ANGIOTENSIN II STIMULATES ENDOTHELIAL CELL MIGRATION VIA SIRT2-MEDIATED DEACETYLATION OF α-TUBULIN

Aiko Hashimoto Komatsu, Tetsuaki Hirase, Machiko Asaka and Koichi Node

Department of Cardiovascular and Renal Medicine, Saga University Faculty of Medicine, Japan

Angiotensin II regulates blood pressure as well as the pathophysiology of vascular injury. Microtubule composed of tubulins controls cell shape and migration of endothelial cells that are implicated in vascular remodeling. Acetylation of tubulin plays an important role in regulating microtubule properties, such as stability and structure, as well as microtubule-based cellular functions.

We studied angiotensin II-induced changes in morphology and acetylation of α-tubulin by immunofluorescence confocal microscopy in human endothelial EA hy.926 cells. In quiescent cells, α-tubulin-positive microtubules were acetylated and showed radial layout from microtubule-organizing center to peripheral edge of cells. Angiotensin II induced obscure microtubule-organizing center and disassembly of α-tubulin-positive microtubules that were deacetylated. Angiotensin II-induced deacetylation of α-tubulin was blocked by pretreatment with losartan that is an angiotensin II type 1 receptor blocker, sirtinol that is an inhibitor for sirtuin class deacetylases, and SIRT2 depletion by RNA interference. We investigated the involvement of SIRT2 in angiotensin II-induced cell migration using Boyden two chamber method. Angiotensin II induced significant increase in cell migration, which was blocked by pretreatment with sirtinol and SIRT2 depletion by RNA interference.

These data suggest that angiotensin II type 1 receptor-SIRT2-mediated deacetylation of α-tubulin is implicated in migration of endothelial cells.
ALDOSTERONE RAPIDLY ENHANCES ENDOTHELium-DEPENDENT RELAXATION OF RAT SMALL MESENTERIC ARTERIES VIA POTENTIATION OF BOTH THE NO AND EDHF COMPONENTS

Cristina Oprisa, Ionela Lacramioara Serban, Ostin Costel Mungiu, Dumitru D Branisteau and Dragomir N Serban

Cell Physiology & Pharmacology Laboratory, Functional Sciences Department, “Grigore T. Popa” University of Medicine and Pharmacy, Roumania

Despite variable vascular effects, the rapid nongenomic action of aldosterone generally attenuates the effect of vasoconstrictor agents, due to nitric-oxide-synthase activation (via mineralocorticoid receptors and the HSP90/PI3K/PKB pathway), but also potentiates vasoconstriction in absence of endothelium. We investigated for the first time the effect of aldosterone (1 nM to 1 microM) upon the EDHF response, using isometric myography of rings (1 mm wide) from mesenteric artery and its first order branches, obtained from male Wistar rats (200-250 g). After 1 h equilibration under a tension of 1 g the vessels were checked for the absence of myogenic response to stretch and for complete EDR induced by carbachol (0.01 mM) when precontracted by 0.01 mM phenylephrine. We tested the EDR induced by carbachol (100 nM to 0.1 mM) in phenylephrine-precontracted rings, as global EDR and its EDHF component (in presence of 0.1 mM L-NAME and 0.01 mM indomethacin). Results were expressed as residual active tension (% of precontraction level; mean ± SEM; n=6). We observed that aldosterone induces contraction in the presence of L-NAME. It NOS-dependently inhibits the contractions induced by phenylephrine (10 nM to 10 microM) or high extracellular K (30 to 90 mM), but far from the pronounced effects in renal arterioles which result from NOS activation by aldosterone. Moreover, aldosterone potentiates both the global EDR and its EDHF component, but these effects are also relatively weak and they are visible only for concentrations above 10 nM aldosterone (which is beyond the physiological range). Supported by Romanian Grant CNCSIS-A1222/2007-2008.
IMPAIRMENT OF ENDOTHELIAL FUNCTION IN ATHEROSCLEROTIC MICE LACKING VASCULAR ENDOTHELIAL ENDOTHELIN-1

Dyah Wulan Anggrahini\textsuperscript{a}, Noriaki Emoto\textsuperscript{a}, Kazuhiko Nakayama\textsuperscript{a}, Bambang Widyantoro\textsuperscript{a}, Kazuya Miyagawa\textsuperscript{a}, Vita Yanti Anggraeni\textsuperscript{a}, Hirowati Ali\textsuperscript{a}, Yaz Y Kisanuki\textsuperscript{b}, Masashi Yanagisawa\textsuperscript{b} and Kenichi Hirata\textsuperscript{a}

\textsuperscript{a}Division of Cardiovascular Medicine, Kobe University Graduate School of Medicine, Japan
\textsuperscript{b}Howard Hughes Medical Institute, University of Texas Southwestern Medical Centre, USA

The interaction between endothelin-1 (ET-1) and nitric oxide is an important mechanism in maintaining endothelial function. Previous studies revealed the upregulation of ET-1 marks endothelial dysfunction and involves in the development of atherosclerosis. However, we demonstrated here the beneficial role of ET-1 in vascular protection in atherosclerosis condition.

In this study, we crossbred ApoEKO mice with Vascular Endothelial-cell Endothelin-1 Knockout (VEETKO) mice. These mice showed significantly lower ET-1 expression as compared to ApoE/WT mice. No differences of blood pressure, plasma cholesterol or lipid profiles were observed in both mice. Surprisingly, chronic inhibition of ET-1 in endothelial cells resulted in an impaired endothelium-dependent relaxation in association with lower eNOS mRNA level in DKO mice despite similar level of ETB receptor expression.

To observe the consequences of reduced endothelial function, mice were given western diet for 8 weeks. Reduction in vascular protection increased inflammation, macrophage recruitment, and lipid retention. Consequently, the DKO mice exhibited higher atherosclerotic lesion in aortic sinus, descendent aorta and brachiocephalic artery.

Taken together, we demonstrated the impaired endothelial function followed by exaggeration of atherosclerosis in our model. This further suggests that ET-1 produced from vascular endothelial cells is required for protective mechanism in vascular wall in balance with nitric oxide production. Our data imply for the careful monitoring in the use of ET receptor antagonist in clinical setting.
ANGIOTENSIN II ATTENUATES VASODILATATION OF ANP IN HUMAN: ROLES OF CGMP BIOAVAILABILITY AND AT1 RECEPTOR

Shinichiro Ueda and Yoko Azekoshi

Department of Clinical Pharmacology & Therapeutics, University of the Ryukyus, Japan

Purpose: Vascular and renal effects of endogenous and exogenous natriuretic peptides are attenuated in patients with heart failure, which might be caused by an activated renin-angiotensin system. We investigated the effect of angiotensin II on vasodilation induced by atrial natriuretic peptide (ANP) and associated cyclic guanosine monophosphate (cGMP) spillover in humans.

Methods: We measured forearm blood flow by venous occlusion strain gauge plethysmography during intra-arterial ANP infusion (1.62-16.2 pmol/min) with or without co-infused angiotensin II (1 or 5 pmol/min) or noradrenalin (50 or 250 pmol/min). This experiment was repeated after a single dose of candesartan, valsartan, nifedipine or sildenafil. Forearm cGMP spillover was also estimated during ANP infusion.

Results: Angiotensin II, but not noradrenalin significantly and dose-dependently attenuated ANP-induced vasodilation and cGMP spillover. Both candesartan and valsartan, but not nifedipine abolished these inhibitory effects of angiotensin. Sildenafil also restored reduced cGMP spillover and attenuated response to ANP by ANG II.

Conclusion: Angiotensin II at a concentration similar to that in patients with heart failure attenuated the vasodilating effect of ANP presumably resulting from decreased cGMP bioavailability by enhancement of PDE5 activity, which was mediated by AT1 receptors. This interaction may be implicated in ANP resistance in patients with heart failure.
HIGH SODIUM AUGMENTS ANGIOTENSIN II-INDUCED PROLIFERATION OF RAT VASCULAR SMOOTH MUSCLE CELL THROUGH ERK1/2-DEPENDENT PATHWAY

Hirofumi Hitomi\textsuperscript{a}, Gang Liu\textsuperscript{a}, Naohisa Hosomi\textsuperscript{b}, Hideyasu Kiyomoto\textsuperscript{b}, Daisuke Nakano\textsuperscript{a}, Shoji Kimura\textsuperscript{a}, Masakazu Kohno\textsuperscript{b} and Akira Nishiyama\textsuperscript{a}

\textsuperscript{a}Department of Pharmacology, Kagawa University, Japan
\textsuperscript{b}Department of Cardiorenal and Cerebrovascular Medicine, Kagawa University, Japan

Previous studies demonstrate that angiotensin II (Ang II)-induced vascular injury is exaggerated by a high salt diet. The aim of this study was to examine the effects of high sodium on Ang II-induced cell proliferation in cultured rat vascular smooth muscle cells (VSMCs). VSMCs were cultured in a standard medium containing 137.5±1 mM sodium. High sodium medium were made by simply adding sodium chloride. Cell proliferation was determined by \[^3\text{H}\] thymidine incorporation. Extracellular signal-regulated kinases (ERK1/2) phosphorylation was determined by Western blot with an anti-phospho-specific antibody. Ang II (100 nM) significantly increased ERK1/2 phosphorylation and cell proliferation in the both medium containing standard sodium, and high sodium. However, high sodium augmented Ang II -induced cell proliferation and ERK1/2 phosphorylation. Pretreatment with 1 \(\mu\)M candesartan (AT\(_1\) receptor antagonist), 10 \(\mu\)M PD98095 (MEK inhibitor) abolished the proliferative effect and ERK1/2 activation induced by high sodium/Ang II. On the other hand, pretreatment with 30 \(\mu\)M 5-N,N hexamethylene amiloride (Na\(^+\)/H\(^+\) exchanger type 1 inhibitor), but not 10 \(\mu\)M SN-6 (Na\(^+\)/Ca\(^{2+}\) exchanger inhibitor) or 1 mM ouabain (Na\(^+\)/K\(^+\)-ATPase inhibitor) greatly attenuated ERK1/2 activation and cell proliferation by high sodium/Ang II. If osmotically equivalent amount of mannitol or choline chloride were substituted for sodium chloride, there were no effects on Ang II-induced ERK1/2 activation and cell proliferation. This study indicates that high sodium directly augments Ang II-induced VSMCs proliferation through ERK1/2-dependent pathway via AT\(_1\) receptor and Na\(^+\)/H\(^+\) exchanger type 1.
P3-7
EFFECTS OF ANGIOTENSIN II TYPE 2 RECEPTORS ON CALCITONIN GENE-RELATED PEPTIDE NEURITES OUTGROWTH IN APOLIPROPOTEIN-E-DEFICIENT MICE

Narumi Hobara\textsuperscript{a}, Naoya Hashikawa\textsuperscript{a}, Chikao Yutani\textsuperscript{a} and Hiromu Kawasaki\textsuperscript{b}

\textsuperscript{a}Department of Science, Okayama University of Science, Japan
\textsuperscript{b}Department of Clinical Pharmaceutical Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan

The apoE-deficient (ApoE/\textendash/-) mice spontaneously develop hypercholesterolemia, atherosclerotic lesions in large arteries and exhibits hypertension. The decrease of calcitonin gene-related peptide (CGRP), a potent vasodilator neuropeptide, containing perivascular nerves may result in hemodynamic changes such as hypertension. The density of CGRP-like immunoreactivity (LI) nerve fibers of mesenteric arteries in ApoE/\textendash/- mice were markedly decreased by approximately 50% compared age-matched wild type (WT) mice. To assess the interaction with angiotensin II type 2 receptors (AT2R) and CGRPergic, neurites outgrowth of CGRPergic were examined by activating AT2R in primary cultures of dorsal root ganglia (DRG) neurons. Treatment with CGP42112, which is AT2R agonist at 10 nM, caused significant increase CGRP-LI neurite outgrowth in both ApoE/\textendash/- and WT mice on DRG cells. However, the effect of CGP42112 did not inhibit by AT2R antagonist, PD123,319 in ApoE/\textendash/- mice. Next, we asked whether the ApoE deficient could influence the expression of AT2R in DRG. To determine the expression of AT2R, mRNA and protein were measured by using real-time quantitative RT-PCR and Western blotting, respectively. The AT2R mRNA and protein expression detected in ApoE/\textendash/- mice DRG were much lower than WT mice. In conclusion, CGRPergic disordering happened in ApoE/\textendash/- mice and reduction of AT2R expression might be involved in CGRPergic remodeling.
INHIBITORY EFFECT OF VASCULAR ENDOTHELIUM ON AGONIST-INDUCED VASOCONSTRICTION IN RAT MESENTERIC RESISTANCE ARTERIES DISAPPEARS WITH AGEING

Xin Jin, Yukiko Satoh-Otonashi, Yoshito Zamami, Toshihiro Koyama, Peng Yuan Sun, Narumi Hobara, Yoshihisa Kitamura and Hiromu Kawasaki

Department of Clinical Pharmaceutical Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan

Department of Life Science, Okayama University of Science, Japan

Department of Pharmaceutical Care and Health Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan

Vascular endothelium regulates vascular tone by releasing endothelium-derived vasoactive substances such as nitric oxide (NO), prostaglandin I2 (PG I2) and endothelium-derived hyperpolarizing factor (EDHF). This study investigated characterization of inhibitory effect of the endothelium on vasoconstrictor stimuli and the age-related changes in endothelial regulation of vasoconstriction in rat mesenteric vascular beds. Changes in perfusion pressure induced by continuous perfusion of Krebs solution containing methoxamine (α1-adrenoceptor agonist) or high KCl were measured over 180 min. In preparations with intact endothelium from 8 week-old rats, methoxamine-induced vasoconstriction was time-dependently decreased to cause 60-80% reduction of initial vasoconstriction level, while no reduction was observed in high KCl-induced vasoconstriction. Endothelium removal significantly blunted the time-dependent reduction of methoxamine-induced vasoconstriction without affecting high KCl-induced vasoconstriction. Neither NO synthase inhibitor (L-NAME) nor indomethacin (cyclooxygenase inhibitor) altered the time-dependent reduction of vasoconstriction. High KCl, K+-channel inhibitors tetraethylammonium and apamin plus charybdotoxin, and 18α-glycyrrhetinic acid (gap junction inhibitor), significantly inhibited the time-dependent reduction of methoxamine-induced vasoconstriction. In contrast, in preparations with intact endothelium from 16 week-old rats, the time-dependent reduction of methoxamine-induced vasoconstriction was not observed. Removal of the endothelium in 16 week-old rat's preparation significantly attenuated methoxamine-induced vasoconstriction, but it did not cause the time-dependent reduction. These findings suggest that EDHF in mesenteric resistance arteries is mainly responsible for counteracting long-lasting vasoconstriction induced by methoxamine, and the inhibitory effect of vascular endothelium on agonist-induced vasoconstriction markedly decreases with ageing.
Arginine-vasopressin (AVP) acts on three distinct receptors, V1a, V1b, and V2, and is important for both osmotic and cardiovascular homeostasis. Although it is well known that a potent vasoconstrictor effect of intravenously administered AVP is mediated by V1a, the physiological contribution of V1a and V1b to basal blood pressure (BP) is ill-defined. We investigated the functional roles of the V1a and V1b in cardiovascular homeostasis using gene targeting. The basal BP of conscious mutant mice lacking the V1a receptor gene (V1a-/-) was significantly lower compared to the wild-type mice without a notable change in heart rate. AVP-induced vasopressor responses were abolished in the V1a-/- mice; rather, AVP caused a decrease in BP, which occurred in part, through V2 receptor-mediated release of nitric oxide from the vascular endothelium. In V1a-/-, arterial baroreceptor reflexes were markedly impaired and a significant 9% reduction in circulating blood volume was noted. In contrast, V1b knockout mice (V1b-/-) showed the elevated systolic BP at rest. Pressor responses to intravenous administration of AVP or phenylephrine were preserved in V1b-/-.

Cardiac contractile functions assessed by echocardiography were enhanced and heart weight/body weight ratio was increased in V1b-/-. Taken together, these results indicate that both V1a and V1b receptors are critically involved in the maintenance of resting arterial BP within the physiological range.
ROLE OF EXTRACELLULAR SIGNAL-RELATED KINASES IN THE PATHOPHYSIOLOGY OF VASCULAR DYSFUNCTION IN AGING AND HYPERTENSION

Susan WS Leung, Eva YW Ho, Godfrey SK Man, George PH Leung and Ricky YK Man
Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong, China

Recent evidence suggests that extracellular signal-regulated kinase-1/2 (ERK 1/2) affect vascular tone. The present study examined the role of this enzyme in vascular control under physiological and pathological conditions. Using tissue bath technique, vascular responses were measured in terms of changes in isometric tension in aortic rings that were isolated from normotensive Wistar-Kyoto (WKY) rats and hypertensive spontaneously hypertensive rats (SHR) at different ages, 36-39 week old (adult group) and 72-75 week old (aged group). Inhibition of ERK 1/2 by U0126 (10 µM) reduced phenylephrine-induced contraction in aortae. The inhibitory effect of U0126 on contraction was smaller in aged WKY rats compared to the other groups. While U0126 did not affect relaxation to acetylcholine (1 µM) in adult WKY rats, it enhanced acetylcholine-induced relaxation in aged WKY rats and in adult and aged SHR. The enhanced relaxation was abolished by L-NAME (a nitric oxide synthase inhibitor, 300 µM) in WKY and SHR of all age groups, and reduced by indomethacin (a cyclooxygenase inhibitor, 10 µM) in aged WKY rats. Therefore, our results indicate that ERK 1/2 contributes to the contraction induced by phenylephrine to a similar degree in adult rats. On the other hand, ERK 1/2 is activated in aged and hypertensive rats, leading to the suppression of nitric oxide-mediated relaxation. As such, ERK 1/2 appears to contribute to vascular dysfunction in aging and hypertension.

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A NEW VIEW OF ATHEROSCLEROSIS RELATED PHARMACOGENETICS BASED GENE ANALYSIS IN JAPANESE ELDERLY WOMEN

Koichiro Ina\textsuperscript{a}, Toshio Hayashi\textsuperscript{a}, Jun Funami\textsuperscript{a}, Asako Watanabe-Ishizuka\textsuperscript{a} and Akihisa Iguchi\textsuperscript{b}

\textsuperscript{a}Department of Geriatrics, Nagoya University Graduate School of Medicine, Japan
\textsuperscript{b}Department of Health Science, Aichi Syukutoku University, Japan

\textbf{Background:} To evaluate genetic mutation factor(s) in relation to thrombosis or atherosclerotic risk in Japanese elderly women.

\textbf{Methods:} This is observational study which 104 Japanese postmenopausal women with a mean age of 80.9 years recruited from outpatient clinics of Nagoya University Hospital and related hospitals attended. A total of 10 single nucleotide polymorphisms (SNPs), where each gene acts in blood coagulation (factor V Leiden, prothrombin G20210A, factor XIII Val34Leu, factor VII Arg353Gln, MTHFR C677T, \(\beta\)-fibrinogen G-455A, PAI-1 4G/5G), metabolic syndrome related pathways (PPAR\(\alpha\) Leu162Val), or endothelium/estrogen system (eNOS Glu298Asp, ER\(\alpha\) IVS1-401), were analyzed in relation to clinical values related on lipid, B-type natriuretic peptide (BNP), fasting plasma glucose, tumor necrosis factor-\(\alpha\), interleukin-6, cyclic GMP, and nitric oxide metabolites.

\textbf{Results:} Comparison between the distributions of different genotypes and clinical values showed three relations. First, factor VII Arg353Gln and HDL-cholesterol (HDL-C) were linked to Arg/Arg carriers on higher levels (P=.049). The HDL-C to LDL-cholesterol (LDL-C) ratio supported this link (P=.027). Second, eNOS Glu298Asp and triglycerides were linked to Glu/Glu carriers on higher levels (P=.031). A chi-square test revealed that different genotypes were independent factors (P=.021). Last, ER\(\alpha\) IVS1-401 and BNP were related to the CC genotype on lower levels (P=.031). Here also, different genotypes were independent factors (P=.031).

\textbf{Conclusion:} Relations of factor VII Arg353Gln to HDL-C and eNOS Glu298Asp to triglycerides were newly shown. We have demonstrated that polymorphism in the endothelium/estrogen system and the heart failure marker BNP are correlated, with ER\(\alpha\) IVS1-401 being the first. SNP is helpful for understanding pathophysiology of atherosclerotic diseases in elderly women.
Background: Progranulin (PGRN) is a unique growth factor that plays an important role in cutaneous wound healing. It has an anti-inflammatory effect and promotes cell proliferation. However, when it is degraded to granulin peptides (GRNs) by neutrophil proteases, a pro-inflammatory reaction occurs. Since injury, inflammation and repair are common features in the progression of atherosclerosis, it is conceivable that PGRN plays a role in atherogenesis.

Results: Immunohistochemical analysis of human carotid endoatherectomy specimens indicated that vascular smooth muscle cells (vSMCs) in the intima expressed PGRN. Some macrophages in the plaque also expressed PGRN. We assessed the effect of PGRN on a human monocytic leukemia cell line (THP-1) and human aortic smooth muscle cells (HASMCs). PGRN alone had no effect on HASMC or THP-1 proliferation or migration. However, when THP-1 cells were stimulated with MCP-1, the number of migrated cells decreased in a PGRN-dose-dependent manner. TNF-α-induced HASMC migration was enhanced only at 10nM of PGRN. IL-8 secretion from HASMCs was reduced by forced expression of PGRN and increased by RNAi-mediated knockdown of PGRN. While exogenous treatment with recombinant PGRN decreased IL-8 secretion, degraded recombinant GRNs increased IL-8 secretion from HASMCs.

Conclusions: The expression of PGRN mainly reduces inflammation and its degradation into GRNs enhances inflammation in atherosclerotic plaque and may contribute to the progression of atherosclerosis.
BASIC AND CLINICAL CARDIOVASCULAR ACTIONS INDUCED BY GINKGO
BILOBA EXTRACT AND ITS CONSTITUENTS IN RAT AORTA

Seiichiro Nishida and Hiroyasu Satoh

Department of Pharmacology, Nara Medical University, Japan

Ginkgo biloba extract (GBE) has been known the usefulness for many clinical applications; cerebral insufficiency, Alzheimer’s disease and peripheral vascular diseases. GBE is composed of terpenoids (containing bilobalide, ginkgolide A, B and C) and flavonoids (containing quercetin and rutin). The cardiovascular actions of these compounds were examined using the cardiomyocytes and the aorta ring strips of guinea pig and rat. Furthermore, clinical effective actions for the treatment with GBE were also examined. In cardiomyocytes, GBE and its constituents markedly modulated cardiac ionic channels. The automaticity of sino-atrial nodal cells decreased, and the inhibitions of Na⁺, Ca²⁺ and K⁺ channels and the hyperpolarized-activated inward current. In ventricular cardiomyocytes, GBE inhibited the Ca²⁺ and K⁺ channels, whereas bilobalide enhanced them. Also GBE, bilobalide and quercetin exerted the potent vasodilating action due to endothelium-dependent (NO) action, and due to inhibitions of Ca²⁺ channel, and PK-Cδ, and activation SKCa channel. Other containing compounds also caused the almost similar effects. Furthermore, the bilobalide-induced vasodilating actions greatly decreased in according with aging. But multiple compounds (as GBE or terpenoids) did not produce the age-dependent actions at all. In clinical studies, the oral applications of GBE exhibited well effective for peripheral vascular diseases. These results indicate that GBE and its constituents cause the potent actions on cardiovascular tissues, presumably leading to the clinical effectiveness. GBE exerts the potent vasodilating action due to both endothelium-dependent and -independent mechanisms. The age-independency of vasodilating action induced by multiple compounds would be responsible for the complicated interactions of the constituents.
EFFECTS OF ANTIVIRAL NUCLEOSIDE ANALOGS ON RELAXATION OF RAT BASILAR ARTERIES

Rachel Wai Sum Li and GPH Leung
Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong, China

A large prospective cohort study has found that abacavir, a nucleoside analog used in the treatment of HIV infection, can increase the risk of cardiovascular events such as myocardial infarction and stroke (Post and Campbell) but the underlying mechanism is not known. We hypothesized that abacavir may impair the endothelium-dependent vasorelaxation. Therefore, we sought to study the effect of abacavir on vascular contractility. To examine if other antiviral nucleoside analogs exhibit similar effect, 2',3'-dideoxyadenosine (2',3'-ddA) and zidovudine (AZT) were also studied.

Wire myograph was used to study the contractility of rat basilar arteries. Our results showed that pre-incubation of rat basilar arteries with abacavir, 2',3'-ddA and AZT did not affect the endothelium-dependent relaxation caused by acetylcholine. Interestingly, abacavir and 2',3'-ddA could relax basilar arteries directly in a dose-dependent manner (but AZT did not). Removal of endothelium attenuated the vasorelaxing response to abacavir but it has no effect on 2',3'-ddA. Immunoassays revealed that 2',3'-ddA increased the cGMP level in rat basilar arteries.

In conclusion, the present findings are contrary to our expectation because abacavir, 2',3'-ddA and AZT did not impair endothelium-dependent vasorelaxation. Surprisingly, abacavir and 2',3'-ddA exert direct vasorelaxing effect. The mechanisms of vasorelaxation elicited by them are different. The vasorelaxing effect of abacavir relies on the presence of endothelium but 2',3'-ddA can relax vascular smooth muscle directly, probably through the increase in cGMP level.

Reference:
P5-1
CORRELATION OF THE PATENCY OF MYOENDOTHELIAL CONNECTIONS WITH ENDOTHELIAL VASODILATOR RESPONSES IN SPONTANEOUSLY HYPERTENSIVE RATS

John Christie McGrath, Claire Hamill, Craig J Daly and Laura Methven
Integrative and Systems Biology, University of Glasgow, UK

Cellular projections traversing the internal elastic lamina via fenestrations are the only routes for contact between endothelial and smooth muscle cells, bringing their cell membranes into close apposition at myoendothelial gap junctions proposed as involved in the EDHF response. The current study demonstrates the patency of myoendothelial connections passing through fenestrae in the IEL of pressurised arteries, by observing the passage of the fluorescent marker calcein AM between cells and correlates this with functional vasodilator responses to acetylcholine, separated pharmacologically into NO and EDHF components. This aimed to elucidate differences between normotensive rats and the endothelial-compromised SHR strain.

**Methods/Results:** CLSM and pressure myography were combined to study the transfer of luminal loaded calcein AM from endothelium to smooth muscle in live third order mesenteric resistance arteries. The gap junction inhibitors 37,43Gap27 and 40Gap27 prevented the transfer of calcein from endothelial cells to smooth muscle cells in normotensives, but in SHR dye transfer was minimal even without blockers. This was correlated with the inability to demonstrate endothelial responses in SHR by wire myography.

**Conclusion:** Fluorescent dye transfer with gap junction inhibitors has the advantage of indicated the patency of intercellular connections in live pressurized vessels, hence facilitating analysis of their pharmacological manipulation and, through correlation with functional responses, allowing a new explanation for the loss of the EDHF response, in terms of loss of the ability to transfer small molecules via the myoendothelial route.

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COMBINATION THERAPY WITH ANGIOTENSIN RECEPTOR BLOCKER AND CALCIUM CHANNEL BLOCKER IMPROVES EDHF-MEDIATED RESPONSES IN DIABETIC APOLIPOPROTEIN E-DEFICIENT MICE

Maki Hosoya, Aya Takaki, Ayuko Sawada, Junko Ohashi and Hiroaki Shimokawa
Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan

Background: We have previously demonstrated that hypercholesterolemia and diabetes mellitus, when combined, markedly impair endothelium-derived hyperpolarizing factor (EDHF)-mediated relaxations. In this study, we examined whether the combination therapy with an angiotensin receptor blocker (ARB) and a calcium channel blocker (CCB) improves endothelial dysfunction in diabetic ApoE-/- mice.

Methods and Results: We used male C57BL/6N mice (control) and diabetic ApoE-/- mice that were made diabetic by intraperitoneal injection of streptozotocin (STZ) at the age of 8 weeks. Two weeks after STZ injection, animals received oral administration of vehicle (DM-ApoE/-), olmesartan (OLM, 30 mg/kg/day), azelnidipine (AZL, 10 mg/kg/day) or their combination (OLM-AZL) in drinking water for 5 weeks (n=6 each). Systolic blood pressure was significantly higher in DM-ApoE/- group compared with other groups. In control mice, endothelium-dependent relaxations were largely mediated by EDHF. In DM-ApoE/- group, EDHF-mediated relaxations were significantly reduced. EDHF-mediated relaxations were partially improved in the OLM and AZL groups, and were further improved in the OLM-AZL group. Endothelium-dependent hyperpolarizations were also markedly reduced in DM-ApoE/- group, and were recovered in the OLM-AZL group (n=4 each). By contrast, endothelium-independent relaxations to sodium nitroprusside or NS-1619 (a direct opener of KCa channels) were unaltered in any groups.

Conclusions: These results indicate that long-term combination therapy with an ARB and a CCB ameliorates EDHF-mediated responses in diabetic ApoE-/- mice.
P5-3

HYPERTENSION AND EDHF-MEDIATED RESPONSES IN MESENTERIC ARTERIES OF THE RATS

Billy Wing Cheung Kong, Ricky YK Man, Paul M Vanhoutte and Susan WS Leung

Department of Pharmacology and Pharmacy, University of Hong Kong, Hong Kong, China

Hypertension is a risk factor for endothelial dysfunction which is associated with reduced bioavailability of endogenous vasodilators. The present study examined the hypothesis that responses attributed to endothelium-derived hyperpolarizing factors (EDHF) are reduced in hypertension. Endothelium-dependent relaxations to acetylcholine were examined in superior mesenteric arteries isolated from 8-month-old spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats. Inhibition of the EDHF signaling cascade with a combination of TRAM-34 and UCL 1684 did not affect acetylcholine-induced relaxations in arteries of both WKY rats and SHR. NO-mediated relaxations were comparable in arteries of both strains. In the combined presence of indomethacin and L-NAME, acetylcholine induced full relaxation in WKY rats but a reduced relaxation in SHR. The EDHF-mediated response was not affected by the gap junction inhibitors (carbenoxolone and GAP-27) but was inhibited by the Na\(^+\)/K\(^+\)-ATPase inhibitor, ouabain. The present findings suggest that EDHF-mediated relaxation is impaired in hypertension. In addition, Na\(^+\)/K\(^+\)-ATPase, but not gap junctions, is involved in the EDHF-mediated relaxation of the mesenteric artery of the rats. The expression and/or activity of calcium-activated potassium channels and Na\(^+\)/K\(^+\)-ATPase may be altered in hypertension.
P5-4

ROLES OF ENDOTHELIAL OXIDASES IN ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR RESPONSES IN MICE

Junko Ohashi\textsuperscript{a}, Aya Takaki\textsuperscript{a}, Keiko Morikawa\textsuperscript{b}, Yoshinori Murayama\textsuperscript{b}, Hiroto Yamagishi\textsuperscript{a}, Maki Hosoya\textsuperscript{a} and Hiroaki Shimokawa\textsuperscript{a}

\textsuperscript{a}Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan
\textsuperscript{b}Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Japan

Background: The endothelium synthesizes and releases several vasodilator substances, including prostacyclin, nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). We have demonstrated that endothelium-derived hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) is an EDHF in mouse and human mesenteric arteries and porcine coronary microvessels and that superoxide anions derived from endothelial nitric oxide synthases (NOSs) system are an important precursor for EDHF/H\textsubscript{2}O\textsubscript{2} in mice. There are several intracellular sources of superoxide anions other than NOSs, including NAD(P)H oxidase, xanthine oxidase, lipoxygenase, and mitochondrial electron transport chain. In this study, we examined the possible role of endothelial oxidases other than NOSs in the EDHF-mediated responses.

Methods and Results: In angiotensin II-infused mice (subcutaneous, 2 mg/kg/min, 1 week), both EDHF-mediated relaxations and hyperpolarizations to acetylcholine were significantly reduced, nitric oxide-mediated relaxations were rather enhanced, and vascular smooth muscle responses were preserved. Antihypertensive treatment normalized blood pressure but failed to improve EDHF-mediated responses in those mice. Acute inhibition of endothelial oxidases other than NOSs, including NAD(P)H oxidase, xanthine oxidase, lipoxygenase, or mitochondrial electron transport chain, had no inhibitory effects on EDHF-mediated responses. Furthermore, in p47phox-knockout mice, EDHF-mediated responses were unaltered.

Conclusion: These results suggest that endothelial oxidases other than NOSs are not involved in EDHF/H\textsubscript{2}O\textsubscript{2} responses in mice, suggesting a specific link between endothelial NOSs system and EDHF responses under physiological conditions.
DISTINCTIVE LOCALIZATION AND OPPOSED ROLES OF VASOHIBIN-1 AND VASOHIBIN-2 IN THE REGULATION OF ANGIGENESIS

Hiroshi Kimura\textsuperscript{a}, Hiroki Miyashita\textsuperscript{a}, Yasuhiro Suzuki\textsuperscript{a}, Miho Kobayashi\textsuperscript{a}, Kazuhide Watanabe\textsuperscript{a}, Hikaru Sonoda\textsuperscript{b}, Hideki Ohta\textsuperscript{b}, Takashi Fujiwara\textsuperscript{c}, Tooru Shimosegawa\textsuperscript{d} and Yasufumi Sato\textsuperscript{a}

\textsuperscript{a}Department of Vascular Biology, Institute of Development, Aging and Cancer, Tohoku University, Japan
\textsuperscript{b}Discovery Research Laboratories, Shionogi & Co., Ltd., Japan
\textsuperscript{c}Department of Biological Resources, INCS, Ehime University, Japan
\textsuperscript{d}Department of Gastroenterology, Tohoku University Graduate School of Medicine, Japan

We recently isolated a novel angiogenesis inhibitor, vasohibin-1 and its homologue vasohibin-2. In this study we characterize the role of these 2 molecules in the regulation of angiogenesis. In a mouse model of subcutaneous angiogenesis, the expression of endogenous vasohibin-1 was low in proliferating ECs at the sprouting front, but high in non-proliferating ECs in the termination zone. In contrast, endogenous vasohibin-2 was preferentially expressed in mononuclear cells mobilized from bone marrow that infiltrated the sprouting front. When applied exogenously, vasohibin-1 inhibited angiogenesis at the sprouting front where endogenous vasohibin-1 was scarce, but did not influence vascularity in the termination zone where endogenous vasohibin-1 was enriched. Exogenous vasohibin-2 prevented the termination of angiogenesis in the termination zone and increased vascularity in this region. Angiogenesis was persistent in the termination zone in the vasohibin-1 knockout mice, whereas angiogenesis was deficient at the sprouting front in the vasohibin-2 knockout mice. Supplementation of deficient proteins normalized the abnormal patterns of angiogenesis in the vasohibin knockout mice. These results indicate that vasohibin-1 is expressed in ECs in the termination zone to halt angiogenesis, whereas vasohibin-2 is expressed in infiltrating MNCs in the sprouting front to promote angiogenesis.
P5-6

EFFECT OF DIENOGEST ON ESTROGEN-INDUCED NITRIC OXIDE PRODUCTION IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS AND ENDOTHELIUM-DEPENDENT VASODILATATION IN POSTMENOPAUSAL WOMEN

Noriko Henmi, Kazuhiro Takahashi, Hizuru Yamatani, Takayuki Yoshida, Keiko Takata and Hirohisa Kurachi

Department of Obstetrics and Gynecology, Yamagata University, Japan

Objectives: Estrogen induces endothelium-dependent vasodilatation in postmenopausal women. Medroxy-progesterone acetate (MPA), commonly used with estrogen in hormone therapy, inhibits the favorable effects of estrogen on the endothelium. We investigated the effects of dienogest (DNG) on the favorable effects of estrogen in endothelial function.

Methods: In vitro study: human umbilical vein endothelial cells were treated with MPA, DNG, progesterone (P4) with or without estradiol (E2), then examined NO production using with NO-sensitive fluorescent dye, and phosphorylation of eNOS and Akt by western blot analysis. Clinical study: sixteen surgically menopaused women were randomly divided into four groups (control, E2, E2+MPA, E2+DNG). Except for the control group, the other three groups were treated with transdermal E2 (0.72 mg) /two days, or with E2 plus MPA (2.5 mg/day), or with E2 plus DNG (2 mg/day) for a week, starting 1 week after the surgery. The flow-mediated dilatation (FMD) was measured before, one and two weeks after the surgery.

Results: MPA attenuated the E2-induced phosphorylation of eNOS and Akt, and NO production. However, DNG as well as P4 did not inhibit the E2 effect. A significant decrease in FMD was observed a week after the surgery. FMD was significantly recovered in the E2 and E2+DNG groups. However, the decrease in FMD was sustained in the control and E2+MPA groups.

Conclusions: These results suggest that DNG did not attenuate the favorable effects of E2 on the endothelial function. DNG may have an advantage compared to MPA on the endothelial function in postmenopausal women receiving hormone therapy.
AGING AND PROSTACYCLIN RESPONSES IN AORTA AND PLATELETS FROM WKY AND SHR RATS

Michel Feletou, Elodie Gomez, Cedric Schwendemann, Severine Roger, Serge Simonet, Jerome Paysant, Christine Courchay and Tony J. Verbeuren

Department of Angiology, Institut de Recherches Servier, France

In SHR, prostacyclin is an endothelium-derived contracting factor contributing to the endothelial dysfunction. This study was designed to determine whether the impairment of the prostacyclin response is influenced by aging and whether such a dysfunction is observed in platelets. Isometric tension was measured in aortic rings and aggregation was studied in platelet-rich plasma taken from 3, 6 and 15 month-old WKY and SHR. In aorta from 3 and 6 month-old WKY, the IP-receptor agonists, prostacyclin and beraprost, produced relaxations that were enhanced by terutroban, a TP-receptor antagonist. In 15 month-old WKY, the relaxations to beraprost were maintained, but not those to prostacyclin. In SHR aorta, prostacyclin or beraprost produced no or minor relaxations, which, in younger SHR, were enhanced by terutroban. In both strains, the relaxations were inhibited by CAY-10441, an IP-receptor antagonist. The relaxations to forskolin and isoproterenol were reduced with aging. When compared to WKY, the relaxations to isoproterenol were reduced in 3 but not in 6 or 15 month-old SHR, whereas those to forskolin were consistently diminished at any given age. Whatever the age, prostacyclin and beraprost produced CAY-10441-sensitive inhibitions of ADP-induced platelet aggregation. Both agonists were more potent in SHR than in WKY. Therefore, in platelets from WKY and SHR the IP-receptor-dependent antiaggregant response is functional and maintained during aging. In aorta from WKY those responses are reduced by aging and, in SHR, are already compromised at 3 months. This dysfunction of the IP-receptor is only partially explained by a general dysfunction of the adenylate-cyclase pathway.
MECHANICAL STRETCH AUGMENTS INSULIN-INDUCED VASCULAR SMOOTH MUSCLE CELL PROLIFERATION BY UPREGULATION OF INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR

Hirofumi Hitomi\textsuperscript{a}, Gang Liu\textsuperscript{a}, Naohisa Hosomi\textsuperscript{b}, Daisuke Nakano\textsuperscript{a}, Hideyasu Kiyomoto\textsuperscript{b}, Shoji Kimura\textsuperscript{a}, Masakazu Kohno\textsuperscript{b} and Akira Nishiyama\textsuperscript{a}

\textsuperscript{a}Department of Pharmacology, Kagawa University, Japan
\textsuperscript{b}Department of Cardiorenal and Cerebrovascular Medicine, Kagawa University, Japan

Objectives: Insulin resistance and hypertension have been implicated in the pathogenesis of cardiovascular disease; however, little is known about the roles of insulin and mechanical force in vascular smooth muscle cell (VSMC) remodeling. In the present study, we investigated the contribution of mechanical stretch to insulin-induced VSMC proliferation.

Methods and Results: VSMCs grown on a flexible membrane were stretched by a Flexcell culture system. DNA synthesis and glucose metabolism were assessed according to [\textsuperscript{3}H]-labeled thymidine and 2-deoxy-glucose incorporation, respectively. Thymidine incorporation was stimulated by insulin in stretched VSMCs, but not in un-stretched VSMCs. Insulin also increased 2-deoxy-glucose incorporation in both cell types, but there was no significant difference between stretched and un-stretched VSMCs. Mechanical stretch augmented insulin-induced extracellular signal-regulated kinase (ERK) and Akt phosphorylation. Inhibitors of ERK kinase and phosphatidylinositol 3-kinase attenuated insulin-induced thymidine incorporation in stretched VSMCs. Inhibitors of epidermal growth factor (EGF) receptor tyrosine kinase and Src also attenuated insulin-induced ERK and Akt phosphorylation, and thymidine incorporation, whereas 2-deoxy-glucose incorporation was not affected by these inhibitors. Moreover, stretch augmented insulin-like growth factor (IGF)-1 receptor protein expression, although it did not alter the expression of insulin receptor and insulin receptor substrate-1. Insulin-induced ERK and Akt activation, and thymidine incorporation were inhibited by siRNA for the IGF-1 receptor in stretched VSMCs.

Conclusions: Mechanical stretch augments insulin-induced VSMC proliferation via upregulation of IGF-1 receptor, and downstream Src/EGF receptor-mediated ERK and Akt activation. These results provide a basis for clarifying the molecular mechanisms of vascular remodeling in hypertensive patients with hyperinsulinemia.
ROLE OF RHO-KINASE IN THE PATHOGENESIS OF CORONARY HYPERCONstrictING RESPONSES INDUCED BY PACLITAXEL-ELUTING STENT

Takashi Shiroto\textsuperscript{a}, Satoshi Yasuda\textsuperscript{a}, Ryuji Tsuburaya\textsuperscript{a}, Yoshitaka Ito\textsuperscript{a}, Jun Takahashi\textsuperscript{a}, Kenta Ito\textsuperscript{a}, Hatsue Ishibashi-Ueda\textsuperscript{b} and Hiroaki Shimokawa\textsuperscript{a}

\textsuperscript{a}Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan
\textsuperscript{b}Department of Pathology, National Cardiovascular Center, Japan

**Background:** Recent studies showed that coronary vasoconstricting responses are enhanced at the edge of coronary segment implanted with drug-eluting stent (DES) as compared with bare-metal stent (BMS) in humans. We have previously demonstrated that activated Rho-kinase pathway plays a central role in the molecular mechanism of coronary vasospasm in animals and humans. Thus, we examined whether Rho-kinase pathway is involved in the pathogenesis of coronary hyperconstricting responses induced by DES in pigs in vivo.

**Methods:** Human coronary artery smooth muscle cells (hCASMC) were co-incubated with various concentrations of paclitaxel ($10^{-9}$-$10^{-6}$ mol/L, corresponding levels reported in DES-implanted arterial tissue) for 24 hours. A paclitaxel-eluting stent (PES) and a BMS were randomly implanted in the left coronary arteries in pigs for 4 weeks.

**Results:** In hCASMCs, paclitaxel significantly enhanced Rho-kinase expression (n=9) and activity (n=6). In a porcine model, coronary vasoconstricting responses to serotonin (10 and 100 \textmu g/kg, IC) were significantly enhanced at the PES site compared with the BMS site ($45 \pm 4\%$ vs. $30 \pm 3\%$, P<0.01; n=12 each), and were abolished by hydroxyfasudil (90 and 300 \textmu g/kg, IC), a selective Rho-kinase inhibitor. PES enhanced inflammatory responses and microthrombus formation at the stent edge, where immunoreactivities for Rho-kinase expression and activity (phosphorylated form of myosin phosphatase target subunit 1) were significantly enhanced in rings from PES-edge site compared with BMS-edge site (n=12).

**Conclusions:** These results suggest that Rho-kinase pathway plays an important pathogenetic role in the DES-induced coronary hyperconstricting responses.
P6-3
SERUM RAGE LIGANDS INDUCE OSTEOBLASTIC DIFFERENTIATION OF VASCULAR SMOOTH MUSCLE CELLS VIA RAGE-Notch-Msx2 PATHWAY

Toshihiro Suga\textsuperscript{a}, Tatsuya Iso\textsuperscript{a}, Takehisa Shimizu\textsuperscript{a}, Toru Tanaka\textsuperscript{a}, Sho-ichi Yamagishi\textsuperscript{b}, Masashi Arai\textsuperscript{a}, Tsutomu Imaizumi\textsuperscript{b} and Masahiko Kurabayashi\textsuperscript{a}

\textsuperscript{a}Department of Cardiology, Gunma University, Japan
\textsuperscript{b}Kurume University, Japan

Receptor for advanced-glycation end products (RAGE) plays an important role in development of atherosclerosis in patients with diabetes and chronic renal failure (CRF). However, little is known about effects of RAGE on calcification of vascular smooth muscle cells (VSMC). Our RT-PCR analysis revealed that expression of alkaline phosphatase (ALP) was up-regulated in human aortic SMC (HASMC) under RAGE overexpression. The ALP up-regulation was decreased when fetal bovine serum (FBS) was pre-treated with soluble-RAGE, a decoy receptor, or when 1% FBS was used instead of 15%, suggesting FBS contains RAGE ligands. The ALP mRNA induction was accompanied by ALP activity, calcium deposition, and expression of osteogenic transcription factor, Msx2, and Notch components, Jagged1 and Notch1. Consistent with our previous report that Notch signaling induces Msx2-dependent calcification of VSMC, the induction of Msx2 and ALP by RAGE was blocked by Notch signal inhibitor. Msx2 siRNA blocked Notch-induced ALP expression in HASMC. We further studied whether human serum from patients with diabetes and CRF induces ALP activity. The serum from healthy volunteers didn’t up-regulate ALP while those from the patients did it under RAGE overexpression. The ALP activity was positively co-related with serum creatine level, but not hemoglobinA1c, suggesting that RAGE ligands accumulate in serum in GFR-dependent manner. These findings suggest that serum RAGE ligands promote CRF-associated vascular calcification via RAGE-Notch-Msx2 pathway.
THE RELAXING EFFECT OF OKADAIC ACID ON CANINE BASILAR ARTERY INVOLVES PHOSPHORYLATION OF THE MYOSIN LIGHT CHAIN AT THREONINE-9

Kazuo Obara\textsuperscript{a} and Koichi Nakayama\textsuperscript{b}

\textsuperscript{a}Department of Pharmacology, University of Shizuoka, Japan
\textsuperscript{b}Department of Molecular and Cellular Pharmacology, Iwate Medical University, Japan

The phosphorylation of 20-kDa myosin light chain (MLC\textsubscript{20}), depending on a balance between myosin light chain kinase (MLCK) and myosin phosphatase (MLCP), is a primary step in the contraction of smooth muscle. Recently, we reported that stretch-induced triphosphorylation of MLC\textsubscript{20} counteracted a contraction in the canine basilar artery. On the other hand, low concentration of okadaic acid (OA), a potent protein phosphatase inhibitor, relaxed vascular smooth muscles. In the present study, we investigated the relationship between relaxing effect of OA and multiple phosphorylation of MLC\textsubscript{20} in canine basilar artery.

OA (1 \textmu M) relaxed 80 mM KCl-induced contraction of the artery and this relaxant effect was partially inhibited by Go6976, a conventional protein kinase C (PKC) inhibitor, and calphostin C, an inhibitor of conventional and novel PKCs. Rottlerin, a specific inhibitor of PKC\textdelta, did not influence effect of OA. KCl increased phosphorylation of MLC\textsubscript{20} at Ser-19. OA additionally increased MLC\textsubscript{20} phosphorylation at Thr-18 and Thr-9, resulting in triphosphorylation of MLC\textsubscript{20}. This phosphorylation was inhibited by Go6976. OA stimulated phosphorylation of PKCa and 17-kDa PKC-potentiated inhibitory phosphoprotein (CPI-17), and Go6976 inhibited these phosphorylations. These results suggest that relaxant effect of OA involves MLC\textsubscript{20} triphosphorylation through a direct phosphorylation by PKCa and an indirect phosphorylation by inhibition of myosin light chain phosphatase through PKCa-mediated CPI-17 phosphorylation. Of the triphosphorylated amino acid residues of MLC\textsubscript{20}, the phosphorylated Thr-9 via PKCa has an inhibitory action on the contraction.
IMPORTANCE OF DUAL INDUCTION TESTS FOR CORONARY VASOSPASM AND VENTRICULAR FIBRILLATION IN PATIENTS SURVIVED FROM OUT-OF-HOSPITAL CARDIAC ARREST

Yusuke Takagi, Satoshi Yasuda, Jun Takahashi, Morihiko Takeda, Masaharu Nakayama, Kenta Ito, Masanori Hirose, Yuji Wakayama, Koji Fukuda and Hiroaki Shimokawa

Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan

Background: The prevalence of sudden death in the absence of organic heart disease (termed as Pokkuri disease in Japan) is high in Asian males. However, the pathogenesis of such out-of-hospital cardiac arrest (OHCA) remains to be elucidated. It is conceivable that both ventricular fibrillation (VF) and coronary vasospasm play key roles in this disorder.

Methods: In the present study, we enrolled consecutive 12 patients who survived from VF and OHCA but had no organic heart disease, as confirmed on clinical, hemodynamic and angiographical evaluation (M/F, 12/1; age, 44±12 [SD] years). We performed dual induction tests for both coronary vasospasm with intracoronary acetylcholine and VF with programmed stimulation on separate days at 1 month after the event.

Results: Left ventricular ejection fraction was preserved (66±7%). All patients were positive for either test; vasospasm alone in 3, VF alone in 2, and both of them in 7. Importantly, 7 of the 10 patients positive for coronary vasospasm also had inducible VF even under intensive medical therapy including calcium channel blockers. All patients subsequently underwent implantation of implantable cardioverter defibrillator (ICD). During the follow-up period of 19 months (mean), the appropriate ICD shock for VF was documented in one patient.

Conclusions: These findings indicate the heterogeneity in the pathogenesis of OHCA and therefore the importance of dual induction tests for coronary vasospasm and VF. Multicenter study is needed to clarify whether ICD could improve the prognosis of OHCA patients positive for coronary vasospasm.
Rho kinase is involved in the pathogenesis of hypertension which favors the occurrence of endothelium-dependent contractions. The present study was designed to determine the effects of two Rho kinase inhibitors, HA1077 and Y27632 on endothelium-dependent and -independent contractions. Isometric tension of one year old SHR and WKY aortae were measured. In the presence of L-NAME, HA1077 and Y27632 reduced endothelium-dependent contractions caused by acetylcholine and the calcium ionophore A23187. The Rho kinase inhibitors did not significantly affect prostacyclin production measured as 6-keto prostaglandin F₆. They nearly abolished endothelium-independent contractions to U46619, prostaglandin F₆ and phenylephrine. Western blotting revealed a comparable expression of Rho kinase in the aortae of the two strains. The reduction by Rho kinase inhibitors of endothelium-dependent contractions is mainly due to their direct effect on the vascular smooth muscle cells.
ENDOGENOUS UREA AS A SUBJECT AND AS AN OBJECT OF β-ADRENOCEPTOR CONTROL IN ANIMAL ORGANISM

Nikolai Dimitrov Temnyalov
Preclinical and Clinical Pharmacology, Medical University - Varna, Bulgaria

Introductorily, it is to be underlined on our IN VIVO and IN VITRO studies, we showed that urea at concentrations - physiological and pathophysiological in humans, could act as a nonspecific and nonselective antagonist of beta-1,2-AR in the cardiovascular system of mammals. In other words, it can be evaluated as an example that urea possesses properties of a subject.

Is it possible the endogenous urea to possess some and/or determined properties of an object - just one opposite position, even in evolution aspect?

In a searching the available literature it was and it is still my happiness to find just necessary data of the group of Italian physiologists of C. Lippe on β-adrenergic regulation of urea permeability of the Bufo Bufo blader (1988-1994). Authors conclude for discovered “two complementary mechanisms for absorption of urea: via β-2-, but not with β-1-, alpha-1- and alpha-2- adrenergic receptors - from the one side and from the other side via vasopressin (ADH) receptors. In quantitative aspect at terrestrice Bufo Bufo predominate the role of vasopressine receptors - responsible for 19-times transfer of 14C urea flow in comparison with 14 times increased transfer with β-2-adrenergic receptors after the stimulation with forskoline 1mM. It is clear that such mechanism did not exist in water Bufo Bufo.

In conclusion we interpret that in evolution in different animal organism the endogenous Urea can be regulated and efficacy controlled at least in two direction - as a subject and as an object depending from different animal organism.
We previously reported that chronic hyperinsulinemia and insulin resistance induced by high level fructose drinking results in abnormal neuronal regulation of vascular tone, which partly contributes to development of hypertension, and that pioglitazone ameliorates these changes. In this study, to assess effects of pioglitazone on the altered neuronal function in the hyperinsulinemic state, we investigated functional and innervation changes in vasoconstrictor adrenergic neuropeptide Y (NPY)- and vasodilator calcitonin gene-related peptide (CGRP)-containing perivascular nerves using isolated mesenteric vascular beds in fructose-drinking rats (FDR). Male Wistar rats received 15% fructose solution in drinking fluid for 4 weeks, which resulted in significant increases in plasma levels of insulin and systolic blood pressure, but not blood glucose levels, compared with those in control rats. In perfused mesenteric artery of FDR, adrenergic nerve-mediated vasoconstriction was enhanced and CGRPergic nerve-mediated vasodilation was reduced. Immunohistochemical studies showed increased density of NPY-like immunoreactive (LI) fibers and decreased density of CGRP-LI fibers in mesenteric arteries of FDR. Oral administration of pioglitazone to FDR for 4 weeks markedly decreased plasma levels of insulin and blood pressure. In FDR preparations, pioglitazone opposed the enhanced adrenergic nerve-mediated vasoconstriction and increased density of NPY-LI fibers, and ameliorated the reduction of CGRPergic nerve-mediated vasodilation and decreased density of CGRP-LI fibers. These results suggest that pioglitazone improves not only insulin resistance, but also dysfunction in neuronal vascular control resulted from abnormal innervation of perivascular nerves in the hyperinsulinemic state.
P7-3
DEXMEDITOMIDINE RELAXES ISOLATED PORCINE CORONARY ARTERIES
BY ACTIVATING ENDOTHELIAL 2A, 2B AND 2C ADRENOCEPTORS

Kwok Fu Jacobus Ng, Matthew L.Y. Chan, Ricky Y.K. Man and Paul M. Vanhoutte
Pharmacology & Pharmacy, The University of Hong Kong, Hong Kong, China

Introduction: Dexmedetomidine is an intravenous anaesthetic agent which relaxes isolated porcine coronary artery by activation of endothelial α2 adrenoceptors. The present study aimed to determine the α2 adrenoceptor subtypes involved.

Methods: Porcine coronary arterial rings with (EC+) or without (EC-) endothelium were suspended in organ chambers filled with Krebs-Ringer solution, maintained at 37°C and aerated with 95%O2 and 5% CO2. Isometric tension was measured. After equilibration and viability testing, indomethacin was added and the rings were contracted with prostaglandin F2α. Dexmedetomidine was then added in cumulative fashion in the absence or presence of increasing concentrations of the selective 2A antagonist BRL44408, 2B antagonist ARC239 or 2C antagonist MK912. One way ANOVA was used to compare the concentration-response curves.

Results: Dexmedetomidine caused a dose dependent relaxation of porcine coronary artery (EC50=3.9±1.2 nM, Emax=44±5%, n=10). The relaxation was abolished by removal of endothelium and by L-NAME (100 μM). The EC50 of the relaxation curves was shifted to the right by 0.3 μM of BRL44408 (EC50=0.21±0.09 μM, n=10, P <0.01), by 1 μM of ARC239 (EC50=97±15 nM, n=10, P <0.01) and by 3 nM of MK912 (EC50=85±31 nM, n=9, P <0.05). Lower doses of these antagonists did not attenuate the relaxations. In addition, 3 nM of MK912 also significantly reduced the Emax (28±5%, n=8, P <0.05) of dexmedetomidine.

Conclusion: All three α2-adrenoceptor subtypes contribute to the endothelium-dependent relaxation to dexmedetomidine in the porcine coronary artery.
PROTONS MEDIATE PERIVASCULAR ADRENERGIC NERVE-MEDIATED
VASODILATION IN THE RAT MESENTERIC ARTERY

Kazuhiro Hirai\textsuperscript{a}, Satoko Miyashita\textsuperscript{a}, Narumi Houbara\textsuperscript{b}, Yoshito Zamami\textsuperscript{a},
Yoshihisa Kitamura\textsuperscript{c} and Hiromu Kawasaki\textsuperscript{a}

\textsuperscript{a}Department of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan
\textsuperscript{b}Department of Life Sciences, Okayama University of Science, Japan
\textsuperscript{c}Department of Pharmaceutical Care Health Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan

Previous studies showed that nicotine stimulates presynaptic nicotinic ACh receptors in adrenergic nerves and activates TRPV1 located on CGRP-containing vasodilator nerves (CGRPergic nerves) resulting in vasodilation. The aim of this study is to investigate whether proton acts as an axo-axonal transmitter in perivascular nerves. Rat perfused mesenteric vascular beds without endothelium were contracted by perfusion with Krebs solution containing methoxamine and the pH levels of perfusate were measured with a pH meter. Nicotine perfusion (1-100 uM) for 1 min and periarterial nerve stimulation (PNS; 2, 4, 8 Hz) for 30 s lowered pH levels of the perfusate concomitant with long-lasting vasodilation. Cold-storage denervation (4 °C for 72 h) of the preparation abolished vasodilation and pH lowering induced by nicotine and PNS. Guanethidine inhibited PNS- and nicotine-induced lowering of pH levels, nicotine-induced vasodilation, but not PNS-induced vasodilation. Nicotinic receptor antagonist mecamylamine blunted nicotine-induced pH lowering and vasodilation, but TRPV1 antagonist capsazepine inhibited only nicotine-induced vasodilation. In the study using fluorescent pH indicator, nicotine applied outside the isolated small mesenteric artery caused pH lowering. Immunohistochemical study showed dense innervation of adrenergic neuropeptide Y (NPY)-like immunoreactivity (LI) and CGRP-LI-containing nerves and both appeared in the same neuron. CGRP-LI and TRPV1-LI-containing nerves also appeared in the same neurons. Application of HCl in denuded preparations induced vasodilation, which was inhibited by denervation, capsazepine, capsaicin pretreatment and CGRP receptor antagonist (CGRP8-37). These results suggest that excitation of adrenergic nerves releases protons to activate TRPV1 in CGRPergic nerves and thereby induce vasodilation.
LOCAL-COOLING EFFECTS ON THE SKIN BLOOD FLOW IN MICE AND RATS, THE MOST MAJOR RODENTS IN EXPERIMENTAL ANIMALS

Koichi Nakayama\textsuperscript{a} and Tomohisa Ishikawa\textsuperscript{b}

\textsuperscript{a}Department of Molecular & Cellular Pharmacology, Iwate Medical University, Faculty of Pharmaceutical Sciences, Japan
\textsuperscript{b}Department of Cellular and Molecular Pharmacology, University of Shizuoka, Japan

Cooling reduces the skin blood flow to protect body from heat loss. This response results from a reflex increase in sympathetic output and a local enhancement of vasoconstriction to noradrenaline in cutaneous vessels (Vanhoutte, 1980, Handbook of Physiology, The Cardiovascular System II, pp 443-474). Of mechanisms hitherto suggested for the effect of cooling, the well-accepted one is the cooling-induced augmentation of alpha2-adrenoceptor reactivity. The aim of the present study was undertaken to dissolve the question whether the same sequence of events for the cooling effect on the skin blood flow in vivo occurred in mice and rats. Male Wistar rats and ddY mice, anesthetized with pentobarbital, were treated with tetrodotoxin and artificially ventilated. The plantar skin blood flow by cooling air temperature was measured by laser Doppler flowmetry. The decrease in plantar blood flow was similarly induced by local cooling in both rats and mice. However, the mechanism involved in mice is apparently different from that in rats; the response in mice primarily results from increased reactivity of alpha2-adrenoceptors to circulating catecholamines, in which the Rho/Rho kinase pathways is involved (Honda et al., Brit. J. Pharmacol., 152: 91-100, 2007), whereas the local cooling-induced reduction of skin blood flow in rats induces the release of ATP, which stimulates presynaptic P2 purinoceptors on sympathetic nerve terminals and facilitates the release of noradrenaline, thereby leading to contractions of skin blood vessels mediated by alpha1-and alpha2-adrenoceptors (Koganezawa et al., Brit. J. Pharmacol., 148: 579-586, 2006).
DIFFERENTIAL EFFECTS OF ALPHA-ADRENOCEPTOR AGONISTS ON RELAXATION IN THE MESENTERIC ARTERY AND AORTA OF THE RAT

Emily S.W. Wong\textsuperscript{a}, Ricky Y.K. Man\textsuperscript{a}, Paul M. Vanhoutte\textsuperscript{a} and Kwok F.J. Ng\textsuperscript{b}

\textsuperscript{a}Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong, China
\textsuperscript{b}Department of Anaesthesiology, The University of Hong Kong, Hong Kong, China

Introduction: Dexmedetomidine, UK14304 and clonidine are \(\alpha\)-adrenergic agonists, with different selectivity for \(\alpha_1\) and \(\alpha_2\) adrenoceptors. The present experiments were designed to compare their effects in the mesenteric artery and aorta of the rat.

Methods: Mesenteric arteries and thoracic aortae with endothelium were isolated from male Sprague Dawley rats (10 week old), and suspended in organ chambers for isometric tension recording. The arteries and aortae were contracted with U46619. Cumulative concentrations of dexmedetomidine, UK14304 and clonidine were added during these contractions. Relaxations were expressed as a percentage of the contractions.

Results: In mesenteric arteries, dexmedetomidine caused concentration-dependent relaxations with a maximal response of about 50%. UK14304 and clonidine induced relaxations that were greater and smaller than those to dexmedetomidine, respectively. These relaxations were inhibited by rauwolscine, a \(\alpha_2\) adrenergic antagonist. Prazosin (\(\alpha_1\) adrenergic antagonist) increased relaxations to dexmedetomidine and clonidine but did not affect those to UK14304. In aortae, relaxations to the three adrenergic agonists were smaller (maximal response averaging 10-20%) than in mesenteric arteries. These relaxations were potentiated by prazosin.

Conclusions: Dexmedetomidine, UK14304 and clonidine caused relaxation through \(\alpha_2\) adrenoceptor activation in the rat aorta and mesenteric artery. These relaxations were masked by concomitant \(\alpha_1\) adrenoceptor stimulation. The differential relaxing effects of \(\alpha\) adrenergic agonists in the two vascular beds studied suggest that in the rat the mesenteric artery possess a higher density of \(\alpha_2\) adrenoceptor than the aorta resulting in greater effects in the mesenteric artery.
P7-7
WITHDRAWN
BENEFICIAL BIPHASIC EFFECTS OF ADENOSINE ON RAT AFFERENT AND EFFERENT ARTERIOLES

Hiroshi Nakamoto, Yasuo Ogasawara and Fumihiko Kajiya

Department of Medical Engineering and Systems Cardiology, Kawasaki Medical School, Japan

The purpose of this study was to evaluate the effects of adenosine augmented by dilazep under physiological conditions in rats. We measured afferent and efferent arteriolar diameter changes using an intravital videomicroscope and renal blood flow. We administered dilazep at a dose of 300 μg/kg intravenously. If adenosine is administered from a peripheral route, i.e., intravenously, it is eliminated within a half-life of seconds by carrier-mediated uptake, which occurs in most cell types, and subsequent metabolism by adenosine deaminase. Dilazep, whose half-life is 4 hours, is preferable to examine the acute local effect of adenosine. To further investigate, rats were pre-treated with a nonselective adenosine receptor antagonist, an A1 receptor antagonist, or an A2 receptor antagonist. Dilazep constricted the afferent and efferent arterioles at the early phase and dilated them at the later phase. A1 blockade abolished vasoconstriction and A2 blockade abolished vasodilatation. Non-selective blockade abolished both early vasoconstriction and later vasodilatation. In summary, adenosine augmented by dilazep constricted the afferent and efferent arterioles of the glomeruli at the early phase and dilated both arterioles at the later phase via A1 and A2 adenosine receptor activation, respectively. That the ratio of afferent to efferent arteriolar diameter was fairly constant suggests that intraglomerular pressure is maintained in the acute phase by adenosine despite the biphasic flow change. It is postulated that early vasoconstriction may be beneficial to maintain blood flow for other important organs in the first few minutes of acute ischaemia followed by recovery.
ROLE OF HYDROGEN PEROXIDE, AN ENDOGENOUS EDHF DURING CORONARY OCCLUSION AND INJECTION OF ERYTHROPOIETIN IN CANINE CORONARY NATIVE COLLATERAL MICROCIRCULATION

Toyotaka Yada\textsuperscript{a}, Hiroaki Shimokawa\textsuperscript{b}, Osamu Hiramatsu\textsuperscript{a}, Masami Goto\textsuperscript{a}, Yasuo Ogasawara\textsuperscript{a} and Fumihiko Kajiya\textsuperscript{a}

\textsuperscript{a}Department of Medical Engineering and Systems Cardiology, Kawasaki Medical School, Japan
\textsuperscript{b}Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan

Background: We examined the role of H\textsubscript{2}O\textsubscript{2} as an EDHF in the vasodilatation of collaterals after coronary occlusion and injection of erythropoietin (Epo) in canine coronary microcirculation in vivo.

Methods: Canine subepicardial collateral small coronary arteries (CSA, $\geq$100 $\mu$m) and arterioles (CA, <100 $\mu$m) were continuously observed by a microscope during coronary occlusion under cyclooxygenase blockade (ibuprofen). Experiments were performed after LAD occlusion under the following 7 conditions (n=5 each): control, low-dose and high-dose Epo (L-Epo 100 and H-Epo 1000 IU/kg), catalase (a decomposer of H\textsubscript{2}O\textsubscript{2})+H-Epo, NO synthase inhibitor (L-NMMA)+catalase+H-Epo, L-NMMA+tetraethylammonium (TEA, KCa channels blocker)+H-Epo and wortmannin (WTMN, PI3-kinase inhibitor)+H-Epo.

Results: CA dilated (16±3\% vs. baseline) after 80 min of LAD occlusion, but CSA did not (1±1\%). The coronary vasodilatation was significantly increased after H-Epo in both-sized arteries (CSA 6±1\%, CA 22±4\%) compared with control and L-Epo (CSA 2 ±1\%, CA 18±3\%). Catalase+H-Epo significantly decreased the vasodilatation in CA (12 ±2\%). The vasodilatation was markedly attenuated after L-NMMA+catalase+H-Epo (CSA -4±1\% and CA 6±1\% vs. control, both P<0.01), L-NMMA+TEA+H-Epo (CSA -5 ±1\% and CA 6±1\%, both P<0.01) and WTMN+H-Epo (CSA -5±1\% and CA 6±1\%, both P<0.01) in both-sized arteries. H-Epo group after ischemia/reperfusion (90 min/5 hrs) significantly improved CPK-MB (29±8 ng/ml, P<0.05) compared with control (77±24 vs. baseline 10±1, P<0.05) and L-Epo (43±10, P<0.05). CPK-MB was significantly increased in L-NMMA+catalase+H-Epo (95±10), L-NMMA+TEA+H-Epo (99±10) and WTMN+H-Epo (131±10) (all P<0.05).

Conclusions: H\textsubscript{2}O\textsubscript{2} plays an important role in the dilatation of collaterals after coronary occlusion and injection of Epo in canine coronary microcirculation in vivo.
P8-3
CAPILLARY-SPECIFIC EXPRESSION OF FABP4 AND FABP5 (FATTY ACID BINDING PROTEINS): A POSSIBLE ROLE OF FATTY ACID TRANSPORT THROUGH CAPILLARY ENDOTHELIUM

Tatsuya Iso and Masahiko Kurabayashi
Department of Medicine and Biological Science, Gunma University, Japan

Fatty acids are major source of energy metabolism in various organs including heart, but it is uncertain how they can reach interstitial space through endothelial layer of capillary. Fatty acid binding proteins (FABP) are cytosolic fatty acid chaperones whose biological role is not fully understood. FABP4, also known as aP2, is believed to be exclusively expressed in adipocytes and macrophages while FABP5, also called mal1, is detected more widely. Here we re-examined tissue distribution of FABP4 and FABP5. Systemic immunohistochemistry of mice revealed that in addition to highly expressing organs already reported, both FABP4/5 were strongly expressed in capillary endothelium, but not in any arteries, in heart, skeletal muscle, adipose tissue and medulla of kidney. RT-PCR analysis showed that they were obviously detected in heart, skeletal muscle, and kidney even after removal of adipose tissues. Their expression in capillary of liver and brain was almost absent, suggesting diverse function of capillary endothelium in different organs. We observed the same capillary-specific expression of FABP4/5 in human heart and adipose tissue. Along with the previous report showing dramatic prevention from obesity and the type 2 diabetes in mice doubly deficient for FABP4/5, their capillary-specific expression strongly suggest that capillary endothelial cells play an important role in transport of energy substrates including fatty acids as well as glucose via endothelial FABP4/5.
Impaired coronary metabolic dilation in metabolic syndrome

Takahiko Kiyooka\textsuperscript{a}, Akira Nikaidoh\textsuperscript{a}, Michio Takikawa\textsuperscript{a}, Nami Okamoto\textsuperscript{a}, Takeaki Kasai\textsuperscript{a}, Keiko Oikawa\textsuperscript{a} and William M Chilian\textsuperscript{b}

\textsuperscript{a}Department of Cardiology, Tokai University Hachioji Hospital, Japan
\textsuperscript{b}Integrative Medical Sciences, Northeastern Ohio Universities College of Medicine, USA

Metabolic regulation of coronary vascular tone results from a balance in the production of vasodilators and vasoconstrictors by cardiac myocytes. We hypothesized that derangement in the production of vasodilator metabolites by cardiac myocytes from obese Zucker-rats (OZR) underlies insufficient coronary metabolic vasodilation. Isolated cardiac myocytes (CM) in OZR and lean Zucker-rats (LZR) were stimulated at 400 beats/min, and supernatant from these preparations was added to isolated coronary arterioles (53-156 \textmu m). Administration of the supernatant from LZR-CM to the arterioles from LZR or OZR produced similar dose-dependent vasodilation (42\pm6\% vs 39\pm5\%). However, administration of supernatant from OZR-CM to arterioles from OZR and LZR produced less vasodilation of 11\pm2\% [OZR] and 28\pm3\% [LZR] compared with the responses to the supernatant from LZR-CM. Treatment of LZR arterioles with catalase significantly reduced vasodilation to LZR supernatant: (20\pm1\%, respectively vs control of 42\pm6\%). Catalase reduced, but did not completely inhibit dilation to supernatant from LZR-CM, and the remaining component was blocked by 8-parasulfophenyltheophylline (purinergic A1/A2 antagonist), suggesting the vasodilatory activity in LZR was mediated by H2O2 and another vasodilator(s). Conversely, dilation of LZR arterioles to OZR-CM supernatant was almost completely inhibited by catalase, suggesting the metabolic vasodilation produced OZR was mediated by almost by H2O2. We conclude that abrogated coronary metabolic dilation in the metabolic syndrome results from impaired production of vasodilators by cardiac myocytes, rather than diminished responsiveness of arterioles to the vasoactive metabolites.
IMPACT OF THE PRESENCE OF METABOLIC SYNDROME ON CORONARY MICROVASCULAR RESPONSE

Hiroki Teragawa\textsuperscript{a}, Kenji Nishioka\textsuperscript{a}, Naoya Mitsuba\textsuperscript{a}, Shinsuke Mikami\textsuperscript{a}, Yuichi Fujii\textsuperscript{a}, Noritaka Fujimura\textsuperscript{a}, Takayuki Hidaka\textsuperscript{a}, Takenori Okada\textsuperscript{a}, Futoshi Tadehara\textsuperscript{a}, Yukihito Higashi\textsuperscript{b} and Yasuki Kihara\textsuperscript{a}

\textsuperscript{a}Department of Cardiovascular Medicine, Hiroshima University Graduate School of Biomedical Sciences, Japan  
\textsuperscript{b}Department of Cardiovascular Physiology and Medicine, Hiroshima University Graduate School of Biomedical Sciences, Japan

**Background:** Endothelial dysfunction is thought to be involved in the pathogenesis of metabolic syndrome (MS)-induced cardiovascular events, however, it remains to be clarified how is coronary endothelial function in patients with MS. We investigated this relationship in patients with normal coronary arteries.

**Methods:** Seventy-seven men with angiographically normal coronary arteries were enrolled. Acetylcholine (ACh, 3 and 30 \( \mu \)g/min) and nitroglycerin were infused into the left coronary ostium over 2 min. Coronary blood flow (CBF) was calculated by quantitative angiography and Doppler flow velocity measurements. The change in CBF in response to ACh was expressed as percent change from baseline value. Coronary flow reserve (CFR) was also calculated as the ratio of coronary flow velocity after injection of adenosine triphosphate (20 \( \mu \)g) to baseline value. The Japanese MS criteria was used to identify MS.

**Results:** There were 16 patients with MS (21\%). The increase in CBF in response to the infusion of ACh was impaired in patients with MS (MS; 3 \( \mu \)g/min: 33.7\( \pm \)12.1\%, 30 \( \mu \)g/min: 90.4\( \pm \)24.2\%, without MS; 3 \( \mu \)g/min: 66.5\( \pm \)6.2\%, 30 \( \mu \)g/min: 176.91\( \pm \)2.4\%, \( p = 0.0012 \)). Multivariate regression analysis demonstrated that the presence of MS (\( p = 0.0115 \)) as well as a reduced CFR (\( p = 0.0009 \)) were significant factors associating with a reduced change in CBF induced by ACh at 30\( \mu \)g/min (\( r^2 = 0.32 \)).

**Conclusion:** These findings suggest that coronary microvascular endothelial dysfunction is present in patients with MS and normal coronary arteries, indicating that this may be involved in pathogenesis of MS-induced cardiovascular events.
A HYPOTHESIS: VIRCHOW-ROBIN SPACE AS AN INTEGRATING “CLEFT” OF CEREBROCORTICAL LOCAL ARTERIOLAR RESPONSE

Minoru Tomita\textsuperscript{a}, Yutaka Tomita\textsuperscript{b}, Haruki Toriumi\textsuperscript{a}, Miyuki Unekawa\textsuperscript{a}, Hidenori Hattori\textsuperscript{a} and Norihiro Suzuki\textsuperscript{a}

\textsuperscript{a}Department of Neurology, Keio University School of Medicine, Japan
\textsuperscript{b}Department of Neurology and Department of Preventive Medicine for Cerebrovascular Disease, School of Medicine, Keio University, Japan

The presence of Virchow-Robin space is a matter of controversy. The majority view is that the space ends as a cul-de-sac at the arteriolar level with coalescence of the vascular and parenchymal basal laminae, while others (Hirano and Matsui, 1978; Pollock et al., 1997) claim that there are still two distinct basal laminae in apposition, forming a perivascular space between them. Cancilla et al. (1993) reported that the astroglial foot process is in contact with the basal lamina, which separates the cytoplasmic membrane of the astroglia from that of the endothelium. Cauli et al. (2004), observing that stimulation of interneurons can contract/dilate cortical microvessels, claimed that these nerves directly innervate intraparenchymal arterioles across the closed perivascular space or the coalescent basal laminae. Here we show that astroglial endfeet stained with sulforhodamine cover arterioles continuously implies that the perivascular space does exist as a site of glio-vascular interface. The space may appear closed, but be present as an ultra-thin empty space under physiological conditions. Vesicles containing terminals of central axons could be separated from the basal laminae by delicate intervening astrocytic endfeet. We speculate that the thin space serves as a “cleft” for an astroglial tripartite synapse, that the space is capable of synaptic and nonsynaptic neurohumoral dispersion, and that there is no specific postsynaptic structure for transmitter(s) released into this space. Therefore, we suggest that the space serves as the site to transmute incoming neuronal afferent signals into appropriate vascular responses and acts as a local integrator of neurovascular coupling.
Astrocytes play a crucial role in vasodilatory responses to neuronal activities. Glutamate released from activated neurons binds to astroglial metabotropic glutamate receptors (mGluR) and triggers the synthesis and release of PGE₂, a potent vasodilator, eliciting functional hyperemia. Importantly, the activation of astroglial mGluR also causes vasoconstriction. Recent findings indicating that the metabolic state of glucose (glycolytic or oxidative) dictates vascular responses (Gordon et al, Nature 456: 745-9, 2008) prompted us to investigate the effects of glutamate, D-aspartate (a non-metabolizable analogue), and (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (tACPD; an mGluR agonist) on astroglial glucose metabolism.

**Methods:** Astroglial cells were prepared from newborn SD rats, and cultured in the presence of high (23 mM) or low (5 mM) concentrations of glucose for 2 weeks. Total glucose consumption was assessed by measuring [¹⁴C]deoxyglucose phosphorylation. The oxidative metabolism was assessed by measuring [¹⁴C]CO₂ production from [¹⁴C]glucose/lactate/glutamate, and the glycolytic metabolism was assessed by measuring the lactate concentrations in the assay solution.

**Results:** tACPD (50 µM) did not alter either the oxidative or glycolytic metabolism of glucose. Glutamate (500 µM) increased [¹⁴C]deoxyglucose phosphorylation without affecting the glucose oxidation, while it stimulated [¹⁴C]CO₂ production from [¹⁴C]glutamate, suggesting TCA cycle activation. D-aspartate did, indeed, activate the oxidative metabolism of glucose. A high glucose medium increased lactate production and decreased glucose oxidation in astroglia, but preserved the glutamate-induced CO₂ production.

**Conclusions:** Vasodilatory signals mediated by mGluR might be enhanced by the co-production of CO₂ from glutamate. High glucose environments may affect the basal and function-driven vasodilation by astrocytes.
SYNTHETIC PROSTACYCLINE AGONIST, ONO-1301, AMELIORATE LEFT VENTRICULAR DYSFUNCTION AND CARDIAC FIBROSIS IN CARDIOMYOPATHIC HAMSTERS

Yoichiro Hirata\textsuperscript{a}, Hiroshi Iwata\textsuperscript{b}, Kazuto Nakamura\textsuperscript{c}, Yoshiki Sakai\textsuperscript{d} and Masataka Sata\textsuperscript{a}

\textsuperscript{a}Department of Cardiovascular Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, Japan
\textsuperscript{b}Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo, Japan
\textsuperscript{c}Department of Internal Medicine II, Yamanashi University, Faculty of Medicine, Japan
\textsuperscript{d}Ono Pharmaceutical Co. LTD. Research Headquarters, Japan

\textbf{Background:} Impairment of cardiac function in cardiomyopathy has been postulated to be related to decrease blood flow and increased collagen synthesis. Administration of growth factors has been shown to attenuate left ventricular (LV) remodeling and dysfunction in animal models of dilated cardiomyopathy. We previously reported that ONO-1301, a synthetic prostacyclin agonist with thromboxane-synthase inhibitory activity, promotes production of hepatocyte growth factor and vascular endothelial factor from various cell types.

\textbf{Methods and Results:} We evaluated therapeutic efficacy of ONO-1301 in the Syrian hamster (TO-2), a model of genetically determined dilated cardiomyopathy. Either vehicle, ONO-1301 (orally twice a day, 1 mg/kg/day), or ONO-1301PLGA•MS, a slow releasing form of ONO-1301 (subcutaneously every 3 weeks, 10 mg/kg), was administered to TO-2 hamsters from 24 to 32 weeks of age (n=12 for each group). Echocardiographic study demonstrated that LV ejection fraction was significantly improved in the ONO-1301 group (23±3\%, p<0.01) or ONO-1301 PLGA group (25±4\%, p<0.01) compared with that in the vehicle group (19±2\%). Azan-Mallory staining demonstrated that cardiac fibrosis was significantly reduced by ONO-1301 (p<0.01) or ONO-1301PLGA•MS (p<0.05).

\textbf{Conclusion:} ONO-1301 improves LV dysfunction and reduced cardiac fibrosis in the hamster model of dilated cardiomyopathy. ONO-1301 might hold a therapeutic potential in the treatment of dilated cardiomyopathy.
EXTRACORPOREAL CARDIAC SHOCK WAVE THERAPY AMELIORATES LEFT VENTRICULAR REMODELING AFTER MYOCARDIAL ISCHEMIA-REPERFUSION INJURY IN PIGS IN VIVO

Yoshitaka Ito, Kenta Ito, Takashi Shiroto, Ryuji Tsuburaya, Jun Yi Gao, Yoku Kikuchi, Kentaro Aizawa, Morihiko Takeda, Satoshi Yasuda and Hiroaki Shimokawa

Department of Cardiovascular Medicine, Tohoku University, Japan

**Background:** We have previously demonstrated that low-energy extracorporeal cardiac shock wave (SW) therapy induces angiogenesis and improves cardiac function in a porcine model of chronic myocardial ischemia and that of acute myocardial infarction with permanent coronary ligation. However, in the clinical setting, most of the patients with AMI receive reperfusion therapy. In this study, we thus examined whether our SW therapy ameliorates LV remodeling after myocardial ischemia-reperfusion (I/R) injury in pigs.

**Methods:** Sixteen pigs were subjected to myocardial ischemia (90 min occlusion of the left anterior descending coronary artery) and reperfusion using a balloon catheter. Three hours after the reperfusion, they were randomly assigned to the 2 groups with or without the SW therapy to the ischemic border zone (n=8 each) on day 1, 3 and 5.

**Results:** Four weeks after I/R, LV end-diastolic volume (LVEDV), LV end-diastolic pressure (LVEDP) and LV ejection fraction (LVEF) were markedly deteriorated in the control group, but were significantly ameliorated in the SW group (LVEDV, 130±9 vs. 100±7 ml; LVEDP, 11±2 vs. 4±1 mmHg; LVEF, 28±2 vs. 36±3 %, P<0.05). Furthermore, wall thickening fraction (15±2 vs. 24±4 %), increase in regional myocardial blood flow (-0.06±0.11 vs. 0.36±0.13 ml/min/g), capillary density (1227±48 vs. 1614±50 /mm²) and endothelial nitric oxide synthase activity (0.24±0.03 vs. 0.41±0.05) in the ischemic border zone were all increased in the SW group compared with the control group (all P<0.05).

**Conclusions:** These results indicate that our SW therapy also is effective to ameliorate LV remodeling after myocardial I/R injury in vivo.
EXOGENOUS ERYTHROPOIETIN PROTECTS LEFT VENTRICLE AGAINST PRESSURE OVERLOAD-INDUCED DYSFUNCTION IN MICE

Wanting Wang\textsuperscript{a}, Yutaka Kagaya\textsuperscript{b}, Yasuhide Asaumi\textsuperscript{a}, Shigefumi Fukui\textsuperscript{a}, Morihiko Takeda\textsuperscript{a} and Hiroaki Shimokawa\textsuperscript{a}

\textsuperscript{a}Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan
\textsuperscript{b}Graduate Medical Education Center, Tohoku University Hospital, Japan

\textbf{Background:} Erythropoietin (Epo) receptors (EpoRs) are expressed in the heart. We have recently demonstrated that endogenous Epo-EpoR system plays an important protective role in pressure overload-induced left ventricular (LV) dysfunction in mice. In the present study, we tested our hypothesis that exogenous Epo also elicits a protective effect on LV with pressure-overload.

\textbf{Methods and Results:} Mice were subjected to transverse aortic constriction (TAC) or sham operation. They were randomly assigned to either the treatment with vehicle (TAC, n=19) or recombinant human Epo (2000 u/kg twice a week) (TAC-Epo, n=21) 24 hours after the surgery. Treatment with Epo resulted in a significant increase in hematocrit as compared with sham and TAC groups (69% vs. 43% and 45%, respectively; P<0.001 for each). The survival rate of TAC-Epo group was significantly increased compared with that of TAC group during the 8-week treatment period (82% vs. 47%, P<0.05). Post-mortem examination revealed severe pulmonary congestion in most of the mice that had died within 8 weeks of the treatment in both TAC groups. Echocardiography at 8-week of the treatment revealed that both LV end-diastolic and end-systolic diameters of TAC-Epo were significantly decreased (P<0.05), and LV fractional shortening was significantly increased as compared with TAC (P<0.05). Cardiac catheterization performed at 8 weeks of the treatment demonstrated that both LV +dp/dt max and LV -dp/dt min tended to be improved in TAC-Epo as compared with TAC (P=0.08 and 0.07, respectively).

\textbf{Conclusion:} These results suggest that exogenous EPO protects LV against pressure overload-induced dysfunction in mice.
TRANSCRIPTOMIC ANALYSIS FOR CARDIAC HYPERTROPHY

Masaharu Nakayama, Naomi Yamaki, Morihiko Takeda, Yasuhide Asaumi, Tatsuya Komaru and Hiroaki Shimokawa

Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan

Background: Our aim is to identify the transcriptional activity that is specific to a stimulus inducing cardiac hypertrophy as an early phase of heart failure.

Methods and Results: We used two different models of cardiac hypertrophy in mice, including pressure overload by banding of the transverse aorta (TAC) and neurohormonal activation by angiotensin II infusion (AngII). One week after the operation, the expression profiling in the left ventricles was performed by microarray analysis in the both models. We extracted 425 significant up-regulated genes in the TAC model and 12 in the AngII model, respectively. We next collected the promoter sequences of these genes from DBTSS and searched motifs of transcription factor binding sites retrieved from the TRANSFAC database. We identified significantly frequent transcription factor binding sites in the promoter regions of the co-regulated genes in each model (P <0.05, binomial probability). Twenty binding sites, including AP4, were identified in the TAC model whereas four sites, including Evi1, were in AngII model. GATA binding sites were noted in the both models. Finally, we directly injected a firefly luciferase vector plasmid containing each selected binding site into the left ventricle in both models. During the progression of cardiac hypertrophy, we were able to confirm the enhancement of the transcriptional activity by using the in vivo imaging system (IVIS) in living mice during the progression of cardiac hypertrophy.

Conclusion: Our novel approach is useful to identify the unique transcription factors, in order to characterize different models of cardiac hypertrophy.
ENDOTHELIN-1 LEVELS IN CHRONIC CONGESTIVE HEART FAILURE

Minoru Ohmae

Department of Cardiology, Kochi General Rehabilitation Hospital, Japan

Background: Endothelin-1 levels in patients with chronic congestive heart failure were evaluated with respect to the severity of cardiac dysfunction. The relationship among neurohormones (BNP, HANP, and endothelin-1) was evaluated.

Methods: 39 patients (16 males and 23 females, aged 64-98 years old) with chronic congestive heart failure were studied. In each patient, echocardiography was recorded, and the ejection fraction was calculated. Titers of HANP, BNP, and endothelin-1 in serum were measured. Exclusion criteria consisted of acute myocardial infarction, unstable angina, or renal dysfunction (serum creatinine < 2.5 mg/dl).

Results: Endothelin-1 levels were correlated with HANP levels (r = 0.70, p < 0.0001) and BNP levels (r = 0.57, p < 0.0001). Endothelin-1 levels were not correlated with the LV ejection fraction nor end-diastolic volume.

Conclusion: Endothelin-1 levels were correlated with HANP and BNP levels. These findings indicate that endothelin-1 interacts with HANP and BNP in patients with chronic congestive heart failure.
UREA AND MANGANESE AS CARDIOVASCULAR β-ADRENOCEPTORS ANTAGONISTS IN VIVO SUPPORTING DATA IN ANIMAL ORGANISM

Nikolai Dimitrov Temnyalov

Preclinical and Clinical Pharmacology, Medical University - Varna, Bulgaria

The chief purpose of this paper is to give further data - mainly in support of already described capability of urea and manganese to antagonize β-adrenergic receptors in cardiovascular system.

Experiments were performed on anaesthetized cats, mongrel dogs and Wistar rats, all of male origin (in Faradey cage). After single urea 17 mmol/kg infusion - immediately before and at the end of 1st, 2nd, 3rd, 4th and 5th hour have been electronically registered the ISO 1-2-4 nmol/kg i.v. positive chronotropic and vasodepressor responses in adequate series - with a respect to the initial blood pressure level. In similar experimental conditions the influence of manganese dichloride 2 mg/kg intraperitoneally has been investigated in different series - before and after it.

Results obtained show that both urea and manganese antagonize significantly the studied ISO (+)-chronotropic and vasodepressor effects in dose- and time-dependent manner. The character of antagonism is a nonspecific, nonselective and a noncompetitive in nature.

On conclusion it is clear that both urea and manganese are members of a new antagonizing system, which is presented according our developing concept as an endogenous beta-adrenergic receptor antagonists (EBARA). More precisely it is a nonspecific system - ENBARA. Because of that in RESBARA we include only beta-arrestin 1 and beta-arrestin 2, namely, as relative endogenous specific factors - cytosolic proteins. It is known that the group of R. Lefkowitz first shows that beta-arrestin is co-factor of 5-specific β-adrenergic receptor kinases engaged in the final phosphorylation of “three of four” of agonis-activated β-adrenoceptors.
P9-7

UREA AND MANGANESE AS CARDIOVASCULAR β-ADRENOCEPTORS ANTAGONISTS - IN VITRO PROOFS IN HEART AND VASCULAR TISSUES

Nikolai Dimitrov Temnyalov
Preclinical and Clinical Pharmacology, Medical University - Varna, Bulgaria

The aim is to search urea and manganese - in correspondence of cardiovascular data could modify alpha- and beta-adrenergic receptor mediated responses.

All experiments performed on rabbit thoracic aorta and pulmonary artery and rat thoracic aorta rings or portal vein and guinea pig atria. Urea 5 and 50 mM and manganese 0.5 and 5 mM solved ex tempore in PSS, is added prior and after control series with ISO 1-10 nM or phenoterol 5 -50 mM. cDRC registered (“Gemini”, Ugo Basile, Italy) isometrically.

Results show:
2. Both urea and Mn antagonize adrenaline positive inotropic effects.
3. The character of the antagonism of urea and of Mn is nonspecific, nonselective and noncompetitive - using method of Linewiver-Burk with double-reciprocal plots.
4. According to recommendations of R. Furchgot calculating KB value in the presence of 5 mM urea and comparatively with 2 nM butoxamine (selective-β-2-adrenergic blocker) the stability of ISO-vascular relaxation diminish significantly.
5. However, the stability of the complex of noradrenaline with alpha-adrenergic receptor in the presence of 5mM urea and comparatively with phentolamine 500 nM is not changed significantly, keeping in mind that the stable value of phentolamine in this concentration is between 60 to 90 min. exposure time in agreement with R. Furchgott data.

On concluding - it is necessary to be underlined that both endogenous urea and Mn in concentrations, corresponding to physiological and pathophysiological ones are able to antagonize nonspecifically and non selectively cardiac and vascular adrenergic responses.
POLYMORPHISMS OF CYP4A11, A PRODUCING ENZYME OF 20-HETE, ARE ASSOCIATED WITH HYPERTENSION

Ken Sugimoto, Hiroshi Akasaka, Tomohiro Katsuya, Osamu Yasuda, Tomomi Fujisawa, Kazuaki Shimamoto and Hiromi Rakugi

Geriatric Medicine, Osaka University Graduate School of Medicine, Japan
The Second Department of Internal Medicine, Sapporo Medical University, Japan

Introduction: CYP4A11 oxidizes arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE), a metabolite with renovascular and tubular function. 20-HETE plays a dual role in the regulation of blood pressure; prohypertensive and antihypertensive. A previous study demonstrated a significant association between the CYP4A11 gene (CYP4A11) polymorphism and hypertension. However, the precise mechanism of the association has not been clarified. To assess the involvement of CYP4A11 in the pathogenesis of hypertension, we sought to identify a functional polymorphism of CYP4A11 and examined its impact on predisposition to hypertension in the Tanno-Sobetsu Study.

Methods or Results: The -845A/G polymorphism was identified in the promoter region of CYP4A11 by direct sequencing. Luciferase expression driven by the promoter of CYP4A11 containing the wild-type -845GG genotype was 30% lower than expression with the variant -845AA genotype. Gel mobility shift assays with nuclear protein extracts showed specific binding to probes containing the variant -845GG. To assess the effect of CYP4A11 polymorphisms on hypertension, we also carried out a case-control study using four SNPs (-845A/G, -366C/T, 7119C/T, and 8590T/C) in the Tanno-Sobetsu Study. The odds ratio (OR) for hypertension in participants with the AG+GG genotype of -845A/G was 1.42 (P=0.008). The haplotype-based case-control analysis using four SNPs revealed a significant haplotype (G-C-T-T) that was significantly associated with hypertension: OR=1.44 (P=0.006).

Conclusion: We have identified a functional variant (-845A/G) of CYP4A11 that is significantly associated with hypertension. Further investigations will be needed to clarify whether this mutation is involved in renal vasodilation or inhibition of sodium reabsorption in renal tubules.
P10-2
RETINA DERIVED RELAXATIONS ARE MAINTAINED IN CAROTID AND MESENTERIC ARTERIES OF L-NAME-INDUCED HYPERTENSIVE RATS

Fulya Gezerler\textsuperscript{a}, Selcuk Takir\textsuperscript{a}, F. Ilkay Alp\textsuperscript{a}, Bulent Ergin\textsuperscript{a}, Cihan Demirci\textsuperscript{b}, Oaman Ozdemir\textsuperscript{c} and B. Sonmez Uydes-Dogan\textsuperscript{a}

\textsuperscript{a}Department of Pharmacology, Faculty of Pharmacy, Istanbul University, Turkey
\textsuperscript{b}Department of Biology, Faculty of Science, Istanbul University, Turkey
\textsuperscript{c}Sanovel Pharmaceutical Company, Turkey

Retinal relaxing factor (RRF) is a novel transferable factor released from the retinal tissue and suggested to involve in the regulation of retinal arterial tone. It’s nature and mechanism of action as well as effectiveness in pathological conditions affecting retinal circulation is still unknown. Herein, we aimed to investigate whether hypertension influences RRF response by determining its reactivity on rat carotid and mesenteric arteries. Hypertension was induced by giving NO synthase inhibitor, L-NAME (daily intake of 60mg/kg in drinking water) to male Wistar rats (200-250g) for 5-6 weeks. Thereafter, isolated carotid and mesenteric arteries of hypertensive and control rats were studied parallely in a wire myograph system. Retinas were placed in close proximity to the precontracted arteries to maintain retinal relaxation. In the arteries of L-NAME hypertensive rats, endothelium-dependent relaxations were significantly reduced whereas, endothelium-independent relaxations remain unchanged compared to control arteries. Placement of retinal tissue on top of carotid and mesenteric arteries produced acute relaxations which generally displayed a biphasic character. Retinas from hypertensive rats elicited comparable relaxations to that of control retinas in both arteries (carotid arteries; hypertensive: 70.26±6.84\%, n=5 vs control: 76.48±3.28\%, n=10, p>0.05 ; mesenteric arteries ; hypertensive: 88.74±2.78\%, n=5 vs control: 89.79±1.78\%, n=10, p>0.05). Our results showed that retinal relaxation is maintained in hypertensive conditions where endothelial reactivity is impaired and suggested that RRF may play a regulatory role in the maintenance of retinal vascular tone in hypertension. The present work was supported by the Research Fund of Istanbul University. Project No. T-3136.
MECHANICAL STRETCH POTENTIATES ANGIOTENSIN II-INDUCED PROLIFERATION IN SPONTANEOUSLY HYPERTENSIVE RAT VASCULAR SMOOTH MUSCLE CELLS

Hirofumi Hitomi\textsuperscript{a}, Gang Liu\textsuperscript{a}, Naohisa Hosomi\textsuperscript{b}, Hideyasu Kiyomoto\textsuperscript{b}, Daisuke Nakano\textsuperscript{a}, Shoji Kimura\textsuperscript{a}, Masakazu Kohno\textsuperscript{b} and Akira Nishiyama\textsuperscript{a}

\textsuperscript{a}Department of Pharmacology, Kagawa University, Japan
\textsuperscript{b}Department of Cardiorenal and Cerebrovascular Medicine, Kagawa University, Japan

Although angiotensin II (Ang II) has been shown to stimulate proliferative and hypertrophic growth in vascular smooth muscle cells (VSMCs), little is known about the effect Ang II on proliferation in the presence of mechanical force. We examined the mechanical stretch regulation of cell proliferation in VSMCs from normatensive and hypertensive rats. VSMCs from the thoracic aorta of 8-week-old WKY rats and SHR grown on a flexible membrane base were stretched by vacuum. Mechanical stretch (5\% or 15\% elongation, 2 h) significantly upregulated AT\textsubscript{1} receptor, EGF receptor, mitogen-activated protein kinase phosphatase-1 protein expression of both SHR and WKY VSMCs, however there was no significant difference between the two cell lines before and after stretch. Meanwhile, mechanical stretch augmented Ang II (100 nM) induced-activation of ERK, MEK and EGF receptor and [\textsuperscript{3}H] thymidine incorporation in SHR, but suppressed them in WKY. Furthermore, the augmentation of Ang II-induced ERK activation and cell proliferation in SHR were blocked by pretreatment with candesartan (100 nM), and PD98059 (10 \mu M), a special inhibitor of MEK. Moreover, pretreatment with an EGF receptor tyrosine kinase inhibitor, AG1478 (25 nM), also blocked the upregulation of Ang II-induced ERK activation by stretch in SHR. We demonstrate for the first time that mechanical stretch directly regulates Ang II-induced mitogenic response in VSMCs and the AT\textsubscript{1}/EGF receptor/ERK dependent signaling pathway is involved in the upregulation of Ang II-induced VSMCs proliferation in SHR. These findings provide new insights into the signaling mechanisms whereby angiotensin II exerts its growth-promoting effects on the vasculature.
NEW ADDITIONAL PROGNOSTIC FACTORS OF PULMONARY HYPERTENSION -LESSONS FROM LONG-TERM FOLLOW-UP STUDY-

Yutaka Miura, Yoshihiro Fukumoto, Makoto Nakano, Kimio Satoh, Kohichiro Sugimura, Minako Oikawa and Hiroaki Shimokawa

Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan

Background: Pulmonary hypertension (PH) still remains a serious disease, for which several factors have been identified as prognostic factors, including plasma levels of brain natriuretic peptide (BNP) and uric acid (UA), hemodynamic values (cardiac output, CO and right atrial pressure, RAP), 6 minutes walk distance (6MWD), and WHO functional class. In this study, we aimed to identify new additional prognostic factors of PH in our relatively large cohort.

Methods: Our cohort consisted of 139 consecutive PH patients (34 male, 105 female) who admitted to our hospital from July 1974 to August 2008 with a long-term follow-up.

Results: During the follow-up (mean 68.1 months), 47 patients died of cardiopulmonary causes. Among the complications, thyroid dysfunction was most common (40%). Although patients with thyroid dysfunction had higher levels of BNP than in those without it (450 ±120 vs. 177±39 pg/mL, P<0.05), thyroid dysfunction itself did not have prognostic impact. However, low CO at the diagnosis was a poor prognostic factor for the death within one year after the diagnosis (cardiac index (CI) 1.84±0.22 vs. 2.65±0.08 L/min/m², P<0.01). Importantly, patients with low CO at the diagnosis (CI<2.0) but with a subsequent normalization after the treatment (CI>2.5) survived significantly longer than those with normal CO at the diagnosis (CI>2.5) (115.3±47.2 vs. 59.7±10.1 months, P<0.05). Furthermore, in the 39 PAH patients, dilated main pulmonary artery (PA) diameter (>36 mm) was significantly correlated with better prognosis (P<0.05).

Conclusion: These results suggest that CO normalization and PA dilatation during long-term treatment are new additional prognostic factors for PH.
ROLE OF SOLUBLE EPOXIDE HYDROLASE IN FLOW-INDUCED DILATIONS OF MOUSE MESENTERIC ARTERIES

Kathryn M Gauthier\textsuperscript{a}, Yuttana Chawengsub\textsuperscript{a}, Darryl C. Zeldin\textsuperscript{b} and William B. Campbell\textsuperscript{a}

\textsuperscript{a}Pharmacology and Toxicology, Medical College of Wisconsin, USA
\textsuperscript{b}National Institutes of Health, National Institute of Environmental Health Sciences, USA

Epoxide containing metabolites of arachidonic acid (AA) including the cytochrome 450 metabolites, the epoxyeicosatrienoic acids (EETs), and the lipoxygenase metabolites, the hydroxyepoxyeicosatrienoic acids (HEETAs), are substrates for soluble epoxide hydrolase (sEH). In many vascular beds, sEH hydrolysis of EETs results in the formation of less vasoactive dihydroxy metabolites. However, the vascular consequence of sEH hydrolysis of HEETAs to the trihydroxyeicosatrienoic acids (THETAs) remains poorly defined. We examined flow-induced dilations of mesenteric arteries from male wild-type (WT) and sEH knockout (KO) mice. Second order mesenteric arteries were cannulated, pressurized (60 mmHg) and constricted with U46619 (50-400 nM) in the presence of indomethacin (10 \textmu M). Stepwise increases in flow from 0 to 29 ul/min increased internal diameter 38\% (97±11 to 139±14 m\mu) in arteries from WT mice and 55\% (99±16 to 170 ±10 m\mu, P<0.05 vs WT) in arteries from sEH KO mice. Using reverse phase-HPLC, arterial \textsuperscript{14}C-AA metabolites from WT and KO mice co-migrated with HEETAs, THETAs and hydroxyeicosatetraenoic acids (HETEs). EET synthesis was not detected. In \textsuperscript{14}C-AA incubations of WT arteries, treatment with the sEH inhibitor AUDA (1 \textmu M) increased the formation of the HEETAs. Western blot confirmed sEH expression in arteries from WT mice but not in arteries from KO mice. These results suggest that HEETA hydrolysis by sEH represents an inactivation mechanism to decrease vasodilation in mouse arteries.
P10-6
ENDOTHELIUM-DEPENDENT VASODILATION IN AORTA FROM STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS MADE HYPERGLYCEMIA WITH STREPTOZOCIN

Hong Chen, Mei-Fang Zhong and Wei-Li Shen
Department of Pharmacology, Shanghai Jiao Tong University School of Medicine, China

Background: Sustained hypertension is detrimental to endothelium-dependent vasodilation but the effect of hyperglycemia is controversial. This experiment was designed to examine the influence of hyperglycemia on aortic endothelium-dependent vasodilation in normal and hypertensive rats.

Methods: Stroke-prone spontaneously hypertensive rats (SHR-SP) at age of 3 months and age-matched control Wistar-Kyoto rats (WKY) were injected with streptozocin (60 mg/kg) to induce hyperglycemia or injected with solvent as controls. Aorta was isolated 12 weeks after hyperglycemia and endothelium-dependent relaxation was examined. Aortic nitric oxide synthase (NOS) and heme oxygenase (HO) were examined with Western blotting. Aortic endothelial morphology was observed with transmission electron microscopy.

Results: While aorta from SHR-SP demonstrated significantly impaired endothelium- and non-endothelium-dependent relaxation, aorta from hyperglycemic WKY showed no significant impairment of relaxation. Endothelium-dependent relaxation in SHR-SP made hyperglycemia by streptozocin showed a paradoxical enhancement in endothelium-dependent relaxation as compared with SHR-SP without hyperglycemia. There was significant increase of catalase, endothelial NOS and HO in endothelium layers of both SHR-SP and hyperglycemic WKY aorta. Aortic endothelium of SHR-SP but not hyperglycemic WKY showed increased mono-phagocytes infiltration.

Conclusions: SHR-SP had significantly impaired endothelium-dependent relaxation and endothelium inflammation. Hyperglycemia did not impair endothelium-dependent relaxation whether combined with hypertension or not.
BH4 is a major cofactor required for NOS3 activity. When deficient, NOS3 becomes “uncoupled” and generates superoxide instead of NO. These changes are observed together with an increase in intracellular Ca2+ in aged endothelial cells. The question was addressed whether supplementation of BH4 may improve NOS3 activity and calcium homeostasis in dysfunctional porcine aortic endothelial cells obtained in vitro by successive passages for one month, where intracellular BH4 is reduced.

Under resting conditions, NOS3 expression was unchanged while phospho-Ser1177-NOS3 and NOS3 activity were reduced in aged cells. After BK stimulation, the calcium response and the NOS3 activity were decreased in aged cells. Exogenous BH4 at 10μM, inducing a two fold increase in its cellular bioavailability, improved basal NOS3 activity by 58%. It also partially restored the BK-induced calcium response but failed to normalize the BK-stimulated NOS3 activity. Because exogenous NO (DETA NONOate) was also able to normalize the calcium response under stimulated conditions, by activating SERCA pumps and thus refilling intracellular calcium stores, it is speculated that the effects of BH4 supplementation on cytosolic calcium are a consequence of the increased NO production.

Therefore, strategies that would increase endogenous levels of BH4 in dysfunctional aged cells, as well as in many cardiovascular diseases, thus preventing the decrease in NO, should normalize calcium dependent functions.
PROSTACYCLIN ANALOGS RAPIDLY INDUCE NITRIC OXIDE PRODUCTION THROUGH ENDOTHELIAL NITRIC OXIDE SYNTHASE PHOSPHORYLATION IN VASCULAR ENDOTHELIAL CELLS

Masataka Kudo\textsuperscript{a}, Akira Sugawara\textsuperscript{b}, Akiko Saito\textsuperscript{c}, Fumitoshi Satoh\textsuperscript{a}, Akira Uruno\textsuperscript{b} and Sadayoshi Ito\textsuperscript{a}

\textsuperscript{a}Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Medicine, Japan
\textsuperscript{b}Department of Advanced Biological Sciences for Regeneration, Tohoku University Graduate School of Medicine, Japan
\textsuperscript{c}Department of Pediatrics, Tohoku University Graduate School of Medicine, Japan

Introduction: Prostacyclin (PGI2), a prostanoid derived from endothelium, demonstrates anti-atherosclerotic effects including platelet aggregation inhibition, vasoconstriction, and antagonizing to thromboxane A2. PGI2 and nitric oxide (NO) are both endothelium-derived relaxing factors (EDRFs) with similar vascular actions. Although PGI2 has recently been reported to stimulate endothelial NO synthase (eNOS) gene transcription in long-term, little is known regarding mutual interaction between PGI2 and NO in short-term.

Methods: We examined rapid effects (0-60min) of PGI2 analogs (beraprost, iloprost, and carbaprostacyclin) on eNOS phosphorylation and NO production in various doses using bovine aortic endothelial cells (BAECs).

Results: Short-term (30 min) treatment with PGI2 analogs induced cAMP production in BAECs. Thereafter, a significant (~200%) increase of intracellular cGMP as a result of NO production was observed. Moreover, PGI2 analogs rapidly induced eNOS phosphorylation (Ser-1179) (5~10 min) without changing its protein expression. We next examined prostacyclin signals for the PGI2 analogs-induced eNOS phosphorylation. PGI2 analogs did not affect Akt phosphorylation (Ser-473). Moreover, disruption of endogenous Akt using siRNA did not affect the eNOS phosphorylation. In contrast, pre-treatment with either adenylate cyclase inhibitor SQ-22536, cAMP antagonist Rp-cAMP, or protein kinase A (PKA) inhibitor H89 almost completely inhibited the eNOS phosphorylation. Disruption of endogenous PKA c-\(\alpha\) using siRNA significantly (-80%) decreased the eNOS phosphorylation.

Conclusion: PGI2 analogs induce eNOS phosphorylation through cAMP/PKA pathway, but not through Akt pathway. Our study thus first demonstrated the rapid effects of PGI2 on NO production/eNOS phosphorylation. PGI2 analogs may therefore be useful for cardiovascular disorders complicated with endothelial damages and/or dysfunctions.
PLASMA TETRAHYDROBIOPTERIN / DIHYDROBIOPTERIN RATIO: A POSSIBLE MARKER OF ENDOTHELIAL DYSFUNCTION

Masafumi Takeda, Tomoya Yamashita, Masakazu Shinozaka, Kenji Nakajima, Naoto Sasaki, Ken-ichi Hirata and Seinosuke Kawashima

Department of Cardiology, Kobe University, Japan
Osaka Saiseikai Nakatsu Hospital, Japan

Background: Although endothelium-dependent vasodilatation has been used as a marker of endothelial dysfunction (ED), there have been no reliable plasma markers for ED. Oxidative stress, which is a major determinant of ED, oxidizes tetrahydrobiopterin (BH4), an essential cofactor of eNOS, and resulted in relative deficiency of BH4.

Method and Results: In 163 patients with cardiovascular disorders, we measured plasma levels of BH4 and BH2 by HPLC and compared them with flow-mediated vasodilatory response of the brachial artery (FMD) measured by ultrasonography. The effects of atorvastatin on plasma pteridine levels and FMD were examined in patients with multiple coronary risk factors.

There was a positive relationship between FMD and plasma BH4 levels and a negative relationship between FMD and plasma BH2 levels. Subsequently, we found a strong positive relation between FMD and BH4/BH2 ratio (r=0.585, p<0.0001). Although we didn’t find significant relations between pteridine levels and individual traditional risk factors, BH4/BH2 ratio in patients with risk factors more than 2 showed significant reductions compared with that in those without risk factors. Statin treatment improved FMD in association with an increase in plasma BH4/BH2 ratio.

Conclusion: Plasma pteridine levels were associated with endothelial dysfunction in cardiovascular disorders.
CHARACTERIZATION OF VASCULAR FUNCTION IN MICE LACKING ENTIRE NITRIC OXIDE SYNTHASE SYSTEM

Osamu Suda\textsuperscript{a}, Masato Tsutsui\textsuperscript{b}, Hiroaki Shimokawa\textsuperscript{c}, Sei Nakata\textsuperscript{d}, Tsuyoshi Morishita\textsuperscript{d}, Ken Sabanai\textsuperscript{b}, Nobuyuki Yanagihara\textsuperscript{b} and Yutaka Otsuji\textsuperscript{d}

\textsuperscript{a}Health Care Center Central Japan Railway Company, Japan
\textsuperscript{b}Department of Pharmacology, School of Medicine, University of Occupational and Environmental Health, Japan
\textsuperscript{c}Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan
\textsuperscript{d}Second Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Japan

Background: We have recently succeeded in developing mice deficient in the entire nitric oxide synthase system (triply nNOS/iNOS/eNOS-KO mice). The present study was designed to characterize vascular function of those mice.

Methods and Results: Experiments were performed in isolated aortas of 2-month-old wild-type and triply-KO mice (n=5-7). Vascular function was examined by isometric force recording. Relaxations to forskolin ($10^{-9}$ to $10^{-5}$ mol/L), which is an activator of adenylate cyclase, were comparable between the two genotypes. In contrast, relaxations to diethylamine NONOate ($10^{-9}$ to $10^{-5}$ mol/L), which is a NO donor, were significantly more enhanced in the triply-KO than in the wild-type mice ($P<0.05$), suggesting hypersensitivity to exogenous NO in the vasculature of the triply-KO mice. Importantly, relaxations to acetylcholine ($10^{-9}$ to $10^{-5}$ mol/L), which is an endothelium-dependent vasodilator, were completely lacking in the triply-KO mice ($P<0.05$), and contractions to phenylephrine ($10^{-9}$ to $10^{-5}$ mol/L), which is an $\alpha_1$ adrenergic receptor agonist, were markedly potentiated in the triply-KO than in the wild-type mice ($P<0.05$), indicating that vascular reactivity of the triply-KO mice is contraction-predominant.

Conclusions: These results provide the first evidence that genetic disruption of the whole NOS system results in hypersensitivity to exogenous NO, loss of endothelium-dependent relaxation to acetylcholine, and hypercontractility to phenylephrine in mice, demonstrating the critical role of the endogenous NOS system in regulating vascular tonus.
BLOCKAGE OF NOS EXAGGERATES OXYGEN EXTRACTION OVER BLOOD FLOW REDUCTION IN HUMAN SKELETAL MUSCLE AS MEASURED DIRECTLY WITH PET

Ilkka Heinonen\textsuperscript{a}, Bengt Saltin\textsuperscript{b}, Jukka Kemppainen\textsuperscript{a}, Vesa Oikonen\textsuperscript{c}, Juhani Knuuti\textsuperscript{c}, Pirjo Nuutila\textsuperscript{d}, Kari Kalliokoski\textsuperscript{c} and Ylva Hellsten\textsuperscript{e}

\textsuperscript{a}Turku PET Centre & Clinical Physiology and Nuclear Medicine, University of Turku, Finland
\textsuperscript{b}Copenhagen Muscle Research Center, University of Copenhagen, Copenhagen, Denmark
\textsuperscript{c}Turku PET Centre, University of Turku, Finland
\textsuperscript{d}Turku PET Centre & Department of Medicine, University of Turku, Finland
\textsuperscript{e}Department of Exercise and Sport Sciences, Section of Human Physiology, University of Copenhagen, Denmark

In vitro studies suggest that nitric oxide (NO) strikingly inhibits mitochondrial respiration. Human studies however oppose this view. We applied the most natural mean, muscular contractions, to enhance the need to increase muscle oxygen consumption and blood flow (BF), and measured these parameters in eight healthy young men directly from human thigh skeletal muscles by Positron Emission Tomography (PET). Muscle BF and differences in femoral arterial-venous oxygen content were measured at rest and during exercise in control situation and alone under L-NMMA infusion or in combination with indomethacin to inhibit the synthesis of NO and cyclo-oxygenase (COX), respectively. NOS blockade that reduced thigh muscle BF by \~35 \% at rest had in line with majority of previous studies any effect on BF during exercise, but combined NOS and COX blockade that reduced thigh muscle BF by \~44 \% at rest decreased BF significantly and solely in exercising muscle by \~13 \%. On the other hand, NOS blockade alone increased oxygen extraction fraction at rest by \~96 \% and during exercise by \~9 \% and double blockade by \~105 \% at rest and \~26 \% during exercise. Muscle oxygen consumption was however increased (\~22 \%) only during L-NMMA infusion at rest (p = 0.01). In conclusion, blockade of NOS exaggerates oxygen extraction over blood flow reduction in resting human skeletal muscle as measured directly within the muscle by PET suggesting that NO indeed has potential to directly alter mitochondrial respiration of the major oxygen consuming tissue of the human leg.
EFFECT OF CHRONIC TREATMENT WITH ATORVASTATIN ON NITRIC OXIDE SYNTHASE, RHO-KINASE AND AKT IN THE KIDNEY OF SPONTANEOUSLY HYPERTENSIVE RATS

Daisuke Ito, Osamu Ito, Naoyoshi Mori, Yoshikazu Muroya, Peng-Yu Cao, Kenta Takashima, Masayuki Kanazawa and Masahiro Kohzuki

Department of Internal Medicine and Rehabilitation Science, Tohoku University Graduate School of Medicine, Japan

**Background:** HMG-CoA reductase inhibitors, statins improve endothelial dysfunction by up-regulating the expression and the activity of nitric oxide synthase (NOS). However, the effect of statins on NOS in the kidney remains to be elucidated. Thus, the aim of this study was to examine chronic treatment with atorvastatin (ATV) on NOS, Rho-kinase (ROCK) and Akt in the kidney of spontaneously hypertensive rats (SHR).

**Method:** Five-week old, male SHR were divided into a control group and an ATV group and given vehicle or ATV (20 mg/kg) for 8 weeks. The expression of NOS isoforms was determined by Western blot, reverse transcription-polymerase chain reaction (RT-PCR). The activity of ROCK and Akt was also determined by the phosphorylation of ezrin-radixin-moesin (ERM, Thr567-564-558) and Akt (Ser473).

**Results:** The systolic blood pressure was lower in the ATV group. The expression of endothelial NOS (eNOS) protein was elevated by in the cortex and the medulla of the ATV group. The expression of neuronal NOS (nNOS) protein was also elevated in the medulla of the ATV group. The expression of inducible NOS (iNOS) protein was not different among the groups. The mRNA levels of eNOS and nNOS were not different among the groups. The activity of ROCK was decreased and the activity of Akt was increased in the kidney of ATV group.

**Conclusions:** Chronic treatment with ATV increases the expression of eNOS and nNOS at the protein levels with the suppression of ROCK and the activation of AKT in the kidney of SHR.
P12-2
WITHDRAWN
ATORVASTATIN REDUCES SYMPATHETIC NERVE ACTIVITY THROUGH THE INHIBITION OF RAC/NAD(P)H OXIDASE AND UPREGULATION OF Mn-SOD IN BRAIN

Takuya Kishi, Yoshitaka Hirooka and Kenji Sunagawa

Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Japan

We demonstrated that oxidative stress in rostral ventrolateral medulla (RVLM) increases sympathetic nerve activity (SNA) and that oral administration of atorvastatin decreases SNA through the anti-oxidant effect in RVLM of stroke-prone spontaneously hypertensive rats (SHRSP). The aim of the present study was to clarify the mechanism by which atorvastatin reduces oxidative stress in RVLM of SHRSP. In SHRSP, continuous intracerebroventricular infusion of atorvastatin (2 µg/kg/day) (ATROVA) for 14 days significantly reduced systolic blood pressure, heart rate (-48±4 mmHg, -56±7 bpm, n=5 for each, P<0.01), and SNA (-28±3 %, n=5, P<0.01) in comparison with vehicle infusion (CNT). In the RVLM, ATROVA decreased oxidative stress, Rac1 and NAD (P) H oxidase activity. ATROVA also significantly lowered the expression of Rac1, gp91phox, p22phox in membrane fraction, and p40phox, p47phox and p67phox in cytosolic fraction. In contrast, the expression level of Rac1 in cytosolic fraction was significantly increased in ATORVA. Furthermore, Mn-superoxide dismutase (SOD) activity was significantly increased in ATORVA, however, that of Cu/Zn-SOD was not different. these results suggest that atorvastatin inhibits membrane translocation of Rac1 and NAD (P) H oxidase subunits and upregulates Mn-SOD activity in the RVLM of SHRSP, which contribute to the inhibition of oxidative stress in the RVLM and the sympato-inhibitory effect.
EFFECT OF PPAR AGONISTS ON RELAXATIONS AND CONTRACTIONS OF THE SHR AORTA

Chen Qu, Susan W.S. Leung, Paul M. Vanhoutte and Ricky Y.K. Man

Department of Pharmacology and Pharmacy, University of Hong Kong, Hong Kong, China

**Background:** Metabolic syndrome is a combination of disorders that increase the risk of developing diabetes and cardiovascular diseases, and is associated with endothelial dysfunction. The present study was designed to determine whether or not activation of peroxisome proliferator-activated receptor (PPAR) improves endothelial dysfunction.

**Methods:** Isometric tension was recorded in isolated thoracic aortic rings with and without endothelium of spontaneously hypertensive rats (SHR) in conventional organ chambers. The effects of Wy14643 (a non-specific PPAR agonist), clofibrate (PPAR\(\alpha\)) and ciglitazone (PPAR\(\gamma\)) were tested.

**Results:** Wy14643 produced endothelium-dependent relaxations but also endothelium-independent relaxations and appeared to involve calcium-activated potassium channels. Endothelium-dependent contractions evoked by acetylcholine in the presence of L-NAME (a nitric oxide synthase inhibitor) were reduced by Wy14643. Wy14643 did not affect contractions produced by KCl, phenylephrine, U46619 (a thromboxane A\(_2\) analogue) and endothelin-1. Reductions of endothelium-dependent contractions were also observed in the presence of clofibrate and ciglitazone.

**Conclusions:** Activation of PPAR produced relaxation. PPAR\(\alpha\) and PPAR\(\gamma\) agonists also reduced endothelium-dependent contractions. Thus PPAR activation may exert protective effect against endothelial dysfunction in SHR and may have beneficial effects in the treatment and/or prevention of vascular complications in patients with metabolic syndrome and/or diabetes.

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PPARγ AUGMENTS ANGIOTENSIN II-INDUCED AT₂ RECEPTOR MEDIATED RELAXATION IN RAT THORACIC AORTA OF HIGH FAT DIET FED RATS

B Viswanad and P Ramarao

Department of Pharmacology & Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), India

Objective: To assess the role of PPARγ on angiotensin II type 2 receptor (AT₂) mediated responses in thoracic aorta of high fat diet (HFD) fed rats.

Methods: The concentration-dependent relaxation response (in the presence of an AT₁ receptor blocker and phenylephrine (PE) precontraction) to angiotensin II (Ang II) and its receptor radioligand binding characteristics were studied in rat thoracic aorta isolated from PPARγ agonist and/or antagonist treated HFD-fed rats.

Results: Ang II-induced relaxation response (% relaxation) was unaltered between control (24%) and HFD-fed rats (25%). Whereas, pioglitazone (10 mg/kg, p. o., 7 days) augmented the relaxation response from HFD (70%) when compared with HFD-fed control (25%) rats. N⁶-nitro-L-arginine methyl ester (L-NAME) (100-300 μmol/l) [a nitric oxide (NO) synthase inhibitor] partially inhibited the relaxation response in pioglitazone treated HFD-fed rats. However, L-NAME (100 μmol/l) and glipizide (1 μmol/l) (ATP sensitive K⁺ channel blockers) together completely blocked the relaxation response. [³H]Ang II saturation binding at the AT₂ receptor was enhanced in aortic membranes from pioglitazone treatment [maximum binding capacity, (Bₘₐₓ) 1.36 ± 0.06 fmol/mg protein] compared to HFD-fed control rats (Bₘₐₓ 0.84 ± 0.04 fmol/mg protein), with no change in the dissociation equilibrium constant (Kₐ) value (2.11 ± 0.14 versus 1.98 ± 0.11 nmol/l). GW9662 (PPARγ antagonist) co-treatment abrogated the pioglitazone effects.

Conclusions: The results suggest enhanced AT₂-receptor density and function [mediated by a nitric oxide and ATP sensitive K⁺ channel-dependent relaxation response (in presence of an AT₁ receptor blocker)] in thoracic aorta isolated from pioglitazone treated HFD-fed rats.
P12-6
ENHANCED MACROPHAGE INFILTRATION IN EPICARDIAL ADIPOSE TISSUE IN PATIENTS WITH CORONARY ARTERY DISEASE.

Yoichiro Hirata\textsuperscript{a}, Toshiyuki Niki\textsuperscript{a}, Kenya Kusunose\textsuperscript{a}, Koji Yamaguchi\textsuperscript{a}, Shusuke Yagi\textsuperscript{a}, Kunihiro Koshiba\textsuperscript{a}, Takashi lwase\textsuperscript{a}, Hirotugu Yamada\textsuperscript{a}, Takeshi Soeki\textsuperscript{a}, Tetsuzo Wakatsuki\textsuperscript{a}, Masashi Akaike\textsuperscript{a}, Hirotugu Kurobe\textsuperscript{b}, Humio Chikugo\textsuperscript{c}, Takaki Hori\textsuperscript{d}, Tetsuya Kitagawa\textsuperscript{b} and Masataka Sata\textsuperscript{a}

\textsuperscript{a}Department of Cardiovascular Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, Japan
\textsuperscript{b}Department of Cardiovascular Surgery, Institute of Health Biosciences, The University of Tokushima Graduate School, Japan
\textsuperscript{c}Department of Cardiovascular Surgery, Tokushima Prefectural Central Hospital, Japan
\textsuperscript{d}Department of Cardiovascular Surgery, Ehime Prefectural Central Hospital, Japan

\textbf{Background:} Infiltration of inflammatory cells has been reported to be predominant in human atherosclerotic lesions. Adipose tissue may function as an endocrine organ that contributes to inflammatory burden in patients at risk of cardiovascular complications. In this study, we immunohistochemically investigated infiltration of inflammatory cells into epicardial and subcutaneous adipose tissues in patients with or without coronary artery disease.

\textbf{Methods and Results:} Pare samples of epicardial and subcutaneous adipose tissues were obtained during elective cardiac surgery (CABG, n=4; non-CABG, n=4). We performed immunohistochemical study for CD68 to determine macrophage infiltration. There was no significant difference in age, BMI, and lipid profiles between the 2 groups. The number of CD68-positive cells in epicardial adipose tissue in the CABG group (170.3 ±40.5 cells/mm\textsuperscript{2}) was more than that in the non-CABG group (60.2±26.7). There was no significant difference in the number of CD68-positive cells in subcutaneous adipose tissue.

\textbf{Conclusion:} This result suggests that macrophage infiltration into epicardial adipose tissue is enhanced in patients with coronary artery disease.
Objective: Hydrogen sulfide (H$_2$S) is known as a suicide gas, but it is identified as an endogenous vasodilator. Recently the activation of ATP-sensitive potassium channels (IKATP) in H$_2$S-induced relaxation was reported. Although many of thiol (SH)-containing substrates causes vasodilatation, the mechanism for activation of IKATP in SH-mediated relaxation is unknown. In this study, we characterized metabolic changes in SH-induced relaxation using vascular metabolome analysis.

Methods and results: Aortic rings from rats were equipped with organ chamber for the isometric-tension measurement. After the constriction with phenylephrine, aortic rings were relaxed with a cysteine (Cys) donor, N-acetylcysteine (NAC: 0.3-10 mM) or a H$_2$S donor, NaHS (0.1-1.0 mM). A CSE inhibitor, propargylglycine (PAG: 10 mM) decreased NAC-induced relaxation suggesting the participation of H$_2$S. Neither a NO synthase inhibitor, L-NAME (100-200 μM) nor a guanylyl cyclase inhibitor, ODQ (10 μM) inhibited the relaxations to NAC/NaHS. An IKATP inhibitor, glybenclamide (GLB: 10 μM), and a sarcoplasmic reticulum Ca$^{2+}$ ATPase (SERCA) inhibitor, thapsigargin (10 μM), prolonged relaxation half time (RHT) to NAC/NaHS. An AMP-activated kinase inhibitor, compound C (10 μM) and catalase (3,000U) prolonged RHT to NaHS. Aortic metabolome analysis indicated decreases in intravascular branched chain amino acids levels and increases in AMP levels with H$_2$S.

Conclusion: SH-mediated relaxation was not involved with NO/cGMP but partially with the activation of IKATP and SERCA. In addition, AMP-kinase, and H$_2$O$_2$ may contribute to H$_2$S-induced relaxation. An increase in vascular AMP levels with the energy consumptions can contribute to SH-mediated relaxation.
C-type natriuretic peptide (CNP) is a vasodilator peptide produced by endothelial cells. Nitric oxide (NO), a major endothelium-derived mediator, reduces the relaxation to CNP in arteries of different animal models. The present experiments were designed to determine whether or not NO modulates the relaxation to CNP in coronary arteries. Rings (with and without endothelium) of porcine coronary arteries were suspended in organ chambers for isometric tension recording. Concentration-relaxation curves to CNP were obtained during contractions to prostaglandin F2α or endothelin-1. Experiments were performed in the absence or presence of the inhibitor of NO synthase ω-nitro-L-arginine methyl ester hydrochloride (L-NAME), the NO donor sodium nitroprusside (SNP), the inhibitors of soluble guanylyl cyclase ODQ and NS2028, or the cell permeable analog of cyclic GMP (cGMP) 8-Br-cGMP. Endothelial denudation significantly potentiated CNP-induced relaxations. In rings with, but not in those without endothelium, L-NAME potentiated the CNP-induced relaxations. Conversely, SNP inhibited the CNP-induced relaxation only in the absence of endothelium. ODQ and NS2028 inhibited the relaxation to CNP in rings without endothelium. On the other hand, 8-Br-cGMP enhanced the relaxation to CNP significantly only in arteries without endothelium. These findings demonstrate that, in the porcine coronary artery, CNP is an endothelium-independent vasodilator which activates soluble guanylyl cyclase. Endothelium-derived NO and NO donors inhibit the CNP-induced relaxation.
ROLE OF MYOSIN LIGHT CHAIN PHOSPHATASE IN DEVELOPMENT OF NITRATE TOLERANCE

Yuansheng Gao, Dou Dou, Huijuan Ma and Xiaoxu Zheng

Department of Physiology and Pathophysiology, Peking University Health Science Center, China

Objective: Myosin light chain phosphatase (MLCP) is an important mediator for the actions of nitroglycerin (NTG) and nitric oxide (NO). The present study was to determine the role of MLCP in the development of nitrate tolerance.

Methods: Nitrate tolerance was developed by treatment of C57 mice with subcutaneous injection NTG (20 mg/kg, tid) for 3 days and by incubation of isolated porcine coronary arteries (PCA) with NTG (10-4 M) for 24 hour. The relaxation responses were studied with organ chamber technique and protein levels of MYPT1-LZ, MYPT1 or PP1cδ determined with Western blotting. MLCP activity was determined by measuring the ratio of MLC20-p over MLC20.

Results: Treatment with NTG significantly reduced the response of mice aorta or PCA and the protein levels of MYPT1-LZ, but not that of PP1cδ of these vessels. Incubation with NTG, DETA NONOate or 8-Br-cGMP for 24 hour reduced MYPT1-LZ protein expression of PCA. These effects could be prevented with ODQ or PKG inhibitor. Decrease in MYPT1-LZ expression induced by incubation with NTG or 8-Br-cGMP was prevented with MG-132 (an inhibitor of proteasome), but not cyclohexmide (an inhibitor of protein synthesis). MLCP dephosphorylation of PCA by NTG decreased after incubation with NTG. Decreased MLCP dephosphorylation and relaxation of PCA caused by NTG incubation was partly prevented with MG-132.

Conclusion: Continuous exposure to NTG decreases protein expression of MYPT1-LZ and MLCP activity via NO/cGMP/cGMP-dependent protein kinase pathway, which may contribute to the development of nitrate tolerance.
VASCULAR SMOOTH MUSCLE RELAXATION IN SOLUBLE GUANYLYL CYCLASE β1 HIS 105 PHE MUTANT MICE.

Johan Van de Voorde\textsuperscript{a}, Sofie Nimmegeers\textsuperscript{a}, Kelly Decaluwe\textsuperscript{a}, Rob Thoonen\textsuperscript{b} and Peter Brouckaert\textsuperscript{b}

\textsuperscript{a}Department of Pharmacology, Ghent University, Belgium
\textsuperscript{b}Department of Molecular Biomedical Research, Gent University, Belgium

Binding of NO on the heme group of soluble guanylyl cyclase (sGC) induces vascular smooth muscle relaxation, thereby controlling blood pressure, blood flow and erection. The sGCα1β1 and sGCα2β1 are the physiologically active heterodimers, in which the histidine residue at position 105 of the β1 subunit functions as axial ligand for the heme prosthetic group. Substitution of histidine by phenylalanine abolishes the heme-dependent activation of sGC. This is the case in sGCβ1ki/ki mice from which aortic, femoral artery and corpora cavernosa (CC) segments were mounted for isometric tension recording. In comparison with the preparations isolated from the wild type littersmates, the responses to endogenous NO (released from the endothelium by acetylcholine (ACh)) and exogenous NO (from the NO-donor sodium nitroprusside (SNP)) were abolished in the aorta from the sGCβ1ki/ki mice, but not in the femoral arteries. In CC the relaxation response to ACh (releasing endothelial NO) and electrical field stimulation (releasing neuronal NO) was abolished, while SNP response was only reduced. The response to the NO-independent sGC-stimulator (BAY 41-2272) was also significantly reduced in the different preparations of sGCβ1ki/ki mice, indicating that the heme group plays a role in the BAY 41-2272-induced activation of sGC. Our results demonstrate the importance of sGC as the sole target for NO in regulating vasodilatation in mice aorta but not in femoral artery. Furthermore, the remaining relaxing effect of BAY 41-2272 in the sGCβ1ki/ki mice, suggests that the heme-binding pocket is very important but not indispensable for the interaction of BAY 41-2272 with sGC.
BICARBONATE-DEPENDENT EFFECT OF HYDROGEN SULPHIDE ON VASCULAR CONTRACTILITY IN RAT AORTIC RINGS

Jinsong Bian and Yihong Liu
Pharmacology, National University of Singapore, Singapore

Hydrogen sulfide (H₂S), an endogenous gaseous mediator, produces both vasorelaxation and vasoconstriction at different concentrations. We found in the present study that NaHS, a H₂S donor, produced stronger vasorelaxant and weaker vasoconstrictive effects in HEPES solution compared with those achieved in Krebs solution. We further screened the buffer components and found that bicarbonate was the ion to influence the effect of H₂S. After examining the vasorelaxant effects of acetylcholine, a vasodilator via releasing nitric oxide, and isoprenaline, a β-adrenoceptor agonist, in HEPES and Krebs buffers, we found the bicarbonate-dependent effect was specific to H₂S. Blockade of anion exchanger (AE2) activity with 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) or with bicarbonate-free solution abolished the vasoconstrictive effect of NaHS. Moreover, NaHS stimulated AE2 activity and decreased NO production in the rat aorta. Both blockade of AE2 with bicarbonate-free solution and removal of superoxide anion radicals (O₂⁻.) with its scavenger, superoxide dismutase, increased NO production. In summary, we find for the first time that H₂S stimulates AE2 to transport extracellular bicarbonate ions in exchange for intracellular O₂⁻., which may further inactivate NO and induces vasoconstriction.
HYDROGEN SULFIDE INDUCED RELAXATION OF ISOLATED RENAL ARTERY OF RABBITS

Yuming Wu, Jing Zhang, Sheng Jin and Shaobin Liu

Department of Physiology, Hebei Medical University, China

Background and Aim: Hydrogen sulfide (H2S), a new endogenous mediator, produces both vasodilatation and vasoconstriction. This study was designed to examine the effect of H2S on renal artery of rabbits and explore the underlying mechanisms.

Methods: The concentration-response curves of NaHS (H2S donor) was measured by recording the tension changes of rabbit renal artery rings which mounted between two wire hooks and suspended in organ bath chambers. Then, pretreatment with some drugs, we observed their influence on the effect of H2S.

Results: (1) NaHS (50, 100, 200, 400 and 800 μmol/L) induced significant relaxation of renal artery rings with intact endothelium in a concentration-dependent manner preshrunk by KCl (50 mmol/L) as a control. The IC50 of the concentration-response relaxation curve was 281.46 ± 17.26 μmol/L. A maximum relaxation of 89.97% was attained at 800 μmol/L of NaHS. Exogenous H2S could endothelium-dependently relax rabbit renal artery through opening of KATP channels further closing the calcium channels in vascular smooth muscles. NO and PGI2 possible have a synergism with H2S on the effect of vasodilatation. After inhibited endogenously H2S induced an increasing contraction effect of the vascular. But we also found that H2S relaxed vascular tissue independent of the activation of the cGMP pathway or KCa channels. We determined that the physiological level of H2S in rabbit plasma was 44.39 ± 4.9 μmol/L, and the endogenous level of H2S in rabbit renal artery was 143.94 ± 4.80 pmol/mg proteins/ minute.
ELEVATED cGMP PLAYS IMPORTANT ROLE IN ACUTE VASODILATORY EFFECT OF SILDENAFIL

Akihiro Tsuji, Norikazu Yamada, Satoshi Ota, Ken Ishikura, Mashio Nakamura and Masaaki Ito

Objective: Sildenafil produces pulmonary arterial vasodilation by promoting an enhanced and sustained level of cyclic guanosine monophosphate (cGMP). However, it has been unknown whether the increase of cGMP is associated with the magnitude of arterial vasodilatation in human. The purpose of this study is to investigate the association between the increase of cGMP and the magnitude of arterial vasodilatation.

Methods: Acute sildenafil response tests were performed in the patients with idiopathic pulmonary arterial hypertension (n=5) and collagen associated pulmonary arterial hypertension (n=2). We evaluated acute hemodynamic effect (n=7) and the change of cyclic adenosine monophosphate (cAMP) and cGMP between at baseline and one hour after sildenafil administration (n=6).

Results: Mean pulmonary arterial pressure (mPAP) decreased from 46.1±9.3 mmHg at baseline to 41.4±8.8 mmHg one hour after drug administration. Total pulmonary vascular resistance (TPR) decreased from 1239±548 dynes sec/cm5 to 995±419 dynes sec/cm5. cAMP was changed from 13.4±2.9 pmol/ml to 14.8±4.1 pmol/ml, cGMP increased from 5.6±3.3 pmol/ml to 7.1±3.8 pmol/ml. TPR decreasing rate was defined as (1h TPR - baseline TPR)/baseline TPR respectively. cGMP increasing rate was defined with (1h cGMP - baseline cGMP)/baseline cGMP. TPR decreasing rate correlated strongly with cGMP increasing rate (r=0.817) but not cAMP (r=0.327).

Conclusion: The improvement of TPR correlated strongly with the increase of cGMP in acute response of sildenafil.
NO interacts with the reduced heme of the soluble guanylate cyclase (sGC). The oxidation of sGC completely prevents its effects. Stimulators of sGC stimulate the reduced form of sGC and potentiate the effect of NO. Activators of sGC directly interact with the oxidized and/or the heme-free form of the sGC. The purpose of this work was to clarify the mechanisms of action of BAY41-2272 and BAY58-2667, purported stimulator and activator of sGC, respectively. Isometric tension was recorded in isolated aortic rings from normotensive Wistar-Kyoto rats and in some instances cGMP levels were determined. Acetylcholine and sodium nitroprusside elicited ODQ-sensitive concentration-dependent relaxations. BAY41-2272 evoked slowly developing, long-lasting, concentration-dependent and ODQ-sensitive relaxations that were endothelium-dependent and correlated with cGMP generation. BAY58-2667 evoked also slowly developing, long-lasting, concentration-dependent relaxations, which, in contrast to BAY41-2272, were endothelium-independent, potentiated by ODQ and only associated with a delayed cGMP generation. The presence of BAY41-2272 but not that of BAY58-2667 produced a small potentiation of the relaxation to SNP whereas both compounds enhanced the effects of isoproterenol. In conclusion 1) BAY41-2272 is a potent vasodilator that requires the reduced form of sGC and the presence of endothelial NO; 2) BAY58-2667 is also a potent vasodilator, which, in an NO-independent manner, preferentially activates the oxidized sGC; 3) Early relaxations to BAY58-2667 are not associated with measurable cGMP generation, suggesting that the activation of sGC might not be the sole mechanism of action of this compound; 4) a potent synergism is observed between the cAMP- and cGMP-dependent pathways.
QUERCETIN EXERTS A GENDER-SELECTIVE RELAXANT EFFECT ON PHENYLEPHRINE CONTRACTED NORMAL AND DIABETIC RAT AORTA.

Aloysius Iguegbe Umelo, Achike FI Francis and Mustafa MR Rais

Department of Pharmacology, University Malaya, Malaysia
International Medical University, Malaysia
Faculty of Medicine, Department of Pharmacology, University Malaya, Malaysia

Elevated levels of reactive oxygen species (ROS) are associated with cardiovascular disease. ROS incapacitates endothelium derived nitric oxide (NO) leading to altered vascular responses. Antioxidants, including the flavonoid, quercetin (Q) have been advocated for the management of cardiovascular disorders, including diabetes and metabolic syndrome for which gender is a risk factor. We explored possible gender differences in Q relaxant effect on phenylephrine (PE)- contracted thoracic aortic rings isolated from age- and sex-matched euglycemic and STZ induced diabetic WKY rats. The tissues were mounted in organ chambers containing Krebs physiological salt solution for isometric tension recording. Q (10 μM, 20 minutes) significantly attenuated PE (10-12-10-5M) contraction in diabetic male and female tissues, endothelium-intact or denuded normal male tissues, but only caused attenuation in endothelium-denuded normal female tissues. L-NAME, methylene blue and indomethacin inhibited Q relaxant effect in PE- treated euglycemic male aorta, but not in euglycemic female tissues. These results agree with our earlier study which suggested that Q exerts its relaxant effect in tissues undergoing oxidative stress, such as the euglycemic male (but not female) and the diabetic male or female tissues. The present data further suggest that quercetin exerts its vasodilatory effect via eNOS/sGC/cGMP- sensitive and -insensitive pathways with the latter dominant in the euglycemic female tissues.
INNOVATIVE THERAPIES OF ENDOTHELIAL DYSFUNCTION IN EXPERIMENTAL DIABETIC RATS

Irina Camelia Chis, Doina Baltaru, Marius Ionut Ungureanu, Ramona Simedrea, Adriana Marton, Adriana Muresan, Andreea Cozma, Anca Dumitrovici, Rami Ababneh, Nicoleta Decea, Mahdi Juhar and Monica Maier

Physiology Department, "IULIU HATIEGANU" University of Medicine and Pharmacy, Roumania

Increasing evidence in both experimental and clinical studies suggest that oxidative stress is involved in the pathogenesis and progression of diabetic tissue damage. The present work examined the effect of chronic oral administration of quercetin, a flavonoid antioxidant, on blood glucose, vascular function and oxidative stress in streptozotocin-induced diabetic rats. Male Wistar rats were divided into 4 groups: control rats, control rats treated orally with quercetin (15 mg/kg body weight), untreated diabetic rats, and diabetic rats treated with quercetin for 4 weeks. Diabetes was induced by a single i.p. injection of streptozotocin (50 mg/kg). For weeks later we measured MDA and carbonilatet proteins (CP) levels in plasma as markers of oxidative stress, and the activities of the antioxidant enzymes catalase, SOD, and glutathione peroxidase, as well as the serum NO and eNOS by ELISA. The plasmatic glucose concentration was significantly increased in diabetic rats and was decreased by quercetin. Streptozotocin administration induced significant increases in plasma MDA and CP concentration, and decreased SOD, catalase and glutathione peroxidase activities. Quercetin treatment significantly decreased the elevated MDA and CP (P<0.05), while increasing the antioxidant enzyme activities (P<0.05). Activation of NO's increased degradation was also observed in streptozotocin-treated rats. NO was associated with a marked increase of eNOS. All these effects were abolished by quercetin. From the present study, it can be concluded that quercetin administration to diabetic rats restores vascular function, probably through an enhancement in the bioavailability of endothelium-derived NO coupled with reduced blood glucose and oxidative stress levels.
LOSARTAN REVERSES THIAZIDE DIURETICS-EXACERBATED INSULIN RESISTANCE THROUGH MODULATION OF MUSCULAR CAPILLARY DENSITY IN FRUCTOSE-FED RATS

Qi Guo, Takefumi Mori, Chunyan Hu, Yusuke Osaki, Yoshimi Yoneki, Takashi Nakamichi, Takuma Hosoya, Hiroshi Sato and Sadayoshi Ito
Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Japan

Impairment in glucose metabolism is associated with a high risk of cardiovascular diseases, and antihypertensive drugs are expected to have a role on insulin sensitivity (IS). Although the combination of angiotensin II receptor blockers (ARB) and diuretics have been recommended for treatment of hypertension, usage of diuretics is concerned in patients with reduced IS. Therefore, the present study was designed to determine whether ARB could improve skeletal muscle capillary density and IS in fructose-fed rats (FFR).

Six-week-old male Sprague-Dawley rats were fed either normal rat chow (Control) or fructose-rich chow for 8 weeks. For the last 4 weeks, fructose-fed rats were allocated to 4 groups: FFR group, and groups treated with hydrochlorothiazide (HCTZ), losartan (LOS), and combinations of drugs. IS was evaluated by euglycemic hyperinsulinemic glucose clamp technique in the eighth week. Capillary density in extensor digitorum longus muscle was examined.

Blood pressure in FFR was significantly higher than that of the controls. HCTZ or/and LOS significantly lowered blood pressure in FFR. IS was significantly lower in FFR than in the controls. LOS alone or a combination of HCTZ and LOS significantly increased IS associated with a significant increase in capillary density, however HCTZ alone significantly reduced IS in FFR associated with a significant reduction in capillary density. There were significant correlations between IS and capillary density in all the rats (P<0.0001).

These results indicate that angiotensin II receptor blockade by LOS reverse HCTZ-exacerbated insulin resistance through the modulation of muscular circulation in rats with impaired glucose metabolism.
DIABETES- AND HYPERTENSION-INDUCED UPREGULATION OF CONCENTRATIVE NUCLEOSIDE TRANSPORTER-2 IN ENDOTHELIAL CELLS

George PH Leung\textsuperscript{a}, Eva YW Ho\textsuperscript{a}, Rachel WS Li\textsuperscript{a}, SW Seto\textsuperscript{b} and YW Kwan\textsuperscript{b}

\textsuperscript{a}Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong, China
\textsuperscript{b}Department of Pharmacology, The Chinese University of Hong Kong, Hong Kong, China

Adenosine modulates a variety of vascular functions and adenosine homeostasis is controlled by nucleoside transporters. Nucleoside transporters are divided into 2 classes. The equilibrative nucleoside transporters (ENTs) are Na\textsuperscript{+}-independent and are subdivided into 2 types based on their sensitivities to 6-[(4-nitrobenzyl)thiol]-9-\textbeta-D-ribofuranosylpurine (NBMPR). The concentrative nucleoside transporters (CNTs) are Na\textsuperscript{+}-dependent and are subdivided into 3 types based on the substrate selectivity. In this study, the nucleoside transporters in vascular cells were characterized and their changes in diabetes and hypertension were investigated.

Functional studies showed that 35\% of the \textsuperscript{3}H adenosine transport in human brain vascular endothelial cells (HBECs) was Na\textsuperscript{+}-dependent. In the Na\textsuperscript{+}-independent component, 80\% was inhibited by NBMPR (10 nM, which inhibits ENT1 but not ENT2). However, the \textsuperscript{3}H adenosine transport in vascular smooth muscle cells (HBSMCs) was totally Na\textsuperscript{+}-independent and 95\% of it was inhibited by NBMPR (10 nM). RT-PCR revealed the presence of mRNA of ENT1, ENT2 and CNT2 in HBECs, but no CNT2 was found in HBSMCs. The unique presence of CNT2 in endothelial cells was confirmed by the observation that mRNA of ENT1, ENT2, and CNT2 were present in rat basilar arteries but no CNT2 was detected after removal of endothelia. The mRNA expression of CNT2, but not ENT1 and ENT2, was higher in basilar arteries of spontaneously hypertensive rats and streptozotocin-induced diabetic rats compared with those in normal rats. Such increase in CNT2 in hypertension and diabetes may affect the availability of adenosine in the vicinity of adenosine receptors of endothelial cells and, thus, alter vascular functions.
P14-6
WITHDRAWN
METFORMIN IMPROVES IMBALANCE BETWEEN VASODILATOR AND VASOCONSTRICTOR ACTIONS OF ENDOTHELIUM-DERIVED FACTORS IN MESENTERIC ARTERIES FROM TYPE 2 DIABETIC RATS

Takayuki Matsumoto, Tsuneo Kobayashi and Katsuo Kamata

Department of Physiology and Morphology, Hoshi University, Japan

We previously reported that in mesenteric arteries from aged Otsuka Long-Evans Tokushima Fatty rats (OLETF) (a type 2 diabetic model), the endothelium-derived hyperpolarizing factor (EDHF)-type relaxation is impaired, while the endothelium-derived contracting factor (EDCF)-mediated contraction is enhanced (Am J Physiol Heart Circ Physiol 293: H1480-H1490, 2007). Here, we investigated whether metformin, a biguanide derivative (dimethylbiguanide), might improve this imbalance between the effects of the above endothelium-derived factors in mesenteric arteries isolated from OLETF rats. In acute studies on OLETF mesenteric arteries, the acetylcholine (ACh)-induced relaxation was impaired and the relaxation became weaker at high ACh concentrations. Both metformin and AICAR (an AMP-kinase activator, which is also activated by metformin): (1) diminished the tendency for the relaxation to reverse at high ACh concentrations, and (2) suppressed both the ACh-induced EDCF-mediated contraction and production of prostanoids (TXA₂ and PGE₂). In studies on OLETF arteries from chronically treated animals, metformin treatment (300 mg/kg/day for 4 weeks): (1) improved the endothelium-dependent relaxation (i.e., NO or EDHF) and COX-mediated contraction, (2) reduced the EDCF-mediated contraction, (3) suppressed the production of prostanoids, and (4) reduced superoxide generation. Metformin did not alter the protein expressions of eNOS, phospho-eNOS (Ser1177), or COX1, but it increased COX2 protein. These results suggest that metformin improves endothelial functions in OLETF mesenteric arteries by suppressing vasoconstrictor prostanoids and by reducing oxidative stress. Our data suggest that within the time-scale studied here, metformin improves endothelial function through this direct mechanism, rather than by improving metabolic abnormalities.
P14-8
BLOCKADE OF THE ENDOTHELIAL NF-κB PATHWAY PREVENTS OBESITY- AND AGE-RELATED INSULIN RESISTANCE AND PROLONGS LONGEVITY

Yutaka Hasegawa\textsuperscript{a}, Tokuo Saito\textsuperscript{a,b}, Takehide Ogihara\textsuperscript{b}, Toshio Fujita\textsuperscript{c}, Yoshitomo Oka\textsuperscript{a} and Hideki Katagiri\textsuperscript{b}

\textsuperscript{a}Division of Molecular Metabolism and Diabetes, Tohoku University Graduate School of Medicine, Japan
\textsuperscript{b}Division of Advanced Therapeutics for Metabolic Diseases, Tohoku University Graduate School of Medicine, Japan
\textsuperscript{c}Department of Nephrology and Endocrinology, Faculty of Medicine, University of Tokyo, Japan

The transcriptional factor nuclear factor-kappa B (NF-κB) signaling plays critical roles in a variety of physiological and pathological processes, such as responses to inflammation and oxidative stress. To examine the role of endothelial NF-κB signaling in vivo, we attempted to block endothelial NF-κB signaling by generating transgenic mice expressing the dominant-negative form of IκB under the tie2 promoter/enhancer (E-DNIκB mice). While these transgenic mice exhibited mild metabolic phenotypes when young under normal chow conditions, they showed marked resistance to the development of both insulin resistance and blood pressure elevation with high fat diet feeding. Protection from insulin resistance and hypertension was more remarkable in genetically obese (Ay) mice. Obesity-induced macrophage infiltration into adipose tissue was markedly inhibited in E-DNIκB mice. In addition, blood flow and mitochondrial biogenesis were increased in muscles of E-DNIκB mice. These findings suggest that endothelial inflammation is involved in the development of obesity-induced insulin resistance in various processes and organs. Furthermore, blockade of endothelial NF-κB signaling prevented age-related insulin resistance and vascular senescence and thereby prolonged longevity. Thus, the endothelial NF-κB signaling plays important roles in various pathological processes of obesity- and age-related disorders and is a potential target for the treatment of metabolic syndrome as well as anti-aging strategies.
P15-1
RETROVIRAL GENE DELIVERY OF RECEPTOR FOR ADVANCED GLYcation END PRODUCTS (RAGE) INTO RAT VASCULAR SMOOTH MUSCLE CELLS

Eri Hayakawa, Takanobu Yoshimoto, Naoko Sekizawa, Kyoichiro Tsuchiya, Masayoshi Shichiri and Yukio Hirata

Department of Clinical and Endocrinology, Tokyo Medical and Dental University Graduate School, Japan

It is well known that advanced glycation end products (AGE) play a pivotal role in diabetic microangiopathy through its receptor (RAGE). The pathophysiological role of RAGE in diabetic macroangiopathy, however, has not been fully understood due to its very low expression levels in vascular smooth muscle cells (VSMC) under physiological condition. To study whether redox-sensitive signaling is involved in RAGE action, the present study was undertaken to establish the rat VSMC cell line (A10) which stably expresses human RAGE using bicistronic retrovirus vector (pMX-IP). After positive selection by puromycin and subsequent colony isolation and expansion, we established two independent A10 clones (RAGE-A10-2 and -11) which stably expressed RAGE protein as confirmed by immunoblot analysis. Empty retroviral vector-transduced A10 (pMX-A10) was used as control. Semi-quantitative RT-PCR analysis revealed that gene expression of monocyte chemoattractant protein-1 (MCP-1) significantly (P<0.05) increased both in RAGE-A10-2 (13-fold) and RAGE-A10-11 (5-fold) after stimulation with a RAGE ligand (S100B protein; 10 microg/ml) for 4h, while the stimulation with S100B did not affect MCP-1 expression in pMX-A10 or wild type A10. Luciferase assay using reporter constructs containing rat MCP-1 region showed that S100B did not caused a significant increase (P<0.05) in MCP-1 promoter activity in RAGE-A10-2 (6-fold) and RAGE-A10-11 (4-fold). In conclusion, we established VSMC cell lines stably expressing RAGE with enhanced RAGE-mediated MCP-1 gene expression, which could be a useful cell model for investigating the pathophysiological role of RAGE in the vasculature.
COMPARISON OF CALCIUM CHANNEL BLOCKER AND AT1 RECEPTOR BLOCKER FOR THE PROTECTION OF ENDOTHELIAL FUNCTION IN DIABETES

Nobukazu Ishizaka, Kan Saito, Kyoko Furuta, Gen Matsuzaki and Ryozo Nagai
Cardiovascular Medicine, University of Tokyo, Japan

Background: Recent clinical studies indicate that the appropriate control of the blood pressure is the most important goal for the protection of atherosclerosis, and thus for the protection of vascular endothelial function.

Purpose: We have investigated the effects of a long-acting calcium antagonist (CCB), and an angiotensin AT1 receptor antagonist (ARB) on the vascular endothelial function observed in the Otsuka Long-Evans Tokushima Fatty OLETF rats, an animal model of hypertension and diabetes.

Methods and Results: At 34 weeks of age, OLETF rats were treated with either CCB, benidipine (3 mg/kg/day, per os) or ARB, losartan (25 mg/kg/day, per os) for 8 weeks. There were not significant differences in the extent of blood pressure reduction between both groups (systolic blood pressure; untreated OLETF 149±4 mmHg, CCB 138±3 mmHg, ARB 135±3 mmHg). Aortic tension study showed that endothelium-dependent vascular relaxation was blunted in the aorta from OLETF rat compared with that from non-diabetic counterpart. On the other hand, both CCB and ARB improved the endothelial function to a similar extent. In addition, both CCB and ARB suppressed iNOS expression and retained eNOS expression in the aorta of OLETF.

Conclusion: In diabetic hypertensive animal models, both CCB and ARB improved arterial endothelial function to a similar extent, supporting the concept that controlling the blood pressure is the most important in hypertension also in the setting of diabetes.
EFFECT OF SPECIFIC T-TYPE CALCIUM CHANNEL BLOCKER R(-) EFONIDIPINE ON THE AMELIORATION OF RENAL MEDULLARY CIRCULATION IN RATS

Chunyan Hu a, Takefumi Mori b, Qi Guo a, Ying Sun a, Yoshimi Yoneki a, Yusuke Osaki a, Takashi Nakamichi a, Hiroshi Sato a and Sadayoshi Ito a

aNephrology, Endocrinology and Vascular Medicine, Tohoku University, Japan
bHealth Administration Center, Tohoku University, Japan

Blockade of T-type calcium channel (TCC) has been shown to protect from renal injury. TCC is expressed in renal efferent arterioles of juxtamedullary nephron and vasa recta. Present study was designed to determine whether blockade of TCC with specific TCC blocker R(-) efondipine (R(-)EFO) would ameliorate renal medullary circulation (RMC) during the reduction of mean arterial pressure (MAP) in rats.

Renal medullary blood flow (MBF) was simultaneously monitored through an optical fiber attached to the laser-Doppler flowmetry implanted in anesthetized male Sprague-Dawley rats. MAP was monitored through a catheter implanted from the left femoral artery. After 30 minutes’ baseline period, vehicle or R(-)EFO (1.25mg/h or 0.25mg/h) was intravenously infused from femoral vein for 90 minutes. Urine was collected for hydrogen peroxide (H2O2) measurement as an indicator of oxidative stress.

Acute intravenous infusion of high dose R(-)EFO (1.25mg/h) significantly reduced the MAP over 90 minutes (n=6, P<0.05), while no significant change was found in MBF. However, MBF was significantly increased by 24.0±7.0% (n=8, P<0.05) compared with baseline and vehicle group (n=8) over 90 minutes’ infusion of low dose R(-)EFO (0.25mg/h) without change of MAP. Urinary H2O2 excretion was significantly reduced in R(-)EFO infused group compared to the vehicle group.

The results in the present study indicate that blockade of TCC would ameliorate RMC even with the reduction of MAP by altering renal oxidative stress. We conclude that R(-)EFO may protect from ischemic renal injury in the renal medullary region.
NATRIURETIC PEPTIDES ENHANCE THE PRODUCTION OF ADIPONECTIN

Masashi Fujita\textsuperscript{a}, Osamu Tsukamoto\textsuperscript{a}, Mahoto Kato\textsuperscript{b}, Satoru Yamazaki\textsuperscript{b}, Yoshihiro Asano\textsuperscript{a} and Masafumi Kitakaze\textsuperscript{b}

\textsuperscript{a}Department of Cardiovascular Medicine, Osaka Graduate School of Medicine, Japan  
\textsuperscript{b}National Cardiovascular Center, Japan

**Background:** Natriuretic peptides are promising candidates for the treatment of congestive heart failure (CHF) because of their wide range of beneficial effects on the cardiovascular system. Adiponectin is a cytokine derived from adipose tissue with various cardiovascular-protective effects that has been reported to show a positive association with plasma BNP levels in patients with heart failure. We investigated the functional relationship between natriuretic peptides and adiponectin by performing both experimental and clinical studies.

**Methods:** The expression of adiponectin mRNA and its secretion were examined after ANP or BNP was added to primary cultures of human adipocytes in the presence or absence of HS142-1 (a functional type A guanylyl cyclase receptor antagonist). Changes of the plasma adiponectin level were determined in 30 patients with CHF who were randomized to receive intravenous ANP (0.025 μg/kg/min hANP for 3 days, \(n=15\)) or saline (\(n=15\)).

**Results:** Both ANP and BNP dose-dependently enhanced the expression of adiponectin mRNA and its secretion, while such enhancement was inhibited by pretreatment with HS142-1. The plasma adiponectin level was increased at 4 days after administration of hANP compared with the baseline value (from 6.56±0.40 to 7.34±0.47 μg/mL, \(p<0.05\)), while there was no change of adiponectin in the saline group (from 6.53±0.57 to 6.55±0.56 μg/mL).

**Conclusions:** Natriuretic peptides enhance adiponectin production by human adipocytes in vitro and even in patients with CHF, which might have a beneficial effect on cardiomyocytes in patients receiving recombinant natriuretic peptide therapy for heart failure.
RAPID, NON-GENOMIC VASCULAR ACTIONS OF GENISTEIN INVOLVES A G-PROTEIN COUPLED RECEPTOR

Amanda HY Lin, George PH Leung, Susan WS Leung and Ricky YK Man
Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong, China

Background: Genistein enhances endothelial function in a receptor-mediated manner. The present studies were designed to identify the putative receptor and related signaling pathways in the rapid vascular actions of genistein.

Methods: Isometric tension was measured in isolated aortic rings from 32-weeks-old male spontaneously hypertensive rats. Estrogen receptor-α66, estrogen receptor-α46, estrogen receptor-α36 and G protein-coupled receptor 30, identified as the potential candidates of the putative receptor, were cloned and expressed using a cell-free expression system (MembraneMax Protein Expression Kit).

Results: Genistein acutely potentiated acetylcholine-induced relaxation. This effect was insensitive to the transcription and translation inhibitors, actinomycin D and cycloheximide respectively. The potentiation of acetylcholine and A23187-induced relaxation by genistein was inhibited by NF023 and GP antagonist-2A, the selective G_{i/o} and G_{q} α-subunit antagonists, respectively, but not by NF449, a selective G_{s} α-subunit antagonist. In order to identify the putative receptors, the cDNA of candidates were successfully amplified from MCF7 cell using PCR and further cloned into the expression vector (pEXP5-NT/TOPO) which was confirmed by sequencing. Soluble receptor proteins were expressed in vitro, whose molecular sizes were confirmed by Western blotting and purified for use in radioligand binding assay.

Conclusion: Our results demonstrate that rapid effect of genistein in potentiating relaxation is non-genomic and G_{i} and G_{q}, but not G_{s}, were involved in the effect of genistein in potentiating acetylcholine and A23187-induced relaxations. Involvement of G-proteins suggests that genistein exerts its effect through a putative G-protein coupled receptor. Radioligand binding assays will be performed to confirm the identity of this putative receptor.
A vasodilator, bradykinin, plays a key role in the contrast media induced nephropathy in mice

Jun-ichi Suzuki\textsuperscript{a}, Masahito Ogawa\textsuperscript{a} and Mitsuaki Isobe\textsuperscript{b}

\textsuperscript{a}Department of Advanced Clinical Science and Therapeutics, University of Tokyo, Japan
\textsuperscript{b}Tokyo Medical and Dental University, Japan

Background: Iodinated contrast media (CM) has been broadly used for diagnostic procedures; CM-induced nephropathy (CIN) affects morbidity and mortality of patients. Although CIN is mediated by several factors including the renin-angiotensin system (RAS), little is known about the evidence obtained from experimental models. An angiotensin converting enzyme inhibitor (ACEI) has beneficial effects in some works, while other works indicate that ACEI administration is a risk for developing CIN. The effects of a vasodilator, bradykinin, in CIN has been still controversial. Thus, we developed a murine CIN model to evaluate the mechanism.

Methods: We performed 5/6 subtotal nephrectomy (NTX) and administered CM (iopamidol) intravenously into the mice 4 weeks after NTX. We administered an angiotensin converting enzyme inhibitor (ACEI, imidapril), an angiotensin II receptor blocker (ARB, TA606), or ACEI plus a bradykinin B2 receptor antagonist (Hoe-140) into the mice daily.

Results: Serum creatinine levels on day 28 were significantly elevated in the NTX group compared to those in the non-NTX group. A day after CM injection, creatinine levels were significantly elevated in the non-treated group. While ACEI treatment significantly suppressed the creatinine levels, ARB treatment did not decrease the creatinine levels. A bradykinin antagonist Hoe-140 negated ACEI's ability to suppress renal dysfunction.

Conclusion: ACEI treatment is useful for the prevention of CM-induced nephropathy because bradykinin pathway is critical to regulate CIN development.
A NOVEL MAS RECEPTOR IN THE EYE TISSUE

Heikki Vapaatalo\textsuperscript{a}, Anu Vaajanen\textsuperscript{a}, Paivi Lakkisto\textsuperscript{b}, Ismo Virtanen\textsuperscript{c}, Esko Kankuri\textsuperscript{a}, Olli Oksala\textsuperscript{a} and Ilkka Tikkanen\textsuperscript{d}

\textsuperscript{a}Institute of Biomedicine/Pharmacology, University of Helsinki, Finland
\textsuperscript{b}Department of Clinical Chemistry, Helsinki University Central Hospital, Finland
\textsuperscript{c}Institute of Biomedicine, Anatomy, University of Helsinki, Finland
\textsuperscript{d}Minerva Foundation, Institute for Medical Research, Finland

Introduction: All recognized renin-angiotensin system (RAS) components have been identified in the eye, except a novel Mas-receptor, whose endogenous ligand is Angiotensin (1-7) (Ang (1-7)). We investigated especially the expression of Mas receptor in the eye structures.

Materials and Methods: The experiment was conducted by using enucleated eyes of normotensive WKY and SD rats, and arterial hypertensive SHR (spontaneously hypertensive) and double transgenic rats harboring human renin and angiotensinogen genes. The eyes were enucleated, snap frozen and stored at -80°C for in vitro autoradiography and real time RT-PCR analysis. The expression of angiotensin receptors was determined in the ciliary body responsible for formation of aqueous humor and in the retina.

Results: An expression of a novel Mas receptor as well as AT1 and AT2 receptors were detected in the eye in all rat strains. Level of Mas receptors was approximately half of that of AT1 receptors and twice that of AT2. Overall the receptor expression was more pronounced in the retina vs ciliary body.

Conclusion: RAS modifying compounds have previously been shown to lower intraocular pressure and might have a role in the treatment of diabetic retinopathy. Ang (1-7) is a biologically active heptapeptide, a metabolite of Angiotensin I and II. In most situations, Ang (1-7) and angiotensin II exert opposing actions, suggesting a primary role for Ang (1-7) as a counter-regulatory component for the vascular and proliferative actions of Angiotensin II. Expression of Mas receptor in the eye may offer a novel target for development of antiglaucomatous drugs.
WITHDRAWN
INVOLVEMENT OF TRPV4 CHANNEL IN ENDOTHELIAL DYSFUNCTION

Yoshiko Munehisa\textsuperscript{a}, Hiroyuki Watanabe\textsuperscript{a}, Kyoichi Ono\textsuperscript{b}, Takayoshi Ohba\textsuperscript{b}, Kiyoshi Nobori\textsuperscript{a}, Manabu Murakami\textsuperscript{b} and Hiroshi Ito\textsuperscript{a}

\textsuperscript{a}Division of Cardiovascular, Department of Internal Medicine, Akita University School of Medicine, Japan
\textsuperscript{b}Department of Physiology, Akita University School of Medicine, Japan

Endothelial dysfunction is an initial step in the pathogenesis of cardiovascular diseases. It has been known that Ca\textsuperscript{2+} influx plays a crucial role in endothelial nitric oxide production. However, the ionic mechanism underlying the endothelial dysfunction remains unclear. Transient receptor potential V4 channel (TRPV4) has been shown to be involved in endothelial Ca\textsuperscript{2+} transport induced by shear stress and resultant NO production. The purpose of this study was to explore the role of TRPV4 channels in the endothelial dysfunction induced by angiotensin II (Ang II). We studied human coronary artery endothelial cells (hCAECs). By using the most specific ligand 4 alpha-phorbol 12,13-didecanoate (4\textalpha PDD) as a tool to stimulate endothelial TRPV4, we evaluated TRPV4 mediated Ca\textsuperscript{2+} entry in impaired endothelial cells which were treated with Ang II (100 nM) for 48 h. 4\textalpha PDD induced an increase in [Ca\textsuperscript{2+}] in Ang II treated hCAECs but was 50\% less effective than in control hCAECs. Ang II exerted the inhibitory effects upon TRPV4 activity in a dose dependent manner (10 nM - 1 \mu M) and even by using other TRPV4 stimuli, 30\% hypotonic stress and 5',6'-epoxycisatrienoic acid. Likewise, 4\textalpha PDD induced TRPV4 current density at -80mV was significantly decreased in Ang II treated hCAECs. In NO measurement using DAF2, 4\textalpha PDD induced NO production was markedly attenuated in Ang II treated cells. The protein expression of TRPV4 was decreased in Ang II treated hCAECs.

Conclusion: Down-regulation of TRPV4 might contribute to endothelial dysfunction induced by Ang II.
LONG-TERM TREATMENT WITH EICOSAPENTAENOIC ACID SUPPRESSES ISCHEMIA-INDUCED VENTRICULAR FIBRILLATION IN PIGS IN VIVO. POSSIBLE INVOLVEMENT OF ATP-SENSITIVE POTASSIUM CHANNEL

Ryuji Tsuburaya, Satoshi Yasuda, Yoshitaka Ito, Takashi Shiroto, Jun Yi Gao, Kenta Ito and Hiroaki Shimokawa

Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan

Background: Although epidemiologic studies demonstrated that eicosapentaenoic acid (EPA) reduces sudden cardiac death, the antiarrhythmic mechanisms remain to be elucidated. It was reported that activated ATP-sensitive potassium channels (KATP channel) could cause fatal ventricular arrhythmia during early myocardial ischemia. We thus hypothesized that long-term treatment with EPA suppresses ischemia-induced ventricular fibrillation (VF) by modulating myocardial KATP channels.

Methods: Male pigs treated with either a control chow or EPA (600 mg/kg/day, PO) for 3 weeks (n=8 each) were subjected to myocardial ischemia (90 min) by occlusion of the left circumflex coronary artery. Monophasic action potential (MAP) that reflects electrical activity of the left ventricle in vivo was measured.

Results: In the EPA group, EPA content in red blood cells was increased to 4.5±0.3 mol%, a comparable level in the clinical studies (~5.8 mol%). The EPA treatment significantly reduced the occurrence of VF (1.5±0.8 vs. 5.1±1.7 times/animal, P<0.05), with a resultant marked improvement in mortality (EPA 0% vs. control 50%, P<0.05). Ischemia-induced shortening of MAP duration (measured at 90% repolarization) was attenuated in the EPA group compared with the control group (EPA; -19.5±1.1% vs. control; -27.6±2.9%, P<0.05), whereas hemodynamics and myocardial perfusion at ischemic region were not different between the two groups. Pre-treatment of Diazenoxide, a KATP channel opener ( 4mg/kg for 20min, ic) abolished the effect of EPA on the ischemia-induced shortening of MAP (EPA+diazenoxide -27.9±6.4%, P<0.05 vs. EPA).

Conclusions: These results indicate that EPA improves survival and ventricular stability during ischemia partly through suppressing KATP channels.
ADENOSINE INDUCES VASODILATATION OF SMALL MESENTERIC ARTERY BY INHIBITING STORE-OPERATED CALCIUM CHANNELS

Sheng-Peng Wang\textsuperscript{a}, W. Gil Wier\textsuperscript{b}, Yan Zhang\textsuperscript{c}, Ming Zhao\textsuperscript{a}, Xiao-Jiang Yu\textsuperscript{a} and Wei-Jin Zang\textsuperscript{a}

\textsuperscript{a}Department of Pharmacology, Xi'an Jiaotong University School of Medicine, China
\textsuperscript{b}Departments of Physiology and Medicine, University of Maryland School of Medicine, USA
\textsuperscript{c}Key Laboratory of Environment and Genes Related to Diseases, Ministry of Education, School of Medicine, Xi'an Jiaotong University, China

Background: Adenosine has been shown to produce vasorelaxant effect in various arteries, but the mechanisms is not clear. Store-operated Ca\textsuperscript{2+} entry (SOCE) have recently been proposed to contribute to Ca\textsuperscript{2+} influx in vascular smooth muscle cells (VSMCs). We assessed the hypothesis that SOCE have a functional role in adenosine-induced vasodilatation.

Methods: Isolated rat small mesenteric arterial rings were mounted in organ baths and the isometric tensions were measured continuously by a sensitive myograph system. Laser scanning confocal microscopy was used to determine intracellular Ca\textsuperscript{2+} concentration of Fluo-3-loaded VSMCs.

Result: Adenosine produced concentration-dependent relaxation of artery rings precontracted by noradrenaline, U46619 or 5-hydroxytryptamine. The relaxation was unaffected by endothelium denudation, but A\textsubscript{2A} receptor antagonism with SCH58261 and adenylate cyclase (AC) inhibition with SQ22536 attenuated the relaxation to 17 ±4% and 23±5%, respectively. Rise in extracellular K\textsuperscript{+} concentration or pretreatment with glibenclamide reduced adenosine relaxation to 67±2% and 27±1%, respectively. Intracellular Ca\textsuperscript{2+} store depletion by phenylephrine and thapsigargin triggers SOCE via store-operated Ca\textsuperscript{2+} channels. Adenosine showed an 87±6% and 83±4% reduction in the contractions induced by phenylephrine and thapsigargin associated with SOCE. In cultured vascular smooth muscle cells, adenosine showed an 59±3% and 47±2% reduction in phenylephrine and thapsigargin induced increases in cytosolic Ca\textsuperscript{2+} levels via SOCE.

Conclusions: These results indicate a novel mechanism of vasodilatation by adenosine which involves the regulation of SOCE through cAMP signaling pathway due to activation of adenosine A\textsubscript{2A} receptors. In addition, adenosine-induced relaxation does not involve the endothelium, but may partially involve activation of K\textsubscript{ATP} channels.
PROMINENT ROLE OF LARGE CONDUCTANCE CALCIUM-DEPENDENT POTASSIUM CHANNELS IN ENDOTHELium-DEPENDENT RELAXATION OF RAT SMALL MESENTERIC ARTERIES IN ALLOXANIC DIABETES

Bogdan Alexandru Stoica, Ionela-Lacramioara Serban, Dumitru D Branisteanu, Sorin Beschea-Chiriac and Dragomir N Serban

Cell Physiology & Pharmacology Laboratory, Functional Sciences Department, “Grigore T. Popa” University of Medicine and Pharmacy, Iasi, Roumania

Although streptozotocin is more widely used to induce rodents diabetes resembling the human insulin-dependent one, in this study we used alloxan to induce diabetes in rats, as previously described. Both agents are toxic due to their cellular uptake via GLUT2 transporter and subsequent pancreatic beta cells destruction, which involves alkylation and DNA damage in the case of streptozotocin, but a free-radical-generating cycle in the case of alloxan. Others have shown that renal arteries from rabbits with alloxan diabetes do not present endothelial dysfunction, but participation of K(ATP) to endothelium-dependent relaxation is prevented. Based on our previous observations regarding endothelial dysfunction in this model, here we investigate the EDHF phenomenon and participation of certain potassium channels. We used isometric myography of rings (1 mm wide) from mesenteric artery and its first order branches, obtained from male Wistar rats (200-250 g). We used 0.01 mM glibenclamide to block K(ATP) and 10 mM tetraethylammonium to block BK(Ca). We tested the effect of carbachol (100 nM to 0.1 mM) in phenylephrine-precontracted rings, as global endothelium-dependent relaxation and its EDHF component (in presence of 0.1 mM L-NAME and 0.01 mM indomethacin). Endothelium-dependent relaxation in diabetic animals was reduced, but EDHF was enhanced in diabetic vs. control. Moreover, Endothelium-dependent relaxation in diabetic animals was fully inhibited by TEA, in contrast with healthy controls, where K(ATP) are also involved. K(ATP) are not involved in the EDHF component in either diabetic or control animals. Supported by Romanian Grant CNCSIS-A1222/2007-2008.
BESTROPHIN-3-ASSOCIATED CALCIUM-ACTIVATED CHLORIDE CURRENT IS IMPORTANT FOR RHYTHMIC BUT NOT TONIC ACTIVITIES IN RAT MESENTERIC SMALL ARTERIES

Christian Aalkjaer\textsuperscript{a}, Vladimir V. Matchkov\textsuperscript{a}, Torbjoern Broegger\textsuperscript{a}, Donna MB Boedtkjer\textsuperscript{a} and Finn S Pedersen\textsuperscript{b}

\textsuperscript{a}Institute of Physiology and Biophysics, Aarhus University, Denmark
\textsuperscript{b}Department of Molecular Biology, University of Aarhus, Denmark

We have previously characterized a cGMP-dependent, calcium-activated chloride-current \( I_{Cl,cGMP} \) in vascular smooth muscle cells and shown that bestrophin-3 expression is essential for the current.

To study the role of \( I_{Cl,cGMP} \) we transfected rat mesenteric small arteries in vivo with siRNA against bestrophin-3 and for control with mutated bestrophin-3 siRNA. The arteries were tested 3 days later for mRNA and protein expression, membrane conductances and contractility.

Bestrophin-3 siRNA significantly reduced mRNA to 24+/−17%, \( n=3 \), and bestrophin-3 protein to 59+/−9%, \( n=4 \) expression and \( I_{Cl,cGMP} \) to 19.6+/−2.7%, \( n=7 \) without affecting other membrane conductances. In control arteries no significant effects were seen.

Both maximal contractile response 3.7+/−0.5 vs 3.7+/−0.5 N/m and sensitivity to noradrenaline, \(-\log EC_{50} \) 5.89+/−0.02 vs 5.93+/−0.01, \( n=13 \) were similar in arteries transfected with bestrophin-3 siRNA and with mutated bestrophin-3 siRNA. The amplitude of noradrenaline induced vasomotion was, however, significantly lower, 0.08+/−0.01 vs. 0.17+/−0.02 N/m, \( n=11 \) in arteries with bestrophin-3 siRNA. Although, the average vasomotion frequency was not different, 0.22+/−0.01 vs. 0.26+/−0.03 Hz, \( n=11 \), the peak of the frequency spectrum was lower, 16 vs. 23 p<0.05 in arteries treated with bestrophin-3 siRNA compared to control arteries.

Our study confirms that bestrophin-3 is essential for about half of the calcium activated chloride current in vascular smooth muscle i.e. the cGMP dependent chloride current. Importantly the study demonstrates that bestrophin-3 is important for vasomotion and strongly supports our suggestion that \( I_{Cl,cGMP} \) is a key mechanism for development of vasomotion, while it has no importance for the sensitivity or maximal force development to noradrenaline.
INSULIN INHIBITS Na⁺/H⁺-EXCHANGE IN VASCULAR SMOOTH MUSCLE AND ENDOTHELIAL CELLS IN SITU: INVOLVEMENT OF H₂O₂ AND TYROSINE PHOSPHATASE SHP-2

Christian Aalkjaer and Ebbe Boedtkjer

Institute of Physiology and Biophysics, Aarhus University, Denmark

We investigated the effect of insulin on Na⁺/H⁺-exchange activity, intracellular pH (pHi) and reactive oxygen species (ROS) in smooth muscle (VSMCs) and endothelial cells (ECs) of mouse mesenteric arteries using fluorescence microscopy.

In the absence of CO₂/HCO₃⁻, removal of bath Na⁺ produced EC acidification inhibited by the Na⁺/H⁺-exchange inhibitor cariporide. Insulin and H₂O₂ acidified ECs 0.2-0.3 pH-units and reduced the acidification upon Na⁺-removal by ~65%. Cariporide abolished the effect of insulin and H₂O₂.

VSMCs were acidified by H₂O₂ (ΔpH=-0.48±0.06) and insulin (ΔpH=-0.03±0.01). Na⁺/H⁺-exchange activity after an NH₄⁺-prepulse was ~80% attenuated by H₂O₂ and ~40% by insulin.

NHE1 was the only plasma membrane NHE isoform detected by RT-PCR analyses.

In ECs and VSMCs, PEG-catalase abolished the effect of insulin on pH and exposure to insulin increased the concentration of ROS. NSC-87877 and PTP inhibitor IV (selective inhibitors of tyrosine phosphatase SHP-2) reduced steady-state pH, up to 0.3 pH-units and inhibited Na⁺/H⁺-exchange activity 60-80%; when applied in combination with insulin or H₂O₂, the SHP-2 inhibitors had no further effect.

We conclude that Na⁺/H⁺-exchange in ECs and VSMCs is inhibited by insulin and H₂O₂ and propose that insulin signaling involves H₂O₂-mediated inhibition of SHP-2.
STIMULATION OR ACTIVATION OF SOLUBLE GUANYLATE CYCLASE AND AGGREGATION OF WASHED PLATELETS OF WKY RAT

Michel Feletou, Severine Roger, Cecile Badier-Commander, Jerome Paysant and Tony J. Verbeuren

Department of Angiology, Institut de Recherches Servier, France

The purpose of the present study was to determine whether a stimulator or an activator of soluble guanylate cyclase (sGC), BAY41-2272 and BAY58-2667, respectively, inhibits platelet aggregation and to clarify their mechanisms of action. Blood was collected from anaesthetized Wistar-Kyoto rats. The aggregation of washed platelet was measured and the production of cAMP and cGMP was determined. ADP-induced platelet aggregation was concomitantly reduced basal cGMP levels. ADP-induced platelet aggregation was inhibited by BAY41-2272 and to a lesser extent by NO-donors and BAY58-2667. These effects were paralleled with an increase in cGMP, in contrast to the anti-aggregating effect of beraprost associated with an increase in cAMP and no change in cGMP levels. The effects of BAY41-2272 and BAY58-2667 were not affected by L-nitroarginine or ODQ, but was enhanced by hydroxocobalamin, which concomitantly reduced basal cGMP levels. ADP-induced platelet aggregation was inhibited by BAY41-2272 and to a lesser extent by NO-donors and BAY58-2667. These effects were paralleled with an increase in cGMP, in contrast to the anti-aggregating effect of beraprost associated with an increase in cAMP and no change in cGMP levels. The effects of BAY41-2272 and BAY58-2667 were not affected by L-nitroarginine. ODQ prevented the effects of BAY41-2272 but enhanced those of BAY58-2667. Hydroxocobalamin inhibited the effects of the NO-donors and BAY41-2272 but did not affect those of BAY58-2667 or beraprost. A positive interaction was observed between NO-donors and BAY41-2272 but not with BAY58-2667. Beraprost potentiated the anti-aggregating effects of both BAY41-2272 and BAY58-2667. Hydroxocobalamin inhibited the synergy between beraprost and BAY41-2272 without affecting that with BAY58-2667. In conclusion, 1) BAY41-2272 requires the reduced form of sGC and the presence of NO, the origin of which remains unresolved; 2) sGC is mostly under the reduced form but when oxidized, BAY58-2667 becomes a potent and NO-independent anti-aggregating agent; 4) a potent synergism is observed between cAMP- and cGMP-dependent pathways.
THE AMP-ACTIVATED PROTEIN KINASE (AMPK) α2 SUBUNIT IS INVOLVED IN PLATELET SIGNALING, CLOT RETRACTION AND THROMBUS STABILITY

Ingrid Fleming, Voahanginirina Randriamboavonjy, Johann Isaak and Beate Fisslthaler

Institute for Vascular Signalling, Johann Wolfgang Goethe University Frankfurt, Germany

Human and murine platelets express both the AMPKα1 and α2 isoforms and although various stimuli are known to activate the kinase its role in platelet activation has not yet been investigated. We found that the phosphorylation of the AMPK on Thr172 was time- and concentration-dependently stimulated by thrombin (0.01 to 0.3 U/ml). Both the tumor promoter LKB1 and the Ca^{2+}/calmodulin kinase kinase (CaMKK) are reported to phosphorylate the AMPK in other cells. We found that thrombin markedly increased the phosphorylation of LKB1 in platelets. Moreover, the thrombin-induced phosphorylation of the AMPK in platelets was not affected by the CAMKK inhibitor KN93 indicating the involvement of LKB1 but not of CAMKK. In washed human platelets the AMPK inhibitors iodonitrotetrazolium and compound C significantly inhibited thrombin-induced aggregation without affecting the concomitant increase in Ca^{2+}. Moreover, in human samples clot retraction was significantly inhibited by the AMPK inhibitors, and AMPKα2⁻/⁻ mice demonstrated an impaired clot retraction when compared with their wild-type littermates. Furthermore, thrombus stability was also decreased in an in vivo model of FeCl₃-induced injury in the AMPKα2⁻/⁻ mice even though bleeding time was not significantly different between the AMPKα2⁻/⁻ and wild-type animals. Mechanistically, the thrombin-induced phosphorylation of β3 integrin in human platelets was inhibited by AMPK inhibitors and impaired in AMPKα2⁻/⁻ mice.

Altogether, these results demonstrate that the AMPK plays a key role in platelet signaling leading to clot retraction and stability probably by affecting the αIIbβ3 integrin signaling.
CYP2J2 is one of the cytochrome P450 epoxygenases involved in the metabolism of arachidonic acid. CYP2J2 has been identified in several tissues, especially cardiovascular tissues. CYP2J2 has cardiovascular effects, as epoxyeicosatrienoic acid, one of its metabolites, has anti-inflammatory and vasodilative activities. We investigated the expression of CYP2J2 in human leukocytes using reverse transcription-polymerase chain reaction, immunoblotting and immunostaining. Human monocytic cells, but not human neutrophils, exhibited constitutive expression of CYP2J2. Furthermore, the expression of CYP2J2 mRNA increased when the human monocytic cell line THP-1 cells and human monocytes were stimulated with phorbol 12-myristate 13-acetate and macrophage-colony stimulating factor in combination with granulocyte/macrophage-colony stimulating factor, respectively. These results suggest that expression of CYP2J2 was upregulated when human monocytes differentiated into macrophages and that human monocytic cells and macrophages have a pathway to metabolize arachidonic acid using CYP epoxygenases.
ADMA, AN ENDOGENOUS NOS INHIBITOR IS METABOLIZED ACTIVELY IN RAT ERYTHROCYTES

Miyuki Yokoro, Makiko Suzuki, Yoshitaka Takahashi, Hiromi Yamashita, Miki Hiemori, Hideaki Tsuji and Masumi Kimoto

Department of Nutritional Science, Okayama Prefectural University, Japan

Background: Synthesis of the vasodilator nitric oxide (NO) can be inhibited by the endogenous $N^G$, $N^G$-dimethylarginine (ADMA) and $N^G$-monomethylarginine (MMA), which are post-translationally synthesized in nuclear proteins and then released in body fluids during protein turnover. Recently, plasma ADMA level (PAL) was shown to be elevated in a number of cardiovascular disorders, in which NO availability is reduced, suggesting that the impaired NO production may contribute to the initiation and progression of their diseases. However, the regulatory mechanism of PAL is poorly understood. The purpose of this study was to examine the possibility whether the circulatory blood system may contribute to the elevation of PAL. First, we investigated the characterization of the metabolic systems of ADMA in blood cells.

Methods and Results: Platelets, leukocytes and erythrocytes were prepared from rat blood by centrifugation. The expression of DDAHs, ADMA-degrading enzymes and PRMT1, which methylates specifically arginine residues in protein moiety, was determined by RT-PCR and western blotting. Although DDAH1 and PRMT1 were shown to express in all blood cell fractions, the DDAH2 expression was not observed in any cells. The DDAH activity occurred predominantly in erythrocyte fraction. Some proteins incorporated ADMA were detected in erythrocyte cells by western blotting with ASYM24 antibody. One of them was identified "catalase" by LC/MS/MS analysis.

Conclusions: These results demonstrate that ADMA, an endogenous NOS inhibitor is actively metabolized by enzyme system in erythrocytes, suggesting that the cells might regulate the NO-NOS system by modulating plasma ADMA.
Objective: Little information is available about the effects of serotonergic receptors in vein grafts. We examined whether chronic administration of 5-hydroxytryptamine (5-HT) type 2A receptor antagonist, sarpogrelate hydrochloride (SH) modulates the expression of these 5-HT receptors in vein grafts.

Methods: Male rabbits were divided into a control and SH-treated group (SH group). Jugular vein was interposed in carotid artery in reversed fashion. Isometric tension was examined using vein grafts after 4-weeks. Concentration-response curve (CRC) for 5-HT was obtained in each group in the absence and presence of the NO synthase inhibitor L-NG-nitroarginine (L-NNA). The expression of 5-HT2A and 5-HT1B receptors was examined immunohistochemically.

Results: 5-HT induced a concentration-dependent contraction in both groups. L-NNA did not significantly modify this contraction in the control but enhanced this in the SH group. The 5-HT1B receptor antagonist GR55562 sifted CRC for 5-HT to the right in control. On the other hand, in SH group, this shifted CRC for 5-HT to the left and this action was inhibited by L-NNA. The expressions of 5-HT2A receptors in medial region were significantly less in SH group than in control. The expression of 5HT1B receptors in endothelium was not significantly different between the two groups.

Conclusions: The chronic administration of SH reduces 5-HT-induced contraction, which is due to reduction of 5-HT2A receptors in smooth muscle cells of rabbit vein graft. The increased function of NO in SH group may contribute to the 5-HT1B receptor-mediated endothelium-dependent inhibition of 5-HT-contraction.
POSSIBLE ROLE OF ORGANIC CATION TRANSPORT-3 IN SEROTONIN UPTAKE IN HUMAN BRAIN VASCULAR SMOOTH MUSCLE CELLS

Eva YW Ho, Rachel WS Li, Paul M Vanhoutte, Ricky YK Man, Susan WS Leung and George PH Leung

Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong, China

Serotonin (5HT) is a vasoconstrictor. It has been reported that 5HT can be taken up by vascular smooth muscle cells of rat aortas through the serotonin transporters (SERT). This 5HT uptake mechanism plays a crucial role in fine-tuning the availability of 5HT at its cognate receptors. However, it is unclear if SERT or other transporters are responsible for the 5HT uptake in vascular smooth muscle cells of human resistance arteries. The aim of this work was to characterize the 5HT uptake in human brain vascular smooth muscle cells (HBVSMCs). The $[^{3}H]5HT$ uptake in HBVSMCs was increased with time and was saturable with a Michaelis-menten constant of 50.36 ± 10.2 mM. The $[^{3}H]5HT$ uptake was enhanced when the extracellular medium was changed alkaline. Moreover, the $[^{3}H]5HT$ uptake was resistant to citalopram, a SERT inhibitor, up to 1 μM, but it could be inhibited by citalopram at higher concentrations (nearly 40% inhibition by 1 mM). Tetraammonium (TEA, at 1 mM) and 1-methy-4-phenylpyridinium (MPP, at 1 mM), which are substrates of organic cation transporters (OCTs), inhibited 5HT uptake by 17 and 26%, respectively. The low affinity of 5HT transport, pH dependence and inhibition by organic cations are typical characteristics of OCTs. In addition, the results of RT-PCR demonstrated the presence of mRNA of OCT-3, but the absence of OCT-1, OCT-2, organic anion transporters (OATs) and SERT. Therefore, we suggest that the 5HT uptake in HBVSMCs is different from that in rat aorta. It is partly mediated by OCT-3, but does not involve SERT.
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CELL SURFACE EXPRESSION OF HISTAMINE H3 RECEPTOR IS REGULATED BY PROTEINS INTERACTING WITH THE CARBOXY-TERMINUS OF THE RECEPTOR

Kazuki Kinoshita\textsuperscript{a}, Shiori Takayanagi\textsuperscript{a}, Takeya Sato\textsuperscript{a}, Kay Maeda\textsuperscript{a}, Mituya Haraguchi\textsuperscript{b}, Kazuhiko Yanai\textsuperscript{c}, Kohji Fukunaga\textsuperscript{d}, Teruyuki Yanagisawa\textsuperscript{a} and Jun Sukegawa\textsuperscript{a}

\textsuperscript{a}Department of Molecular Pharmacology, Tohoku University Graduate School of Medicine, Japan
\textsuperscript{b}Mitsubishi Tanabe Pharma Corporation, Japan
\textsuperscript{c}Department of Pharmacology, Tohoku University Graduate School of Medicine, Japan
\textsuperscript{d}Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Japan

Histamine H3 receptor (H3R) is a Gi protein-coupled receptor expressed mainly at presynaptic membrane of nerve terminals in various parts of central and peripheral nervous systems. As an auto- or hetero-receptor, the receptor activities negatively modulate release of not only histamine, but also a variety of neurotransmitters in the nervous systems. Especially, activation of H3R located on the postganglionic sympathetic nerve terminals inhibits noradrenalin release and causes vasodilatory effects on various organs. Therefore, H3R has been recognized as a unique drug target for treating various pathological conditions including myocardial ischemia. As reported previously, we identified CLIC4 (chloride intracellular channel 4) as an H3R interacting protein that enhanced cell surface expression of the receptor. Introduction of small interfering RNA (siRNA) against CLIC4 decreased cell surface H3R, and revealed enhanced signals from the receptor retained at endoplasmic reticulum (ER). In the continuing search for the proteins that interact with H3R, we have identified yet another protein that interacts directly with the carboxy(C)-terminus of H3R. Cell ELISA experiments and flow cytometric analyses demonstrated that overexpression of the protein decreased not only the cell surface receptor, but also the total amount of H3R in cells. The protein shares the binding site with CLIC4 on the C-terminus of H3R, but showed less affinity than that of CLIC4 for the receptor. We will discuss possible mechanisms of the regulation of H3R cell surface expression by this protein.