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PROTEOMIC CHARACTERIZATION OF BIOMARKERS FOR SALT SENSITIVE STROKE-PRONENESS OF STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS (SHRSP/IZM)

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Background: SHRSP is a good model for human essential hypertension and dies shortly with stroke after salt loading. Our previous study showed that clofibrate attenuated developing stroke and significantly prolonged survival through elevation of CYP2C11 expression and brain blood flow improvement in salt-loaded SHRSP/Izm. To explore biomarkers related to salt sensitive stroke-proneness, we directly analyzed cerebral cortex proteins in salt-loaded SHRSP/Izm by MALDI-MS/MS system. *Design:* Male SHRSP/Izm (9 weeks) were fed a regular diet or with 1 % NaCl solution for drinking water, salt water and 0.25 % clofibrate for 10 days. Three groups of SHRSP/Izm and age-matched SHR/Izm brain were frozen and 10 μm sections were mounted on film-coated glass slides and fixed. For MALDI-MS analysis, specific cerebral cortex regions were detached from slides and mounted on a stainless steel target through double-sided electrically conductive tape. Forty mg/ml of Sinapic acid solution were directly spotted onto the section and analysed by MALDI-MS. The interested portions were also cut out of thin section by laser capture micro dissection and dropped directly into lysis buffer containing 0.1 % Triton X-100 for protein extraction. The extracted proteins were analyzed by MALDI-MS/MS system. *Results and Conclusions:* Several protein-mass peaks were obtained by in situ mass spectrum analysis. Principal component analysis revealed three peaks out of 60 peaks clearly separated those groups. Genetic algorithm showed 93.75 % recognition capability, using five peaks to classify those models. MS/MS analysis was performed to each picked peaks for identification. We suppose those markers might be involved in salt-sensitive stroke-proneness of SHRSP/Izm.

EFFICACY OF A RENIN-ANGIOTENSIN SYSTEM (RAS) VACCINE FOR PREVENTION OF PROTEINURIA AND RENAL INJURY IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

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Objectives: A phase IIa clinical trial testing vaccine against RAS showed vaccination significantly decreased blood pressure without serious adverse events (Tissot *et al.,* Lancet 2008). However few reports are available on the renal-protective effect provided by vaccination. Therefore in this study, we examined the effects of vaccination against RAS to evaluate its renal-protective effect. *Methods:* The peptide, eight-amino-acid sequence from rat angiotensin II type 1A (AT1) receptor, was synthesized as reported by Zhu *et al.* (*Cell Mol Immunol* 2006). Keyhole Limpet Haemocyanin (KLH) was used as carrier protein. 3-week-old male SHR were randomly divided into 6 groups as follows: SHR in group 1 (n=5) and in group 2 (n=5) were control SHR. SHR in group 3 were injected subctaneously with KLH vehicle (n=10) and in group 4 were injected with AT1 receptor vaccine (n=10) at 4, 6, and 8 weeks of age. SHR in group 5 were administered hydralazine (n=7) and in group 6 were administered candesartan (n=6) from 5 weeks of age. Ten weeks after the final vaccination, all rats except those in group 1 were administered the nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME) to induce proteinuria and renal injury. Systolic blood pressure (SBP) was monitored weekly. The animals were euthanized at 21-week-old. *Results:* In comparison with SBP of group 1, 2 and 3, there was significant decrease in SBP of group 4 from 10 weeks of age to the end of study. SBP level of group 5 and 6 were almost equal to that of group 4 during whole study. Urine protein of group 2, 3 and 5 increased after the administration of L-NAME, but that of group 4 and 6 did not significantly increased. *Conclusions:* These results suggest that vaccination against AT1 receptor not only decreases BP, but also significantly attenuates the development of L-NAME-induced proteinuria and renal injury in SHR and this renal-protective effect was independent on anti-hypertension effect.

DIETARY RESTRICTION SUPPRESSES INFLAMMATION AND DELAYS THE ONSET OF STROKE IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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Background: It is not clear whether inflammation is cause or effect of stroke, however, inflammatory events are important in onset or development of stroke. Dietary restriction (DR) suppresses systemic inflammation in animal models. We studied whether DR delays the onset of stroke in stroke-prone spontaneously hypertensive rats (SHRSP). *Design:* SHRSP were assigned to either control (*ad libitum*) or DR (50 % diet of control), and the onset of stroke and lifespan were examined. After two week of DR, plasma inflammatory cytokine levels, gene expression levels in several tissues, and adhesion molecule expression levels in cerebrovascular endothelial cells (CVECs) were measured. Macrophage infiltration into brain was assessed immunohistochemically. *Results:* DR markedly delayed the onset of stroke and extended their lifespan in SHRSP without affecting blood pressure compared to control. Plasma concentrations of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and monocyte chemoattractant protein-1 (MCP-1), and their mRNA levels in adipose tissue were significantly lower in DR. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) mRNA expressions in CVECs, and macrophage infiltration into brain in DR were lower than control. However, DR did not affect mRNA expression levels of anti-oxidative enzymes such as catalase, glutathione peroxidase, CuZnSOD, and MnSOD in brain and CVECs. In cultured CVECs, IL-1βand TNF-α treatment increased MCP-1, CRP, ICAM-1, and VCAM-1 mRNA and their protein levels. *Conclusion:* These data indicate that suppression of inflammation in response to DR may lead to a delay in the onset of stroke in SHRSP.

TRANSCRIPTIONAL REGULATION OF PROGRESSIVE RENAL DISEASE BY THE GENE SILENCING PYRROLE-IMIDAZOLE POLYAMIDE TARGETED TO THE TGF-β1 PROMOTER

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Background: Pyrrole-imidazole (PI) polyamides are novel gene silencers that inhibit promoter activity by strong binding on DNA to block binding of transcription factor. We evaluated long term effects, pharmacological properties and specificity of PI polyamide targeted to TGF-β1 promoter on the progress renal injury, and investigated novel factors and mechanisms in the TGF-β1-related renal injury by the transcriptional down-regulation of TGF-β1 with PI polyamide in Dahl-S rats with high salt-loading. *Results*: FITC-labeled PI polyamide was distributed and strongly bound nucleus in kidney, aorta and liver after iv injection. This PI polyamide completely inhibited the increases in proteinuria and albuminuria in salt-loaded Dahl-S rats along with suppression of TGF-β1 expression. This PI polyamide specificically inhibited TGF-β1 in assessment by the microarray analysis. This PI polyamide down-regulated 13 in 681 growth factor transcripts, 18 in 685 cytokine transcripts, and 11 in 397 ECM transcripts on these array plates. Almost genes are relating with TGF-β1, indicating the specific inhibition of TGF-β1 with PI polyamide. In the renal injury in Dahl-S rats by transcriptional down-regulation of TGF-β1 with PI polyamide, we found TGF-β1-associated novel molecules including ECM molecules, TGF-β1-related cytokines, angiogenic factors, cell stabilizing factors, proteinases, renal injury-related factors (cathepsin W, Ngal, and clusterin) and unknown functional factors (stratifin, enolase 2γ megsin, IgG Fc receptor, synaptotagmin XIII, complement 3, and plectin 1). *Conclusion*: PI polyamides will be a next generation of gene therapeutic agent to ameliorate diseases that are not treatable with current medicines by transcriptional gene regulation.

TAURINE AND ESTROGEN IMPROVES ENDOTHELIAL PROGENITOR CELL SENESCENCE IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR/IZM)

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Background: Endothelial cell is an important target organ of hypertension. In SHR acetylcholine-induced vaso-dilation was blunted, which was improved by taurine. Bone marrow-derived endothelial progenitor cells (EPCs) shown to be incorporated into sites of physiological and pathological neo-vascularization *in vivo*. Present study was carried out to clarify the effects of taurine and estrogen on EPC senescence. *Design and Results:* EPCs were isolated and cultured from peripheral blood and evaluated EPC senescence in 6-week old spontaneously hypertensive rats (SHR/Izm). Dual-stained cells positive for DiLDL and lectin were judged to be EPC. After *ex vivo* cultivation, we detected senescent EPCs by acidic beta-galactosidase staining. Telomerase activity was measured using PCR-ELISA based assay. Number of senescent EPCs in SHR/Izm was high and telomerase activity was blunted compared with that in WKY/Izm. Senescence associated beta -galactosidase positive EPCs were significantly reduced and telomerase activity was improved after treatment of taurine in SHR/Izm and WKY/Izm. 17beta-Estradiol (E2) significantly increased the number of adherent BM-EPCs from SHR/Izm, which was almost completely inhibited by 3-kinase (PI3-K) blockers, Wortmannin or LY294002. E2 treatment inhibited the senescence and improved telomerase activity of BM-EPCs from SHR/Izm and WKY/Izm. E2 activated the phosphorylation of Akt in BM-EPCs in both rats. *Conclusion:* Present study shows that E_2 augmented the differentiation in EPCs derived from SHR/Izm through PI3-K/Akt pathway. Taurine and estrogen improves the accelerated EPC senescence in hypertension.

ROLE OF EXTRACELLULAR MATRIX (ECM) REMODELING IN HIGH-DOSE ANGIOTENSIN BLOCKER-INDUCED REGRESSION OF HYPERTENSION AND GLOMERULO-SCLEROSIS

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Background: Previously, we reported that prehypertensive treatment of spontaneously hypertensive rats (SHR) with a renin-angiotensin system inhibitor resulted in prevention of hypertension. The aims of this study were to examine the effects of high-dose angiotensin receptor blocker (ARB) on regression of hypertension and glomerulosclerosis in two separate animal models, and their molecular mechanisms. *Design and Results:* (Experiment 1) 16-week-old SHR with established hypertension were treated transiently with high-dose ARB (candesartan 50 mg/kg/d) or calcium-channel blocker (CCB). Treatment of SHR with ARB but not CCB caused a regression of established hypertension, and decreased renal microvascular media/lumen ratios associated with changes in arteriolar matrix metalloproteinase (MMP) activity and expression. (Experiment 2) Mice were administered adriamycin (18 mg/kg) to induce glomerulosclerosis. High-dose ARB treatment of mice with established glomerulosclerosis caused a significant regression of glomerulosclerosis and albuminuria and an increase in glomerular MMP-2 activity. The regression of glomerulosclerosis was partially attenuated by pretreatment with the MMP inhibitor doxycycline, as well as in MMP-2-knockout mice. *Conclusion:* These results suggest that high-dose ARB causes marked regression of renal arteriolar hypertrophy and glomerulosclerosis in the SHR model of hypertension, and ADM model of glomerulosclerosis, in part by mechanisms involving MMP-mediated remodeling of the ECM. Clinical confirmation of these results may result in new strategies for the therapy of hypertension and glomerulosclerosis.

FRONTOCORTICAL FUNCTION OF SHRSP, AN ANIMAL MODEL OF ATTENTION-DEFICIT/ HYPERACTIVITY DISORDER

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Background: Attention-deficit/hyperactivity disorder (ADHD), one of the most common childhood psychological disorders, is known to induce a phenotypically heterogeneous symptom characterized by inattention, hyperactivity and impulsivity, which can give rise to social and affective impairments. The pathogenesis of ADHD remains elusive although cortical hypofunction involving dopaminergic systems has been postulated. *Design:* In the present study, we have evaluated synaptic plasticity expressed as long-term potentiation (LTP), as a functional measure of the medial prefrontal cortex (mPFC), in a symptomatic animal model of ADHD, juvenile stroke-prone spontaneously hypertensive rats/Ezo (SHRSP/Ezo). *Results:* LTP induction in the hippocampal CA1-mPFC pathway was impaired in SHRSP/Ezo, whereas LTP in the Schaffer collaterals-CA1 pathway was induced. A monoamine reuptake inhibitor methylphenidate (1 mg /kg, i.p.) or a selective noradrenaline (NA) reuptake inhibitor atomoxetine (3 mg/kg, i.p.), which are clinically available for ADHD treatment, reversed synaptic impairment in the mPFC, while a selective dopamine (DA) reuptake inhibitor GBR12909 (3 mg/kg, i.p.) failed. In addition, methylphenidate and atomoxetine, but not GBR12909, significantly increased the cortical extracellular NA and DA levels in SHRSP/Ezo. Pharmacological effects of monoamine reuptake inhibitors were also observed on the impaired behavioral response to contextually conditioned fear. *Conclusion:* The present study demonstrated that synaptic plasticity was impaired in the hippocampus-mPFC pathway but not in the intrahippocampus, possibly indicating that frontocortical dysfunction underlie the symptomatic feature of an ADHD animal model, SHRSP/Ezo. Pharmacological findings further support the notion that monoaminergic mechanisms may be critical for the hypofrontality observed in SHRSP/Ezo, as postulated in ADHD patients.

METABOLIC SYNDROME AND REACTIVE OXYGEN SPECIES IN SHRSP.*Z-LEPR*$^{FA/}$IZM DMCR RATS, THE MODEL OF METABOLIC SYNDROME

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Background: Metabolic syndrome (MS) is associated with overproduction of reactive oxygen species (ROS). Uncoupling proteins (UCPs) are a family of mitochondrial transporters that can uncouple oxidative phosphorylation through a dissipation of the proton gradient across the inner mitochondrial membrane. It is reported that UCP2 may play a role in protection against ROS. This study was designed to clarify the contribution of UCP linked ROS in SHRSP.*Z-Lepr*$^{fa/}$Izm Dmcr rats (SP·ZF). *Design:* At 12 weeks of age, male Wistar-Kyoto (WKY) rat, lean phenotype (Lean) and SP·ZF were used in this study. The 24h urinary NO_2^-+NO_3^- and 8-OHdG excretions were measured by Griess and ELISA, respectively. The mRNA expression of UCP2 was determined by real-time PCR in heart. *Results:* Urinary NO_2^-+NO_3^- excretions was significant greater ($p<0.05$) in SP·ZF than in other strains, Lean and WKY. Urinary 8-OHdG excretions in SP·ZF was higher than in Lean ($p<0.05$). In heart, the mRNA expression of UCP2 was significant greater ($p<0.05$) in SP・ZF than in Lean. *Conclusion:* The increase of UCP2mRNA expression may depend on increased oxdative stress in SP·ZF. The increase of UCP2 mRNA expression may relate to the phenotype in metabolic syndrome status in SP·ZF.

COMPLEMENT 3 IS A PRIMARY FACTOR FOR THE CARDIOVASCULAR AND RENAL REMODELINGS IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background: We have demonstrated that vascular smooth muscle cells (VSMCs) from spontaneously hypertensive rats (SHR) show the

synthetic phenotype that induces the angiotensin (Ang) II production and the exaggerated growth. We found that complement 3 (C3) is expressed only in VSMCs from SHR, not in cells from Wistar Kyoto (WKY) rats, by microarray analysis. We also demonstrated that C3 changes VSMCs to the synthetic phenotype through KLF-5. In the present study, we investigated contributions of C3 on phenotypic change and the exaggerated growth of VSMCs and mesangial cells (MCs) from SHR and SHR-SP *in vitro*, and cardiovascular and renal organ growth in SHR-SP *in vivo*. *Design:* We examined C3a inhibitor SB290157 on proliferation, expression of phenotype marker and KLF-5 mRNAs in VSMCs or MCs from WKY rats, SHR and SHR-SP. We examined effects of SB290157 on Ang II production in VSMCs or MCs measured by radioimmunoassay in conditioned medium. We examined effects of SB290157 on PCNA staining in aortic and kidney from three strains *in vivo*. *Results*: SB290157 significantly inhibited proliferation, expression of phenotype markers and KLF-5 mRNAs and significantly decreased Ang II production in VSMCs and MCs from SHR and SHR-SP. C3a inhibitor significantly suppressed PCNA staining in kidney from SHR/Izm and SHR-SP/Izm *in vivo*. *Conclusion:* C3 is a primary factor that genetically involved in the cardiovascular and renal remodelings in hypertension.

CAFFEIC ACID PHENETHYL ESTER SUPPRESSES ADIPOCYTE DIFFERENTIATION IN 3T3-L1

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Background: Obesity is associated with many diseases, diabetes, dyslipidemia and atherosclerosis which become risk factors of metabolic syndrome. It is a condition in which excess body fat has accumulated due to a charge of lipids into adipocyte and an increase by number of differentiated mature cells. *Design:* In this study we investigated the inhibitory effect of CAPE for differentiation 3T3-L1 cells to adipocyte. 3T3-L1 was differentiated to adipocytes with dexamethasone, isobutylmethylxanthine and insulin among 7 days. After differentiation, cells were stained with Oil Red-O to detect oil droplets in adipocyte. Additionally the cells were lysed and measured for triglyceride content. Total RNA was harvested on days 4 and 7. Then RNA was analyzed using RT-PCR. The effect to protein expression was analyzed by western blotting. *Results:* Staining with Oil Red-O cells, CAPE dose-dependently suppressed oil droplet accumulation and reduced the size of droplets. CAPE at concentrations of 25 to 50 μM could significantly inhibited triglyceride deposition ($P<0.05$). PPARgamma mRNA expression was significantly suppressed by CAPE at concentrations. In addition C/EBPalpha was decreased at dose-dependently. Fas and aP2 are known to associate for lipid metabolism on mature adipocyte, were also suppressed by CAPE. Insulin bind to insulin receptor following activates the IRS and Akt pathway. In this study, CAPE treated cells were inhibited the phospholylation of Akt. *Conclusion:* These findings suggested that CAPE suppressed lipid accumulation at 3T3-L1 differentiation to adipocytes through down regulation of PPARgamma and C/EBPalpha. In addition CAPE has a potential for inhibitory effect on activate the Akt pathway.

INFLUENCE OF DIETARY CUSTOM AND EFFECTS OF TAURINE SUPPLEMENTATION ON OXIDATIVE STRESS AND ENDOTHELIAL PROGENITOR CELL FUNCTION IN HUMANS

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Background: Long-term ingestion of foods that contain high levels of antioxidants act to protect against production of excessive oxidation. Endothelial progenitor cells (EPCs) repairing cardiovascular damages are pivotal cells to prolong life span, which are suppressed by oxidative stress. We evaluated influence of life-style and effects of taurine supplementation on oxidative stress and endothelial progenitor cell function in humans. *Design*: Twenty healthy men aged 20-30 years were participated in this study. As baseline study, we evaluated vascular endothelial function, nutritional intake according to the WHO-CARDIAC (Cardiovascular Diseases and Alimentary Comparison) Study protocol. Peripheral blood mononuclear cells were separated to quantify EPC colony formation. Oxidative stress in peripheral blood were evaluated by d-ROMs and BAP tests by FRAS4, and TBARS assay. As nutritional intervention study, participants received 3 g of taurine or placebo peroraly for 2 weeks as a double blind test, than the oxidative stress and EPC colony formation were evaluated. *Results:* Smoking significantly suppressed EPC colony formation. Taurine supplementation significantly increased EPC colony formation. Taurine supplementation significantly decreased oxidative stress markers as d-ROMs and TBARS in peripheral blood and urinary 8-OHdG. *Conclusion:* As anti-oxidative nutrition taurine supplementation increase EPC function. Thus the anti-oxidative nutritional custom may lead prolongation of life span of human with activation of repairing cells.

GENOME-WIDE PHARMACOGENOMIC RESPONCE TO ANTIHYPERTENSIVE MEDICATION: THE HOMED-BP-GENE STUDY

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Background: To elucidate the genetic background underlying antihypertensive drug responsiveness, we carried out a genome-wide association study (GWAS) using the subjects under home blood pressure monitoring. *Design:* The subjects studied were recruited from the participants of HOMED-BP study after obtaining the informed consent for the genetic analysis. Home blood pressure (HBP) was measured every day within 1 hour after wake-up using HEM747-IC-N (Omron) for one month. Half million single nucleotide polymorphisms (SNPs) were determined using GeneChip Genome-Wide Human SNP5.0 Array (Affymetrix). The study protocol was approved by the ethical committee of Osaka University. *Results:* We excluded the SNPs from the analysis which showed low call rate and minor allele frequency less than 0.05. Ultimately, 278 subjects were used for the genetic and statistical analysis. Approximately 20 days of initial dose of anityhypertensive medication, Ca antagonist, ACE inhibitor or angiotensin II receptor blocker (ARB), decreased mean HBP of the objectives from 148.7/88.3 mm Hg to 142.2/85.5 mm Hg. After adjustment of confounding factors, 15 SNPs distributed on 8 chromosomes were significantly ($p<0.0001$) associated with mean HBP reduction in whole subjects (n=278). We also carried out the same investigation to each type of 3 antihypertensive drugs, resulted that SNPs correlated with responsiveness of medication were completely different according to the type of drug. The only positive association observed in both all subjects and subjects under Ca antagonist treatment was SNPs in storkhead box 2 gene. *Conclusion:* The genetic background of the responsiveness of antihypertensive medication is significantly different according to the type of drugs.

CARDIOVASCULAR RESPONSES TO MICROINJECTIONS OF ENDOMORPHIN-2 INTO THE NUCLEUS TRACTUS SOLITARIUS ARE ATTENUATED IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Endomorphin-2 (EM2) is an endogenous ligand of μ_1-opioid receptors (OPRM1) presenting in the nucleus tractus solitarius (NTS). EM2-containing neurons in the hypothalamus, key region in cardiovascular responses to stress, project to the NTS. On the other

hand, spontaneously hypertensive rats (SHRs) showed augmented cardiovascular responses to stress. The study was aimed to examine whether effects of EM2 in the NTS differ between SHR and Wistar-Kyoto rats (WKYs). *Design and Results:* Urethane-anesthetized, 14-week-old male SHR/Izumo strain (SHR/Izm) and age-matched WKY/Izm were used. Depressor and bradycardic responses and inhibition of the greater splanchnic nerve activity elicited by microinjections of EM2 (40 pmol) into the medial subnucleus of NTS (mNTS) of SHR/Izm were significantly smaller than in WKY/Izm. Attenuation effects by microinjection of EM2 into the mNTS of SHR/Izm on depressor and bradycardic responses elicited by electrical stimulations of the aortic baroreflex nerve were significantly smaller than in WKY/Izm. Microinjection of naloxonazine (200 pmol), OPRM1 antagonist, alone into the mNTS elicited neither BP nor HR response in both groups of rats. Expression levels of OPRM1 mRNA in the NTS were also investigated by real-time RT-PCR. The expression of OPRM gene was assessed in relation to glyceraldehyde-3-phosphate dehydrogenase gene. The level of OPRM mRNA in the NTS of SHR/Izm was significantly lower than in WKY/Izm. *Conclusions:* EM2 exerts no tonic effects on the mNTS of both groups of anesthetized rats. In the mNTS of SHR/Izm, the attenuation of depressor and bradycardic responses and inhibitory action of sympathetic vasomotor tone elicited by EM2, and the reduction of effect of EM2 on stimulation of the aortic baroreflex might be due to a quantitative decrease of OPRM1 compared to WKY/Izm.

A NEW DEVICE FOR MILK COLLECTION FROM LACTATING RATS: APPLICATION TO THE ANALYSIS OF AMINO ACID COMPOSITION OF MILK IN SHR AND WKY

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Background: Nutrition in the neonatal period through mother's milk may be an important environmental factor influencing the development of hypertension in spontaneously hypertensive rats (SHR). However, it is difficult to obtain rats' milk samples enough for analyses of various nutrients. In this study, we have developed a new device for sampling milk from rats, which was applied in the comparison of the amino acid-composition between SHR and Wistar-Kyoto rats (WKY). *Design:* Within 48 h after delivery, parturition litters were reduced to 9 for each mother rats. Milk samples were obtained using the newly developed device on 2nd, 9th, 16th and 23rd postpartum days. Pups were separated from their mother 6 h before sampling. The mother rat was administrated with oxytocin (0.02U/100 gBW) just before sampling milk. *Results:* Milk yield increased during the lactation period and reached a maximal on the 23rd day (780±190 and 760±50 ul for WKY and SHR, respectively). No inter-strain difference was observed in the milk volume examined. Tau, b-Ala and Mea content in the milk was significantly greater in SHR than in WKY, while Pea, Glu, Gln, Gly, Cit and Lys was lower in SHR than in WKY. Total amino acids content was not significantly different. *Conclusion:* The newly developed device was useful in the sampling of milk in rats. Further study is necessary to clarify the significance of the difference on amino acids composition between the two strains.

BLOOD PRESSURE, HEART RATE, LOCOMOTOR ACTIVITY AND ANALYSIS OF VARIATIONS OF THESE PARAMETERS IN CONGENIC RATS

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Introduction: The congenic rats (SHRSPwch1.0) were derived from stroke-prone SHR/Izumo (SHRSP/Izm) and Wistar-Kyoto rat/Izumo (WKY/Izm). The blood pressure, heart rate and locomotor activity and variations of these parameters in SHRSPwch1.0 have not been fully studied yet. Therefore, systolic arterial pressure (SAP), heart rate (HR) and locomotor activity (ACT) of SHRSPwch1.0, SHRSP/Izm and WKY/Izm were studied. *Methods:* Male mature SHRSPwch1.0 were used and age-sex matched SHRSP/Izm and WKY/Izm for the controls. They were housed in controlled (22 °C), 12-hour light-cycled (07:00 on 19:00 off) environment. The SAP, HR and ACT were monitored using telemetry, and the variations of these parameters were analyzed using the maximum entropy method. *Results:* As expected, SAP in SHRSPwch1.0 was lower than in SHRSP/Izm but higher than WKY/Izm (194±7 vs. 229±7 vs. 117±4 mm Hg, P=0.0098, Kruskal-Wallis). HR in SHRSPwch1.0 was lower than in SHRSP/Izm but did not differ from that in WKY/Izm (310±24 vs. 381±16 vs. 313±30 beats/min, P=0.0001). ACT in SHRSPwch1.0 was higher than those in SHRSP/Izm and WKY/Izm (62±47vs. 26±26 vs.13±10 counts/10 sec, P=0.0001). The variations of SAP, HR and ACT in SHRSPwch1.0 were more clearly observed in 24-h periodicity than those in SHRSP/Izm and WKY/Izm. The variations of both HR in SHRSP/Izm and ACT in WKY/Izm were not clearly observed in 24-h periodicity. *Summary:* The SAP in SHRSPwch1.0 demonstrated intermediate value between SHRSP/Izm and WKY/Izm. Its variation exhibited most clearly 24-h periodicity amongst SHRSP/Izm and WKY/Izm. However, the variation of HR in SHRSPwch1.0 is more likely to be that of WKY/Izm, and the variation of ACT in SHRSPwch1.0 is more likely to be that of SHRSP/Izm.

PRAVASTATIN, STEROID HORMONE, OSTEONECROSIS, FEMORAL HEAD, SHRSP RATS

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The main causes of steroid-induced osteonecrosis of the femoral head (ONFH) are considered to be increased oxidative stress, lipid metabolism disturbance, and peripheral circulatory failure due to steroid hormones. The aim of this study was to evaluate the effectiveness of statin in preventing steroid-induced ONFH in stroke-prone spontaneously hypertensive rats (SHRSP). Seventy nine 13-week-old male SHRSP/Izm were divided into four groups: a control group (group C), pravastatin-administered group (group P), steroid-administered group (group S), and group administered pravastatin plus steroid (group PS). The steroid hormone was administered at 15 weeks of age. Pravastatin as a statin was continuously administered with drinking water from 13 weeks of age for 4 weeks. Rats were sacrificed at 17 weeks of age. Osteonecrosis was diagnosed based on histopathological examination. Oxidative stress was detected by immunostaining. Improvement was not seen with pravastatin for lipid metabolism. The incidence of histological ONFH indicated that pravastatin markedly inhibits ONFH. Immunohistochemical staining for oxidative stress showed that the staining was significantly lower in the group PS than in the group S. The ratio of adipocyte area in the bone marrow in group PS decreased more than in group S significantly. Pravastatin, which showed no improvement of lipid metabolism, seems to reduce the incidence of steroid-induced ONFH in SHRSP by decreasing oxidative stress and the ratio of adipocyte area at sites. Pravastatin is useful for prevention of steroid-induced osteonecrosis.

OBESE HYPERTENSIVE SHR.CG-*LEPR*^{CP}/NDMCR AND SHRSP.*Z-LEPR*^{FA}/IZMDMCR AS MODELS FOR ATHEROSCLEROTIC DISEASE

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Background: Atherosclerosis is commonly observed in obesity. Obese atherosclerosis-prone animal models may be a promising tool for understanding the pathophysiology of obesity-associated atherosclerosis and for developing effective therapeutic strategies. However, most rat strains are resistant to atherosclerosis. This study aimed to assess the susceptibility of 2 obese hypertensive rat models, SHRSP.Z-*Lepr*^{fa}/IzmDmcr rats (SHRSP-fatty) and SHR.Cg-*Lepr*^{cp}/NDmcr rats (SHR-cp), to arterial lipid deposition, a characteristic feature in atherosclerosis, by comparing these strains with nonobese stroke-prone spontaneously hypertensive rats (SHRSP). *Design*: Eight-week-old male SHRSP, SHRSP-fatty, and SHR-cp were fed a high-fat and high-cholesterol diet containing 20 % palm oil, 5 % cholesterol, and 2 % cholic acid for 5 weeks. Body weight, blood pressure, and fasting serum total cholesterol were measured in 12-week-old rats. Oil red O staining was conducted to visualise lipid deposition in the mesenteric artery. *Results:* The body weight of 12-week-old SHRSP-fatty and SHR-cp was higher than that of SHRSP (p<0.005). The systolic blood pressure of SHRSP and SHRSP-fatty was higher than that of SHR-cp (p<0.005). Serum total cholesterol was elevated in SHRSP-fatty (p<0.005) and SHR-cp (p<0.05) compared with those in SHRSP. Lipid deposition area in the mesenteric artery was more extensive in SHRSP-fatty (37.7±4.9 %; p < 0.005) than in SHRSP (13.1±2.8 %), but was markedly less in SHR-cp (1.8±0.4 %; p < 0.05). *Conclusion:* These results indicate that SHRSP-fatty is highly susceptible to arterial lipid deposition, whereas SHR-cp is resistant to such deposition. SHRSP-fatty may be a useful obese rat model for studying atherosclerotic processes.

CAROTENOIDS AND CARDIOVASCULAR DISEASE RISKS IN JAPANESE ELDERLY AND ADOLESCENTS－IMPLICATION FOR RISK REDUCTION

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Background: Various antioxidants were proven in 1990's to prevent stroke in SHRSP, which showed increased urinary 8-OHdG excretion as oxidative stress markers. Concomitantly WHO-coordinated CARDIAC Study demonstrated increased 24 hour urinary (24U) 8-OHdG excretion in hypertensive and /or diabetic Africans without medication, indicating the involvement of oxidative stress in the pathogenesis of lifestyle-related diseases. *Design:* To prove the association of antioxidants with cardiovascular diseases (CVD) risks, health survey similar to CARDIAC Study was conducted in 43 male and female Japanese elderly aged 56-83 and 49 male and female adolescents aged 18-22 and total carotenoids (TC) including lutein, β-cryptoxanthin, α and β-carotene and lycopene in the blood were assayed. Elderly and adolescents were randomized into 2 groups, recommended to eat vegetables with or without 200 ml of vegetable juice daily for 2 months. *Results:* Baseline CVD risks such as systolic blood pressure, triglycerides, insulin resistance (HOMA-IR) were significantly inversely related with TC in the elderly and significantly positively related with HDL in the adolescents. Two month intervention significantly increased TC particularly in vegetable juice plus groups in the elderly and adolescents, and significantly decreased CVD risks in the elderly with higher baselines of triglycerides and HOMA-IR, and significantly reduced LDL cholesterol and abdominal circumference in the adolescents. *Conclusion:* Blood TC was significantly associated with CVD risks in Japanese elderly and adolescents, and 2 month interventions to increase vegetables with or without vegetable juice significantly increased TC, concomitantly with significant CVD risk reduction.

ALLEVIATION OF SALT-SENSITIVITY BY SOY AND FISH INTAKES IN HUMANS AND SHRSP

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Background: Soy and fish protein diets containing isoflavones (I) and taurine (T), respectively, extended the life-expectancy of salt-loaded SHRSP. In WHO-CARDIAC Study, 24-hour urinary (24U) salt (Na) excretion was significantly positively associated with blood pressure (BP) in males and post-menopausal females, suggesting estrogenic influence on salt-sensitivity, and the structure equation analyses showed 24UT to creatinine ratio was inversely related with stroke and coronary heart disease (CHD) mortalities. *Design:* Population strategy to eat well-balanced low salt and high soy diets were enforced for 5.6 million inhabitants of Hyogo Prefecture and CARDIAC Study health survey were carried out for 894-1241 inhabitants aged 40-80 for 3 years. In 7 Japanese CARDIAC populations, BP in high or low salt eaters (24UNa ≥ or <10g) was compared in relation to 24UT. *Results:* Average 2 g salt intake reduction from 12.0g with the concomitant significant fall by 3.5 mm Hg systolic BP from 131.9±18.7 mm Hg and 1.7 times 24UI increase from 22.2±1.6 mg were observed. BP in higher 24 UNa excreters (≥10g salt) was significantly higher than in lower Na excreters (<10g) in the individuals taking less than 25 mg I daily, but no significant Na-related BP rise was observed in individuals taking over 25 mg I. Similarly among males excreting upper 45 % of 24UT, BP in high and low salt eaters showed no significant difference in contrast to significant Na-related BP rise in males excreting lower 45 % of 24 UT. *Conclusion:* Enough soy I and fish T intakes alleviate the adverse BP effect of Na intake also in humans.

DEVELOPMENT OF NEW RAT MODEL FOR NONALCOHOLIC FATTY LIVER DISEASE IN SHRSP5/DMCR FED A HIGH-FAT AND HIGH-CHOLESTEROL DIET

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Background/Aims: Patients with nonalcoholic fatty liver disease (NAFLD) are increasing worldwide, and preventive measures are an urgent need and primary concern today. The purpose of this study is to develop and clarify the usefulness of the SHRSP5/Dmcr rat, derived from a stroke-prone spontaneously hypertensive rat, as an animal model for human NAFLD. *Designs:* Ten-week-old male SHRSP5/Dmcr rats were divided into six groups. Half were fed a high-fat and high-cholesterol containing diet (HFC diet), and the others were fed the control SP diet for 2, 8 and 14 weeks. *Results:* Serum transaminase activity, tumor necrosis factor alpha level, and serum and hepatic total cholesterol levels were significantly higher in rats fed the HFC diet than those of the SP diet group after 2-week feeding, and the levels further elevated after 8 and 14 weeks of the HFC diet. In contrast, the levels of serum glucose, adiponectine and albumin were lower in the HFC group than the SP group. Histopathologically, the HFC diet increased steatosis and fibrosis in the liver after 2 weeks, and produced macrovesicular steatosis, ballooned hepatocytes with Mallory's body formation in some, and inflammatory cell infiltration in the liver after 8 weeks. Interestingly, this fibrosis formed a honeycomb fibrosis after 14 weeks. According to the progression from steatosis to the fibrosis, expressions of TGF-β, NF-κB subunit and α-SMA mRNA and protein increased. *Conclusions:* SHRSP5/Dmcr rats fed the HFC diet appear to be a useful model for inducing all stages of NAFLD *via* a continuous feeding experiment.

NEUROPROTECTION ROLE OF CLOFIBRATE IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Stroke-prone spontaneously hypertensive rats (SHRSP) are characterized by severe spontaneous hypertension and the development of cerebrovascular diseases, and a useful animal model to investigate the mechanism responsible for salt-induced stroke. Peroxisomal proliferator-activated receptor α (PPARα) agonist, clofibrate is used clinically to treat dyslipidemia. We previously reported that clofibrate significantly prolonged the lifespan of salt-loaded SHRSP/Izm without affecting blood pressure. To explore a role of reactive oxygen species in salt-sensitive stroke-prone SHRSP/Izm, we studied in salt-loaded SHRSP/Izm with clofibrate. *Design:* At 9 weeks of age, male SHRSP/Izm were fed normal chow with or without 0.25 % clofibrate and given water containing 1 % NaCl for 10 days. The 24-hour urinary total protein was measured by Pyrogallol Red method. Serum TBARS and 24-hour urinary 8-OHdG were measured as an oxidative stress biomarker. Cerebral cortex samples were obtained by laser capture microdissection. The mRNA expression of UCP2, UCP4 and UCP5/BMCP1 were determined by real-time PCR in cerebral cortex. *Results:* There was no significant difference of blood pressure between SHRSP with and without clofibrate (284±3 vs 272±6 mm Hg). The 24 h urinary total protein was significantly lower in SHRSP/Izm with clofibrate than without clofibrate ($p<0.05$). The serum concentration of TBARS was significantly lower in SHRSP/Izm with clofibrate than without clofibrate ($p<0.05$). In cerebral cortex, the mRNA expression of UCP2 was attenuated in SHRSP/Izm with clofibrate than without clofibrate (NS). *Conclusion:* These results suggest that the induction of reactive oxygen species, suppressed by clofibrate partly relates to salt-sensitive stroke-proneness in SHRSP/Izm.

EXPRESSION OF HYPOXIA-INDUCIBLE FACTOR (Hif-1α) AND RELATED FACTOR GENES IN THE SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Hypoxia is the critical conditions to initiate many adaptive responses in pathophysiological processes, such as angiogenesis, inflammation, atherosclerosis, endothelial dysfunction and hypertension. Hif (hypoxia-inducible factor) is the main transcription factor activated of many genes by low oxygen conditions. Hif-1α is rapidly degraded by the ubiquitin-proteasome pathway under normoxic conditions. Angiotensin (Ang) II has been show to produce ROS (reactive oxygen species) following stabilization of Hif-1α nonhypoxic condition. And recent studies have shown that NF-κB (nuclear factor κB) regulated Hif-1α expression and precedes development of atherosclerosis. In these ways, Hif-1α is believed linked to pathological condition of hypertension. *Design:* To elucidate etiology of hypertension and to characterize pathophysiological mechanism hypoxia to hypertension and/or lifestyle disease, we analyzed Hif-1α and related factor genes of spontaneously hypertensive (SHR/kpo, SHRSP/kpo and M-SHR/kpo) and normotensive Wister-Kyoto rat (WKY/kpo). Total RNA was extracted from tissues (heart, brain, liver, kidney and lung). Amplified DNA fragments by RT-PCR were cloned, and there nucleotide sequences were determined. Expression levels of genes are measure by real time PCR and the SNP genotyping was determined by high-resolution melting analysis using the same apparatus (qiagen: Rotor-Gene Q). *Results and Conclusion:* Expression levels of Hif-1α, NF-κB, IL-1β and NADPH oxidase genes were correlated each other with organs and rat species. The results suggest these factors play sequential roles in the rats. The distribution patterns of expression of genes in each organ and species of rats were differed and did not correlated blood-pressure levels of rats. It was known angiotensin II type 1 receptor (AT1) stimulates Hif-1α expression by posttranscriptional mechanism *via* AT1. As our data of the expression of AT1 gene were not correlated well with that of Hif-1α gene, it has another mechanism related to hypoxia in SHRs. Although, under both the hypoxia and oxidative stress conditions expression of the Hif-1α was induced, the mechanisms of ROS in the induction of Hif-1α is arguably, inflammatory conditions are correlated with the pathogenesis of hypertension profoundly.

EXPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS AND OXIDATIVE STRESS RELATED FACTOR GENES IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Oxidative stress has been shown to play an important role in the development of hypertensive heart failure and inflammatory reactions are involved in the pathological changes of hypertension. Peroxisome proliferator-activated receptors (PPARs) are the family of ligand-activated transcription factors that include PPAR-alpha, PPAR-gamma and PPAR-delta. PPAR-ligand complex has been shown to have anti-inflammatory effects. One of the effects of PPAR was mediated by decrease of the increased activity of NAD[P]H oxidase activity and generation of reactive oxygen species (ROS). PPARs have been found to regulate diverse aspects of lipid metabolism, including fatty acid oxidation, adipocytes development, lipoprotein metabolism, and glucose homeostasis. As hypertension is one of risk factors of metabolic syndrome, it must be some factors to be actor as the common etiologic factors of other diseases such as diabetes and hyperlipemia. To concern PPARs are one of important factors serve as an intermediary of hypertension between metabolic syndrome, we examined expression levels of PPARs and related factor genes of rats. *Design:* We evaluated inflammatory status in organs (heart, brain, liver, kidney and lung) in normotensive rat (WKY/kpo) and in spontaneously hypertensive rats (SHR/kpo, SHRSP/kpo and M-SHRSP/kpo), the genetic animal models of hypertension. Amplifications of genes of PPARs, superoxide dismutases (SODs), NFκB, GATA transcription factors and retinoid X receptors were carried out using KOD FX (TOYOBO) DNA polymerase. *Results and Conclusion:* Expression levels of PPAR-alpha in liver and kidney of SHR and M-SHRSP were lower than that of WKY and SHRSP. The same results were obtained for SOD gene expression. Although expression levels of PPAR and other genes from each organs or species of rats were differed, no obvious relationship between the severities of hypertension to the levels of these gene expression. It is unknown whether pathophysiological disorder is a compensatory and adaptive process or a primary event. ROS induce inflammatory status and related to progress of hypertension, diabetes and hyperlipemia. The balance of ROS and the antioxdative effects of the factors such as SOD and GPX (glutathion peroxidase) are important to keep from diseases and from our data of expression levels of these factor genes the importance was confirmed. But to declare etiology of hypertension needs further genes identification and validation.

EFFECTS OF FOOD RESTRICTION ON LIPID AND GLUCOSE METABOLISM IN OBESE SHRSP

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Background: To clarify the beneficial effects of food restriction (FR) on the metabolic syndrome, several factors related to lipid and glucose metabolism were investigated in obese stroke-prone SHR (SHRSP/IDmcr-*fa*), a model of human metabolic syndrome. *Design:* Male SHRSP/IDmcr-*fa*s at ten weeks of age were treated with 85 % FR for 2 weeks. Metabolic parameters, serum adipocytokines and

distribution of serum adiponectin multimers were investigated. In addition, the expression of adipokines and PPARs in epididymal adipose tissue and Glucose transporter (GLUT) in liver and skeletal muscle were analyzed. We further compared the Sirt1 expression in these tissues. *Results:* In FR group, serum arachidonic acid and Leukotrien B4 levels were significantly higher than in the Controls. Furthermore, serum adiponectin level was also higher in FR group. The ratio of high molecular weight form adiponectin in serum was increased in FR group. In epididymal adipose tissue, downregulation of leptin and upregulation of adiponectin and PPARγ mRNA were found in FR group, which was well coincident with serum levels. While upregulation of Sirt1 mRNA was found in the adipose tissue in FR group, it was inversely lower in liver. Although upregulation of liver PPARα and GLUT-2 were found in FR group, no difference was found in Sirt1, PPARs and GLUT-4 expression in skeletal muscle. *Conclusion:* These results indicate that FR ameliorates lipid and glucose metabolism in obese SHRSP through upregulation of adiponectin in adipocytes and GLUT-2 in hepatocytes. Sirt1 may have a key role in signal transduction in lipid and glucose metabolism.

DEVELOPMENT OF PI POLYAMIDE TARGETING ABCA1 GENE

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Background: Pyrrole-imidazole polyamides (PI polyamides) are effective inhibitors of tissue specific and general transcription factors as well as viral repressors and trans-activators. HDL plays a central role in cholesterol transport from extra hepatic tissues to the liver. HDL is thought to be anti-atherogenic by removing cholesterol form atherosclerotic vascular lesion. The process of the cholesterol removal thought to be mediated by a membrane protein ABCA1. In this work, we have designed the PI polyamide against mouse ABCA1 gene, and evaluated the effect on the cholesterol removal in monocyte/macrophage cell-line and in C57BL/6J mouse. *Design and Results:* PI polyamide was designed to target the AP-2 binding site of mouse ABCA1 gene. Binding of the PI polyamide against target sequence of ABCA1 promoter was confirmed by the gel shift assay. ABCA1 mRNA expression increased by PI polyamide at the concentration of 1μM in RAW264.7 cells determined by the real-time PCR method. HDL biogenesis was determined by apolipoprotein AI mediated cellular lipid release. Treatment for 24 hours in ABCA1 PI polyamide increased cholesterol and phospholipid concentration of medium after incubation of RAW264.7 cells with apolipopritein AI. Finally, PI polyamide increased mouse plasma HDL concentration and peripheral blood cell ABCA1 mRNA expression. *Conclusion:* In this study, we have demonstrated the effect of PI polyamide targeting ABCA1 gene *in vivo*. This novel therapeutic agent has a possibility to be a useful tool for the new strategy for transcription regulation therapy.

DYSLIPIDEMIA AND ALTERED HEPATIC GENE EXPRESSION IN A NEW RAT METABOLIC SYNDROME MODEL (SHRSP. ZLepr^{fa} IsmDprc)

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Background: We investigated lipid and lipoprotein abnormalities in SHRSP fatty rats as a new animal model of metabolic syndrome and examined differentially expressed genes in the liver, one of the major tissues contributing to lipid metabolism. *Design:* Plasma lipoprotein profiles were analyzed by HPLC using a gel-permeation column. Gene expression profiling of liver tissue was examined by DNA microarray method. *Results:* Increased cholesterol concentrations of small particle size LDL fractions were observed in SHRSP fatty rats, whereas the Zucker Fatty strain did not show a similar elevation of cholesterol content. Existence of apolipoprotein B in these fractions was confirmed by western blotting. Seventeen genes were significantly upregulated in liver tissue in SHRSP fatty rats compared with SHRSP rats. Ten genes were significantly reduced (less than 0.3 fold) in the liver tissue of SHRSP fatty rats compared with that in SHRSP rats. *Conclusion:* The SHRSP fatty rat is the first one involving small LDL, which is frequent in the presence of this disorder, and may be involved in the onset of arteriosclerosis. This model may be useful for investigating small LDL in the future. Three of seventeen genes upregulated in liver tissues of SHRSP fatty rats play a role in this metabolic abnormality and seem to be a therapeutic target of this metabolic syndrome. These genes are candidate genes for the metabolic abnormality in the metabolic syndrome.

ADIPOCYTOKINES EXPRESSION IN SHR/NDMCR-CP RATS, A MODEL OF METABOLIC SYNDROME

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Background: Adipocytokines including leptin, adiponectin, and monocyte chemoattractant protein-1(MCP-1), play important roles in development of cardiovascular disease in obesity. However, molecular mechanisms underlying the relationship between obesity and its complications have not been fully elucidated yet. SHR/NDmcr-cp (fa^k/fa^k) rats (CP) which have a nonsense mutation of leptin receptor gene, are characterized by occurrence of obesity, hypertension, dislipidemia, hyperinsulinemia, and hyperglycemia. To elucidate the pathophysiological mechanisms of the metabolic syndrome, we examined metabolic parameter and adipocytokines expression in CP. *Design:* We measured metabolic parameter including adiponectin, leptin and MCP-1 in CP and compared with those in their lean littermates (Lean). We examined mRNA expressions (adiponectin, MCP-1) by using real-time PCR method in different fat depots such as epididymal white adipose tissue (WAT), retroperitoneal WAT, mesenteric WAT. *Results:* In 12 weeks of age, both animals had hypertension because of their background of spontaneously hypertensive rats. Body weight was higher in CP compared with Lean. CP showed abdominal obesity, hyperglycemia, hyperinsulinemia, and hyperlipidemia. Circulating leptin and MCP-1 levels were higher in CP. MCP-1 mRNA expressions in epididymal WAT is up-regulated in CP. There were no significantly differences between CP and Lean in plasma adiponectin levels. *Conclusion:* In this study, CP showed several metabolic changes which resemble to human metabolic syndrome. Changed adipocytokine expressions were observed, which may affect the metabolic status in CP.

EFFECTS OF ANGIOTENSIN (1-7) ON INSULIN RESISTANCE AND HYPERTENSION INDUCED BY ANGIOTENSIN II STIMULATION IN PREPUBERTY

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Background: We investigated effects of angiotensin (1-7) on insulin resistance and hypertension induced by angiotensin II (AII) infusion to young rats. *Methods:* Mini-pumps loaded with angiotensin II were implanted in 4-week-old Sprague-Dawley (SD) rats for 4 weeks (AII). In some AII, mini-pumps loaded with angiotensin (1-7) were simultaneously implanted (AII&(1-7). At the age of 8 weeks, the mini-pumps were removed. At the age of 12 weeks, euglycemic hyperinsulinemic glucose clamp was performed to evaluate insulin sensitivity (GIR). In ex-vivo experiment, soleus muscle from 3-week-old SD rats was incubated with or without angiotensin II and/or angiotensin (1-7) in addition of insulin and 2-Deoxy-glucose (2-DOG) to evaluate skeletal muscle insulin sensitivity. *Results:* Blood pressure was robustly ($p<0.05$) elevated during angiotensin II infusion (8-week-old: 172.0±6.3 vs. 121.6±2.6 mm Hg, AII and controls, respectively), and angiotensin (1-7) did not significantly lower the higher blood (AII&(1-7): 153.5±9.0). In AII, blood pressure measured even 4 weeks

after the termination of angiotensin II infusion was still higher than that of controls (12-week-old: 138.5±9.4 vs. 125.7±5.8 mm Hg, AII and controls, respectively, $p<0.05$), and angiotensin (1-7) co-treatment significantly ($p<0.05$) decreased the AII-induced higher blood pressure (AII&(1-7): 129.0±3.4). GIR was significantly ($p<0.05$) decreased in AII rats (11.1±0.7 vs. 17.6±0.5 mg/kg/min, AII and controls, respectively), and angiotensin (1-7) significantly reversed it (AII&(1-7): 15.6±1.3). In the *ex-vivo* experiment, angiotensin II significantly ($p<0.05$) reduced insulin-induced 2-DOG uptake to 56 % of the controls, and angiotensin (1-7) co-incubation recovered it to 89 %. *Conclusion:* Angiotensin (1-7) may have antagonistic effects of angiotensin II-induced insulin resistance and hypertension.

EVIDENCE FOR A MARINOBUFAGENIN-LIKE IMMUNO-REACTIVITY IN CENTRAL NERVOUS SYSTEM

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Background: The digitalislike factors (DLFs) are secreted from the adrenal gland, pituitary and hypothalamus. Ouabain is a modulator of blood pressure *via* the sodium pump inhibition not only in the peripheral circulation but also in the central nervous system, and it could be related in a renin-angiotensin-aldosterone system. Recently marinobufagenin (MBG) and its related compounds belonged to bufadienolide were isolated in mammals, and they were implicated in hypertension. However, it is not fully elucidated the tissue distribution and the secretory mechanism, particularly in the brain. *Design:* We sampled tissue of the adrenal gland, pituitary, hypothalamus, heart, liver and kidney in three 10-week-old male Wistar rats. Each tissue was homogenized, and then the tissue supernatants were collected. MBG-like immunoreactivity (MBGi) in the tissue supernatants was measured with our enzyme-linked immunosorbent assay (ELISA) using anti-MBG monoclonal antibody. Moreover, culture supernatant of immortalized hypothalamic cell line, N1 cells, was collected at 0.5, 2, 4, 24 hours. The MBGi in the N1 culture supernatant was also measured. *Results:* The MBGi in the adrenal gland (260.0±21.9 pg/g), hypothalamus (63.0±40.3 pg/g) and pituitary (136.7±47.8 pg/g) were higher levels than them in the liver (15.3±6.2 pg/g), heart (7.3±2.7 pg/g) and kidney (28.3±12.4 pg/g). The MBGi concentration in N1 culture supernatant was increased at 24hours compared at 0.5 hour. *Conclusion:* We found that bufadienolides were secreted from the hypothalamus. Our results may be a clue to elucidate the mechanism of DLFs in the brain.

A MINERALCORTICOID RECEPTOR BLOCKER, EPLERENONE, DECREASES PLASMA LEVELS OF MARINOBUFAGENIN-LIKE IMMUNO-REACTIVITY AND INHIBITS ITS SECRETION FROM ADRENAL GLAND IN RATS

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Background: Mariobufageninn (MBG)-related endogenous compounds are believed to be endogenous digitalislike factors implicated in cardiovascular regulations. Since both Renin-Angiotensin-Aldosterone System (RAAS) and endogenous digitalis have been released more with sodium loading in the central nervous system in rats, we explored the possible interactions between the two even in the systemic circulation. *Design:* Twenty 10-week-old male Sprague-Dawley rats fed with normal diet were further divided into two groups with and without eplerenone administration for four weeks. Systolic blood pressure was measured at each week. MBG-like immunoreactivity (MBGi) was measured with our enzyme-linked immunosorbent assay (ELISA) using anti-MBG monoclonal antibody. Urinary MBGi was measured at each week, and plasma MBGi was measured at the 4th week. Each group of the adrenal gland was sampled at the 4th week. Each tissue was homogenized with phosphate-buffered saline, and then the tissue supernatants were collected after centrifugation. MBGi in the tissue supernatants was measured. *Results:* The eplerenone treatment group significantly lowered systolic blood pressure at the 3rd and 4th weeks. There was no difference in urinary MBGi levels throughout the observation period in both groups. Plasma levels of MBGi were 22±1.2 pg/ml in the control and 16±0.9 pg/ml in the eplerenone group. The difference between the control and eplerenone group was significant. The tissue supernatant of MBGi in eplerenone group was significantly lower than that in the control group. *Conclusion:* Aldosterone may be a secretagogue of DLFs. This may be a clue to elucidate the mechanism of hypertension where DLFs and RAAS are implicated.