140±2.8/84±2.1 to 131±3.9/80±3.9 mmHg during chlorthalidone vs 130±2.8/79±2.6 mmHg during spironolactone). In contrast, SNA increased significantly with chlorthalidone (from 39±3 to 45±4 bursts/min, $p < 0.05$ vs baseline and < 0.01 vs spironolactone), while it was unaffected by spironolactone in the same subjects (38±3 bursts/min). Baroreflex gain was unaffected by treatment with either drug (from baseline -13.2±2.1 to -9.8±0.8 total activity/beat/mmHg during chlorthalidone vs -10.5±1.4 total activity/beat/mmHg during spironolactone, $p > 0.05$), indicating that failure of SNA to increase during spironolactone was not due to impaired baroreflex function. In conclusion, our data suggest that thiazide-type diuretics, the first-line drug therapy for hypertension, cause persistent activation of sympathetic nervous system in hypertensive patients. This side effect is avoided by MR antagonists, at the doses equally effective in reducing BP. Because sympathetic overactivity contributes to poor prognosis in patients with cardiovascular diseases, MR antagonists may constitute a safer alternative to thiazide-type diuretics for treatment of hypertension without compromising antihypertensive efficacy.

1416

Chronic TNF-Alpha Infusion Induces Mitochondrial Damage in the Heart and Kidney of Rats by Depleting Mitochondrial Membrane Permeability Proteins

Nithya Mariappan, Srinivas Sriramula, Masudul Haque, Francis Joseph; Louisiana State Univ, Baton Rouge, LA

TNF-α is multifunctional cytokine and plays an important role in cardiovascular regulation. In this study, we demonstrate that chronic TNF-α induces mitochondrial oxidative stress, and depletes superoxide dismutase in heart and renal medulla of rats. Methods: Rats were treated with TNF-α (40μg/kg, subcutaneously), TNF-α + Apocyanin (APO, 200 micromolar/kg, intraperitoneally), TNF-α +Tempol (TEMP, 300 micromolar, orally) or vehicle for 5 days. On day 5 left ventricular (LV) function was measured using echocardiography, followed by conscious renal sympathetic nerve activity (RSNA) measurement. Subsequently, rats were sacrificed the LV and renal medullary tissue was removed for gene expression studies and mitochondrial assay. The structural integrity of mitochondrial membrane was measured using swelling assay and Western blot, and the antioxidant status in the mitochondria and the tissues were measured using enzymatic assay. Results: TNF-α treatment induced significant increase in RSNA, increased the expression of NADPH oxidase subunits, AT-1 receptor and induced mitochondrial damage to the LV and kidney medulla and decreased the membrane permeability transitional pore proteins ANT, and VDAC. The SOD2 mRNA and enzyme activity decreased, compared with vehicle treated animals. Treatment with TNF-α+TEMP prevented mitochondrial damage, and restored antioxidant enzymes and improved SOD2 activity and restored RSNA. It also decreased AT1 receptor expression and NADPH oxidase subunits. Treatment with TNF-α+APO, decreased NADPH oxidase subunits, AT-1 receptor and attenuated mitochondrial damage but did not restore antioxidant defense system in the mitochondria. These results suggest that TNF-α causes damage to both the cell membrane and mitochondrial membrane by increasing superoxide production both in the cytosol and in the mitochondria of the heart and kidneys. Apocyanin partially restores cell membrane damage by preventing induction of NADPH oxidase and does not restore mitochondrial membrane damage. Conclusion: TNF-a induced mitochondrial damage in the heart and kidney could contribute to the pathophysiology of cardiovascular disease. Preventing excessive production of TNF-a might benefit patients with cardiovascular disease.

1417

Contribution of Angiotensin II in the Increased Reactive Oxygen Species in Rostral Ventrolateral Medulla and Enhanced Central Sympathetic Outflow in Stroke-Prone Spontaneously Hypertensive Rats

Yoji Sagara, Yoshitaka Hirooka, Masatsugu Nozoe, Yasuaki Koga, Kenji Sunagawa; Kyushu Univ, Fukuoka, Japan

Background: We demonstrated that reactive oxygen species (ROS) are increased in the rostral ventrolateral medulla (RVLM) in the brainstem, where the vasomotor center is located, in stroke-prone spontaneously hypertensive rats (SHRSP). The brain renin- angiotensin system plays an important role in the neural mechanism of hypertension. It is suggested that angiotensin II (Ang II) increases ROS production via the NADPH oxidase pathway, which is regulated by the small GTP-binding protein Rac1. The aim of the present study was to evaluate whether brain Ang II contributes to increase the ROS in the RVLM and to enhance central sympathetic outflow in SHRSP. Methods: SHRSP were treated orally with telmisartan (10 mg/kg per day) for 30 days. In other SHRSP, we transfected adenovirus vectors encoding dominant-negative Rac1 (AdN17Rac1) into the RVLM to inhibit the NADPH oxidase/Rac1 pathway. Blood pressure and heart rate were measured. Urinary norepinephrine excretion was measured as a marker of sympathetic nerve activity. We measured ROS using the salicylate trapping technique to quantify 2,3-dihydrobenzoic acid by in vivo microdialysis. Levels of ROS in the RVLM of SHRSP were measured and compared with those of WKY. The changes of ROS levels in the RVLM were evaluated after treatment with telmisartan or transfection of AdN17Rac1. Furthermore, the effects of infusion of Ang II into the RVLM on blood pressure and ROS production were observed before and after treatment. Results: Both treatment with telmisartan and transfection of AdN17Rac1 in RVLM decreased blood pressure, heart rate, and urinary norepinephrine excretion in SHRSP. ROS levels in the RVLM measured by in vivo microdialysis were greater in SHRSP than in WKY. ROS levels were decreased by treatment with telmisartan or transfection of AdN17Rac1 in SHRSP. Blood pressure and ROS production were increased during infusion of Ang II into the RVLM, and the increases in these variables were greater in SHRSP than in WKY. These effects were attenuated by both treatment with telmisartan and transfection of AdN17Rac1 in SHRSP. Conclusions: These results suggest that brain Ang II contributes to increase the ROS in the RVLM of SHRSP and might also contribute to the activation of the central sympathetic outflow and hypertension.

Preconditioning and Postconditioning to Protect the Heart

Subspecialty: Myocardial Ischemia/Function/Metabolism
Wednesday Morning
McCormick Place, E352
Abstracts 1418–1427

1418

Distinct Cardioprotective Mechanism of Cyclosporin A and Ischemic Preconditioning Against Ischemia/Reperfusion Revealed by Real-Time Two-Photon Imaging of Perfused Rat Hearts

Madoka Matsumoto-Ida, Masaharu Akao, Toshihiro Takeda, Masashi Kato, Moritake Iguchi, Toru Kita; Kyoto Univ Graduate Sch of Medicine, Kyoto, Japan

Background: We recently established a real-time imaging system to monitor mitochondrial function in the perfused heart subjected to ischemia/reperfusion, using two-photon laser scanning microscopy. The loss of mitochondrial membrane potential ($\Delta\Psi_m$) is a critical step of cardiomyocyte death during ischemia/reperfusion, which is mediated by the opening of the mitochondrial permeability transition pore (MPTP). We tested the effect of a MPTP blocker, cyclosporin A (CsA), and that of ischemic preconditioning (IPC) on $\Delta\Psi_m$ loss. *Methods and Results:* The rat heart was cannulated and perfused with Tyrode's solution in Langendorff mode. After loading with a fluorescent indicator of $\Delta\Psi_m$, tetramethylrhodamine ethyl-ester, it was transferred onto the microscope stage. Under the two-photon excitation with 810 nm line of a Ti:Sapphire laser, the heart was subjected to ischemia/reperfusion by clamping the perfusion line and releasing the clamp. Spatio-temporal changes of $\Delta\Psi_m$ in response to ischemia/reperfusion were monitored at subcellular level. During ischemia/reperfusion, cells maintained a constant $\Delta\Psi_m$ for the cell-to-cell specific period of latency, followed by a rapid and irreversible $\Delta\Psi_m$ loss. CsA (0.2 μmol/L) did not affect the latency period, but slowed the process of $\Delta\Psi_m$ loss and blunted its severity. In contrast, IPC (3 cycles of 5 min ischemia and 5 min reperfusion) not only decreased the number of cells undergoing $\Delta\Psi_m$ loss but also delayed the onset of $\Delta\Psi_m$ loss, whereas it did not change the duration of $\Delta\Psi_m$ loss in unprotected cells. Moreover, $\Delta\Psi_m$ level was fully polarized in the protected cells. Although CsA and IPC achieved the similar level of protection, the mechanism of action was suggested to be distinct, as evidenced by the differential kinetics of $\Delta\Psi_m$ loss in each individual cell. *Conclusions:* This novel two-photon imaging provides deeper insights into anti-ischemia/reperfusion therapy targeting mitochondria.

1419

Ischemic Postconditioning Against Ischemia/Reperfusion Injury Beyond the Myocardium

Sandra E McAllister, Homa Ashrafpour, Ning Huang, Peter C Neligan, Cho Y Pang; Hosp For Sick Children, Toronto, Canada

In trauma surgery, skeletal muscle is subjected to sustained ischemia before surgery and ischemia/reperfusion (I/R) injury is a common complication. We tested the hypothesis that postconditioning (PostC) skeletal muscle with 4 cycles of 30 sec I/R at the beginning of reperfusion salvages ischemic skeletal muscle from I/R injury (infarction), and the mechanism involves inhibition of mitochondrial permeability pores (mPTPs). Pigs (~18 kg) with bilateral latissimus dorsi muscle flaps were assigned to 6 groups ($n = 5$) to undergo 4h ischemia/48h reperfusion with the following treatments: (1) control; (2) PostC muscle flaps at the beginning of reperfusion; (3) intravenous (IV) injection of the mPTP inhibitor NIM-811 (10 mg/kg) 5 min before reperfusion; (4) IV injection of the mPTP inhibitor cyclosporin A (CsA, 10 mg/kg) 5 min before reperfusion; (5) IV injection of the mPTP opener atractyloside (ATS, 5 mg/kg) 5 min before PostC; and (6) IV injection of ATS (5 mg/kg) 5 min before reperfusion. Muscle infarction was 46 ± 2% after 4h ischemia/48h reperfusion. Muscle infarction was reduced ($p < 0.05$) by PostC, NIM-811 and CsA to 21 ± 1, 26 ± 2, and 27 ± 1%, respectively when given at the beginning of reperfusion. ATS abolished the infarct protection of PostC, but alone it did not induce infarction. Muscle free mitochondrial Ca2+ concentration (m[Ca2+]), ATP contents and myeloperoxidase (MPO) activity were similar in all groups before and after 4h of ischemia. At the end of 2h of reperfusion, the muscle free m[Ca2+] were lower ($p < 0.05$) in the PostC and CsA treatment groups than in the control (372 ± 55, 379 ± 43 and 538 ± 41 mmol/mg mitochondrial protein, respectively); muscle ATP contents were higher ($p < 0.05$) in PostC and CsA groups than in the control (19 ± 4, 19 ± 3, and 8 ± 1 umol/g protein, respectively); and MPO activities were lower ($p < 0.05$) in the PostC and CsA groups than the control (0.8 ± 0.3, 0.9 ± 0.3 and 2.1 ± 0.3 U/g wet wt., respectively). This is the first report that the efficacy and mechanism of PostC in salvage of ischemic skeletal muscle from infarction are similar to those of ischemic cardiac muscle reported by others. Importantly, our findings provide insights into the use of the clinical mPTP inhibitor CsA for salvage of ischemic skeletal muscle from I/R injury in trauma surgery.

1420

Differential Interaction of p38mapk-alpha and p38 mapk-beta with Caveolin-1 and Caveolin-3 Regulates Cardioprotection by Ischemic Preconditioning

Manika Das, Dipak K Das; Univ Connecticut Sch Med, Farmington, CT

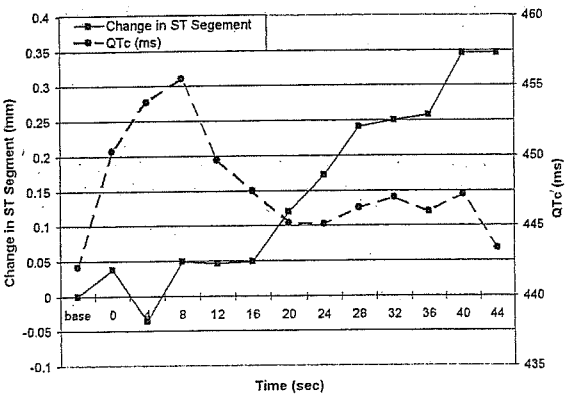
Activation of p38MAP kinase (p38MAPK) appears to be essential for preconditioning (PC); however, its mechanism of action remains controversial. Based on the recent reports that MAPKs can be translocated into caveolins, we hypothesized that caveolins could regulate

932

Temporal Relationship between QTc Prolongation and ST-segment Elevation in Early Transmural Myocardial Ischemia Measured by a Continuous 12-lead ECG Monitor

Marcin Kowalski, James Dobbs, Mohamad Sinno, Henry Ford Hosp, Detroit, MI; David N Kenigsberg, Med College of Virginia, Richmond, VA; Joel Xue, General Electric Med System, Menomonee Falls, WI; Subramaniam C Krishnan, Univ of California at Irvine Sch of Medicine, Irvine, CA; Sanjaya Khanal; Henry Ford Hosp, Detroit, MI

Introduction: We have previously reported that prolongation in the corrected QT interval (QTc) is the most consistent ECG abnormality seen during early transmural ischemia. However, the time course of QTc compared to ST segment (ST) changes during early transmural ischemia had not been investigated. **Methods:** Fourteen consecutive patients undergoing elective percutaneous coronary interventions (PCI) had electrocardiograms (ECGs) continuously recorded using 12-lead Holter Recorder (SeerMC^{tm} ,GE Healthcare). QT intervals and ST segment changes were digitally measured using the Interval Editor (GE Healthcare). The mean QTc and changes in ST segments were plotted over time during the first balloon occlusion. ST changes were measured in the lead with maximum change. QT interval was corrected for the heart rate using the Bazett's formula. **Results:** The average QTc prolonged from 442±16 ms at baseline to 455±18 ms (p=0.01) at a mean of 8 seconds from the balloon occlusion. The maximum ST-elevation was 0.34±0.86 mm and it occurred at mean of 40 sec from balloon inflation. ST elevations were consistently found to occur only after the maximum QTc prolongation (Figure). **Conclusion:** By continuous ECG monitoring, QTc prolongation occurs very early during the initial stages of transmural ischemia. The more commonly used marker for transmural ischemia, ST elevation, occurs only after QTc prolongation. **Figure:** QTc and Changes in ST-segments during Early Transmural Ischemia



Autonomic, Reflex, and Neurohormonal Control of Circulation I

Subspecialty: Integrative Biology

Tuesday Morning

McCormick Place, E353b

Abstracts 933–936

933

Increased Reactive Oxygen Species in the Nucleus Tractus Solitarius are Involved in the Neural Mechanisms of Hypertension in Stroke-Prone Spontaneously Hypertensive Rats

Masatsugu Nozoe, Yoshitaka Hirooka, Yasuaki Koga, Shu-ichiro Araki, Takuya Kishi, Kenji Sunagawa; Dept of Cardiovascular Medicine, Kyushu Univ Graduate Sch of Medicine Sciences, Fukuoka, Japan

Background: Reactive oxygen species (ROS) in the central nervous system (CNS) are thought to contribute to sympathoexcitation in cardiovascular diseases such as hypertension and heart failure. NAD(P)H oxidase is a major source of ROS in the CNS, and mediates angiotensin II signaling in neurons. The nucleus tractus solitarius (NTS), which receives afferent input from baroreceptors, have an important role in cardiovascular regulation. The role of ROS in the NTS, however, is not understood. The aim of the present study was to examine 1) whether ROS generation is increased in the NTS of stroke-prone spontaneously hypertensive rats (SHRSP), 2) whether ROS scavenging is decreased in the NTS of SHRSP, 3)and whether ROS in the NTS is involved in the neural mechanisms of hypertension. ***Methods:*** ROS generation in the NTS of SHRSP and WKY was evaluated by measuring thiobarbituric acid-reactive substances (TBARS) levels. NADPH oxidase activity was measured by lucigenin luminescence and Cu/Zn supeoxide dismutase (Cu/SnSOD) activity was assayed by reduction of cytochrome c. The role of ROS in the NTS of SHRSP in cardiovascular regulation was evaluated using two different methods; transfection of either adenovirus encoding human Cu/Zn superoxide dismutase (AdCu/ZnSOD) to scavenge cytosolic superoxide or adenovirus encoding dominant-negative Rac1 (AdDN17Rac1) to suppress ROS generation by NAD(P)H oxidase in the NTS. Mean blood pressure (MBP) and heart rate (HR) were measured using a radio-telemetry system. Urinary norepinephrine excretion for 24 hours was measured as an indicator of sympathetic nerve activity. ***Results:*** TBARS levels and NAD(P)H oxidase activity in the NTS were greater in SHRSP than in WKY. Cu/ZnSOD protein expression and SOD activity in the NTS was lower in SHRSP than in WKY. Transfection of either AdCu/ZnSOD or AdN17Rac1 into the NTS decreased MBP, HR, and urinary norepinephrine excretion in SHRSP. ***Conclusions:*** These results suggest that the increased ROS in the NTS of SHRSP contribute to the neural mechanisms of hypertension. Furthermore, both the accelerated ROS production and suppressed scavenging of cytosolic ROS might contribute to increased oxidative stress in the NTS of SHRSP.

934

Nerve Growth Factor is Critical for Cardiac Sensory Innervation and Rescues Neuropathy in Diabetic Hearts

Masaki Ieda, Hideaki Kanazawa, Kensuke Kimura, Yasuyo Ieda, Keisuke Matsumura, Yuichi Tomita, Shinji Makino, Motoaki Sano, Satoshi Ogawa, Keiichi Fukuda; keio Univ, tokyo, Japan

Background Molecular mechanisms regulating the cardiac sensory nervous system remain poorly understood. Cardiac sensory nerve impairment causes silent myocardial ischemia, the main cause of sudden death in diabetes mellitus (DM). The present study focused on the roles of nerve growth factor (NGF) in the regulation of the cardiac sensory nervous system and analyzed the mechanism of silent myocardial ischemia in DM. **Methods and Results** (1) We screened neurotrophic factors, and found that cardiac sensory nerves develop sequentially after birth in parallel with NGF synthesized in the heart. (2) In NGF-deficient mice, the cardiac sensory nervous system, composed of cardiac nociceptive sensory nerves immunopositive for calcitonin gene-related peptide (CGRP), dorsal root ganglia (DRG) and dorsal horn, was markedly retarded. (3) Transgenic mice over-expressing NGF under a control of α-MHC promoter (NGFTG) were used to rescue NGF defects in NGF$^{-/-}$ mice (NGF$^{-/-}$/TG) hearts. A strong NGF expression of 22 fold was detected in NGF$^{-/-}$/TG hearts compared with NGF$^{+/+}$, and NGF$^{-/-}$/TG mice demonstrated complete restoration of cardiac sensory deficits. (4) DM was induced by an intraperitoneal injection of streptozotocin in wild-type and NGFTG mice. Both wild-type and NGFTG mice had similar hyperglycemia, and had lost body weight at 16 weeks. NGF mRNA expression in the heart was significantly down-regulated by 48% in DM-induced wild-type mice, and cardiac sensory denervation and atrophic changes in DRG were observed. (5) In contrast, a strong NGF expression was detected in DM-induced NGFTG hearts, and the sensory deterioration was completely rescued. (6) To analyze cardiac sensory function, the mice were subjected to myocardial ischemia and c-Fos expression in DRG, a marker of pain perception, was measured. Myocardial ischemia induced c-Fos expression in DRG, which was down-regulated by DM to 50% in the wild-type mice. (7) In contrast, DM-induced NGF transgenic mice did not show down-regulation of c-Fos induction in myocardial ischemia. **Conclusions** These findings demonstrate that the development and regulation of the cardiac sensory nervous system are dependent on NGF synthesized in the heart, and that DM-induced NGF reduction causes cardiac sensory neuropathy.

935

A Novel Protective Mechanism Underlying Endotoxin-Induced Hypotension: Role of the TRPV1 Receptor

Youping Wang, Michigan State Univ, East Lansing, MI; Martin Novotny, Veronika Quaiserova-Mocko, Greg M Swain, Donna H Wang; Michigan State Univ, East Lansing, MI

The transient receptor potential vanilloid type 1 (TRPV1) channels, expressed primarily in sensory nerves, function as a molecular integrator of multiple stimuli and trigger the release of sensory neurotransmitters such as substance P (SP) upon activation. This study was designed to test the novel hypothesis that TRPV1 and SP play a protective role against endotoxic hypotension and mortality induced by systemic administration of lipopolysaccharide (LPS). LPS (10 mg/kg, iv) elicited tachycardia and hypotension in anesthetized male Wistar rats, which peaked at 10 min with a fall in mean arterial pressure (MAP, mmHg) up to 66 ± 4 and gradually recovered 1 hr after the injection with a smaller fall of 25 ± 4 in MAP. Blockade of TRPV1 with its selective antagonist, capsazepine (CAPZ, 3 mg/kg, iv), impaired recovery given that the fall in MAP was greater 1 hr after CAPZ plus LPS injections (47 ± 5) compared to LPS injection alone (25 ± 4, $p<0.05$). Degeneration of TRPV1-positive sensory nerves caused by neonatal capsaicin treatment elicited similar MAP responses to LPS as that of CAPZ. Blockade of the neurokinin-1 (NK1) receptor with its selective antagonists, RP67580 (5 mg/kg, iv) or L-733,060 (4 mg/kg, iv), prevented recovery considering that falls in MAP were not different 1 hr after injections of NK1 antagonists plus LPS from their peak decreases (66 ± 9 vs 74 ± 5, and 61 ± 8 vs 69 ± 3, respectively, $p>0.05$). LPS increased plasma SP (pg/ml), norepinephrine (NE, ng/ml), and epinephrine (EP, ng/ml) levels compared to vehicles (SP: 29.4 ± 5.1 vs 17.3 ± 3.2; NE: 2.75 ± 0.24 vs. 1.63 ± 0.28; EP: 10.53 ± 0.88 vs 5.27 ± 0.89, $p<0.05$), and increases in plasma NE and EP were inhibited by RP67580. The survival rate during the first 24 hrs after LPS injection (20 mg/kg, ip) was lower in conscious rats pretreated with CAPZ (22.2%) or RP67580 (11.1%) compared to rats treated with LPS alone (55.5%, $p<0.05$). Thus, our results show for the first time that the TRPV1, possibly via triggering release of SP which activates the NK1 and stimulates the sympathetic axis, plays a protective role against endotoxin-induced hypotension and mortality. Our data indicate that intact TRPV1 and TRPV1-postive sensory nerve function are essential in protecting vital organ perfusion and survival during the endotoxic condition.

936

Gender Differences in the Vasoconstrictor Effect of Sympathetic Neural Activity

Andrew J Hogarth, Alan F Mackintosh, David A Mary; Dept of Cardiology, St James's Univ Hosp, Leeds, United Kingdom

Background: Sympathetic activation and its vasoconstrictor effect have been associated with myocardial infarction (MI) and hypertension (HT). However women, when compared to age-matched men, are considerably less likely to develop MI or HT until much later years when