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EARNEST

EARly Nutrition programming- long term follow up of Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research

Instrument: Integrated Project

Thematic Priority 5.4.3.1: Food Quality and Safety

Final public report on activity 3.3.2:

Uncoupling of nitric oxide synthase and the pathogenesis of hypertension

Period covered from 15.04.2005 to 14.10.2010

Start date of project: 15.04.2005 Duration: 5,5 Years

Organisation Name of Lead Contractor for this report: UMCU

Within EARNEST we participated in theme 3, that was primarily devoted to animal science. Besides our own contribution, that was focussed on perinatal interventions that could lower blood pressure in a genetic model of hypertension, we also collaborated with other participants in this theme who were interested in the effects of perinatal interventions on kidney function and structure. All in all, participation in EARNEST was very rewarding for us because it allowed us to establish new collaborations with others in the field.

Activity 3.3.2

The first study showed that maternal supplementation with citrulline increases renal nitric oxide (NO) in young spontaneously hypertensive rats (SHRs) and has long-term antihypertensive effects. NO deficiency is associated with development of hypertension. Defects in the renal citrulline-arginine pathway or arginine reabsorption potentially reduce renal NO in prehypertensive SHRs. Hence, we investigated genes related to the citrulline-arginine pathway or arginine reabsorption, amino acid pools, and renal NO in 2-week-old prehypertensive SHRs. In addition, because perinatally supporting NO availability reduces blood pressure in SHRs, we supplemented SHR dams during pregnancy and lactation with citrulline, the rate-limiting amino acid for arginine synthesis. In female offspring, gene expression of argininosuccinate synthase (involved in renal arginine synthesis) and renal cationic amino acid Y-transporter (involved in arginine reabsorption) were both decreased in 2-day and 2-week SHRs compared with normotensive Wistar-Kyoto (WKY) rats, although no abnormalities in amino acid pools were observed. In addition, 2-week-old female SHRs had much less NO in their kidneys but not in their heart. Furthermore, perinatal supplementation with citrulline increased renal NO at 2 weeks and persistently ameliorated the development of hypertension in females and until 20 weeks in male SHR offspring. Defects in both the renal citrulline-arginine pathway and in arginine reabsorption precede hypertension in SHRs. We propose that the reduced cationic amino acid transporter disables the developing SHR kidney to use arginine reabsorption to compensate for reduced arginine synthesis, resulting in organ-specific NO deficiency. This early renal deficiency and its adverse sequels can be corrected by perinatal citrulline supplementation persistently in female and transiently in male SHRs [Koeners et al. *Hypertension.* 2007;50: 1077-84].

The second study was directed at the consequences of perinatal treatment with L-arginine and antioxidants for the renal transcriptome in spontaneously hypertensive rats. Treating SHRs with L-arginine, taurine, and vitamins C and E (ATCE) during nephrogenesis (2 weeks before to 4 weeks after birth) persistently lowers blood pressure. Hypothetically, differential gene expression in kidney of SHR vs. normotensive WKY rats is partially corrected by maternal ATCE in SHR. Differential gene expression in 2-days, 2-weeks, and 48-week-old rats was studied using oligonucleotide chips. Transcription factor binding sites (TFBS) of differentially expressed genes were analyzed in silico. Differential gene expression varied between SHR+ATCE and SHR, suggesting both direct and indirect effects; but, few genes were modulated toward WKY level and there was little overlap between ages. TFBS analysis suggests less Elk-1-driven gene transcription in both WKY and SHR+ATCE vs. SHR at 2 days and 2 weeks. Concluding, in SHR, persistent antihypertensive effects of maternal ATCE are not primarily due to persistent corrective transcription. Less Elk-1-driven transcription at 2 days and 2 weeks may be involved [Wesseling et al. *Pflugers Arch.* 2009;458:513-24].
Subsequently, we also showed that renal glutathione S-transferase µ type 1 expression is already reduced in new-born SHRs and not responsive to anti-hypertensive treatment in adult SHRs [Wesseling et al. *J Hypertens.* 2010;28:633-4]. Furthermore, we also wrote an editorial

about the various mechanisms that could be involved in the alleged anti-atherosclerotic effects of dietary taurine [Wesseling et al. *Hypertension.* 2009;53:909-11].

The third study demonstrates that perinatal inhibition of nuclear factor-kappa B (NF-κB) has long-term antihypertensive effects in SHRs. Excessive reactive oxygen species (ROS) activate the inflammatory transcription factor NF-κB. ROS-induced inflammation appears to be an early event in the development of hypertension in different models. In SHRs we investigated whether perinatal inhibition of NF-κB persistently decreases blood pressure. To probe antihypertensive mechanisms we studied natriuresis and sensitivity of blood pressure and renal hemodynamics to the superoxide dismutase mimetic Tempol. Perinatal PDTC (a NF-κB inhibitor), administered during pregnancy and lactation to SHR dams, persistently ameliorated hypertension up to 28 wk of age in their offspring. Furthermore, after perinatal treatment with PDTC natriuresis was temporarily doubled at 4 wk of age in both females and males. Urinary excretion of thiobarbituric acid reactive substances (an indirect measure of oxidative stress) was decreased by perinatal PDTC, persistently in females and temporarily in males. At 28 wk, Tempol reduced arterial pressure in all groups, but had opposite effects in control and perinatal PDTC rats on renal vascular resistance (RVR), namely decreased RVR in controls and increased RVR in perinatal PDTC rats. These data indicate that increased activity of NF-κB very early in life, presumably in conjunction with oxidative stress, can lead to the development of hypertension. Perinatal inhibition of NF-κB has persistent antihypertensive effects. This could be related to a short phase of enhanced sodium excretion at an early age, and persistent changes of intrarenal vasoreactivity to ROS. [Koeners et al. submitted].

Finally we reviewed developmental and gender effects –related to salt sensitivity of blood pressure [Wesseling et al. *Am J Clin Nutr.* under review]. Epidemiological studies have convincingly shown that drastically reducing salt intake in the community is accompanied by blood pressure reductions that are comparable to those achieved by anti-hypertensive medication. Many subjects with hypertension are salt-sensitive, implying that their blood pressure is more responsive to changes in salt intake than in subjects with normal blood pressure. Presence of conventional risk factors associated with the metabolic syndrome correlate with salt sensitivity. However, women appear to be more salt-sensitive than men. Sparse data indicate that salt sensitivity of blood pressure is increased in subjects with low birth weight. Studies in rats have shown that hypertensive offspring of mothers maintained on low protein diets throughout or in late pregnancy are salt-sensitive. This is accompanied by increased expression of the thick ascending limb Na-K-2Cl symporter (NKCC2). Perinatal interventions aimed at persistently lowering blood pressure in genetically hypertensive rats have proven to be effective. In fawn-hooded hypertensive rats (FHH) this was not accompanied by a change in salt sensitivity in either females or males. However, blood pressure is far more salt-sensitive and renal NKCC2 gene expression is much higher in female than in male FHH. Summarizing, besides conventional metabolic risk factors for cardiovascular disease, low birth weight and its sequels such as catch-up growth, are modifiable risk factors for salt-sensitivity of blood pressure. Female gender may be a non-modifiable risk factor for salt-sensitivity. Experimental data indicate that the thick ascending limb Na-K-2Cl symporter may be a determinant of salt-sensitivity, both in acquired (developmental) and genetic hypertension.

Conclusions

The main findings of our project were that several interventions directed at inhibiting factors that promote vasoconstriction and inflammation in the kidney during early life were effective in persistently reducing blood pressure in spontaneously hypertensive rats. Interestingly, several of these interventions also induced a wave of sodium excretion (natriuresis) at weaning (4 wk of age) suggesting a reset of extracellular fluid volume or sodium stores. This should be studied in a future project. Moreover, in future studies related to developmental influences on blood pressure regulation, more attention should be directed towards salt sensitivity.

Activities with other partners in theme 3

In collaboration with the department of Clinical Biochemistry, University of Cambridge, UK, we studied whether protein restriction in lactation confers nephroprotective effects in the male rat and is associated with increased antioxidant expression. Telomere shortening has been implicated in the aging process and various age-associated disorders, including renal disease. Moreover, oxidative stress has been identified as an initiator of accelerated telomere shortening. Our collaborators had shown previously that maternal protein restriction during lactation leads to reduced renal telomere shortening, reduced albuminuria, and increased longevity in rats. Here we address the hypothesis that maternal protein restriction during lactation is nephroprotective and associated with increased expression of antioxidative enzymes and decreased age-dependent renal telomere shortening. Newborn rats were suckled by a dam fed either a control (20% protein) or low-protein (LP, 8% protein) diet. All animals were weaned onto standard chow. Offspring that had been suckled by LP mothers had reduced albuminuria, N-acetyl-glucosaminidase, and urinary aldosterone excretion. These animals also did not show significant age-dependent renal telomere shortening and hence had significantly longer telomeres at 12 mo of age. This lack of renal telomere shortening was associated with increased levels of the antioxidant enzymes manganese superoxide dismutase, glutathione peroxidase, and glutathione reductase. These findings suggest that beneficial effects of slow growth during lactation are associated with increased antioxidant capacity and prevention of age-dependent telomere shortening in the kidney [Tarry-Atkins et al. Am J Physiol Regul Integr Comp Physiol. 2007;293:R1259-66]. Subsequently, we also showed that this level of maternal protein restriction did not impede nephrogenesis [Joles et al. Am J Physiol Regul Integr Comp Physiol. 2008;294: R277-8].

In collaboration with the department of Nutrition, School of Biosciences, Nottingham, UK, we studied whether a low-protein diet during mid-gestation results in proteinuria in aging rats. Nephrogenesis in the rat starts mid-gestation and continues into lactation. Maternal low protein intake (LP) leads to renal injury in rats and associates with mild renal injury in humans. We hypothesized that LP during early nephrogenesis or throughout gestation would induce more renal injury in rat offspring than when LP was only present before nephrogenesis. Pregnant rats were fed LP diet (9% casein) at early gestation (day0-day7, LPE), mid (day8-day14, LPM), late (day15-day22, LPL), or throughout gestation (day0-day22, LPA) and compared to controls on 18% casein diet. Offspring were studied at 18 months. Renal injury was assessed by 24h proteinuria, plasma urea, antioxidant enzyme activities, and apoptosis (Bax/Bcl2). Proteinuria was higher in LPM males and LPE and LPM females. In LPM males glutathione peroxidase activity was lower, while in LPE males catalase activity was higher. Antioxidants were not much affected in females. Bax expression was higher in LPM males and females, while Bcl2 expression was higher in LPA females. Thus even before nephrogenesis (day0-day7), LP impacted on renal integrity in adult life,

while LP during a later phase (day15-day22) or throughout gestation had less effect. In summary, for aging rat kidney LP poses the greatest threat when restricted to early nephrogenesis [Joles et al. *J Developmental Origins Health Disease* 2010;1: 75-83].

Conclusions

In combination to these two studies show that, at least in the rat, LP during early nephrogenesis is deleterious for the kidney, although it may not actually reduce nephron numbers, while LP during lactation, i.e. late nephrogenesis, may actually be beneficial with respect to renal injury.