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THE EFFECTS OF ENERGY AND/OR SALT RESTRICTION OF HYPERTENSION AND KIDNEY INJURY IN OBESE SALT-SENSITIVE HYPERTENSIVE RATS

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To clarify the role of obesity and salt excess in hypertension during young age, we examined the effects of energy- and/or salt-restricted diets in hypertension and kidney injury of Dahl's Z-Lept/Slc (DS/faf) rats, which were produced by crossbreeding Zucker obese (ZO) and Dahl salt-sensitive (DS) rats. Firstly, we examined the characteristics of DS/faf rats, comparing blood pressure (BP), kidney function, metabolic parameters, and plasma renin activity (PRA), aldosterone concentration (PAC) and weight of adrenal glands. And then, the effects of energy-(60 % restriction compared to ad libitum feeding) and/or salt- (0.05 % salt) restricted diets on the above-mentioned parameters in DS/faf rats. Body weight gain was almost similar between DS/faf and ZO rats, which was extremely greater than body weight of DS rats. Systolic BP was higher in DS/faf rats compared to DS and ZO rats. Fasting blood sugar (BS) and insulin (IR) was similarly higher in DS/faf and ZO rats than DS rats. On the other hand, dyslipidemia was accelerated in DS/faf rats than ZO rats, which showed higher serum total cholesterol (TC) and triglyceride (TG) than DS rats. Especially, serum TG was extremely greater in DS/faf rats. Urinary protein and blood urea nitrogen (BUN) were increased in DS/faf rats. Also, renal histological damage was progressed in DS/faf rats. PAC was increased in ZO rats compared to DS rats but it was similar between DS/faf and DS rats. Compared to DS rats, PRA was increased in ZO rats but suppressed in DS/faf rats. As a result, PAC/PRA ratio was increased in DS/faf rats but almost same between ZO and DS rats. Weight of adrenal glands was greater in DS/faf rats than ZO and DS rats. Thus, aldosterone production may be enhanced in DS/faf rats than ZO rats, which showed higher serum total cholesterol (TC) and triglyceride (TG) than DS rats. We used 6-7 and 16-17 week old SHRSP and WKY as control rats.

RENAL SYMPATHETIC ACTIVITY AND RENAL CORTICAL AND MEDULLARY BLOOD FLOW DISTRIBUTION IN SHRSR

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In the previous experiments, we showed that high frequency stimulation of renal nerve caused a transient increase of cortical blood flow and a sustained decrease of blood flow in WKY. In contrast, blood flow in renal medulla showed no significant changes. Young SHRSR showed a decrease of cortical and medullary blood flow by renal nerve stimulation. The magnitude of cortical blood flow reduction in aged SHRSR was similar to young SHRSR and that of medullary blood flow reduction was more than that in young SHRSR. LNAME augmented the reduction of medullary blood flow by electrical stimulation in aged WKY and young SHRSR. LNAME showed no effects on blood flow reduction in medulla in aged SHRSR. These results indicate that renal nerve flow is protected through the nitric oxide production in normotensive rats and this mechanism is blunted in hypertensive rats. Reduction of nitric oxide production in renal medulla might be contributed to this phenomenon. Our aim of this study was to clarify that chronic treatment of taurine can improve the altered renal cortical and medullary blood flow distribution by renal nerve stimulation in SHRSR.

We used 16-17 weeks old SHRSR/Zam which were divided to two groups, taurine group and control group. Three percent of taurine was given to taurine group for 3 weeks and tap-water was given to control group. Renal nerve around left renal artery was stimulated by 2, 5, 10 and 15 Hz under the anesthesia of isoflurane. Renal blood flow was measured using laser tissue blood flow meter with noncontact probe for cortical blood flow and contact glass-fiber needle type of probe for medullary blood flow. After a series of electrical stimulation, 1 mg of LNAME was injected and same protocol was repeated. Renal blood flow distribution was similar to the previous study. Cortical and medullary blood flow in aged SHRSR was reduced by electrical stimulation. Chronic treatment of taurine suppressed the reduction of blood flow by electrical stimulation in renal medulla but not in renal cortex of aged SHRSR. Injection of LNAME showed no effects on renal blood flow distribution in aged SHRSR. In conclusion, taurine can improve the reduction of renal medullary blood flow in aged SHRSR but this phenomenon was not counteracted by the treatment of LNAME. Mechanism of the improvement of renal medullary blood flow by taurine is unclear but the suppressive effect of taurine on sympathetic nerve activity as shown in our previous data might be related to these mechanisms.
ACTIVATION OF THE CALDIAH HYPERTOPHY-RELATED CALCINEURIN BY CALPAIN IN THE SPONTANEOUSLY HYPERTENSIVE RATS

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Metabolic syndrome patients had accumulation of the visceral fat, and prone to high blood pressure, dyslipidemia or diabetes. Morbidity of these diseases rise with the aging, and besides many patients have been under therapy, there are much more people suspected of suffering those diseases. It has become serious economic and social problems, in not only developed countries and in the developing countries. One of the main causes of the heart diseases and the cerebro-vascular diseases are the high blood pressure. The mortality of the ischemic diseases followed to a cancer, and that prominent symptom arteriosclerosis lead to the cardiac hypertrophy, finally cause the heart failure, the end-stage of chronic cardiopathy. To establish the preventive method and the development of the cure, elucidation of the molecular mechanism of the onset of those diseases is required, and our objective of our study is to elucidate mechanisms of cardiac hypertrophy. It is known that many calcium dependent intracellular signaling factors are pertinent to the cardiomyocyte remodeling, shrinking imperfection, cell death and hypertrophy. Calcineurin (CN) is a Ca\(^{2+}\) -dependent serine/threonine kinase. It is a multifunctional regulator of the cardiac hypertrophy caused by the Ca\(^{2+}\)-calcineurin pathway, activation mechanisms of CN in kidney differed from that of other tissues, not only leading to their activation, but also causing intracellular pathological changes. These result show it is useful for study each molecular mechanisms as tissues individually to elucidate etiology of diseases.

TRANSLATIONAL RESEARCH BASED ON THE ANALYSES OF CELL-SPECIFIC (PRO) RENIN RECEPTOR KNOCKOUT MICE

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Prorenin generated from renin gene is an inactive precursor of renin and secretes into the blood freely. Ten percentages of prorenin generated in juxtaglomerular cells are processed to active renin in the secretory granules and thereafter secrete into the blood. Consequently, plasma concentrations of prorenin are nine times higher than those of renin. In 2002, (pro)renin receptor [(P)RR] is identified as one-transmembrane protein from human kidneys. The (P)RR binds prorenin and renin in tissues, not only leading to their activation, but also causing intracellular MAPK signaling. As a key player in the tissue renin-angiotensin system (RAS), (P)RR activation plays an important role in the development of end-organ damage in the RAS dependent diseases such as hypertension and diabetes. We developed the cell-specific (P)RR knockout mice to elucidate physiological roles of (P)RR in cardiomyocytes, glomerular epithelial cells, and vascular smooth muscle cells. (P)RR includes the domain, consisting of transmembrane part and C-terminal part, called ATPase domain because it has been shown to be associated with vacuolar H\(^{+}\)-ATPase (V-ATPase). The V-ATPase is a multi-subunit proton pump involved in diverse and fundamental cellular physiology, including receptor-mediated endocytosis and recycle, processing of proteins and signaling molecules, membrane sorting and trafficking, and activation of lysosomal/autophagosomal enzymes. Recent studies using the cell-specific (P)RR knockout mice showed that (P)RR is essential for the formation and activity of the V-ATPase. Furthermore, the novel function of the (P)RR as an adaptor protein between the Wnt receptor complex and the V-ATPase was reported. Based on these findings, the (P)RR is a multi-functional molecule that regulates the tissue RAS under pathological conditions and contributes to the development, growth, and maintenance of cells under physiological conditions. Based on the insights to the (P)RR as a modulator of the tissue RAS and V-ATPase, translational research for developing a new diagnostic marker and novel therapy has been ongoing.

THE METABOLIC CONTROL FOXO RELATED GENE ANALYSIS IN SPONTANEOUSLY HYPERTENSIVE RATS

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Metabolic syndrome and cancer are the multifactorial diseases. Environmental factors are involved in the development of disease in addition to genetic factors. Diabetes, hypertension, and lipid abnormalities are risk factors of the metabolic disorder. When the patient have supervene those diseases, pathologic condition will be more severe. There are networks of the diseases through the various factors related to the onset of them. Many factors associated with metabolic diseases have important roles in different diseases. It is conceivable that there are some key factors related to onset and supervision. There are differences of the metabolic and the intracellular signaling state between the healthy person and the patient. It is known that the inflammation, oxidative stress, and apoptosis are basis of many diseases. Post-translational modification of proteins, transcription factors, and enzymes such as phosphatase, phosphorylase, deacetylase and acetylase, etc., affect to the physiological and pathological states of the cell. Pathogenesis of multifactorial diseases that are affected by various factors in this manner has not been clarified. Forkhead box proteins (FOXO) are a family of transcription factors that play important roles in regulation of expression of genes involved in proliferation, differentiation, apoptosis and
longevity. Sirtuin (SIRT), NAD+-dependent deacetylase possesses important roles in regulation of many transcription factors and other proteins. Deacetylation of FOXO and histone by SIRT regulates transcriptional rates. And SIRT has been noted to be involved in various diseases in addition to cancer and aging. Both FOXO and SIRT functions are related metabolic state. It was known that regulation of each function of the FOXO and SIRT are related to the metabolic state and known there are many factors effect on them and affected by them. However, there are many unclear points relevant to the SIRT and FOXO. Purpose of this study is to clarify the roles of factors related FOXO and SIRT in a disease of metabolic syndrome such as the high blood pressure. Expression levels of genes of the hypertensive rats (SHR/kpo, SHRSP/kpo and M-SHRSP/kpo) and normotensive rats (WKY/kpo) were measured by RT-PCR methods. Although expression of FOXO1 gene of lung is correlated to the blood pressure levels of rats, the levels of liver are almost constant. As the expression patterns of FOXO family genes were resemble, the regulation mechanisms of FOXO family may be the common ways. Nuclear localization of FOXO was inhibited by phosphorylation by AKT. Expression patterns of AKT were resembled to that of FOXO. Effects on transcription activity of phosphorylation and deacetylation by AKT and SIRT of a FOXO molecule are still unclear. Further studies of FOXO related genes are needed to elucidate pathophysiological mechanism of disease.

**P38 MAP KINASE MEDIATES ALDOSTERONE-INDUCED PODOCYTE INJURY IN NARIURETIC PEPTIDE RECEPTOR (GC-A)-DEFICIENT MICE**

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Aldosterone plays an important role in the pathogenesis of renal injury. We have already reported that natriuretic peptide receptor/guanylyl cyclase-A (GC-A) signaling exerts renoprotective effects in subtotal nephrectomy, anti-GDM glomerulonephritis and streptozotocin-induced diabetes mouse models by eliciting natriuresis, reducing blood pressure, and inhibiting fibrosis. Furthermore, we demonstrated that uninephrectomized GC-A knockout (KO) mice with aldosterone and sodium overload exhibited accelerated hypertension with massive proteinuria. These mice exhibited increased phosphorylation of p38 MAPK kinase (MAPK) mainly in podocytes. To explore the interaction between p38 MAPK and GC-A signaling, we examined the effects of p38 MAPK inhibition on renal findings of GC-A-KO mice with aldosterone administration. GC-A KO mice or wild-type mice were uninephrectomized and then fed for 4 weeks with high salt diet and infused with aldosterone (0.2 μg/kg/min) subcutaneously using an osmotic minipump. They were administered with p38 MAPK inhibitor (FR167653, 33 mg/kg/day) or hydralazine in drinking water. We examined BP, urinary albumin excretion, renal histology, electron microscopy and immunohistochemical study for nephrin, podocin and p53 which is one of downsteam targets of p38 MAPK. Administration of FR167653, but not hydralazine, remarkably suppressed urinary albumin excretion by 90% in GC-A KO mice (p<0.01). Glomerular hypertrophy, mesangial expansion and interstitial fibrosis were decreased in FR167653-treated aldosterone-treated GC-A-KO mice. In electron microscopic analysis, FR167653 treatment normalized GBM thickening and foot process widening. Immunofluorescence study showed that this treatment kept high intensity of nephrin and podocin in glomeruli. Furthermore, administration of FR167653 reduced the number of glomerular TUNEL-positive cells and p53 staining. In vitro study, overexpression of MKK3 in cultured human podocytes upregulated the phosphorylation of p38 MAPK and p53 expression, an apoptosis-related gene. Such induction was significantly inhibited by the administration of ANP. These results suggest that renoprotective properties of the endogenous natriuretic peptide/GC-A system may result from the local inhibition of aldosterone/p38 MAPK pathway and p53 signaling in podocytes.

**GENOMICS OF HYPERTENSION IN DAILY MEDICAL PRACTICE**

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Four and a half years have passed since I started my life as a physician of general practice. In Osaka University, I spent most of my time for the research of hypertension genomics, and the main aim of research was “identification of causative genes for hypertension”. Even though we carried out genome-wide association study (GWAS) using several different panels including huge number of subjects, it seems to be impossible to make a certain diagnosis of essential hypertension using only genetic information. In contrast, Prof. Lifton and his colleagues in Yale University have succeeded in identifying the causative genes of monogenic blood pressure abnormality, such as Liddle syndrome, etc. They also revealed that specific types of hypertension are caused by rare variants. In addition, recent investigations revealed that the epigenetic modification was also important in the pathogenesis of hypertension. For general physicians, genomic information concerning antihypertensive drug responsiveness, salt sensitivity and susceptibility of cardiovascular complications should be useful. In HOMED-BP-GENE study, we revealed that the responsiveness for the treatment was different among antihypertensive drugs. We also revealed high salt sensitivity of Japanese from our genetic association studies using classical candidate gene approach. In addition, beta-adrenergic receptor gene (ADRB2) polymorphism was revealed to be involved in obese hypertension and altered the responsiveness of life style modification in a cohort study. ADRB2 was also involved in the pathogenesis of salt sensitive hypertension via epigenetic modification of WNK family. Our recent investigation examining of the effect of ANRIL (antisense non-coding RNA in the INK4a locus) on multi-disease susceptibility suggested the future direction of genomic research in polygenic diseases. In this symposium, I would like to review my previous investigations and discuss the advantages and disadvantages of using genetic information in general clinical practice.

**PRENATAL CORTISOL EXPOSURE AFFECTS THE BLOOD PRESSURE IN LATER LIFE**

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It is known that the prenatal stress is a risk factor for the metabolic disease and emotional disorders. This change is permanent and induced by epigenomic changes. The aim of this study is to investigate whether fetal stress would affect the blood pressure control in the central nervous system. We injected dexamethasone (200 mg/kg/d, Dex group) or saline (Control group) intraperitoneally to pregnant Sprague-Dawley rat (from F15 to F20). The body weight of Dex group (14.8±0.5 g) at the 7 post-natal day (P7) is lower than Control group (15.2±0.4 g, P<0.05), whereas it is much higher at the 12 weeks (Dex:552.5±39.9 g, S:393.8±9.4, p<0.001). To investigate whether the blood pressure is elevated in the Dex group, we measured blood pressure using telemetry system or direct measurement via carotid artery with normal, 8% salt (HS), and 0.1% salt (LS) diet loading at the 12 weeks. Blood pressure is elevated after salt loading in Dex group (Dex_HS 159.5±3.4 mm Hg, Dex_NS group:142.8±1.9 mm Hg, P<0.05). Next we evaluated urine catecholamine, which is depressed by salt loading, and catecholamine decrease is weaker in the Dex group than Control group. Importantly suppression of catecholamine decrease is one of the causes of salt sensitivity. To examine whether DNA methylation status is affected by dex, we evaluated the methylation enzyme (DNMT1, DNMT3a, 3b) in hypothalamus and mRNA expressions of the DNMT3a and 3b were downregulated in the Dex group. Further, we injected demethylating agent, 5-Aza-2’-deoxycytidine intracranially to male SD rats, measured the blood pressure loading HS and LS by telemetry system. Importantly DNA demethylation in the CNS induced salt sensitive hypertension. We hypothesized that renin-angiotensin system and ROS would be affected by methylation enzyme, because they are one of the key regulators of the blood pressure in the CNS. However, mRNA expressions of the AT1a, AT1b, NR3C1 and NR3C2 were not different among antihypertensive drugs. We also revealed high salt sensitivity of Japanese from our genetic association studies using classical candidate gene approach. In addition, beta-adrenergic receptor gene (ADRB2) polymorphism was revealed to be involved in obese hypertension and altered the responsiveness of life style modification in a cohort study. ADRB2 was also involved in the pathogenesis of salt sensitive hypertension via epigenetic modification of WNK family. Our recent investigation examining of the effect of ANRIL (antisense non-coding RNA in the INK4a locus) on multi-disease susceptibility suggested the future direction of genomic research in polygenic diseases. In this symposium, I would like to review my previous investigations and discuss the advantages and disadvantages of using genetic information in general clinical practice.
between Control and Dex group, nor NOX2 or NOX4, NADPH oxidase component, though the mRNA change by salt loading were different between groups. The DNA methylation state in the hypothalamus changed with the stress exposure in an embryo and a possibility that it had contributed to salt susceptibility high blood pressure was suggested.

EXAMINATION AS THE DIETARY NON-ALCOHOLIC FATTY LIVER DISEASE MODEL OF SHRSP5/Dmcr

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Non-alcoholic fatty liver disease includes a chronic progressive liver disorder from non-alcoholic fatty liver to non-alcoholic steatohepatitis with chronic inflammation and fibrosis of the liver. We developed SHRSP5/Dmcr for these models, which is a strain derived from stroke-prone spontaneously hypertensive rat (SHRSP), the so-called "Arteriolipidosis-prone rats (ALP)" established in Kyoto University and Kinjo Gakuen University. When the rats were fed the high-fat and high-cholesterol containing (HFC) diet, their liver became white in color. Therefore, we examined the effects of the HFC diet intake period and the dose on liver damage of SHRSP5/Dmcr. Ten-week-old male SHRSP5/Dmcr rats were fed control (SP) or HFC diet for 2, 8 and 14 weeks. In another experiment, male SHRSP5/Dmcr rats were divided into groups and fed four different diets, respectively: control diet (HFC: SP = 0.10%), low-HFC diet (HFC: SP = 10%), medium-HFC diet (HFC: SP = 50%) and high-HFC diet (HFC: SP = 100%) for 8 weeks. We examined the body and liver weight, blood pressure, several serum indices of liver function and hepatic histopathological changes. Body weights of rats fed HFC diet were lower compared with those of the control group. The blood pressure of HFC group was significantly lower than control at 14 weeks. The HFC diet time-dependently increased serum ALT, AST, γ-GTP levels. HFC diet increased microvesicular steatosis and inflammatory cell infiltrations at 2 weeks, Mallory-Denk body and bridging fibrosis in addition to macrovesicular steatosis, and inflammatory cell infiltrations at 8 weeks. At 14 weeks, honeycomb fibrosis was observed in HFC-fed rats. In another experiment, we found that the various liver damage indices mentioned above became more evident in a dose-dependent fashion on the HFC diet. Interestingly, no liver damage occurred in rats fed the control and low-HFC diet. Thus, HFC diet time- and dose-dependently induces lipid accumulation, inflammation and severe fibrosis in the liver of SHRSP5/Dmcr. Their histological and biochemical changes are very similar to those of non-alcoholic fatty liver disease in human. Therefore, SHRSP5/Dmcr is thought to be a progressive model of the non-alcoholic fatty liver disease without the obesity.

RENOVASCULAR EFFECT OF VASOPRESSIN V2 RECEPTOR ANTAGONIST TOLVAPTAN IN DAIL RATS WITH HEART FAILURE

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Tolvaptan is the highly selective and orally effective arginine vasopressin V2 receptor antagonists, and is potentially useful for treatment of heart failure (HF) patients. However, the renoprotective effect of long-term tolvaptan therapy and its underlying mechanisms remain unknown. We evaluated the effects of chronic treatment with tolvaptan on renal dysfunction, podocyte injury, inflammation, and oxidative stress, Rho-kinase, epithelial-mesenchymal transition (EMT), and extracellular signal-regulated protein kinase (ERK1/2) pathway in the renal cortex of Dahl salt-sensitive hypertensive (DS) rats with end-stage severe HF. DS and Dahl salt-resistant rats were fed a high-salt diet at 6 weeks of age. DS rats were treated with vehicle and tolvaptan (0.05 % concentration in diet) from the age of 11 to 18 weeks. Vehicle-treated DS rats developed proteinuria, renal dysfunction, glomerulosclerosis, and interstitial fibrosis, which were ameliorated by tolvaptan without changing blood pressure. Decreased expression of nephrin and podocin and increased desmin-positive area in failing rats were restored by tolvaptan. Upregulation of NAD(P)H oxidase p22phox, p47phox, and gp91phox, EMT marker such as α-SMA, TGF-β1, vimentin, and fibronectin expression, and Rho-kinase and ERK1/2 phosphorylation in DS rats was significantly suppressed by tolvaptan. Tolvaptan administration resulted in significant inhibition in TNF-α, MCP-1 and RANTES expression, and upregulation in klotho, sirt1 expression and CD34/Fklt-positive cells by FACS. We concluded that long-term DPO therapy may improve renal dysfunction, glomerulosclerosis, podocyte injury, and inflammation associated with oxidative stress, EMT, senescent gene and EPC in failing heart of DS rats. Thus, DPO may be a potential therapeutic strategy in CHF.

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Tolvaptan is the highly selective and orally effective arginine vasopressin V2 receptor antagonists, and is potentially useful for treatment of heart failure (HF) patients. However, the renoprotective effect of long-term tolvaptan therapy and its underlying mechanisms remain unknown. We evaluated the effects of chronic treatment with tolvaptan on renal dysfunction, podocyte injury, inflammation, and oxidative stress, Rho-kinase, epithelial-mesenchymal transition (EMT), and extracellular signal-regulated protein kinase (ERK1/2) pathway in the renal cortex of Dahl salt-sensitive hypertensive (DS) rats with end-stage severe HF. DS and Dahl salt-resistant rats were fed a high-salt diet at 6 weeks of age. DS rats were treated with vehicle and tolvaptan (0.05 % concentration in diet) from the age of 11 to 18 weeks. Vehicle-treated DS rats developed proteinuria, renal dysfunction, glomerulosclerosis, and interstitial fibrosis, which were ameliorated by tolvaptan without changing blood pressure. Decreased expression of nephrin and podocin and increased desmin-positive area in failing rats were restored by tolvaptan. Upregulation of NAD(P)H oxidase p22phox, p47phox, and gp91phox, EMT marker such as α-SMA, TGF-β1, vimentin, and fibronectin expression, and Rho-kinase and ERK1/2 phosphorylation in DS rats was significantly suppressed by tolvaptan. Tolvaptan administration resulted in significant inhibition in tumor necrosis factor-α and monocyte chemoattractant protein-1 expression, and nuclear factor-κB phosphorylation. We concluded that long-term tolvaptan therapy may improve renal dysfunction, glomerulosclerosis, podocyte injury, and inflammation associated with oxidative stress, EMT, ERK, and Rho-kinase pathway in failing heart of DS rats. Thus, tolvaptan may be a therapeutic strategy for end-stage severe HF.

RENOVASCULAR MECHANISMS OF TELMISARTAN ON RENAL INJURY AND INFLAMMATION IN SHRSP-Z.LEPF(RA)Z/DMCR RATS

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SHRSP-Z.Lepf/RaZ/Dmcr (SHRSP fatty) rats create a new animal model of metabolic syndrome. However, the renoprotective effect of telmisartan therapy and its underlying mechanisms in SHRSP fatty rats remain unknown. We evaluated the effects of long-term telmisartan
therapy on renal dysfunction, podocyte injury, inflammation, and transforming growth factor-β1 (TGF-β1)/Smad, epithelial-mesenchymal transition (EMT), mitogen-activated protein kinase (MAPK), Rho kinase, and cell-cycle progression pathway in the renal cortex of SHRSP fatty rats. Seven-week-old male SHRSP fatty rats were treated with vehicle, telmisartan, and hydralazine for 8 weeks. Age-matched male Wistar-Kyoto/Lezno rats served as a control group. Vehicle-treated SHRSP fatty rats developed proteinuria and renal dysfunction, which in the telmisartan group was less than the vehicle and hydralazine group without changing blood pressure. Glomerulosclerosis and interstitial fibrosis were impaired in SHRSP fatty rats, and these renal damages in the telmisartan group was less than the vehicle and hydralazine group. Decreased expression of nephrin and podocin and increased desmin-positive area in SHRSP fatty rats were restored by telmisartan but not hydralazine. TGF-β1/Smad, EMT marker, MAPK, Rho-kinase, and cell-cycle progression pathways were upregulated in SHRSP fatty rats, and these increased proteins in the telmisartan group were less than the vehicle and hydralazine group. Telmisartan administration resulted in significant suppression in tumor necrosis factor-α expression and nuclear factor-κB phosphorylation. Long-term telmisartan therapy may improve renal dysfunction, glomerulosclerosis, podocyte injury, and inflammation associated with EMT, TGF-β1/Smad, MAPK, Rho-kinase pathway in SHRSP fatty rats. Thus, telmisartan may have significant therapeutic potential for metabolic syndrome.

**RENAL DAMAGE AFTER MYOCARDIAL INFARCTION IS PREVENTED BY LINAGLITIN, A DIPEPTIDYL PEPTIDASE INHIBITOR, FOR CARDIO-RENAL PROTECTION**

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Myocardial infarction (MI) aggravates preexistent mild renal damage that is elicited by unilateral nephrectomy in rats. However, cardiac-renal protection after MI is unclear. We evaluate whether linagliptin, a dipeptidyl peptidase inhibitor, improves cardiovascular remodeling and renal damage after MI in unilateral nephrectomized (UNX) Wistar rats. MI was induced after UNX by ligation of the left coronary artery (UMI). Vehicle and linagliptin were administration in UMI rats for 12 weeks. Impaired glomerulosclerosis and interstitial fibrosis in UMI rats were ameliorated by linagliptin. Podocyte injury such as decreased expression of nephrin and podocin and increased desmin-positive area in the renal cortex of UMI rats were restored by linagliptin. Upregulation of tumor necrosis factor-alpha expression and nuclear factor-kappaB phosphorylation and CD68-positive area in the renal cortex were suppressed by linagliptin. Increased cell-cycle progression marker such as cyclin D1/DCK4 and cyclin E/CDK2 expression in the renal cortex was blocked by linagliptin. Decreased 5'-FS and increased fibrosis area in the LV was improved by linagliptin. UMI rats in the renal cortex and LV were characterized by increased NADPH oxidase p22phox, p47phox, gp91phox, and decreased eNOS phosphorylation, and linagliptin improved these indices. These findings suggest that cardio-renoprotective effect of linagliptin in cardiac-renal damage after MI may play an important role in UMI rats. Thus, linagliptin may be a therapeutic strategy in the kidney and heart after MI.

**RRX AGONIST HX630 MAY BE A NOVEL CANDIDATE FOR THE THERAPEUTICS AGAINST CUSHING’S DISEASE**

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Cushing’s disease is a disorder caused by excessive adrenocorticotropic hormone (ACTH) secretion from corticotroph adenomas in the anterior pituitary gland. So far, effective medications against Cushing’s disease have not been established yet. Since nuclear retinoid X receptor (RXR) has been shown to be expressed in human pituitary corticotroph adenomas, we focused on the effects of RXR agonists on proopiomelanocortin (POMC) gene expression, mRNA expression, ACTH secretion, cell proliferation, and cell apoptosis using mouse pituitary corticotroph AtT20 cells. We incubated AtT20 cells with various concentrations of RXR agonists HX630, PA024, and CD3254, and analyzed POMC promoter activity by luciferase assay, POMC mRNA expression by real-time PCR, ACTH secretion by ELISA, cell proliferation by Cell Counting Kit-8, and cell apoptosis by Caspase Assays. HX630 and PA024, but not CD3254, inhibited AtT20 cell proliferation and induced AtT20 cell apoptosis. On the other hand, HX630, but not PA024 and CD3254, inhibited POMC gene transcription, POMC mRNA expression, and ACTH secretion. RXR agonist HX630 may therefore be a novel candidate for the therapeutics against Cushing’s disease.

**THERAPEUTIC ANGIOGENIC POTENTIAL OF DEDIFFERENTIATED FAT CELLS TO TREAT ISCHEMIC CARDIOVASCULAR DISEASES**

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Mature adipocytes are the most abundant cell type in adipose tissue. Using an in vitro dedifferentiation strategy, referred to as ceiling culture method, we established differentiated fat (DFAT) cells from mature adipocytes. DFAT cells were capable of differentiating along multiple lineages and had significant expansion capability as similar as mesenchymal stem cells (MSCs). In the present study, we examined the potential impact of DFAT cells on angiogenesis and therapeutic angiogenesis. Mouse DFAT cells were cocultured with mouse vascular endothelial cells (GFP-MS1) for 7 days and immunostained for the pericyte markers α-smooth muscle actin (ASMA) and NG2. Nude mice were subcutaneously implanted with Matrigel plugs mixed with GFP-labeled DFAT cells for 2 weeks. Subsequently, gels were removed and processed for immunohistochemistry to detect GFP vascular cells. Human DFAT cells, adipose-derived stem cells (ASCs), and bone marrow MSCs were cultured with 0.5% FBS in DME for 3 days and conditioned media were taken and analyzed for cytokines using a cytokine array and ELISA. Twenty-four-hour combined immune deficiency (SCID) mice underwent right femoral artery dissection. Subsequently, DFAT cells (DFAT group, n = 8) or peripheral mononuclear cells (PB-MNC group, n = 8), or saline (Control group, n = 8) were injected into ischemic muscle tissue. Blood flow was measured every week by a laser doppler blood flow meter. At 4 week after transplantation, ischemic limbs were processed for immunohistochemistry and number of isletin B4 (IB4) capillaries and ASMA/IB4+ capillaries were counted. In coculture conditions with mouse DFAT cells and mouse vascular endothelial cells (MSC), DFAT cells frequently differentiated into α-smooth muscle actin (ASMA)- NG2+ pericyte-like cells. In Matrigel plug assay, transplanted DFAT cells differentiated ASMA+ vascular smooth muscle cells in nude mice. In cytokine analysis of conditioned media, DFAT cells secreted several angiogenic cytokines, such as VEGF and HGF. The expression profiles of DFAT cells were very similar to those of ASCs and bone marrow MSCs. In a mouse ischemic hindlimb model, Blood flow was significantly increased in DFAT group compared to that in PB-MNC group or Control group from 2 to 4 weeks after transplantation (p<0.05). Density of IB4+ capillaries and ASMA/IB4+ capillaries in the ischemic muscle tissue was significantly increased in DFAT group compared to that in PB-MNC group or Control group (p<0.05). In conclusion, these data demonstrated that DFAT cells have the ability to differentiate to vascular cells in vivo and in vitro, and secrete several angiogenic factors. In addition, transplantation of DFAT cells led to neovascularization and improve blood flow in mice with ischemic hind limbs. We propose that DFAT cells represent a promising candidate cell source for angiogenic cell therapy in patients with ischemic cardiovascular diseases.
DIABETES PATIENTS WITH A HYPERTENSION RESPONSE TO EXERCISE HAVE IMPAIRED LEFT VENTRICULAR DIASTOLIC FUNCTION

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Differences in cardiac contractility compared with non-lactating PAH mice. These results show that cardiac remodeling induced by hypertension during pregnancy is improved in the postpartum period except fibrosis, whereas lactation induces cardiac contractile dysfunction in mice with a history of pregnancy-associated hypertension.

CHANGES OF CHOLESTEROL AND BILE ACID KINETICS IN LIVER OF SHRSP5/Dmcr FED A HIGH FAT-CHOLESTEROL DIET

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High-fat cholesterol (HFC) diet induced fibrotic steatohepatitis in liver of Stroke-Prone Spontaneously Hypertensive 5/Dmcr rat (SHRSP5/Dmcr). In this study, we aimed to clarify the molecular mechanism by analyzing the cholesterol and bile acid (BA) kinetics in the liver. Ten-week-old male SHRSP5/Dmcr rats were divided into two groups. One was fed SP (control) diet, and another was fed HFC diet. After feeding these diets for 2, 8 and 14 weeks, respectively, molecular markers involved in cholesterol and BA kinetics were investigated by measuring the protein and mRNA levels. Unlike the control diet, the HFC diet deposited much cholesterol in the livers, where 3-hydroxy-3-methylglutaryl CoA reductase, low-density lipoprotein (LDL) receptor and LDL receptor-related protein-1 were expectedly downregulated, especially at 8 and 14 weeks, suggesting that cholesterol synthesis and uptake may be inhibited in response to liver cholesterol accumulation. HFC diet increased BA synthesis by upregulating cholesterol 7 alpha-hydroxylase at 8 and 14 weeks. Unexpectedly, HFC diet did not inhibit feedback of BA synthesis by farnesoid X receptor. However, HFC diet suppressed canaliculai BA excretion by decreasing the expression of bile salt export pump and multidrug resistance-associated protein-2 (MRP2) from 2 weeks because of the marked liver cholesterol accumulation. In contrast, HFC diet compensatorily increased basolateral BA excretion by increasing the expression of MRP3 at 8 and 14 weeks. The disturbed BA detoxification may correlate with suppressed pregnane X receptor and constitutive androstane receptor. The HFC diet increased the expressions of liver fibrosis makers such as transforming growth factor (TGF)-β1 (cytosol fraction) and $\alpha$-smooth muscle actin (liver homogenate) at 8 and 14 weeks corresponding to liver fibrotic changes. HFC diet promoted BA synthesis, but decreased those of CYP27A1, CYP8B1 and bile salt export pump.

INFLUENCE OF LACTATION ON POSTPARTUM CARDIAC FUNCTION IN PREGNANCY-ASSOCIATED HYPERTENSIVE MICE

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Pregnancy-induced hypertension (PIH) is a serious complication during pregnancy and recent epidemiological studies indicate the association between PIH and cardiac morbidity and mortality during the postpartum period. Although lactation is essential for the mammalian reproduction, the role of lactation in the heart with a prior of PIH remains unclear. In this study, we investigated postpartum changes in cardiac remodeling and function of pregnancy-associated hypertensive (PAH) mice with and without lactation. The systolic blood pressure was increased in PAH mice at day 19 of gestation (E19), and was reduced to normal levels in both lactating and non-lactating groups in the postpartum period. Histological analyses of heart revealed that hypertrophy and macrophage infiltration in PAH mice at E19 were improved in both lactating and non-lactating groups at 4 weeks postpartum (4W-PP), while marked fibrosis was remained. Increased mRNA expression of profibrotic genes and proinflammatory cytokines in PAH mice at E19 was significantly reduced in both lactating and non-lactating groups at 4W-PP. Echocardiographic analysis found no significant differences in fractional shortening between PAH mice and C57BL/6J mice at E19. On the other hand, at 4W-PP, non-lactating PAH mice showed normal fractional shortening, but lactating PAH mice exhibited significant decreases in cardiac contractility compared with non-lactating PAH mice. These results show that cardiac remodeling induced by hypertension during pregnancy is improved in the postpartum period except fibrosis, whereas lactation induces cardiac contractile dysfunction in mice with a history of pregnancy-associated hypertension.

BILE ACID ACCUMULATIONS IN THE LIVER OF RATS FED HIGH-FAT-CHOLESTEROL DIET AND THEIR RELATIONSHIPS WITH STEATOSIS, INFLAMMATION AND FIBROSIS INDICES

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High-fat and -cholesterol (HFC) diet increased expressions of cytokine P450 (CYP) 7A1 and CYP7B1 in the liver of stroke-prone spontaneously hypertensive 5/Dmcr (SHRSP5/Dmcr) rats, but decreased those of CYP27A1, CYP8B1 and bile salt export pump. These results suggested that HFC diet may dysregulate bile acids (BAs) in the liver. In this study, we measured 21 bile acids in the liver of rats fed HFC diet and investigated the relationships between each bile acid and macrovesicular score, serum alanine aminotransferase (ALT) level and
fibrotic area. Male SHRSps/Dmcr rats were fed with SP (control) or HFC diets for 2, 8, and 14 weeks, respectively. Hepatic BA levels were determined by ultra-performance liquid chromatography-tandem mass spectrometry, using a published method. In the HFC group, the predominant BAs were CA, followed by deoxycholic acid (DCA) and CDCA species at 2 weeks. Cholic acid (CA) and chenodeoxycholic acid (CDCA) remained major species thereafter, but CA and DCA species dramatically decreased at 8 and 14 weeks; CDCA tended to increase after 2 weeks. As a result, CA/CDCA was 4 ~ 6 in control group, and about 1 in HFC group at 8 and 14 weeks. Unlike control, ursodeoxycholic acid (UDCA) species was a minor BA in the HFC group. Lithocholic acid (LCA), dehydrocholic acid and ursodeoxycholic acid species were present in trace amounts in the liver of control and HFC groups. When glyco-BAs were compared with tauro-BAs, the HFC diet dramatically increased the former species but decreased the latter except for UDCA and LCA. CDCA species correlated positively, whereas total CA/total CDCA correlated negatively, with macrovesicular steatosis score, serum ALT and quantified fibrotic area. Total UDCA and tauro-CDCA also negatively correlated with these indices. Moreover, total glyco-BAs/total tauro- BAs positively correlated with each parameter of liver injury, whereas inverse associations were detected for total tauro-BAs. Hepatic BA accumulation may not predict liver disease. CDCA plays a pivotal role in the pathogenesis of fibrotic steatohepatitis.

COMBINING IRBESARTAN AND TRICHLOROMETHAZIDE ELICITS STRONG BLOOD PRESSURE REDUCTION VIA INHIBITING SYMPATHTIC ACTIVITY WITHOUT ADVERSE EFFECTS ON METABOLISM IN HYPERTENSIVE RATS WITH METABOLIC SYNDROME

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Sympathoexcitation plays a pivotal role in hypertension with metabolic syndrome. We have demonstrated that the oxidative stress in the rostral ventrolateral medulla (RVLM) increases sympathetic activity in hypertensive rats. The aim of the present study was to determine whether orally administered angiotensin II receptor blocker (irbesartan; IRB) and diuretics (trichlormethiazide; TCM) decreases blood pressure (BP) via inhibiting sympathetic activity through anti-oxidant effects in the RVLM of spontaneously hypertensive/CDoem-cr rats (SHR-cp). We also investigated the effects of IRB/TCM therapy on the metabolic profile. IRB (25 mg/kg/day), TCM (1 mg/kg/day), IRB/TCM (25/1 mg/kg/day) or vehicle; VEH were orally administered for 28 days to SHR-cp. IRB/TCM treatment decreased BP to a greater extent than IRB monotherapy. Both urinary norepinephrine excretion and oxidative stress evaluated by TBARS levels in the RVLM were decreased in both IRB and IRB/TCM groups compared with VEH group, but not in TCM group. IRB/TCM treatment did not exert an adverse effect on the metabolic profile, such as fasting blood glucose, total cholesterol, LDL- cholesterol, and HDL-cholesterol, but it decreased the triglyceride levels of SHR-cp compared with VEH group. These findings suggest that IRB/TCM profoundly decreases BP by inhibiting sympathetic activity via anti-oxidant effect in the RVLM in SHR-cp.

‘SALT MEMORY’ FOR DEVELOPMENT OF HYPERTENSION IN HYPERTENSIVE ANIMALS

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We and others have shown that treatment of spontaneously hypertensive rats (SHR) or Dahl salt-sensitive rats with an renin-angiotensin-aldosterone (RAA) system inhibitor at the developmental phase of hypertension causes a sustained reduction of blood pressure. But the efficacy of salt intake in hypertension development is still unclear. The aim of this study was to examine the effects of temporary exposure to a high-salt diet on blood pressure and markers of end-organ damage in hypertensive rat models. (Experiment1) Dahl salt-sensitive rats were divided into 4 groups, and fed during an early stage of development (from age 6 to 14 weeks) with low-salt (0.12 % NaCl), normal-salt (0.8 % NaCl), high-salt (7 % NaCl) or high-sodium/normal-chloride (12.7 % NaAA) diet. After these treatments, all groups were returned to a normal-salt diet. (Experiment 2) Male SHRs were divided into five groups, and fed from age 6 to 14 weeks with a low-salt, normal-salt, or high-salt diet. Other groups were given a 12.7 % NaAA or normal-sodium/high-chloride diet (11.6 % AACI). After these treatments, all groups were returned to a normal-salt diet. The effects on systolic blood pressure and urine protein excretion were examined regularly until age 24-28 weeks. (Experiment 1) Transient treatment with a high-salt diet caused an elevation in blood pressure not only during the treatment period, but also after returning to the normal-salt diet. 3 months after the treatment cessation, blood pressures were still elevated in the rats transiently exposed to a high-salt diet. Similarly, urine protein excretion was elevated in the high-salt rats at the end of the study. No such effect was seen in the NaAA or AACI groups. An increase in proteinuria and renal arteriolar hypertrophy was recognized in the high salt group, together with a marginal elevation in systematic and renal RAA system activity. In conclusion transient high dietary salt intake in the critical stage in the development of hypertension induces a sustained elevation of blood pressure, suggesting the presence of ‘salt memory’ in hypertensive animals. Both sodium and chloride ions were found to be essential for the development of this memory phenomenon. The mechanism of salt memory is suggested that high-salt diet intake in critical period of hypertension induces renal arteriolar remodeling through blood pressure rising, and elevates systematic and renal RAA activity.

VASCULAR PROTECTIVE EFFECTS OF ELASTIN PEPTIDE FROM BONITO BULBUS ARTERIOSUS IN SPONTANEOUSLY HYPERTENSIVE RATS

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It is well known that the hypertrophy of vascular smooth muscle cells and degeneration or degradation of elastic fiber in the arterial wall is responsible for the development of hypertension and vascular injuries. In this study, we examined the effects of elastin peptide from bonito bulbus arteriosus on vascular walls in Spontaneously hypertensive rats (SHR/Izm). Male SHR were divided into two groups at 15 weeks of age. Elastin peptide (3 g/kg, in Funabashi SP) from bonito bulbus was administered for 5 weeks. Plasma prolyl glycin (a degradation product of elastin peptide) levels were measured and endothelial changes were observed using scanning electron microscope (SEM). We further investigated the vascular function and expression of endothelial nitric oxide synthase (eNOS), intercellular adhesion molecule1 (ICAM-1) in endothelial cells and connective tissue related gene in the aorta by realtime PCR analysis. No significant differences were found in blood pressure, body weight, vascular function and connective tissue-related gene expression between the control and the elastin treated groups. Morphologically, the surface of the endothelium was smoother and endothelial changes such as blebs or crater-like structures were much less intense as compared to those in the control group. The expression of ICAM-1 was significantly lower in the elastin treated group than in the control, but not the expression of eNOS. These results indicate that PG (degradation product of elastin peptide) has protective effects against endothelial injuries through the inhibition of adhesion in endothelial cells and leukocytes. We plan to do more research to elucidate the mechanisms of such beneficial effects of elastin peptide, such as regarding elastin receptor, signal transduction and so on, using cultured endothelial cells and smooth muscle cells.
THE DIFFERENCE OF THE INFLUENCE OF AUTONOMIC NERVOUS ACTIVATION CAUSED BY REACTIVE HYPEREMIA ON TWO DIFFERENT ENDOTHELIAL FUNCTION TESTS

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While reactive hyperemia is used for the assessment of endothelial function in clinical settings, this maneuver is stressful and may affect autonomic nervous system activity. Two different methods to assess endothelial function are available (i.e., conduit arterial endothelial function: flow-mediated vasodilatation (FMD), micro-vascular endothelial function: reactive hyperemia index assessed by both (left/right) finger-tips arterial tonometry (RHIendPAT)), but influence of the changes of autonomic nervous activity related with reactive hyperemia on these two methods has not been clarified. The present study was conducted to examine the influence of the changes of autonomic nerve activity caused by reactive hyperemia on micro-vascular and conduit arterial endothelial function. In 108 hypertensive patients, FMD and RHIendPAT were measured simultaneously. The analysis of heart rate variability (HRV) (high frequency domain (HF), low frequency domain (LF) and their ratio (LF/HF)) were performed throughout this reactive hyperemia in these patients. By reactive hyperemia, LF was increased in 60 patients (LFinc group) and was not increased in 48 patients (LFdec group). RHIendPAT was lower in the former group (LFinc group) (2.2±0.5) than that in the latter group (LFdec group) (2.5±0.7) (p<0.05). However, no difference was found in FMD between these groups (3.4±2.0 % vs 3.9±2.5 %, p=0.28). Multivariate linear regression analysis demonstrated that the change of LF by reactive hyperemia was a significant determinant for RHIendPAT (β= -0.23, p<0.05), but not for FMD(β= -0.10, p=0.33), independent of conventional risk factors for cardiovascular disease. The influence of the changes of autonomic nerve activity by reactive hyperemia might be significant on RHIendPAT, but not on FMD, even with the adjustment of simultaneous both sides (left and right fingers) measurements of finger-tip tonometry.