Ergebnisse des «AstraZeneca-Forschungspreises 2006»

The role of COUP-TF and c-fos in the process of angiotensin II-induced cardiac hypertrophy

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Cardiomyocyte hypertrophy is a major cause of morbidity and mortality worldwide. The development of left ventricular hypertrophy involves cardiomyocyte growth, accumulation of extracellular matrix and fibrosis. Upon binding to the AT_1 receptor subtype in cardiomyocytes, Angiotensin II (AngII), the principal effector of the renin-angiotensin-aldosterone system, participates in the pathogenesis of heart failure through induction of cardiac hypertrophy. In the present study, we propose to investigate the mechanisms of the hypertrophic action of AngII, by focusing specifically on the role of two transcription factors whose expression is modulated by AngII, COUP-TF and c-fos.

The COUP-TF-family, consisting of two structurally related proteins, COUP-TFI and COUP-TFII belongs to the orphan member of the steroid/thyroid hormone receptor superfamily. In particular, the expression of COUP-TFII during embryogenesis suggests that it may participate in mesenchymal-epithelial interactions required for organogenesis. Targeted deletion of the COUP-TFII gene results in embryonic lethality with defects in angiogenesis and heart development. Thus, COUP-TFII may be required for bidirectional signaling between the endothelial and mesenchymal compartments, essential for proper angiogenesis and cardiac development. On the other hand, COUP-TFI is highly expressed in the developing nervous systems and act as an important regulator of neuronal development and differentiation.

We are investigating the possibility that COUP-TFs may be implicated in AngII-mediated cardiomyocyte hypertrophy, as assessed by ^{3}H-leucine incorporation and by changes in cell size. In another major target cell for AngII, the adrenal glomerulosa cell, we have shown that COUP-TFI expression is modulated by AngII. Our current data suggest that COUP-TF levels are also regulated in neonatal rat cardiomyocytes in primary culture. Indeed, COUP-TFI is induced by AngII in a time- and concentration-dependent manner. This induction of COUP-TF involves in part protein kinase C activation but neither ERK1/2 nor p38 MAP kinases appear to be involved. Moreover, protein neo-synthesis is required for AngII-mediated COUP-TFI induction.

We now plan to identify some of the genes whose expression is controlled by COUP-TF during cardiac hypertrophy. To this end, we will use the so-called “Chip on ChIP” (chip on chromatin immunoprecipitation) methodology. Furthermore, using adenoviral vectors, we are presently introducing siRNAs against COUP-TF, in order to evaluate the effect of COUP-TF silencing on the hypertrophic response to AngII.

We are also exploring in the involvement of c-fos in AngII-mediated cardiomyocyte hypertrophy. The AP-1 family of transcription-factors is composed of heterodimers of Jun and Fos members. Generally, AP-1 activity regulates transcription of genes associated with cell growth. In cultured rat cardiomyocytes, AngII causes induction of various immediate-early genes. Specifically, we observe that AngII treatment of neonatal cardiomyocytes is associated with a rapid, important and transient induction of c-fos expression. The ERK1/2 MAPK pathway plays a critical role in c-fos induction by Ang II.

Abbildung 1
Der Präsident der Schweizerischen Hypertonie-Gesellschaft, Prof. Jürg Nussberger, (rechts aussen) mit den Preisträgern des AstraZeneca-Forschungspreises 2006.
Von links nach rechts: Dr. Andreas Schönenberger, Prof. Alessandro Capponi, Prof. Hans Imboden.
Auf dem Bild fehlt der 4. Preisträger: Dr. Grégoire Wuerzner.

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In order to assess the role of c-fos in AngII-induced cardiac hypertrophy, we now plan to knock down c-fos by using both a dominant negative mutant of c-fos and anti-fos siRNA and adenoviral vectors. We will also apply the Chip on ChIP methodology.

We expect that these studies performed with the support of the SSH AstraZeneca grant-in-aid will help us gain further insight into the physiopathological mechanisms of angiotensin II-induced cardiac hypertrophy.

Angiotensinerge Innervierung von Blutgefässen: ein neues Konzept für das Renin-Angiotensin-System

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Angiotensin II (Ang II), das Haupteffektorpeptid des Renin-Angiotensin-Systems (RAS), ist neben dem Neurotransmitter Noradrenalin des Sympathischen Nervensystems (SNS) eine der wichtigsten Substanzen für die Regulierung und die Aufrechterhaltung eines normalen Blutdruckes. Ausgangsstoff des Ang II ist das in der Leber produzierte Angiotensinogen. Das Enzym Renin, das in Nierenzellen gebildet und ins Blut abgegeben wird, spaltet davon in einem ersten Schritt Angiotensin I (ANG I) ab. In einem weiteren Schritt trennt ein u.a. in der Lunge hergestelltes Angiotensin-Konversionsenzym (ACE) zwei Aminosäuren von ANG I, wodurch ANG II gebildet wird (Abb. 2).

Überdies wird beschrieben, dass das Hormon Ang II mit dem SNS, zum Beispiel im Gehirn, mit den sympathischen Ganglien und was äusserst wichtig ist, direkt bei den sympathischen Synapsen bei den Blutgefässen interagiert. Hier soll das zirkulierende Ang II die Noradrenalin-Ausschüttung aus den sympathischen Axonendigungen fördern [1–4]. Dadurch wird eine verstärkte Vasokonstriktion ausgelöst und der Blutdruck steigt an. Bluthochdruck entsteht, wenn die komplexen Vorgänge zur Regulierung des normalen Blutdrucks gestört werden [5].

Neben diesem klassischen RAS in der Zirkulation konnten mit molekularbiologischen Verfahren in verschiedenen Organen Komponenten für das RAS, z.B. Gehirn, Herz, Nieren und Nebennieren, nachgewiesen werden [6].

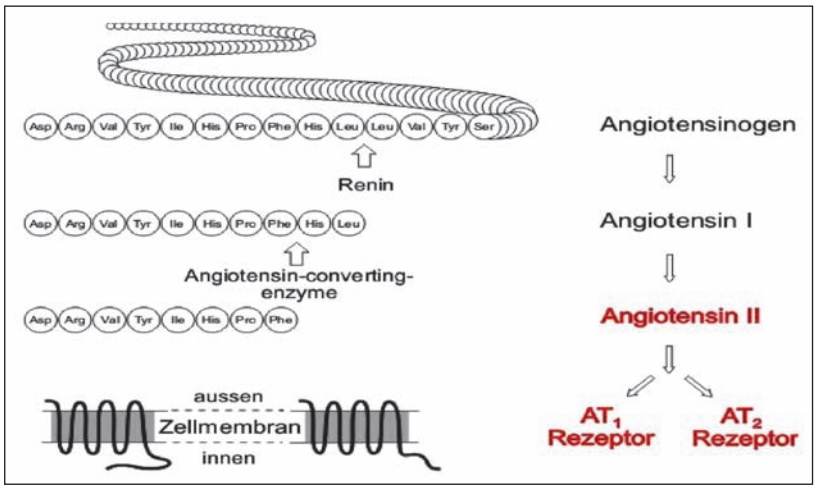

Abbildung 2
Komponenten des Renin-Angiotensin-Systems.

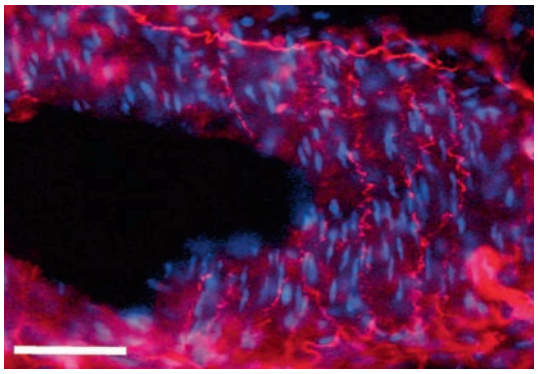

Abbildung 3
Angiotensinerge Innervierung von mesenteriellen Blutgefässen bei der Ratte. Rot = Angiotensin II-Immunreaktivität; blau = Kernfärbung aller Zellen mit DAPI; Balken = 50 µm.

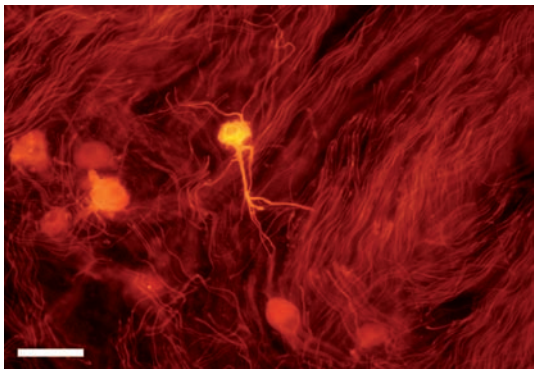

Abbildung 4
Angiotensinerge Nervenzellen mit Fortsätzen im Bauchganglion (Ganglion coeliacum) beim Menschen. Rot = Angiotensin II-Immunreaktivität; Balken = 50 µm.

Meiner Arbeitsgruppe ist es gelungen, monoklonale Antikörper gegen Komponenten des RAS, insbesondere gegen Ang II, herzustellen und damit eine angiotensinerge Innervierung von Blutgefässen (Abb. 3) nachzuweisen. Alle Abklärungen zur Prüfung der Spezifität dieser monoklonalen Antikörper gegen Ang II sind positiv ausgefallen [7]. Zusätzlich konnten im Ursprungsgebiet dieser angiotensinergen Nervenendigungen, im Bauchganglion, Ang-II-enthaltende Nervenzellen nachgewiesen werden (Abb. 4). Dieser Nachweis konnte sowohl bei der Ratte als auch beim Menschen erbracht werden. *Es handelt sich um neue Resultate, welche bis anhin noch niemand dokumentieren konnte.*

Weil eine angiotensinerge Innervierung bei Blutgefässen bis jetzt noch nie gezeigt werden konnte, haben wir totale RNA-Extrakte aus den Bauchganglien (Ursprungsnervenzellen für Ang II) von Ratten hergestellt und diese nach dem Vorhandensein von Angiotensinogen m-RNA, dem Ausgangsprodukt für das RAS, untersucht. Als positive Kontrolle wurde die Rattenleber verwendet.

Vorläufige Resultate mit der Methode der «real time PCR» zeigen, dass in Extrakten von Bauchganglien tatsächlich Angiotensiongen m-RNA vorhanden ist. Weil in einem Totalextrakt von Bauchganglien ebenfalls andere Zelltypen (z. B. Mantelzellen) enthalten sind, kann mit dieser Methode nicht ausgesagt werden, welche Zelltypen m-RNA für Angiotensinogen enthalten.

Um die zelluläre Lokalisierung der Angiotensinogen m-RNA abzuklären haben wir uns der Methode der *In-situ*-Hybridisierung bedient. Dank der Anwendung dieser Methode konnten wir in den Bauchganglien das Vorläuferprodukt für Ang II im Zytoplasma von Neuronen nachweisen. Diese vorläufigen, viel versprechenden Ergebnisse müssen noch eingehend analysiert werden. Bei den Resultaten handelt es sich um Untersuchungen an Gewebe von normotensiven Individuen. Interessant wird sein, diese Untersuchungen auch in der Pathologie, z. B. an Gewebe von hypertensiven Individuen, durchzuführen (Abb. 2–4).

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Prediction of hypertensive crises based on 24-hour ambulatory blood pressure monitoring: use of average blood pressure and a measure of entropy

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Hypertensive crises are a common problem in emergency medicine. Most hypertensive crises appear in a setting of pre-existent hypertension, but the factors leading to the rapid rise in blood pressure (BP) are poorly understood. Ineffective outpatient BP control has been identified as an independent risk factor for hypertensive crisis in retrospective case-control studies. However, the predictive value of potential predictive measures has not been assessed in prospective studies. Considering the severe organ damage that potentially follows hypertensive crisis, current knowledge of measures predicting hypertensive crisis has to be improved.

At the Annual Meeting of the Swiss Society of Hypertension 2006, we presented results from a first longitudinal study in 53 patients. In that study, we hypothesised that approximate entropy (ApEn), a nonlinear statistical measure of chaos theory describing irregularity of a BP time series, might predict hypertensive crisis. We showed that average and ApEn of systolic BP during 24-hour ABPM allow prediction of hypertensive crisis. However, a replication study in a larger sample is needed to confirm the validity of the study findings for clinical applications.

The replication study will include patients consecutively examined in a 24-hour ambulatory blood pressure monitoring (ABPM) at the medical outpatient unit of the University Hospital of Berne during a certain time period. The study aims at 100 included patients. Average and ApEn will be calculated within the time series of systolic and diastolic BP values of the 24-hour ABPM. In addition to ABPM, baseline information comprises cardiovascular risk factors, comorbidities (in particular cardiac or renal involvement), drug therapy, and laboratory findings. All patients will be followed-up for the development of hypertensive crisis. For

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a diagnosis of hypertensive crisis, the three following criteria have to be fulfilled simultaneously: (1.) systolic blood pressure exceeds 200 mm Hg, (2.) diastolic blood pressure exceeds 120 mm Hg, and (3.) the patient is symptomatic (headache, dizziness, or chest pain). Follow-up will be obtained from several sources: outpatient medical records will be reviewed and structured patient interviews will be conducted. Statistical analysis will include bi- and multivariate Cox proportional-hazards modelling.

In conclusion, we believe that average and ApEn of a BP time series are potential predictors of hypertensive crisis. If a replication study in a larger and independent sample confirms our first results, average and ApEn of BP based on 24-hour ABPM might be utilised to optimise surveillance and antihypertensive treatment of patients who are prone to hypertensive crisis.

Clinical, biological and pharmacological investigation of a polymorphism of Nedd 4–2, a regulatory protein of the amiloride sensitive epithelium sodium channel

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Background: Sodium homeostasis is an important mechanism in blood pressure regulation. Among the different transporters implicated in sodium balance, the amiloride sensitive epithelial sodium channel (ENaC) plays a major role [1]. Mutation affecting subunits of the ENaC have been shown to be responsible for Liddle's syndrome. Nevertheless, genetic polymorphism of the ENaC has not been proven functional in vivo so far [2]. Still, other polymorphisms may play important role in controlling the ENaC. Nedd 4–2 (neural precursor cell expressed developmentally downregulated) seems a potential candidate, since this ubiquitin ligase affects cell expression of the ENaC [3]. The polymorphism affects the splicing between the exon 1 and 2 of the Nedd 4–2 gene and leads to the production of isoforms devoided of the C2 domain of the protein. This domain is crucial for the insertion of the protein Nedd 4–2 to the membrane and thus for its interaction with ENaC. So far, Nedd 4–2 has been associated with hypertension [4], therefore it may be hypothesised that a polymorphism of Nedd 4–2 may induce a gain in function of ENaC and by this mean promote salt sensitivity and increase the risk of hypertension.

Objective: The objective of this study will be to investigate the clinical, biological and pharmacological effect of a polymorphism of Nedd 4–2 (A/G) in contrasted conditions of sodium diet. The main criteria of judgement will be the chronic readjustment of the renin-angiotensin system (RAS) from a low sodium diet to a high sodium diet. Additional criteria of judgement will be the acute (first 72 first hours) adjustment of the RAS from a low to a high sodium diet, indirect measurement of ENaC activity at the nasal epithelium of the inferior turbinate using transepithelial potential difference, indirect measurement of ENaC activity using a vasopressin analogue, indirect measurement of ENaC activity using urinary prostasin and finally the hormonal, natriuretic and pressure response to amiloride, a selective ENaC inhibitor.

Methods: This monocentric study will be performed in 48 healthy volunteers at the Clinical Investigation Center (CIC) of the European Hospital Georges Pompidou in Paris. Sixteen subjects will be selected per genotype (AA, AG, GG). Given the different genotype frequency, the estimated number of subjects needed to be screened is 300. This double blinded study will consist of 3 one week periods (low sodium: 20 mmol/24h, high sodium: 250 mmol/24h, amiloride) of investigation at the CIC. Clinical (blood pressure using ambulatory blood pressure measurement, nasal transepithelial potential difference) and biological (plasma renin, plasma and urinary aldosterone, urinary Na/K ratio, urinary prostasin) will be measured during and/or at the end of each period.

Results: The inclusion of healthy volunteers has started on May the 1st 2007. The expected end of the study will be in June 2008.

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