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THE EFFECTS OF EXERCISE TRAINING ON GLUCOSE INTOLERANCE IN SHRSP

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Spontaneously hypertensive stroke-prone rats (SHRSP) exhibit not only hypertension and stroke but also insulin resistance and glucose intolerance, which have been shown to be pathologies of diabetes. One of the features of glucose intolerance in SHRSP is hypoinsulinemia. Although exercise training is reported to improve diabetes, the effects of exercise training on glucose intolerance as seen in SHRSP are not known. Therefore, the present study was conducted to evaluate the effects of exercise training on glucose tolerance, and to elucidate detailed responses to exercise through analyzing insulin signaling-related factors in exercise-trained SHRSP. Male SHRSP at 11 and 4 weeks of age were used as adult and young rats, respectively. Preliminary experiments from our laboratory have shown that young SHRSP exhibit normal glucose tolerance, while adult SHRSP exhibit glucose intolerance. SHRSP of each age were divided into two groups, the control (Cont) group and the exercise training (Tr) group. Each Tr rat was subjected to swimming training with a river-pool device (spending 60 min/day, 5 days/week in the pool). At 14 and 9 weeks of age, each rat was given an oral-glucose tolerance test (OGTT). One week later, each rat was sacrificed under anesthesia. Isolated gastrocnemius muscles were used for the analyses of the expression of insulin signaling-related factors, including insulin receptor β subunits (IR-β), Akt, glucose transporter 4 (GLUT4) and hexokinase 2 (HXK2). Adult SHRSP: The body weight of rats in the Cont group was not different from that of rats in the Tr group. The systolic blood pressure of the Tr group was lower than that of the Cont group. Although the insulin-area under the curve (AUC) of the Cont group did not differ from that of the Tr group, glucose-AUC of the Tr group was lower than that of the Cont group. The protein content of Akt, GLUT4 and HXK2 in gastrocnemius muscle was increased by swimming training. Young SHRSP: The body weight of rats in the Tr group was lower than that of rats in the Cont group. The systolic blood pressure, glucose and insulin-AUC were not different between the Cont and Tr groups. The protein content of IR-β and Akt in gastrocnemius muscle in the rats that underwent swimming training were higher than those in the Cont group. The mRNA expression of GLUT4 in gastrocnemius muscle of the Tr group tended to be higher than that in the Cont group. These results suggested that exercise training ameliorates glucose intolerance in adult SHRSP through up-regulation of insulin signaling-related factors. The results also suggested that normal glucose tolerance as seen in young SHRSP was responsible for the lack of any effect of exercise training on glucose tolerance. The results of this study should help researchers to clarify the benefit of exercise for hypertensive diabetics.

THE EXAMINATION OF INHIBITORY EFFECT OF CAFFEIC ACID PHENETHYL ESTER (CAPE) TO HYPERTROPHIC ADIPOCYTES

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Obesity is one of the risk factors of the metabolic syndrome. And it is thought to be the cause of the adipocyte accumulates a lot of fats, it is enlarged, and the number of adipocytes increases. The aim of this study was to investigate the inhibitory effect of Caffeic Acid Phenethyl Ester (CAPE) to hypertrophic adipocytes which differentiated from 3T3-L1 mouse fibroblasts. 3T3-L1 cells were seeded in 6-well plate and grown to confluence at 5 % CO_2 in DMEM with 10 % FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. 2 days after confluence, cells were fed high-glucose DMEM supplemented with 10 % FBS, 1 μM DEX, 500 μM IBMX, 5 μg/ml insulin for 3 days (Day 0-2). Medium was then changed to high-glucose DMEM with 10 % FBS, 5 μg/ml insulin for 3 days (Day 3-5) in respective cultures. Cells subsequently re-fed every 2 days with DMEM or high-glucose DMEM with 10 % FBS until day14. Cells were maintained in differentiation medium with variable concentrations of CAPE (10, 25, 50, 75 μM) from day14. The protein was extracted at 3 hours or 6 hours after treatment of CAPE, the change in the protein expression using Western blotting, and after 24 hours, cells were stained with DAPI. Moreover, staining with Oil-Red-O on the 18 days after induced differentiation to adipocytes and the subcellular component has been extracted. Additionally, the cells were lysed and measured for triglyceride content. Total RNA was isolated from differentiated cells on day18. Then RNA was analyzed using RT-PCR to determine the expressions of several adipogenesis-related factors. Staining with Oil-Red-O on day18 after induced differentiation, CAPE dose-dependently suppressed oil droplet accumulation and reduced the size of droplets into adipocytes. The CAPE concentrations of 50 to 75 μM could significantly inhibit triglyceride deposition. Resistin mRNA expression was significantly suppressed by CAPE at concentrations of 50 to 75 μM on 18 days after induced differentiation cells. Adiponectin expression was maintained with variable concentrations of CAPE. Staining with DAPI on 24 hours after treatment of CAPE, CAPE dose-dependently increased chromatin cohesion. The changed the protein levels of cleaved PARP, caspase-3, caspase-9 at 3 hours or 6 hours after treatment of CAPE. The cleaved PARP and cleaved caspase-3 expression was significantly increased by CAPE at concentrations of especially 50 and 75 μM on 3 hours and 6 hours CAPE in addition later. In this study showed that CAPE controls the fat accumulation in the 3T3-L1 hypertrophic adipocytes, and influenced the expression of the adipocytokines. In addition, we suggested that CAPE induces the apoptosis of the hypertrophic adipocytes and inhibits the hypertrophy of adipocytes.

EFFECT OF TAURINE ON IMPARAIED COGNITIVE FUNCTION INDUCED BY IMMOBILIZATION STRESS IN SPONTANEOUSLY HYPERTENSIVE RATS

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We have reported in the previous studies that taurine had a potent blood pressure lowering effect in spontaneously hypertensive rats (SHR/Izm). Mechanisms of this effect include suppression of sympathetic nervous system and improvement of baro-reflex sensitivity in SHR. In clinical experience cognitive function was often impaired after psychological stress in elderly subjects. Present study was designed to clarify that acute immobilization stress impaired cognitive function and chronic treatment of taurine can improve immobilization stress-induced cognitive dysfunction in SHR/Izm and Wistar Kyoto Rats (WKY/Izm). SHR/Izm and age matched WKY/Izm was used at 6 weeks of age. These rats were divided to two groups, taurine and control group. Taurine group was taken 3 % taurine as drinking water for 3 weeks and control group drank tap water *ad libitum*. Moving were restricted for 1 hour as immobilization stress. Morris water maze test was performed at 9 weeks-age of SHR/Izm and WKY/Izm after free moving or restricted condition with or without treatment of taurine. Avoidance time and distance were measured in each group. Blood pressure was decreased in SHR/Izm after treatment of taurine (193.2±9.0 mm Hg vs. 173.2±6.2 mm Hg, mean ± S.E.M.) but not in WKY/Izm (124.3±4.5 mm Hg vs. 126.2±2.6 mm Hg). Avoidance time was elongated by immobilization stress in WKY/Izm (7.6±0.9 s vs. 22.0±4.1 s, p=0.01). Avoidance times in SHR/Izm were short compared with those of WKY/Izm. Avoidance times were elongated by immobilization stress (5.7±0.3 s vs. 21.9±7.6 s, p=0.05). Treatment of taurine improved avoidance time in WKY/Izm (12.3±1.8 s vs. 21.9±4.7 s, p=0.08) and SHR/Izm (7.6±1.2 s vs. 7.3±1.6 s). Improvement of avoidance time extended by immobilization stress was marked in SHR/Izm compared with WKY/Izm. Avoidance distance was extended after immobilization stress in both WKY/Izm (286.3±37.0 cm vs. 1432.1±234.9 cm, p=0.002) and SHR/Izm (144.7±8.4 cm vs. 447.9±148.0 cm, p=0.04). Taurine improved the extended avoidance distance by immobilization stress in both WKY/Izm (207.1±25.1 cm vs. 1307.2±168.0 cm, p=0.002). In SHR extended avoidance distance was almost normalized by the treatment of taurine in SHR (111.9±43.1 cm vs. 227.5±113.9 cm). In conclusion, acute immobilization stress impairs cognitive function in WKY and SHR. Taurine improves impaired cognitive function by immobilization stress and this effect is augmented in SHR.

EFFECTS OF SALT INTAKE ON THE DEVELOPMENT OF HYPERTENSION IN OBESE HYPERTENSIVE SHRSP.Z-*Lepr*^{fa}/IzmDmcr RATS

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Excessive salt intake is a risk factor for the development of hypertension and renal injury. The association of dietary salt with hypertensive cardiovascular and renal disease is well recognized, but there are still many controversies regarding the role of salt in the pathogenesis of hypertension and cardiovascular and renal injury. Obese persons with metabolic syndrome are often associated with salt-sensitive hypertension, microalbuminuria, and cardiac dysfunction. In this study, we investigated the effects of excessive salt intake in SHRSP.Z-*Lepr*^{fa}/IzmDmcr rats (SHRSP.ZF), which showed obesity and hypertension. Eight-week-old male SHRSP.ZF were divided into two groups, a salt-loading group and a tap-water group as a control. Salt-loaded SHRSP.ZF were given water containing 1 % NaCl for 6 weeks. Body weight and systolic blood pressure were measured weekly. Twenty-four-hour urine samples were collected at 12 weeks of age using a metabolic cage. Urinary albumin and angiotensinogen (AGT) were measured by ELISA. The mRNA expressions of AGT, transforming growth factor beta (TGF-β), type-1 plasminogen activator inhibitor (PAI-1), cyclooxygenase-2 (COX2), and 12/15-lipoxygenase (12/15-LO) were determined by real-time PCR in kidneys. Systolic blood pressure in the salt-loading group was significantly higher from 2 weeks after salt loading than in the control group (2 weeks after salt loading 195±2.3 vs. 179±2.5 mm Hg, $p<0.05$). The 24-h urinary albumin was significantly higher in salt-loading group than in the control group (30.2±6.9 vs. 16.2±4.8 mg/day, $p<0.05$). The mRNA expressions of AGT and TGF-β in the kidneys were up-regulated in the salt-loading group ($p<0.05$), and mRNA expressions of PAI-1, COX2, and 12/15LO showed higher tendency. In this study, increased blood pressure and microalbuminuria were observed in salt-loaded SHRSP.ZF. Excess salt intake may encourage the development of renal injury in patients with metabolic syndrome.

POSSIBLE CORRELATES OF SYNAPTIC PLASTICITY IN THE MEDIAL PREFRONTAL CORTEX AND FEAR-RELATED BEHAVIOR IN SHRSP

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Emotional responses based on fear memory consisting of some processes, i.e., acquisition, consolidation and retention, are expressed *via* limbic and prelimbic systems such as medial prefrontal cortex (mPFC), hippocampus and amygdala. Particularly, mPFC is a critical region to integrate and express fear-related responses, however, the functional role in fear memory was not fully understood. The juvenile SHRSP shows the symptomatic features of attention deficit/hyperactivity disorder (ADHD), i.e., inattention, hyperactivity and impulsiveness. Moreover, fear-related response is likely to be impaired in SHRSP. The present study elucidated synaptic function in the mPFC underlying contextual fear memory of SHRSP. Six-8 weeks old male-SHRSP and sex- and age-matched Wistar Kyoto rat (WKY) as a genetic control were used. Fear-related behavior, freezing, was evaluated using contextual fear conditioning (CFC) test. As a measure of the evaluated synaptic plasticity expressed as long-term potentiation (LTP), the population spike amplitude (PSA) of field potentials was determined in the hippocampal CA1/subicular-the mPFC pathway. Monoamine reuptake inhibitors were administrated before behavioral and synaptic evaluation in SHRSP. In the SHRSP, extracellular DA levels in the mPFC were increased by stimulation of ventral tagmental area (VTA), however, the increasing rate was significantly reduced as compared with WKY. Freezing behavior attenuated during acquisition and retention period in CFC test. LTP induction in the hippocampus-mPFC pathway was impaired in SHRSP. 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, or a selective NA reuptake inhibitor desipramine (3 mg/kg, i.p.) reversed synaptic impairment in the mPFC, while a selective dopamine (DA) reuptake inhibitor GBR12909 (3 mg/kg, i.p.) failed. In addition, methylphenidate, atomoxetine and desipramine, but not GBR12909, significantly increased the cortical extracellular NA and DA levels in SHRSP. Pharmacological effects of monoamine reuptake inhibitors were also observed on the impaired fear-related behavior. The present study possibly indicates that synaptic plasticity in the hippocampal-mPFC pathway is critical for expressing fear-related behavior. Pharmacological findings further support the notion that monoaminergic mechanisms may underlie hypofrontality observed in SHRSP, as postulated in ADHD patients.

ASSESSING METABOLIC SYNDROME AMONG PRE- AND POST-MENOPAUSAL WOMEN OF BANGLADESH

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Metabolic syndrome is the clustering of metabolic disorders commonly related to insulin resistance that increase the risk of cardiovascular disease (CVD) and also type 2 diabetes mellitus, the most common causes of morbidity and mortality in many communities. MetS is now considered as a global epidemic. Gender differences in prevalence and consequences of the metabolic syndrome (MetS) as a strong predictor of CVD are challenging problems. Post-menopausal (post-M) status may explain in part the cause of acceleration of CVD with aging. The present study was aimed at identifying the principal components of risk variables associated with the metabolic syndrome in pre-M and post-menopausal (post-M) Bangladeshi rural women. A total of 1802 apparently healthy rural Bangladeshi women aged ≥15 years were studied using a community based cross-sectional survey. The present study presents an interim report of ongoing study conducted in rural women of Bangladesh. This sample size was sufficient enough to test the research hypothesis at 5 % level of significance with a statistical power of 90 % (beta=0.90). The study uses the World Health Organization's STEPS approach (modified), which entails a stepwise collection of the risk factor data based on standardized questionnaires covering socio-demographic characteristics, somatic illnesses, somatic and mental symptoms, medications, life style, and health-related behavior (step 1), basic physical measures (step 2) and basic biochemical investigations, such as levels of blood glucose and cholesterol (step 3). All participants gave informed consent. Subjects receiving any drugs were excluded. Venous samples were collected for necessary investigations and analyzed at the hospital central laboratory. MetS was measured following the US National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATPIII) criteria. 1802 subjects (1094 per-M and 708 post-M) with a mean age of 39.9 (per-M 31.36, post-M 53.31) years were studied. Prevalence of the modifiable cardiovascular risk factors screened were as follows: generalized BMI ≥30kg/m2 2.8 % (pre-M 3.0 %, post-M 2.5 %, p=0.333), truncal obesity 8.4 % (pre-M 8.4 %, post-M 8.4 %, p=0.51), hypertension 28.2 % (pre-M 14.5 %, post- M 49.4 %, $p<0.001$), elevated fasting blood glucose 35.4 % (pre-M 26.2 %, post-M 49.6 %, $p<0.001$), hypercholesterolaemia 35.5 % (pre-M 32.8 %, post-M 39.7 %, p=0.002), elevated LDL-cholesterol 42.7 % (pre-M 41.5 %, post-M 44.0 %, p=0.208), low HDL-cholesterol 84.0 % (pre-M 80.7 %, post-M 87.3 %, $p<0.001$), hypertriglyceridaemia 28.7 % (pre- M 23.2 %, post-M 37.1 %, $p<0.001$), MetS (NCEP ATPIII criteria) 25.8 % (pre-M 17.4 %, post-M 38.8 %, $p<0.001$) and atherogenic index (HDLc/TC<0.18) 32.8 % (pre-M 23.4 %, post-M 42.2 %, $p<0.001$). We found a prevalence of MetS that was highly age-dependent. Low HDL seems to be the

commonest component of MetS in the study subjects. The mean level of HDL was 45.74 mg/dl in pre-M women and 35.83 mg/dl in post-M women. The mean levels of the following components were significantly higher in post-M subjects compare to pre-M subjects: Insulin (pre-M 7.72 mU/l, post-M 11.31 mU/l, p<0.001), HOMA-IR (pre-M 2.16, post-M 4.1, p<0.001), Systolic blood pressure (pre-M 110.56 mm Hg, post-M 126 mm Hg, p<0.001), Diastolic blood pressure (pre-M 73 mm Hg, post-M, 79.44 mm Hg, p<0.001), Triglyceride (pre-M 114.79 mg/dl, post-M 153.6 mg/dl, p<0.007), Cholesterol (pre-M 176.15 mg/dl, post-M 188.75 mg/dl, p<0.001), fasting blood glucose (pre-M 5.77 mg/dl, post-M 6.91 mg/dl, p<0.001) and VEGF (pre-M 458.53 pg/ml, post-M 604.45 pg/ml, p<0.001). However, mean values of the following components were significantly higher in pre-M compared to post-M women: VEGF-R2 pre-M (9853.39 pg/ml, post-M 9377.09 pg/ml, p=0.005), non-HDL (pre-M 143.07 mg/dl, post-M 134.09 mg/dl, p<0.013) and waist circumference (pre-M 76.89 cm, post-M 76.73 cm, p<0.001). The prevalence of MetS and the associated modifiable cardiovascular risk factors were higher in post-menopausal women compared to those in pre-menopausal women in rural Bangladesh. The correlation of VEGF needs further investigations to define the clinical utility and predictive value of serum VEGF, VEGF-R1 and VEGF-R2 levels in cardiovascular complications of MetS. At the same time, there is a dire need for policy implications of health awareness and preventive measures. Funding source: Grant-in-Aid for Scientific Research and Overseas Scientific Research Grant Kiban B (2010-2012), and research grant from Uehara Foundation (2011), Tokyo, Japan.

TRANSPLANTED BONE MARROW STROMAL CELLS PROTECT NEUROVASCULAR UNITS AND AMELIORATE BRAIN DAMAGE IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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This study was aimed to assess whether the bone marrow stromal cells (BMSC) could ameliorate brain damage when transplanted into the brain of stroke-prone spontaneously hypertensive rats (SHR-SP). The BMSC or vehicle was stereotactically engrafted into the striatum of male SHR-SP at 8 weeks of age. Daily loading with 0.5 % NaCl-containing water was started from 9 weeks. MRI and histological analysis were performed at 11 and 12 weeks, respectively. As the results, T2-weighted images demonstrated neither cerebral infarct nor intracerebral hemorrhage, but identified abnormal dilatation of their lateral ventricles. HE staining demonstrated selective neuronal injury in their neocortex. Double fluorescence immunohistochemistry revealed that they had a decreased density of the collagen IV-positive microvessels and a decreased number of the microvessels with normal integrity between basement membrane and astrocyte end-feet. BMSC transplantation significantly ameliorated the ventricular dilatation and the breakdown of neurovascular integrity. These findings strongly suggest that long-lasting hypertension may primarily damage the neurovascular integrity and neurons, leading to tissue atrophy and ventricular dilatation prior to the occurrence of cerebral stroke. The BMSC may ameliorate these damaging processes when directly transplanted into the brain, opening the possibility of prophylactic medicine to prevent microvascular and parenchymal damaging processes in hypertensive patients.

THE SDF-1α/CXCR4 SYSTEM ATTRACTS NEURAL STEM CELLS IN THE CEREBRAL CORTEX FOLLOWING STROKE IN A STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RAT MODEL

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Stroke-prone spontaneously hypertensive rats (SHRSP) suffer from spontaneous cerebral stroke. The number of neural stem cells (NSCs) and new neurons increase in the penumbral area and ipsilateral subventricular zone in the acute phase after cerebral stroke in SHRSP, and the stromal cell-derived factor-1α (SDF-1α)/CXCR4 system contributes to neurogenesis following rat traumatic brain injury (TBI). However, the relationship between NSCs and SDF-1α/CXCR4 following cerebral stroke remains unknown. The present study addressed the temporal relationship between NSCs and SDF-1α/CXCR4 in the penumbral area and stroke lesion following cerebral stroke in SHRSP. Molecular biology techniques and immunohistochemistry were used to determine the relationship between SDF-1α/CXCR4 expression and NSCs in the penumbra following cerebral stroke in SHRSP. SDF-1α mRNA and protein expression was not significantly increased. However, SDF-1α leaked from the injured area and diffused into the cortex, and SDF-1α protein levels significantly increased in the cerebral spinal fluid. Subsequently, CXCR4 mRNA and protein expression significantly increased. CXCR4-positive cells also expressed nestin. Moreover, a proportion of CXCR4-positive fibers expressed neurofilaments. Leaked SDF-1α attracted CXCR4-positive NSCs and elongated nerve fibers. The SDF-1α/CXCR4 system in the brain contributed to increased numbers of NSC and neural regeneration after cerebral stroke.

CHANGING IN MATRIX METALLOPROTEINASES AND HEPATIC FIBROSIS IN SPONTANEOUSLY HYPERTENSIVE HYPERLIPIDEMIC RATS

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Nonalcoholic steatohepatitis (NASH) is regarded as a hepatic manifestation of lifestyle-related diseases which can progress to hepatic cirrhosis and hepatocellular carcinoma. It is thought that matrix metalloproteinases (MMPs) play an important role in hepatic fibrosis. We previously reported a correlation between oxidative stress in plasma and MMP-9 expression in white blood cells. However, the expression of MMPs and tissue inhibitors of metalloproteinases (TIMPs) in the progression of NASH is unclear. Spontaneously hypertensive hyperlipidemic rats (SHHRs) were developed as a stable model of early vascular degeneration. Moreover, invasive changes occur in the subendothelium of SHHR, followed by a high fat diet and 30 % sucrose water treatment (HFDS). We have found fibrosis and cell inflammation around the central vein, and fat accumulation in hepatocytes on liver histology in SHHR-HFDS; thus we use SHHR-HFDS as a rat model of NASH. In this study we used SHHR- HFDS as a model for NASH, in order to clarify the relationships between hepatic fibrosis and MMP family at various time-points in the progression of NASH. Male SHHR and Sprague-Dawley (SD) rats were divided into four groups; SHHR-normal diet (ND), SHHR-HFDS, SD-ND and SD-HFDS. Until the age of 5 months, the regular diet was available to all groups *ad libitum* and then HFDS diet *ad libitum* for 4, 6 and 8 months, thus we obtained rats of 9, 11 and 13 months of age. Oxidative stress was measured in rat plasma using the diacron reactive oxidative metabolites (d-ROMs) test. Quantitative real-time polymerase chain reaction (Q-PCR) was used to quantify the mRNA levels of MMPs, TIMPs and superoxide dismutase 1 (SOD1) in rat liver. MMP-2 and MMP-9 were immunostained in the liver. Data were analyzed using the Mann-Whitney U-test. Severe fibrosis and cell inflammation around the central vein were observed and clearly increased at 13 months in SHHR-HFDS, resembling NASH. Liver weight (LW) and the d-ROMs test in plasma were increased in SHHR-HFDS compared to the other groups. The d-ROMs test was correlated with LW in all rats (r=0.71, P<0.0001). Expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 mRNA by Q-PCR was increased in the liver of SHHR-HFDS at 13 months. SOD1 mRNA expression in SHHR-HFDS decreased at 13 months. In SHHR-HFDS, staining of MMP-2 was detected mainly in extracellular spaces, while staining of MMP-9 was observed in the WBCs, hepatocytes, bile ducts and biliary canaliculi. This study demonstrates that the increased LW associated

with oxidative stress markers progressed to liver fibrosis. In addition, MMP-2 and MMP-9 expression in SHHR-HFDS increased dramatically at 13 months with the progression of NASH. Regulation of the MMP family may lead to suppression of the progression of hepatic fibrosis. The SHHR model may help in the elucidation and treatment of NASH.

DISRUPTION OF VEGF SYSTEM IN METABOLIC SYNDROME

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Metabolic syndrome (MS) is associated with impaired angiogenesis where vascular endothelial growth factor (VEGF) plays a key role in angiogenesis through binding to its specific receptor, sVEGF-R1 and sVEGF-R2. The purpose of the present study was to assess circulating levels of VEGF, sVEGF-R1, and sVEGF-R2 in subjects with Metabolic Syndrome (MS) or without metabolic syndrome (non-MS) and further examined their association with clinical and metabolic parameters. A total of 1485 rural Bangladeshi women aged ≥15 years were studied using a population based cross-sectional survey. The prevalence rates of MS were 25.05 % (NCEP ATP III). VEGF levels were significantly increased in MS subjects (MS vs. non-MS: 575 vs. 490, $p<0.001$). There were no significant relation of sVEGF-R1 and sVEGF-R2 with MS (sVEGF-R1, MS vs. non-MS: 446 vs. 667, $p=0.093$; sVEGF-R2, MS vs. non-MS: 8943 vs. 9400, $p=0.0.344$). In multivariable analyses, we found that VEGF had significant positive associations with fasting blood glucose ($r=0.181$, $p<0.001$), BMI ($r=0.143$, $p<0.001$), cholesterol ($r=0.101$, $p<0.001$), non-HDL cholesterol ($r=0.091$, $p<0.001$), LDL cholesterol ($r=0.079$, $p=0.007$), insulin($r=0.075$, $p=0.022$), DBP ($r=0.064$, $p=0.025$) and SBP ($r=0.057$, $p=0.048$) even after adjusting for age. Multiple regression analysis revealed that fasting blood glucose (beta=0.158, $p=0.001$) and BMI (beta=0.083, $p=0.017$) were independent determinants of VEGF. We also found that mean VEGF levels increased in proportion to the accumulation of components of MS. The correlation of VEGF needs further investigations to define the clinical utility and predictive value of serum VEGF levels in MS.

DEVELOPMENT OF ONE-HANDED MILKER FOR MILK COLLECTION FROM LACTATING RATS: ANALYSIS OF COMPONENTS OF MILK IN SHR AND WKY

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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 sufficient amounts of rats' milk 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). Within 48 h after delivery, parturition litters were reduced to 9 for each mother rat. Milk samples were obtained using the newly developed device on the 2nd, 9th, 16th and 23rd postpartum days. Pups were separated from their mother 6 h before sampling. The mother rat was administrated oxytocin (0.02 U/100 gBW) just before sampling milk. 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. In nutrient composition in the milk, the densities of carbohydrates and sodium were greater in SHR than in WKY. Protein and fat was slightly lower in SHR than in WKY. On the 9th day, Tau, Ala, Mea and Orn content in the milk was significantly greater in SHR than in WKY. On the 23rd day, 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 acid content was not significantly different. The newly developed one-handed milker device was useful in the sampling of milk in rats. Further study is necessary to clarify the significance of the difference in amino acid composition between the two strains.

SYNAPTIC RESPONSES OF CORICO-LIMBIC SYSTEM IN SHRSP/Ezo

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Clinical and animal studies have shown that "Attention Deficit Hyperactivity Disorder (ADHD)" may involve dysfunctions in cortical glutamatergic NMDR transmission and monoaminergic modulation. Spontaneously hypertensive rats (SHR) have been accepted as an animal model of ADHD, however, there are liability differences among substrains. Previous reports showed SHRSP/Ezo, an SHR substrain rat, develop ADHD-like behaviors with male predominance in prehypertensive childhood period (six-week old). As hippocampus (an old cortex) and prelimbic medial prefrontal cortex (mPFC) are responsible to emotional expression, it was considered that functional changes in these areas may be possibly related with ADHD. In this symposium, we introduce pathophysiological evidences for ADHD in SHRSP/Ezo, by observation of cortical synaptic function using long-term potentiation (LTP) as an index of synaptic plasticity, and microdyalysis measurement of dopamine (DA) which is critical for cortical LTP formation. In anesthetized Wistar Kyoto rats (WKY) and SHRSP/Ezo, single electrical pulse stimulation (ES) of Schaffer collaterals evoked population spike (PS) in hippocampal CA1. High frequency stimulation increased PS amplitude for long period indicating the development of LTP both in WKY and SHRSP/Ezo. In WKY mPFC, single ES of CA1 evoked PS and high frequency stimulation increased PS amplitude indicating the development of LTP. However, in SHRSP/Ezo mPFC, high frequency stimulation failed to induce LTP indicating the synaptic plasticity is impaired in SHRSP/Ezo. Methylphenydate, an amine transporter inhibitor, which is beneficial in ADHD patients, recovered the LTP formation with an increase of DA release in SHRSP/Ezo mPFC. Thus, DA regulation of synaptic plasticity is damaged in SHRSP/Ezo mPFC. The notion is supported by the finding that DA release by ES of the ventral tegmental area (VTA) was also absent in SHRSP/Ezo. Patch-clamp study with mPFC slice under inhibition of $GABA_A$ receptor showed the single ES of layer II developed excitatory postsynaptic potential (EPSP) in layer V pyramidal neuron of WKY and SHRSP/Ezo. In some recordings, high frequency stimulation increased the slope (LTP) of EPSP in WKY but scarcely in SHRSP/Ezo, indicating that basal inhibition of LTP in SHRSP/Ezo mPFC. Long period (40 min) perfusion of the slice with DA facilitated LTP formation at 3 μmol/l in WKY and at 10 μmol/l in SHRSP/Ezo, indicating that DA sensitivity in synaptic plasticity is also decreased in SHRSP/Ezo mPFC. The above evidences indicated DA mechanism is critical for mPFC synaptic plasticity. The synaptic plasticity in SHRSP/Ezo mPFC may be inhibited by a decrease in VTA-mediated DA release and DA sensitivity. These abnormalities in DAergic regulations in mPFC synaptic plasticity may be involved in the pathophysiology of ADHD-like behaviors of SHRSP/Ezo.

POTENTIAL AS NON-ALCOHOLIC FATTY LIVER DISEASE MODEL IN SHRSP5/DMCR

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Non-alcoholic fatty liver disease (NAFLD) includes a progressive liver disorder from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH). Patients with nonalcoholic fatty liver disease (NAFLD) are increasing worldwide, and preventive measures are an urgent need. Therefore, establishment of appropriate animal models are needed. SHRSP5/Dmcr, formerly called the arteriolipidosis-prone rat, is the 5th substrain of stroke-prone spontaneously hypertensive (SHRSP) rat. Pregnant female rats were given from Y. Yamori, Emeritus Professor of Kyoto University, and this model has been maintained at Kinjo Gakuin University. This substrain has been produced by selective brother-sister inbreeding of SHRSP with stronger hypercholesterolmic responses to the high-fat and high-cholesterol (HFC) containing diet and lower blood pressure than SHRSP. We had found marked enlargement liver with whitish color by HFC diet. Therefore, we aimed to clarify the usefulness of the SHRSP5/Dmcr rat, as a novel animal model for time-course analysis of liver disease. Ten-week-old male SHRSP5/Dmcr rats were divided into six groups. Half were fed a HFC diet, and the others were fed the control SP diet for 2, 8 and 14 weeks. Body weight was lower in the HFC group than the SP group. Histopathologically, microvesicular steatosis and lymphocyte infiltrations were observed in the liver of rats fed HFC diet for 2 weeks, and macrovesicular steatosis, ballooned hepatocytes with eosinophilic Mallory-Denk bodies formation in some, inflammatory cell infiltrations and bridging fibrosis at 8 weeks. Interestingly, this fibrosis formed a honeycomb fibrosis after 14 weeks. Thus,we developed a new NASH model animal SHRSP5/Dmcr rat with severe fibrosis simply caused by HFC feeding alone for 14 weeks. However this strain did not accompany obesity, therefore, this model may be useful to investigate the mechanism and treatment of human NASH without obesity.

THE RENOPROTECTIVE EFFECTS OF TELMISARTAN ACT VIA THE PPARγ/ HGF PATHWAY

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Angiotensin (Ang) II type 1 receptor (AT1R) blockers (ARBs) have demonstrated beneficial effects beyond blood pressure (BP) control in the treatment of chronic kidney disease. There is clinical evidence that telmisartan is superior to losartan in reducing proteinuria in hypertensive patients with diabetic nephropathy, as it is a partial agonist of perioxisome-proliferator activated receptor γ (PPARγ) as well as an AT1R blocker (AMADEO study). However, the mechanism that explains why telmisartan has better renoprotective effects than losartan is not fully understood. We examined the role of PPARγ activation in the renal protective actions of telmisartan using AT1R-deficient mice. Renal injury was induced in AT1R-deficient mice by producing unilateral ureteral obstruction (UUO), which exhibited severe renal interstitial fibrosis and inflammation. We compared the renoprotective effects of telmisartan with that of losartan. Telmisartan, but not losartan, significantly prevented hydronephrosis induced by UUO. Importantly, the prevention of renal atrophy and fibrosis by telmisartan was significantly attenuated by GW9662, a PPARγ antagonist. Interestingly, the downstream effector of PPARγ activation by telmisartan is hepatocyte growth factor (HGF), a well known anti-fibrotic factor, as renal HGF expression was significantly increased by telmisartan, and a neutralizing antibody against HGF diminished the renal protective action of telmisartan. These beneficial changes by telmisartan were associated with a decrease in the expression of TGF-β1 and other pro-inflammatory and pro-fibrotic cytokine genes through PPARγ/HGF activation. Our findings provide evidence of organ protective actions of telmisartan through the PPARγ/HGF pathway, independently of AT1R blockade. Further development of the next generation of ARBs with added organ protective actions such as PPARγ activation might provide new beneficial drugs to treat renal and cardiovascular diseases.

EFFECT OF 5-HYDROXYTRYPTAMINE ON CYCLO-OXYGENASE-2 EXPRESSION IN VASCULAR SMOOTH MUSCLE CELLS ISOLATED FROM WKY AND SHRSP

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In the cardiovascular system, 5-hydroxytryptamine (5-HT) is known to cause vasoconstriction, vascular smooth muscle cell (VSMC) proliferation, and platelet aggregation. At the site of local vascular injury, 5-HT may be abundantly released from platelets upon stimulation. Cyclooxygenases (COXs) are essential enzymes for producing prostanoids, and play important roles in cardiovascular pathophysiology. In VSMCs, COX-2 is induced by cytokines or by vasoactive substances. COX-2-derived prostaglandin I_2 (PGI_2) is thought to have antithromobotic actions in the presence of a systemic inflammatory response and/or vascular inflammation. We previously reported that 5-HT induces COX-2 expression in rat VSMCs, and that protein kinase C, Src-family tyrosine kinase, and mitogen-activated protein kinase (MAPK) activations are each essential for the full expression of COX-2 pathways. In this study, we investigated the effect of 5-HT on COX-2 expression in VSMCs isolated from stroke-prone spontaneously hypertensive rats (SHRSP) and Wistar Kyoto rats (WKY). VSMCs were enzymatically isolated from aortic media of 6- to 7-week-old SHRSP or WKY. Primary VSMCs were used throughout the experiments. Twenty-four hours before the experiment, the medium was replaced with serum-free medium containing 0.1 % bovine serum albumin. 5-HT was dissolved in physiological saline and further diluted with serum-free culture medium containing 0.1 % bovine serum albumin. COX protein expression, MAPK and Akt activations were evaluated by Western blot analysis. mRNA expression was evaluated by real-time RT-PCR. This study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals by the Animal Research Committee of the Health Sciences University of Hokkaido. Basal COX-2 expression was significantly lower in SHRSP cells than in WKY cells. The 5-HT-induced COX-2 expression was also lower in SHRSP cells than in WKY cells. There was no difference in COX-1 expression between SHRSP cells and WKY cells regardless of the presence or absence of 5-HT. The effects of 5-HT on extracellular signal-regulated kinase and c-Jun N-terminal kinase activations were significantly stronger in SHRSP cells than in WKY cells. LY 294002, a phosphatidil-inositol-3-kinase inhibitor, inhibited 5-HT-induced COX-2 expression in rat VSMCs. However, an obvious Akt activation by 5-HT stimulation was not observed in SHRSP cells. Our results suggest that the reduction in Akt activation may be attributable to the reduction of COX-2 expression in 5-HT-stimulated SHRSP cells. On the other hand, since 5-HT-induced MAPK activation was potentiated in SHRSP cells compared to WKY cells, the signaling pathway leading to COX-2 protein expression may be impaired in SHRSP cells. In conclusion, the present study demonstrated that 5-HT-induced COX-2 expression was lower in SHRSP cells than in WKY cells, which may have relevance to the initiation and establishment of hypertension and vascular disorders in SHRSP.

RECENT PROGRESS OF GENE-TARGETING TECHNO-LOGIES IN RATS

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Although the laboratory rat is a widely used animal model in biomedical sciences, there was no simple technology for producing *in vivo* genetically engineered mutations equivalent to knockout or knock-in mice for several decades. Zinc finger nucleases (ZFNs) have been recently used to create site-specific DNA double-strand breaks, and thereby stimulate targeted gene mutations in a wide variety of organisms including plants, xenopus, drosophila, zebrafish. Using the ZFN technology, we recently demonstrated ZFN-stimulated gene-targeting at an endogenous rat gene, for which human and mouse mutations are known to cause a severe combined immunodeficiency (SCID). Co-injection of mRNAs encoding the custom-designed ZFNs into pronucleus of fertilized oocytes yielded 25-50 % gene-modified

offspring, including a wide variety of deletion or insertion mutations. ZFN-modified founders faithfully transmit these genetic changes to the next generation with the SCID phenotypes. Furthermore, we have also succeeded to generate double knockout mutant rats. This was archived by co-injection of two different ZFNs targeting two different genes. The high frequency of gene-targeting and the rapid creation of gene knockouts indicate that ZFN technology can provide a new strategy not only in rats but also in other species for creating animal models of human diseases. In addition, SCID rats, due to their ten times larger body size compared to the mouse, could be an important experimental model for pre-clinical testing during drug development as well as it could provide hosts for allogeneic and xenogeneic tissue grafts and stem cell transplantation.

SUPPRESSION EFFECT OF CAFFEIC ACID PHENETHYL ESTER (CAPE) FOR THE LIPID ACCUMULATION OF THE MATURE ADIPOCYTES

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To deposit oil droplets into adipocytes, free fatty acid (FFA) is taken in adipo tissue as for triglyceride (TG). Recently, adipocytes are recognized to be endocrine cell which secreted the various bioactive substances. Propolis particular attention as a healthy food. It is known that Caffeic Acid Phenethyl Ester (CAPE) is the main active ingredient of propolis shows bactericidal activity, anticancer and immunostimulation. We reported that CAPE has inhibition effect of adipocytes differentitation. Furthermore, in this study we investigated the suppression effect of CAPE on oil droplets accumulation in adipocytes after differentiation and adipocytokine expression. Differentiation of 3T3-L1 mouse fibroblast to adipocytes were grown to confluence in Dullbecco's Modified Eagle's medium (DMEM) with 10 % fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured for two days after having reached confluent. differentiation medium was including 5 μg/ml insulin 1 μM dexametazone, 500 μM isobutylmethylxanthine in high-glucose DMEM. Cells were stained with Oil-Red-O to detect oil droplets and cells were lysed at 7 days after. Following that medium was then changed to a high-glucose DMEM with 10 % FBS with or without at CAPE 50 μM in respective cultures. The cell lysate was isolated to measured for TG content. Total RNA was isolated at day 7 and day 14. The expressions of adipocytokines or hormone-sensitive lipase (HSL) mRNA were analyzed using RT-PCR. The mRNA levels were corrected using GAPDH as an internal standard. The accumulation of TG in cells were chronologicall increased at day 7 to day 14 but treatment of CAPE decreased the TG accumulation in cells. In addition, at day 14, expression of adiponectin mRNA has increased more than at day 7. Treatment of CAPE, the mRNA expression of adiponectin was increased more than control. But mRNA expression of leptin and IL-6 were decreased by CAPE. These fingings suggested that CAPE suppresses the oil droplets accumulation in mature adipocytes. Decrease of TG by CAPE is associated with mRNA expression of adipocytokine and hormone sensitivity lipase (HSL) in mature adipocytes. In addition, CAPE may have important implications for elucidate mechanism of decrease of TG in mature adipocytes.

ELUCIDATION OF THE MECHANISM FOR REDUCED SERUM CHOLESTEROL IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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Epidemiological studies have reported a positive association between lower serum cholesterol and the incidence of cerebral hemorrhage in humans. The spontaneously hypertensive rat (stroke-prone) (SHRSP) is a widely used animal model for hypertension and stroke. The serum cholesterol of this rat is also lower than that of normotensive Wistar Kyoto rats (WKY); therefore, a low level of serum cholesterol may participate in cerebral stroke in this rat and humans. We previously reported that lower mevalonate pyrophosphate decarboxylase (MPD), which is a cholesterol biosynthetic enzyme, is responsible for reduced serum cholesterol in SHRSP; however, it remains unclear whether cholesterol biosynthetic enzymes downstream of MPD in SHRSP fell, or whether lower serum cholesterol in SHRSP is caused by increasing the receptor involved in the uptake of cholesterol into the liver from serum. In this study, we investigated the change in the expression of cholesterol biosynthetic enzymes and low density lipoprotein (LDL) receptor (LDL-R) involved in the uptake of LDL or scavenger receptor class B type-1 (SR-B1) involved in the uptake of high density lipoprotein (HDL). Total cholesterol, very low density lipoprotein (VLDL)/LDL- or HDL-cholesterol in serum and cholesterol content in the liver were measured using a kit. Messenger RNA and protein levels were analyzed by real-time PCR and immunoblot analysis, respectively. Total cholesterol, VLDL/LDL- and HDL-cholesterol in the serum of SHRSP were statistically lower than those of WKY, but cholesterol content in the liver of SHRSP was similar to that of WKY. When the mRNA level of 9 cholesterol biosynthetic enzymes containing MPD in the liver were examined, the mRNA of MPD, farnesyl pyrophosphate synthase (FPS), squalene epoxidase (SQE) or lanosterol 14-α dimethylase (CYP51) in SHRSP was significantly low as compared with WKY. The protein level as well as the mRNA level of MPD, SQE and CYP51 in SHRSP were also statistically significant, but not FPS. The mRNA and protein levels of LDL-R were similar between WKY and SHRSP, but those of SR-B1 were significantly higher than those of WKY. From these data, it was suggested that lower serum cholesterol in SHRSP was caused by acceleration of the uptake of HDL from serum by increasing of SR-B1 in the liver, supplementing the reduction in cholesterol biosynthesis due to low levels of MPD, SQE and CYP51 in the liver.

SAFE AND EFFICIENT IMMUNOTHERAPY FOR ANGIOTENSIN II

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Vaccine against self-antigen needs to be efficient and safe to achieve clinical application: present adequate humoral immune response (antibody production) from B cell activation for efficiency while eliminating adverse cellular immune responses caused by T cell activation for safety. In this study, we examined the B cell and T cell response toward AngII in vaccine for hypertension. AngII was conjugated with the classical immunogenic carrier Keyhole Limpet Hemocyanin (KLH) to form AngII-KLH, and was subcutaneously injected in C57Bl/6 male mice three times every two weeks with Freund's adjuvant. In terms of B cell activation, AngII-KLH injection successfully induced high titers of anti-AngII antibody. As an evaluation of neutralizing effect of the produced antibody, co-treatment of AngII and serum from immunized mice attenuated AngII-induced ERK phospholylation and *c-fos* promoter activation in HASMC. In a therapeutic model of continuous AngII infusion, immunization with AngII-KLH resulted in lower systolic blood pressure (101.8±9.3 mm Hg) compared to control mice (142.8±9.9 mm Hg) accompanied with reduced cardiac hypertrophy and fibrosis. The antibody titer was gradually decreased until day 98, and was not increased by larger amount of AngII administration, which indicates endogenous AngII itself does not stimulate the antibody production. In terms of T cell activation, stimulation with AngII did not increased the proliferation and the lymphokine production of T cells from mice immunized with AngII-KLH. Taken together, we showed AngII-KLH vaccination induces efficient antibody production against AngII from B cell activation with reversibility, without the risk of T cell activation mediated autoimmune toward AngII.

CAFFEIC ACID PHENETHYL ESTER SUPPRESSES OXIDATIVE STRESS IN 3T3-L1 ADIPOCYTES

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Abdominal obesity is the principal causative factor in the development of metabolic syndrome. Adipocytes in obese patients exhibit increased oxidative stress *via* the activation of reactive oxygen species (ROS)-producing systems and inactivation of antioxidant enzymes. Caffeic acid phenethyl ester (CAPE) is a component of propolis, which is a substance taken from the hives of honeybees, and is known to exhibit anti-inflammatory activity. In this study, the suppressive effect of CAPE on oxidative stress in 3T3-L1 adipocytes was investigated. 3T3-L1 cells were seeded in 6-well plates and grown to confluence in DMEM with 10 % FBS. Two days after confluence, cells were fed a high-glucose DMEM supplemented with 10 % FBS, 1mM dexamethasone, 500 mM isobutylmethylxanthine, and 5 mg/ml insulin for 3 days. Medium was then changed to high-glucose DMEM with 10 % FBS, and 5 mg/ml insulin with 25 and 50 mM CAPE for 2 days in respective cultures. In addition, after two days, the medium was changed to only high-glucose DMEM with or without CAPE for the last 3 days. Cells were harvested 7 days after initiation of the differentiation protocol and triglyceride contents were measured. ROS was measured as the evaluation of oxdative stress in cells by NBT assay. The mRNA expressions of subunit genes of NADPH oxidase (gp91 phox, p47 phox), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase were measured in 3T3-L1 with or without CAPE. Furthermore, SOD enzyme activity was detected. CAPE suppressed the accumulation of triglycerides in 3T3-L1 on day 7. ROS were increased by differentiated adipocytes, and CAPE significantly inhibited ROS production in 3T3-L1 adipocytes. It was observed the mRNA expressions of SOD, GPx, and catarase were up-regurated in the cells with CAPE, although the expressions of NADPH-oxidase subunits gene (pg91 phox and p67 phox) were no significant differences. The enzyme activity of SOD was increased by the addition of CAPE. CAPE demonstrated inhibition of intracellular ROS *via* activation of antioxidant enzymes in 3T3-L1 adipocytes. These results suggested that CAPE reduces ROS production in adipocytes. CAPE might have anti-oxidative effects in 3T3-L1 adipocytes.

TREATMENT OF MCAO-INDUCED BRAIN INFARCTION BY ANTI-HMGB1 MONOCLONAL ANTIBODY

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High mobility group box-1 (HMGB1) , originally identified as a non-histone nuclear protein, is now thought to be a representative damage-associated molecular pattern (DAMP). Once released in to extracellular space, HMGB1 exerts pro-inflammatory cytokine activity through the stimulation of plural receptors, such as receptor for advanced glycation endproduct and toll-like receptor-4/2. Increasing evidences suggest that HMGB1 may be involved in the pathogenesis of many types of inflammatory diseases. In the present study, we raised monoclonal antibody (mAb) against HMGB1 and examined the effects of the mAb on middle cerebral artery occlusion (2 h)/reperfusion (MCAO)-induced brain infarction in rats. The mAb was administered after MCAO. In the control rats, marked translocation of HMGB1 from nuclei to cytosolic compartment was observed in neurons in ischemic core. The treatment of MACO rats with anti-HMGB1 mAb significantly reduced the infarction size, associated with the marked improvement of motor paralysis. The disruption of blood-brain barrier (BBB) was apparent 3 hour after reperfusion in the control rats. The swelling of astrocyte endfeet and the detachment of endfeet from lamina propria were most prominent by electron microscopic observation. The anti-HMGB1 mAb maintained BBB structurally and functionally. In contrast, intracerebroventricular injection of HMGB1 exacerbated the brain infarction induced by MCAO. The mAb treatment also inhibited the activation of microglia and the expression of inflammatory molecules such as TNF-α and iNOS. Using *in vitro* BBB system composed of vascular endothelial cells, pericytes and astrocytes, we demonstrated that recombinant human HMGB1 can directly induced contractile changes in vascular endothelial cells and pericytes, leading to the increase in the permeability of BBB that were inhibited by the addition of anti-HMGB1 mAb. These results as a whole indicated that HMGB1 plays crucial role in the inflammatory responses in the brain after ischemic insult and that anti-HMGB1 mAb may provide a novel and excellent therapy for ischemic stroke.

PROTECTIVE EFFECTS OF EPLERENONE ON BLOOD-BRAIN BARRIER INJURY AND COGNITIVE DECLINE IN HIGH SALT-TREATED TYPE 2 DIABETIC MICE

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We examined the effects of selective mineralocorticoid receptor antagonist, eplerenone, on blood-brain barrier (BBB) disruption and cognitive impairment in high salt-treated type 2 diabetic KKAy mice. Thirteen-week-old KKAy mice were treated with normal salt diet (0.8 % NaCl, n=6), high-salt diet (6 % NaCl, n=7), high-salt diet treated with eplerenone (100 mg/kg/day, p.o., n=6), and high-salt diet treated with hydralazine (50 mg/kg/day in drinking water, n=6) for 8 weeks, respectively. KKAy mice exhibited hypertension, leakage of macromolecule from brain microvessels into hippocampus, and impaired cognitive functions, which were further enhanced by a high salt diet. In high salt-treated KKAy mice, eplerenone and hydralazine showed similar blood pressure reduction. On the other hand, eplerenone restored the cognitive decline and ameliorated leakage from brain microvessels, but hydralazine did not. These data suggest that BBB disruption and cognitive impairment are induced during the development of type 2 diabetes, which are deteriorated by high salt diet. Treatment with a selective mineralocorticoid receptor antagonist, eplerenone, may elicit neuroprotective effects in cognitive disorders by preventing BBB permeability through mechanisms that are not simply explained by its blood pressure lowering effect.

EARLY TREATMENT WITH OLMESARTAN PREVENTS JUXTAMEDULLARY GLOMERULAR PODOCYTE INJURY AND THE ONSET OF MICROALBUMINURIA IN TYPE 2 DIABETIC RATS

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In this study, we aimed to determine if early treatment with an angiotensin II (Ang II) receptor blocker (ARB), olmesartan, prevents the onset of microalbuminuria by attenuating glomerular podocyte injury in Otsuka Long-Evans Tokushima Fatty (OLETF) rats with type 2 diabetes mellitus. OLETF rats were treated with either a vehicle, olmesartan (10 mg/kg/day) or a combination of non-specific vasodilators (hydralazine 15 mg/kg/day; hydrochlorothiazide 6 mg/kg/day; and reserpine 0.3 mg/kg/day; HHR) from the age of 7 to 25 weeks. OLETF rats were hypertensive and had microalbuminuria from 9 weeks of age. At 15 weeks, OLETF rats had higher Ang II levels

in the kidney, larger glomerular desmin-staining areas (an index of podocyte injury), and lower gene expression of nephrin and podocin in juxtamedullary glomeruli, than non-diabetic Long-Evans Tokushima Otsuka (LETO) rats. At 25 weeks, OLETF rats showed overt albuminuria, and higher levels of Ang II in the kidney and larger glomerular desmin-staining areas in superficial and juxtamedullary glomeruli compared to LETO rats. Reductions in mRNA levels of nephrin and podocin were also observed in superficial and juxtamedullary glomeruli. Although olmesartan did not affect glucose metabolism, it decreased blood pressure and prevented the renal changes in OLETF rats. HHR-treatment also reduced blood pressure, but did not affect the renal parameters. The present study demonstrated that podocyte injury occurs in juxtamedullary glomeruli prior to superficial glomeruli in type 2 diabetic rats with microalbuminuria. Early treatment with an ARB may prevent the onset of albuminuria through its protective effects on juxtamedullary glomerular podocytes.

DIABETIC KIDNEY DISEASE IS ACCELERATED BY TEMPORAL PROTEIN RESTRICTION DURING GROWING PERIOD IN OTSUKA LONG-EVANS TOKUSHIMA FATTY (OLETF) RATS

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Protein malnutrition in early stages of life causes growth retardation in animals partly due to the reduction of plasma insulin and insulin like growth factor-1 (IGF-1) levels. The effect of protein malnutrition during growing period on the health in later life is not well studied. In this study, we investigated the effects of protein restriction during growing period on glucose metabolism and diabetic nephropathy in OLETF rats. OLETF rat is an animal model of type II diabetes that develops mild obesity and diabetic nephropathy. Male OLETF rats were divided into a control group (CN, receiving a 20 % casein diet) and a protein restriction group (PR, receiving a 9 % casein diet) at 5 weeks of age. Dietary manipulations were performed from 5 to 10 weeks of age. Rats of both groups were fed 20 % casein diet from 10 weeks of age. Oral glucose tolerance tests (OGTT) were performed at 10 and 15 weeks of age. After 16 hours of fasting, the rats were orally administered with glucose (2 g/kg/day). Serum IGF-1 concentrations were determined. Urine was collected at 16 weeks of age. Mean body weight of the PR group was significantly lower than that of control OLETF rats during protein restriction. There was no difference in body weight between the control group and the PR group after termination of protein restriction. On 10 weeks of age, plasma glucose levels after glucose loading in the PR rats were significantly lower than those in the control rats. There was no difference in plasma insulin levels between two groups. On 15 weeks of age, *i.e.* 5 weeks after the termination of protein restriction, no difference was observed in plasma glucose and insulin between the groups. Interestingly, urinary protein excretion was increased in the PR group compared with the CN group. Renal expression of Type IV collagen (col4a1) and monocyte chemotactic peptide-1 (MCP-1) was significantly increased in the PR group compared with the CN group. There was no significant difference in serum IGF-1 concentration between the CN and the PR groups at 10 and 15 weeks of age. In the PR group, serum IGF-1 concentration at 15 weeks of age was lower than that at 10 weeks. Present study demonstrates that temporary dietary protein restriction during growing period leads to deterioration of diabetic nephropathy in OLETF rats. It was suggested that dietary protein in growing period affects not only developmental growth but also programmed diabetic nephropathy.

EFFICACY OF RENIN-ANGIOTENSIN VACCINE FOR THE PREVENTION OF HYPERTENSION AND RENAL INJURY IN SPONTANEOUSLY HYPERTENSIVE RATS

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It has been shown that renin-angiotensin system (RAS) vaccines may be effective for the prevention of hypertension, but their role in the prevention of renal disease is unclear. In this study, we compared the effects of an angiotensin II type 1 (AT1) receptor vaccine with an angiotensin II receptor blocker (ARB) and a vasodilator on blood pressure and renal injury in an animal model of hypertensive nephrosclerosis, the NG-nitro-L-arginine methyl ester (L-NAME) nephropathy model. Male spontaneously hypertensive rats (SHR) were divided into 6 groups and treated transiently with 3 injections of vehicle or AT1 receptor vaccine (0.1 mg) at age 4, 6, and 8 weeks, or continuously with candesartan cilexetil (0.1 mg/kg/day) or hydralazine hydrochloride (5 mg/kg/day), then administered NG-nitro-L-arginine methyl ester (L-NAME) from age 18 to 21 weeks to induce renal injury. It was found that vaccination against the AT1 receptor caused a significant increase in AT1 receptor titers, and a sustained decrease in blood pressure. Moreover, L-NAME treatment resulted in a marked increase in proteinuria in the control groups, which was completely suppressed in the AT1 vaccine-treated group, and glomerular injury scores were also significantly decreased. Real-time RT-PCR and immunofluorescence studies revealed increased renin mRNA, and increased glomerular expression of nephrin. Comparable results were seen in rats treated continuously with the ARB candesartan, but not with hydralazine. To confirm the production of AT1 receptor blocking antibodies, both *in vivo* and *in vitro* studies were performed. Infusion of angiotensin II in vehicle-treated rats resulted in an increase in systolic blood pressure which was attenuated in the AT1 vaccine-treated rats. Moreover, addition of serum from AT1 vaccine-treated rats to cultures of vascular smooth muscle cells resulted in an attenuation of angiotensin II-induced ERK phosphorylation *in vitro*. It was found that the antibody titers were increased equally by 3 and 6 vaccinations of the AT1 vaccine, and that the antibody titers decreased over several months, resulting in a decrease in the hypotensive effect after 9 months. These results suggest that 3 injections of the AT1 vaccine are as effective as continuous treatment with ARB, not only for the attenuation of hypertension, but also for the prevention of L-NAME-induced nephropathy in SHR.

LEFT VENTRICULAR EXPRESSION OF LOX-1 REFLECTS CARDIAC FUNCTION IN HYPERTENSIVE HEART FAILURE IN RATS

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Lectin-like oxidized low-density lipoprotein (oxLDL) receptor-1 (LOX-1) was originally identified as an endothelial receptor for oxLDL, and now is recognized as a multi-ligand receptor. LOX-1 expression in cardiomyocytes can be induced by oxidative stress and various hormonal stimuli, subsequently facilitating apoptosis. In this study, the relationship between left ventricular (LV) expression of LOX-1 and cardiac function in chronic heart failure (CHF) was analyzed by using salt-sensitive Dahl (DS) rat model of hypertension. DS and control salt-resistant Dahl (DR) rats were fed a high-salt diet from the age of 6 weeks. Their LV expression of LOX-1 was dramatically increased in proportion to the extent of heart failure. Compared with DR rats, LV mRNA levels of LOX-1 in DS rats increased by 4.7-fold at the age of 11 weeks with LV hypertrophy (LVH), and by 32-fold at 18 weeks with decreased systolic function. LV mRNA levels of LOX-1 were significantly ($p<0.0001$) correlated with the decrease in ejection fraction (EF) ($r= -0.772$), the increases in plasma ($r=0.744$) and LV mRNA ($r=0.814$) levels of brain natriuretic peptide (BNP), and LV mRNA levels of inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1) ($r=0.943$), interleukin-1β (IL-β) ($r=0.760$), and transforming growth factor-β1 (TGF-β1) ($r=0.936$). LV mRNA levels of a macrophage marker, F4/80, were also correlated with those of LOX-1 ($r=0.560$, $p=0.0019$). These results suggest that LV expression of LOX-1 is involved in the progression of heart failure associated with inflammation. The membrane proximal extracellular domain of LOX-1 can be proteolytically cleaved and released into the blood stream as a soluble form (sLOX-1). To determine whether sLOX-1 serves as a biomarker of heart failure in human, the levels of sLOX-1 in sera from CHF patients with LVH were measured. ELISA assay reveled that

serum sLOX-1 levels were significantly increased in the CHF group compared with the control group with normal LV size and systolic function (948±208 vs 435±57 pg/ml, p=0.021). In addition, serum sLOX-1 levels were significantly correlated with the decrease in EF (r= -0.495, p=0.037). Animal and human studies thus respectively indicated that LV expression of LOX-1 and serum levels of sLOX-1 reflect the cardiac function, suggesting that sLOX-1 is a potential marker for the development of heart failure in human.

INFLUENCE OF THE SENSITIVITY TO OXIDATIVE STRESS IN ADULT LIFE ON FETAL EXPOSURE TO MILD PROTEIN RESTRICTION IN SHRSP

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Restricted nutrition during pregnancy induces dyslipidemia, obesity, hypertension, hyperinsulinemia and hyperleptinemia in offspring. We have already reported that maternal protein restriction induced high salt sensitivity, severe hypertension and development of hypertensive end-organ damage in spontaneously hypertensive stroke-prone rat (SHRSP) Furthermore, we have confirmed that these phenomena were continued for several generations. Oxidative stress is one of the most important risk factor for the occurrence of these metabolic dysfunctions. In the present study, we investigated the influencce of protein restriction on the sensitivity to oxidative stress in the offspring of SHRSP. Virgin female SHRSP at 10 weeks of age were mated with a single stud male rat. Pregnant female rats were fed 20 % casein diet in the control group, and 9 % casein diet in the low protein (LE) group throughout gestation. After the birth, all dams were fed Funabashi SP diet during lactation. All offspring were weaned at 28 days and fed Funabashi SP diet. Beginning at 12 weeks of age and continuing for 4 weeks, all offspring received phorbol 12-myristate 13-acetate (PMA, NADPH oxidase activator, 10 nmol/h) intravenously by osmotic mini pump. At the end of the experiment, we measured plasma d-ROM level (an oxidative stress marker) and the antioxidant enzyme activities level in erythrocytes (an indicator of SOD activity and GSH-Px activity). Endothelial nitric oxide synthase (eNOS) and soluble guanylate cyclase (sGC) protein level in the thoracic aorta were detected by western blot analysis. Although the birth weight was significantly lower in the LE group than in the control group in both males and females, the weight gain and blood pressure elevation showed the same levels throughout the experimental period. At 16 weeks of age, the blood pressure in both groups reached over 220 mm Hg with severe hypertension. Despite the high d-ROM level, no difference was found in the antioxidant enzyme activities level in erythrocytes. The endothelial-dependent vascular relaxation in the thoracic aorta was reduced in the LE group compared with the control group. The down-regulation of eNOS and up-regulation of sGC were observed in the aorta of the LE group. In summary, SHRSP with fetal exposure to mild protein restriction during the pregnancy appear to show high sensitivity to oxidative stress and experience endothelial dysfunction.

EFFECTS OF ELASTIN PEPTIDE ON VASCULAR STRUCTURE AND FUNCTION IN SHR

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Connective tissue is an important structural component and contributes to biomechanical function in the vascular wall. Among these functions, elastin plays a major role in vascular elasticity. It is well known that the degeneration or degradation of aortic elastin fiber is responsible for vascular dysfunction such as hypertension. In this study, we examined the effects of elastin peptide from bonito bulbus arteriosus on vascular morphology and function using spontaneously hypertensive rats (SHR/Izm). [Experiment 1] Effects of elastin peptide: Male SHRs were divided into two groups at 20 weeks of age. Elastin peptide from bonito bulbus was orally administered (600 mg/kg) to one group for 5 weeks. [Experiment 2] Effects of Proryl glysin (PG), a degradation product of elastin peptide: Male SHR/Izm were divided into two groups at 15 weeks of age. PG was infused intravenously by osmotic mini pump to one group for 4 weeks (104 nmol/hr). [Experiment 1] No significant differences were found in blood pressure, body weight and connective tissue-related gene expression (lysyl oxidase, type III collagen, elastin) in the aorta between the control and elastin treated group. However, the contractile response to L-Phenylephirine Hydrochroride was suppressed by the treatment with elastin peptide. [Experiment 2] The vasodilating response to increasing perfusion pressure was higher in the PG group than in the control. Morphologically, the endothelium of the control group was very rough due to the disarrangement of endothelial cells and a lot of crater-like structures. On the other hand, the surface of the endothelium was smooth and the endothelial changes were less intense in the PG group as compared to that in the control group. These results indicate that elastin peptide from bonito bulbus has beneficial effects for vascular functions due to the protective effects against endothelial injuries. We plan to do more research to elucidate the mechanisms of such beneficial effects of elastin peptide, such as changes in elastin receptors, signal transduction and so on, using cultured endothelial cells and smooth muscle cells.

EPIGENETIC REGULATION OF THE EXPRESSION OF GENES RELATED TO CARDIAC HYPERTROPHY BY THE CALCINEURIN IN SPONTANEOUSLY HYPERTENSIVE RATS (SHRS)

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Hypertension is one of the major underlying diseases of hypertrophy leading to heart failure. Although cardiac hypertrophy is essentially compensatory for workload in pathological conditions like hypertension, prolongation high-blood pressure leads to congestive heart failure, arrhythmia and sudden death. During cardiac hypertrophy and the following heart failure, cardiomyocytes show abnormal Calcium state, oxidative stress and mitochondrial DNA damage. Calcineurin (CN) is Ca^{2+}- and calmodulin (Calm)-dependent serine/threonine protein phosphatase, regulate hypertrophy related transcription factors (e.g. nuclear factor of activated T-cell (NFAT)). It is known that calmodulin kinase CaMK upregulate transcription of the cardiac hypertrophy-related genes *via* phosphorylation of the histone deacetylase (HDAC). We cloned and analyzed expression levels of CN, Calm and other related genes of spontaneously hypertensive rats (SHR/kpo, SHRSP/kpo and M-SHRSP/kpo) and normotensive WKY/kpo. The levels of CN and NFAT are correlated with the blood pressure levels of rats. And of 14-3-3, transporter of the phosphorylated HDAC from the nuclear altered same as them. Other genes are not consistent with the pressure. These results indicate the cardiac hypertrophy related hypertension. Although blood pressure in the SHRSP is moderate level between those in SHR and M-SHRSP, SHRSP tend to onset of the cardiac hypertrophy rather than others. These results showed that the hypertrophy is not only related to blood pressure. From our results of expression o HDAC may crucially regulate hypertrophy in rats. Cloned genes were sequenced and confirmed existence of the genetic variants of CN subunits, lacked autoinhibitory domain and the EF-hand motifs of CnA and CnB, respectively. The variant proteins showed low calcium binding activity revealed the physiological activity will be altered. Although it was thought that calcineurin works mainly up-regulated the transcription of hypertrophy related genes *via* NFAT, the molecular mechanisms of the morbidity of hypertension and hypertrophy are not apparent. Above the results of expression and variants structures of CN show that the dephosphrylation of HDAC is important to prevent hypertrophy as the counter effects of NFAT. In the multifactorial disease such as hypertension, diabetes and cancer caused by not only genetical backbone the environmental factors are crucial. It is not apparent that pathological conditions were regulated by epigenetically, yet. Epigenetic regulations and calcium signaling are important as the key steps in the disease network.

ANGIOTENSIN CONVERTING ENZYME INHIBITOR DURING LACTATION PERIOD LESSENS EXACERBATION OF GLUCOSE METABOLISM DISORDER IN FETUSES OF UNDERNOURISHED PREGNANT STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RAT DAMS

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Prenatal nutrient restriction presenting with low birth weight is linked to the metabolic syndrome consisting of insulin resistance and type II diabetes mellitus. We previously demonstrated that maternal nutrient restriction (NR) during pregnancy enhanced glucose intolerance in the offspring of stroke-prone spontaneously hypertensive rats (SHRSP). The results of various investigations have demonstrated that an angiotensin II type-1 receptor blocker (ARB) and an angiotensin-converting enzyme inhibitor (ACEI) can improve insulin resistance. The present study was conducted to investigate the participation of the rennin-angiotensin system in the development of glucose intolerance in NR-SHRSP and to examine. The effect of administering ACEI during lactation on glucose intolerance in NR-SHRSP. Pregnant SHRSP received 80 % of their daily food intake during full gestation. At birth, dams had access to food *ad libitum*. Offerings were divided into two groups, receiving a daily oral treatment of either captopril (3 mg/kg) dissolved in evaporated milk (captopril group) or only evaporated milk (control group) for 4 weeks. At 8, 12 and 16 weeks of age, the two groups were subjected to glucose tolerance tests. Insulin receptor signaling (insulin receptor, Akt, phospho-Akt, glucose transporter 4) in skeletal muscle and fatty tissue and the pancreatic and duodenal homeobox 1 (PDX 1) in the islet protein level were detected by western blot analysis. Glucose transporter 2 (Glut 2) and sirtuin 1 (Sirt 1) mRNA level in the liver were detected by real-time PCR. The body weight of the captopril group during lactation was slightly lower than that of the control group, but treatment of NR-SHRSP with captopril did not influence the body weight or the systolic blood pressure after weaning. The captopril group showed attenuation of the increase in the plasma concentration of glucose after a glucose load at 12 and 16 weeks of age. At 12 weeks, the plasma concentration of insulin in the captopril group was lower than that in the control group. Moreover, we observed that the captopril group showed increased PDX 1 protein expression in islet, Glut 2 mRNA expression in the liver and Sirt1 mRNA expression in the skeletal muscle. However, treatment with captopril did not influence insulin receptor signaling in the skeletal muscle or the fatty tissue. These results indicate that administration of ACEI during lactation might have lessened glucose intolerance in NR-SHRSP.

BEHAVIORAL CHARACTERIZATION OF SHR SUBSTRAINS AS AN ADHD ANIMAL MODEL

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The spontaneous hypertensive rat (SHR) is widely used to elucidate the pathogenesis of not only essential hypertension itself but also the complications associated with hypertension and metabolic diseases. Furthermore, SHR (SHR/N), the inbred SHR from National Institute of Health (NIH), has been applied as an animal model for psychiatric disorder of "attention deficit hyperactivity disorder (ADHD)". Recently, SHR substrains have been deposited to National BioResource Project-Rat in Japan (NBRP-Rat, Project manager: Professor Tadao Serikawa, Kyoto University). In the project, SHR substrain was comprehensively characterized by the phenotypes including behavioral observations and the genotypes determined by global analysis of single nucleotide polymorphism. On the other hand, we have previously proposed 6-weeks-old “SHRSP/Ezo”, an inbred SHR in our laboratory, as more appropriate animal model of ADHD than SHR/N, because symptomatic features in SHRSP/Ezo are different from those in SHR/N. Namely, juvenile SHRSP/Ezo showed the ADHD-like abnormal behaviors, i.e. attentional dysfunction (short-term memory impairment assessed by the Y-maze test), hyperactivity (excessive locomotor activity assessed by the open-field test) and an impulsive action (low anxiety level assessed by the elevated plus-maze test). Especially, attentional dysfunction in SHRSP/Ezo show male preponderance. These behavioral characterizations were similar to symptom of ADHD patients and (were) ameliorated by therapeutic drugs for ADHD such as methylphenidate and atomoxetine. In addition, juvenile SHRSP/Ezo exerted monoaminergic dysfunction in the frontal cortex as reported in ADHD patients. In this symposium, we introduce our research to elucidate the pathogenesis of ADHD, in comparison with behavioral and genetic profiles among SHR substrains (SHRSP/Ezo, SHRSP/Izm, SHRSP/Ngsk, SHR/Izm, WKY/Izm, WKY/Ezo[genetic control]), by ADHD-like-behavior measures of SHRSP/Ezo.

FOCAL ISCHEMIA MODELS IN SHR/IZM AND SHR/KYUSHU

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Almost 50 years have passed since the establishment of spontaneously hypertensive rat (SHR), and there are a variety of substrains of SHR. To our knowledge, six focal ischemia models in 6 substrains of SHR or stroke-prone SHR have been established. We performed an online *PubMed* search (2001-2010), and identified 117 original articles with focal ischemia in SHR. Recently, the intraluminal suture model was frequently used. However, distal middle cerebral artery occlusion (MCAO) is the predominant and relevant method of focal ischemia in hypertensive rats. Surprisingly, I found that physiological parameters of SHR such as age, body weight, and blood pressure were often neglected in recent literature. Prado *et al.* (1996) first used SHR as a photothrombotic distal MCAO model, because SHR develop much larger cortical infarcts after distal MCAO than normotensive rats because of hemodynamically vulnerable collateral function. Subsequently, SHR/Kyushu and SHR/Izm were employed for photothrombotic distal MCAO in Japan. The SHR/Kyushu were from the F10 and F11 generations derived by Okamoto and Aoki. Cai *et al.* (1998) showed the infarct size was larger in male and female SHR/Kyushu than in SHR/Izm. Because blood pressure levels were the same between the two substrains, factors other than hypertension probably account for different lesion size. Although infarct volume in SHR/Kyushu and SHR/Izm with simple Y-shaped MCA were smaller than those of the regular pattern, we found that simple MCA was less prevalent in SHR/Izm compared with SHR/Kyushu. Increased infarct size or stroke sensitivity in SHR/Kyushu than in SHR/Izm is likely to be attributable to not only hypertension but also additional genetic factors specific to SHR/Kyushu.

SATIETY HORMONES IN SHR/NDmcr-cp RATS LACKING A FUNCTIONAL LEPTIN RECEPTOR

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Peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) have been identified as key peptides in appetite regulation and are altered in the obese state. Both hormones are secreted from intestinal L cells along an increasing gradient from the stomach to colon in response to ingested nutrients, and reduce food intake by modulating appetite circuits in the hypothalamus. Recently, such satiety hormones have become targets for the treatment of type 2 diabetes. SHR/NDmcr-cp rats (CP) are an obese animal model, lacking functional leptin and showing metabolic disorders. To characterize the gastrointestinal tract in CP, we investigated the status of satiety hormones. At 12 weeks of age, the oral glucose tolerant test was conducted in CP and their lean littermates

(Lean). Plasma glucose, insulin, GLP-1 and PYY were measured after glucose loading (2 g/kg body weight). Fasting plasma concentrations of glucose, insulin, leptin, GLP-1, and PYY were also determined. The small intestine and stomach were weighed. CP had greater intestine, colon, and liver mass than Lean. Fasting plasma glucose, insulin, and leptin were significantly higher in CP. Fasting PYY level was significantly increased in CP compared to Lean (0.84±0.09 vs 1.23±0.15, $p<0.05$), whereas no significant differences were seen in the fasting plasma GLP-1 level. At 30 minutes after glucose load, the PYY level was similar to the fasting status, and the GLP-1 level had increased. Due to the lack of a functional leptin receptor, SHR/NDmcr-cp rats demonstrate severe obesity as well as severe hyperleptinemia. In the present examination, several defects were detected in the obese gut. Further study is needed.