FROM BEDSIDE TO RESEARCH LAB AND BACK

Renalase – new renal hormone
Relationship between:

scientist = clinician
Translational medicine
Clinical symptoms of disease are macroscopic manifestation of cell dysfunction!
Clinical problem

• Very high mortality of ESRD patient
• Cardio – vascular causes!

• Renal disease – independent risk factors for cv events.
SYMPATHETIC OVERACTIVITY IN PATIENTS WITH CHRONIC RENAL FAILURE

RICHARD L. CONVERSE, JR., M.D., TAGE N. JACOBSEN, M.D., ROBERT D. TOTO, M.D.,
CHARLES M.T. JOST, M.D., FRANK COSENTINO, D.O., FETHI FAOUAD-TARAZI, M.D.,
AND RONALD G. VICTOR, M.D.

Abstract Background. Hypertension is a frequent complication of chronic renal failure, but its causes are not fully understood. There is indirect evidence that increased activity of the sympathetic nervous system might contribute to hypertension in patients with end-stage renal disease, but sympathetic-nerve discharge has not been measured directly in patients or animals with chronic renal failure.

Methods. We recorded the rate of postganglionic sympathetic-nerve discharge to the blood vessels in skeletal muscle by means of microelectrodes inserted into the peroneal nerve in 18 patients with native kidneys who were undergoing long-term treatment with hemodialysis (of whom 14 had hypertension), 5 patients receiving hemodialysis who had undergone bilateral nephrectomy (of whom 1 had hypertension), and 11 normal subjects.

Results. The mean (±SE) rate of sympathetic-nerve discharge was 2.5 times higher in the patients receiving hemodialysis who had not undergone nephrectomy than in the normal subjects (58±3 vs. 23±3 bursts per minute, P<0.01). In contrast, the rate of sympathetic-nerve discharge was similar in the patients receiving hemodialysis who had undergone bilateral nephrectomy (21±6 bursts per minute) and the normal subjects. The rate of sympathetic-nerve discharge in the patients receiving hemodialysis who had not undergone nephrectomy was also significantly higher (P<0.01) than that in the patients with bilateral nephrectomy, and it was accompanied in the former group by higher values for vascular resistance in the calf (45±4 vs. 22±4 units, P<0.05) and mean arterial pressure (106±4 vs. 76±14 mm Hg, P<0.05). The rate of sympathetic-nerve discharge was not correlated with either plasma norepinephrine concentrations or plasma renin activity.

Conclusions. Chronic renal failure may be accompanied by reversible sympathetic activation, which appears to be mediated by an afferent signal arising in the failing kidneys. (N Engl J Med 1992;327:1912-8.)
Microneurography
Central Nervous System

efferent

Nerves

afferent

Kidney damage
Norepinephrine and Concentric Hypertrophy in Patients With End-Stage Renal Disease

Carmine Zocco, Francesca Mallamaci, Giovanni Tripepi, Saverio Parlongo, Sebastiano Cutrupi, Francesco Antonio Benedetto, Alessandro Cataliotti, Lorenzo Salvatore Malatino, on behalf of the CREED investigators

Abstract—We have recently observed that in patients with end-stage renal disease (ESRD) raised plasma norepinephrine (NE) is an independent predictor of incident cardiovascular events but that its prognostic power is reduced when this sympathetic marker is tested in statistical models including also left ventricular mass. Because left ventricular hypertrophy (LVH) may be a mechanism whereby NE contributes to the high rate of cardiovascular events in ESRD, we examined the relationship between plasma NE and echocardiographic parameters of left ventricle mass in a large group of ESRD patients. Mean wall thickness (MWT) was higher in patients in the third NE tertile than in the other 2 tertiles ($P=0.001$), and such an increase was paralleled by a rise in relative wall thickness (RWT) ($P=0.006$). Concentric LVH was more prevalent in patients in the third NE tertile (46%) than in the second (38%) and first (25%) NE tertiles. Multivariate regression analysis confirmed that the association of plasma NE with the muscular component of left ventricle (MWT) and with RWT was independent ($P=0.001$) of other cardiovascular risk factors, and in these models, plasma NE ranked as the second correlate of MWT and RWT. Similarly, multiple logistic regression analysis showed that the association of plasma NE with concentric LVH was strong and again independent of other risk factors ($P=0.003$). Plasma NE is associated to concentric LVH in ESRD patients. These observations constitute a sound basis for testing the effect of anti-adrenergic drugs on left ventricle mass and on cardiovascular outcomes in patients with ESRD. (Hypertension. 2002;40:41-46.)

Key Words: cardiovascular risk ■ dialysis ■ left ventricular hypertrophy ■ norepinephrine ■ renal failure ■ sympathetic activity ■ uremia
Plasma Norepinephrine Predicts Survival and Incident Cardiovascular Events in Patients With End-Stage Renal Disease

Carmine Zoccali, MD; Francesca Mallamaci, MD; Saverio Parlongo, MD; Sebastiano Cutrupi; Francesco Antonio Benedetto, MD; Giovanni Tripepi; Graziella Bonanno, MD; Francesco Rapisarda, MD; Pasquale Fatuzzo, MD; Giuseppe Seminara, MD; Alessandro Cateliotti; Benedetta Stancanelli, MD; Lorenzo Salvatore Malato, MD

Background—Sympathetic tone is consistently raised in patients with end-stage renal disease (ESRD). We therefore tested the hypothesis that sympathetic activation is associated with mortality and cardiovascular events in a cohort of 228 patients undergoing chronic hemodialysis who did not have congestive heart failure at baseline and who had left ventricular ejection fraction >35%.

Methods and Results—The plasma concentration of norepinephrine (NE) was used as a measure of sympathetic activity. Plasma NE exceeded the upper limit of the normal range (cutoff 3.54 nmol/L) in 102 dialysis patients (45%). In a multivariate Cox regression model that included all univariate predictors of death as well as the use of sympathicoleptic agents and β-blockers, plasma-NE proved to be an independent predictor of this outcome (hazard ratio [1-nmol/L increase in plasma NE] 1.07, 95% CI 1.01 to 1.14, P = 0.03). Similarly, plasma NE emerged as an independent predictor of fatal and nonfatal cardiovascular events (hazard ratio [1-nmol/L increase in plasma NE] 1.08, 95% CI 1.02 to 1.15, P = 0.01) in a model that included previous cardiovascular events, pulse pressure, age, diabetes, smoking, and use of sympathicoleptic agents and β-blockers. The adjusted relative risk for cardiovascular complications in patients with plasma NE >75th percentile was 1.92 (95% CI 1.20 to 3.07) times higher than in those below this threshold (P = 0.006).

Conclusions—Sympathetic nerve overactivity is associated with mortality and cardiovascular outcomes in ESRD. Controlled trials with antidiurenergic drugs are needed to determine whether interference with the sympathetic system could reduce the high cardiovascular morbidity and mortality in dialysis patients. (Circulation. 2002;105:1354-1359.)

Key Words: nervous system, sympathetic ■ norepinephrine ■ kidney ■ risk factors ■ nervous system, autonomic
Zoccali et al. Sympathetic Activity and Survival in ESRD

**All cause death**

- Log rank 7.75
- P=0.005

Cumulative survival

Time (months)

**Cardiovascular events**

- Log rank 8.10
- P=0.004

Cumulative events free survival

Time (months)
Sympathetic Nerve Activity Is Inappropriately Increased in Chronic Renal Disease

INGE H.H.T. KLEIN,* GERRY LIGTENBERG,* JUTTA NEUMANN,* P. LIAM OGEY,† HEIN A. KOOMANS,* and PETER J. BLANKESTIJN*
Departments of *Nephrology and †Clinical Neurophysiology, University Medical Center Utrecht, The Netherlands
Figure 1 | Kaplan-Meier curve for all-cause mortality during 24-month follow-up in hemodialysis patients with cardiomyopathy according to the use of carvedilol. Used with permission.

CiCE G., A.J.Card., 2003
Hypertension in HD patients:

- hypervolemia!
- hypervolemia!
- hypervolemia!
DOGMA IN SCIENCE!
Obstructive Sleep Apnea
Implications for Cardiac and Vascular Disease

Abu S. M. Shamsuzzaman, MBBS, PhD
Bernard J. Gersh, MBChB, DPhil
Virend K. Somers, MD, DPhil

Along with the epidemic of obesity, there is a growing awareness of sleep-disordered breathing as a potential and treatable risk factor for cardiovascular disease. The repetitive nocturnal hypoxemia experienced by patients with obstructive sleep apnea (OSA) is associated with activation of a number of neural, humoral, thrombotic, metabolic, and inflammatory disease mechanisms, all of which have also been implicated in the pathophysiology of cardiac and vascular disease. Activation of these mechanisms is often evident even in patients who are free of overt cardiovascular disease, suggesting that OSA may conceivably contribute to the initiation and progression of cardiovascular disease (Figure 1).

Sleep apnea can be categorized as OSA, in which there is preserved and increased respiratory effort despite partial or complete occlusion of the upper airway; or as central sleep apnea (CSA), in which there is absence of both respiratory efforts and airflow. The apnea-
Figure 2. Neural and Circulatory Changes in Obstructive Sleep Apnea

Awake

- Sympathetic Nerve Activity
- Respiration
- Blood Pressure, mm Hg

Constant Positive Airway Pressure Therapy During REM Sleep

Obstructive Sleep Apnea (OSA) During REM Sleep

- Sympathetic Nerve Activity
- Respiration
- Blood Pressure, mm Hg

Recordings of sympathetic nerve activity, respiratory rate, and intra-arterial blood pressure in the same individual when awake, with OSA during rapid eye movement (REM) sleep, and with elimination of OSA episodes by continuous positive airway pressure (CPAP) therapy during REM sleep. Sympathetic nerve activity is very high during wakefulness, but increases even further secondary to obstructive apnea during REM sleep. Blood pressure increases from 130/65 mm Hg when the individual is awake to 250/110 mm Hg at the end of the apneic episode. Elimination of apneic episodes by CPAP therapy results in decreased sympathetic activity and prevents blood pressure surges during REM sleep. Reproduced with permission from Somers et al. 19
Urinary catecholamines before and after tracheostomy in patients with obstructive sleep apnea and hypertension.

Fletcher EC, Miller J, Schaaf JW, Fletcher JG.

Obstructive apnea (asphyxia) is accompanied by acute elevation of systemic blood pressure. The usual nocturnal fall in blood pressure seen during sleep in normals may be absent in patients with repetitive apneas, and daytime systemic hypertension is reported to occur in up to 90% of such patients. Increased sympathetic activity in response to repetitive nocturnal episodes of asphyxia could explain the reversal of the diurnal pressure variation but not the daytime systemic hypertension in this setting. We examined diurnal variation in urinary catecholamines in eight subjects with severe apnea before and after tracheostomy. Five obese hypertensive subjects without apnea served as controls. Three urine specimens, two awake (7 a.m. to 3 p.m. and 3 p.m. to 11 p.m.) and one asleep (11 p.m. to 7 a.m.) were collected preoperatively and again 10-14 days postoperatively when the patient was free of pain and signs of stoma infection. All specimens were analyzed for epinephrine, norepinephrine, metanephrine, and normetanephrine by liquid chromatography with electrochemical detection. Urinary epinephrine and metanephrine were not different between subjects and controls. Norepinephrine and normetanephrine were significantly higher in apneic subjects pretracheostomy as compared either with controls or with their own values posttracheostomy. Diurnal variation was not seen before or after tracheostomy. Only two of the controls showed significant diurnal variation in norepinephrine. We conclude that the absence of diurnal variation in catecholamines prior to tracheostomy reflects increased nocturnal sympathetic activity. Elevation of daytime norepinephrine and normetanephrine with return to control levels following tracheostomy implies increased sympathetic activity throughout the day.

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Norepinephrine-induced human platelet activation in vivo is only partly counteracted by aspirin.

Larsson PT, Wallén NH, Hjemdahl P.

Department of Clinical Pharmacology, Karolinska Hospital, Stockholm, Sweden.

BACKGROUND: Epinephrine and mental stress may, via platelet stimulation, enhance the risk of thrombus formation. Norepinephrine is more likely than epinephrine to activate platelets in vivo because of higher levels in plasma but is less well studied in this respect. The antiplatelet drug of choice for patients with coronary artery disease, aspirin, may be less effective during sympathoadrenal activation. We therefore investigated platelet responses in vivo to exogenous norepinephrine with and without aspirin pretreatment. METHODS AND RESULTS: Platelet aggregability in vivo was assessed in 11 healthy male subjects, by filtragometry ex vivo (which reflects platelet aggregability in vivo) and by measurements of plasma beta-thromboglobulin (beta-TG, which reflects platelet secretion). Norepinephrine infusions elevated venous plasma norepinephrine from 1.5 to 4 and 15 nmol/L, respectively, and enhanced platelet aggregability (filtragometry) concentration dependently (P < .001). Platelet secretion (beta-TG levels) increased during high-dose infusion (P < .01). Aspirin pretreatment (500 mg orally 12 hours earlier) reduced the excretion of 11-dehydrothromboxane B2 by 62 +/- 5% (P < .001) and attenuated platelet aggregability at rest (P < .05) but not the effect of norepinephrine infusion on platelet aggregability. Conversely, resting plasma beta-TG levels and the urinary excretion of high-molecular-weight beta-TG were not altered by aspirin pretreatment, whereas the norepinephrine-induced increase in plasma beta-TG was abolished. CONCLUSIONS: Norepinephrine, at plasma levels easily attained during exercise, enhances platelet aggregability and platelet secretion in vivo in healthy humans. Aspirin may be less effective as an antithrombotic drug during sympathoadrenal activation in humans.
Platelet function in patients with obstructive sleep apnoea syndrome

B.M. Sanner*, M. Konermann*, M. Tepel*, J. Groetz*, C. Mummenhoff*, W. Zidek*

ABSTRACT: Patients with obstructive sleep apnoea syndrome (OSAS) are subject to an increased cardiovascular morbidity including myocardial infarction and stroke. Platelets play an important role in the pathogenesis and triggering of acute cardiovascular syndromes. So far, the influence of OSAS on platelet function is not fully understood.

Platelet aggregability to epinephrine, collagen, arachidonic acid, and adenosine diphosphate in vitro was measured in 17 consecutive male patients (53.0±2.1 yrs) with polysomnographically verified OSAS and compared with that of 15 male controls (50.1±3.6 yrs) at 20:00 h, 24:00 h, and 06:00 h. In addition, the long-term effects of continuous positive airway pressure (CPAP) therapy on platelet aggregability were assessed after 6 months.

Platelet aggregation in vitro induced by epinephrine showed a slight increase overnight in the untreated OSAS patients (ns) whereas it decreased slightly (ns) in the controls and in the treated OSAS patients. Pretherapeutic platelet aggregability was significantly lowered by CPAP therapy both at 24:00 h (64.0±6.5 versus 55.3±6.7%, p<0.05) and at 06:00 h (64.1±6.5 versus 45.8±7.6%; p=0.01). Platelet aggregability during sleep in the controls resembled that found in patients with OSAS during CPAP therapy.

The results suggest that obstructive sleep apnoea syndrome contributes, at least in part, to platelet dysfunction and that long-term continuous positive airway pressure treatment may reduce platelet aggregability.

Spontaneous Platelet Activation and Aggregation During Obstructive Sleep Apnea and Its Response to Therapy With Nasal Continuous Positive Airway Pressure

A Preliminary Investigation

George Bokinsky, MD; Michael Miller, MD, PhD; Kenneth Ault, MD; Philip Husband, MD; and Jane Mitchell, MT

Study objective: To determine whether alterations of platelet reactivity occur during obstructive sleep apnea (OSA) and, if so, whether therapy with nasal-continuous positive airway pressure (N-CPAP) alters this reactivity.

Design: Patients with suspected moderate to severe OSA had blood drawn for spontaneous platelet aggregation (sAGG) and activation (sACT) measurements at hourly intervals during diagnostic polysomnography (PSG) and, in those with confirmed OSA, on a separate night during which N-CPAP was applied.

Setting: Tertiary care center sleep laboratory.

Patients: Six patients with OSA had matched blood samples drawn on both diagnostic and N-CPAP treatment nights. Five patients without confirmed OSA served as controls.

Interventions: N-CPAP was applied to those patients with OSA and pressures adjusted with goals of eliminating apneas; N-CPAP was then maintained through the night.

Measurements and results: sACT and sAGG were measured using flow cytometric determination of P-selectin expression using a monoclonal antibody. Platelet aggregation was assessed by measuring the proportion of platelets larger than resting platelets by light scatter techniques. Mean values for sACT and sAGG were higher on the diagnostic night compared with treatment night (p=0.001 and p=0.003, analysis of variance, respectively). The mean baseline supine sACT compared with completion supine sACT for both diagnostic and N-CPAP nights also revealed significant differences (mean=16.6±3.5% vs 36.9±7.5%, p=0.04; and 11.9±3% vs 39.5±9.1%, p=0.04). Platelet activation during sleep in five subjects without OSA resembles that found in patients with OSA during N-CPAP.

Conclusions: Increased platelet sACT and sAGG occur during sleep in patients with OSA. This effect is greatly reduced by N-CPAP. (CHEST 1995; 108:623-30)
Search for gene!
The Mammalian Gene Collection (MGC) project is a new effort by the NIH to generate full-length complementary DNA (cDNA) resources. This project will provide publicly accessible resources to the full research community. The MGC project entails the production of libraries, sequencing, and database and repository development, as well as the support of library construction, sequencing, and analytic technologies dedicated to the goal of obtaining a full set of human and other mammalian full-length (open reading frame) sequences and clones of expressed genes.

It is not yet routine to identify all possible mammalian genomic regions that are transcribed. This is in part because much of the DNA does not encode gene transcripts, and the rules of transcription and transcript processing are not yet fully understood. A particularly powerful material for studying gene expression, therefore, is cDNA, which is DNA reverse-transcribed from a complete RNA molecule that represents the full-length, expressed gene transcript. Indeed, one of the most effective and widespread manifestations of the genomics revolution has been the ready public access to cDNA libraries, sequences, and clones. The value of having such resources has been recognized since the early planning phases of the Human Genome Project (HGP) (1). However, it was also clear at that time that the development of an annotated and complete catalog of full-length human cDNAs (with sizes ranging from <1 to >10 kb for the array of human genes) would require advances in methodology and strategy, as well as improved reagents. Moreover, cost-effective DNA sequencing of tens to hundreds of thousands of full-length cDNAs would require technological advances not available at the start of the HGP.

In 1991, Venter and colleagues (2) developed a conceptually different approach to the establishment of systematic cDNA resources, termed the expressed sequence tag (EST) strategy. Although the sequence tags covered only a segment of the gene, and the clones were generally not full length, their utility for gene iden-
Figure 2: Structural motifs of renalase

Structural motifs detected in renalase protein. aa, amino acid; FAD, flavin adenine dinucleotide binding domain; SP, signal peptide.
Criteria which new protein must fulfill:

1. HAS TO BE NEW!

2. HAS TO CONTAIN SP (signal peptide) SEQUENCE!

3. SHOULD NOT CONTAIN TRANSMEMBRANE DOMAIN SEQUENCE!

4. HAS TO BE EXPRESSED PREDOMINANTLY IN KIDNEY!!
12 500 candidate genes

- 114 gene

- 1
Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure

Jianchao Xu,1,2,3 Guoyong Li,1,2,3 Poili Wang,1,2,3 Heino Velazquez,1,2,3 Xiaqiang Yao,4 Yanyan Li,1,2,3 Yanling Wu,1,2,3 Aldo Peixoto,1,2,3 Susan Crowley,1,2,3 and Gary V. Desir1,2,3

1Section of Nephrology, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut, USA.
2Veteran Administration Connecticut Health Care System Medical Center, West Haven, Connecticut, USA.
3Chinese University of Hong Kong, Sha Tin, New Territories, Hong Kong, People’s Republic of China.
gene transfer

renalase gene

cell cultures

generation of recombinant renalase in Escherichia coli and function test in vitro
degrades specifically: dopamin, noradrenalin, adrenalin
Clinical problem at bedside

new therapies or/and diag. procedures

research lab

understanding at cellular and molecular level
Thanks for attention !!
ŽIVOJIN STEVANOVIC, MD

Fresenius Medical Care
Slovenia
Figure 4. Correlation between epinephrine-mediated hemodynamic changes and reninase activation. A. Epinephrine (dose ranging from 0.1 to 100 µg/kg) is infused over a 2-minute period, and changes from baseline in systolic blood pressure and plasma reninase activity 30 seconds into the infusion period are plotted (n=7 normotensive rats, reninase activity measurements are carried out in triplicate). B. As in A, except that changes in diastolic pressure are plotted vs reninase activity.