8 ± 15.6</td>
<td>33.7 ± 17.5</td>
<td>49.5 ± 20.0</td>
<td>66.5 ± 18.9</td>
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<td>No CV events -15.1 ± 20.0</td>
<td>-28.0 ± 24.0</td>
<td>-40.0 ± 25.9</td>
<td>-52.4 ± 26.4</td>
<td>-62.2 ± 25.9</td>
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Effects in Extracellular Matrix in Small Subcutaneous Resistance Arteries of Patients with Primary Aldosteronism

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Introduction: It has been previously demonstrated that aldosterone may possess a strong profibrotic action in vitro and in animal models of genetic or experimental. Hypothesis: To investigate the presence of such a profibrotic action in the human microcirculation is presently available. Methods: We investigated 13 patients with primary aldosteronism, 7 patients with essential hypertension (EH) and 10 normotensive controls (NT). All subjects were submitted to a biopsy of gluteal or abdominal subcutaneous fat tissue. Small resistance arteries were dissected and mounted on an isometric myograph and the tunica media to internal lumen ratio (ML) was measured. The total collagen content within the tunica media was detected (Sirius red staining and image analysis) and collagen subtypes were evaluated using polarized light microscopy; under this condition thicker type I collagen fibers appear orange or red, while thinner type III collagen fibers are yellow or green. Results: ML was significantly increased in primary aldosteronism (0.99 ± 0.02) and in essential hypertension (1.01 ± 0.03) compared with normotensive controls (0.97 ± 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 III vascular smooth muscle actin was significantly increased in primary aldosteronism (7.81 ± 2.91 and 5.92 ± 1.73, P<0.01) than in essential hypertension (7.00 ± 2.46 and 5.25 ± 1.64, respectively). Normotensive controls had less total and type III collagen in respect with the two hypertensive groups (2.32 ± 1.63 and 1.86 ± 0.64, P<0.001). Type I collagen was less in primary aldosteronism (2.91 ± 1.01) than in normotensive controls (2.71 ± 1.03, P<0.05) with an intermediate result in essential hypertension (2.31 ± 1.06). Conclusions: Our results indicate that in small resistance arteries of patients with primary aldosteronism a pronounced fibrosis may be detected, even more evident that in blood-pressure matched patients with essential hypertension.

Table

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<tr>
<th>Table</th>
<th>Basal NE mol/L</th>
<th>NE 10^{-5} mol/L + insulin hd</th>
<th>NE 10^{-5} mol/L + insulin hd</th>
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<tr>
<td>NT, n=12</td>
<td>1154 ± 7735</td>
<td>783 ± 7375</td>
<td>737 ± 691</td>
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<td>EH, n=11</td>
<td>838 ± 735</td>
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<td>NIDDM, n=8</td>
<td>8730 ± 943</td>
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<td>7039</td>
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<tr>
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<td>9415 ± 8348</td>
<td>9039</td>
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Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase (NOS). Our previous studies have shown that endothelial dysfunction, mediated by down-regulation of NOS and a decreased nitric oxide (NO) is present in autosomal dominant polycystic kidney disease patients even before the hypertension and renal insufficiency. But the relationship between ADMA and NOS in ADPKD is unclear. We hypothesized that raised plasma ADMA concentration (P_{ADMA}) contributes to the endothelial dysfunction of ADPKD. P_{ADMA}, symmetric dimethylarginine (P_{SDMA}) and L-arginine (P_{ARG}) were determined and in relation to plasma nitrate levels in ADPKD patients, vessels NOS activity (vNOS) and endothelium dependent relaxation (EDR) in subcutaneous resistance vessels from 8 ADPKD patients with normal renal function compared with 5 healthy control subjects. P_{ADMA}, P_{ARG}, and P_{ARG} were determined by HPLC. vNOS, P_{ADMA} and P_{SDMA} were determined by related analysis kits. EDR was determined in isolated small subcutaneous resistance arteries using Mulvany-Halpern myograph. The results show that P_{ADMA} and P_{ARG} concentrations in ADPKD patients were significantly higher than those in control group (0.64 ± 0.16 mol/L vs 0.41 ± 0.07 mol/L in P_{ADMA}, 0.69 ± 0.06 mol/L vs 0.40 ± 0.06 mol/L in P_{SDMA}, all with p<0.002). P_{ARG} concentrations were not significantly different between the two groups. P_{ADMA} and P_{SDMA} concentrations in ADPKD patients were significantly higher than those in controls (55.7 ± 73.13 mol/L in P_{ADMA}, 49.16 ± 33.99 mol/L in P_{SDMA}, all with p<0.05). P_{ADMA} was inversely correlated to vNOS (r=-0.59, p<0.001) and EDR (r=-0.32, p<0.05), but not correlated with P_{ARG}. P_{ADMA} and P_{ARG} concentrations inversely correlated to vNOS and EDR. In conclusion, patients with ADPKD, ADMA is significantly increased before renal dysfunction, and may contributes to the development of endothelial dysfunction and cardiovascular disease.

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

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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-alpha) decrease blood pressure and IL-6 production. Therefore, this study tested the hypothesis that the deletion of PPAR-alpha receptors would augment the hypertensive response to chronic AngII infusion. Male PPAR-alpha knockout (PPAR-alpha KO) mice and their wild-type (WT) controls, 129S1/SvImj, 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-alpha KO and WT mice, respectively. AngII (50 ng/min, s.c.) caused a rapid increase in MAP in both groups, averaging 144 ± 6 mmHg (PPAR-alpha KO) and 135 ± 5 mmHg (WT) by day 2. Although blood pressure plateaued at this level in WT mice (143 ± 5 mmHg on day 4), MAP was significantly higher in PPAR-alpha 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-alpha KO and WT mice, respectively. These data suggest that PPAR-alpha-dependent mechanisms may 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.
homolog, 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 angiotensin II (Ang II)- like vasodilator that 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 hepatocyte may participate in cardiac hypertrophy and fibrosis. Since aldosterone induces cardiac hypertrophy and fibrosis, we investigated its effects on the extracellular matrix (ECM). We isolated from neonatal rat hearts and pretreated for 24 h in growth media depleted of serum or hormones, to study the transcriptional regulation of ACE2. Treatment of myocytes with aldosterone [5 μM] caused a significant decrease in ACE2 mRNA (relative gene expression of 38.86, 13.5 of control, n = 3, p < 0.001). The majority of each protein caused a significant up-regulation of ACE mRNA (1200% ± 170 control, n = 3, p < 0.05). These results suggest that aldosterone alters the ratio of ACE/AEC in the heart, increasing ACE while concomitantly decreasing ACE2. Moreover, aldosterone-induced imbalances in Ang II relative to Ang-(1-7), secondary to changes in expression, favor increased fibrosis and attenuated anti-fibrotic effects. These studies further suggest that blockade of the aldosterone-induced increase in ACE/AEC by mineralocorticoid antagonists such as spironolactone may participate in their attenuation of the accumulation and structural remodeling of the collagen matrix.

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

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Vascular superoxide (O2−) levels are increased in DOCA-salt rats. The purpose of this study was to investigate the sources of the ET-1-induced reactive oxygen species (ROS) production in conduit (aorta-AO) and resistance arteries (mesenteric arteries-MA) of DOCA-salt rats, and the implication of ET1 receptor in ROS generation. DOCA-salt rats (n = 8) received 5 different treatments: apocynin (NADPH oxidase inhibitor, 1.5 mM/L), biliverdin (xanthine oxidase inhibitor, 100 μg/kg/day), bosentan (ET receptor antagonist, 100 mg/kg/day), BMS182874 (ET1 antagonist, 40 mg/kg/day), and hydralazine (25 mg/kg/day). Data were compared to uninephrectomized rats (UnNx). After 3 weeks treatment, systolic blood pressure in DOCA-salt rats was reduced by apocynin, BM and hydralazine, (P < 0.01). TBARS levels (lipid peroxidation) were increased in DOCA-salt rats (2.8 ± 0.1 μM/L) compared to control (1.9 ± 0.1 μM/L) and hydralazine (2.0 ± 0.3 μM/L), bosentan (2.2 ± 0.5 μM/L) and hydralazine (1.9 ± 0.4 μM/L) prevented lipid peroxidation increase. Fluorescence confocal microscopy showed reduced O2− production in MA and AO from bosentan-treated DOCA-salt rats. As well, chemiluminescence analysis of increased xanthine oxidase activity in MA and AO from DOCA-salt rats compared to controls (413.85 ± 116.51 ± 118.10 vs 60 ± 1 × 109 cpd/mg dry weight, respectively). All treatments reduced or prevented the increase of xanthine oxidase activity (P < 0.01) in MA whereas bosentan and BM had no effect in AO. In addition, confocal microscopy showed reduced O2−, when tissues were treated in situ by TFA and COP (inhibitors of mitochondrial electron transport complexes II and IV). Rotenone (mitochondrial complex I inhibitor) had no effect in MA. Our findings suggest involvement of multiple ROS-generating systems, some of which are ET1-sensitive, in the development of hypertension and vascular inflammation in DOCA-salt rats.

The Role of Heme Oxygenase in the Regulation of the Renal Afferent Arteriole

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Heme oxygenases (HO-1, HO-2) catalyze the conversion of heme to carbon monoxide, iron, and biliverdin. Carbon monoxide (CO) has been shown to cause vasorelaxation via stimulation of guanylate cyclase (GC) and/or activation of K+ channels. We used the blood-perfused juxtamedullary nephron preparation to test the effects of CO on renal afferent arterioles and their interaction with sodium nitroprusside (SNP). Afferent arterioles (AA)s were superfused with either 15 μM Chromium mesoporphyrin (CrMP, HO inhibitor) or Tricyanovinyl(dichlororuthenium) (II) dimer, known as carbon monoxide releasing molecule or CO-RM-2. Experimental data were performed on AAs that were pretreated with 1 μM nitro-L-arginine (L-NAME) to inhibit nitric oxide synthase (NOS). Sodium nitroprusside (SNP) was superfused over control preparation and increased AA diameter averaged 17.1 ± 0.7 μm, n = 5, inhibition of HO by superfusing CrMP over control preparation did not affect AA diameter (17.1 ± 0.8 μm, n = 5, p < 0.05). CORM-2 (150 μM) caused a significant vasodilatation after 5 minute of treatment (20.8 ± 0.6 μm, 18.9 ± 0.9 μm, n = 4, p < 0.05). The afferent arteriole continued to dilate during treatment with 300 μM CORM-2 with maximal vasodilatation reached after 6 minutes (23.5 ± 1.2 μm, n = 4). Inhibition of NO production by NLA caused a significant vasoconstriction by 14.2 ± 4.3%, n = 6, p < 0.05, which was significantly exacerbated by concurrent H9 inhibition (29.1 ± 5.0%, n = 6, p < 0.05). These results indicate that CO acts as a vasodilator in the renal microcirculation. We conclude that heme oxygenase enzymes do not seem to regulate the afferent arteriole diameter under normal conditions, but exert a vasodilatory influence when NO production is inhibited. Supported by COSEHC postdoctoral fellowship and NHLBI 18426.
Renal Venous Oxygen Tension After ACE-inhibition Predicts a Functional Renal Artery Stenosis in Two-kidney, One Clip Hypertension

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Clinicians require an immediate, functional test of the significance of renal artery stenosis (RAS) when this is detected incidentally by angiography to provide guidance about the need for angioplasty. We evaluated renal venous oxygen tension (RVpO2) and its response to acute ACE inhibition in the two-kidney, one clip hypertensive (2K1C) model. Three weeks after left renal artery clipping or sham surgery (sham), rats were inactin-anesthetized and split kidney function was assessed in the two-kidney, one clip hypertension (2K1C) model. Three weeks after left renal artery clipping or sham surgery (sham), rats were inactin-anesthetized and split kidney function was assessed in the two-kidney, one clip hypertension (2K1C) model. Three weeks after left renal artery clipping or sham surgery (sham), rats were inactin-anesthetized and split kidney function was assessed in the two-kidney, one clip hypertension (2K1C) model.

Renal autoregulation is mediated by two intrinsic mechanisms, tubuloglomerular feedback and myogenic vasoconstriction of the preglomerular microvasculature. While the myogenic response is an inherent property of arteriolar vascular smooth muscle cells, several factors may modulate its intensity. Chronic heart failure (HF) is a progressive disorder that leads to intense intrarenal vasoconstriction, elevated renal vascular resistance, and reduced renal blood flow. We hypothesized that pressure-induced contraction of arterial smooth muscle was reduced in HF. We induced HF by ligating the left anterior descending coronary artery in male C57BL/6J mice 4 to 6 weeks old. Sham-ligated mice (SHAM) served as controls. Systolic blood pressure (SBP) was measured by tail cuff, and cardiac function was evaluated by echocardiography. To determine whether the reduction in systolic blood pressure persists when renin-angiotensin-aldosterone system (RAAS) was eliminated, we treated the mice with an ACE inhibitor (enalaprilat). In particular, the reduction in RVpO2 after acute ACE-inhibition in patients with a RAS may be a useful method to diagnose functionally significant stenosis requiring angioplasty.

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

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The nucleus of the solitary tract (NTS) is the central site of termination of baroreceptor afferents. We hypothesize that changes occur in voltage-gated calcium channels (VGCCs) within NTS neurons as a consequence of hypertension. Whole cell patch clamp recordings were obtained from adult normotensive (NT, 109 ± 2 mmHg; n = 63) and 4-week, renal wrap hypertensive (HT, 150 ± 20 mmHg; n = 24) rats. In some experiments the tracer DIA was applied to the aortic nerve to visualize NTS neurons receiving baroreceptor synaptic contacts. Cell size, as estimated by membrane capacitance, was not different comparing neurons from HT (7.8 ± 0.5 pF; n = 26) and NT (7.2 ± 0.5 pF; n = 28) rats. This was also observed in NTS neurons receiving arterial baroreceptor inputs as determined by the presence of somatic DIA labeled appositions. Membrane capacitance was not different comparing DIA labeled NTS neurons from HT (9.0 ± 0.2 pF; n = 9) and NT (9.0 ± 0.1 pF; n = 7) (p > 0.05). 

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

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Endothelin A (ETa) receptor blockade enhances the integrated pressor response to acute environmental stress in Dahl salt-resistant (DR) rats, but the mechanism is unknown. We therefore tested the hypothesis that ETa receptor antagonism enhances sympathetic nerve activity, as measured by catecholamine release, in a supersensitive dependent manner. Stress was induced by restraint and administration of air jet pulses (3min) in rats maintained on a normal salt diet before and after 3-day treatment with either the ETa receptor antagonist ABT-267 (5 mg/kg/day) or the superoxide dismutase mimetic, tempol (1 mM). Blood draws were accomplished using indwelling catheters, and basal and stress-induced increases of microalbumin and catecholamines were 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 thus is a polygenic trait attributable to variation at multiple points in the adrenergic pathway. Further genetic linkage/association studies in larger numbers of twins and siblings are ongoing to better characterize the role of adrenergic traits and their genetic polymorphisms in subtle glomerular injury.

Afferent Arteriole Myogenic Response in Mice with Chronic Heart Failure

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Renal autoregulation is mediated by two intrinsic mechanisms, tubuloglomerular feedback and myogenic vasoconstriction of the preglomerular microvasculature. While the myogenic response is an inherent property of arteriolar vascular smooth muscle cells, several factors may modulate its intensity. Chronic heart failure (HF) is a progressive disorder that leads to intense intrarenal vasoconstriction, elevated renal vascular resistance, and reduced renal blood flow. We hypothesized that pressure-induced contraction of arterial smooth muscle was reduced in HF. We induced HF by ligating the left anterior descending coronary artery in male C57BL/6J mice 4 to 6 weeks old. Sham-ligated mice (SHAM) served as controls. Systolic blood pressure (SBP) was measured by tail cuff, and cardiac function was evaluated by echocardiography. To determine whether the reduction in systolic blood pressure persists when renin-angiotensin-aldosterone system (RAAS) was eliminated, we treated the mice with an ACE inhibitor (enalaprilat). In particular, the reduction in RVpO2 after acute ACE-inhibition in patients with a RAS may be a useful method to diagnose functionally significant stenosis requiring angioplasty.
Renal and Heart ACE2 Activity in Models of ACE2 and ACE Ablation and Diabetic Mice

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ACE2 is the only known and enzymatically active homologue of ACE in the human genome. ACE2 activity may counterbalance the angiotensin II promoting effects of ACE by preventing angiotensin II accumulation in tissues, particularly in the kidney and heart where ACE is predominantly expressed and may exert protective actions. To determine tissue ACE2 activity, we utilized a microplate based fluorometric method using ACE2 specific substrate and specific inhibitors for ACE2. ACE2 activity (RFU/ug protein/hr) was examined in models of ACE and ACE2 gene ablation (ACE2[−/−] and ACE2 knock-out mice) to investigate ACE2 activity over a wide range of ACE and ACE2 protein expression, respectively. ACE2 activity had a strong positive correlation with renal cortex ACE2 protein expression (90 kDa band recognized by our non-commercial ACE2 antibody) in both knockout models and their respective wild-type littersmates (r = 0.94, p < 0.01). In the ACE2 knockout, the 90 kDa band was absent and ACE2 activity was barely detectable despite the presence of another 67 kDa band detected by our ACE2 antibody. Renal cortex ACE2 activity had no correlation with renal cortex ACE2 protein expression (r = 0.02, n = 6, 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 (264 ± 3 vs 1.27 ± 0.24 RFU/ug protein/hr, respectively). In renal cortex of 8 weeks old db/db mice, ACE2 activity was increased as compared to db/m controls (db/db 46.7 ± 4.4 vs db/m 22.0 ± 4.7 RFU/ug protein/hr, p < 0.01), which is in concordance with the higher levels of ACE2 protein in the renal cortex of the db/db model of type 2 diabetes. We conclude that ACE2 enzymatic activity reflects the relative abundance of the ACE2-immunoreactive protein at this time in the life of the animal. Profiling ACE2 gene expression by qPCR on PR stimulated (1, 4, 24 & 48 hours) samples (Rosetta Resolver), among which were osteoprotegerin, Timp1, Hsp27, Anp and Best5. Among these, mRNA pool of 5 isolations, dye-swap) detected 259 regulated genes (p < 0.001). qPCR on PR stimulated (1, 4, 24, 24 & 48 hours) samples (n = 7) revealed temporal regulation of osteoprotegerin, Timp1, Hsp27, Anp and Best5. This regulation was not altered in presence of a renin inhibitor, or an AT1 receptor antagonist. Conclusion: In cardiomyocytes, PR directly (i.e., independently of Ang II) activates p38 MAP kinase-induced activation of 2nd messenger systems. Polyclonal antibodies detected membrane bound Ang I generation (Prescott et al., 2002), but could also lead to angiotensin-independent pathways. Local Ang I generation (Prescott et al., 2002), but could also lead to angiotensin-independent pathways. Local Ang I generation (Prescott et al., 2002), but could also lead to angiotensin-independent pathways.
Angiotensin II Induced Hypertensive Response is Modulated Through Tumor Necrosis Factor-alpha: Role of Nox1, Nox4 and Gp91phox

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Angiotensin II (ANG) and Tumor necrosis factor-α (TNF-α) play an important role in the pathogenesis of cardiovascular disease. Recent evidence suggests that both ANG II and TNF-α induce hypertension and exert stress to contribute to development of heart disease. In this study, we examined whether ANG II induced hypertensive response 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−/− mice were subjected to osmotic minipumps containing ANG II (1 μg/kg/min) or saline for 14 days. In a group of TNF−/−−/− mice, human recombinant TNF 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 measurement of gp91phox, Nox1 and Nox4 using real time PCR. Results: The real time PCR values show that ANG II (1 μg/kg/min) increased the expression of TNF-α and MCP-1 and VCAM-1. In conclusion, in DOC-salt hypertensive mice, the ANG II induced hypertension is contributed by TNF-α and MCP-1 and VCAM-1.

Conclusions: 1) ANG II-induced hypertensive effect is at least in part mediated through TNF-α. 2) ANG II-induced hypertensive response is also in part mediated through ANG II and TNF-α. 3) ANG II-induced expression of MCP-1 and VCAM-1 in mesenteric arteries suggests a role for these homologues in hypertensive response.

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

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Peroxisome proliferator-activated receptor (PPAR)-γ activators increase insulin sensitivity and prevent Ang II-induced vascular remodeling. The aim of the present study was to evaluate the effects of PPAR-γ activators on cell growth and Ang II signaling pathways. Vascular smooth muscle cells (VSMCs) derived from mesenteric arteries were treated with Ang II (10−7M) with or without PPAR-γ activators: prostaglandin J2 (PGJ2) and rosiglitazone for 24 hours. PGJ2 (5μM) decreased Ang II-induced protein synthesis by 82% (p<0.001), while both PGJ2 and rosiglitazone (10μM) decreased DNA synthesis induced by Ang II, respectively by 67% and 56% (p<0.001). Western blot analysis was performed to evaluate PPARs isoform, ERK1/2, P38, Akt/PI3K, SHP-2, and Ang II receptors A1 and A2 expression. Activity of PPAR-γ, ERK1/2, Akt/PI3K and P38 was also evaluated. Rosiglitazone treatment significantly increased nuclear PPAR-γ expression and activity in VSMC 2-fold (p<0.01). Rosiglitazone decreased ERK1/2 peak activity by 59% (p<0.001), while PGJ2 peak activity by 58% (p<0.001), both of which were induced by Ang II. Ang II-induced SHP-2 activity was also decreased (p<0.01) by rosiglitazone. In conclusion, PPAR-γ activators PGJ2 and rosiglitazone reduced Ang II-induced VSMC growth associated with inhibition of ERK1/2, Akt/PI3K. We demonstrate for the first time that PPAR-γ activation SHP-2 activity and decrease Ang II-induced VSMC growth. These results indicate that PPAR-γ activation may hold promise for vascular remodeling in diabetic and hypertensive patient.

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

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We have previously reported that DOCA-salt hypertension (HTN) (Z1-21 days), results in enhanced cardiac and renal damage and inflammation in ccr5 KO compared to WT mice, despite an equal increase in MAP in the two groups. Because the KO mice have a 15–20 mmHg higher baseline MAP, they consistently have a higher BP than the WT mice during DOCA-salt induced HTN. The aim of this study was to determine the role of the higher BP to the DOCA-salt HTN induced increase in cardiac and renal damage in the absence of ccr5 KO. DOCA-salt HTN was induced in telemetry probe implanted 8–10 week old ccr5 KO and WT mice by standard technique. To equalize the BP to that of the DOCA-salt WT mice, a separate group of DOCA-salt KO mice were given 0.025% hydralazine (HDY) to drink. Basal MAP was significantly elevated in KO (117±4 mmHg) compared to WT (101±3 mmHg) mice. The DOCA-salt protocol increased the MAP in the KO (142±1 mmHg) and WT (129±2 mmHg) mice. The MAP of the HYD treated DOCA-salt KO mice was (126±2 mmHg). Using a subjective 0 to 4+ scoring system, cardiac and renal sections from DOCA-salt KO mice exhibited marked histopathologic damage, which was absent in DOCA-salt WT as well as control KO and WT mice. HYD treatment reduced the cardiac (+25%) and renal (+25%) damage compared to DOCA-salt KO mice. Plasma C reactive protein (CRP), a marker for inflammation, was significantly elevated (50%) in DOCA-salt KO mice compared to DOCA-salt WT mice and control KO and WT mice. HYD treatment in the DOCA-salt KO mice lowered (~20%) but did not normalize the elevated CRP levels compared to untreated DOCA-salt KO mice. Cardiac hypertrophy in DOCA-salt KO mice treated with a standard technique for MCP-1 and V-CAM-1, showed a significant but equal elevation of both markers in DOCA-salt WT and DOCA-salt KO mice compared to their controls. HYD treatment had no effect on these elevated levels in the DOCA-salt KO mice. Our data demonstrate that equalization of BP with HYD between the DOCA-salt KO and DOCA-salt WT mice significantly attenuates but does not normalize the increased cardiac and renal damage and plasma CRP levels without an effect on the elevated levels of MCP-1 and V-CAM-1. In conclusion, in DOCA-salt hypertensive ccr5 KO mice, both the elevated BP and absence of ccr5 KO contribute to the cardiac and renal damage and inflammation observed in this setting.

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

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Evidence suggests a relationship between short-term blood pressure (BP) variability and cardiovascular target-organ damage. Although a blunted nocturnal decrease in BP and reduced heart rate variability have been shown to be associated with cardiovascular morbidity in diabetic patients, little information is available on short-term BP variability. In this study, short-term BP variability was assessed in 36 subjects with type 2 diabetes and overt nephropathy who underwent antecedent BP monitoring for one BP cycle that consisted of short-term BP variability examination. The incidence of coronary artery disease (CAD) was significantly greater in the patients with increased 24-h systolic BP variability (67% versus 11%; p<0.0005), while that of cerebrovascular disease was not significantly affected (61% versus 50% not significant). Multiple regression by stepwise regression analysis did reveal that serum cholesterol and plasma norepinephrine (p25E) were significant and independent contributors to
nighttime systolic BP variability (partial R² = 0.490, p < 0.001; and partial R² = 0.470, p < 0.001), and demonstrated that body mass index and p-NE were primary determinants of nighttime diastolic BP variability (partial R² = 0.539, p < 0.0005; and partial R² = 0.504, p < 0.05). Diabetic nephropathy patients with CAD had significantly increased daytime systolic (17.8 ± 5 mm Hg vs. 13.1 ± 5 mm Hg, p < 0.0005) and nighttime systolic (15.4 ± 5 mm Hg vs. 7.2 ± 3 mm Hg, p < 0.05) variability. Furthermore, logistic regression analysis demonstrated that nighttime systolic BP variability was an independent risk factor for CAD odds ratio 3.13 (95% CI 1.02 to 9.61; p < 0.05). The increase in nighttime BP variability is associated with a proportional sympathetic activation in diabetic nephropathy. Elevated short-term BP variability combined with relative sympathetic prevalence during the night might represent an important risk factor for cardiovascular events in the diabetic population.

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

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Activation of cardiac AT1R signaling plays an important role in cardiac hypertrophy. We previously cloned a novel molecule interacting with AT1R, ATRAP (for AT1R-associated protein), using the yeast two-hybrid strategy. In this study, we tested the hypothesis that cardiomyocytes express ATRAP and that ATRAP modulates Ang II-induced hypertrophic responses in cardiomyocytes. We identified that the ATRAP mRNA and protein were endogenously expressed in cardiomyocytes. There was a partial co-localization of the AT1R and ATRAP on immunofluorescent staining under baseline conditions. Additionally, ATRAP expression was 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 adenovirus transduction significantly decreased the number of AT1R on the surface of cardiomyocytes (95% of lacZ control), suggesting an inhibitory effect of ATRAP on the recycling of AT1R. Interestingly, overexpression of ATRAP specifically inhibited Ang II-mediated phosphorylation of p38 MAPK (p < 0.05, n = 6) but not that of ERK or JNK in cardiomyocytes. Furthermore, this phenomenon was accompanied by inhibition of Ang II-induced activation of c-fos promoter transcription (p < 0.05, n = 6), and amino acid incorporation (p < 0.05, n = 6). These results indicate that ATRAP significantly promotes down-regulation of the AT1R and further attenuates certain Ang II-mediated hypertrophic responses in cardiomyocytes, and may suggest a novel strategy to inhibit cardiomyocyte hypertrophy.

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

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Growing evidence indicates that the endothelin (ET) system is important in the initiation and maintenance of salt-sensitive hypertension. Sex hormones have been shown to play a role in the development of hypertension in Dahl salt sensitive (DS) rats. The aim of this study was to examine the role of salt sensitivity in DS rats and the participation of renal endothelin system in sex hormone modulated hypertension. To achieve this goal, we examined the effects of castration and ovariotomy in DS rats in low (0.3%), LS and 3 weeks of high (6%) HS diet sodium diet. Mean arterial pressure (MAP) was followed by telemetry. Pretreatment of ET-1 and endothelin receptors (ETA-R, ETB-R) mRNA levels were measured in the right and left kidneys, ANG II levels were measured in the renal cortex, and the urinary excretion of HO-1 by ELISA. We found a significant increase in MAP in females (from 110 ± 2 to 168 ± 4 mm Hg, p < 0.0002; and partial R² = 0.470, p < 0.05). Development of salt sensitive hypertension in male DS rats was associated with a proportional sympathetic activation in diabetic nephropathy.

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

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The initiating event in preeclampsia is thought to be reduced uteroplacental perfusion which leads to widespread dysfunctions of the maternal vascular endothelium. Circulating factors such as inflammatory cytokines, VEGF receptor antagonists (sflt1), and agonistic autoantibodies to the antibodies are reported to be important in the development of type 1 (AT1) receptor linked mechanisms of preeclampsia. We found that serum from pregnant rats exposure to chronic reductions in uterine blood flow (UBF) results in an increase in ET production in vitro in human umbilical vein endothelial cells (HUVEC). These data indicate that serum from pregnant rats exposed to chronic reductions in uterine blood flow after exposure to RUPP serum (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 ± 3.5 pg/ml from cells exposed to normal pregnant rat serum (n = 9). Pretreatment of HUVEC with an AT1 receptor antagonist, Losartan (15uM), markedly attenuated the increased endothelin production observed in 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 ± 3.3 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 reduces ET production by endothelial cells, an effect that is, in part, mediated by AT1 receptor activation.

Heme Oxygenase-1 Induced by Serum From Pregnant Rats Exposed to Chronic Reductions in Uterine Perfusion

LB44

LB45

The Link Between Nitric Oxide System and Aldosterone: Long Term Oral Supplementation of L-arginine Elevates Arterial Elasticity and Hemodynamic Variables

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Background: Dietary Supplementation with L-arginine enhances activity of the NO system. We aimed to evaluate the effect of L-arginine, in a double-blind randomized, placebo-controlled, multicenter, long-term oral supplementation of L-arginine on arterial properties, humoral and hemodynamic variables. Patients and Methods: We evaluated the effects of 6 months oral supplementation of 6 gms/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 evidence of vascular cut-out from L-arginine treatment with L-Argetine. Large vessel elasticity, including compliance were derived from radial artery waveforms, obtained using a calibrated tonometer (model CR-2000, H&I Inc, Eagan, MN). Results: Systolic blood pressure did not change in the placebo group, 146.4 ± 25.3 before and 146.4 ± 20.3 mm Hg after 6 months of treatment. In the placebo group (Tx) group hypertension from 116.2 ± 19.8 to 116.2 ± 19.8 mm Hg (p > 0.007). Large artery elasticity (LAE) decreased in the placebo group from 11.62 ± 19.8 to 11.62 ± 19.8 but in the Tx group L-Argetine increased arterial elasticity from 10.03 to 12.76 ± 3.48 ml/mmHg/p<0.001). Systemic vascular resistance (SVR) increased slightly in the placebo group from 1759.6 ± 371.98 to 1826.8 ± 389.08 dynes/cm² cm (-0.07; p < 0.01). In the placebo group from 1696.33 ± 300.97 to 1577 ± 222.11 dynes/cm².

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treatment also significantly increased insulin index and HDL's values in blood in these groups of various risk factors. Conclusions: Supplementation of L-Arginine activates the NO system and exerts positive remodeling on the arterial system, improves arterial elasticity, hemodynamic and humoral parameters.

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

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α-calcitonin-gene-related peptide knockout (α-CGRP KO) mice display a significant increase in basal blood pressure compared to their wild type (WT) counterparts. Initial observations from our laboratory demonstrated that aged (~2 yr old) α-CGRP KO mice, fed a normal diet, were significantly heavier compared to WT controls (48.1 ± 3.4 vs 34.2 ± 1.8 g). In the present study, we examined the phenotype of α-CGRP KO mice with obesity. We found that α-CGRP KO mice were significantly heavier than WT controls (201 ± 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 antagonism.

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

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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 ± 13 vs 103 ± 2 mm Hg, P < 0.01). In addition, bilateral renal denervation abolished hypertension in LBW offspring (104 ± 1 mmHg, P < 0.01 vs. LBW SD, but did not alter MAP in control offspring (102 ± 3 mmHg). Adequacy of renal denervation was verified by a greater than 90% reduction in renal neuropeptide content (LBW: 22 ± 4 (BRD) vs. 301 ± 22 (SD) ng/g, P < 0.01 and control: 31 ± 6 (BRD) vs. 193 ± 13 (SD) ng/g, P < 0.01). In addition, renal neuropeptide 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 mmHg, P < 0.01 vs. LBW SD, but did not alter MAP in control offspring (102 ± 3 mmHg). Adequacy of renal denervation was verified by a greater than 90% reduction in renal neuropeptide content (LBW: 22 ± 4 (BRD) vs. 301 ± 22 (SD) ng/g, P < 0.01 and control: 31 ± 6 (BRD) vs. 193 ± 13 (SD) ng/g, P < 0.01). In addition, renal neuropeptide content was significantly elevated in intact LBW offspring as compared to intact control offspring (P < 0.01, SD LBW vs. SD control, respectively). Thus, the renal nerves may participate in the etiology of hypertension in LBW offspring induced by placental insufficiency in the rat.

The Genomic Signatures of Hypertension

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In a set of 120 hypertensive and dyslipidemic French-Canadian families from the Saguenay-Lac-St-Jean region of Quebec, Canada, in which we have recently described 46 loci significantly linked to hypertension (HT) and its metabolic components, we searched for the genomic signatures (specific haplotype sets) of HT. After performing a rigorous phenotyping with battery of >50 anthropometric, metabolic and humoral traits, 274 individuals (168 hypertensive) from 25 families presenting HT with extremes of high and low density of metabolic syndrome were genotyped using Affymetrix GeneChip Human Mapping 50K Array XbaI. These were performed on a subset of 140 subjects of hypertensive 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 of the 3 haplotypes increases the aOR (up to 3.1 for set of 3,16 and 18). The degree of risk of HT increased with age, was independent of sex, and was seen in most pedigrees analyzed. In addition to higher BP (SBP 146 ± 19 vs. 118 ± 10 mmHg, P < 0.01), the subjects with the highest aOR have also increased total and LDL cholesterol, triglycerides and extracellular/intracellular water volume compared to subjects with none of these haplotypes. Only the chr. 11 and 16 haplotypes localize to known genes, none of which previously associated with HT though being involved in apoptosis (Fatso gene on chr. 16) and protein folding (zinc finger CSL3 on chr.11). At the depth of 12 layers/germances, the ancestors of families whose members carry any of the 4 haplotypes showed a higher transmission to the current generation in contrast to families in which at least one patient carries all 4 haplotypes (80% vs. 62%) with overall ancestral separability by 42%. In conclusion, using a genome-wide SNP typing approach in an extensively annotated population in terms of genealogy, genetic makeup and phenotype, we have identified genomic signatures providing strong predictive value of hypertensive status.
Primary Aldosteronism Contributes to Poorly Controlled Hypertension in Diabetic Subjects

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Background: Data from large clinical trials suggest that most diabetic subjects will require multiple medications for adequate blood pressure (BP) control. Based on these findings, diabetic subjects are rarely screened for secondary forms of hypertension. In non-diabetic subjects primary aldosteronism (PA) is present in a large number (9%-14%) of patients with poorly controlled BP on multiple drugs. Accordingly, we aimed to determine the prevalence of PA in diabetics with poorly controlled hypertension.

Methods: Diabetic subjects with a BP ≥140/90 mm Hg on ≥3 anti-hypertensive medications in Diabetes Care Centers were consecutively screened for PA with a plasma aldosterone concentration to plasma renin activity ratio (PAC/PRA). Except for aldosterone inhibitors, patients were continued on their usual BP medications. Subjects with a PAC/PRA ≥ 30 ng/dl (35 nmol/l) 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/collection were given a renin stimulation test after the 3-day salt load was considered as having PA.

Results: Sixty-two subjects were screened for PA by the study protocol. Seventeen subjects (27.4%) had a positive screen. Eight of the subjects with a positive screen have ruled in for PA as defined by study criteria. The results for 6 others are still pending and 3 patients have ruled out for PA. The prevalence of PA is 12.9% (95%CI: 8.9-19.5).

Clinical and experimental 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 hypolglycemic and antihypertensive agents were randomized to double-blind treatment for one year with valsartan (80-160 mg) or atenolol (50-100 mg) once daily, added to previous therapy. Healthy, age-matched controls (n=12) were also studied. Serum levels of cytokines (IL-6, IL-1β, chemokines (MCP-1), adhesion molecules (soluble ICAM and soluble E-selectin) were measured by ELISA before and one year after treatment. One year after therapy, BP was similarly controlled by valsartan and atenolol (123±2/74±2 vs 114±3/84±2 mmHg, p<0.005; 128±3/75±2 vs 114±2/83±2 mmHg, p<0.005, respectively). Glycemic control was identical in the two groups. Serum levels of IL-6, IL-1β, sICAM and sE-selectin were increased (2-4-fold) in patients before treatment compared with controls (p<0.05). IL-6 and IL-1β levels were reduced by valsartan (3-fold, p<0.01) and comparable to levels in controls. Our findings indicate that 1) proinflammatory mediators are increased in hypertensive type 2 diabetic patients, 2) BP reduction is associated with improved inflammatory status and 3) valsartan and atenolol have differential anti-inflammatory actions. In conclusion, antihypertensive treatment, particularly with valsartan, ameliorates inflammatory processes in hypertensive diabetic patients. Such effects may contribute, in part, to cardiovascular protection by these agents.
Chromosome 2p Shows Genome Wide Significant Linkage to Anti-Hypertensive Medication Response in the British Genetics of Hypertension (BRIGHT) Study.

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Introduction
generome-wide linkage studies have found evidence for loci influencing blood pressure and hypertension status on almost all chromosomes. We hypothesised that drug responsiveness to antihypertensive drugs is used in a strictly genomewide fashion, and may be used to identify and map loci on chromosome 2 in the same region as the AB only group (LOD 1.61 at 90.68 cM). This is the first study to identify significant genome wide linkage by partitioning different hypertension trials into different groups 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). This suggests that the region may contain a gene for the response 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.

Results

2142 severely hypertensive Caucasian ASP (BRIGHT). Anti-hypertensive therapy was classified into two groups - those that inhibit RAS (A-ACEAR, B-beta-blockers) or not (C-CCB, D-diuretics). Non-responders had a BP <140/90 or a BP reduction of <20mmHg. 298 sibling pairs (ABCD) were identified who were non-responders on A/B/CD therapy only. Of them 89 pairs (AB) were on AB only, and 76 pairs (CD) were on C/D only. NPL analysis was performed on a 10 cM genome scan in the three groups using MERLIN in combination with MLSix. Results Significant linkage was observed in the AB group on chromosome 2 (Figure, multipoint LOD 4.84 at 90.90 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 a significant genome wide linkage by partitioning different pathways of hypertension based on drug response. The locus on chromosome 2p in a subset of Caucasian hypertensives 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.

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

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Angiotensin converting enzyme 2 (ACE2) is a homologue of ACE that preferentially converts the potent vasoconstrictor and growth stimulator angiotensin II (Ang II) to Ang-(1-7), a peptide with vasodilator and anti-proliferative properties. We previously reported ACE2 mRNA and protein in distinct brain regions from both neonatal and adult Sprague-Dawley rats as well as primary cultures of rat astrocytes isolated from the medulla oblongata and cerebellum of neonatal rat brain. Since central components, such as Ang II and the precursor protein angiotensigen, were found 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 reactive band. No difference in ACE2 activity was observed in the CSF from male or female Sprague-Dawley rats. We found that the ACE2 was biologically active in CSF collected from male SD rats (0.47 ± 0.06 fmol/L/min) 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/L/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-(1-9), although the latter reaction was less robust. In summary, the current study is the first to demonstrate the endogenous presence of an enzymatically active form of ACE2 in the CSF. We conclude that astrocytes may constitute a novel paracrine system that maintains the balance of extracellular Ang II and Ang-(1-7) in the brain in part through the secretion of ACE2 into the CSF and interstitial space.

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

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Endothelium-derived hyperpolarizing factor (EDHF) is reduced in diseases such as hypertension and diabetes. Epoxyeicosatrienoic acids (EETs) are several EDRFs in many vascular beds and regulate vascular tone. EETs actuate smooth muscle calcium-activated K' channels that cause hyperpolarization and relaxation of coronary arteries. However, whether EETs act through a membrane receptor is not known. Here, we developed a stable iodinated 14,15-EET agonist, 14,15-EET-phenyldosulfonfluoramide (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 expression in the SHR RVLM and its enhanced signaling may contribute to hypertension in light of the fact that PI3-kine activity and ROS generation in this area have been implicated in hypertension. Supported by NIH grants HL33610 and HL76312.
concentration-dependent relaxations, with maximal relaxation of 94.1 ± 5.4% and ED50 of 10⁻⁶ mol/L. It was equipotent with 14,15-EET. The relaxations to 14,15-EET-PISA were inhibited by the K⁺ channel inhibitor iberiotoxin (100 nmol/L; max relaxation 39.3 ± 12.8%); 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 7.8 ± 12.8%). Relaxations to 14,15-EET were similarly inhibited by these treatments. 14,15-EET-PISA binding to human U937 cell membranes was time- and concentration-dependent. The specific binding reached equilibrium by 15 min at 4°C and remained unchanged at 30 min. With 50 μg of protein, the estimated Kd of 14,15-EET-PISA was 33 nM. When 14,15-EET-PISA was incubated with myocardial or coronary arterial membranes, a 49kD protein was detected on SDS-PAGE gels. The radiolabeling of the 49kD 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**

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The Zucker diabetic fatty (ZDF) obese rats (ZDFO) are a model of type 2 diabetes and metabolic syndrome based on impaired glucose tolerance caused by the inherited insulin-resistance gene. ZDFO exhibit progressive nephropathy; however, the mechanisms have remained unclear. A recent study indicates that a temporary blockade of the renin-angiotensin (Ang) system (RAS) during the prediabetic stage attenuates renal injury in another model of type 2 diabetes, suggesting the activated renal RAS in type 2 diabetes. The present study was performed to examine the possible involvement of AGT in diabetic nephropathy of ZDFO. Genetic pairs of male ZDFO and ZDF lean rats (ZDFL) (N = 6 each) were maintained on a diet containing 16.7% fat from 12 to 17 weeks of age. ZDFO showed an increased body mass compared to ZDFL (378 ± 18.0 vs 288 ± 7.6 g at 17 weeks). Fasting blood glucose levels were also significantly higher in ZDFO compared with ZDFL (148 ± 36 vs 47 ± 5 mg/dl). Urinary levels of 8-isoprostane were significantly increased in ZDFO compared with ZDFL (38.5 ± 4.1 vs 15.7 ± 3.4 ng/day). Systolic BP (SBP) was progressively increased in ZDFO from 120 ± 1 to 137 ± 1 mmHg during this period. In contrast, SBP did not increase in ZDFL. Kidney AGT protein levels were significantly increased in ZDFO compared with ZDFL (1.83 ± 0.34 vs 1.00 ± 0.26, relative ratio). Kidney Ang II contents tended to increase in ZDFO compared with ZDFL (348 ± 44 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 ± 0.25 vs 1.00 ± 0.17, relative ratio). Previous papers reported that ZDFO show renal injury at around 25 weeks of age. However, at 17 weeks of age, measured indices of renal damage in the present study (glomerular sclerosis, macrophage infiltration, interstitial expansion, and renal arterial hypertrophy) were not significantly different between these 2 groups. We have previously shown that reactive oxygen species (ROS)-associated AGT enhancement plays an important role in renal damage of genetic salt-sensitive hypertension and the present data suggest that elevated ROS and ROS-induced intrarenal AGT augmentation are present prior to the development of diabetic nephropathy in ZDFO.
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