CLOSING IN ON A MOLECULAR DESCRIPTION OF THE SHR

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Remarkable advances in genomics have occurred in the past 5 years including the advent of whole genome association studies and the ability to re-sequence entire mammalian genomes at a tiny fraction of the cost of the original sequences. The spontaneously hypertensive rat has a distinguished track record as a model of hypertension and metabolic syndrome. Scores of quantitative trait loci (QTLs) have been mapped to distinguish track record as a model of hypertension and metabolic syndrome. Scores of quantitative trait loci (QTLs) have been mapped to discriminate regions of the genome over the past two decades and a small number of the underlying molecular defects (quantitative trait nucleotides or QTNs) have been identified. With advances in genome technology and integrative approaches that use expression profiling and informatics, the genome sequence of the SHR is now in our hands, and the opportunity to identify QTNs for large numbers of QTLs presents itself. This talk will address the present state of the art in SHR genomics, and will look to the possibilities for discovery of SHR disease genes and pathways, and their potential translation to human biology, over the next 5 years.

PRODUCTION OF GAMMA-AMINOBUTYRIC ACID (GABA)-ENRICHED RICE GRAINS AND THEIR HYPOTENSIVE EFFECT BOTH IN SHR/Nrler/l AND SHR/Ndmc-r-cp/cp

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Recently, lifestyle-related diseases such as obesity, high blood pressure and hyperlipemia have getting severe problems rest of the world regarding not only human health but also increase in medical expenses. GABA, one of the ubiquitous non-protein amino acids and known to be an inhibitory neurotransmitter, has been focusing due to its effect on blood pressure decrease. GABA itself is synthesized from L-glutamate by glutamate decarboxylase in the cells. Here we report an effect of GABA-enriched rice grains in SHRs. Based upon both molecular approaches of protein engineering and over-expression of a rice GAD gene in the seeds, we have successfully established transgenic rice lines that produce GABA-enriched rice grains: amino acid analysis indicated three- to thirty-fold GABA content increase, compared with those of wild-type. Interestingly, most of the free protein amino acids were also increased in the seeds. GABA-enriched rice was administered to ten weeks old of SHR/Nrler/ or eight weeks old of SHR/Ndmc-r-cp/cp everyday for six weeks (GABA dose of 0.5 mg/kg body weight) and systolic blood pressure was measured. At three, four, and five weeks after administration to two different kinds of SHRs, blood pressure lowering effect was each observed with a statistically significant level compared with the control group. These results suggest that GABA-enriched rice grains developed in this study have a hypotensive effect in SHRs through a long-range action. Transgenic rice grains enriched with free amino acids as well as GABA have a blood pressure lowering effect reproducibly on SHRs.

FUNCTIONAL ANALYSIS OF CARDIOVASCULAR GENES BY TARGETED GENE KNOCKDOWN IN RATS

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The rat is the preferred animal model in several areas of research including the study of cardiovascular diseases. However, gene targeting technology is not established for this species due to the lack of germline competent embryonic stem cells. Therefore, our goal was to evaluate different methods to attenuate or ablate expression of a specific gene in transgenic rats. First, we successfully generated a transgenic rat expressing antisense RNA against angiotensinogen exclusively in the brain. These animals showed a 90% reduction in angiotensinogen concentration in the brain with no change in other organs. As a consequence, the rats developed reduced blood pressure, a mild diabetes insipidus and alterations in autonomic control confirming the important function of angiotensin in central cardiovascular control. As a second model we generated transgenic rats expressing a dominant negative mutant of the NPR-B receptor for C-type natriuretic peptide. These rats spontaneously developed cardiac hypertrophy providing evidence for a central role of this hormone in cardiac growth control. Recently, we used shRNA technology to knockdown genes in rats. We employed a novel tetracycline inducible shRNA expression system targeting the insulin receptor (InsR) and successfully generated shRNA transgenic rats. Doxycycline (DOX) treatment of these animals led to a dose-dependent increase in blood glucose caused by a nearly total inhibition of InsR expression, which was reversible. We could neither detect an interferon response nor any disturbances in microRNA processing in the transgenic rats under DOX treatment indicating that there are no toxic side effects of the shRNA-based gene suppression. Thus, these rats represent a new model for diabetes mellitus type 2. In conclusion, we have developed novel technologies allowing the specific suppression of genes in the rat.

BRAIN DAMAGE IN AN ANIMAL MODEL OF HYPERALDOSTERONISM

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Aldosterone exerts a number of effects in the central nervous system. Facilitated by its lipophilic properties, aldosterone can cross the blood-brain barrier, and causes acute and long-term effects on brain vascular structures. Aldosterone has been implicated in the pathogenesis of vascular disease and elevated plasma aldosterone is considered as a risk factor for stroke, although mechanism(s) underlying this role remain(s) to be elucidated. The present study was designed to assess possible neuronal, neuroglial and blood-brain-barrier changes induced by aldosterone in an animal model of hyperaldosteronism. Male Wistar rats of 8 weeks of age were treated for 28 days with aldosterone (40 μg/kg/day) via osmotic minipumps with or without 0.9 % NaCl. In control animals, minipumps contained vehicle alone and no salt was added to drinking water. Microanatomical aspects of intracerebral and pial artery, nerve cell number, phosphorylated 200-kDa neurofilament immunoreactivity were assessed by microanatomical, and immunohistochemical techniques. Rats treated with aldosterone alone or plus salt load developed arterial hypertension and displayed microanatomical changes of the vascular tree, nerve cell loss and cytoskeletal breakdown. Neuronal changes occurred primarily in the frontal cortex, striatum and CA1 subfield of hippocampus. These data suggest that aldosterone causes brain injury that can be related with blood pressure rise or with neurotoxicity linked to specific mineralocorticoid receptor activity. Supported by the grant MIUR-COFIN No. 2006060985_003.

PANEL DISCUSSION: A TRANSGENIC VIEW ON PHYSIOLOGY

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When physiologists ask me whether their discipline has a future in the times of -omics, I tell them that it will become even more important. Transgenic researchers such as myself can generate animals with all kinds of alterations in single genes. In a lot of cases the animals were these handicaps. And then tedious work is necessary to detect the consequences of the genetic alteration, if any. To this purpose, classical
physiology is essential. In particular, system physiology is of greatest importance. Most researchers have a limited focus on a single cell type or organ, and analyze their transgenic animal models only with a few established technologies focussing on this tissue. I am sure, hundreds of transgenic lines still bear a secret which would enhance our knowledge about the physiological function of the affected gene. It was not yet uncovered due to an incomplete phenotyping of the animals. Therefore, there are more and more public and commercial labs offering a broad check up of transgenic and knockout mouse lines. And in these labs classical physiology revives. In my lab, we generate animals with alterations in hormone coding genes. If we would not analyze the animals with a multitude of collaboration partners disposing of very divergent expertise in physiology, we would miss the most important phenotypes. For example our mice lacking the enzyme tryptophan hydroxylase 1, which is responsible for serotonin synthesis in the periphery, would have never been comprehensively phenotyped without such partners. We found out that the mice have a defect in hemostasis but our partners discovered functions in the physiology of the mammary gland, the liver, and the pulmonary vasculature. And for sure there are still phenotypes to be discovered in these animals. The most promising and also most important in the respect of this meeting is that the mice are slightly hypertensive. Thus surprisingly, peripheral serotonin exerts a tonic antihypertensive effect. By employing classical systems physiology of cardiovascular regulation we try to clarify the implicated mechanisms in the moment. In conclusion, classical physiology will not die out. In the opposite, it becomes an increasingly indispensable tool in the analysis of genetically altered animal models.

CHRONIC CROWDING STRESS PRODUCES NITRIC OXIDE DEFICIENCY AND ENDOTHELIAL DYSFUNCTION IN SHR

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This study investigated cardiovascular stress response in normotensive Wistar and SHR rats induced by chronic crowding. Twelve-week-old males were divided into the control (four rats/cage, 480 square cm/rat) or crowding-stress-exposed (five rats/cage, 200 square cm/rat) group for eight weeks. Blood pressure (determined by tail-cuff plethysmography) of Wistar and SHR rats at the end of experiment was 111±2 and 184±3 mm Hg, respectively. Control nitric oxide (NO) synthase activities (determined by [3H]-L-arginine conversion) in the aorta and left ventricle were significantly higher in SHR vs. Wistar. Control acetylcholine-induced vasorelaxation of the femoral artery (measured by Mulvany’s myograph in isometric conditions) was significantly elevated in SHR vs. Wistar. Crowding increased significantly blood pressure (193±3 mm Hg) and reduced aortic and left ventricular NO synthase activity only in SHR rats by approximately 30% and 24% vs. control. Acetylcholine-induced vasorelaxation of the femoral artery of stress-exposed rats was improved in Wistar but reduced in SHR rats. Additionally, crowding significantly elevated noradrenaline-induced vasoconstriction in both phenotypes while serotonin-induced vasoconstriction was reduced in crowded Wistar and unchanged in SHR rats. Thus, the results showed the absence of NO deficiency and endothelial dysfunction in uncrowded SHR, while both reduced NO production and endothelial dysfunction occurred in crowded SHR. Chronic social stress might significantly modulate vascular function in rats depending on their phenotype. Hypertensive rats developed endothelial dysfunction, NO insufficiency and greater level of blood pressure in stressful conditions. Supported by grants APVT-51-0180004 and VEGA 2/7064/27.

TWO COMMON POLYMORPHISMS WITHIN THE ADIPOGENIC GENE ARE ASSOCIATED WITH FOOD PREFERENCES IN EXTREMELY OBESE CZECH INDIVIDS

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Considering the pivotal role of adiponectin in white adipose tissue metabolism and signaling, we assessed whether the 45T/G and 94T/G polymorphisms within the adiponectin gene influence the food preferences along with basic anthropometrical characteristics in the Czech extremely obese population. The total of 44 extremely obese subjects was enrolled in the study (BMI 46.03±5.63; % of body fat 50.33±5.13). Basic anthropometrical characteristics associated with obesity were measured and the food intake was monitored using 7-day record method. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to establish the distribution of allele and genotype frequencies of the 45T/G (exon 2) and 94T/G (exon 2) polymorphisms. ELISA was used to determine plasma levels of leptin and leptin receptor. Both polymorphisms studied were significantly associated with various body size measurements including weight, waist and hip circumference, percentage of body fat and body mass index. Furthermore, the 45T/G was linked to the plasma levels of leptin receptor (β = −0.36, p = 0.002). A strong effect of 94T/G on the food preferences of the morbidly obese individuals was observed, the 94T/G GG genotypes being significantly associated with the increased protein and carbohydrates intake (β = −3.2, p = 0.02, β = −5.5, p = 0.002, respectively). The obtained data well fit into a framework for food intake regulation and could provide possible targets for further nutrigenomical research and intervention focused on the food preferences.

ROLE OF CYP3A5*1 FOR SALT-SENSITIVE BLOOD PRESSURE REGULATION IN YOUNG MALE NORMOTENSIVE CAUSIAN INDIVIDUALS

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CYP3A5*1-allele status has been associated with salt-sensitive hypertension particularly in black patients while its contribution to salt-sensitive blood pressure regulation in Caucasian individuals is not established. We therefore determined the association of the CYP3A5 genotype with blood pressure and salt-sensitivity in a group of young normotensive male Caucasians. Following a standardized low-salt (20 mmol NaCl/d) or high-salt (240 mmol NaCl/day) diet intake for 7 days each resting blood pressure was measured in 310 young white normotensive Caucasians using an oscilometric blood pressure device. Salt-sensitivity was defined as a decrease in mean arterial blood pressure (MAP) of 3 mmHg after switching from high-salt to low-salt diet. Genotyping was performed by real-time PCR. Cortisol and 6β-OH-cortisol excretion in 24 h-urine was determined by HPLC in a subset of individuals (n=136) under low-salt diet. Patient characteristics between CYP3A5*1-carriers (n=44) and non-carriers (CYP3A5*3/n=266) were similar. Salt-sensitivity was more common in the CYP3A5*1*1 carriers (22% vs. 14% CYP3A5*1-carriers) but this was not statistically significant. CYP3A5 genotype was not related to blood pressure.
plasma renin activity, and aldosterone levels under either diet but heart rate was lower in CYP3A5*/1-carriers under low-salt diet (55.1±13.3 vs. 57.8±6.5, p=0.05). A significant effect of CYP3A5*/1-allele status on urinary 6β-OH-cortisol was observed (CYP3A5*/1-carriers: 154.7±15.4 μg/24h vs. CYP3A5*/3/3: 102.7±5.7 μg/24h, p<0.01). Although CYP3A5*/1-carriers exhibit significantly higher urinary 6β-OH-cortisol excretion rates no significant effect of CYP3A5* genotype on salt-sensitive blood pressure regulation was detected in young normotensive male Caucasians under standardized salt intake.

CHRONIC ADMINISTRATION OF TWO TYPES OF NO-SYNTHESE INHIBITORS: DIFFERENT EFFECTS ON VASCULAR FUNCTION AND STRUCTURE OF RAT THORACIC AORTA

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We compared the effects of chronic treatment with 7-nitroindazole (neuronal NO-synthase inhibitor) and N^2-nitro-L-arginine methylster (L-NAME, general NO-synthase inhibitor) on blood pressure (BP) and on the function and structure of thoracic aorta (TA). Male 10 weeks old Wistar rats were divided into groups: controls (n=10), L-NAME (50 mg/kg BW/day) and 7-nitroindazole (10 mg/kg BW/day), both in drinking water for 6 weeks. BP was measured noninvasively. Vasoactive responses on isolated TA were recorded and geometry of TA was measured. After 6-weeks of treatment BP was increased in L-NAME group, however, not in 7-nitroindazole group. Relaxation of TA induced by acetylcholine (10^-7-10^-6 M) was inhibited in L-NAME group compared to controls. The treatment with 7-nitroindazole decreased acetylcholine-induced relaxation but this depression was significantly smaller than in L-NAME group. The contraction of TA to noradrenaline (10^-6-10^-5 M) was augmented in L-NAME group compared to controls. On the other hand the treatment with 7-nitroindazole inhibited noradrenaline-induced contraction. Wall thickness and wall cross-sectional area of TA were increased in L-NAME group whereas in 7-nitroindazole group the decrease in both parameters from two constitutive forms of NO-synthase could participate in regulation of cardiovascular tone by distinct mechanisms.

WHOLE GENOME SURVEY OF COPY NUMBER VARIATION BETWEEN THE SPONTANEOUSLY HYPERTENSIVE RAT AND THE WISTAR-KYOTO RAT

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The spontaneously hypertensive rat (SHR) is one of the most widely used genetic models of hypertension. The association of Copy number variation (CNV) is increasingly recognized as a source of inter-individual differences in disease but has not been previously investigated in the SHR. We performed experiments to determine whether CNVs exist between the SHR and the normotensive Wistar-Kyoto (WKY), which may possibly play a role in the disease progression in the SHR. We performed a 2X2 comparative genomic hybridization (CGH) using a whole-genome array with a 5303bp median probe to detect alterations in DNA copy number between the SHR model and the non-hypertensive WKY model from which the SHR was genetically derived. A criteria of 5 or more probes in a segment, mean amplitude of log2 shift across segment >±0.5 were used to define the final set of high confidence CNV calls. To validate CNVs detected by CGH, qPCR assays were used to measure copy number in altered regions relative to a control region of invariant copy number between the two strains. CNVs were detected on various rat autosomes and varied in size from 27 kb to 190 Kb. Interestingly, most of these variations were located in areas where previous QTLs for cardiovascular risk factors reside. CNVs were detected on autosomes 1, 3, 4, 6, 7, 10, 14 and 17. CNVs detected in this study seem to be located near or within blood pressure QTLs, more often than would be expected by chance, which supports the hypothesis that CNVs are causally linked. Importantly, many of the CNVs contain known genes and thus may underlie both gene expression and phenotypic variation between the rat models. Further studies and finer tiling arrays are warranted.

FLAX OIL FEEDING REDUCES OXIDATIVE STRESS AND LIVER LIPIDS IN ADULT SHR/NDmer-cp RATS

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Flax oil feeding has been associated with a beneficial effect on the outcome of metabolic syndrome. However, the underlying mechanisms remain unclear. To understand and explore these mechanisms, we compared the effects of flax oil vs. lard feeding in a genetic model of metabolic syndrome. 8-week old male SHR/NDmer-cp rats were fed a high fat diet enriched with flax oil, lard and rodent chow for a period of 4 weeks. Both homozygous fatty as well as lean animals were compared on each diet leading to six dietary groups, which were designated as FF: flax fatty, FL: flax lean, LF: lard-fatty, LL: lard-lean, CF: chow fatty and CL: chow lean. Blood and tissues were collected at the age of 12 weeks after overnight (0N) fasting. Fatty groups (FF, LF and CF) were significantly heavier than their lean counterparts (FL, LL and CL), however, no effects on body weights were observed for different diets. Similarly, serum lipids, liver lipids and TBARS content were increased in the fatty groups when compared with their lean counterparts. Paradoxically, serum TG was increased in case of FF group as compared to LF and CF. However, liver TG, total-cholesterol and 24-hr urine TBARS were significantly reduced in FF when compared with both LF and CF. m RNA levels of PPAR-alpha and PPAR-gamma were reduced in FL when compared with LL and CL, however, no significant differences were observed between the fatty groups. Our data indicate that although flax feeding increased serum TG, it lowered liver lipids and urine TBARS content in adult fatty SHR/NDmer-cp rats, thus suggesting a beneficial effect. Mechanistically, flax oil altered the oxidative stress status and the liver lipid metabolism in these adult SHR/NDmer-cp rats.

PERIVASCULAR DELIVERY OF DOMINANT NEGATIVE N19RHOA INHIBITS NEOINTIMAL HYPERPLASIA IN BALLOON-INJURED RAT CAROTID ARTERIES

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Inhibition of RhoA-Rho kinase pathway has potential to prevent vascular adventitial fibroblasts to myofibroblasts in vitro at our previous report. However its efficiency has not been demonstrated in animal models. We therefore tested whether perivascular delivery of dominant negative N19RhoA (Ad-N19RhoA) prevents luminal narrowing after balloon injury by inhibiting intimal hyperplasia. 12- to 14-week-old male adult SHR/NDmcr-cp rats were fed a high fat diet enriched with flax oil, lard and rodent chow for a period of 4 weeks. Both homozygous fatty as well as lean animals were compared on each diet leading to six dietary groups, which were designated as FF: flax fatty, FL: flax lean, LF: lard-fatty, LL: lard-lean, CF: chow fatty and CL: chow lean. Blood and tissues were collected at the age of 12 weeks after overnight (0N) fasting. Fatty groups (FF, LF and CF) were significantly heavier than their lean counterparts (FL, LL and CL), however, no effects on body weights were observed for different diets. Similarly, serum lipids, liver lipids and TBARS content were increased in the fatty groups when compared with their lean counterparts. Paradoxically, serum TG was increased in case of FF group as compared to LF and CF. However, liver TG, total-cholesterol and 24-hr urine TBARS were significantly reduced in FF when compared with both LF and CF. m RNA levels of PPAR-alpha and PPAR-gamma were reduced in FL when compared with LL and CL, however, no significant differences were observed between the fatty groups. Our data indicate that although flax feeding increased serum TG, it lowered liver lipids and urine TBARS content in adult fatty SHR/NDmer-cp rats, thus suggesting a beneficial effect. Mechanistically, flax oil altered the oxidative stress status and the liver lipid metabolism in these adult SHR/NDmer-cp rats.
Sprague-Dawley rat artery was subjected to balloon angioplasty. Immediately after the injury procedures, 200 μl pluronic gel containing Ad-N19RhoA-hrGFP (2×10^9 pfu/ml) or Ad-hrGFP (inactive controls) was applied to the adventitial surface of the artery. The neointimal formation was quantified after 7 and 14 days with morphometry and histology, immunohistochemistry and morphometric analysis were also performed. Injured nontreated arteries exhibited a pronounced intimal hyperplasia (0.10±0.06 mm² at 7 days and 0.23±0.04 mm² at 14 days) and a marked reduction in luminal area (19% at 7 days and 44% at 14 days) with a strong α-SM-actin expression in neointima (81.4±6.6 % at 14 days). Perivascular delivery of Ad-N19RhoA potently decreased neointimal formation (0.06±0.04 mm² at 7 days and 0.14±0.07 mm² at 14 days) and luminal narrowing (13% at 7 days and 24% at 14 days) after balloon injury whereas administration of Ad-hrGFP had no effect. As well, a potentially reduced neointimal α-SM-actin expression (58.5±14.3%) at 14 days was observed after balloon injury in N19RhoA treated arteries compared with levels in non-treated arteries. These results suggested that perivascular release of N19RhoA inhibits intimal hyperplasia after balloon injury, which associated with RhoA signaling involved in the differentiation of adventitial fibroblasts to myofibroblasts.

**ADRENOCORTICAL FUNCTION IN STRESS SENSITIVE HYPERTENSIVE RAT STRAIN**

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The aim of this study was uncovering the adrenocortical mechanisms probably underlying hypertension development in the ISIAH (inherited stress-induced arterial hypertension) rats. ISIAH rat strain was developed by selection for increased response of systolic arterial blood pressure (ABP) to a mild emotional stress produced by 0.5 h restraint in a small wire mesh cylindrical cage. The ABP in anesthetized ISIAH rats is 160-170 mm Hg and it elevates to about 200 mm Hg under the stress. The mRNA level was measured by RT-PCR method. Hormonal secretion rate was measured in anesthetized ISIAH and WAG rats by cannulation of the left adrenal vein and subsequent blood sampling. The hormone concentrations in blood and adrenal gland were measured by HPLC. It was found that the production of CRH-mRNA in hypothalamus and POMC-mRNA in pituitary in ISIAH rats was increased in comparison with normotensive WAG rats. No significant differences were found between the resting levels of plasma corticosterone and aldosterone in the ISIAH and WAG rats. But the plasma corticosterone and aldosterone responses to some stressful stimuli were enhanced in ISIAH rats. The adrenal weight and corticosteroids contents in adrenals were significantly higher in ISIAH rats as compared to WAG rats. Secretion rate of corticosterone, aldosterone, 11-dehydrocortistosterone and deoxycorticosterone were significantly higher in ISIAH than in WAG rats. The genetic predisposition to development of the stress sensitive arterial hypertension in the ISIAH rats is associated with a total increase in the functional activity of hypothalamic pituitary adrenocortical system.

**PROTECTION OF RENAL MEDULLARY OXIDATIVE STRESS IN DAHL SALT-SENSITIVE RAT BY SUBSTITUTION OF CHROMOSOME 13 OF THE BROWN NORWAY RAT**

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The development and physiological study of consomic rat strains have begun to reveal functional pathways involved in the development of salt-sensitive hypertension in Dahl salt-sensitive (SS) rats. Measurement of renal outer medulla (ROM) interstitial oxyethidium levels in dialysate and superoxide (O_2\(^-\)) production in tissue homogenates showed that O_2\(^-\) from the ROM in prehypertensive SS rats (0.4 % NaCl diet) was twice that of consomic SS-13BN rats. NADPH oxidase inhibition reduced O_2\(^-\) levels in SS but not SS-13BN rats while inhibition of xanthine oxidase, nitric oxide synthase, and cyclooxygenase had no effect on either strain. NADPH oxidase is therefore the source of the difference in O_2\(^-\) production between SS and SS-13BN rats. Chronic infusion of the NADPH oxidase inhibitor, apocynin (3.5 μg/kg/min), directly into the ROM, significantly reduced arterial pressure in the SS rats by 18 mmHg and ROM interstitial O_2\(^-\) fell 60%. SS-13BN rats were unaffected. Time-resolved fluorescent microscopy studies of thin tissue strips obtained from the SS RM showed increased production of O_2\(^-\) in the medullary thick ascending limb of Henle that diffuses to surrounding vasa recta (VR) and attenuates nitric oxide (NO) cross-talk from the tubule to the VR. We conclude that diffusion of O_2\(^-\) from mTAL to surrounding tissue contributes to reduced bioavailability of NO, reductions of medullary blood flow and interstitial fibrosis in the ROM of SS rats. Studies using narrow congenic strains are currently underway to identify the gene(s) within chromosome 13 responsible for the excess production of O_2\(^-\) in SS rats.

**THE RECENT PROGRESS OF GWAS STUDIES IN HYPERTENSION AND CARDIOVASCULAR DISEASE**

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Among the common complex diseases, hypertension has been particularly unlucky in the recent surge of positive results from genome-wide association studies. We summarize the evidence that would support continuing effort in the hunt for a genetic basis for hypertension. The problems facing the genetic studies for hypertension are not unique, but phenotypic characterization, heterogeneity and high prevalence make it a special case requiring a more individualized approach. We argue that even in the presence of a strong environmental component to hypertension risk, the common disease common variant model is relevant for hypertension and discuss the issues involved in designing a genome-wide association study for hypertension. It is likely that the individual odds ratios for disease variants will be less than 1.3, and although individually these effect sizes are minor, the combination of even a few such common polymorphisms can have substantial population attributable risks. The identification of hypertension gene variants should provide new insight into the disease susceptibility, progression and severity. This will lead to identification of potential targets for lifestyle and pharmacological interventions, with the ultimate goal of improving prevention, diagnosis and treatment.

**CHANGES IN MITOCHONDRIAL RESPIRATION SHR COMPARED WITH THAT OF RENAL HYPERTENSIVE RATS**

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Recently, it was shown that decrease of mitochondrial ATP synthesis in different tissues may be considered as a source of systemic arterial hypertension. The aim of this study was to compare respiratory functional parameters of mitochondria in SHR, rats with renal hypertension and control normotensive WKY rats. The experiments were carried out with male SHR (180 mmHg) and WKY (90 mm Hg) rats, body weight 230 to 280 g. Systolic blood pressure was measured with noninvasive method on the rat tail artery. Renal hypertension (RH) by Goldblatt (one clip-one kidney) was induced by placing a clip (0.4 mm) on one of the renal arteries. The systolic blood pressure increased in two weeks after this procedure and reached 160 mmHg.
The liver mitochondria were isolated by differential centrifugation. Mitochondrial respiration (state 3, state 4, respiratory control ratio (RCR)) was measured in suspension (2 mg of protein) by Clark-type oxygen electrode. The results for respiratory characteristics of mitochondria isolated from rats liver in renal hypertension (RH), spontaneously hypertensive rats (SHR), and normotensive rats (WKY) are presented in the table. The results demonstrate that despite of a similar decrease in ATP content and synthesis rate in SHR and in rats with renal hypertension, the mechanisms of mitochondrial dysfunction may be different. In spontaneous hypertension ATP synthesis decrease is realized through altered Ca$^{2+}$ exchange leading to mitochondrial Ca$^{2+}$ overload. In renal hypertension the decrease of ATP production is conditioned by the depression of mitochondrial respiration probably due angiotensin II action. More detailed study should be done.

<table>
<thead>
<tr>
<th></th>
<th>State 3 (µM min$^{-1}$mg$^{-1}$ of O$_2$)</th>
<th>State 4 (µM min$^{-1}$mg$^{-1}$ of O$_2$)</th>
<th>RCR (State 3/State 4)</th>
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<tr>
<td>WKY (n=10)</td>
<td>33.85±8.71</td>
<td>10.12±2.49</td>
<td>3.42±0.62</td>
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<td>SHR (n=5)</td>
<td>33.32±4.61</td>
<td>9.92±2.07</td>
<td>3.43±0.54</td>
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<tr>
<td>RH (n=5)</td>
<td>22.31±5.12**</td>
<td>7.83±0.99**</td>
<td>2.87±0.74</td>
</tr>
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</table>

Mean value ± SD. *p<0.05, **p<0.01

**CHOLINERGIC INFLUENCE ON RENIN-ANGIOTENSIN SYSTEM ACTIVITY**

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The regulatory influence of the cholinergic system on the renin-angiotensin system is poorly known. Cholinergic/adrenergic dysbalance is one of key factors for hypertension development. In this study cholinergic influences on different components of the renin-angiotensin system were investigated. Anesthetized rats with sympathetic overactivity were selected by previous testing. Corticosterone, norepinephrine and epinephrine plasma levels, arterial pressure reactivity on epinephrine and acetylcholine injections, and difference between carotid and tail arterial pressure were used as tests. I.v. administration of acetylcholine with a preliminary ß1-adrenergic blockade decreased plasma renin activity (PRA) of arterial blood in animals with an increased sympathetic reactivity and a high sensitivity to acetylcholine (the “accentual antagonism” state). PRA did not change in animals with sympathetic overactivity without accentual antagonism state. I.v. administration of stable analogue of acetylcholine (carbachol) decreased angiotensin-converting enzyme (ACE) activity in arterial plasma and lung and ACE secretion by kidney in dose-dependent manner. Angiotensin I pressor response and conversion of angiotensin I to angiotensin II were decreased by carbachol or acetylcholine in patients with arterial hypertension and ACE secretion by kidney is less active in hypertension. Angiotensin I pressor response and conversion of angiotensin I to angiotensin II were decreased by carbachol or acetylcholine in patients with arterial hypertension and ACE secretion by kidney is less active in hypertension. Angiotensin I pressor response and conversion of angiotensin I to angiotensin II were decreased by carbachol or acetylcholine in patients with arterial hypertension and ACE secretion by kidney is less active in hypertension.

**PARACRINE ROLE OF PERIADVENTITIAL ADIPOSE TISSUE ON THE REGULATION OF VASCULAR TONE**

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In the last years a paracrine role for periadventitial adipose tissue (PVAT) in the regulation of vascular function has been uncovered by several studies. Solís and Cassis were the first to demonstrate that PVAT significantly attenuates vascular responsiveness of rat aortic rings to norepinephrine. More recent reports have confirmed the inhibitory action of PVAT on the contractile response to a variety of vasoconstrictors on rat aortic and mesenteric arteries, as well as on human thoracic arteries. The anti-contractile action is induced by a still undefined transferable factor released by periadventitial fat, called adipocyte-derived relaxing factor (ADRF). Its anticontractile effect seems to be mediated by different mechanisms depending on the type of vascular bed and species. In addition, PVAT is a source of leptin, which participates in the regulation of vascular tone. Vascular effects of leptin are the net result of two different actions: i) indirect vasoconstriction through stimulation of sympathetic activity at hypothalamic level, and ii) direct vasodilatation that depends on intact and functional endothelium through mechanisms that vary between different vascular beds. Moreover, PVAT also releases several vasoconstrictors. It expresses the components of the renin-angiotensin system and releases angiotensin II. Other factors with vasoactive effects, such as superoxide anion and inflammatory cytokines, have also been described. Changes in the amount of PVAT in different situations such as hypertension and obesity are related to an altered balance of vasoconstrictors and vasodilators, thus contributing to changes of arterial blood pressure. Supported by grants SAF2005-05180 and SESCAMET.

**GENETIC MECHANISMS OF THE EXAGGERATED GROWTH OF VASCULAR SMOOTH MUSCLE CELLS FROM SHR**

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SHR-derived VSMCs show the exaggerated growth. We have demonstrated that SHR-derived VSMCs change to the synthetic phenotype that produced Ang II and growth factors. We found that only VSMCs from SHR produce complement 3 (C3), not from WKY rats, by microarray analysis. We investigated mechanisms underlying the involvement of C3 on the exaggerated growth and the synthetic phenotype of VSMCs from SHR. We examined the effects of C3 and antisense ODN to pre-pro-C3 on DNA synthesis and expression of phenotype marker mRNAs in VSMCs from SHR and WKY rats. We examined the effects of C3 on the expression of transcription factors that affect VSMC phenotype. We examined the effects of C3 on KLF5 mRNA expression in VSMCs and the KLF5 promoter activities. C3 increased DNA synthesis and proliferation of VSMCs from WKY rats and SHR. C3 changed VSMCs to the synthetic phenotype. C3 increased expression of KLF5 mRNA and activated KLF5 promoter activity. Antisense ODN to C3 inhibited the exaggerated growth and the synthetic phenotype in VSMCs from SHR. C3-increased KLF5 promoter activity was completely inhibited by a MEK inhibitor. C3 is produced only in VSMCs from SHR, not in VSMCs from WKY rats, independent of complement systems. C3 activates KLF5 to change SHR-derived VSMCs to the synthetic phenotype that is associated with increases in Ang II production and growth factors. Thus, C3 may be a genetic and a primary factor of the exaggerated growth of VSMCs from SHR.
ESTABLISHMENT OF THE CONSERVATIVE REGENERATIVE MEDICINES FOR CARDIOVASCULAR AND RENAL DISEASES USING SHR AND SHR-SP

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Cellular and molecular mechanisms of vasomotor function due to the synthesis and release of vasoactive mediators, such as leptin and the adipocyte-derived relaxing factor (ADRF), play a role in the regulation of vascular tone. In the current study, we aimed to investigate the effects of perivascular adipose tissue (PVAT) on vasomotor function in SHR and SHR-SP. We established EPC assays in rats by colony formation. Oxidative stress in cells was evaluated by TBARS. Abundance of cardiac stem cells was evaluated by expression of c-kit mRNA. Label-retaining cell as renal stem cell was evaluated by slow cycling cells in renal medulla by BrDu incorporation. EPC colony formation was significantly reduced in SHR-SP or SHR than in WKY rats. TBARS in EPCs was significantly higher in SHR-SP or SHR than in WKY rats. ARBs improved the reduced colony formation with inhibition of oxidation in EPC from SHR-SP. Celiprolol and atorvastatin increased colony formation with inhibition of oxidation in EPC from SHR. Candesartan increased expression of c-kit mRNA in left ventricle from SHR-SP. Varsaltan significantly increased number of label-retaining cells from SHR-SP. Thiazide did not affect the reduced colony formation in EPC. ARB, antioxidant-beta-blocker and statin improved EPC function, and increased cardiac stem cells and renal stem cells in hypertensive rats, suggesting these agents will be feasible for cardiovascular and renal diseases as the conservative regenerative medicines.

A LOWER AMOUNT OF PERIVASCULAR ADIPOSE TISSUE IN SHR IS RELATED TO A REDUCED RELEASE OF VASODILATORY ADIPOKINES

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Perivascular adipose tissue (PVAT) plays a role in the regulation of vasomotor function due to the synthesis and release of vasoactive factors, such as leptin and the adipocyte-derived relaxing factor (ADRF). This study aims to establish a possible link between the amount of PVAT and cardiovascular function in SHR. We characterized mesenteric PVAT from 3-months-old Wistar Kyoto (WKY) and aged-matched SHR. Vascular function was analyzed in the perfused mesenteric bed (MB, at a constant flow of 2 ml/min) and in isolated mesenteric arteries. MB weight and MB adipocyte diameter were smaller in SHR as compared to WKY. Accordingly, MB total lipid content, plasma and mesenteric leptin were lower in SHR correlating with the quantity of mesenteric PVAT. Vasodilatation induced by leptin (10⁻⁷ to 10⁻⁴ M) was significantly reduced in mesenteric rings from SHR (p<0.05). The antcontractile effect of ADRF was analyzed in the presence of the K⁺ channel blocker 4-aminopyridine (4-AP, 2 mMol/l). The increase in perfusion pressure induced by 4-AP was lower in the MB from SHR and was directly correlated with the mesenteric fat amount. In isolated mesenteric artery rings, 4-AP (2 mMol/l) induced a contractile effect that was lower in SHR compared to WKY. Our findings demonstrate that lower amounts of PVAT observed in SHR go along with a local deficiency in vasodilatory adipokines, such as ADRF and leptin. These results suggest that elevated peripheral resistance in SHR may be linked to the low amounts of visceral fat.

CONDITIONED MEDIUM FROM PERIVASCULAR ADIPOSE TISSUE STIMULATE MIGRATION AND DIFFERENTIATION OF VASCULAR ADVENTITIAL FIBROBLASTS

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Perivascular adipose tissue is an important component of vascular adventitia. The effects of adipocytes secreted from perivascular adipose tissue on vascular smooth muscle cell (SMC) were emerging as regulators of vascular function. However, the effects of adipocytes on vascular adventitial fibroblasts have not yet been investigated. In this study, we investigated whether Perivascular Adipose Tissue Conditioned Medium (PVATCM) can affect cellular function in cultured rat aortic adventitial fibroblasts. We found that PVATCM stimulated migration of fibroblasts. ARBs improved the reduced migration of fibroblasts, which was used as characteristic marker of fibroblast-to-myofibroblast differentiation. Furthermore, ARBs improved the reduced migration of fibroblasts, which was revealed by a transwell technique. Next we tested the mRNA and protein level of Adipo-R1 and R2, and found that adiponectin receptors including Adipo R1 and R2 were expressed in fibroblasts but not adiponectin. Moreover, expression level of Adipo R1 and R2 were increased after ARVM treatment, and the increased expression of adiponectin receptors occurred before α-SM-actin expression. Further experiment demonstrated that PVATCM also quickly activated the mitogen activated protein kinase (MAPK) pathway represented by phosphorylation of extracellular signal-regulated kinases (ERK1/2) and JNK. Our studies demonstrated that PVATCM stimulate α-SM-actin expression and migration of vascular adventitial fibroblasts, which suggest that the effect of perivascular adipose tissue may be involved in vascular adventitial remodelling associated with differentiation and migration.

REDUCED LEFT VENTRICULAR MASS INDEX AND INCREASED VENTRICULAR FUNCTION IN A HYPERTENSIVE CONGENIC STRAIN

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Previously we identified a left ventricular mass index (LVMI) quantitative trait locus (QTL) on rat chromosome 14. This study aimed to confirm the QTL by generating a congenic strain. A chromosome 14 congenic strain (SP.WKYGl14a) was produced by introgression of a 63cM WKY region into the SHRSP genetic background. Systolic blood pressure (SBP) was measured by radiotelemetry. Echocardiography was used to examine 5 and 16-weeks-old animals. LVMI (mg/g), relative wall thickness (RWT), ejection fraction (EF-%) and cardiac output (CO) were calculated using m-mode images. Left ventricular (LV) function was assessed using pulse wave Doppler. SBP was not significantly different between SHRSP (198.7±3.7 mmHg; n=5) and SP.WKYGl14a (191.6±6.1 mmHg; n=5) at 16 weeks of age. Cardiac parameters are shown in Table. LVMI, RWT, isovolumetric relaxation time (iVRT) and myocardial performance index (MPI) were significantly reduced in SP.WKYGl14a without significant age difference time-dependently compared to SHRSP with CO reduced in 5 week SHRSP versus SP.WKYGl14a. Diastolic
function was impaired in 16 week SHRSP, with reduced early to atrial rever- 
sal flow (E/A) ratio. A QTL for LVMI independent of SBP was confirmed 
in SP.WKY/Gla14a. LV function was also improved significantly in SP.WKY/Gla14a versus SHRSR. Subsequent dissection of 
the QTL region to produce congenic substrains is required to identify candidate genes contributing to the observed phenotype.

![Table Image]

<table>
<thead>
<tr>
<th></th>
<th>SHRSP</th>
<th>SP.WKY/Gla14a</th>
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<tbody>
<tr>
<td></td>
<td>5 wk (n=7)</td>
<td>6 wk (n=6)</td>
</tr>
<tr>
<td>LVM (mg/g)</td>
<td>3.03±0.13</td>
<td>3.00±0.06</td>
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<tr>
<td>RWT</td>
<td>0.49±0.01</td>
<td>0.62±0.01</td>
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<tr>
<td>CO (ml/min)</td>
<td>127.6±5.4</td>
<td>276.6±3.7</td>
</tr>
<tr>
<td>EF (%)</td>
<td>89.8±0.8</td>
<td>88.6±0.2</td>
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<tr>
<td>IVRT (ms)</td>
<td>20.28±1.31</td>
<td>23.44±0.62</td>
</tr>
<tr>
<td>E/A (m/s)</td>
<td>1.42±0.04</td>
<td>1.19±0.02</td>
</tr>
<tr>
<td>MPI</td>
<td>0.45±0.01</td>
<td>0.47±0.01**</td>
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*p<0.01, **p<0.005, #p<0.001 compared to SHRSP

EVALUATION OF THE IMPORTANCE OF CHROMOSOME 13
IN LYN HYPERTENSIVE RATS USING CONSOMIC STRAINS

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Since a full genome scan showed the existence of Quantitative Trait Loci (QTLs) influencing Blood Pressure (BP) and kidney renin content on chromosome 13 (RNO 13) of Lyon Hypertensive (LH) rats, we thought of interest to use reciprocal consomic strains to determine their influence in the pathophysiology of our model. Therefore, we generated two reciprocal consomic strains by substituting LH RNO 13 by that of BN rats (BN-13 LH). The characterisation of these strains includes radio-telemetric measurement of BP during normal and elevated salt intake as well as the determination of renal, metabolic and morphological parameters. Compared to LH parents, LH-13BN rats showed increased body weight and markedly decreased mean BP (-13 mmHg) and of proteinuria and increased level of antioxidant coenzyme Q (CoQ). The positive correlation between the OH- level and the LV hypertrophy demonstrates the involvement of free-radical oxidative processes in the pathogenesis of myocardial hypertrophy.

INCREASED HYDROXYL RADICAL GENERATION AND DEFICIT OF COENZYME Q IN MYOCARDIUM OF SHRSR

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The oxidative stress is of great importance in the pathogenesis of arterial hypertension and myocardial remodeling. The aim of the present study was to estimate the generation of hydroxyl radical (OH·) and the level of antioxidant coenzyme Q (CoQ) in hypertrophied myocardium of SHRSR. Experiments were carried out on anesthetized open-chest male SHRSP and age-matched WKY rats (300-400 g). The OH· level was evaluated using microdialysis technique with sodium salicylate. 2,3-Dihydroxy-xybenzoic acid (DHBA) produced in vivo in the reaction of OH· with salicylate was used as a marker of OH· generation and was measured by HPLC. DHBA concentration in dialysates was expressed for each animal as percentage of the DHBA concentration in perfusing solution in vitro. The content of CoQ10 and CoQ7 in left ventricle (LV) myocardium was measured by HPLC with electrochemical detection. The content of DHBA in dialysates of SHRSR myocardium was higher (157±12.5%) than in WKY (106±12%, p=0.005) rats. Increased DHBA level in SHRSR was positively correlated (r=0.831, p<0.05) with the degree of LV hypertrophy expressed as a ratio of LV weight to the body weight. CoQ10 and CoQ7 myocardial content in SHRSR (101±39 and 6.2±2.1 µkg/g) was lower (p<0.05 and p<0.0001) than in WKY (143±31 and 11.5±2.6 µkg/g) rats. LV hypertrophy of SHRSR is accompanied by enhanced tissue production of OH· and decreased level CoQ. The positive correlation between the OH· level and the LV hypertrophy demonstrates the involvement of free-radical oxidative processes in the pathogenesis of myocardial hypertrophy.
Our previous study demonstrated that transforming growth factor β1 (TGF-β1) and angiotensin II (Ang II) were able to induce the differentiation of vascular adventitial fibroblasts (AFs) to myofibroblasts (MFs), which is a key step in vascular remodeling. The aim of this study was to demonstrate that complement C3 might be responsible for the cell phenotypic change. Cultured rat AFs were treated with TGF-β1 (10 ng/ml) and Ang II (10 μM). TGF-β1-induced gene expression profiling was studied using Affymetrix oligonucleotide microarrays. Expression profile of complement C3 was verified by real-time RT-PCR. Then, the role of complement C3 in differentiation of AFs to MFs induced by Ang II was investigated using Western-blot and MTT array. Complement C3 expression in AFs from spontaneously hypertensive rats (SHR), Wistar-Kyoto (WKY) rats and Sprague-Dawley (SD) rats. Using SD AFs, microarray analysis identified 2121 genes with a 2-fold change or above post-TGF-β1 stimulation. Among 1231 genes with known function, we found that the gene expression of complement C3 was significantly up-regulated which was validated later by real-time RT-PCR and Western-blot. Complement C3 also increased in MFs induced by Ang II. SB290157 C3a inhibitor reduced proliferation of AFs from SD rats and WKY rats post treatment of Ang II. C3 may be the gene underlying the differentiation of MFs and exaggerated growth of MFs. C3 may be a new target for the treatment of hypertension.

### ROLE OF LOSARTAN AND SPIRONOLACTONE IN THORACIC AORTIC WALL COMPOSITION OF SPONTANEOUSLY HYPERTENSIVE RATS AT DIFFERENT HYPERTENSIVE STAGE

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The composition of thoracic aortic media and adventitia from 16- and 32-week-old WKY and SHR and their responsiveness to drug treatment was examined. 16-week-old SHR were randomly divided into 3 groups for treatment with either 0.9 % NaCl, losartan (20 mg/kg/day), or spironolactone (200 mg/kg/day) for 16 weeks. Thoracic media was examined. 16-week-old SHR were randomly divided into 3 groups for treatment with either 0.9 % NaCl, losartan (20 mg/kg/day), or spironolactone (200 mg/kg/day) for 16 weeks. Thoracic media was made primarily of elastin and less collagen, adventitia contained collagen comparable to that of the media but had less elastin. Thoracic medial mass, collagen and elastin content was increased in SHR than in age-matched WKY, and increased with age in both strains. However, there was no significant difference in aortic adventitial mass, collagen or elastin content between SHR and age-matched WKY, although adventitial collagen also increased with age. Thoracic aortic medial mass, collagen and elastin content were positively related to pulse pressure (PP) in WKY and SHR, but adventitial collagen or elastin was not. Losartan and spironolactone decreased aortic medial mass and collagen content, while adventitial mass and collagen content remained unchanged. The thoracic media and adventitia of large-conduit vessels has different biochemical composition, and showed different responsiveness to blood pressure and RAAS antagonists.

### TAURINE IMPROVES VASCULAR TONE THROUGH THE SUPPRESSION OF PERIPHERAL SYMPATHETIC TONE AND ENDOTHELIAL PRECURSOR CELL SENESCENCE IN HYPERTENSION

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Taurine, sulfur-containing amino acids, has shown to reduce blood pressure in spontaneously hypertensive rats (SHR/Izm). To clarify the antihypertensive mechanisms of taurine, we investigated the effects of taurine on peripheral sympathetic nerve tone and endothelial function in...
mesenteric artery. We also investigated bone-marrow derived endothelial progenitor cells (EPCs) differentiation and senescence. Mesenteric arterial preparations of six-week old SHR/Izm were made. Arterial contraction and norepinephrine overflow induced by electrical stimulation to sympathetic nervous plexus around mesenteric arterial trunk and acetycholine-induced vasodilation were observed. We counted number of EPCs assessed by both DilLDL uptake and lectin binding and EPCs senescence was detected by acidic β-galactosidase staining. Taurine had a direct suppressive effect on norepinephrine overflow by electrical stimulation, which was more marked in SHR/Izm than that in WKY/Izm. Magnitude of acetycholine-induced vascular dilation in mesenteric artery pre-contracted with norepinephrine was augmented in SHR/Izm treated with 3% taurine for 3weeks compared with control SHR given top water. The ratio of senescence associated β-galactosidase positive EPCs to total cells was significantly reduced in SHR/Izm treated with taurine. Treatment of taurine suppresses vascular contraction through the inhibition of peripheral sympathetic tone and improves endothelial function through the reduction of EPCs senescence in SHR/Izm.

WHERE ARE ALL THE BLOOD PRESSURE GENES?

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Promises that the genome project will change the face of human disease have been uttered for at least 20 years. The public and funding bodies are becoming somewhat jaded by this message and doctors and patients wonder when something useful will emerge. There is general agreement that about 40% of variance of blood pressure is genetically determined. Therefore, it was presumed that modern molecular techniques would quickly reveal the whereabouts of genes influencing blood pressure. Yet despite many millions of dollars spent, no new clinical test and no new drug have emerged as a result of genetic discovery. In the literature there is ongoing debate about the importance of well-known candidate genes and their variants. Even genome wide association studies have been unable to provide evidence of significant loci influencing blood pressure. Where then do the problems lie? The possibilities are likely to relate to heterogeneity – heterogeneity of populations, of phenotypes and of polymorphisms. The assumption that populations would share gene variants is unlikely to be the case. Even if the functional DNA variants are shared, patterns of linkage disequilibrium mean that the common markers might not be shared. Mixing populations is not taken to obtain representative measures, power of the genetic analyses will be diminished. The second relates to the specific blood pressure measure. For example, the physiological determinants of mean arterial and pulse pressure differ, and presumably their genetic influences are diverse also. Are the genetic controls of lying, siting and standing blood pressure likely to be identical? One can extend this argument to sub-phenotypes, in which blood pressure (usually hypertension) is subgrouped according to underlying physiological characteristics in an attempt to define groups more closely linked with specific candidate genetic mechanisms. The degree to which such “splitting” (as opposed to “lumping”) is effective depends on the balance between phenotypic authenticity and its relevance insofar as the proportion of a population represented. The heterogeneity of polymorphisms refers to the potential complexity of genetic and allelic contributors to blood pressure. The existence of many alleles of small effect is not a good substrate for discovery. This is further confounded if substantial effects on blood pressure are predicated on allelic interaction, for which statistical methods are not well equipped. Finally, the positional answer to the “where” question is likely to be non-coding DNA. Quantitative variation of blood pressure might be explained by variable expression of key genes under the control of a small number of “master” non-coding DNA sequences. The emerging challenge is to recognise such sequences and devise experiments to define the developmental stage and tissue specific expression they might orchestrate. The exciting potential of these discoveries more that makes up for the false starts and disappointments of blood pressure genes to date.

LONG-TERM VOLUNTARY EXERCISE DECREASED THE INCIDENCE OF APOPEXY AND ELONGATED THE LIFESPAN THROUGH ACTIVATION OF eNOS AND INHIBITION OF INFLAMMATORY SIGNALING PATHWAY IN STROKE-PRONE SHR

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Clinical evidences show that exercise exerts atheroprotective or beneficial effects on cardiovascular events. Precisely causative mechanisms, however, are still unknown. Therefore, the hypothesis that endurance voluntary exercise decreases the inflammatory signaling through eNOS induction and ROS inhibition was assessed in SHRSP. Males SHRSP aged 6-week-old were divided into two groups; voluntary wheel-running (EX) and sedentary (SED) rats. EX rats were allowed to run voluntarily in a wheel (2.5~4 km/day). SED rats were placed in the cage without running. After 8 wks of experiment, the rats were sacrificed under anesthesia. Isolated aortas were used for the analyses of ROS, NO, eNOS, NAD(P) oxidase, and several molecules related to inflammation and cell proliferation. Plasma was collected for the measurement of inflammatory cytokines using ELISA. Voluntary exercise significantly attenuated the changes of vascular remodeling, delayed stroke events and elongated the lifespan in EX rats. Isolated aortas of EX rats showed a decrease of superoxide production, an increase of NO production concomitant with elevation of eNOS activity, and decreases of nox1 and several protein molecules related to inflammation and cell proliferation such as AT1R, p-JNK, and p38MAPK concomitant with inhibition of AS1K. Plasma in EX rats showed significant decreases of TNF-alfa, sICAM-1, MCP-1, and high sensitive CRP. These data showed that exercise could protect oxidative stress-induced cell injury or inflammation by an interaction with signaling molecules such as AS1K /JNK/p38MAPK through NO production and inhibition of superoxide production.

INCREASED REACTIVE OXYGEN SPECIES IN BRAINSTEM CONTRIBUTE TO NEURAL MECHANISMS OF HYPERTENSION IN STROKE-PRONE SHR

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The involvement of reactive oxygen species such as superoxide and hydroxyl radicals is implicated in the pathogenesis of hypertension. Thiobarbituric acid-reactive substances (TBARS), end products of lipid peroxidation and an indirect marker of oxidative stress, are increased in the brainstem of stroke-prone spontaneously hypertensive rats (SHRSP) compared with those of Wistar-Kyoto rats (WKY). In addition, the intensity of electron spin resonance signals taken from the rostral ventrolateral medulla (RVLM), a cardiovascular center, decreases more rapidly in SHRSP than in WKY. To confirm the role of reactive oxygen species in the RVLM in SHRSP, we transfected adenovirus vectors encoding the manganese superoxide dismutase (MnSOD) gene (AdMnSOD) bilaterally into the RVLM. After the gene transfer, blood pressure and heart rate of SHRSP monitored by radio-telemetry system were significantly decreased compared with non-treated SHRSP, but not WKY. Urinary norepinephrine excretion was significantly decreased in AdMnSOD-transfected SHRSP, but not in WKY. Taken together, these results suggest that the increased oxidative stress in the RVLM contribute to the central nervous system mechanisms underlying
hypertension in SHRSP. We also found that some anti-hypertensive drugs or statin have actions of reducing oxidative stress in the brain associated with sympatho-inhibitory effects. Furthermore, we found that activation of NAD(P)H oxidase via Rac1 is a source of reactive oxygen species generation in the brainstem of hypertensive rats.

EFFECT OF A GENETIC LOCUS ON RAT CHROMOSOME 20 ON METABOLIC RATE IN HIGH-FAT DIET-INDUCED OBESITY

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We have shown previously that a segment of rat chromosome 20 (RNO20) contains a gene or several genes that regulate body-weight (BW) gain and an adipocyte-size increase in response to a chronic high-fat diet (HFD). The aim of our present investigation was to determine whether this effect of the RNO20 segment is mediated by its impact on HFD-induced changes in energy intake or energy expenditure. Sixteen-week-old male spontaneously hypertensive rats (SHR) and a congenic strain SHR.1N in which the RNO20 segment is transferred from the Brown Norway rat onto the SHR background were placed on HFD (F3282, Bio-Serv: 5.3 kcal/g; SHR: n=4; SHR.1N: n=4) for six weeks. Prior to (week 0) and at the end (week 6) of the dietary intervention, metabolic rate (measured with indirect calorimetry), locomotor activity, and food intake were recorded with the Comprehensive Lab Animal Monitoring System (Columbus Instruments) for at least two consecutive 24-hour periods. Similar to our previous study, BW gain was significantly greater in SHR.1N than in SHR. When changes between weeks 0 and 6 were compared, 24-hour energy intake was higher and did not differ between SHR and SHR.1N. Total ambulatory activity was also higher and did not differ between the two strains. Twenty-four-hour metabolic rate, calculated from oxygen consumption and carbon dioxide production, was higher and showed a trend towards being greater in SHR than in SHR.1N. Our results suggest that the RNO20 segment regulates BW gain in response to HFD and that this effect may be mediated by its influence on metabolic rate. Further studies are currently in progress to confirm these findings.

THE RETINA AND THE METABOLIC SYNDROME – A STUDY IN SPONTANEOUSLY HYPERTENSIVE SHR/N-cp RATS

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Diabetic retinopathy, one of the leading causes of blindness, emerges with increasing prevalence as a co-morbidity of the metabolic syndrome. However, the effects of the metabolic syndrome on the retina are poorly understood. Therefore, we focused on the corpulent rat strain SHR/cp(-/-) in comparison to the lean SHR/cp(+/+) controls (n=9, each). Systolic blood pressure was measured by a non-invasive tail-cuff method. Urine was collected via metabolic cages, urine and blood parameters and light microscopic analysis were determined using standard procedures. mRNA expression levels were analyzed with the GeneChip® Rat Genome 230 2.0 Array and validated by real-time PCR. Obese SHR/cp(-/-) were hypertensive and showed significant increases in body weight, serum levels of glucose, triglycerides, total cholesterol, and urinary glucose excretion (p<0.01, respectively) compared to lean controls. Histological analysis of the retina showed a regular laminar structure and no proliferation of microvessels. On the molecular level, microarray analysis revealed regulated genes involved in apoptosis, signal transduction and glutamate metabolism. Adult SHR/cp(-/-) show characteristics of pronounced metabolic syndrome. The integrity of the retina remains intact, however is accompanied by the regulation of genes affecting retinal homeostasis. These may direct to a complex molecular network which underlies retinopathy in the course of metabolic syndrome. Future studies will explore the functional consequences of these dysregulated networks in the framework of genetically determined metabolic syndrome.

GENETIC VARIATION IN THE RAT GENOME – FROM THE BN REFERENCE GENOME TO SHR & FROM SNPS TO COPY NUMBER VARIATION

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Inbred laboratory rat strains originated from limited Rattus norvegicus founder populations, and the inherited genetic variation provides an excellent resource for the identification of quantitative trait loci (QTL) in the rat genome. The STAR consortium has identified almost 3 millions novel SNPs in the rat genome and obtained accurate and complete genotypes for a subset of more than 20,000 SNPs across 167 distinct inbred rat strains, two rat recombinant inbred panels, and an F2 intercross. This dataset encompasses several SHR derived substrains and the SHR x BN panel of rat recombinant inbred strain originally established in Prague. I will describe the initial characterization of the population structure and the degree of linkage disequilibrium in the rat genome and provide a summary of the detailed SNP map and demonstrate its utility for mapping of quantitative trait loci. Moreover, I will provide an overview on the challenges of the abundance and dynamics of copy number variants (CNVs) in the rat genome that poses new challenges in the identification of their impact on natural and disease phenotypes. These characteristics make the rat an excellent model for studying phenotypic effects of natural genetic and structural variation in relation to human complex traits and disease and that complete sequence information on several rat strains would help achieving this goal.

INTERACTION BETWEEN GENES AND DIETARY FACTORS-EPIGENETICS IN BLOOD VESSEL OF SHR

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Histone modifications play critical roles in the epigenetic regulation of gene expression. Epigenetic regulation associates gene expression with environment. In the present investigation, we tested the hypothesis that SHR aorta displayed senescent phenomenon and epigenetic modifications, and that some dietary factors was able to regulate histone methylation and SirT1 modification. The aortas from 28-week old SHR were compared with those from the age-matched Wistar rats. Senescent phenomenon was determined by β-galactosidase staining, which had higher level of p53 and p21 expression. SHR aortas also displayed higher level of p22phox, PAI-1 expression and lower level of eNOS than those of Wistar rats. In the SHR aortas, histone H3 exhibited high intensity of methylation by Western blot assay, as observed in H3 Lys9 methylation, trimethylation and dimethylation of histone H3 Lys4, and dimethylation of histone H3 Lys79, but the trimethylation of histone H3 Lys79 was not enhanced. The suppression of Ser27 and Ser47 phosphorylations of SirT1, which was aging-related and NAD(+)-dependent histone deacetylase, was firstly proved in the blood vessels of SHR. The SirT1 activity in SHR vessels was lower than in Wistar rats.
as assayed by SirT1/Sir2 deacetylase fluorometric assay. Vascular smooth muscle cells from SHRSP aorta treated with angiotensin II for 5 days exhibited suppression in Ser27 and Ser47 phosphorylations of SirT1. Moreover, we found that some dietary factors depressed histone methylation at H3 Lys4 or H3 Lys9 assayed by the global histone H3-K9 and H3-K4 methylation assay kits. In the present study, our data showed that SHR aortas displayed senescence accompanied with epigenetic regulation, and that some dietary factors depressed histone methylation.

DISTURBANCE OF CIRCADIAN RHYTHM IN HEART RATE AND BLOOD PRESSURE WITH A RAPID RISE OF SERUM NOX AT STROKE-ONSET IN MALIGNANT SHRSP (M-SHRSP)

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Malignant M-SHRSP separated from SHRSP siblings by Okamoto et al., spontaneously develops stroke at early ages. Using this model, the influence of stroke-onset on the autonomic nervous system and serum NOx were investigated. Heart rate (HR), systolic and diastolic blood pressure (SBP, DBP) and locomotive activity were continuously measured from 5 weeks of age to the post-stroke using a telemetry system. A transmitter for radiotelemetric measurement was surgically placed into the peritoneum of male M-SHRSP at 4 or 7 weeks of age, and a catheter was inserted into the descending aorta. Continuous 24-hour ambulatory HR, SBP, DBP and locomotive activity were monitored in freely moving animals using Dataquest IV system. Blood was collected from tail vein and serum NOx was measured using ENO-10. Stroke-onset was assessed by neurologic symptoms, changes in body weight and fluid intake, and confirmed by MRI. SBP (1-day average) at 5 weeks of age was approximately 150 mmHg and increased to 260 mmHg at 12 weeks of age. Rats displayed a nocturnal pattern of circadian rhythms. HR rapidly increased by approximately 100 bpm at stroke onset. Circadian variation in HR was blunted or reversed at post stroke. The variation of SBP, DBP and locomotive activity changed similarly at stroke-onset. There was a marked increase in serum NOx level at stroke-onset. A rapid change in serum NOx level and HR, and the disturbance of circadian rhythm in HR, SBP, DBP and locomotive activity serve as reliable and accurate markers for stroke onset.

THE COMPOSITION OF ADIPOGENIC MULTIMERS IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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One of the unique characteristics of SHRSP is atrophy of the adipose tissues with age. Adipose tissue is known as an important endocrine organ secreting adipokynes. Adiponectin is an adipocyte-derived hormone, which plays an important role in insulin sensitivity, anti-atherosclerosis and anti-hypertension, and functions as trimer, hexamer and 12-18mer via disulfide bonds. We hypothesized that difference of the adiponectin multimer composition would be one of the causes of severe hypertension and its sequelae in SHRSP. To detect adiponectin multimers, sera from 12-week-old male SHRSP and age-matched normotensive male Wistar-Kyoto rats (WKY) were analyzed by Western blotting under condition without reducing and heating. Realtime PCR and ELISA were employed to quantify adiponectin mRNA and serum concentration, respectively. There was no significant difference in adiponectin mRNA in adipose tissue and serum adiponectin concentration between SHRSP and WKY. However, Western blotting for adiponectin multimers showed that WKY had a more intense band at ~200 kDa than SHRSP did. This band disappeared under reducing condition, indicating that ~200 kDa protein was the adiponectin multimer. These results suggest that SHRSP and WKY have the different adiponectin multimer composition. There was a significant difference in ~200 kDa adiponectin multimer between SHRSP and WKY. Because the composition of adiponectin multimers in serum is much more important than serum adiponectin concentration, the drastic decrease of ~200kDa in SHRSP would be one of the factors resulting in severe hypertension and stroke.

NEURAL STEM CELL AND NEWLY NEURONS APPEARANCE IN THE CEREBRAL CORTEX OF STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS AFTER STROKE

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SHRSP is the only one animal model which has a spontaneous cerebral stroke. However, the appearance of neural stem cells (NSCs) and newly neurons around a lesion area has not yet been elucidated. In this study, we studied the appearance of NSCs and newly neurons around a lesion area after cerebral stroke in SHRSP. Male SHRSP (aged 16 weeks) before cerebral stroke were injected with BrdU intraperitoneally for 2 weeks. Brain sections were immunostained with BrdU, which is cell division and cell proliferation marker, SOX-2, which is NSCs marker, nestin, which is NSCs and immature astrocytes marker, doublecortin (DCX), which is immature neuron marker, and NeuN, which is mature neuron marker. There were many SOX-2+/nestin+ double-labeled cells after stroke in acute phase group. Moreover, there were many DCX-positive cells and some nucleus of DCX-positive cells also showed immunopositivity for BrdU (DCX+/BrdU+). At the subventricular zone (SVZ) in acute phase group, there were many SOX-2+/nestin+ double-immunopositive cells and DCX-positive cells. There were NeuN+/BrdU+ double-labeled cells after stroke in chronic phase group. It was shown that there were many immature cells (SOX-2+/nestin+) which seem to be NSCs and newly neurons (DCX+/BrdU+) in SVZ right after a cerebral stroke. Furthermore, NSCs and newly neurons appeared in early stage after cerebral stroke, and these cells differentiated into mature neurons (NeuN+/BrdU+). These results indicate that promotion of the maturation and differentiation of NSCs and newly formed immature neurons around a cerebral lesion area may improve the brain dysfunction after cerebral stroke.

CHANGING ROLES OF GENETIC AND GENOMICS STUDIES IN THE RAT

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In 2008, animal models are selected primarily on one of two criteria: 1) a gene or its expression is modified or can be modified and its implication in a human disease can be studied in a particular model system; 2) the physiological characteristics of the model systems reflect some aspect of the clinical picture. There has been some movement towards other considerations such as genome background and effects of the environment (e.g. diet). However, it appears that the added benefit of comparative genomics is seldom considered when a model is selected. With a large number of QTL genetically mapped in many different species, and many mapping to the same evolutionarily conserved regions, it seems reasonable that many of the same genes will play a role in the same disease process. For many years the role of animal systems in genetic studies has been predicated on the increased heritability, flexibility and statistical power of experimental crosses over corresponding studies in humans. The new human genome wide association studies (GWAS) and re-sequencing programs are now able to sift the genome of the human without the need of animal model data, therefore raising a question concerning the continuing value of animal
CoQ9 myocardial content was the same in both strains; CoQ10 myocardial content: CoQ9 level was lower by 44% (p<0.001) and 46% (p<0.0001) in 12 and 24 weeks old rats than in WKY rats. In the right ventricle of SHRSP the CoQ depletion in comparison with age-matched WKY rats was revealed later: for CoQ9 - by 29% (p<0.05) in 24 weeks old rats; for CoQ10 - by 43% (p<0.05) and 44% (p<0.005) in 12 and 24 weeks old rats correspondingly. The content of CoQ in RV was always 2-4 times less than in LV in the both strains. The deficit of CoQ is characteristic sign of SHRSP myocardium.

ANGIOTENSIN II IS CRITICAL IN THE DEVELOPMENT OF ABNORMAL VASORELAXATION IN SHRSP.ZF RATS, AN ANIMAL MODEL OF METABOLIC SYNDROME

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Metabolic syndrome, a cluster of lifestyle-related diseases, is known to facilitate the development of cardiovascular disease. We have already reported that mesenteric arteries of SHRSP.ZF (SHR fatty) rats display impaired vasorelaxation response. To elucidate the mechanism of this dysfunction, we examined whether the condition could be alleviated by treatment with telmisartan, an angiotensin II type 1 (AT1) receptor antagonist with PPAR-gamma activating properties, and pioglitazone, a PPAR-gamma agonist. Telmisartan (5 mg/kg/day) or pioglitazone (2.5 mg/kg/day) was orally administered to male SHRSP fatty rats for 8 weeks (from 9 to 17 weeks of age). Serum triglyceride and cholesterol levels were determined using commercial kits, and the oral glucose tolerance test was performed to evaluate insulin resistance. Vasorelaxations in response to sodium nitroprusside (SNP) were determined by the organ bath method, protein expressions of soluble guanylyl cyclase (sGC) in mesenteric arteries by western blot, and the contents of 3-nitrotyrosine in aortas by the HPLC-ECD method. Telmisartan exerted antihypertensive effects. Pioglitazone ameliorated metabolic abnormalities in SHRSP fatty rats. Telmisartan restored the impaired SNP-induced relaxation, decreased sGC protein expression in mesenteric arteries and increased 3-nitrotyrosine content in aortas, but pioglitazone displayed no such alleviating effects. These findings indicate that telmisartan shows vascular protective effects by AT1 blocking, but not PPAR-gamma activation, probably via decreased oxidative and nitrate stress in SHRSP fatty rats. AT1 receptor antagonists may be beneficial for preventing cardiovascular events in metabolic syndrome.

DEPLETION OF COENZYME Q CONTENT IN MYOCARDIUM OF SHRSP DURING AGING

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The chronic oxidative stress accompanying the arterial hypertension can lead to age-related depletion of the antioxidant defense system. The aim of the present study was to estimate the levels of second-line antioxidant coenzyme Q (CoQ) in myocardium of SHRSP during aging. Experiments were carried out on male SHRSP and age-matched WKY rats at the age 6, 12 and 24 weeks. The content of CoQ in hypertrophied left (LV) and non-hypertrophied right (RV) ventricle myocardium was measured by HPLC with electrochemical detection. In the left ventricle of 6 weeks old rats CoQ9 myocardial content was the same in both strains; CoQ10 myocardial content in SHRSP was lower by 28% than in WKY. During aging the LV myocardial level of CoQ9 in SHRSP was decreased by 36% (p<0.01) and 30% (p<0.02) in 12 and 24 weeks old rats to compare with age-matched WKY rats. Similar changes were observed for CoQ10. LV myocardial content: CoQ9 level was lower by 44% (p<0.001) and 46% (p<0.0001) in 12 and 24 weeks old rats than in WKY rats. In the right ventricle of SHRSP the CoQ depletion in comparison with age-matched WKY rats was revealed later: for CoQ9 - by 29% (p<0.05) in 24 weeks old rats; for CoQ10 - by 43% (p<0.05) and 44% (p<0.005) in 12 and 24 weeks old rats correspondingly. The content of CoQ in RV was always 2-4 times less than in LV in the both strains. The deficit of CoQ is characteristic sign of SHRSP myocardium.

DISSECTION OF PHENOTYPIC TRAITS ASSOCIATED WITH METABOLIC SYNDROME AND CARDIO-VASCULAR COMPLICATIONS IN SHR AND SHRSP OF JAPANESE COLONY

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To elucidate the genetic basis underlying the metabolic syndrome and cardiovascular complications towards the possible identification of causative molecular variations in the model organism, we have developed a series of consomic/congenic rat strains derived from SHR and stroke-prone SHR of Japanese colony. We developed 14 consomic rat strains by performing repeated backcrossing between a recipient SHR and/or SHRSP and a donor WKY. In each consomic rat strain, we examined the presumed phenotypic impacts of QTLs thus trapped in the corresponding chromosomal fragments by measuring blood pressure, body and fat weight, lipid and glucose levels and further investigated mRNA levels with and without pharmacological and dietary interventions. In the consomic rat strains developed by speed congenic strategy using >170 microsatellite markers, we found significant changes in metabolic phenotypes between consomic rat strains and the progenitor strains. For example, as for consomic rat strains derived from SHR (n=30–45 in both sexes), we verified QTLs for blood pressure on rat chromosomes (RNO) RNO3, 15 and 19, body weight on RNO1, 2 and 19, total cholesterol on RNO2, 15 and 19, blood glucose on RNO1, 15 and 19, and fat weight on RNO3 and 19. We have constructed a panel of 14 consomic rat strains in which principal QTLs for blood pressure, dyslipidemia and related traits are successfully trapped. Metabolic phenotypes have been separated en masse into the same chromosomal fragment in several consomic rat strains. These strains should constitute useful molecular tools for the dissection of complex genetic interplay.

GENE-ENVIRONMENTAL INTERACTION IN HYPERTENSION IN JAPANESE

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Essential hypertension (EH) has a multifactorial origin arising from an interaction between susceptibility genes and environmental factors. Even though the genome-wide association (GWA) studies succeeded to identify potential new susceptibility loci for Crohn’s disease and type II diabetes, the susceptible gene for hypertension has not been identified yet. In contrast, large genetic epidemiological study is a way to reveal a small but certain effect of SNPs (single nucleotide polymorphisms) on the hypertension susceptibility under the specific environmental condition. For example, the high frequency of salt sensitive allele of angiotensinogen (AGT), G protein beta 3 subunit (GNB3), aldosterone synthase (CYP11B2) and alpha adducin (ADD1) genes in Japanese suggested the importance of strict restriction of salt intake for the prevention of cardiovascular disease. In the Amagasaki Study, two SNPs involved in alcohol metabolism, ADH1B and ALDH2, significantly associated with blood pressure via plasma drinking behavior; suggested that the modification of drinking volume might be altered by individual genotype. In our cohort study, the
gene polymorphisms of beta 2 (ADRB2) and beta 3 adrenoceptor (ADRB3) related to subsequent weight gain and blood pressure elevation through leptin-mediated sympathetic activation. On the other hand, SNP in methylenetetrahydrofolate reductase gene (MTHFR) significantly associated with plasma homocysteine level and the carotid atherosclerosis depending on smoking status in the Suita Study. Finally, our results in the Ohasama Study suggested the usefulness of the genetic cohort approach to detect the effect of SNPs on the increase of blood pressure along with aging. I would like to introduce recent progress of ‘gene-environmental interaction in hypertension’ based on Japanese epidemiological studies and discuss the feasibility of ‘optimal environmental setting based on individual genotype’.

EFFECTS OF FOOD RESTRICTION ON A RAT METABOLIC SYNDROME MODEL

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The SHR/Ndmer-cp/cp (SHR-cp) rat is a new model for obesity, hypertension, hyperlipidemia and insulin resistance. In the present study, we examined the influence of food restriction on SHR-cp rats. Eight-week-old male SHR-cp rats and Wistar-Kyoto rats (WKY) obtained from the Disease Model Cooperative Research Association (Kyoto, Japan) were used. Food restriction was performed by supplying food (MF, Oriental Yeast Co, Tokyo, Japan) every other day from 8 weeks of age to 20 weeks of age. Control rats were fed the same diet every day. Marked obesity, reaching 600 g, was observed in the control SHR-cp, while mean body weight of the SHR-cp with food restriction was about 400 g at the end of the experiment. The body weight of WKY was 446 g without food restriction, and 342 g with food restriction. The liver weight per 100 g of body weight in the SHR-cp with the food restriction was about half of that in those without the food restriction. Histological examination indicated that fat deposition in the liver was less in the SHR-cp with food restriction than in those without. Plasma GOT, GPT, glucose, triglycerides, and the total cholesterol levels in the SHR-cp with food restriction were significantly lower than those of SHR-cp without the food restriction. As for WKY, there was no difference between the two groups with regard to liver weight or histological findings. Blood biochemical values, except for glucose and triglycerides, were within the normal range and, interestingly, comparable with those in the restricted SHR-cp. Food restriction improved the symptoms of metabolic syndrome in SHR-cp. It is also suggested that SHR-cp will be the useful model in studies on metabolic syndrome.

ANALYSIS OF CIRCADIAN VARIATIONS IN SYSTOLIC ARTERIAL PRESSURE, HEART RATE AND LOCOMOTOR ACTIVITY IN CONGENIC RATS

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The congenic rats (SHRSPhwch1.0) were derived from stroke-prone SHR/Izumo (SHRSPhw/Izm) and Wistar-Kyoto rat/Izumo (WKY/Izm). We studied the circadian variations of systolic arterial pressure (SAP), heart rate (HR) and locomotor activity (ACT) in SHRSPhwch1.0. We also studied the effect of central selective noradrenergic neurotoxin, DSP-4 (N-2-chloroethyl-N-ethyl-2-bromobenzylamine HCl), on the circadian variations of SAP, HR and ACT. We used ten male mature SHRSPhwch1.0 and six age-sex matched SHRSPhw/Izm for the control. SAP, HR and ACT were monitored using radiotelemetry, and circadian variations in SAP, HR and ACT were analyzed using the maximum entropy method. SHRSPhwch1.0 were heavier than SHRSPhw/Izm (352±15 vs. 279±15 g, P<0.001). As expected, SAP in SHRSPhwch1.0 was lower than in SHRSPhw/Izm (194±9 vs. 229±15 mm Hg, P<0.001). HR in SHRSPhwch1.0 was slower than in SHRSPhw/Izm (310±9 vs. 381±45 beats/min, P=0.004).

Both daytime and nighttime SAP and HR in SHRSPhwch1.0 were lower than those in SHRSPhw/Izm. SHR, HR and ACT in SHRSPhw/Izm were clearly evident, compared to SHRSPhw/Izm. 24-hour periodicities were dominant in SAP, HR and ACT of SHRSPhwch1.0, compared to those in SHRSPhw/Izm. DSP-4 did not affect the 24-hour periodicities of SAP, HR and ACT in SHRSPhwch1.0. The circadian variations of SAP, HR and ACT appear better maintained in SHRSPhwch1.0, compared to SHRSPhw/Izm. Further work is needed to confirm the effect of noradrenergic neurons on circadian variations of SAP, HR and ACT.

GENETIC LOCUS ON RAT CHROMOSOME 20 REGULATES DIET-INDUCED ECTOPIC FAT STORAGE AND GLUCOSE INTOLERANCE

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Previous study suggests that a 21-Mb telomeric segment of rat chromosome 20 (RNO20) contains a gene(s) regulating diet-induced obesity and glucose intolerance. The aim of this study was to narrow down the chromosomal region mediating this effect. Sixteen-week-old male SHR, a congenic strain (SHR.IN), and two subcongenic strains (SHR.BN1, SHR.BN2) were subjected to a 12-week high-fat diet (HFD). SHR.IN, SHR.BN1, and SHR.BN2 differ from SHR by overlapping segments of RNO20 originating from Brown Norway rats. At the end of the 12-week HFD, glucose tolerance, body-weight (BW) gain, organ/tissue weights, lipid content and fibre cross-sectional area of skeletal muscles and heart weights (p=0.01-0.003), but not for epididymal fat pads weight (p=0.46). Skeletal muscles demonstrated the presence of intra-myoecellular fat droplets and a trend towards increased fibre size in SHR.IN and SHR.BN1 versus SHR and SHR.BN2 (p=0.07). Expression of the peroxisome proliferator-activated receptor (PPAR)γ-gene, located within the differential chromosomal segment of SHR.BN1 and encoding a transcripti on factor regulating fat burning, showed an opposite tendency. The 10-Mb differential segment of SHR.BN1 regulates HFD-induced increases in BW associated with ectopic fat storage in muscle and glucose intolerance. Whether the PPAR-γ gene is the gene mediating these effects requires further investigation.

CONGENIC AND SUBCONGENIC BLSHR RATS: MAPPING OF CANDIDATE GENES FOR FACETS OF THE METABOLIC SYNDROME

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Congenic BB.SHR (D4Got1-Npy-Tacr1); BB.4S) rats develop an incomplete metabolic syndrome with obesity, hyperleptinemia, and

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dyslipidemia as compared to their progenitor strain, the diabetes-prone BB/OK rats. In order to increase the chance of identifying relevant gene(s) the introgressed chromosomal segment of BB.4S rats was systematically narrowed down to generate recombinants and new subcongenic lines carrying smaller and overlapping segments. The gene expression analysis was used to favor genes, which may underlie facets of the metabolic syndrome. BB.4S rats were crossed with BB/OK rats, their F1 hybrids were intercrossed and genetically analysed for markers on chromosome 4. By this procedure 7 subcongenic BB.SHR rat lines – briefly termed BB.4Sb, c, d, e, f and g – were generated. 20 males of BB.4S and 20 males of each subcongenic line were longitudinally characterized for facets of the metabolic syndrome. In addition, the expression of 20 genes was analyzed in blood, liver, subcutaneous and epididymal adipose tissue located in the exchanged region on chromosome 4. A potential candidate gene was sequenced in parental strain BB/OK and chromosomal donor as well as in additional 7 rat strains (F344, BN, DA, LEW, hHTg, WOKW and their founders WOK-F and 4 wild rats). Body weight gain was comparable between BB.4S and their subcongenic derivatives except for male BB.4S rats which showed body weight gain found in the parental strain BB/OK. Serum lipids, insulin and leptin varied more or less between BB.4S and their subcongenic derivatives. Gene expression analysis showed significant differences between BB.4S and its subcongenic derivatives favouring a region of 1 Mb on chromosome 4. Sequencing of the candidate gene, Repin1 (replication initiator 1) a SNP and a triplet repeat in the 3′ untranslated region (3′UTR) was found. Wild rats were heterozygous for the SNP (C/T), all inbred strains were homozygous for C or T. The shortest triplet repeat was found in SHR (5) and highest in hHTg and WOKW (11) developing metabolic disorders. The repeat number correlated with most phenotypic traits studied and relative expression of neighboring genes of Repin1, Rarres2 and a hypothetical protein (Hp). However, using linear multiple regression analysis with the repeat size as dependent variable and considering all data of this study it was clearly demonstrated that not only body weight, serum insulin, VLDL cholesterol and the relative expression of Hp in visceral adipocytes and liver, but also the expression of Repin1 in the liver was significantly associated with the repeat size of the 3′UTR. Based on the phenotype and genotype in BB.4S and its subcongenic derivatives, the most important region for body weight, serum lipids, insulin and leptin can be mapped between D4Rat28 and D4Rat168 (ca. 1 Mb) on rat chromosome 4. It is concluded that the triplet repeat expansion in 3′UTR of Repin1 lead to metabolic disorders as found in hHTg and WOKW rats and that both functional unknown genes, Repin1 and Hp, could be novel candidate genes in the development of metabolic disorders which could be confirmed by studies in human analyzing SNPs of Repin1 in obese and lean probands.

ANTIOXIDANT THERAPY FOR METABOLIC SYNDROME WITH ALLYLMERCAPTOCAPTOPRIL IS NEPHROPROTECTIVE

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The obese SHR (SHROB) is a model of metabolic syndrome which spontaneously develops end stage renal disease. Allylmercaptocaptopril (AMC) is a conjugate of captopril with allicin, an active component of garlic. We sought to determine if oxidative stress is increased in SHROB relative to SHR and if antioxidant actions contribute to nephroprotective actions of AMC and captopril. Male SHROB were treated for 60 days with vehicle, AMC (53.5 mg/kg/d) or an equimolar dose of captopril (40 mg/kg/d), orally via chow. Plasma and kidney peroxides were assayed as markers of oxidative stress using the ferric orange xylene (FOX) method (Sigma). Both agents lowered tail cuff blood pressure, but AMC was more effective (vehicle: 195±1; AMC: 139±3; captopril: 157±2 mm Hg). AMC lowered free fatty acids, but captopril had no effect (vehicle: 0.9±0.05; AMC: 0.6±0.04; captopril: 0.8±0.1 mmol/l). AMC showed significant nephroprotection, as indicated by reductions in urinary protein loss (vehicle: 179±26; AMC: 86±17; captopril: 134±29 mg/day), urinary protein/creatinine, and plasma creatinine. Oxidative stress was increased in untreated SHROB rats relative to age-matched lean SHR, as indicated by a 3-fold increases in levels of peroxides in the plasma (19±2 versus 6±0.5 μmol/l) as well as in the kidney. Antioxidant antihypertensive therapy with either AMC (11±0.5 μmol/l) or captopril (12.0±0.7 μmol/l) both lowered oxidative stress in SHROB by nearly 2-fold. Both AMC and captopril are effective in attenuating metabolic syndrome, possibly in part through antioxidant actions. AMC has additional effectiveness on blood pressure and free fatty acids, thereby further retarding renal disease.

THE DEVELOPMENT OF MALIGNANT HYPERTENSION IN Cypl1a-REN-2 TRANSGENIC RATS IS ATTENUATED BY INHIBITION OF SOLUBLE EPOXIDE HYDROLASE

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We tested the hypothesis that increasing the level of epoxyeicosatrienoic acids (EETs) by inhibition of soluble epoxide hydrolase (sEH) would attenuate the development of angiotensin II (ANG II)-dependent hypertension in transgenic rats with inductive expression of the mouse Ren-2 renin gene. Hypertension was induced in Cypl1a-REN-2 rats through dietary administration of the xenobiotic indole-carbinol (I3C; 0.3% in rat chow) for 11 days. The sEH inhibitor, cis-4-[4-(3-Adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (c-AUCB), was given in drinking water (0.5 mg/day) starting 48 hours before I3C administration. Blood pressure (BP) was monitored by radiotelemetry in conscious animals. At the end of the experiment rats were decapitated to determine plasma and kidney ANG II levels by RIA. I3C administration (n=12) resulted in severe hypertension with characteristics of malignant hypertension including a substantial loss of body weight (BW) of 46 g when compared with basel BW. These I3C-induced changes were associated with marked elevations of plasma (64 ± 11 vs. 20 ± 6 mmol/ml, p<0.05) and intraneural (466 ± 59 vs. 81 ± 12 mmol/g, p<0.05) ANG II levels when compared with non-induced rats (n=8). Treatment with c-AUCB (n=12) attenuated the development of hypertension when compared with untreated rats (systolic BP 165 ± 6 vs. 198 ± 5 mmHg, p<0.05) and prevented the loss in BW but did not alter ANG II levels. These data demonstrate that sEH inhibition attenuates the development of hypertension in this model of inducible ANG II-dependent malignant hypertension and further support the notion that EETs possess substantial antihypertensive properties.

PANEL DISCUSSION:
CIRCULATORY CONTROL IN ESSENTIAL HYPERTENSION

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Many molecular geneticists feel that progress in defining the genes that raise blood pressure (BP) in essential hypertension (EH) has been inadequate. The search for mutant genes has been linked to BP...
regulatory systems that are often poorly defined and of uncertain relevance to EH, but they represent the approaches studied in monogenic types of hypertension. It may be a legacy of G.W. Pickering's view on the nature of EH: he thought that an individual's BP was determined by a large number of genes and environmental influences. He thought that EH was simply a quantitative deviation from the norm, which made him doubt that EH was caused by "a unique and specific fault which distinguishes those with the disease from those without it" (1). This conjecture may have been too pessimistic. Some geneticists believe that the dearth of progress in EH genetics has been due to the ambiguity of the high BP phenotype (2,3). Such ambiguity is inevitable because BP is affected by all that goes on in the circulation. This makes it ideal for predicting prognosis in EH, for deciding when to institute treatment, and for biometrical estimates of BP inheritance. But BP alone provides very little information about underlying mechanisms. It must be related to variables that provide information about specific components of the cardiovascular control system. One well known system, developed by Guyton about 40 years ago, does not explain EH. It regards all hypertension as due to volumed overload in a dysfunctional kidney with a limited capacity to excrete salt and dismisses a role for the autonomic nervous system (ANS) in long-term circulatory regulation and (4). In contrast, a recent analysis indicates that brain and periphery both contribute to the development of EH (5). The circulatory control system has non-linear "adaptive" properties, so that its parameters are altered actively when operating conditions exceed certain limits. Both environmental and genetic influences are necessary for developing EH. This also applies to the borderline hypertensive rat (BHR: F; hybrid of SHR x WKY), which is a good model for EH. In SHR, with double the high BP genes of BHR, hypertension develops in both "healthy" and "adverse" environments. The main environmental causes of EH are mental stress, high salt intake and obesity, giving rise to the syndromes of: 1) Stress-and-salt related EH (SSR-EH) and 2) Hypertensive obesity, i.e. stress-related EH + normotensive obesity. Smoking and high alcohol intake may contribute to each syndrome. Stress is sensed through thalamocortical and limbic neurons and raises the activity of dopaminergic (DA) neurons that affect a pathway linking the prefrontal cortex to the hypothalamus. This raises BP through the "getting-ready-for action" variant of the defense response, with characteristic sympathetic-adrenal changes. Chronic stress raises BP in both normal persons and those susceptible to EH. When the stress is over the response subsides rapidly in the former. However, in those developing EH there is gradual sensitization of DA synapses linking prefrontal cortex to the hypothalamus, so that the defense response is elicited by ever lower levels of stress. This property resembles that of memory neurons during learning. The defense response also raises plasma cortisol. This inhibits nitric oxide synthase (NOS), reducing availability of endothelial NO and raising the amount of reactive oxygen species in the vasculature. Both accentuate the neural vasoconstriction. Remodelling of the structure of the larger resistance vessels further enhances constriction and BP. Downstream there is accentuation of heterogeneity of microcirculatory perfusion, with closure of some microvessels (rarefaction) and raised perfusion in others. These changes contribute to the reduction in blood volume in SSR-EH and to functional deterioration of vital organs. In older people there is preferential elevation of systolic BP as the conduit arteries become stiffer. The stress-induced vasoconstriction increases BP-sensitivity to high salt, by raising blood-brain barrier permeability to sodium. Sodium activates a pressor pathway to the hypothalamic defense area via neurons that release cardiotoxic steroids. Persons with SSR-EH tend to eat more, but are only slightly overweight, because the system regulating energy balance responds well to feedback signals from the accumulating fat. However, these are ignored by the brain of some individuals, who become obese. About 50% of obese persons remain normotensive, and the rest develop hypertensive obesity (SSR-EH + normotensive obesity). This may be a behavioural response to alleviate stress-related anxiety. Their blood volume is greater than in SSR-EH, owing to the renal actions of hyperinsulinaemia. Their sympathetic pattern is similar to that in SSR-EH, but the insulin-induced peripheral vasodilatation masks the vasoconstriction. Perfusion heterogeneity is smaller in most tissues than in SSR-EH, except in the kidney. In obesity signals from adipocytes and several other factors impair tissue glucose transport. This accounts for the gradual development of non-insulin dependent diabetes mellitus, atherosclerosis and left ventricular and renal failure. In both syndromes of EH, the forebrain and hypothalamus play key roles in the autonomic responses to stress, diet and other lifestyle factors in EH. Cardiopulmonary load also increases and accounts for the tonic changes in baroreflex properties. Later the non-neural changes are superimposed on the neural effects. As regards to treatment of EH, the beneficial effects of most anti-hypertensive drugs is largely due to non-specific lowering of BP. In contrast, non-pharmacological management through exercise, reduction of salt and calorie intake, and cessation of smoking specifically antagonise the mechanisms that initiate EH. From the evolutionary viewpoint there may be a short-term advantage to "get-ready-for action" more easily through sensitization of the defense pathway. However, the marked prolonged vasoconstriction and large cardiopulmonary loads are long-term disasters. Probably the best prescription for preventing EH is to encourage a healthy lifestyle from an early age.


ANALYSIS OF RENAL PHENOTYPE-GENOTYPE RELATIONS TO PREVENT CARDIOVASCULAR DISEASE

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A large body of evidence accumulated during the last decade that supported a link between impairment of renal function and cardiovascular risk. Although the increasing incidence of end stage renal disease (ESRD) is without any doubt a major health problem worldwide, moderate renal impairment in the absence of ESRD is of probably far greater epidemiological importance due to its high prevalence and the associated cardiovascular disease. Moderate renal impairment reflects disturbances of the complex network of renal physiology at several levels governing the regulation of glomerular filtration. This leads to an impairment of glomerular filtration rate (GFR) or an increased permeability of the glomerular filtration barrier for macromolecules including albumin, or a combination of both. Thus, in the clinically setting the measurement of serum creatinine or more recently cystatin C concentrations in the blood to estimate GFR levels and the determination of increased urinary albumin excretion (UAE) rates are established and simple laboratory tests to assess moderate renal impairment. Numerous studies have been reported using these parameters demonstrating a consistent link between moderate reductions in GFR or increases in UAE and increased cardiovascular risk. It is hoped that the development of genomic systems biology strategies, e.g. in appropriate model systems including the rat, will allow a better understanding of the functional links between moderate renal impairment and cardiovascular disease. Moreover, to unravel the genotype-phenotype relations for this important connection may lead us to the development of more powerful means for early detection and prevention of cardiovascular disease.
PHARMACOGENETIC NEXUS OF POLYDACTYL- LUXATE AND METABOLIC SYNDROMES

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Polydactylyous rat strain PD/Cub is an inbred model of the metabolic and polydactyly-luxate syndromes. We have previously shown that predisposition for both syndromes colocalizes on rat chromosome 8. We tested the hypothesis of a pharmacogenetic link between metabolic and polydactyly-luxate syndromes. Adult male rats (n=12 strain) of SHR and congenic SHR.PD-(D8Rat42-D8Arb23)/Cub (SHR-Lx PD5) strains, differing in ca 1.4 Mb region of chromosome 8 of PD/Cub origin conferring susceptibility both to metabolic and polydactyly-luxate syndromes, were fed a high sucrose diet (HSD) for two weeks and subsequently treated with retinoic acid (RA, 15 mg/kg) for 16 days, while still on HSD. We contrasted metabolic profiles (incl. oral glucose tolerance test, insulin, adiponectin, free fatty acids, triglyceride and cholesterol in 20 lipoprotein fractions) between SHR and SHR-Lx PD5 under conditions of standard, HSD and HSD + RA administration. We observed noticeable division in effect of RA between SHR and SHR-Lx PD5 strains. SHR.Lx PD5 reacted with significant impairment of glucose tolerance and less favorable distribution of cholesterol and triglycerides into the lipoprotein fractions in comparison with SHR. Significant interactions between strain and diet/RA factors were found for free fatty acids, insulin and several morphometric parameters. We demonstrated interaction of retinoic acid with a 14- gene region of rat chromosome 8 affects the features of metabolic syndrome. Our results support the notion of interconnection of morphogenetic and metabolic processes, which are assumed in several human syndromes such as Bardet-Biedl or Smith-Lemli-Opitz.

GENETIC DISSECTION OF THE LEXF/FXLE RECOMBINANT INBRED STRAINS

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The FXLE and LEXF strain panel consists of 34 recombinant inbred strains (26 + 8 sublines) and was derived by reciprocal crossing of F344/Stm and LE/Stm. Although these RI lines were historically generated to study genes involved in tumor genesis, their value for the detection of non-cancer related quantitative traits has already been proven. Here, we report on genetic dissections of complex traits using this RI set and their parental strains. The origin conferring susceptibility both to metabolic and polydactyly-luxate syndromes, were fed a high sucrose diet (HSD) for two weeks and subsequently treated with retinoic acid (RA, 15 mg/kg) for 16 days, while still on HSD. We contrasted metabolic profiles (incl. oral glucose tolerance test, insulin, adiponectin, free fatty acids, triglyceride and cholesterol in 20 lipoprotein fractions) between SHR and SHR-Lx PD5 under conditions of standard, HSD and HSD + RA administration. We observed noticeable division in effect of RA between SHR and SHR-Lx PD5 strains. SHR.Lx PD5 reacted with significant impairment of glucose tolerance and less favorable distribution of cholesterol and triglycerides into the lipoprotein fractions in comparison with SHR. Significant interactions between strain and diet/RA factors were found for free fatty acids, insulin and several morphometric parameters. We demonstrated interaction of retinoic acid with a 14- gene region of rat chromosome 8 affects the features of metabolic syndrome. Our results support the notion of interconnection of morphogenetic and metabolic processes, which are assumed in several human syndromes such as Bardet-Biedl or Smith-Lemli-Opitz.

OSTEONECROSIS OF FEMORAL HEAD IN THE STROKE PRONE SPONTANEOUSLY HYPERTENSIVE RATS - ESPECIALLY IN OLD RATS

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The SHRSP is an animal model of idiopathic osteonecrosis of the femoral head (ION). The judgment of ION in rats can be diagnosed on the femoral heads by the sacrifice. No investigation was performed on the situation and frequency of the middle and older aged rats. The average life span of Stroke Prone Spontaneously Hypertensive Rats (SHRSP) is about eight months. By using 40-week-old SHRSPs, the ION was researched. Nineteen male 34-week-old SHRSP/Nagasaki rats were classified into two groups by the steroid hormone administering. The rats of S group were subcutaneously injected with methylprednisolone acetate 4 mg on the back at the age of 38 weeks. At 40 weeks, rats were sacrificed. About 40 % of old necrosis and 20 % of early necrosis was seen in the control group. With the steroid hormone administering, the increasing and degeneration of adipocyte in bone marrow, 20 % of fresh necrosis were recognized. Furthermore, the adipocyte change and the early necrosis among the old necrosis with steroid hormone administering were observed. On the serum biochemistry, the steroid hormone administering group increased T-CHOL, HDL, LDL, and TRG significantly compared with the control group and presented hyperlipemia. From this study, the normal femoral head has firstly adipocyte hyperplasia, secondly adipocyte degeneration, thirdly the early necrosis, the fresh necrosis and finally the old necrosis in long-term passage. The steroid hormone administration accelerated the above process and makes the old necrosis accompany with adipocyte hyperplasia, adipocyte degeneration, the early necrosis, and the fresh necrosis.

PANEL DISCUSSION:
THE REASON WHY PHYSIOLOGISTS NEED GENETICS

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Although physiologists have successfully identified a host of mechanisms involved in the short term and long term regulation of blood pressure, they have been unable to determine which mechanisms are primarily responsible for initiating the pathogenesis of essential hypertension. Because of the multitude of complex feed back interactions involved in the regulation of blood pressure, the quest by physiologists to unearth this holy grail of hypertension has been, and will remain, futile. Thus, notwithstanding the enormous role of physiological research in uncovering the mechanisms that regulate blood pressure and in identifying valuable targets for therapeutic intervention, we must turn to other approaches to uncover the prime movers of spontaneous hypertension. Based on the undeniable success of geneticists in uncovering the fundamental causes of monogenic forms of hypertension together with the extraordinary technical advances occurring in the identification of genes that underlie complex traits, it is apparent that genetic strategies provide the best hope for elucidating the primary mechanisms instigating essential hypertension. Of course, while recognizing the preeminence of genetic approaches for pinpointing the principal mechanisms that initiate hypertension, the scientific community will continue to critically depend on physiologists for complex phenotypic analyses and therapeutic studies of blood pressure and related cardiovascular traits.
NUCLEAR AND MITOCHONDRIAL GENE VARIANTS THAT INFLUENCE HYPERTENSION AND THE METABOLIC SYNDROME: LESSONS FROM THE SHR

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The SHR was first described by Okamoto and Aoki more than four decades ago and is one of the most widely studied genetic models of hypertension and the metabolic syndrome. The recent identification of specific genes that regulate blood pressure and associated metabolic phenotypes in the SHR has confirmed that this model can be successfully used to identify QTL at the molecular level that influence the pathogenesis of hypertension and related metabolic disorders. This presentation will discuss how the application of emerging rat genome tools and resources in combination with physiological studies in SHR recombiant inbred, congenic, transgenic, and comprolasic strains has enabled the identification of specific nuclear and mitochondrial genes including Cd36, Srebf1, and mt-Co1 that influence an assortment of biochemical and hemodynamic features of the metabolic syndrome. The relevance of these genes to mechanisms and therapies for insulin resistance and hypertension in humans will also be discussed. The results in SHR together with corresponding findings in humans illustrate an important role for inherited variation in lipid metabolism and ectopic accumulation of fat in risk for multiple components of the metabolic syndrome.

USING ENGINEERED HEART TISSUE TO ACCELERATE POSITIONAL CLONING

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The polygenic nature of resistance/sensitivity of the heart to ischemia is generally accepted. Unfortunately, little is known about gene(s) involved in response to this insult. The goal of the present study was to introduce new tool, engineered heart tissue (EHT), for accelerating positional cloning and for providing novel functional assays. EHT were generated from rat cells isolated from BN, SS, and conomisc SSBN6 hearts. EHT were produced by combining 2.5 million cells and 1 mg collagen per mL of tissue with smaller amounts of Matrigel, horse serum. Functional measurements related to force of contraction were conducted with a probe attached to a force transducer. The EHT was then tested after exposure to hypoxia and the results were compared to both in vitro Langendorf heart preparations, and in vivo animal studies. The EHT was studied for the following phenotypes-transition of fibroblast to myofibroblast, frequency and Frank-Starling mechanism of the beating EHT. Interestingly twitch force measured before and after a 6 hour bout of hypoxia in BN and SSBN6 was significantly higher in comparison with SS rats. These data correlate with findings in isolated hearts and in vivo. Our results indicate that the phenotypic differences between rat strains observed in isolated hearts correlate well with mechanical measurements in engineered heart tissue. We see the application of EHT and other models of engineered tissue as a new paradigm for in vivo physiology and genomics research as we can conduct phenotyping in neonates and using gene therapy vectors transduce genes into a functional segment of heart tissue.

BRAIN RAAS, SYMPATHETIC ACTIVITY AND SALT-SENSITIVE HYPERTENSION

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The presence of enzymes involved in steriodogenesis (including aldosterone synthase), mineralocorticoid receptor (MR) and 11P-HSD-2 and of all components of the RAS cascade has been well documented in several parts of the CNS including the hypothalamus. Both Ang II and aldosterone acting in the CNS may be circulation-derivered or locally synthesized. Evidence for a functional role for locally in the CNS produced aldosterone is recently emerging. Sympatho-excitatory and hypertensive responses to central infusions of aldosterone are MR mediated and enhanced by CFIP[Na+]↑. MR activation by aldosterone appears to activate CNS pathways by enhancing ENaC activity followed by “ouabain” release and AT1-receptor stimulation, associated with a decrease in NOS activity and increase in ROS in eg, the PVN. These same CNS mechanisms appear to be activated by central infusions of Na+-rich artificial cerebrospinal fluid (aCSF) and by high salt intake in Dahl S and SHR. Both strains show an increase in CFIP[Na+]↑ on high salt intake and exhibit enhanced sympatho-excitatory and pressor responses to CFIP[Na+]↑. Both components appear to relate to abnormal MR-Enac regulation and central infusions of either a MR blocker or ENaC blocker prevent an increase in hypothalamic “ouabain”, sympathtico-excitation and hypertension on high salt intake. Recent studies with central infusions of an aldosterone synthase inhibitor suggest that in the CNS Na+ increases aldosterone biosynthesis and in Dahl S rats most of the MR activation appears to be due to an increase in locally produced aldosterone. In conclusion, in a remarkable parallel to Ang II, it has become apparent that beyond its classical role as a hormone regulating renal Na-transport, aldosterone exerts profound effects on arteries and the heart. Its central actions are more slowly emerging, but may play a crucial role in cardiovascular homeostasis, when challenged by high salt. Activation of the brain RAAS plays a pivotal role in the salt-induced sympatho excitation and hypertension in genetic models of salt-sensitive hypertension. Further research on the genetic variants and molecular and cellular pathways mediating the central responses to high salt intake in Dahl S or SHR may provide new perspectives into salt induced hypertension.

THE CHANGE OF KYNURENE AMINOTRANSFERASE AND KYNURENIC ACID IN SPONTANEOUSLY HYPERTENSIVE RATS

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Kynurenic acid (KYN) converted from L-kynurenine (L-KYN) by kynurenine aminotransferase (KAT) is an important metabolite involved in the tryptophan metabolism and represents the only known endogenous compound acting as an antagonist to excitatory amino acid receptors. It had been suggested that endogenous KNYA in the brain might play a role in the central regulation of blood pressure. This study was intended to analyze the role of KNYA in kidney by comparing the urinary KNYA concentration, as well as the enzyme activity and gene expression of KAT in spontaneous hypertensive rats (SHRs) and Wistar-Kyoto rats (WKYS). 12 SHRs and 12 WKYS aged 4 weeks, 16 or 24 weeks old (4 rats in each age group) were used. Urinary KNYA concentration was measured by high performance liquid chromatography (HPLC). The activity of KAT was assayed by the conversion of L-KNYA to KNYA and quantitated by HPLC with fluorescence detection. The mRNA expression of KAT was detected by real time polymerase chain reaction (RT-PCR). The activity of KAT was higher in renal medulla than in renal cortex in both SHRs and WKYS. The KAT activity of SHRs was lower compared to WKYS. The KNYA concentration was significantly lower in SHR compared to WKYS (p=0.045 for 4-week-old; p=0.017 for 16-week-old). The mRNA expression of KAT in renal medulla and renal cortex had the same tendency. The KNYA concentration was significantly lower in SHRs compared to WKYS (p=0.005 for all groups). The renal KAT activity and urinary KNYA concentration was lower in SHR compared to WKY, together with altered renal mRNA expression of KAT in SHR, which suggested that there might be a deficiency in renal regulation of blood pressure in SHR.
The vascular reactivity of hypertensive animals is characterized by enhanced contraction to norepinephrine (NE) and impaired acetylcholine-induced relaxation. Chronic treatment of spontaneously hypertensive rats (SHR) with angiotensin converting enzyme (ACE) inhibitors is often used to prevent the development of hypertension. The effect of this treatment on vascular reactivity of isolated vessels is still not fully understood. The aim of our study was to investigate the influence of chronic captopril treatment on NE-induced contraction in the presence or absence of endothelium. We used 10-week-old Wistar Kyoto (WKY) and SHR, half of them being treated with captopril (100 mg/kg/day) for 6 weeks from the age of 4 weeks. Contractions of isolated femoral arteries were measured using Mulvany-Halpern myograph. The presence of intact endothelium shifted NE dose-response curve of WKY femoral arteries to the right whereas this effect was attenuated in arteries from SHR. The removal of endothelium abolished this strain difference and enhanced the NE dose-response curve in WKY. Chronic captopril treatment reduced NE dose-response curve of vessels with intact endothelium from both WKY and SHR, but the effect was greater in arteries from SHR. On the contrary, NE dose-response curve of deendothelialized arteries was not influenced by captopril treatment. Chronic captopril treatment attenuated norepinephrine dose-response curve by improving the ability of endothelium to oppose smooth muscle contraction, this effect being greater in hypertensive than normotensive rats. This work was supported by grants no. LM0510 and 305/08/0139.

Oxidative Stress and Transcriptional Regulation of Angiotensin AT1 Receptors in Hypertension

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Antioxidant responsive transcription factor Nrf2, plays a major role in the activation of proteins, which provide protection from oxidative stress-induced cell damage. There is increased angiotensin AT1 receptor density in conditions associated with oxidative stress, such as hypertension and diabetes, which could be a main contributor to high blood pressure (BP). However, the molecular mechanisms responsible for oxidative stress-mediated AT1 receptor upregulation are not understood. Following animal groups were studied for responsiveness to vasoactive agents: SHR and WKY rats and Sprague Dawley (SD) rats treated with L-buthionine sulfoximine (BSO), glutathione pathway inhibitor, with or without antioxidant tempol for 3 weeks. SD rats kept on tap water served as control. There was increased oxidative stress, NF-xB activation and AT1 receptor upregulation in SHR than WKY rats. SHR showed decreased response to vasorelaxant compounds while the contractile response to Ang II was exaggerated and prolonged. Similar to SHR, the BSO-treated SD rats exhibited oxidative stress, NF-xB activation, AT1 receptor upregulation, endothelial dysfunction and high BP. Treatment of rats with tempol activated Nrf2, induced phase II antioxidant enzymes, which reduce oxidative stress, prevent NF-xB activation, normalize the AT1 receptor signaling and contribute to high BP. More importantly, antioxidants via Nrf2 activation induce phase II antioxidant enzymes, which reduce oxidative stress, prevent NF-xB activation, normalize the AT1 receptor signaling and lower BP.

Chronic Captopril Treatment Impairs Norepinephrine-Induced Contraction of Femoral Arteries of SHR by Its Effect on Endothelium

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Our previous results demonstrate that SHR, but not Brown Norway (BN) rats, decrease kidney weight and increase blood pressure in response to prenatal exposure to nicotine (PEN). We further examined PEN effects on renal morphology and genome-wide gene expression. Nicotine (6 mg/kg body weight) or saline was administered to dams via subcutaneous osmotic minipumps throughout gestation. Kidneys were removed from nine-week-old male SHR (nicotine: n=13; saline: n=12) and BN (nicotine: n=12; saline: n=12) offspring. Five kidneys per group were randomly selected to assess renal morphology using stereology. Total RNA and protein was extracted from kidneys. RNA samples from individual animals were pooled within each group to perform microarray analysis (Affymetrix; Genespring, GX). Protein expression was verified with Western blotting. Data were analyzed using two-way ANOVA with Strain and PEN as factors. SHR, but not BN, rats treated with nicotine vs. saline showed a trend towards decreased total kidney volume. No significant effect of PEN on cortical volume or on glomerular number or size was observed. Microarray analysis showed significant up-regulation of the angiotensin type 1 receptor b (Agtr1b) in SHR but not in BN rats treated with nicotine vs. saline. A similar trend was observed in total Agtr1 protein levels. In SHR, but not in BN rats, PEN decreases overall kidney size without altering glomerular number or size and up-regulates intra-renal expression of the Agtr1b. The latter may be related to the increase in blood pressure in response to PEN observed only in SHR.

Effects of Prenatal Exposure to Nicotine on Renal Morphology and Gene Expression in Two Genetically Distinct Strains of Rats

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The multi-potential mesenchymal stem cells (MSCs) are ideal source for clinical cell therapy and tissue engineering. This study aimed at determining whether the rat bone marrow mesenchymal stem cells (rMSCs) have differential potential into vascular endothelial cells or smooth muscle cells under specific induced conditions in vitro. MSCs were obtained from femurs and tibias of male Sprague-Dawley (SD) rats using previously described method and the cells isolated by density gradient centrifugation have typical MSCs phenotypes and multiple differentiation potentials. Induced by VEGF and bFGF after three days, the morphology of rMSCs changed to cobblestone-like and expressed specific markers of the endothelial cell lineage including von Willebrand factor (vWF), Flk-1 and CD31. After 14 days, the expression of endothelial phenotype markers is more obvious and the differentiation cells can form capillary-like structures on Matrigel (tube-formation assay). As well the part of cells can uptake Dil-Ac-LDL. However, induced by PDGF-BB after three days, rMSCs expressed smooth muscle cell specific surface markers, including a-SM-actin, Calponin and SM-MHC. These results suggested that rMSCs have potentials to differentiate into vascular endothelial-like and smooth muscle-like cells induced by different cultural condition in vitro, which may provide useful information for clinical cell therapy of vascular diseases.

Rat Bone Marrow Mesenchymal Stem Cells Differentiate Into Vascular-Like Cells

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The vascular reactivity of hypertensive animals is characterized by enhanced contraction to norepinephrine (NE) and impaired acetylcholine-induced relaxation. Chronic treatment of spontaneously hypertensive rats (SHR) with angiotensin converting enzyme (ACE) inhibitors is often used to prevent the development of hypertension. The effect of this treatment on vascular reactivity of isolated vessels is still not fully understood. The aim of our study was to investigate the influence of chronic captopril treatment on NE-induced contraction in the presence or absence of endothelium. We used 10-week-old Wistar Kyoto (WKY) and SHR, half of them being treated with captopril (100 mg/kg/day) for 6 weeks from the age of 4 weeks. Contractions of isolated femoral arteries were measured using Mulvany-Halpern myograph. The presence of intact endothelium shifted NE dose-response curve of WKY femoral arteries to the right whereas this effect was attenuated in arteries from SHR. The removal of endothelium abolished this strain difference and enhanced the NE dose-response curve in WKY. Chronic captopril treatment reduced NE dose-response curve of vessels with intact endothelium from both WKY and SHR, but the effect was greater in arteries from SHR. On the contrary, NE dose-response curve of deendothelialized arteries was not influenced by captopril treatment. Chronic captopril treatment attenuated norepinephrine dose-response curve by improving the ability of endothelium to oppose smooth muscle contraction, this effect being greater in hypertensive than normotensive rats.

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Antioxidant responsive transcription factor Nrf2, plays a major role in the activation of proteins, which provide protection from oxidative stress-induced cell damage. There is increased angiotensin AT1 receptor density in conditions associated with oxidative stress, such as hypertension and diabetes, which could be a main contributor to high blood pressure (BP). However, the molecular mechanisms responsible for oxidative stress-mediated AT1 receptor upregulation are not understood. Following animal groups were studied for responsiveness to vasoactive agents: SHR and WKY rats and Sprague Dawley (SD) rats treated with L-buthionine sulfoximine (BSO), glutathione pathway inhibitor, with or without antioxidant tempol for 3 weeks. SD rats kept on tap water served as control. There was increased oxidative stress, NF-xB activation and AT1 receptor upregulation in SHR than WKY rats. SHR showed decreased response to vasorelaxant compounds while the contractile response to Ang II was exaggerated and prolonged. Similar to SHR, the BSO-treated SD rats exhibited oxidative stress, NF-xB activation, AT1 receptor upregulation, endothelial dysfunction and high BP. Treatment of rats with tempol activated Nrf2, induced phase II antioxidant enzymes hez oxygenase-1 and glutathione S-transferase, which reduce oxidative stress, prevent NF-xB activation, normalize the AT1 receptor signaling and lower BP.

Effects of Prenatal Exposure to Nicotine on Renal Morphology and Gene Expression in Two Genetically Distinct Strains of Rats

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The multi-potential mesenchymal stem cells (MSCs) are ideal source for clinical cell therapy and tissue engineering. This study aimed at determining whether the rat bone marrow mesenchymal stem cells (rMSCs) have differential potential into vascular endothelial cells or smooth muscle cells under specific induced conditions in vitro. MSCs were obtained from femurs and tibias of male Sprague-Dawley (SD) rats using previously described method and the cells isolated by density gradient centrifugation have typical MSCs phenotypes and multiple differentiation potentials. Induced by VEGF and bFGF after three days, the morphology of rMSCs changed to cobblestone-like and expressed specific markers of the endothelial cell lineage including von Willebrand factor (vWF), Flk-1 and CD31. After 14 days, the expression of endothelial phenotype markers is more obvious and the differentiation cells can form capillary-like structures on Matrigel (tube-formation assay). As well the part of cells can uptake Dil-Ac-LDL. However, induced by PDGF-BB after three days, rMSCs expressed smooth muscle cell specific surface markers, including a-SM-actin, Calponin and SM-MHC. These results suggested that rMSCs have potentials to differentiate into vascular endothelial-like and smooth muscle-like cells induced by different cultural condition in vitro, which may provide useful information for clinical cell therapy of vascular diseases.
PHARMACOGENOMICS AND PHARMACOGENETICS OF HYPERTENSION: UPDATE AND PERSPECTIVES: THE ADDUCIN PARADIGM

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The ultimate goal of personalized medicine is to comprehensively identify genetic differences among persons and to correlate specific genetic features (or combinations of genetic features) with the differential risk of human diseases or the efficacy of certain therapeutic interventions. This goal is likely to be achieved in salt-sensitive hypertension with the adducin paradigm. High salt intake raises blood volume and vascular contraction, increases the workload of the heart, and induces natriuresis – excretion of excess sodium into the urine – which is a counterregulatory system involving the kidneys that restores normal osmotic pressure. In the kidneys, natriuresis occurs through the inhibition of sodium reabsorption from the tubular lumen in the nephron as well as through changes in the glomerular filtration rate and pressure. Among many, two mechanisms have been clearly implicated in salt-dependent hypertension: 1) a genetic one, related to polymorphisms of adducin (ADD1), a heterodimeric protein of the cytoskeleton, and 2) a humoral one sustained by endogenous ouabain (EO), a hormone released from the adrenal gland and the hypothalamus. Both lead to increased activity and expression of the Na-K ATPase, activate signal transduction through the Src-EGFr-ERK pathway resulting in cardiovascular and renal hypertrophy. Furthermore, to date, ADD1 mutated allele associates to cardiovascular risk in 8 distinct hypertensive populations and EO plasma levels are related to ventricular hypertrophy in hypertensives. Rostafuroxin is a new oral antihypertensive agent able to selectively antagonize adducin and EO hypertensive effects: at molecular level, normalizes the enhanced activity of the Na-K pump induced by ADD1 mutation and antagonizes the EO triggering of the Src-EGFr-dependent signaling pathway. In the vasculature, it normalizes the increased myogenic tone caused by ouabain. Such an approach has the potential to regulate blood pressure without the hypertensive side effects that occur with drugs that block all blood pressure regulation mechanisms. Together with initial evidence of high tolerability and efficacy in hypertensive patients, indicate Rostafuroxin as the first example of a new class of antihypertensive agents designed to antagonize adducin and EO-hypertensive mechanisms.

CLONING OF NOVEL HUMAN NUCLEAR FACTOR WHICH DOWNREGULATES THE NATHRIURETIC RECEPTOR/ GUANYLYL CYCLASE A GENE EXPRESSION

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GCA, the ANP receptor, can downregulate the transcription of its gene via a cGMP-dependent mechanism. We previously identified a consensus sequence for a cGMP-response element (cGMP-RE) in the GCA promoters of human, rat and mouse. Our goal was to identify DNA-binding proteins specific to the cGMP-RE of GCA human promoter. Using yeast one hybrid technique, we screened a cDNA library of human kidney to detect cGMP-RE binding proteins. We performed DNA binding and electromobility shift assays (EMSA) to confirm the DNA binding capacity of positive clone(s). Cultured cells were stimulated with ANP and gene expression was analysed by RT-PCR. Fluorescence microscopy was used to determine the cellular localization of the GFP-tagged clone. Functional studies were performed by co-transfecting cells with luciferase-coupled GCA promoter and a plasmid coding for the clone. We detected a positive clone, which interacts with the human cGMP-RE. It corresponds to a yet unidentified transcript of 1083bp. Fluorescence microscopy detected a nuclear localization of the GFP-fusion protein. DNA binding assays showed a 5-fold increase in binding capacity compared to controls and EMSA proved the specificity of the interaction. Functional studies showed that over-expression of the clone inhibited GCA promoter activity by 60% through the cGMP-RE. ANP increased the expression level of the factor by 50% while decreasing GCA mRNA levels by half. We identified a nuclear protein which binds to the cGMP-RE, controls GCA promoter activity and its mRNA levels. The characterization of this protein will help understand the transcriptional regulation of GCA.

PRODIABETOGENIC EFFECT OF TRANSGENIC RESISTIN EXPRESSION IN THE OLD SPONTANEOUSLY HYPERTENSIVE RAT

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Previously we have shown that adipose tissue-specific expression of the resistin transgene in the spontaneously hypertensive rats (SHR), was associated with increased serum free fatty acids, skeletal muscle triglyceride accumulation, glucose intolerance and resistance to insulin action. In this study we investigated if increased expression of resistin in adipose tissue has affected age-related change in glucose tolerance and insulin resistance of peripheral tissues in old transgenic SHR. 16 months old male SHR expressing the mouse resistin gene under control of fat-specific aP2 promoter were used. Control group comprised age-matched genetically identical rats with the absence of the transgene. All animals were fed a diet with 60% fructose for 2 weeks. Tissue sensitivity to insulin action and glucose oxidation were measured in vitro without or with insulin (250 μU/ml) according to basal and insulin-stimulated [14C]-U-glucose incorporation into muscle glycogen, CO2 or adipose tissue lipids. Old transgenic rats displayed higher body weight and elevated epididymal fat pad weight. Serum triglyceride concentrations were increased before administration of high-fructose diet and in both fasted and postprandial state (1.99±0.15 vs 1.34±0.11 mmol/l, p<0.01) after the fructose diet. The transgenic expression of resistin impaired the tolerance to the oral glucose load (AUC0-120: 1026±131 vs. 725±120 mmol/l/120 min, p<0.02), increased hyperinsulinemia (339±21 vs 270±24 pmol/l, p<0.05) and was associated with almost total adipose tissue resistance to insulin action. In soleus muscle, glucose oxidation was lower in transgenic rats whereas basal and insulin-stimulated glycogen synthesis was not different between SHR transgenic and control rats. Our data suggest possible involvement of resistin in age-induced development of insulin resistance and diabetes. This study was supported by grant 9/9387-3 from IGA MH CR.

ALTEDERED OXIDATIVE STRESS AND GLUTATHIONE METABOLISM PATHWAYS DURING DEVELOPMENT OF HYPERTENSION IN THE SHRSP

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The objective was to utilise congenic strains and microarray gene expression profiling during the development of hypertension to identify candidate genes and pathophysiological pathways underlying oxidative stress and hypertension in young SHRSP. Renal microarray profiles were compared with Affymetrix RGU34 genome chips from 5 week old SHRSP, WKY and the SP.WKY/Glaac2* (2c*) congenic strain containing Gstm1, a previously identified positional candidate gene for hypertension. Significant differential expression was determined by Rank Products (false discovery rate (FDR) of 5%) and analysed with Ingenuity Pathway Analysis. Superoxide was measured in whole kidney homogenates and glutathione (GSH) levels measured and analysed with ANOVA or T-test. Renal SHRSP, 2c* and WKY expression profiles were compared identifying significantly
differentially expressed genes involved in glutathione metabolism including Gstm1 (2c*vsSHRSP, FDR=0.0, FC=9.4, WKYvsSHRSP FDR=0.0, FC=7.5) and solute carrier 7, member12-like (Scl7a12-like) (2c*vsSHRSP, FDR=0.0, FC=2.9, WKYvsSHRSP, FDR=0.0, FC=2.3). Differential expression was confirmed by qRT-PCR and sequence analysis identified single nucleotide polymorphisms in the promoters. There was a significant increase in NADH stimulated superoxide levels in the SHRSP compared to 2c* and WKY (SHRSP 10.8±1.3, 2c* 8.5±1.1, WKY 8.3±0.9 µmoles/min/µg protein F=6.5, p=0.016) and GSH levels were significantly increased in WKY (SHRSP 18.3±3.4, 2c* 15.4±2.7, WKY 29.1±5.0 µmoles/mg protein F=20.9, p=0.001). At 16 week of age there was a significant increase in GSH levels only in the 2c* strain (5wk 15.4±2.7 v 16wk 20.5±1.6 p=0.006). These findings identify significantly regulated genes and disrupted pathways affecting oxidative stress and glutathione metabolism in the SHRSP during the development of hypertension.

CHARACTERISATION OF METABOLIC SYNDROME PHENOTYPES IN THE SHRSP USING CHROMOSOME 2 CONGENIC STRAINS

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We have previously shown that two weeks of 60% fructose feeding (FF) can result in metabolic changes to the SHRSP, relative to the WKY reference strain. Rat chr2 harbours genetic loci linked to a number of metabolic phenotypes. The aim of this study was to utilise a range of chr2 congenic substrains to extend characterisation of the FF SHRSP. SHRSP, WKY and seven congenic strains (N = 4 to 15) received FF diet for 2 weeks. An echocardiogram, fasted blood sampling and an i.p. glucose tolerance test (IPGTT) were performed prior to sacrifice. Hepatocyte lipid content was determined by Oil Red O staining. Fructose-induced changes in IPGTT, adiposity and left ventricular mass index (LVMI) are summarised in Table. We also observed changes in cardiac geometry and function (as altered relative wall thickness and reduced myocardial performance & ejection fractions, respectively). Lipid deposition was markedly increased in FF SHRSP livers. No exacerbation of phenotypes occurred in SHRSPs exposed to extended (6weeks) FF diet. However, WKY rats exhibited exaggeration of phenotypes following 6 weeks of FF feeding. We have confirmed the involvement of chr2 in metabolic changes occurring in the FF SHRSP. Taken together with the SHRSP’s cardiovascular traits, this model may prove useful in the identification of candidate genes and pathways in common with human metabolic syndrome.

<table>
<thead>
<tr>
<th></th>
<th>IPGTT (AUC)</th>
<th>Adiposity (mg/g)</th>
<th>LVMI (mg/g)</th>
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<tbody>
<tr>
<td>WKY</td>
<td>1059±36</td>
<td>19±1.1</td>
<td>2.48±0.06</td>
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<tr>
<td>SHRSP</td>
<td>1242±45***</td>
<td>24±0.9**</td>
<td>2.86±0.04***</td>
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<td>1259±62</td>
<td>27±10</td>
<td>2.72±0.01‡</td>
</tr>
</tbody>
</table>

**P<0.01 vs. WKY; ***P<0.001 vs. WKY; #P<0.05 vs. SHRSP; ##P<0.01 vs. SHRSP

RENAI FUNCTIONAL AND MORPHOLOGICAL DERANGEMENTS IN Cyp1a1-Ren2 Transgenic Rats with Inducible Ang II-Dependent Hypertension

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Recently, a transgenic rat line [TGR(Cyp1a1Ren2)] was created that allows the induction of various degrees of angiotensin (ANG) II-dependent hypertension. This transgenic rat line was generated by inserting the mouse Ren2 gene, fused to the promoter of the cytochrome P450 1a1 (Cyp1a1) into the genome of the Fischer rat. Induction of the Cyp1a1 promoter by chronic dietary administration of the aryl hydrocarbon, indole-3-carbinol (I3C) results in a fixed level of Ren2 gene expression and in the development of ANG II-dependent hypertension. At a dose of 0.3% (wt/wt), chronic dietary I3C administration induces malignant hypertension, characterized by pronounced loss of body weight, polyuria, polydipsia, lethargy, and piloerection. Cyp1a1-Ren2 rats with malignant hypertension also exhibit reduced renal plasma flow, increased renal vascular resistance and filtration fraction, augmented tubuloglomerular feedback responses, elevated PRA, and elevated plasma and intrarenal ANG II levels. Administration of the AT1 receptor antagonist, candesartan, prevented the development of hypertension, the associated changes in renal hemodynamics, and the augmented intrarenal ANG II levels. These findings indicate that AT1 receptor activation by ANG II, generated as a consequence of induction of the Cyp1a1-Ren2 transgene, mediates the increased arterial pressure, the associated changes in renal hemodynamics, and the enhancement of intrarenal ANG II levels in Cyp1a1-Ren2 rats with malignant hypertension. Many of the effects of ANG II in this form of malignant hypertension are mediated indirectly via activation of other systems that amplify its hypertensinogenic actions. In this regard, administration of either the superoxide anion dismutase mimetic, tempol, or the selective COX-2 inhibitor, nimesulide, markedly decreased arterial blood pressure in hypertensive Cyp1a1-Ren2 rats, indicating that both superoxide anion and COX-2 derived vasoconstrictor metabolites contribute to the elevated arterial pressure in this form of malignant hypertension. The elevated ANG II levels also activate counteracting compensatory mechanisms that dampen its effect. Administration of either the selective COX-2 inhibitor, nimesulide, or the selective neuronal NOS (nNOS) inhibitor, L-SMTG, decrease RPF and increase RVR indicating that both nNOS-derived NO and COX-2-derived NO and COX-2-derived vasodilator prostanoids exert pronounced renal vasodilator influences in hypertensive Cyp1a1-Ren2 rats. Such maintained renoprotective effects of COX-2-derived vasodilatory prostanoids and nNOS-derived NO act to prevent excessive renal vasoconstriction in Cyp1a1-Ren2 transgenic rats with malignant hypertension. The renal functional derangements in hypertensive Cyp1a1-Ren2 rats are accompanied by pronounced renal morphological changes, including myointimal hyperplasia and tubular dilation, glomerulosclerosis, and tubulointerstitial inflammation and proliferation, particularly in the perivascular area. Such renal morphological changes, together with the renal functional derangements would likely contribute to the inability of the kidney to maintain normal rates of sodium excretion at normotensive arterial pressures, and attenuate the natriuretic response to the ANG II-mediated elevation of arterial pressure. In this manner, the renal functional and morphological derangements would contribute importantly to the development of malignant hypertension in Cyp1a1-Ren2 transgenic rats.
GENETIC DEFICIENCY IN THE RENAL EXPRESSION OF CD36 INCREASES BLOOD PRESSURE IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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To search for quantitative trait loci (QTL) involved in the renal pathogenesis of essential hypertension, we combined genome wide expression QTL and quantitative trait transcripts (QTT) analyses of the kidney transcriptome in recombinant inbred strains derived from the spontaneously hypertensive rat (SHR/N). This strategy identified inherited variation in the renal expression of Cd36 encoding fatty acid translocase as a possible determinant of inherited variation in the risk for hypertension. In renal cross transplantation studies in SHR progenitor, transgenic, and congenic strains, selective genetic deficiency or congenic expression of wild type Cd36 in the kidney restored both renal and urine cGMP to normal levels and decreased blood pressure. These data provide evidence that a functional deficit exists in the renal nitric oxide system in the SHR/N strain and that this deficit can be repaired by transgenic or congenic expression of wild type Cd36 thereby implicating Cd36 in a novel renal gene pathway influencing blood pressure and the risk for spontaneous hypertension.

RISK FACTOR REDUCTION BY CALCIUM SUPPLEMENTATION IN MODEL RATS FOR METABOLIC SYNDROME, SHR/Ndmcp-cp (SHR-cp)

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We previously proved that Ca supplementation for 8 months significantly increased bone mineral content and decreased body fat in Japanese adolescent females, and epidemiologically observed that adult Masai consuming about 3 l of fermented milk daily demonstrated high bone mineral density, less body fat and increased muscle mass with higher resting energy expenditure. Therefore, we investigated the effect of Ca supplementation on bone and fat metabolism in SHR-cp genetically developing hypertension, hypercholesterolemia, hyperglycemia and obesity. 5-week-old SHR-cp, 18 males and 18 females were randomized into three groups: Funabashi SP diet-fed control group (C), 0.8% calcium carbonate containing SP diet group (CC) and 0.8% calcium lactate containing SP diet group (LC), and fed these 3 different diets for 16 weeks. Tail blood pressure (BP), body weight (BW), and food consumption were checked once a week. Blood and 24-hour urine samples were collected for checking total cholesterol and glucose, urinary protein, sodium and potassium. Visceral fat and all organs were weighed at dissection. LC but not CC showed significantly lower BP and higher bone weight/body weight ratio than C. Urinary protein excretion in LC was significantly lower than in C at 15 weeks. Despite the significantly increased food consumption in LC, the body weight of LC was not significantly different from those of 2 other groups. Dietary supplementation of Ca lactate, but not Ca carbonate could reduce BP, attenuate the increasing urinary protein excretion related to nephropathy noted in SHR-cp and increase significantly bone weight, suggesting enough daily milk product intake would be beneficial for reducing BP and improving not only bone metabolism but also renal functional deterioration related to obesity.

THE INDUCIBLE HYPERTENSIVE TRANSGENIC RAT – ITS ORIGIN AND EXPERIMENTAL POSSIBILITIES

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TGRRen2 transgenic rats, which carry the mouse Ren 2 gene, become hypertensive at an early age, and exhibit the characteristic features of malignant hypertension, including the presence of fibrinoid necrosis in the kidney. Cross-breeding studies using Fischer F344 and Lewis rats showed that the severity of the malignant hypertension was genetically determined and QTLs were identified on Chromosomes 10 and 17. The severe nature of the phenotype prevented a more detailed genetic mapping of the regions responsible for determining susceptibility. To facilitate further studies and identify the gene(s) responsible we therefore developed an inducible strain of hypertensive rat in which expression of the ren-2 enzyme could be controlled. This strain, termed TGRcyp1a1-Ren2, or the Inducible Hypertensive Rat (IHR), was generated on the Fischer F344 inbred genetic background. Expression of Ren-2 from the Cyp1a1 promoter can be induced through administration of the Indole-3-Carboline (I3C), a benign xenobiotic of natural origin. Induction of Ren 2 by I3C is dose-dependent and rats develop hypertension within 24 hours. The ability of the investigator to control the development of hypertension without surgical intervention is allowing the early events to be studied and a number of investigators are currently using this strain for studies of renal and vascular (dys)function. We have now extended our genetic studies to generate congenic rats that, in addition to the transgene, harbour the chromosome 10 locus from either Fischer F344 (sensitive) or Lewis (resistant) strains. Comparative studies demonstrate that we have captured the QTL and microarray and real time PCR studies have established a minimal set of genes that may be causal. The IHR and the recent congenic derivatives will allow detailed mechanistic studies to be undertaken to address both the establishment of end organ damage and its repair, and the molecular basis for differential sensitivity to the effects of a rapid increase in blood pressure.

RAT MODELS–TOOLS FOR UNDERSTANDING GENOTYPE AS AN INTERFACE BETWEEN ENVIRONMENT AND PHENOTYPE

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Hypertension as well as other multifactorial diseases has complex etiologies. Genetic factors for the diseases can be assumed as ‘functions’ modifying environmental inputs to output particular phenotypes. One of the goals of hunting genes responsible for multifactorial diseases is to clarify the ‘norm of reaction’ of genotypes, which is useful for modifying the environmental factors to prevent the diseases. In this context, genetic rat models are good research tool because they have no inter-individual genetic variations and it is easy to modify the environmental factors. Accordingly, one of the final goals of genetic studies on genetic model rats is to identify genes determining the responsiveness to various environmental stimuli. Salt intake is one of the most important environmental factors exacerbating hypertension and the related organ damage. Identification of genes determining the responsiveness to salt-intake has been therefore attempted in SHRSP. Using a series of congenic strains for the blood pressure QTL on chromosome 1, we found that severity of renal damage and incidence of cerebral stroke was clearly dependent on the difference in the genotypes of small chromosomal regions among the congenic strains. Of further interest, the severity of renal damage did not correlate with the blood
pressure, while the incidence of stroke did. This result implied that the renal damage was influenced by the susceptibility gen(s) in this chromosomal region independently of blood pressure. Another important environmental factor is various types of stresses. We showed evidence that the blood pressure QTL on chromosome 1 harbored a gene (or genes) influencing the sympathetic responsiveness to both physical and mental stresses. The same environmental inputs drew responses different between SHRSP and WKY, which may contribute to the difference in blood pressure between the two strains.

EVALUATION OF THE SYMPATHETIC RESPONSE TO THE COLD STRESS IN A SERIES OF RECIPROCAL CONGENIC STRAINS FOR THE CHROMOSOME-1 QTL

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Our previous study on the reciprocal congenic strains for the chromosome (Chr-1) QTL for blood pressure showed that genes responsible for the sympathetic hyperactivity to several stresses were in this region, which might have causal link with hypertension. To narrow down the region, a series of the ‘sub-congenic’ strains were examined in the present study. The Chr-1 QTL region was divided into smaller regions with 4 WKY-based and 8 SHRSP-based congenic strains. As simple markers for sympathetic activity, the urinary NE excretion and the urinary volume during 6 hours of cold stress (4 ºC) as well as the renal norepinephrine (NE) content were examined. Blood pressure (BP) was measured at a room temperature with the tail-cuff method. BP and the renal NE content were fluctuated among the series of congenic strains, implying that multiple loci in this region contributed to these phenotypes. By contrast, the response to cold stress estimated with the urinary volume and the urinary NE excretion seemed controlled largely by one major locus in a reciprocal manner. Our observation implied that the urinary volume and the urinary NE excretion under the cold stress were largely regulated by one locus in the Chr-1 QTL region independent of the genetic background.

DYSLFUNCTION OF ADENOSINE RECEPTOR IN VASORELAXATION RESPONSE OF SHR

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Adenosine is known to perform various physiological functions as an extracellular signaling molecule. We have previously shown that prejunctional Adenosine receptor dysfunction is caused in the caudal arteries of the SHR.Cg-Lepr1/NfDicer and NOS knockout rat, and that the amount of adenyl purine-release from rat caudal artery increased in a blood pressure-dependent manner. However, few reports have examined the relationship between postjunctional adenosine receptor and blood pressure. The present study investigated whether arterial adenosine receptors function is changed in SHR. The effects of adenosine receptor agonist and other relaxants on caudal arteries precontracted by phenylephrine isolated from WKY and SHR were examined. Intracellular calcium levels in caudal artery smooth muscle were also measured using confocal microscopic imaging. Relaxation responses to 2-chloroadenosine (an adenosine receptor agonist) in ring preparations of caudal artery isolated from SHR were significantly smaller than those from WKY. However, relaxation responses to forskolin (an adenylate cyclase activator) or 8-bromo-CAMP (a protein kinase A activator) displayed no significant difference between SHR and WKY. On the other hand, 2-chloroadenosine significantly decreased intracellular calcium ion levels in smooth muscle cells of caudal arteries. This 2-chloroadenosine-induced calcium decrease was significantly smaller in SHR than in WKY. These results indicate that caudal arterial adenosine receptor dysfunction is present in SHR. Insufficiency of vasorelaxation due to adenosine receptor dysfunction may be one of the reasons for hypertension.

GENETIC AND ENVIRONMENTAL INFLUENCES IN THE ASSOCIATION BETWEEN IMPAIRED FETAL GROWTH AND ADULT CARDIOVASCULAR HEALTH

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Epidemiological data have repeatedly shown that associations exist between early life development and risk of disease in adult life, most notably for cardiovascular disease and type 2 diabetes. The underlying mechanisms are not entirely understood, but it seems as if fetal growth retardation in utero followed by a rapid catch-up growth pattern in postnatal life, is deleterious for glucose metabolism, insulin sensitivity and haemodynamic regulation. Another accompanying feature is a less than normal number of capillaries and glomeruli. The original observations by Forsdahl, Barker and others pointed to the importance of growth restriction in utero due to poor maternal diet and caloric intake in association with poor social and hygienic living conditions in early 20th century urban surroundings or in poor countryside areas. Later on this view has been challenged, and the finding of an increased cardiovascular risk also in mothers of growth retarded offspring has suggested the influence of genetic factors acting across generations. One hypothesis based on this view was the so called “Fetal insulin hypothesis”, stating that genetic traits for insulin resistance were active both in the mother and in the feto-placental unit. Modern cohort studies have strengthened the view that these traits run in families and that the increased risk could be due to some gene-environmental influences, most notably on glucose metabolism and growth patterns. Furthermore, the introduction of epigenetics and fetal imprinting has shown that both paternal and maternal genes are of great importance to shape the growth development of the fetus. Some authors have even coined the term “genetic war” to describe the different, and sometimes opposite, genetic traits regulating fetal growth. A small mother could die from delivery of a very large baby, and therefore it is natural that some kind of growth restriction has to be programmed to avoid a deleterious outcome of such a pregnancy. One of the most well studied environmental factors influencing fetal growth is maternal smoking during pregnancy, known to increase the risk not only of growth restriction, but also of fetal loss, malformations, and early complications in neonatal life. In a long-term follow-up of a Swedish cohort of smoking mothers from the early 1960’s, we were able to show an increased mortality risk in offspring sons and an increased risk of small-for-gestational-age daughters. It is likely that some mothers with an increased genetic susceptibility to the negative effects of smoking could be more harmed than other mothers, for example due to the gene-environmental interaction between exposure to smoking and eNOS activity, thus regulating endothelial function and blood supply. In summary, fetal growth restriction in the early developmental hypothesis of adult disease could be a consequence of either gene-environmental or gene-gene interaction, or both, as well as epigenetic imprinting. Further research is needed to better understand these mechanisms, stretching from large-scale epidemiological cohort follow-up analyses to mechanistic studies based on animal experiments under controlled conditions.
A MOLECULAR BASIS FOR THE REGULATION OF CATECHOLAMINE BIOSYNTHESIS, STORAGE AND SECRETION IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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Catecholamines play a pivotal role in the physiological control of blood pressure, heart rate and the response to stress. The spontaneously hypertensive rat (SHR) - a well established experimental model of human hereditary hypertension - exhibits diminished activities of catecholamine biosynthetic enzymes in the adrenal medulla, as first described more than three decades ago. Countless scientific papers have since then tackled the question of whether these adrenomedullary downregulations are secondary to the developing hypertension, or rather primarily genetically determined and thus contributory to the blood pressure increase. Despite such efforts the molecular mechanism of the catecholaminergic dysregulation in the SHR remains elusive. To uncover the genetic underpinnings of the catecholaminergic pathway in the SHR, we integrated gene expression profiling, biochemical and physiological phenotyping, as well as linkage analysis and molecular studies in the HXB/BIXH recombinant inbred (RI) strains, derived from the SHR and the normotensive Brown Norway rat (BN.Lx). Our investigation provides evidence that the enzymatic downregulations of Dbh and Pnmt are primary and caused by genetic variations in the SHR.

AGING ASSOCIATED OXIDATIVE STRESS IN TISSUES OF SPONTANEOUSLY HYPERTENSIVE RATS

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Mechanisms of age-related dysfunction and tissue injury in various organs in consequence of hypertension and insulin resistance remains to be fully elucidated. The aim of the study was to examine the activities of the main antioxidant enzymes, concentrations of reduced glutathione (GSH) and lipid peroxidation products in the renal cortex, myocardium and liver in young and old SHRs. The experiments were carried on 3- and 18-months old male SHRs fed a standard laboratory diet. Antioxidant enzyme activities, thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) and GSH concentrations were determined spectrophotometrically using Sigma and Randox assay kits and were adjusted to protein concentration (P). Lipid peroxidation products, CD and TBARS were markedly elevated in 18-months old SHRs compared with young animals in renal cortex (TBARS: 1.78±0.10 vs. 1.41±0.09 μM GSH/min/mg, p<0.05), liver (TBARS: 1.49±0.08 vs. 0.74±0.05 μM GSH/min/mg, p<0.001) and myocardium (320±19 vs. 425±31 μM H₂O₂/min/mg, p<0.01) and liver (458±46 vs. 582±16 μM H₂O₂/min/mg, p<0.01). Results suggest that impaired antioxidant defense system and higher lipid peroxidation could contribute to the progression of hypertension-associated dysfunction and tissue injury. Supported by grant 00023001 from Ministry of Health of the Czech Republic.

THE EFFECT OF MELATONIN ON VASCULAR FUNCTION IN L-NAME-INDUCED HYPERTENSIVE RATS

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Melatonin was shown to reduce experimental as well as clinical hypertension. However, the mechanisms of its blood pressure lowering effect are not completely understood. We aimed to elucidate the role of NO-pathway in the antihypertensive effects of melatonin. Four groups of male adult Wistar rats were investigated: controls, L-NAME (40 mg/kg), melatonin (10 mg/kg) and L-NAME + melatonin for 5 weeks. Blood pressure was measured non-invasively each week. NO-synthase activity and RNA expression of NO-synthase and cyclooxygenase were determined in the aortas. Acetylcholine-induced responses and their NO-mediated component were evaluated in phenylephrine-preconstricted femoral and mesenteric arteries. Endothelin-derived constricting factor (EDCF)-mediated component of acetylcholine-
induced responses and inner diameter were determined in femoral arteries as well. Chronic L-NAME treatment caused hypertension, impaired acetylcholine-induced relaxations, decreased NO-component, augmented EDCF-component and reduced inner diameter. L-NAME also inhibited NO-synthase activity in the brain and the aorta, in which the endothelial NO-synthase expression was not altered, and cyclooxygenase-2 expression was enhanced. Concomitant treatment with melatonin decreased blood pressure by 15%, failed to improve NO-synthase activity, NO-synthase or cyclooxygenase-2 expression, vascular structure or function. We conclude that the blood pressure reduction after melatonin administration was reduced in L-NAME-induced NO-deficient hypertensive rats compared to our previous experiments on spontaneously hypertensive rats. We therefore suggest that the enhancement of NO pathway (which was prevented by the L-NAME treatment) might represent a major mechanism of the complete antihypertensive effect of melatonin. However other NO-independent mechanisms may be involved in the residual blood pressure lowering effect of melatonin. (grants VEGA 1/3429/06 and 2/6148/26)

THE SAGUENAY YOUTH STUDY: ROLE OF GENE-ENVIRONMENT INTERACTIONS IN SHAPING CARDIOVASCULAR AND METABOLIC HEALTH IN ADOLESCENCE

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In North America and Europe, prenatal exposure to maternal cigarette smoking (PEMCS) is the most common environmental insult to the developing foetus. Beyond the well-established link to adverse pregnancy outcomes, recent research suggests that PEMCS may have also long-lasting effects including an increased risk for individual components of the metabolic syndrome (MS), namely obesity, diabetes mellitus and hypertension. The Saguey Youth Study is a large-scale study aimed at investigating long-term consequences of PEMCS on cardiovascular and metabolic health, and on brain and behaviour, in adolescence. To facilitate the search for genes that modify an individual's vulnerability to PEMCS, the study is family-based (adolescent sibships) and is carried out in a population with a founder effect in Quebec, Canada. A total of 1,000 adolescents, 500 exposed and 500 non-exposed prenatally to maternal cigarette smoking, matched by pair linkage analyses, and fine mapping of identified loci will be done with family-based association analyzes. Phenotypic analysis includes cardiovascular and metabolic health assessments and questionnaires of life habits. To date, we have phenotyped over 500 adolescents. In this dataset, we have demonstrated that PEMCS increases the risk for obesity but not for other components of the MS; this may be due to altered autonomic function. We have also shown that accumulation of intra- abdominal fat promotes development of the MS, affecting the metabolic and inflammatory components similarly in males and females but impacting blood pressure adversely only in males. The latter may be attributed, at least in part, to the augmentation of sympathetic activity that is also seen only in males. Currently, we are examining the effect of candidate genes involved in the regulation of the autonomic nervous system on the above metabolic and cardiovascular phenomena.

INTEGRATED GENOMIC APPROACHES IDENTIFY OSTEOGLYCIN (Ogn) AS A NOVEL DETERMINANT OF LEFT VENTRICULAR MASS

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The spontaneously hypertensive rat (SHR) has been studied for more than 40 years as a model of hypertension and the metabolic syndrome and is the founder of the BxH/BxH recombinant inbred (RI) strains, the largest RI strain panel available for analysis of cardiovascular and metabolic phenotypes. In this panel of RI strains we characterized left ventricular mass (LVM), an important cardiovascular cause of morbidity and mortality, and cardiac gene expression, complex traits regulated by factors both intrinsic and extrinsic to the heart. To dissect the major determinants of LVM, we combined expression QTL and quantitative trait transcript (QTT) analyses of the cardiac transcriptome in the rat RI strains. Using these methods and in vitro functional assays, we identified osteoglycin (Ogn) as a major candidate for rat LVM, with increased Ogn protein expression associated with elevated LVM. We also applied genome-wide QTT analysis to the human heart and observed that, out of ~22,000 transcripts, OGN transcript abundance had
the highest correlation with LVM. A role for *Ogn* in the *in vivo* regulation of LVM was confirmed in the *Ogn* knockout mouse. Taken together, these data implicate *Ogn* as a major determinant of LVM in rats, mice and humans, and suggest that *Ogn* modifies the hypertrophic response to extrinsic factors such as hypertension and aortic stenosis.

**INFLUENCE OF IN VIVO PERTUSSIS TOXIN TREATMENT ON VASOACTIVE BALANCE AND PRESSOR RESPONSIVENESS TO NOREPINEPHRINE IN CONSCIOUS SHR AND WKY**

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G proteins belong to ubiquitous family of proteins playing important roles in various signalling pathways. Overexpression of inhibitory G proteins in vessels of SHR leads to the development and maintenance of high blood pressure (BP), whereas their inactivation by pertussis toxin (PTX) attenuates BP rise. Therefore we focused on the effect of PTX in hypertensive and normotensive rats in which we evaluated the influence of PTX on vasoactive balance as well as on BP responsiveness to norepinephrine (NE). We used conscious 16-week-old WKY and SHR, half of them being injected with PTX (10 µg/kg, i.v., 48 h before the experiment). The contribution of respective vasoactive systems (RAS, SNS) to blood pressure maintenance was assessed using captorpril (10 mg/kg, i.v.) and pentolinium (5 mg/kg, i.v.). BP responsiveness to non-cumulative NE doses (0.01-80 µg/kg, i.v.) was measured after acute RAS inhibition and ganglionic blockade.

PTX significantly lowered basal BP in both strains, the effect being greater in SHR. There was considerably reduced sympathetic vasoconstriction but enhanced angiotensin II-dependent vasoconstriction. Norepinephrine elicited similar BP dose-responses in both strains with a slightly greater slope of the dose-response curve in SHR. However, in PTX SHR pressor responses to lower NE doses were still enhanced compared to PTX WKY. Thus enhanced BP reduction in PTX SHR cannot be explained by greater attenuation of NE pressor responsiveness, but it must be attributed to substantially augmented SNS activity in intact SHR than WKY. Supported by grants 1M0510 (Ministry of Education) and 305/08/0139 (Grant Agency of the Czech Republic)

**EFFECT OF APOCYNIN AND NITRIC OXIDE ON ASCT2 ACTIVITY AND EXPRESSION IN IMMORTALIZED RENAL PROXIMAL TUBULAR EPITHELIAL CELLS FROM SHR RATS**

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Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an important role in several physiological processes and are implied in a wide range of disease processes exerting detrimental and beneficial effects. The aim of this study was to determine the effect of ROS and RNS on the function and the expression of ASCT2 in immortalized renal proximal tubular epithelial (PTE) cells from SHR and WKY rats. Cells were grown in the presence or absence of 100 µM apocynin (APO), an inhibitor of the NADPH oxidase complex, for 4 days or with the nitric oxide donors SNAP 1 mM or SIN1 1 mM for 16 h. Efflux measurements were performed using radiotracier L-[14C]alanine. Abundance of transcript and protein expression were evaluated by real time PCR and immunoblot respectively. In SHR PTE cells APO reduced significantly sodium Km (mM) and Vmax (pmol/mg/6 min) values of the low affinity component of ASCT2 (from 361±7 to 48±6, and 137±2 to 40±2, respectively). The high affinity state of ASCT2 was unaffected by APO. Although ASCT2 protein expression levels were similar for SHR PTE cells treated with APO quantification of ASCT2 transcript showed a significant increase of 18% in SHR cells treated with APO. SNAP and SIN1 did not affect ASCT2 activity or expression. In SHR cells H2O2 may be responsible for keeping ASCT2 in a low affinity state. Nitric oxide may not have a role in ASCT2 regulation in SHR and WKY PTE cells.

**DECREASED MITOCНONDRIAL ATP PRODUCTION AS A COMMON CAUSE OF BP ELEVATION IN SYSTEMIC ARTERIAL HYPERTENSION**

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The immediate cause of stationary elevated blood pressure (BP) of hypertension has not been satisfactorily explained. The presented work deals with the cellular mechanism underlying decreased energy status documented in different tissues from experimental rat models of primary SHR and secondary hypertension as well as the involvement of these abnormalities in the pathogenesis of the disease. The work was carried out using rats with spontaneous (SHR) and secondary of hypertension forms: vasorenal after Goldblatt, DOCA-salt, and Cyclosporine A induced hypertension. The ATP synthesis rate, respiration parameters, mitochondrial calcium induced calcium release parameters were measured in mitochondria isolated from liver and brain. We have found that the studied characteristics of mitochondria in all the investigated forms of hypertension were altered compared to normotensive animals. We hypothesize that founded dysfunction of mitochondrial energy conversion, caused by distinct stimuli, including generalized disturbances of intracellular Ca2+ handling and mitochondrial calcium overload found in primary hypertension, leads to uncoupling of oxidation and phosphorylation and attenuated ATP synthesis. Examples of arterial hypertension accompanied by

**RETROPPOSITION OF ID MOBILE ELEMENT IS A POSSIBLE REASON FOR BLOOD PRESSURE RISE IN SHR RATS**

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Spontaneously hypertensive rats (SHR, Okamoto-Aoki strain) have been employed as a model of human primary essential hypertension (EH). EH is inherited in non-Mendelian manner, being characterized by very high incidence in population and smooth transition in the arterial blood pressure distribution from norm to pathology. These features distinguish EH from classical genetic diseases caused by mutation in unique gene or several genes. We have suggested that genetic determination of primary hypertension is represented by genomic rearrangements of clusters of repetitive sequences. The present study evaluated the role of moderate ID-repeats in the inheritance of spontaneous hypertension in rats. A total of 98 F2(SHRxWKY) hybrids were studied. The genomic DNA isolated from rats liver was analyzed by the original method based on the PCR technique with random primers (SCAN). The PCR product was analyzed by denaturing polyacrylamide gel stained by nitric silver. Hemodynamic parameters including BP were registered by the direct catheter method. 35 polymorphous electrophoresis bands were found in genome of F2 (SHRxWKY) hybrids. It was shown that systolic (SBP) and diastolic (DBP) blood pressure, heart rate and pulse may be predicted by electrophoresis spectra of PCR fragments with statistical methods of piecewise and multiple linear regressions. From 2 to 4 electrophoresis bands were chosen for the prediction of each hemodynamic characteristic. The prediction error was not more than 10%. The described method of the genomic DNA moderate repeats fingerprints allowing to predict the value of certain hemodynamic characteristics in F2 (SHRxWKY) hybrids. This demonstrate possible role of ID mobile element retroposition in the development of chronic hypertension in SHR rats.

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mitochondrial uncoupling and cell ATP depletion (hyperthyroidism, cold hypertension, etc.) may be considered as an additional argument supporting this opinion. It means also that despite of differences in triggering mechanisms of mitochondrial dysfunction in all these models, the final outcome, i.e. decreased mitochondrial ATP production, is similar. Attenuated intracellular ATP content results in the long-term maintenance of elevated BP by increased sympathetic outflow, whereas augmented ROS production following mitochondrial dysfunction lowers the capacity of the NO-dependent vascular relaxation. We suggest that the source of stationary elevated BP in chronic arterial hypertension should be regarded as a compensatory response to decreased mitochondrial ATP synthesis.

CARDIOVASCULAR PHENOTYPE/GENOTYPE RELATIONSHIPS OF THE RI STRAINS

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As clinical disorders, essential (EHT) and genetic (GHT) hypertension in humans and rodents, respectively, constitute equivalent time-varying and progressive syndromes. The physiological phenotype traditionally defining this syndrome is chronic, excessively elevated arterial pressure; however, such a trait may be too encompassing to identify responsible genes. The HXB-BXH recombinant inbred rat strain (RIs) platform, based on the Okamoto and Aoki SHR, is a unique genetic model to explore new defining phenotypes for EHT and GHT. The study was a prospective 12 week, 24 hour radiotelemetry ambulatory blood pressure monitoring analysis of 28 RIs and progenitors in a stress-minimal environment over two levels of dietary salt intake. Diurnal variation of cardiovascular phenotypes was ascertained and two derived quantitative phenotypes, diurnal difference (DdRat) and salt sensitivity (SdRat) were shown to be highly heritable. Strain DdRat for systolic (SP) and diastolic (DP) pressures distributed independent of arterial pressure. Within-strain pulse pressure (PP) was diurnal-invariant. SdRat was independent of baseline pressures but predicted response to high salt intake. The HXB-BXH RIs define a continuous distribution of SP, DP and PP; however, only PP provides a diurnal-independent measure of arterial pressure. No evidence was found that salt diet exerted a causal-connection on the level of hypertension. QTLs for the "diff" phenotypes are different than for basic cardiovascular traits. The HXB-BXH platform may provide a new approach to discovering the origins of GHT and possibly, EHT as well.

ONTOGENETIC FEATURES OF THE BODY WEIGHT GENETIC CONTROL IN ISIAH RATS WITH INHERITED STRESS-INDUCED ARTERIAL HYPERTENSION

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The genetic control of the body weight in the ISIAH rats was studied. This strain was selected for increased response of systolic arterial blood pressure (ABP) to a mild emotional stress caused by 0.5 h restriction in a cylindrical wire-mesh cage. As a result of the selection, the ISIAH rats acquired the number of characteristic features concerned the hypertensive status. QTL analysis was used to identify the genetic loci for ABP, body weight, and related traits. Two F2 populations of 3-4- and 6-month old male rats derived from a cross between normotensive WAG and hypertensive ISIAH rats were used to scan genome with 142 polymorphic markers. In 3-4-month old population the locus for body weight with strong negative effect (-36.09 g) of ISIAH alleles was found near D1Rat76 marker (LOD score 3.23) on Chr.1. In adult rats this locus controls the ABP. In 6-month old population the highest LOD scores for body weight were found on Chr.15 near D15Rat80 marker (LOD score 2.75, effect of ISIAH alleles +31.6 g); and on Chr.X near DXRat140 marker (2.65 Mb) (LOD score 2.2, effect of ISIAH alleles +19.1 g); and in the region DXRat104-DXMco53 (143.4-156.0 Mb) (LOD score 3.88, effect of ISIAH alleles +26.2 g). As a result of selection the ISIAH rat genome acquired alleles for the body weight decrease on Chr.1 in young rats and for the body weight gain in adult rats on Chr.15 and Chr.X. The loci on Chr.X suggest their maternal inheritance.

SNP AND HAPLOTYPE MAPPING FOR GENETIC ANALYSIS IN THE RAT

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Rat inbred strains are a powerful tool for the understanding of molecular complex human diseases. The availability of the BN rat genome sequence has opened possibilities to apply new strategies for the identification of genes underlying complex phenotypes. One of these strategies is the genetic mapping of a disease phenotype to chromosomal regions, and with the discovery of single nucleotide polymorphisms (SNPs) in the genome, mapping has become much more precise. With the access to genomic sequence we are able to present a study on a large set of high-quality candidate SNPs and a first haplotype map of the rat. We have surveyed the transcriptome of 3 million single nucleotide polymorphisms mapped to the draft rat genome sequence. For a portion of these SNPs, we have predicted functional effects by applying several computational approaches, such as estimation of the selective pressure with the Omega value. We have developed SNP genotyping tools for the detection of 20K SNPs. These have been applied for genotyping a large number of commonly used inbred rat strains, including recombinant inbred rat strains and a set of F2 intercross rats. We used the genotype data to construct improved high-resolution genetic maps, and to characterise the population structure of laboratory inbred rat strains. In addition, we estimate on the LD structure and perform first haplotype analysis of the rat genome. In summary, we provide a new detailed SNP map and demonstrate its utility for QTL mapping studies as well as in understanding the population genetics of the rat being a model for human disease. This community resource is openly available and augments the genetic tools for this model of physiological studies.

OXIDIZED LDL RECEPTOR LOX-1 IS INVOLVED IN HIGH FAT DIET-INDUCED LIPID DEPOSITION IN SHR-SP

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The endothelial receptor for oxidized LDL, LOX-1, is implicated in vascular dysfunction. Here we intended to clarify the role of LOX-1 in lipid-deposition in blood vessels under hypertension, utilizing a model of SHR-SP that shows lipid-deposition in mesenteric artery when maintained with high-fat diet and saline. The basal expression level of LOX-1 in the mesenteric artery of SHR-SP was significantly higher than that in WKY. SHR-SP (male, 8 weeks old) fed with high-fat diet for one or two weeks showed strong lipid deposition in mesenteric artery, while WKY did not. Treatment of SHR-SP with a neutralizing anti-LOX-1 antibody (10 mg/kg, i.v. every 3 days) significantly suppressed the lipid deposition. Treatment with AT1 blocker telmisartan (1 mg/kg/day) significantly reduced the lipid deposition as well, which was accompanied by the reduction in the expression level of LOX-1. Increasing vitamin E in the chow from 0.2 mg to 5.2 mg/100g also suppressed the lipid-deposition and reduced the level of oxidized LDL.
Thus, attenuation of LOX-1-LOX-1 ligand interaction effectively suppressed lipid-deposition in SHR-SP. Since oxidized LDL-like immunoreactivity was observed in the vessel wall and LOX-1 is the receptor for oxidized LDL, kinetics of oxidized LDL in vivo was analyzed. Injected labeled oxidized LDL deposited well in mesenteric artery of SHR-SP and the deposition was inhibited by anti-LOX-1 antibody, while WKY did not show such distribution of oxidized LDL. In the mesenteric artery of SHR-SP, lipids might deposit to the arterial wall as the manner of oxidized LDL via LOX-1.

**RECONSTITUTION OF A SALT-SENSITIVE ALBUMINURIA-INDEPENDENT CARDIOVASCULAR PHENOTYPE IN THE SALT-RESISTANT SHR BACKGROUND IN A NEW DOUBLE-CONSONIC RAT MODEL DERIVED FROM DAHL SS RATS**

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In linkage analyses between salt-sensitive Dahl (SS) and salt-resistant spontaneously hypertensive (SHR) rats we mapped QTL linked to salt-sensitive systolic blood pressure (SBP), albuminuria, and left ventricular (LV) hypertrophy on rat chromosome (RNO)3, RNO6 and RNO19, respectively. SS alleles on RNO6 and RNO19 conferred salt-sensitivity by increasing SBP and organ damage in response to high salt, while the SHR allele conferred this phenotype on RNO3. We tested whether renal damage can be expressed in the SHR background by single and double transfer of RNO6 and RNO19 from SS. We generated single consonics by transferring RNO6 and RNO19 from SS into SHR by sequential backcrossing and a double-consonic SHR-6SS19SS, carrying RNO3 from SHR, RNO6 and RNO19 from SS linked to salt-sensitivity. We analysed the effect of high-salt loading for 8 weeks on renal and cardiovascular organ damage in parental rats, single and double-consonics. In single consonics no significant strain differences in albuminuria and cardiovascular damages between consonics and SHR were observed. Similar to SHR, SHR-6SS19SS demonstrated no significant effect on albuminuria. In contrast, a significant increase of SBP (199.0±12.2 vs. 171.5±8.3 mmHg, p<0.0001) and also of the LV weight index (3.2±0.27 vs. 2.6±0.23 mg/g, p=0.007) vs. SHR was detected. Our analysis of the double-consonic SHR-6SS19SS carries RNO3 from SHR, RNO6 and RNO19 from SS linked to salt-sensitivity. We analysed the effect of high-salt loading for 8 weeks on renal and cardiovascular organ damage in parental rats, single and double-consonics. In single consonics no significant strain differences in albuminuria and cardiovascular damages between consonics and SHR were observed. Similar to SHR, SHR-6SS19SS demonstrated no significant effect on albuminuria. In contrast, a significant increase of SBP (199.0±12.2 vs. 171.5±8.3 mmHg, p<0.0001) and also of the LV weight index (3.2±0.27 vs. 2.6±0.23 mg/g, p=0.007) vs. SHR was detected. Our analysis of the double-consonic SHR-6SS19SS shows that in contrast to single-consonics in which two susceptible chromosomes are inherited, an interaction of three susceptible chromosomes converts the salt-resistant phenotype into salt-sensitive hypertensive and cardiac hypertrophy in the SHR strain, independently from renal damage with albuminuria.

**A QUANTITATIVE TRAIT LOCUS ON RAT CHROMOSOME 8 IS CRUCIAL FOR DEVELOPMENT OF ALBUMINURIA WITHOUT AFFECTING THE INHERITED NEPHRON DEFICIT IN MUNICH WISTAR FRÖMTER RATS**

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An important predictor for the development of arterial hypertension, glomerular hyperfiltration and renal disease with age is a reduced nephron number at birth. In a cross between hypertensive Munich Wistar Frömlter (MWF) with an inherited nephron deficit and albuminuria and spontaneously hypertensive rats (SHR) we identified a quantitative trait locus (QTL) on rat chromosome (RNO)6 that is linked to early onset albuminuria in young MWF at 8 weeks, while a second important locus on RNO8 was linked to albuminuria after the age of 14 weeks. Accordingly, we tested the relevance of the QTL on RNO8 for the influence on nephron number and albuminuria. We generated a consonomic strain MWF-8SS8 by transfer of SHR RNO8 into the MWF background. Urinary albumin excretion (UAE) was decreased at 6 time points between 8 and 32 weeks of age (n=9–18). In comparison to SHR the early 55fold increase of UAE in MWF (18±1±2.0 mg/24h) is significantly suppressed in MWF-8SS8 (2.6±2.4 mg/24h) at 8 weeks of age. MWF develops a continuous and progressive increase in UAE between week 8 and 32 (354.6±112.9 mg/24h). In contrast, MWF-8SS8 exhibits only a moderate increase in UAE up to 32 weeks of age (23.2±17.4 mg/24h). Interestingly, the nephron number is similar in MWF-8SS8 compared to MWF and thus significantly reduced compared to SHR (-36%, p<0.0001). Our findings demonstrate that replacement of a QTL on RNO8 in consonic rats prevents the development of overt albuminuria despite an inherited nephron deficit.

**RAT COMPASS FOR NAVIGATING THE PREDICTIVE ECLOGENOMIC LANDSCAPE OF HUMAN DISEASE**

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The common forms of prevalent diseases including metabolic, hemodynamic, neurological, behavioral conditions and cancer are multifactorial (complex) traits with relatively balanced heritable and environmental components. Only recent developments of novel technological and bioinformatic tools including the concept of systems biology, together with the semantic expansion of the environment to include diverse entities from epigenomics to socio-economic status, provide an appropriate conceptual frame for dissection of major causative factors and mechanisms. We are currently at the advent of large collaborative, multi-level projects pursued in order to determine the genomic, transcriptomic and epigenomic factors substantially involved in shaping the ecogenomic landscapes of complex disease. In this context, traditional challenges of dissection of relevant gene–gene–environmental webs of interactions in the general human population are enhanced almost beyond feasibility. Here, the experimental models will play an indispensable role serving as a “compass”, allowing initial orientation and facilitating the charting process. Series of genetically designed inbred, congenic, consomic, multiple congenic and recombinant inbred rat strains should be employed to dissect the distinct and combined contributions of early development, aging as well as ecogenomic aspects of disease pathogenesis. Rather than identifying set of discrete “risk” or “protective” genetic variants, robust statistical models are needed in order to reveal functional genomic signatures, predisposing to particular subsets of complex diseases, present in human population and modeled in the comprehensive experimental sets. Although the experimental findings cannot be directly translated to human biology, significant insights can be obtained through the use of comparative and integrative genomics. The ultimate goal is thus to enhance knowledge of genomic component underlying complex metabolic disease and create a stepping stone in transition to predictive, rather than descriptive, paradigm.

**DISSECTION OF THE DEVELOPMENT OF POLYGENETIC ALBUMINURIA IN THE MUNICH WISTAR FRÖMTER RAT THROUGH A GENOMIC SYSTEMS BIOLOGY APPROACH**

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The identification of susceptibility factors underlying complex diseases is a demanding task despite the discovery of many positional candidate
genes by whole-genome linkage and expression profiling studies. As the majority of complex genetic disorders develop as a consequence of dysregulated pathways, complementary and moderate gene effects in the biological system remain undetected by conventional genetic strategies. We developed an integrated approach to investigate the genetic susceptibility for renal damage in hypertension as a complex disease by combining data from linkage and whole genome expression profiling studies to statistically assess dysregulated pathways as a whole. We chose the polygenetic albuminuria phenotype of the Munich Wistar Frömter (MWF) rat as a model for proof of the concept. We implemented a statistical approach using pathway mapping of genetic data from linkage studies (11 urinary albumin excretion [UA] QTLs including 1307 annotated genes) in combination with expression pattern of the target organ (glomeruli, 939 differentially expressed genes). The pathways were extracted from the KEGG database. Overrepresented pathways were calculated using crosstabulation, statistical significance was tested using chi-square distribution and permutation testing. For the examined UA QTLs, 15 significant (p<0.05) pathways were determined. The pathway mapping of the phenotype associated expression data resulted in 21 overrepresented pathways. Six overlapping pathways appeared, among which structural pathways such as the focal adhesion play a central role. We present a novel approach to determine genetically determined, dysregulated pathways underlying albuminuria in SHR cells through integration of genetic data and expression patterns.

SHORT-TERM REGULATION OF THE Cl--/HCO3-- exchanger in immortalized SHR proximal tubular epithelial cells

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Major mechanisms intervening in renal proximal tubular NaCl absorption, intracellular pH and cell volume regulation have been suggested to occur through the concerted action of Cl-/HCO3- and Na+/H+ exchangers. We evaluated the activity of Cl-/HCO3- exchanger in immortalized renal proximal tubular cells from WKY and SHR and identified the signalling pathways that regulate the activity of the transporter, as well as the abundance of Slc26a6. Cl--/HCO3-- identified the signalling pathways that regulate the activity of the transporter. The HCO3-- sensitive to SB203580 but not to chelerythrine. SHR overexpress SB203580, but not by chelerythrine. Increases in phospho-p38 MAPK activity of Cl--/HCO3-- Slc26a6 protein in the kidney. SHR cells are endowed with an enhanced activity of Cl-/HCO3- exchanger. Cl-/HCO3- exchanger is an effector protein for PKA, PKC and p38 MAPK in both WKY and SHR cells. Supported by grant POCTI/SAU-FCF/59207/2004

PROTECTIVE EFFECTS OF EPIGALLOCATECHIN GALLATE ON SPONTANEOUS STROKE IN MALIGNANT STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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Epigallocatechin gallate (EGCG) is a major polyphenol in green tea. In the present study, we examined the effect of EGCG on the spontaneous stroke in malignant stroke-prone spontaneously hypertensive rats (M-SHRSP). Males M-SHRSP 5-weeks old were given distilled water or 0.3% EGCG solution as drinking water ad libitum for 8 weeks. Blood pressure was measured using tail-cuff method. Stroke onset was temporarily assessed by the appearance of neurological symptoms and the changes in body weight. After the 8th week of administration, rats were sacrificed under anesthesia. Histopathological changes in brain were evaluated in hematoxylin-eosin-stained thin sections. Plasma renin activities, angiotensin II, aldosterone, nitric oxide metabolite (NOx) levels, and urinary bioporphyrins, oxidative metabolites of bilirubin, were measured. Elevation of blood pressure was depressed by EGCG after the 5th week of administration. EGCG ingestion significantly delayed stroke onset compared to the control group. Plasma angiotensin II and aldosterone levels of EGCG-treated rats were decreased in comparison to the control rats. But there was no difference in plasma renin activities. Plasma NOx and urinary bioporphyrins level of EGCG-treated rats were decreased compared to the control rats. These results suggest that long-term administration of EGCG prevents the development of brain lesions in spontaneous stroke model by inhibiting the further development of high blood pressure at later ages, through the inhibition of renin-angiotensin-aldosterone system activity and bilirubin oxidation by NO radicals.

CHARACTERISTICS OF HEART FUNCTION IN SHR/NDmcrc-p RATS WITH METABOLIC SYNDROME

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Metabolic syndrome has been markedly increasing, but whether the functional properties of the heart are affected remains to be clarified. We therefore examined the heart functions of SHR/NDmcrc-p rats (SHR-cp), an animal model of metabolic syndrome. Male 16-week-old SHR-cp, lean littermates (SHR) and normotensive Wistar-Kyoto rats (WKY) were used. Heart rate (HR), blood pressure (BP) and cardiac output (CO) were determined by the tail-cuff method and plethysmogrom method. Atrial rate (AR) and contractile force (CF) were recorded with isolated atria mounted in an organ bath. The BP of SHR-cp and SHR increased compared with that of WKY. The HR and pulse pressure (PP) in vivo study and the positive inotropic response to isoproterenol in the isolated atrium in SHR-cp were significantly lower than those in SHR. These changes were not significantly different from those in WKY. The CO in SHR-cp and SHR was significantly higher than that in WKY, although the degree of increment in SHR-cp was significantly smaller than that in SHR. The ventricles in SHR-cp and SHR were heavier than that in WKY, but the atrium in SHR-cp was heavier than those in SHR and WKY. AR and CF per g (weight of atrium isolated from SHR-cp) were significantly lower than those of SHR and WKY. These results suggest that metabolic syndrome is a risk factor for the development of depressed heart function with atrial hypertrophy that may lead to impairment of peripheral circulation.
ASSOCIATION BETWEEN 24-HOUR VARIATION IN HUMAN AND MOUSE PERIPHERAL BLOOD MONONUCLEAR CELLS CIRCADIAN RHYTHM-RELATED GENES AND OBESITY

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A recent study reported the association between BMAL1, among molecules necessary for the circadian rhythm, and fat accumulation. In this study, we investigated the association between 24-hour variation in circadian rhythm-related genes and obesity using peripheral blood mononuclear cells. The subjects were 27 healthy volunteers (obese: n=12, non-obese: n=15). Blood was collected at 9:00, 12:00, 15:00, 18:00, and 21:00. In blood samples collected at 9:00, we determined blood sugar, IRI, adiponectin, and leptin. At each time point, we measured serum TC, TG, HDL, FFA, cortisol, melatonin and peripheral blood mononuclear cell expressions of BMAL1, PER1, PER2, CRY1, CRY2 and REV-ERBz genes mRNA. 24-hour variation of these genes was also determined in peripheral blood mononuclear cells of C57B6 mouse. In the obese group, the expressions of these genes did not show any marked 24-hour variation. However, in the obese group, their expressions increased between 12:00 and 21:00. In the two groups, PER1 gene expression showed a bimodal pattern, with high values at 9:00 and 18:00. Apparent 24-hour variation of circadian rhythm-related gene was also detected in mouse peripheral blood mononuclear cells. The expressions of circadian rhythm-related genes in peripheral blood mononuclear cells showed marked 24-hour variation, suggesting their usefulness in human circadian rhythm research. There were differences in the 24-hour variation in the expressions of circadian rhythm-related genes between the obesity and non-obese groups, suggesting an association between the onset/aggravation of obesity and circadian rhythm-related genes.

ADIPOKINE SECRETION IS REGULATED BY LOCAL RAS IN SHRSP

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We examined the relationship of renin-angiotensin system (RAS) and adipokine secretion in adipose tissue for the occurrence of hypertensive end-organ damage. Male SHRSP and WKY (at 6, 12 and 20 weeks of age) were used. Some of the SHRSP were administered AT1 receptor blocker (ARB, candesartan 0.5 mg/kg) from 6 to 11 weeks of age and sacrificed at 20 weeks of age. Expression of adipokines and RAS-related molecules in epididymal adipose tissue were examined by real-time PCR and compared to those levels in serum. At 6 weeks of age, serum leptin and adiponectin levels were higher in SHRSP than in WKY. ACE activity and renin mRNA in adipose tissue were higher in SHRSP than in WKY, whereas no significant difference was found in the expression of AII receptors. At 20 weeks of age, marked lipoatrophy and deterioration of serum adipokine levels were found in SHRSP. Expression of RAS-related molecules in adipose tissue was also decreased in both groups and more intensely decreased in SHRSP. However, such age-dependent changes in adipokines and RAS-related molecules were ameliorated in ARB-treated SHRSP and no cerebral lesion was found in these rats. These results indicate that lipoatrophy may be an important factor in the aggravation of hypertension and its end-organ damage, since early blockade of RAS was effective not only for the prevention of cerebral lesions but also lipoatrophy. This may be due to the cross-talk of adipokines and RAS in adipose tissue.

GENOTYPE DETECTION OF ALCOHOLISM USING FINGERNAIL DNA: IDENTIFYING A SINGLE NUCLEOTIDE POLYMORPHISM IN THE ALDEHYDE DEHYDROGENASE 2 GENE

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Aldehyde dehydrogenase 2 (ALDH2) is a key enzyme in alcohol metabolism. A single nucleotide polymorphism (SNP), Glu487Lys in the ALDH2 gene (rs671), which is commonly found in Orientals, is responsible for intolerance to alcohol. The purpose of the study was to clarify the contribution of genetic background and lifestyle to alcoholism using SNPs considered to have functional effects on alcoholism. An individual’s genotype was detected using nail clippings by our method of DNA extraction using an alkaline serine protease derived from fruit, genomic DNA amplification by polymerase chain reaction, and gel electrophoresis of the DNA fragment. The subjects were 149 Japanese university students (female, average age 20.8±7 years; body mass index, 20.4±1.6). The frequency was 0.656 for the typical Glu homozygote, 0.322 for the heterozygote (Glu487Lys), and 0.020 for the atypical Lys homozygote. The typical homozygote (0.071) and heterozygote (0.063) indicated a risk of developing an addiction to drinking. The atypical homozygote indicated non-drinker. For comparison, data for 97 males (average age 31.5±6.41years; body mass index 23.39±10.37) living in Mwanza, Tanzania will also be presented. The method developed, using fingernail DNA, is feasible for routine clinical testing for high throughput genetic screening of ALDH2.

DEVELOPMENTAL PROGRAMMING OF CARDIO-VASCULAR DISEASE IN RODENTS: A CONSEQUENCE OF OVER-NUTRITION IN PREGNANCY

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Converging lines of evidence from epidemiological studies and animal models now indicate that the origins of cardiovascular disease (CVD) lie not only in the interaction between genes and traditional adult risk factors, such as unbalanced diet and physical inactivity, but also in the interplay between genes and the embryonic, fetal and early postnatal environment. Whilst studies in man initially focused on the relationship between fetal under-nutrition and low birth weight on risk of adult CVD and metabolic syndrome, evidence is also growing to suggest that over-nutrition and increased birth weight and/or adiposity at birth can also lead to increased risk for childhood and adult CVD. Hence, there appears to be increased cardiovascular risk at both ends of the birth weight spectrum. Animal models, including both under- and over-nutrition in pregnancy and lactation lend increasing support to the developmental origins of hypertension and CVD. This overview will focus upon the influence of the maternal nutritional and hormonal environment in pregnancy in permanently programming cardiovascular function and the hormonal and neuronal mechanisms that may contribute to the maintenance of elevated blood pressure in the offspring. The potential maternal programming ‘vectors’ shall be discussed and the molecular mechanisms that may lead to persistent pathophysiological changes and subsequent disease.
EFFECT OF NICORANDIL ON SYMPATHETIC NEUROTRANSMISSION VIA ATP-SENSITIVE POTASSIUM CHANNEL IN SHR.Cg-Lep*/NDmer RATS

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Nicorandil, an anti-anginal drug, is known to open ATP-sensitive potassium (KATP) channel on vascular smooth muscle. The present study investigated the effect of nicorandil on the vascular sympathetic neurotransmission and demonstrated the relationship between the KATP channel and metabolic syndrome. We examined the effects of nicorandil on the release of norepinephrine (NE) from the electrically stimulated caudal artery of male Wistar rat, Wistar Kyoto Rat (WKY) and the perfused SHR. In the SHR, NE release was inhibited by ODQ, a soluble guanylate cyclase inhibitor and glibenclamide, KATP channel blocker. Therefore, nicorandil seems to inhibit NE-release via the KATP channel but not the NO pathway. In the caudal artery of WKY, nicorandil inhibited NE-release but did not inhibit the NE-release in SHR-ep. These findings suggest that the KATP channel on the adrenergic nerve terminals plays a role as an inhibitor of NE-release, and is different from the K177 channel of smooth muscle cells. Furthermore, in SHR-ep, this function of KATP in the vascular adrenergic nerve terminals is considered to be impaired.

TREATMENT WITH THE CHOLINERGIC PRECURSOR CHOLINE ALPHOSCRERATE COUNTERS ASTROGLIOSIS IN SHR

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Astrogliosis is a phenomenon defined as hypertrophy, hyperplasia and glial fibrillary acid protein (GFAP) hyperproduction that occurs in several situations of brain damage including neurodegeneration, Alzheimer’s disease and vascular dementia (VaD). This study was designed to assess astrogial reaction in cerebral areas (frontal cortex, striatum and hippocampus) of spontaneously hypertensive rats (SHR), used as a model of hypertensive brain damage and VaD. Choline alphoscerate (alpha-glyceryl-phosphorylcholine; α-GPC) is a phospholipid involved in choline biosynthetic pathways enhancing acetylcholine availability and release. From a clinical side, it improves cognitive dysfunction in VaD. Male SHR of 32 weeks of age were treated for 4 weeks with 150 mg/kg/day of α-GPC. SHR were compared with and without complications using Affymetrix (50K, 500K, 5.0 and 6.0) SNP arrays. Ethnicity was ascertained using STRUCTURE 2.0 and in the brain of SHR with established hypertension. The observation that treatment with α-GPC attenuates the extent of glial reaction in cerebral areas of SHR suggests that the compound may afford neuroprotection in this animal model.

OXIDATIVE STRESS, NITRIC OXIDE AND THE VASCULAR PHENOTYPE IN HYPERTENSION

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Resistance arteries undergo functional and structural changes in hypertension comprising hyperreactivity, endothelial dysfunction, inflammation and vascular remodeling (increased media:lumen ratio). These characteristic changes, known as the "vascular phenotype" are evident in experimental and human hypertension. Cellular changes underlying the vascular phenotype include altered vascular smooth muscle cell growth, migration, differentiation and increased extracellular matrix abundance. Fundamental to such alterations is increased expression of adhesion molecules, increased synthesis of proinflammatory and proatherosclerotic factors, activation of the local renin-angiotensin system and increased ET-1 secretion. Common to these processes is increased generation of reactive oxygen species (ROS), particularly H2O2 and •O2-(oxidative stress) and decreased bioavailability of nitric oxide (NO). A major source for vascular and renal ROS is a family of non-phagocytic NADPH oxidases, including the prototypic Nox2 homologue-based NADPH oxidase, as well as other NADPH oxidases, such as Nox1, Nox4 and Nox5. Upregulation of these Noxes has been implicated in vascular oxidative stress. Reduced NO synthesis by eNOS and increased NO consumption by ROS contribute to decreased NO, which underlies endothelial dysfunction. Superoxide anion and H2O2 stimulate mitogen-activated protein kinases, tyrosine kinases, and transcription factors (NFκB, AP-1, and HIF-1) and inactivate protein tyrosine phosphatases. ROS also increase [Ca2+]i and upregulate protooncogenes and proinflammatory gene expression and activity. These phenomena occur through oxidative modification of proteins by altering key amino acid residues, by inducing protein dimerization, and by interacting with metal complexes such as Fe-S moieties. Changes in the intracellular redox state through glutathione and thioredoxin systems may also influence intracellular signaling events. These findings have evoked considerable interest because of the possibilities that therapies targeted against non-phagocytic NADPH oxidase to decrease ROS generation and/or strategies to increase nitric oxide (NO) availability and antioxidants may be useful in minimizing vascular injury and renal dysfunction and thereby prevent or regress vascular injury and end organ damage in hypertension.

FROM SNPS TO CLINICALLY USEFUL BIOMARKERS FOR HYPERTENSION AND CARDIOVASCULAR COMPLICATIONS

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At present time, diagnostic and prognostic cardiovascular biomarkers are available but there are no widely accepted and validated clinically useful biomarkers for screening prior to outcome appearance. Our general objective is to search for clinically useful biomarkers focusing on SNPs and other DNA sequence and structural variations alone or combined with existing clinical risk factors. Whole genome association studies (WGA) were performed in French-Canadian population affected by hypertension and dyslipidemia and in type 2 diabetic (T2D) patients with and without complications using Affymetrix (50K, 500K, 5.0 and 6.0) SNP arrays. Ethnicity was ascertained using STRUCTURE 2.0 and
principal component analysis with 16K complications-unrelated SNPs. Risk prediction models include SNPs alone or combined to a few uncorrelated clinical biomarkers. ROC curves were drawn to yield a predictive value combining sensitivity and specificity. WGA studies identified a number of SNPs which show strong associations with hypertension, C-reactive protein and gamma glutamyl transferase levels, albuminuria, and myocardial infarction and other outcomes. Odds ratio of being affected increased with the combination of risk alleles. As an example, an area under the ROC curve of 0.73 was obtained with only four SNPs, for T2D vascular complications. The predictive power of these multiple biomarkers will be validated in other populations, functionally assessed in rodent models and optimized by adding classical risk factors and complementary epigenomic markers, to the risk model.

EXAMINATION OF LIPID METABOLISM ABNORMALITIES USING A NEW RAT METABOLIC SYNDROME MODEL (SHRSP. ZLeprfa IsmDprc)

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The clinical features of metabolic syndrome may vary via the complex interactions of genetic/environmental factors. The development of a disease model of this syndrome is important for clarifying the pathogenesis. Small-dense LDL is a lipid profile characteristic of this disease. However, no study has employed any appropriate animal model involving small-dense LDL. In this study, we investigated the lipid profiles of a new animal model with hypertension and obesity (SHRSP. ZLeprfa IsmDprc) fatty rat (ZF) gene mutation to SHRSP rats. The lipoprotein particle size was examined using fast lipid protein liquid chromatography (FPLC). In these rats, a smaller fraction was also detected. This fraction was positive for apoB. Examination using high-sensitivity lipid profiling also showed the marked increases in the numbers of both particles. In these rats, a high density lipoprotein (HDL) particle was noted in SHRSP rats. The SHRSP fatty rats showed metabolic syndrome model with insulin resistance, an increase in the blood neutral fat level, hypercholesterolemia, obesity, and hypertension. We were interested in whether these changes precede hypertension in a model of ANG II-dependent hypertension, in which severe hypertension develops within 24 hours after its induction. Hypertension was induced in Cyp1a1-Ren-2 (iTGR) rats through oral administration of the xenobiotic indole-3-carbinol (IC3, 0.3%) for 12 or 24 hours. Normotensive Cyp1a1-Ren-2 rats fed IC3-free diet served as controls. Evaluations of in vivo renal functions were made at three levels of blood pressure (BP). At the end, rats were decapitated and plasma and kidney ANG II levels were measured by RIA. While mean arterial pressure of 12-h induced iTGR (122 ± 2 mm Hg) did not differ from non-induced iTGR (119 ± 3 mm Hg), that of 24-h induced iTGR was significantly higher (130 ± 3 mm Hg, p<0.05). Plasma ANG II levels were increased gradually in 12-h induced (385 ± 31 fmo/ml) and 24-h induced (512 ± 37 fmo/ml) iTGR versus non-induced iTGR (130 fmo/ml). Similarly, kidney ANG II content increased after 12 h-induction (963 ± 56 fmo/g) and 24 h induction (1571 ± 105 fmo/g) in comparison with non-induced iTGR (691± 61 fmo/g). Basal values of glomerular filtration rate (GFR), renal blood flow (RRBF) and sodium excretion were not significantly different among experimental groups. However, 12-h induced iTGR exhibited impairment of GFR and RRBF autoregulation efficiency. In addition, 24-h induction of renin gene resulted in marked reduction of RBF and GFR. The pressure-natriuresis relationship in 12-h induced iTGR exhibited marked impairment as compared with non-induced iTGR and this was more pronounced in 24-h induced iTGR. The impairment of pressure-natriuresis mechanism precedes the development hypertension in this model of inducible ANG II-dependent hypertension.

EXPANDING THE SYMPATHORENAL INTERACTION IN RATS RECIPROCALLY CONGENIC FOR THE CHROMOSOME 1 BLOOD PRESSURE QUALITATIVE TRAIT LOCUS

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Sympathorenal interaction was examined using reciprocal congenic strains at 6-8 weeks and 10-12 weeks. Two reciprocal congenic strains, WKY.pch1.0 and SHRSPwch1.0, were constructed respectively by introgressing the stroke-prone spontaneously hypertensive rat (SHRSP)-derived fragment for the chromosome-1 blood pressure (BP) quantitative trait locus (QTL) into Wistar-Kyoto rat (WKY) and vice versa. Renal function was determined with and without the acute renal denervation (DNX). Blood pressure (BP) was comparable between the SHRSP and SHRSPwch1.0, and between WKY and WKY.pch1.0 at 6-8-wk. Comparing to SHRSP, SHRSPwch1.0 exhibited lower estimated renal sympathetic nervous activity (RSNA) and renal vascular resistance and the contrary applied to the WKY.pch1.0 in comparison with the WKY. DNX entailed vasodilation and tubular natriuresis in the SHRSP and WKY.pch1.0 but not in the SHRSPwch1.0 and WKY. At 10-12-wk, BP between the individual congenic strain and respective donor strain differed significantly. In addition to the SHRSP and WKY.pch1.0, DNX in the SHRSPwch1.0 also induced renal vasodilation, but without the concomitant tubular functional changes. BP and the estimated RSNA, however, did not differ significantly between the SHRSPwch1.0 cohorts of the two ages. The chromosome-1 QTL region may participate in the BP variations by modulating the sympathetic control of the renal function, whereas it does not seem to be indispensable for an age-dependently enhanced renal vascular reactivity to the sympathetic control. These timely observations may thereby enable an improved understanding of the genetic determinants in the neural regulation of the renal function, especially the renal resistance vessels.
ALCOHOL AND ALDEHYDE DEHYDROGENASE POLYMORPHISMS AND 20-YEAR FOLLOW-UP BLOOD PRESSURE IN JAPANESE

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The effects of the alcohol dehydrogenase-2 (ADH2) and aldehyde dehydrogenase-2 (ALDH2) genotypes on blood pressure (BP) were investigated in a 20-year follow-up study carried out in a Japanese rural community. 580 individuals attending both health examinations (held in 1987 and 2006) were recruited. BP was measured with automatic sphygmomanometers in the two health examinations. Genotypes were determined by the TaqMan method.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) at baseline (in 1987) were comparable among the ADH2 and the ALDH2 genotypes. By contrast, the BP change after the 20-year follow-up significantly differed across the ADH2 but not the ALDH2 genotype; the elevation was most pronounced in subjects with the ADH2*2/*2 (wild type) and comparable between those with ADH2*/2 (superactive heterozygotes) and ADH2*2/*2 (superactive homozygotes) (P < 0.001 by ANOVA). A multiple linear regression analysis showed that the increment in BP was independently affected by the ADH2 genotype (P < 0.001). Meanwhile, the genetic polymorphism in the ALDH2 gene appeared to influence the drinking behavior in our studied population, and accordingly to modulate the effects of ADH2 on the blood pressure. These observations provided a good example of gene-gene and gene-environmental interaction influencing BP.

EFFECT OF THE G994T POLYMORPHISM IN THE LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 (LP-PLA2) GENE ON THE PLASMA OXIDIZED LDL/LDL RATIO IN JAPANESE - THE SHIMANE STUDY

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The G994T polymorphism in the gene of lipoprotein-associated phospholipase A2 (LP-PLA2) is known to have a major effect on the enzyme activity. LP-PLA2 is associated with low-density lipoprotein (LDL) particles and hydrolyzes oxidized phospholipids, a major component of oxidized LDL (oxLDL). LP-PLA2 is thus expected to influence the LDL oxidation in vivo. The effects of the G994T polymorphism on the plasma LDL oxidation were examined in a Japanese population. Genotyping was performed by an allele-specific PCR and the oxLDL level was determined by enzyme immunoassay in 548 individuals recruited at a health examination. Plasma LDL oxidation was estimated by the oxLDL/LDL ratio. The T allele was found to have a lower level of plasma oxLDL/LDL ratio [220 ± 90, 2030 ± 130, and 3230 ± 620 U/mmol (mean ± SEM) for GG, GT, and TT, respectively, P < 0.05 by ANOVA], while it showed a co-dominant effect on Lp-PLA2 activity. A multiple regression analysis revealed that age, the G994T genotype, and the plasma small-dense LDL level were independently associated with the oxLDL/LDL level. The G994T polymorphism in the Lp-PLA2 gene may influence the plasma LDL oxidation and further studies on the effect of this polymorphism in cardiovascular diseases are warranted.

SALT-SENSITIVE HYPERTENSION AND PROGRESSIVE ALBUMINURIA IN SHRSP IS UNDER THE INFLUENCE OF A QUANTITATIVE TRAIT LOCI ON CHROMOSOME 1

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The normotensive Fischer (F344) rat maintains normal blood pressure and cardiac weight in response to high salt loading. We used F344 rats as a contrasting strain to identify quantitative trait loci (QTL) linked to salt-sensitive hypertension and organ damage in stroke-prone spontaneously hypertensive (SHRSP) rats. We performed genome-wide QTL mapping studies and generated a consomic strain in the SHRSP background by sequential backcrossing. We compared the effects of 0.2%-NaCl low-salt (LS) and 4%-NaCl high-salt (HS) diet for 8 weeks on the development of hypertension and organ damage in male animals of the parental strains and the consomic strain. We identified major QTL for left ventricular hypertrophy (LVM) on rat-chromosome 1 (RNO1) at which the F344 allele conferred a protective effect. We introgressed RNO1 from F344 into the SHRSP background, creating the new consomic strain SHRSP-1F344. In contrast to SHRSP, the consomic strain exhibited no significant blood pressure raise in response to the HS-diet. In comparison to SHRSP the left ventricular weight index was significantly reduced in SHRSP-1F344 under both diets (p < 0.05, respectively), but still showed a significant response to the HS-diet (+11% p < 0.05). In addition, the increase in albuminuria under HS-diet was observed in SHRSP was fully suppressed in SHRSP-1F344 (47 mg/d vs. 0.3 mg/d, p < 0.0001). By using F344 as a contrasting strain we confirmed an important QTL on RNO1 affecting salt-sensitive blood pressure regulation and development of albuminuria in SHRSP. The development of left ventricular hypertrophy is also significantly affected but still modulated by high-salt intake.

VASCULAR RELAXATION OF RALOXIFENE ON RAT AORTA CONSTRUCTED BY PHENYLEPHRINE IS MEDIATED BY SCAVENGING MITOCHONDRIA- DERIVED REACTIVE OXYGEN

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The G994T polymorphism in the gene of lipoprotein-associated phospholipase A2 (LP-PLA2) is known to have a major effect on the enzyme activity. LP-PLA2 is associated with low-density lipoprotein (LDL) particles and hydrolyzes oxidized phospholipids, a major component of oxidized LDL (oxLDL). OxLDL is thus expected to influence the LDL oxidation in vivo. The effects of the G994T polymorphism on the plasma LDL oxidation were examined in a Japanese population. Genotyping was performed by an allele-specific PCR and the oxLDL level was determined by enzyme immunoassay in 548 individuals recruited at a health examination. Plasma LDL oxidation was estimated by the oxLDL/LDL ratio. The T allele was found to have a lower level of plasma oxLDL/LDL ratio [220 ± 90, 2030 ± 130, and 3230 ± 620 U/mmol (mean ± SEM) for GG, GT, and TT, respectively, P < 0.05 by ANOVA], while it showed a co-dominant effect on Lp-PLA2 activity. A multiple regression analysis revealed that age, the G994T genotype, and the plasma small-dense LDL level were independently associated with the oxLDL/LDL level. The G994T polymorphism in the Lp-PLA2 gene may influence the plasma LDL oxidation and further studies on the effect of this polymorphism in cardiovascular diseases are warranted.

It is well accepted that raloxifene, a selective estrogen receptor modulator, induces vasorelaxation through inhibiting extracellular calcium influx. This study evaluated the hypothesis that raloxifene relieves constricted vascular smooth muscle by scavenging reactive oxygen species (ROS). On isolated rat thoracic aorta contracted by phenylephrine or potassium, concentration-relaxation curves of raloxifene were constructed by utilizing a variety of drugs. First of all, endothelium denudation, indomethacin (cyclooxygenase inhibitor) or ICI-182,780 (estrogen receptor antagonist) pre-incubation did not attenuate the relaxation effect of raloxifene. Rotenone (mitochondrial oxidase inhibitor) decreased isometric force in phenylephrine-contracted aorta more than apocynin (NADPH oxidase inhibitor) did. Furthermore, rotenone pre-incubation blocked vasorelaxation effect to a significantly profound extent while apocynin pre-incubation failed to. Finally, in cultured vascular smooth muscle cells (VSMC), mitochondria ROS production was significantly inhibited by raloxifene. Each curve was constructed from at least four rats. ROS detection was performed in three independent experiments. Sample t test and one-way ANOVA of SPSS software were used to analyze the data. This study demonstrated that raloxifene does not relax contracted rat aorta in an estrogen receptor-, endothelium-, and prostaglandin-dependent way, but in a pathway involving mitochondria ROS.
Early disease detection and prevention is one of the most promising and yet challenging avenues to population healthcare. A prerequisite to successful prevention is timely identification of the individual who is prone to develop disease. When dealing with the common complex cardiovascular or metabolic diseases, individual susceptibility to disease is composite, and involves a yet poorly understood interaction between the individual’s genetic load (causative genes), genetic susceptibility (susceptibility genes) and environmental or other factors, allowing development of disease. A central task of investigators in the genomic era has been to identify the genes underlying the “genetic load”, the “genetic susceptibility” and the specific nature of the environmental factor. In humans, genome-wide linkage scans and candidate gene association studies have had limited success in identifying the causative or susceptibility genes underlying common complex diseases; currently, genome-wide association studies using SNPs are ongoing, yielding interesting and promising results. The data are, however, only partial. Genome-wide association studies using SNPs are ongoing, yielding improved outcome. As such evidence is not available yet, it is clear that these substitutions are related to hypertension, to declare yet, although the promise and optimism are prevalent and abundant data on metabolic syndrome, a complex of disorders including insulin resistance, hypertension, and dyslipidemia, is associated with the development of obesity. Obesity also induces macrophage accumulation in adipose tissue. Macrophages produce many of the proinflammatory molecules, such as tumor necrosis factor (TNF-α), and interleukin-6. Monocyte chemoattractant protein 1 (MCP-1) is released by adipose tissue. However, the underlying mechanisms in nephropathy with obesity have not been elucidated. We hypothesized that MCP-1 enhances the early nephropathy. We measured metabolic parameters in SHR/NDmc-rat (f6f6/f6f6) rats (CP) and compared them with those in their lean littermates (Lean). Serum MCP-1 level was determined. The level of TNF-α in the kidney was also determined as proinflammatory marker. Furthermore, we examined mRNA expressions (MCP-1, COX2) by using real-time RT-PCR in the kidney and adipose tissue. Protein expressions (PAl-1, PPAR-gamma, eNOS) were measured by western blot analysis. In 12 weeks of age, both groups of animals had hypertension due to their background of spontaneously hypertensive

Molecular cloning and sequencing of cDNAs encoding blood-pressure-related genes of SHR/kpo

Spontaneously hypertensive rats (SHR) are useful to elucidate etiology of essential hypertension in humans. Although QTL (quantitative trait locus) mapping of hypertension phenotypes and genomic region has been identified, discrete gene alterations are not defined. We cloned and sequenced several blood-pressure-related genes of Wistar-Kyoto (normotensive rat), SHR/kpo, SHRSP/kpo, and M-SHRSP/kpo to elucidate the genetic basis of susceptibility to hypertension. Total RNA was isolated using TRIzol (invitrogen) kit from abdominal aorta, liver, kidney and brain of rats. First-strand cDNAs were synthesized from total RNA of each tissue by the Superscript III reverse transcriptase with oligo-dT primer. Amplifications of above blood-related cDNAs by polymerase chain reaction (PCR) were carried out using Phusion High-Fidelity PCR kit (finzymes). Amplified DNA fragments were cloned, and the nucleotide sequences were determined. 5 genes (angiotensin II type I receptor, vasopressin receptor and natriuretic peptides) of WKY, SHR, SHRSP and M-SHRSP and endothelin receptor gene from SHRSP were cloned and sequenced. All cloned genes from SHRs have nucleotide mutations at coding region or flanking region, only CNTF (C-type natriuretic peptide) show no mutation in SHRs. We cloned and sequenced several blood-pressure-related genes of rats. In this work it was confirmed that there were mutations on genes of these factors of SHRs. Angiotensin II type I receptor, BNP (B-type natriuretic peptide) and vasopressin receptor of SHR have amino acid substitution. It is not clear yet that these substitutions are related to hypertension, to declare etiology of hypertension of SHR needs further gene identification and validation.

Enhanced monocyte chemoattractant protein 1 signaling in the early nephropathy of SHR/NDmc-rat: a model of metabolic syndrome

Metabolic syndrome, a complex of disorders including insulin resistance, hypertension, and dyslipidemia, is associated with the development of obesity. Obesity also induces macrophage accumulation in adipose tissue. Macrophages produce many of the proinflammatory molecules, such as tumor necrosis factor α (TNF-α), and interleukin-6. Monocyte chemoattractant protein 1 (MCP-1) is released by adipose tissue. However, the underlying mechanisms in nephropathy with obesity have not been elucidated. We hypothesized that MCP-1 enhances the early nephropathy. We measured metabolic parameters in SHR/NDmc-rat (f6f6/f6f6) rats (CP) and compared them with those in their lean littermates (Lean). Serum MCP-1 level was determined. The level of TNF-α in the kidney was also determined as proinflammatory marker. Furthermore, we examined mRNA expressions (MCP-1, COX2) by using real-time RT-PCR in the kidney and adipose tissue. Protein expressions (PAI-1, PPAR-gamma, eNOS) were measured by western blot analysis. In 12 weeks of age, both groups of animals had hypertension due to their background of spontaneously hypertensive
rats. Body weight was higher in CP compared with Lean. CP showed hyperglycemia, hyperinsulinemia, hyperlipidemia, and proteinuria. Serum MCP-1 level was significantly higher in CP than that in Lean, although renal MCP-1 mRNA expression was not different in both groups of animals. The concentration of TNF-α in the kidney increased in CP. PAI-1 protein and COX2 mRNA expression were upregulated in CP kidney, whereas PPAR-γ and eNOS protein expression were downregulated. In this study, enhanced MCP-1 may increase infiltration of macrophages and secretion of TNF-α, suggesting that they are important sources of inflammation in the early nephropathy.

EVIDENCE OF AN ENDOGENOUS DIGITALIS, MARINOBUBOTOXIN, IN THE SODIUM METABOLISM

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The endogenous digitalis-like factors such as ouabain, and marinobufagenin (MBG) are the most likely modulators of sodium and water metabolism in mammals via the sodium pump inhibition. Marinobufotoxin (MBT), in culture supernatant of Y-1 cells of adrenocortical origin and recently, we found a MBG-related compound, marinobufotoxin (MBT), in culture supernatant of Y-1 cells of adrenocortical origin and its hypertensive activity in rats. But its relationship to sodium metabolism has not been fully elucidated. Herein, we aimed to explore its hypertensive activity in rats. However, sodium chloride was measured with our ELISA. SBP was not different between the two groups during 4 weeks. The plasma concentration and urinary excretion of MBT-like immunoreactivity (MB Ti) were measured with our enzyme-linked immunosorbent assay (ELISA) after 4 weeks. Moreover, the MB Ti concentration in Y-1 culture supernatant of Y-1 cells, subcultured by adding 100 mM sodium chloride was measured with our ELISA. SBP was not different between the two groups during 4 weeks. The plasma concentration and urinary excretion of MBT-like immunoreactivity (MB Ti) were significantly (p<0.05) increased in rats receiving saline (28±2±0.99 vs. 44.9±3.30 pg/ml, and 0.86±0.065 vs. 1.34±0.124 ng/day, respectively). The MB Ti concentration in Y-1 culture supernatant was increased in a dose dependent manner with added sodium chloride. MBT was found to be released by sodium loading, and it may be produced in adrenocortical cells. It may be a clue to elucidate the mechanism of sodium sensitive hypertension.

EFFECTS OF ATYPICAL ANTI PSYCHOTICS ON BEHAVIORAL ABNORMALITIES IN AN ANIMAL MODEL FOR ATTENTION-DEFICIT/HYPERACTIVITY DISORDER, MALE ADOLESCENT SHRSP/Ezo

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Attention-deficit/hyperactivity disorder (AD/HD) is an early-onset neuropsychiatric disorder characterized by inattention, hyperactivity and impulsivity. We have previously proposed adolescent stroke-prone SHR (SHRSP/Ezo) as an appropriate animal model of AD/HD, based on symptomatic features. Recently, clinical reports indicated that some atypical antipsychotics ameliorated psychosisom mot excitation and cognitive dysfunction of schizophrenic patients. In this study, we investigated the effects of atypical antipsychotics on AD/HD-like behavioral abnormalities in adolescent SHRSP/Ezo, in comparison with those of typical antipsychotics. Six-week-old male SHRSP/Ezo (SHRSP) were bred in our laboratory. Age-matched Wistar-Kyoto rats (WKSP) were used as genetic controls. Atypical antipsychotics (olanzapine) and typical antipsychotics (haloperidol) were intraperitoneally administered 30 min before the test. Impulsive-like behavior and attentional dysfunction were evaluated by the elevated plus-maze (EPM) test and the Y-maze test, respectively. In the EPM test, SHRSP showed a significant increase of time spent in the open arms, compared with WKY. Olanzapine but not haloperidol dose-dependently decreased time spent in the open arms in SHRSP. Moreover, SHRSP demonstrated the impaired alternative performance in the Y-maze test, which was also ameliorated by olanzapine but not haloperidol. Both antipsychotics inhibited a marked increase of the total arm entries in SHRSP in the EPM and Y-maze tests. These findings demonstrated that SHRSP showed the impulsive behavior and attentional dysfunction, which was only ameliorated by atypical antipsychotics, olanzapine. Thus, atypical antipsychotics may be beneficial for AD/HD treatment, with different therapeutic spectrums.

SINGLE-NUCLEOTIDE POLYMORPHISMS IN RENIN-ANGIOTENSIN SYSTEM FOR DIAGNOSIS OF LOW-RENNIN HYPERTENSION

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Low-renin hypertension (LRH) affects 10% to 20% of Asia hypertensives. We tested the diagnostic validity of several candidate genes in renin-angiotensin system for LRH. We enrolled 137 patients with LRH and 235 patients with normal-renin hypertension (NRH). LRH was defined as their plasma renin activity (PRA) level on supine position is less than 0.1 ng/ml per h. NRH patients had normal PRA (0.1-5.5 ng/ml per h). Genotypes for 23 single nucleotide polymorphisms (SNPs) of 8 genes of renin-angiotensin system (ACE, ACE2, AGT, AT1R, AT2R, REN, CYH and CYP11B2) were determined by MALDI-TOF mass spectrometry method. Compared to NRH, LRH patients had higher systolic blood pressure, higher levels of serum sodium and urinary sodium, lower HDL level and higher LVMl (all p<0.05). Among the polymorphisms investigated, 1 SNPs located on the CYP11B2 and 1 SNPs in ACE2 were associated with LRH. For CYP11B2 T-344C, the frequency of C allele (0.36 vs. 0.26, OR=1.60, 95%CI 1.16-2.20, p=0.0037) was more prevalent in LRH than in NRH. The CC genotype had the lowest PRA while the TT genotype had the highest PRA (p=0.033). We also observed the significant distribution of ACE2 A8687G variants between LRH and NRH (p=0.006). The AA genotype had lower PRA compared to G carriers (p=0.010). The multifactor dimensionality reduction (MDR) method was performed to assess all possible genetic models and provide the best genetic model for differentiating LRH. The multisite genetic model that best predicted LRH included 4 SNPs of ACE2, CYP11B2, CYH genes. The model including all 4 variants was significantly better than any of the models using individual variants alone and a sensitivity of 73% of LRH. The presence of 4 variants is always associated with high risk of lower PRA (p=0.001). The present study suggested that CYP11B2 and ACE2 gene variants are associated with LRH. A genetic model based on CYP11B2, ACE2 and CYH variants was higher predictive of LRH.

A PROSPECTIVE STUDY ON THE RISK FACTORS AND GENETIC MARKERS FOR THE DEVELOPMENT OF ABNORMAL GLUCOSE METABOLISM IN HYPERTENSIVE PATIENTS

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This hospital-based cohort study was designed to assess the risk factors and genetic markers associated with the development of abnormal glucose metabolism in hypertensives. Three hundreds and thirty-three
hypertensive patients (191 male, 142 female; 58.22±10.74 years old) with normal glucose metabolism between January 2000 and December 2004 were analyzed in this study. For all participants, 20 polymorphisms of 9 candidate genes (TCF7L2, CAPN10, KCNJ11, ADIPOQ, ADIPOR1, ADIPOR2, CD36, LEPR and PPARγ) were determined by MALDI-TOF mass spectrometry method. The prognostic impact of clinical risk factors and these polymorphisms on glucose metabolism was assessed using Kaplan-Meier analysis and Cox’s regression. During a mean follow-up period of 55 months, a total of 33.64% of the study subjects developed an abnormal glucose metabolism (27.63% with impaired fasting glucose and/or impaired glucose tolerance, 9.01% with type 2 diabetes). The patients, who developed to abnormal glucose metabolism had more obvious impaired organ damage and abnormal metabolism, including higher BMI, higher OGTT glucose level, higher HOMA-IR, higher LVMI, higher urinary microalbumin, higher IMT of carotid artery and lower creatinine cleaning rate (all p<0.05). By Kaplan Meier analysis, rs1801282(C/G) G of PPARγ gene (p=0.01), obesity (p=0.04), higher OGTT 30min (p=0.02) and 60min glucose (p=0.01), higher triglycerides (p=0.01), IMT increasing or with plaque (p=0.01) were related with development of abnormal glucose metabolism. Moreover, Cox’s regression revealed that rs1801282 G (p=0.03), OGTT 60min glucose (p=0.01), HOMA-IR (p=0.01) and IMT increasing (p=0.03) were independent risk factors for the development of abnormal glucose metabolism. OGTT 60 min glucose and IMT increasing might be the risk factors, as well as PPARγrs1801282 C/G polymorphism might be the genetic marker for the development of abnormal glucose metabolism in hypertensives.

THE BXH/HXB RECOMBINANT INBRED STRAINS FOR ANALYSES OF COMPLEX TRAITS

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The BXH/HXB recombinant inbred (RI) strains (n=30) were derived from the SHR/Ola and BN-Lx/Cub progenitors. Recently, the RI strains were extensively genotyped and phenotyped. The genome of the RI strains has been covered with over 13,000 SNPs, gene expression profiles have been determined with Affymetrix arrays in kidneys, retroperitoneal fat, adrenals, soleus muscles, left ventricles and whole brains, and strains have been phenotyped for over 200 physiological traits. Owing to the cumulative nature of these results, the RI strains provide a unique model system for the analysis of complex cardiovascular and metabolic traits. Linkage and correlation analyses of physiological traits and transcript abundance (QTL, genetical genomics, and quantitative trait transcripts analyses) can be now used to identify candidate genes underlying physiological QTLs. Recently, blood pressures (BPs) and heart rates were determined in RI strains with radiotelemetry. Significant BP regulatory QTLs were found on chromosomes 10 and 19 (LRS 21.3 and 19, respectively). Several cis regulated expression QTLs on chromosomes 10 and 19 colocalized with BP regulatory QTLs, including genes involved in oxidative stress. In addition, a QTL associated with kidney TBARS levels (LRS=17.5) mapped near the BP QTL on chromosome 19 and kidney TBARS correlated with systolic BP (r=0.63, p=0.0006). These findings suggest that genetic determinants on chromosomes 10 and 19 modulate both oxidative stress and blood pressure and have resulted in the identification of several candidate genes that are now being further pursued in sequencing and functional studies.