Program presentations are eligible for publication in an upcoming issue of Hypertension. The Publications Committee and Editors of Hypertension welcome the opportunity to consider all manuscripts for publication. Manuscripts will be collected at the conference.
Slide Presentations

1 State-of-the-Art Lecture: Finding Genes That Cause Human Hypertension
Richard P. Lifton. Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT

BLOOD PRESSURE FALL IN RESPONSE TO EXOGENOUS NITRIC OXIDE: A BIMODALLY DISTRIBUTED PHENOTYPE SUGGESTING MENDelian INHERITANCE IN HUMAN ESSENTIAL HYPERTENSION

Robert J. Parner, Jessica H. Covenka, Maia T. Kallias, Daniel T. O'Connor. Department of Medicine, VA Medical Center and Univ. of California, San Diego, CA.

Impaired local production of nitric oxide (NO) may contribute to elevated blood pressure in human and experimental hypertension. To further investigate the role of the NO vasodilator system in human essential hypertension and to examine abnormalities in this system as a possible "intermediate (Mendelian) phenotype," we measured blood pressure change (ΔMAPmin) in response to the NO generator, amyl nitrite (inhaled), in 64 age-matched hypertensive and normotensive subjects stratified by the presence (FH+) or absence (FH-) of a family history of hypertension (hypertension occurring before age 60 in a parent or sibling). ΔMAPmin was significantly greater in FH+ subjects (-30.1±2.2 mmHg, n=39) compared to FH- subjects (-17.8±1.8 mmHg, n=25, P<0.001). Multiple regression analysis showed that after controlling for the effects of age, baseline MAP, baseline heart rate, and body mass index, the effect of family history status on ΔMAPmin was still highly significant (P=0.001). Moreover, the distribution of ΔMAPmin in FH+ subjects was clearly bimodal, with discrete frequency peaks at 15 mmHg and 40 mmHg. In contrast, in FH- subjects, only one discrete frequency peak was demonstrated (at 10 mmHg). The exaggerated ΔMAPmin in FH+ subjects is consistent with impaired endogenous NO generation in essential hypertension. In addition, the bimodal distribution of ΔMAPmin in FH+ subjects suggests a Mendelian effect for this parameter. We conclude: 1) ΔMAPmin may be a useful "intermediate phenotype" in essential hypertension; 2) abnormalities in the endogenous NO vasodilator system may be an important hereditary component in the pathogenesis of essential hypertension.

2 Relation of Race and a Polymorphism in the Angiotensin I-Converting Enzyme Gene to Enzyme Levels
Laura J. Bloom, Anita K. Manatunga, Eleanor Boatright, J. Howard Pratt, Indiana University and the VA Medical Center, Indianapolis, IN

A polymorphism for the angiotensin I-converting enzyme (ACE) gene consisting of a 250 bp deletion (D) or insertion (I) is known to be a determinant of plasma ACE in whites. We examined here the influence of race on ACE genotype and plasma ACE.

<table>
<thead>
<tr>
<th></th>
<th>ACE (UI/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>50 81 38</td>
</tr>
<tr>
<td>(%)</td>
<td>29.6 47.9 22.5</td>
</tr>
</tbody>
</table>

Frequencies of alleles were 0.54(D) and 0.46(I) for WH, 0.61(D) and 0.39(I) for BL. A marginally significant association between race and genotype (p=0.088) was observed. In WH, ACE was related to genotype with p=0.0002-0.0001 between genotype groups. In BL, ACE levels were unrelated to genotype. In summary, other mutations within the D allele in WH but not BL result in increased ACE levels. Alternatively, other genetic or environmental factors may modify the effect of the D allele on circulating ACE in BL.

3 A Genetic Polymorphism on the X Chromosome Cosegregates With Blood Pressure in Young Male F2 Dahl/Rapp Rats
James L. Lewis, David G. Warnock. Dept. of Medicine and NRTC, University of Alabama at Birmingham, Birmingham, AL

We developed reciprocal crosses between inbred Dahl/Rapp salt-sensitive (S) and salt-resistant (R) rats and studied the second generation (F2) rats received 8% NaCl chow starting at 35 days of age. Systolic (SBP) and diastolic (DBP) blood pressures were directly measured 10 days later in awake, unrestrained animals. Genomic DNA from each rat, and the polymerase chain reaction were used to amplify microsatellites polymorphic between S and R rats. Two such markers within the intestinal calcium binding protein (CBFP1) locus on the X chromosome cosegregate strongly with DBP in male F2 rats. The strain of Y chromosomal origin, as determined by the direction of the cross, greatly influences the effect of the CBFP1 locus on DBP. No statistically significant differences in allele frequencies were noted for either mutation (X2 of 1.7 and 0.3, respectively). In conclusion, we have not detected candidate mutations of the AT1 gene among 50 hypertensives. Preliminary results from the association study using 2 marker mutations do not support a role for the AT1 gene in human hypertension.

4 A Case-Control Study on Angiotensin II-Type 1 Receptor Gene Polymorphisms in Juvenile Essential Hypertension

The angiotensin II-type 1 receptor (AT1) is a member of the seven transmembrane domain receptor superfamily and the AT1 gene is a candidate for human essential hypertension. Human AT1 is encoded by a single exon spanning 1.2 kb. The purpose of the present study was to search for candidate and marker mutations in the AT1 gene and to investigate whether variants of the AT1 gene might be involved in hypertension. The entire coding and 3' untranslated regions of the AT1 gene were amplified by PCR from genomic DNA extracted from 50 hypertensive subjects with a positive family history into 300 bp fragments, and submitted to non-denaturing electrophoresis to detect single strand conformation polymorphisms (SSCP). Five different base shifts were detected corresponding to non-substitution mutations at nucleotides 573 (T→C), 1062 (A→G), 1166 (A→C), 1517 (G→T), and 1878 (A→G), with respective allele frequencies of 0.53, 0.03, 0.28, 0.02, and 0.15. No substitution mutations were detected. An association study between 298 hypertensive subjects with positive family history and 293 normotensive controls has been performed for mutations at nucleotides 573 and 1878, using allele-specific oligonucleotide (ASO) hybridization. No statistically significant differences in allele frequencies were noted for either mutation (X2 of 1.7 and 0.3, respectively). In conclusion, the polymorphic AT1 allele is not a candidate for human essential hypertension.

5 The SHR-1N Congenic Strain Delineates a Contribution of Chromosome 20 to Blood Pressure in Young Male F2 Dahl/Rapp Rats
Michal Pravenec, Vladimir Kren, Jaroslav Kunes, Theodore W. Kurtz. Czech Academy of Sciences, Charles University, Prague, and Univ. of California, SF

In some humans with essential hypertension and in spontaneously hypertensive rats (SHR), recent linkage studies have suggested that an important blood pressure regulatory gene(s) may be located in or near the major histocompatibility complex (MHC) on human chromosome 6 and rat chromosome 20. According to this hypothesis, we developed and characterized a congenic strain that differs from the SHR strain (166±6 mm Hg) is significantly lower than that of the progenitor SHR strain. The congenic strain has >99.9% probability of being genetically identical to the SHR strain of Y chromosomal origin, as determined by the direction of the cross, greatly influences the effect of the CBPF1 locus on DBP. No statistically significant differences in allele frequencies were noted for either mutation (X2 of 1.7 and 0.3, respectively). In conclusion, we have not detected candidate mutations of the AT1 gene among 50 hypertensives. Preliminary results from the association study using 2 marker mutations do not support a role for the AT1 gene in human hypertension.

6 In conclusion, an X chromosomal locus cosegregates with blood pressure in young male F2 Dahl/Rapp rats on 8% NaCl diet, and interacts with the Y chromosome.

The SHR-1N Congenic Strain Delineates a Contribution of Chromosome 20 to Blood Pressure in Young Male F2 Dahl/Rapp Rats
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In conclusion, an X chromosomal locus cosegregates with blood pressure in young male F2 Dahl/Rapp rats on 8% NaCl diet, and interacts with the Y chromosome.
A neuropeptide Y locus on chromosome 4 cosegregates with blood pressure in the spontaneously hypertensive rat (SHR) strain, we performed a cosegregation analysis between the neuropeptide Y (NPY) locus and blood pressure in the F2 population. Our investigation revealed that the NPY locus on chromosome 4 is a new candidate for the hypertensive effect in original SHR.

### Table 1: ANOVA in blood pressure measurement

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MeanBP (mmHg)</th>
<th>ANOVA p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>132±1.5</td>
<td></td>
</tr>
<tr>
<td>SW</td>
<td>130±1.3</td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>125±1.6</td>
<td></td>
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</tbody>
</table>

P values <0.05 are indicated in bold. ANOVA: one way analysis of variance.

This positive association with blood pressure decreases in proportion to the distance from NPY locus, suggesting that the P receptor (p=0.041) and eNOS (p=0.045) loci are further downstream.

### State-of-the-Art Lecture: Mechanisms of Transcriptional Regulation in Animal Cells

**Robert Tjian**
University of California, Berkeley, CA

**cise-Acting DNA Elements Regulating Mouse Renin Gene Expression**
Thomas A. Black, J. Pablo Abonia, John R. Fabian, Colleen M. Kane, Curt D. Sigmund, Kenneth W. Gross, Roswell Park Cancer Institute, Buffalo, NY 14263.

Previous experiments in transgenic mice have demonstrated that high-level tissue-specific expression of c-mos renin requires distal upstream sequences (1-2.5 kb) relative to the primary CAP site (Biochem. Biophys. Res. Comm. 170:34c; 1990). A cDNA expressing mouse kidney tubular cell line (A4.1) has been developed by transgene targeted organochromosomes (2). The 5'-flanking region of the gene has been completely sequenced. HindIII-Clal fragments in the 5'-flanking region has been analyzed in transient transfection assays of A4.1 and Ltk- cell lines. The addition of 4Kb of 5'-flanking DNA (1-118 to +14Kb) to the 123bp fragment stimulated promoter activity >10 fold in A4.1 cells in an orientation independent manner. No stimulation was seen when 2.5Kb (118 to +2.6Kb) was added to the promoter fragment. This region includes several repetitive DNA insertions not found in other mammalian renin gene promoters. Constructs containing 5'-flanking DNA extending from -4.1Kb to -2.6Kb, -3.1Kb to -2.6Kb, and -2.9Kb to -2.6Kb all showed >10 fold stimulation in A4.1 cells. No stimulation was seen with any of the above constructs in Ltk- cells. DNA-protein interactions examined by mobility shift assays with three overlapping complex fragments identified the -2.9Kb to -2.6Kb fragment as the most efficient cis element in A4.1 normal cells. Currently we are precisely defining relevant DNA-sequence motifs involved in Ren-1 expression.

**Analysis of Human Angiotensin II Type 1 Receptor Promoter**
Deng-Fu Guo and Tadashi Inagami.
Departments of Biochemistry, School of Medicine, Vanderbilt University, Nashville, Tennessee, 37212.

Angiotensin II receptor (AT1) is well known for rapid and intrinsically regulated homologous desensitization. To study the regulatory mechanisms of the gene expression of human AT1, we have cloned genomic DNA for human AT1. It contains at least 5 exons and four introns. To delineate the human AT1 gene promoter structure and functions, a 2.6 kb HindIII-ClaI fragments in the 5'-flanking region has been completely sequenced. Primer extension has determined four transcription initiation sites. Three putative TATA boxes, two CAAT boxes, two AP-2 binding elements, a cyclic AMP induced responsive element, two c-myc binding elements and a HNF 1 binding elements sequences have been found. Expression of human renal promoter/luciferase constructs have shown that the human AT1 is functional, and one CAAT box is enhancer. BFG, IL-1 and forskolin inducible the expression level demonstrated that the the promoter does contain functionally responsive elements.

**Structure of 5'-Flanking Region of Human Endothelin Receptor Genes -- Cis-elements for Transcriptional Regulation --**
Keisuke Arai, Takamiki Yoshimasa, Kazuhiko Takaya, Yoshihiro Ogawa, Yoshihiro Miyamoto, Gota S. Shirakami, Hiroshi Ishibashi, Kazuwa Nakao, 2nd Div., Dept. of Med., Kyoto Univ. Sch. of Med., Kyoto, Japan

We have reported the cDNA cloning and the gene organization of human endothelin-A receptor (ET-AR) and endothelin-B receptor (ET-BR). To elucidate the transcriptional regulation of genes encoding ET-AR and ET-RR, we have sequenced the 5'-flanking regions for approximately 1.2 kilobases upstream of the initiation codon. Both of the genes lack TATA box but contain a sequence of potential 5'-binding site in close proximity to the transcription initiation site, raising the possibility that the 5'-binding site functions as a promoter. There are many consensus sequences for cis-elements. The ET-AR gene contains GATA-motif which has been reported to be essential for cell type-specific expression of preproET-1 gene. CAT box, acute phase regulator element (APRE) and E box, while the ET-RR gene possesses GATA-motif, APRE and E box. These results will lead to further investigations including the functional assay with fusion plasmids to identify cis-elements that act as direct efficient and tissue specific expression and to evaluate the mechanisms underlying transcriptional regulation of the genes encoding ET-AR and ET-RR.
The percentage of the original baseline pressure was AS, 75% transcription. Twelve week old male SHR’s were cannulated intracerebroventicularly (ICV). After recovery, baseline blood pressure was measured by tail angiotensinogen mRNA. The AS-ODN binds to the mRNA inhibiting was greater from dose 1 to dose 3 (p<0.05) 4.36% Angiotensin production, and thereby decreased hypertension in the SHR. AS-ODN shown to be increased in localized brain regions of spontaneously hypertensive in biological systems led us to hypothesize that a specific antisense peptide on proximal tubular ion transport.

Nickolai Dulin, Paul Ernsberger, Zuhayr T. Madhun, Janice G. Tohoku University School of Medicine, Sendai, Japan.

Specific components of the brain renin angiotensin system (RAS), have been shown to be increased in localized brain regions of spontaneously hypertensive rats (SHR). The demonstration of angiotensin mediated gene expression inhibition in biological systems was to hypothesize that a specific antisense oligoglycosyl nucleotide (AS-ODN) to angiotensinogen mRNA would inhibit angiotensin production, and thereby decreased hypertension in the SHR. AS-ODN was made to 18 bases of the 5’ end of the known initiation sequence of angiotensinogen mRNA. The AS-ODN binds to the mRNA inhibiting transcription. Twelve week old male SHR’s were cannulated intracerebroventricularly (ICV). After recovery, baseline blood pressure was measured by tail cuff monitor. Animals were divided into four groups: Antisense (AS) ODN, and control groups: Sense (S) and Sense (SC) ODN and vehicle (C). Each group was injected ICV with (10µg/kg) of ODN or Saline isotonic saline. Three doses were administered at 12 hour intervals. Two hours after the final and doses blood pressure was measured. The AS-ODN treated group was shown to have significantly decreased blood pressure compared to the control groups (P<0.05). The percentage of the original baseline pressure was AS, 90.75% ± 2.31 < 97.49% ± 3.52, 91.97, and 90.55% ± 3.48. The effect of AS-ODN was greater from dose 1 to dose 3 (p<0.05) 4.36% decrease in blood pressure compared to 19.25% ± 2.82 after dose 3. This preliminary data suggests that i.c.v. administration of specific AS-ODN has a cumulative effect on the renal system.

Antisense Inhibition of Central Angiotensin in SHR Reduces Hypertension.

Donna Wielbo, Robert Gyurko and M. Ian Phillips. Dept. of Physiology, College of Medicine, University of Florida, Gainesville, FL, USA.

Specific components of the brain renin angiotensin system (RAS), have been shown to be increased in localized brain regions of spontaneously hypertensive rats (SHR). The demonstration of angiotensin mediated gene expression inhibition in biological systems was to hypothesize that a specific antisense oligoglycosyl nucleotide (AS-ODN) to angiotensinogen mRNA would inhibit angiotensin production, and thereby decreased hypertension in the SHR. AS-ODN was made to 18 bases of the 5’ end of the known initiation sequence of angiotensinogen mRNA. The AS-ODN binds to the mRNA inhibiting transcription. Twelve week old male SHR’s were cannulated intracerebroventricularly (ICV). After recovery, baseline blood pressure was measured by tail cuff monitor. Animals were divided into four groups: Antisense (AS) ODN, and control groups: Sense (S) and Sense (SC) ODN and vehicle (C). Each group was injected ICV with (10µg/kg) of ODN or Saline isotonic saline. Three doses were administered at 12 hour intervals. Two hours after the final and doses blood pressure was measured. The AS-ODN treated group was shown to have significantly decreased blood pressure compared to the control groups (P<0.05). The percentage of the original baseline pressure was AS, 90.75% ± 2.31 < 97.49% ± 3.52, 91.97, and 90.55% ± 3.48. The effect of AS-ODN was greater from dose 1 to dose 3 (p<0.05) 4.36% decrease in blood pressure compared to 19.25% ± 2.82 after dose 3. This preliminary data suggests that i.c.v. administration of specific AS-ODN has a cumulative effect on the renal system.

Identification of des-Asp'Arg'AII Angiotensin II Receptors and Signalling in Opossum Kidney Cells.

Mendonc Dulin, Paul Ernsberger, Subhay T. Madhun, Janice G. Douglas. Case Western Reserve University School of Medicine. Cleveland, OH.

An opossum kidney cell line (OK7A) that expresses a number of proximal tubular properties was employed to evaluate binding of angiotensin II AT1 and AT2 fragments [3H]-BAlol(Ala9) (3-8)AI1(1-7)Alv and (1-7)AII. Among peptides studied only AT1 and AT2 bound to OK7A cells. The binding of Alv was bidirectional, Ka of 3.3±1.51 nM and 2.82±3.5 nM and Bmax of 75.25±2.0 fmol/mg and 17.95±2.80 fmol/mg, respectively. The Ka of BAlol was greater from dose 1 to dose 3 (p<0.05) 4.36% decrease in blood pressure compared to 19.25% ± 2.82 after dose 3. This preliminary data suggests that i.c.v. administration of specific AS-ODN has a cumulative effect on the renin system.

Thus, ACE-inhibition but not AT,-receptor blockade upregulates AT-receptor function in the vascular smooth muscle. Protection of endothelial function by both interventions suggest a role for AT II in CsA-induced endothelial dysfunction.

Modulation of Endothelial Function and Angiotensin-Receptors During Chronic ACE-Inhibition, Angiotensin,-Receptor Blockade, and Cyclosporin A Treatment.

Wolfgang Auch-Schwelk, Ellen Duske, Ulrich Hink, Mathias Betz, Manfred Unkelbach, Eckart Fieck. Dept. of Cardiology, German Heart Institute Berlin, Germany.

The effect of chronic ACE-inhibition (with lisinopril 10 mg/kg/d) and selective angiotensin (AT)-receptor blockade (with D 7971 or DuP 753 10 mg/kg/d) on endothelium-dependent vasodilation and angiotensin (AT) II-induced vasoconstriction was determined in the model of cyclopentia A (CSA 15 mg/kg/d) treated rats 16 weeks, 15 rats/group. Chronic treatment with CSA impairs endothelial function and selectively increases AT 1-receptor expression, whereas AT 2-receptor expression is unaltered. Chronic treatment with CSA decreased maximal responses to phenylephrine or angiotensin II (AT1 and AT2 agonists) in a concentration-dependent manner. No differences were found between CSA and saline treated groups. ACE-inhibition with lisinopril 10 mg/kg/d improved maximal responses to phenylephrine or angiotensin II (AT1 and AT2 agonists) in a concentration-dependent manner. No differences were found between CSA and saline treated groups. ACE-inhibition with lisinopril 10 mg/kg/d improved maximal responses to phenylephrine or angiotensin II (AT1 and AT2 agonists) in a concentration-dependent manner.

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