by aberrantly glycosylated IgA. Conversely, the effects of ACE-I and AT1-RA in modulating the repair mechanisms regulated by cell proliferation and VEGF synthesis are less evident.

M96 DECREASED URINARY INTERLEUKIN 1 RECEPTOR ANTAGONIST EXCRETION IN IgA NEPHROPATHY COMPARED WITH HENOCH-SCHOENLEIN NEPHRITIS

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IgA nephropathy (IgAN) and Henoch-Schönlein nephritis (HSN) have been postulated to have a common pathogenesis and that HSN might be a systemic form of IgAN. Interaction between Interleukin-1β (IL-1β) and Interleukin-1 receptor antagonist (IL-1ra) is important in the regulation of inflammatory responses. Increased activity of IL-1β in urine has been found in IgAN and HSN patients compared with healthy controls.
24h urinary samples were collected from 241 IgAN and 26 HSN patients, and from 33 healthy controls. Excretions of IL-1β and IL-1ra were determined. The results are expressed as cytokine/creatinine (ng/mmol) ratios.
IL-1β excretion was not increased compared to controls either in IgAN or HSN patients, but IgAN patients had decreased urinary IL-1ra excretion ($p<0.05$) compared to controls and to HSN patients ($p<0.01$). In both patients and controls women had significantly higher IL-1ra, IL-1β excretion levels and IL-1ra/IL-1β ratios. However, observed differences in urinary excretions of IL-1ra between controls and IgAN or HSN patients remained, when studied separately in both sexes. Excretions of IL-1β or IL-1ra did not correlate with urinary protein excretion, duration of the disease or any histopathological variables. However, patients with IL-1ra/IL1β ratio above normal limits had significantly milder histopathological changes in renal biopsy than those with low or normal IL-1ra/IL1β ratios. In conclusion, IgAN patients have decreased urinary IL-1ra levels compared with healthy controls and with HSN patients. This finding may indicate differences in pathogenesis of these diseases. Whether male predominance in IgAN and HSN and worse outcome of males reported in several previous studies on IgAN and HSN is related to the lower excretion of IL-1ra and consequently lower IL-1ra/IL-1b ratios in males compared to females remains to be investigated more thoroughly.

M97 DETERIORATION OF ORGAN REDOX STATUS AT THE ONSET OF LUPUS-LIKE GLOMERULONEPHRITIS IN Nrf2-DEFICIENT MICE

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Nrf2-deficient mouse is a newly developed lupus model whose transcriptional factor related to oxidative stress response was defected. Thus, these mice are exposed to high oxidative stress. To reveal the relation between increased oxidative stress and their onset of lupus-like glomerulonephritis, the redox status of Nrf2-deficient female mice was analyzed by an *in vivo* electron spin resonance (ESR) system.
A spin probe carbamoyl-PROXYL (CP) was used. CP was yielded to a hydroxylamine by tissue reduction and lost of its ESR signal. Therefore decay of CP signal reflects organ redox status. CP 200mM, 3ml/kg was administrated to the mice intraperitoneally, and the half-lifes of CP and ESR images were examined.
At 10 weeks of the age, the CP half-life of wild type mice was 12.1±3.4 min and this was significantly lower than that of Nrf2 deficient mice, 20.0±5.1 min ($P<0.05$). At 50 weeks of the age, the CP half-life in wild type and Nrf2-deficient mice were 27.2±2.2 min and 54.0±16.4 min, respectively, both significantly longer than those from each juvenile mice ($P<0.01$). The half-life of elder wild type mice was also significantly lower than that of elder Nrf2-deficient mice ($P<0.05$). ESR imaging revealed that these reduced CP clearance were observed both in the liver and the kidneys of Nrf2-deficient mice.
These results indicate that the diminished organ reducing activity in Nrf2-dificient mice is brought by both Nrf2 deficiency and aging process, and this may induce the onset of lupus-like lesions. A combination of ESR imaging and half-life analysis has advantage in the sequential analysis of *in vivo* redox status.

Hypertension – I

M98 HEART AND KIDNEYS AS TARGET ORGANS FOR ANGIOTENSIN II-INDUCED OXIDATIVE STRESS IN HYPERTENSIVE RATS

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Angiotensin II (ANG II) exerts multiple effects on cardiovascular system including regulation of blood pressure, vascular smooth muscle growth and cardiovascular hypertophy. Among the mechanisms involved in ANG II-induced hypertension and target organ damage, free radical production has been proposed as an additional mechanism of cardiovascular alterations. This study was designed to evaluate the production of reactive oxygen species (ROS) in target tissues for hypertension such as heart, aorta and kidneys after angiotensin II infusion in rats.
Mean arterial pressure (MAP, mmHg), albuminuria (μg/24 h), heart weight index (HWI, mg/g body weight), were measured after 10 days of ANG II (200 mg/kg/min) infusion (osmotic pump) in rats. Reactive Oxygen Species (ROS, mv) production by aorta, kidneys, heart and PMN was determined by chimioluminescence. The results (mean + SEM) are reported in the table 1 ($p<0.05$: *vs vehicle).

	Control	ANG II
Final MAP	122 ± 6	164 ± 4*
HWI	2.97 ± 0.04	3.46 ± 0.09*
Albuminuria	176 ± 20	986 ± 177*
ROS aorta	595 ± 44	951 ± 90*
ROS Kidneys	0,169 ± 0,018	0,440 ± 0,045*
ROS Heart	0,233 ± 0,009	0,321 ± 0,033*
ROS PMN	170 ± 40	533 ± 58*

These results show that functional and morphological changes in target organs such as kidneys, aorta and heart are associated with an enhancement of ROS production. These data suggest that ANGII-induced oxidative stress could be involved in cardiovascular dammages linked to hypertension.

M99 THE DEVELOPMENT OF GENETIC HYPERTENSION IS INDEPENDENT FROM NHE-3, BUT IT IS COUPLED TO UP-REGULATION OF THE Na-K-2Cl mRNA AND PROTEIN ABUNDANCE

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Luminal sodium transport along the proximal tubule (PT) and the thick ascending limb (TAL) is mainly dependent from Na-H exchanger isoform 3 (NHE-3) and bumetanide sensitive Na-K-2Cl co-transport system (rBSC-1), respectively. In the present study we have investigated if the

development of hypertension in the Milano strain of hypertensive rats (MHS) could be dependent from alterations of gene expression and protein abundance of these two transporters. To this end, MHS rats were studied at 25 days after birth, i.e. prior to the onset of overt hypertension. Milano normotensive (MNS) rats of the same age were used as controls. Total RNA was purified from PT and TAL, identified and microdissected from collagenase treated kidneys. NHE-3 and rBSC-1 mRNA abundance were quantified by a competitive PCR using an internal standard of cDNA which differed from the wild-type NHE-3 and rBSC-1 by a deletion of 74 and 86 bp, respectively. The data are expressed as fmol/ng total RNA. At 25 days NHE-3 mRNA abundance in PT was 1.10±0.22 in MNS (n=7) and 0.71±0.15 in MHS (n=8) ($p>0.05$). On the contrary, TAL rBSC-1 mRNA abundance increased from 1.00±0.11 in MNS rats (n=8) to 2.37±0.24 in MHS rats (n=8) ($p<0.001$). These results were reflected by no significant change in NHE-3 protein abundance and by a significant increase ($p<0.001$) in rBSC-1 protein abundance in MHS rats, as detected by Western blot experiments, performed on tissue slices from renal cortex and outer medulla, using b-actin as an internal standard. These data demonstrate that NHE-3 is not significantly affected during the development of hypertension, while rBSC-1 gene is stimulated in young MHS rats as compared to MNS animals. This last effect may contribute to explain, at molecular level, the development of hypertension in this genetic hypertensive strain of rats.

M100 INHIBITOR OF Ca2+ ATPase IN CHRONIC RENAL FAILURE PATIENTS: p-HYDROXY-HIPPURIC ACID

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In patients with end-stage renal failure, disturbances of Ca2+ metabolism are common. Besides hormonal changes, inhibition of cellular Ca2+-ATPase was postulated to contribute to uremic toxicity. We purified a potent inhibitor of the Ca2+-ATPase from the ultrafiltrate of patients with end-stage renal failure by multiple steps of high-performance liquid chromatography to homogeneity and identified the isolated inhibitor by mass spectrometric methods as p-hydroxy-hippuric acid. The enzyme used for the Ca2+-ATPase assay system was isolated from red blood cell by cross-flow filtration. The activity of the Ca2+-ATPase was measured spectrophotometrically as the difference in hydrolysis of ATP in the presence and absence of Ca2+ with different concentrations of ATP and p-hydroxy-hippuric acid. The Ca2+-ATPase was found to be inhibited by p-hydroxy-hippuric acid at a concentration above 11.7 µmol/l. p-Hydroxy hippuric acid inhibited the erythrocyte Ca2+-ATPase by reducing vmax and increasing the KM-value. The EC50 (log mol/l; mean ± SEM) for p-hydroxy-hippuric acid was calculated as 4.82 ± 0.14. In conclusion, p-hydroxy-hippuric acid may play a in disturbed Ca2+ metabolism in end-stage renal failure.

M101 GENERATION OF THE ACE CONGENIC RATS TO ESTABLISH THE CHROMOSOMAL REGION CONTRIBUTING TO MALIGNANT HYPERTENSION

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Our previous study identified the genetic loci encompassing Ace, angiotensin converting enzyme (Ace) gene, on chromosome 10 which contributed to the severity of malignant hypertension in rats (Kantachuvesiri, S. et al. Kidney Int 56, 414-420 (1999). Plasma ACE activity in Fischer rats (highly susceptible strain) was found to be higher than Lewis rats (less susceptible strain). To elucidate the mechanism of hypertension induced vascular injury, we have created transgenic Fischer rats carrying Cyp1a1-Ren2 transgene and MH can therefore be regulated by an inducer, indole-3-carbinol, which bind to Aryl hydrocarbon receptor resulting to transcription of Ren2 gene. By transferring the candidate chromosomal region containing Ace gene from Lewis to Fischer rats and vice versa (congenic strain), the chromosomal region containing the causative gene(s) of MH can be determined. The congenic strains have been produced within 6 generation of backcross by genotype assisted congenic technique with 150 microsatellite marker cover all rat chromosomes. Four congenic sublines with overlapping candidate regions (significant lod score > 3.9) on chromosome 10 were established. The fine mapping was then carried out with the additional microsatellite markers and localised the congenic regions encompassing the Ace gene within 3-5 cM between the outermost primers D10rat15 and Band3A in a pair of congenic strain. The mapping of the other strains revealed congenic regions of 20 cM spanning chromosomal segment between the markers D10rat2 and D10Mgh5. The availability of these congenic strains with fine localisation of the region will permit identifying the causative gene(s) and studying the genetic mechanism governing hypertensive vascular injury.

M102 ANGIOTENSIN II ACTIVATION OF NAD(P)H OXIDASE INDUCES VASOCONSTRICTION

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The importance of oxidative pathways and the membrane NAD(P)H oxidase activity on the angiotensin II-induced vasoconstriction of rat vessels was investigated. The isometric constrictions of thoracic aorta derived from Wistar-Kyoto rats were measured using a vessel myograph. Reactive oxygen species and cytosolic free calcium concentration in aortic smooth muscle cells were measured spectrophotometrically using the fluorescent dyes dichlorofluorescein diacetate and fura2, respectively.
The additon of 1 µmol/L angiotensin II caused a contractile response of the thoracic aorta by increasing the tension to 4.2 ± 0.1 mNewton (mean ± SEM, n=8). In the presence of the superoxide dismutase mimetic, tempol, the angiotensin II-induced contractile response was significantly reduced to 2.5 ± 0.3 mNewton (n=6, $p<0.05$). The addition of specific inhibitors of the NAD(P)H oxidase, apocynin or diphenylene iodonium, both significantly reduced the angiotensin II-induced vasoconstriction to 2.1 ± 0.1 mNewton (n=8, $p<0.05$) or to 2.1 ± 0.1 mNewton (n=14, $p<0.05$), respectively. Angiotensin II significantly increased reactive oxygen species in aortic smooth muscle cells by 393 ± 91% above baseline (n=9, $p<0.01$). Angiotensin II significantly increased cytosolic calcium to from 80 ± 4 nmol/L to 212 ± 29 nmol/L (n=29, $p<0.01$). In the presence of tempol, the angiotensin II-induced calcium increase was significantly reduced to 124 ± 17 nmol/L, n=16, $p<0.05$).
It is concluded that part of the angiotensin II-induced vasoconstriction is mediated by elevation of reactive oxygen species after activation of NAD(P)H oxidase.

M103 A ROLE FOR HEME OXYGENASE IN ERYTHROPOIETIN-INDUCED ERYTHROCYTOSIS AND HYPERTENSION

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Erythropoietin (EPO) has several biological activities that induce not only erythrocytosis, but a pressor effect and the modulation of certain vasoactive substances. Heme oxygenase (HO) is the limiting enzyme that converts heme derived from hemoglobin to biliverdin, iron and carbon monoxide(CO). However, details of the pathophysiological significance of HO/CO system in erythrocytosis and in the pressor mechanism during chronic EPO treatment have remained unclear. In this study, we investigated the role of HO in experimental animal model. Male Sprague-Dawley rats were subcutaneously injected with EPO (300 IU/kg BW) three times a week for 6 weeks. The mRNA and protein expression lev-

els of two isoforms of HO (HO-I and HO-II) in the kidney, liver, spleen and aorta were then measured and the renal function was estimated. The EPO-treated rats had higher hematocrit levels (68 ± 2% in EPO-treated, 43 ±1% in control) and higher systolic blood pressures(160 ± 14 mmHg in EPO- treated, 120 ± 3 mmHg in control) (n=6, p<0.01). The expression of HO-I mRNA was significantly increased in the kidney (1.6 fold), the liver (2.4 fold), the spleen (1.3 fold) and aorta (1.3 fold) in the treated rats, while HO-II was expressed at a constant level. The administration of antisense oligodeoxy nucleotide (ODN) (100 μg/kg BW) increased the mean arterial pressure to a greater degree in the EPO-treated rats than in the control animals (ΔBP 28.9 ±3.5 vs.Δ17.9 ± 2.3%, p<0.01). The expression of HO-I protein was significantly down-regulated in the kidney and spleen but not in the liver. Although the changes in renal function were not observed, serum NOx levels increased after antisense ODN treatment. These observations suggest that HO-I may contribute to a depressor effect in the hypertensive state induced by EPO treatment.

M104 VASOACTIVE HORMONES IN HYPERTENSIVE PATIENTS WITH CRONIC RENAL FAILURE VS ESSENTIAL HYPERTENSIVE PATIENTS: RELATIONSHIP WITH AMBULATORY BLOOD PRESSURE MONITORING

Daniela Angelini, Manuela Parrini, Antonio Carlini, Marcello Neri, Alessandro Antonelli.

We evaluated plasma levels of nitric oxide(NO),cyclic guanosin monophospha-te(cGMP),endothelin-1(ET1)and
cyclic adenosin monophosphate(cAMP)in 27 patients(17M,10F)with essential hypertension(EH)(25-65 years)and in 18 patients(10M,8F)with chronic renal failure(CRF),(29-56 years) and their relationship with mean arterial pressure(MAP)obtained from ambulatory blood pressure monitoring(ABPM: automatic readings at 20'intervals).MAP was evaluated in daytime and nighttime(dMAP,nMAP). We excluded patients with cardiovascular complications and in previous antihypertensive therapy.Thirthythree healthy subjects(21M,12F), 28-62 years,were the control group.The results are expressed as mean±S.D.
NO and cGMP were significantly lower in both groups (EH and CRF patients) than controls (P<0.001, P<0.001), without significant differences between each other. Plasma ET and cAMP were significatly higher in CRF than controls (P<0.003, P<0.003) and EH patients (P<0.001, P<0.001) while we did not find any difference between EH patients and controls. We found a linear correlation between NO and GMP (R=0.67) in all patients and a reverse one between NO and MAP (R=0.4) and GMP and MAP (R=0.4) but only in EH patients. We found a linear correlation between ET,Creat and GFR (R=0.4, R=-0.5). We did not find a significant difference in the correlation between NO, GMP and dMAP and nMAP. We can conclude that plasma ET1 is not involved in the early stage of hypertension without organ damage but it is in hypertension in CRF. The endothelial disfunction instead, with a deficit of NO and cGMP plays an important role in the pathogenesis of both kind of hypertension.

M105 HYPERTENSION,INSULIN RESISTANCE AND NITRATION OF TYROSINE RESIDUES IN PLATELET MEMBRANES

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Hypertension and insulin resistance are associated with endothelial dysfunction and increased cardiovascular risk.It is the diminished bioavailability of nitric oxide that leads to endothelial dysfunction.There may be diminished production of nitric oxide or increased consumption by superoxide radicals leading to the formation of peroxynitrite which causes nitration of tyrosine residues in proteins and hence the formation of stable nitrotyrosine.We measured platelet membrane exposure to peroxynitrite by determining their nitrotyrosine content reflecting the nitric oxide bioavailability.SDS-PAGE of platelet membranes was carried out followed by transfer onto PVDF membrane.Western blotting was performed using a nitrotyrosine antibody.Optical densities of all protein bands were calculated to give each individual a total optical density.

	N	Relative nitrotyrosine content (S.E.M.)	95% CI
Normal control	10	108 (5)	95-120
Hypertension and insulin resistant	16	79 (8)	62-95
Hypertension and insulin sensitive	18	84 (5)	73-94

Mean difference between controls and patients with hypertension/insulin resistance was 29(p<0.05,CI 5-53). Mean difference between controls and patients with hypertension/insulin sensitive was 24(p<0.05,CI 0.2-47). No correlation was found between a person's insulin resistance index and their relative nitrotyrosine.Lower nitrotyrosine values in platelet membranes from patients with treated hypertension compared to normal controls indicates impaired nitric oxide production in this group and consequent endothelial dysfunction. Insulin resistance does not appear to further diminish nitrotyrosine content, possibly as a result of increased superoxide production.

Hypertension – II

M106 EFFECT OF COMBINED ACE AND ALDOSTERON INHIBITION ON PLASMA PLASMINOGEN ACTIVATOR INHIBITOR TYPE–1 (PAI-1) LEVEL IN CHRONIC HYPERTENSIVE PATIENTS

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A possible link between the renin anjiotensin system (RAS) and fibrinolysis has recently been suggested. The systemic infusion of angiotensin II results in increased in plasma plasminogen activator inhibitor type 1 (PAI-1) levels and angiotensin converting enzyme (ACE) inhibitors have been shown to decrease PAI-1 levels. Moreover, recent data indicated that plasma aldosteron levels were positively correlated with plasma PAI-1 levels. Based on this observations we designed this study to compare the effects of ACE inhibitors (Fosinopril) and ACE inhibitörs in conbination with aldosteron antagonist (Fosinopril+spirinolakton) on PAI-1 levels in chronic hypertensive patients.
Patients with hypertension were randomly divided into two groups and were treated with low salt diet+fosinopril (10-20 mg/d) (Group1, n=13, 7M,6F, mean age 48±22) and low salt diet+fosinopril (10-20 mg/d)+spirinolakton (25 mg/d) (Group 2, n=12, 6M, 6F, mean age 50±26) separately. We measured plasma PAI-1 level before and after 24-weeks treatment in both groups.
Results are shown in the table. Although, the mean plasma levels of the PAI-1 were reduced significantly after treatment in both group, the reduction of the PAI-1 levels was more pronounced in group 2 (fo-

Abstract M104 – Table

	ET pg/ml	NO umol/l	cGMP umol/l	cAMP MUI/ml	MAP mmHg	dMAP mmHg	nMAP mmHg	GFR ml/min
EH	2.5±2.8	26.6±3.5	4.4±1.9	11.5±3.6	100.4±23.0	107.4±24	91.2±9.6	101.5±22.8
CRF	6.5±4.3	12.4±4.0	3.8±0.9	17.2±7.7	98.1±11.8	100.2±11.3	90.4±15.7	23.1±8.3
Controls	3.2±2.3	64.6±5.7	6.3±0.5	10.7±4.8	68.1±2.8	78.6±10.9	64.8±8.6	103.6±21.2