cardiovascular and renal system indicate that they may share the functions of cardiovascular regulation.

Key Words: Adrenomedullin, Adrenomedullin 2, Vasoactive Peptide

P-659
CHARACTERIZATION OF THE HUMAN CGRP RECEPTOR SUBTYPES ASSOCIATED WITH RECEPTOR ACTIVITY-MODIFYING PROTEINS
Kenji Kusawasko, Kazuo Kitamura, Yuan-Ning Cao, Yasuko Nagoshi, Tanenao Eto. First Department of Internal Medicine, Miyazaki Medical College, University of Miyazaki, Kiyotake, Miyazaki, Japan.

Calcitonin gene-related peptide (CGRP) is highly potent vasodilator and contains a disulfide bridge between cysteine residues (Cys) at positions 2 and 7, which is required for biological activity. Co-expression of receptor activity-modifying proteins (RAMPs) with calcitonin receptor 2 (CTR2) or calcitonin receptor-like receptor (CRLR) leads to the formation of four functional heterodimeric receptors for human (h) CGRP. CTR2 is the most common splice variant of hCTR and like CRLR, belongs to the Class B family of G protein-coupled receptors. In this study, we transfected hCGRP receptors into human embryonic kidney (HEK)-293 cells and examined their pharmacological profiles using three dominant-negative (DN) RAMP mutants that we identified and various hCGRP analogs. Fluorescence-activated cell-sorting analysis revealed that their co-transfection with CTR2 induced cell surface expression of all three RAMPs, and the three CTR2/RAMP heterodimers mediated equivalent levels of cAMP production in response to hCGRP that were approximately 50-fold greater than were seen with CTR2 alone. By contrast, hCys(Et)²,7hCGRP binding and signaling were markedly weaker with CTR2/RAMP-2 or -3 than with CTR2/RAMP1 or CRLR/RAMP1, whereas 125I-H9251/[Tyr⁰]hCGRP binding and signaling were comparable with that seen with CTR2 alone, indicating that DN-RAMP3 markedly attenuated the activity of endogenous RAMP1 highly expressed in various cultured cells.

Key Words: Calcitonin Receptor, CGRP Receptor Subtype, Receptor Activity-Modifying Proteins

P-660
IDENTIFICATION OF DOMINANT-NEGATIVE HUMAN RAMP1 ABLE TO INHIBIT ENDOGENOUS CGRP, RECEPTOR FUNCTION
Kenji Kusawasko, Kazuo Kitamura, Yuan-Ning Cao, Yasuko Nagoshi, Tanenao Eto. First Department of Internal Medicine, Miyazaki Medical College, University of Miyazaki, Kiyotake, Miyazaki, Japan.

Calcitonin gene-related peptide (CGRP) and adrenomedullin (AM) belong to the calcitonin family of regulatory peptides and are both highly potent vasodilators. Both of these peptides and their specific or common receptors are widely distributed among peripheral tissues and in the central nervous system, enabling them to exert a wide variety of biological effects. The newly identified accessory proteins, receptor activity-modifying protein (RAMP)-1, -2 and -3, form heterodimers with an orphan receptor, calcitonin receptor-like receptor (CRLR), and mediate its translocation to the cell surface. CRLR/RAMP2 or CRLR/RAMP3 comprises an AM receptor. On the other hand, CRLR/RAMP1 comprises the CGRP receptor, which also responds to higher concentrations of AM. To investigate the structural determinants of ligand binding specificity, we examined the extracellular domain of human (h) RAMP1 using various deletion mutants. Co-expression of the hRAMP1 mutants with hCRLR in human embryonic kidney (HEK)-293 cells revealed that deletion of residues 91-94, 96-100, or 101-103 blocked 125I-CGRP binding and completely abolished intracellular cAMP accumulations normally elicited by CGRP or AM. On the other hand, the deletion of residues 78-80 or 88-90 significantly attenuated only AM-evoked responses. In all of these cases, the receptor heterodimers were fully expressed at the cell surface. Substituting alanine (A) for residues 91-103 one at a time had little effect on CGRP-induced responses, indicating that although this segment is essential for high affinity agonist binding to the receptors, none of the residues directly interacts with either CGRP or AM. This finding suggests that RAMPs probably determine ligand specificity by contributing to the structure of the ligand-binding pocket or by allosteric modulation of the conformation of the receptor. Interestingly, the L94A mutant up-regulated surface expression of the receptor heterodimer to a greater degree than wild-type hRAMP1, thereby increasing CGRP binding and signaling. L94A also significantly increased cell surface expression of the hRAMP1 deletion mutant D1100-103 when co-transfected with hCRLR, and expression of a L94A/D1101-103 double mutant markedly attenuated the activity of endogenous RAMP1 highly expressed in various cultured cells.

Key Words: CGRP, CRLR, Receptor, Dominant-Negative RAMP1

P-661
EFFECT OF BQ-123, AN ENDOTHELIN ANTAGONIST, ON RENAL HEMODYNAMICS, TUBULAR FUNCTION, VASOACTIVE HORMONES AND BLOOD PRESSURE IN HEALTHY MAN. A DOSE RESPONSE STUDY
Erling B Pedersen, Ingrid M Thomsen, Lene S Fjorderside. Department of Medicine and Medical Research, Holstebro Hospital, Holstebro, Denmark; Department of Medicine and Medical Research, Holstebro Hospital, Holstebro, Denmark; Department of Medicine and Medical Research, Holstebro Hospital, Holstebro, Denmark.

The importance of endothelin for renal hemodynamics and tubular function during baseline conditions is not clarified, and the interaction with vasoactive hormones is unknown.

The purpose of the study was to measure the effect of the endothelin A antagonist, BQ-123, on renal hemodynamics, tubular function, vasoactive hormones and blood pressure in healthy men.

In a randomized, placebo-controlled, double blind dose response study in 11 healthy men we measured the effect of the endothelin A antagonist BQ-123 on glomerular filtrationsrate (GFR), renal plasmaflow (RPF), fractional excretions of sodium (FENa) and lithium clearance (CLi), blood pressure (BP), and plasma concentrations of renin (PRC) and angiotensin II (Ang II). BQ-123 was infused intravenously in 0.1 mg/kg (low dose), 0.2 mg/kg (medium dose) and 0.3 mg/kg (high dose) during one hour, and the effect variables were measured before, during and after infusion. Renal hemodynamics were determined by the constant infusion clearance method using ⁵¹Cr-EDTA and ¹²⁵I-Hippurane as reference substances. Hormones were measured by radioimmunoassays.

GFR and RPF were not significantly changes by BQ-123, but FENa was increased (20%, medium dose), Clp was unchanged. Systolic BP were unchanged, whereas diastolic BP decreased (-6.3%, medium dose), and pulse rate increased (7.1%, medium dose) during BQ-123 infusion. BQ-123 increased both PRC (62%, medium dose) and Ang II (70%, medium dose). The changes in FENa, diastolic BP, pulse rate and Ang II gradually increased till medium dose.
Infusion of an endothelin A antagonist resulted in an increase in renal sodium excretion despite a stimulation of the renin-angiotensin system and a decrease in diastolic blood pressure. These results show that endothelin plays a role during baseline conditions both in vascular tone in the systemic circulation and in the regulation of renal sodium excretion. Blockade of the endothelin A effects can resist and to some extent antagonize the vascular and renal effects of the renin-angiotensin system on the cardiovascular homeostasis and sodium balance.

Key Words: Endothelin, Renal Hemodynamics, Vasoactive Hormones

P-662
ASSOCIATION OF A NONSYNONYMOUS POLYMORPHISM IN ALOX12 (R261Q) TO ESSENTIAL HYPERTENSION AND URINARY LEVELS OF 12-HETE

Luis F Quintana, Blanca Guzman, Silvia Collado, Joan Claria, Esteban Pich, Nephrology, Hospital Clinic, Barcelona, Spain; Hormonal Laboratory, Hospital Clinic, Barcelona, Spain.

The platelet-type 12-lipoxygenase (12-LO) catalyzes the transformation of arachidonic acid into 12-hydroperoxyeicosatetraenoic acid (12-SHETE), which is reduced to 12-hydroxyicosatetraenoic acid (12-SHETE). These metabolites exhibit a variety of biological activities such as mediation of angiostatin II-induced intracellular calcium transients in cultured rat vascular smooth muscle cells. We previously demonstrated that patients with essential hypertension excrete higher levels of 12-HETE in urine (Hypertension 2001;37:334) than normotensive controls. Several polymorphisms of the human 12-LO gene (ALOX12) have been described, 3 of them being nonsynonymous, of which one, R261Q, is located in a conserved domain of the enzyme. The platelet-type 12-LO polymorphism was evaluated by PCR and restriction digestion and urinary 12(S)-HETE was measured in Sep-Pack-extracted samples using specific ELISA. The distribution of genotypes was significantly different between patients and controls: patients 78 (0.47) GG, 69 (0.42) GA, 19 (0.11) AA vs controls 56 (0.34) GG, 78 (0.48) GA, 30 (0.18) AA (p = 0.03). In addition, there was a trend to lower diastolic blood pressure in AA genotype carriers (mmHg: GG 89 (p = 0.11) AA vs controls 56 (0.34) GG, 78 (0.48) GA, 30 (0.18) AA). The association of this polymorphism with hypertension and urinary levels of 12-HETE. We studied 166 patients with essential hypertension (aged 56±1 years, mean ± SEM, 83 males) and 166 matched controls (aged 54±1 years, 89 males). R261Q polymorphism was evaluated by PCR and restriction digestion and urinary 12(S)-HETE was measured in Sep-Pack-extracted samples using specific ELISA. The distribution of genotypes was significantly different between patients and controls: patients 78 (0.47) GG, 69 (0.42) GA, 19 (0.11) AA vs controls 56 (0.34) GG, 78 (0.48) GA, 30 (0.18) AA (p = 0.03). In addition, there was a trend to lower diastolic blood pressure in AA genotype carriers (mmHg: GG 89±2 vs. GA 85±2, AA 82±2, p = 0.08). Finally, the urinary excretion of 12(S)-HETE was significantly higher in GG homozygous patients than in AA homozygous patients: 12.0±2 vs. 6.8±1 pmol/g creatinine (p = 0.02). These results indicate that a nonsynonymous polymorphism in ALOX12 is associated to essential hypertension and to urinary levels of 12(S)-HETE, thus suggesting a role for this metabolite in human hypertension.

Key Words: Eicosanoids, Lipooxygenase, Polymorphism

P-663
PLASMA EPINEPHRINE DURING MENTAL STRESS IN RELATION TO FITNESS, CARDIOVASCULAR REACTIVITY, AND METABOLIC RISK FACTORS IN YOUNG MEN

Henrik M Reins, Arnljot Flosa, Knut Sever, Eigil Fossman, Harald Mellem, Sverre E Kjeldsen, Heart and Lung Centre, Ullevaal University Hospital, Oslo, Norway; Medical Division, Ullevaal University Hospital, Oslo, Norway; Division of Hypertension, University of Michigan, Ann Arbor.

We studied plasma epinephrine (E) at rest and during mental stress in relation to physical fitness, plasma norepinephrine (NE) and cardiovascular responses, and metabolic risk factors in men (age 21-24 yrs) with high (≥140/90 mm Hg, n = 19) and normal (≥115/75 mm Hg, n = 19) screening blood pressure (BP), E and NE (radioenzymatic method), R-R interval (RR), and finger systolic (SBP) and diastolic (DBP) BP were measured during hyperinsulinemic glucose clamp (rest) and mental arithmetic stress test (MST). E and NE were averaged over 4 samples at rest and during MST. Insulin sensitivity was assessed as insulin-adjusted glucose disposal rate (GDR/I) and fitness as peak oxygen uptake (VO2peak) during treadmill exercise. By multiple regression, E at rest (Erest) was explained by lower body mass index (BMI) (β = 0.56, P < 0.001) and high screening BP (β = 0.37, P < 0.05). Contrast, E during MST (Emst) was independently explained by higher VO2peak (β = 0.54, P < 0.001) and high screening BP (β = 0.40, P < 0.01). BP and RR changes were associated with higher VO2peak but ΔSBP (β = 0.54, P < 0.001), ΔDBP (β = 0.57, P < 0.001), and ΔRR (β = 0.60, P < 0.0001) were independently explained only by Emst. ANE correlated with Emst (r = 0.71, P < 0.0001) but not with VO2peak. HDL was demonstrated that patients with essential hypertension excrete higher levels of 12-HETE in urine (Hypertension 2001;37:334) than normotensive controls. Several polymorphisms of the human 12-LO gene (ALOX12) have been described, 3 of them being nonsynonymous, of which one, R261Q, is located in a conserved domain of the enzyme. The aim of the study was to analyze the association of this polymorphism with hypertension and urinary levels of 12-HETE. We studied 166 patients with essential hypertension (aged 56±1 years, mean ± SEM, 83 males) and 166 matched controls (aged 54±1 years, 89 males). R261Q polymorphism was evaluated by PCR and restriction digestion and urinary 12(S)-HETE was measured in Sep-Pack-extracted samples using specific ELISA. The distribution of genotypes was significantly different between patients and controls: patients 78 (0.47) GG, 69 (0.42) GA, 19 (0.11) AA vs controls 56 (0.34) GG, 78 (0.48) GA, 30 (0.18) AA (p = 0.03). In addition, there was a trend to lower diastolic blood pressure in AA genotype carriers (mmHg: GG 89±2, GA 85±2, AA 82±2, p = 0.08). Finally, the urinary excretion of 12(S)-HETE was significantly higher in GG homozygous patients than in AA homozygous patients: 12.0±2 vs. 6.8±1 pmol/g creatinine (p = 0.02). These results indicate that a nonsynonymous polymorphism in ALOX12 is associated to essential hypertension and to urinary levels of 12(S)-HETE, thus suggesting a role for this metabolite in human hypertension.

Key Words: Eicosanoids, Lipooxygenase, Polymorphism

P-664
COMBINED BENAZEPRIl-AMLODIPINE TREATMENT IMPROVES CARDIAC NITRIC OXIDE AND CGMP PRODUCTION POST CARDIAC ISCHEMIA

Helmy M Siragy, Chun Xue, Randy L Webb. Department of Medicine, University of Virginia, Charlottesville, VA; Novartis Institutes for BioMedical Research, East Hanover, NJ.

Background: We evaluated changes in cardiac interstitial fluid (CIF) levels of NO and cGMP during treatment with the ACE inhibitor Benazepril, calcium channel blocker Amlodipine, individually and combined or hydrochlorothiazide (HCTZ) after induction of myocardial ischemia.

Methods and Results: Utilizing a microdialysis technique, CIF levels of NO and cGMP were monitored at 5 weeks after temporary 30 min occlusion of the left anterior descending coronary artery (LAD) or sham procedure in conscious Lewis inbred rats during oral administration of Benazepril 40 mg/kg/d, Amlodipine 10 mg/kg/d individually and combined, or HCTZ 3 mg/mg/kg/d (n = 10 in each group). In sham animals CIF NO and cGMP levels were 1.8±0.37 mM and 1.3±0.23 pmol/ml respectively. Cardiac NO and cGMP levels decreased after LAD occlusion to 0.44±0.09 mM and 0.68±0.08 pmol/ml (p < 0.001). In animals with ischemia, treatment with Benazepril or Amlodipine caused significant increases in NO levels to 1.29±0.28 mM and 1.09±0.27 mM respectively (p < 0.05 from ischemia). However, Benazepril and not Amlodipine caused significant increase in cGMP to 1.16±0.17 pmol/ml (p < 0.05). Combined administration of Benazepril and Amlodipine caused further increase in both cardiac NO and cGMP to 1.8±0.50 mM.