phenotypes, those mapping in males and/or females, was also assessed. This analysis enabled the comparison of the differences in functional relationships dependent upon genetic variations observed between the male and female F2 rat population. These patterns of correlations (physiological profiles), have provided new insights into the understanding of the gender dependent gene expression and functional relationships. We hope to develop novel hypotheses for mechanism-based physiological studies.

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Mechanisms of Dahl Salt-Sensitive Hypertension Revealed by Temporal Patterns of Renal Medullary Gene Expression

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The introgression of chromosome 13 from salt-resistant BN/Mcw rats into the genome of the Dahl salt-sensitive SS/Mcw rats (consomic SSBN13) substantially reduced blood pressure (BP) salt-sensitivity and renal injury (Hypertension 2001, 37: 456–61). SS.BN13 rats have only 1.95% allelic differences from SS/Mcw (compared to at least 30% in other salt-resistant stains) and thus provide an excellent control for SS/Mcw rats. In the present study, cDNA microarrays (containing ~2,000 currently known rat genes) were used to compare time-related changes of mRNA expression in the renal medulla of SS/Mcw and SS.BN13 rats in response to a high-salt diet. Analysis of 6 replicate arrays with 3 rats at each time point (16 hr, 3 d, or 2 wk) was carried out after the start of 4% salt diet. Differentially expressed genes were identified using a novel analysis based on a threshold determined experimentally using a reference distribution of ratios of all genes between 6 pairs of rats from the same group. This analysis limited the inclusion of no more than one false positive gene in each comparison. Genes exhibiting the most distinct temporal patterns of expression over the entire time course were identified. Analysis of 54 microarrays identified 51 such genes, 30 of which could be assembled into meaningful functional networks. The expression patterns of these genes were consistent with observed levels of lower BP and reduced extracellular matrix formation, and predicted decreased apoptotic activity in SS.BN13 rats. The results generated a series of testable hypotheses and revealed novel insights into the complex mechanistic pathways responsible for salt-sensitive hypertension and renal medullary tissue injury.

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Resistance to Salt-Induced Hypertension in Catechol-O-Methyltransferase (COMT)-Deficient Mice

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Dopamine plays an important role in the regulation of blood pressure and renal sodium excretion. Previous studies suggest that catechol-O-methyltransferase (COMT) may modulate renal dopaminergic tone. Using COMT(-/-)-deficient mice we tested the hypothesis whether COMT blockade could protect against salt-induced hypertension. Blood pressure and heart rate were recorded by radiotelemetry with implants (TA11PA-C20) inserted into the left carotid artery. During normal sodium diet (NaCl 0.8 % w/w) the daytime and nighttime systolic blood pressures in COMT(-/-) mice (121±3 and 130±3 mmHg, respectively) were comparable to those of wild-type COMT(+/+) mice (daytime and nighttime systolic blood pressures 120±3 and 130±4 mmHg, respectively). High sodium intake for 3 weeks (NaCl 6 % w/w) increased nighttime systolic blood pressure in COMT(+/+) mice by 7 mmHg (137±3 mmHg, $p<0.05$ compared to baseline values), whereas no significant change was found in daytime blood pressure (123±3 mmHg). In contrast, high sodium intake did not increase nighttime systolic blood pressure in COMT(-/-) mice (129±4 mmHg, $p<0.05$ compared to control mice on high sodium diet). There was no difference in heart rate between the mice strains during normal sodium diet. High sodium intake decreased daytime heart rate in both mice strains, but nighttime heart rate only in COMT(-/-) mice ($p<0.05$ compared to control mice). To further evaluate the role of COMT inhibition on blood pressure, wild-type COMT(+/+) mice on high sodium diet were treated with COMT inhibitor nitecapone (30 mg/kg i.p.) for 8 days. Nitecapone completely normalized the salt-induced changes in nighttime systolic blood pressure in control mice (139±0.5 before nitecapone treatment vs. 132±1.0 mmHg after 8 days nitecapone treatment, $p<0.05$). Our findings suggest that COMT deficiency in mice is associated with resistance to salt-induced hypertension.

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Genetic Variants That Increase AT_1 Receptor Expression Preferentially Raise Blood Pressure in Females

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Incremental *reductions* in AT_{1A} receptor gene expression implemented through gene targeting cause a proportional lowering of resting blood pressure (BP). For example, in heterozygous *Agtr1a*+/- mice, a 50% reduction in AT_{1A} receptor expression lowers systolic BP (SBP) by 12 mm Hg and the complete absence of AT_{1A} receptors in *Agtr1a*-/- mice is associated with a further lowering of BP. To test whether genetic variants that *increase* AT_{1A} receptor expression would also affect BP, we used gene targeting to generate mouse lines with tandem duplication of the *Agtr1a* gene locus along with more than 10 kb of 5' flanking DNA. By successive breeding, we generated mice with 3 and 4 copies of the *Agtr1a* gene locus on an inbred 129/Sv background. These animals survived in expected numbers, and their body, heart, and kidney weights were similar to wild-type, 2-copy controls. AT_{1A} mRNA expression and AT_1-specific binding of ^{125}I-angiotensin II was increased in proportion to *Agtr1a* gene copy number. Because of known sex differences in regulation of components of the renin-angiotensin system (RAS) and in the epidemiology of hypertension, we performed comparisons of BP and AT1A expression in the entire cohort and separately in male and female mice. In the mixed group, there was a general association between the level of SBP and gene copy number (102±2 vs 104±2 vs 106±2 mm Hg for 2-, 3-, and 4-copy mice, respectively), but these differences were not statistically significant. Similarly, in male mice, there was no correlation between BP and *Agtr1a* gene copy number or AT_{1A} mRNA levels. In contrast, in female mice, there was a highly significant positive correlation between BP and AT_{1A} receptor expression ($p=0.003$). Furthermore, in female but not male mice, there was a significant positive correlation between kallikrein and AT_{1A} receptor mRNA levels, ($p=0.001$), and a significant inverse correlation between renin mRNA and *Agtr1a* copy number ($p=0.009$). Thus, in female but not male mice, increased expression of AT_1 receptors levels affect BP and gene expression programs. These data suggest that gender has strong influences on the physiological impact of genetic variation in the RAS.

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A Targeted Renin Transgene Causes Reversible Hypertension and Kidney Damage in Mice

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Many transgenic animal models have been generated to study the role of the renin-angiotensin system in the pathogenesis of hypertension. These models, developed either by conventional pronuclear microinjection techniques or by gene targeting in embryonic stem cells, have allowed a systematic dissection of the genetic components of the renin-angiotensin system and their roles in the maintenance of basal blood pressure. However, no mouse model of constitutive mouse renin overexpression has been generated in which the renin expression is free from compensatory homeostatic regulation. To provide such a model we have used single-copy chosen-site gene targeting to insert into a liver-specific locus a single copy of a modified mouse renin transgene driven by a liver-specific promoter/enhancer. The resulting RenTg targeted mice express renin at a constant high level in the liver and have elevated plasma levels of prorenin and active renin. Their endogenous production of renin in the kidneys is almost completely suppressed. They develop severe hypertension, increased urine output, proteinuria and kidney damage. Treatment with the angiotensin II type I receptor antagonist, losartan, reversed the hypertension, albuminuria and kidney damage. This RenTg mouse is the first monogenic, intra-species renin model in which the development and treatment of severe hypertension and its complications can be investigated in a high prorenin/renin environment that is not subject to homeostatic compensations.

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Coronary Flow Rate Is Decreased in the α-Calcitonin Gene-Related Peptide Knockout Mouse

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Calcitonin gene-related peptide (CGRP), a potent vasodilator neuropeptide, plays a counter-regulatory role in several models of experimental hypertension. We have reported that α-CGRP null mice display a significant increase in basal mean BP compared to wild type (WT) controls. Additional evidence suggests a critical role for CGRP in the regulation of coronary blood flow. To test this hypothesis, Langendorff-perfused heart preparations were used to compare coronary flow rates between α-CGRP gene knockout (KO) and WT control mice under various pressure loading conditions. Hearts were removed from 33 mice (9 female control, 6 female α-CGRP KO, 10 male control and 8 male α-CGRP KO), and immediately placed in an oxygenated physiologic buffered solution (PBS). Then the aorta was cannulated and the isolated hearts were perfused with PBS at 37°C. Coronary flow rates were measured at multiple perfusion pressures. As shown in the table, deletion of the α-CGRP gene resulted in a significant reduction in the coronary flow at all pressures ($p<0.015$). In addition, coronary flow for both WT and KO mice was consistently lower in female than in male mice. Therefore, these data suggest that α-CGRP is responsible for up to 30% of coronary blood flow. Also, this effect seems to be gender related with less coronary flow noted in the female mice.

Coronary Flow Rate (ml/min/g weight)

Pressure (mmHg)	Control Female	α-CGRP-KO Female	Control Male	α-CGRP-KO Male
50	11.81±0.94	8.62±0.76	13.06±1.54	10.37±1.46
40	9.38±0.86	6.66±0.64	10.30±1.28	8.08±1.1
30	6.32±0.62	4.51±0.59	7.14±1.16	5.41±0.95
20	3.32±0.43	2.39±0.44	3.89±0.67	2.93±0.58

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Birth Weight Impacts on Wave Reflections in Children and Adolescents

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The objective was to study the impact of birth weight (BW) on wave reflections in children and adolescents. Two hundred and nineteen subjects (mean age 11 yr; 126 girls), with a BW ranging from 2 to 5.2 kg were included. The extent of wave reflection was estimated by the augmentation index (AI) defined in two ways: as the amount of and as the ratio of the amplitude of pressure wave above its systolic shoulder to the PP (tonometry, ORourke method). The figure shows that children in the lowest BW group (<3kg, n=67) had higher AI values than those in the other groups (BW 3–3.5 kg, n=61 and BW>3.5 kg, n= 91). These differences remain after controlling for sex, height, heart rate and diastolic blood pressure. A significant inverse correlation was present between BW and AI ($r=-017$, $p=0.01$). In a multiple regression