Hypertension Research (2019) 42:459–468
https://doi.org/10.1038/s41440-018-0157-7

ARTICLE

The exaggerated salt-sensitive response in hypertensive transgenic rats (TGR mRen-2) fostered by a normotensive female

Pavel Svitok[1,2] · Zuzana Husková[2] · Lenka Červenková[2] · Soňa Kikerlová[2] · Zdenka Vaňourková[2] · Lenka Sedláková[2] · Šárka Vacková[2] · Hana Šutovska[1] · Michal Zeman[1] · Libor Kopkan[2]

Received: 5 April 2018 / Revised: 4 September 2018 / Accepted: 1 October 2018 / Published online: 10 December 2018

Abstract

Suboptimal conditions during prenatal and early postnatal development can increase risk of hypertension later in life. We studied consequences of a changed perinatal environment by initiating the cross-fostering of homozygous Ren-2 transgenic rat (TGR) offspring to normotensive, transgene-negative control mothers, and vice versa. We hypothesized that cross-fostering to a normotensive female can attenuate the development of malignant hypertension in TGR offspring (TGRx) and change their salt-sensitive response. Blood pressure (BP) was monitored by the telemetry system under normal salt intake, and BP responses to increased salt intake in the phase of established hypertension. Under normal salt conditions, BP was not markedly different in cross-fostered animals compared with controls. However, BP responses to 2% salt intake led to a stronger BP response in TGRx during the active phase when compared with the control TGR group. The TGRx also exhibited increased albuminuria, lower sodium excretion, and creatinine clearance under higher salt intake compared with control salt intake. Higher salt intake resulted in a significant increase of aldosterone concentrations only in the TGRx group; moreover, TGRx rats exhibited more pronounced renal injury compared with controls. In conclusion, our data indicate that cross-fostering in TGR not only did not attenuate the development of hypertension but, on the contrary, led to the deterioration of BP regulation, particularly due to exaggerated salt sensitivity and sodium retention in TGRx. Results underline the important role of the mother during lactation in postnatal development of the offspring, since these changes reflected different ion content in milk of a particular strain of rats.

Keywords Cross-fostering · developmental programming · hypertension · renin–angiotensin–aldosterone system · renal function

Introduction

Hypertension and other cardiovascular and metabolic events, such as coronary heart disease or diabetes mellitus, can partially result from impaired processes during intrauterine and early postnatal development [1, 2]. Inadequate environmental stimuli during critical periods of development lead to physiological and morphological adaptations of the fetus, which can increase the susceptibility to disease in adulthood [2, 3]. Also, increased blood pressure (BP) in the offspring has been observed after maternal exposure to hypoxia, glucocorticoids, angiotensin II (Ang II), or high- and low-salt diet [4–8]. Vasculature, kidneys, and the sympathetic nerve system are considered to be preferentially affected prenatally, and subsequent changes can increase the risk of hypertension development in adulthood [2, 9, 10].

Rodents, in contrast to humans, are relatively poorly developed at birth, and most of their physiological systems mature postnatally. In rats, critical windows for development of the central nervous system, kidney, or immune system still occur after birth [10–13], and an increased salt intake [14], decreased protein intake [15], or exposure to

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41440-018-0157-7) contains supplementary material, which is available to authorized users.

✉ Libor Kopkan
libor.kopkan@ikem.cz

[1] Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University Bratislava, Bratislava, Slovak Republic

[2] Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

dexamethasone [16] during the lactation period can increase the susceptibility of the offspring to hypertension in adulthood. In contrast, prenatal events causing hypertension in adulthood can be ameliorated by cross-fostering to intact mothers [17, 18], as observed in several hypertensive strains of spontaneously hypertensive rats (SHR) [19] or Dahl salt-sensitive rats [20].

The renin–angiotensin–aldosterone system (RAAS) plays an essential role in the homeostatic control of arterial BP, perfusion of tissues, and control of extracellular fluid [21]. Moreover, RAAS is necessary for a proper development of the fetus. All components of the RAAS are highly expressed in the developing kidney, affecting nephrogenesis, and vascularization and ensuring an adequate development of the structure and function of the kidney [22, 23] as well as the systemic vasculature, brain, and heart [2, 24, 25]. Perinatal manipulation of RAAS has long-term consequences on postnatal offspring development [14, 26–28]. Inhibition of RAAS during nephrogenesis induces gross abnormalities in kidney development and results in renal tissue dysplasia, a decrease in the number and size of glomeruli, dilatation of renal tubules, and atrophy of renal papilla [23]. Increased concentrations of Ang II infused by osmotic minipumps to pregnant rats also resulted in negative consequences on BP regulation and kidney function [8], suggesting that fine tuning of the RAAS during pregnancy is important for both the mother and offspring. However, there is a lack of data about consequences of endogenously enhanced RAAS activity during prenatal and early postnatal development on the kidney function, BP control, and salt sensitivity in mature offspring.

Over-activation of RAAS has been indicated as an important pathophysiological factor in the development of malignant hypertension. The detrimental effects of high levels of renin and Ang II have been demonstrated in transgenic rats harboring the human renin and angiotensinogen genes with all signs of malignant hypertension, such as very high blood pressure, end-organ damage, and mortality [29, 30]. A homozygous transgenic rat model with an inserted mouse *Ren-2* gene (TGR) also exhibited malignant hypertension with a strong salt-sensitive component, accelerating end-organ damage and mortality [31]. Since this model represents a well-defined monogenic model of Ang II-dependent hypertension with endogenous activation of the RAAS [32], it can be effectively used for understanding pathophysiological mechanisms of developmental programming of hypertension and cardiovascular susceptibility in adult offspring.

Therefore, in the present study, we analyzed effects of cross-fostering of homozygous 1-day-old TGR males to normotensive, transgene-negative control strain Hannover Sprague-Dawley (HanSD) mothers, and HanSD pups nursed by TGR mothers. We hypothesized that cross-fostering to normotensive mothers can attenuate the development of malignant hypertension in TGR offspring and reduce salt-sensitive responses. The aim of our study was to establish consequences of cross-fostering in both directions on: (1) blood pressure control, monitored by the telemetry system; (2) salt sensitivity in the phase of established hypertension in cross-fostered TGR; (3) kidney structure and function; (4) activity of selected components of RAAS in offspring; and (5) ion composition of maternal milk.

Materials and methods

This study was approved by the Committee for Animal Care and Use at the Institute of Clinical and Experimental Medicine (IKEM, Prague, Czech Republic) in accordance with guidelines and practices established by EU legislation.

Experimental procedures

Both strains were mated at a ratio of 1 male to 2 females. After 4 days, the females were kept in separate cages and monitored regularly during their entire pregnancies. After delivery, the litter size, birth weight, and sex ratio of the offspring were assessed. Litters were culled to eight animals per dam. After that, half of the offspring was transferred to the mother from the opposite strain. As an effect of cross-fostering, we obtained the following groups: HanSD (HanSD pups nursed by HanSD mothers); HanSDx (HanSD pups nursed by TGR mothers); TGR (TGR pups nursed by TGR mothers); TGRx (TGR pups nursed by HanSD mothers). All analyses were performed on male progeny. Animals were kept in plastic cages under controlled environmental conditions (light:dark regime 12:12 h; temperature 22 ± 1 °C; humidity 45–75%; food and water given ad libitum). Up until week 11, the parental generation and progeny were fed by standard laboratory chow containing 0.4% NaCl. From postnatal week 12, the animals were fed with chow containing an increased salt content (2% NaCl) for an 11-day period.

Blood pressure and heart rate monitoring

The cardiovascular parameters were measured by radiotelemetry (Data Science International, St. Paul, Minnesota, USA) allowing continuous acquisition of BP and heart rate (HR) in freely moving animals. The implantation procedure was performed under isoflurane anesthesia. The pressure transmitters TA11PA-C40 were utilized in 11-week-old animals (six rats per group). Transmitters were implanted into the abdominal aorta, and the catheter was stabilized in the aorta with tissue glue (Histoacryl, B. Braun Surgical, SA, Rubi, Spain) and a cellulose patch (DSI, USA), as

Table 1 Strain differences between control and cross-fostered animals under normal salt (NS) at 12 weeks and high salt (HS) conditions at 14 weeks of age

Group	BW (g)	HW/tibia ratio	KW/tibia ratio	UV (ml/day)
HanSD + NS	483 ± 9	32 ± 1.1	36 ± 1.7	23.5 ± 2.4
HanSDx + NS	446 ± 12	34 ± 1.6	35 ± 1.2	25.3 ± 3.7
TGR + NS	361 ± 11*	40 ± 0.8*	33 ± 1.1	36.2 ± 4.6*
TGRx + NS	369 ± 10*	41 ± 1.2*	32 ± 1.2	38.6 ± 4.9*
HanSD + HS	524 ± 10	39 ± 1.3	41 ± 1.6	29.3 ± 2.7
HanSDx + HS	491 ± 13	37 ± 0.9	40 ± 1.8	28,8 ± 2.5
TGR + HS	403 ± 12*	49 ± 2.4*	37 ± 1.3	44.6 ± 3.5*
TGRx + HS	412 ± 15*	52 ± 2.1*	36 ± 1.5	42.8 ± 2.9*

Means ± SEM. *$p < 0.05$ vs. corresponding HanSD groups

body weight (BW), heart weight (HW)/tibia length ratio, kidney weight (KW)/tibia length ratio, 24 h urine volume (UV)

described previously [33]. After five days recovery, systolic and diastolic BP (SBP and DBP) were monitored continuously.

Metabolic chambers

In another series of intact animals, six animals from every group were placed in metabolic chambers at postnatal weeks 12 (normal salt condition) and 14 (high-salt intake) for determination of creatinine, ions, and albuminuria in urine. Urine was collected for 24 h and then blood samples were taken from the tail vein. Total albumin was determined in urine using the AssayMax Rat Albumin ELISA kit (Assaypro, St. Carles, MO, USA), creatinine concentrations were assessed by Liquick-Cor-CREATININE kit (Cormay, Łomianki, Poland) and urinary aldosterone was determined by radioimmunoassay (Beckman Coulter France S.A.S., Marseille, France). Urinary ions were measured by the flame photometer (BWB Technologies, Essex, UK).

Hormonal analyses and histology

Decapitation of these animals was performed also at postnatal weeks 12 and 14. Organs were weighed and correlated to the tibial length to avoid body weight differences (Table 1). Plasma concentration of angiotensin I (Ang I), and plasma renin activity (PRA) were determined by Angiotensin I RIA kit (Beckman Coulter, Inc., Indianapolis, IN, USA). Plasma and renal Ang II was measured with an Angiotensin II RIA kit (IBL International GmbH., Hamburg, Germany) [31, 32]. Plasma creatinine was measured by FUJI DRI-CHEM 4000i (FUJIFILM Europe GmbH, Düsseldorf, Germany).

For histological analyses of kidneys, samples were fixed in 4% paraformaldehyde and processed. We used PAS staining to assess the glomerulosclerosis index (GSI) score for renal damage and cortical tubulointerstitial injury (CTI), as described previously [33, 34].

Ion determination in breast milk

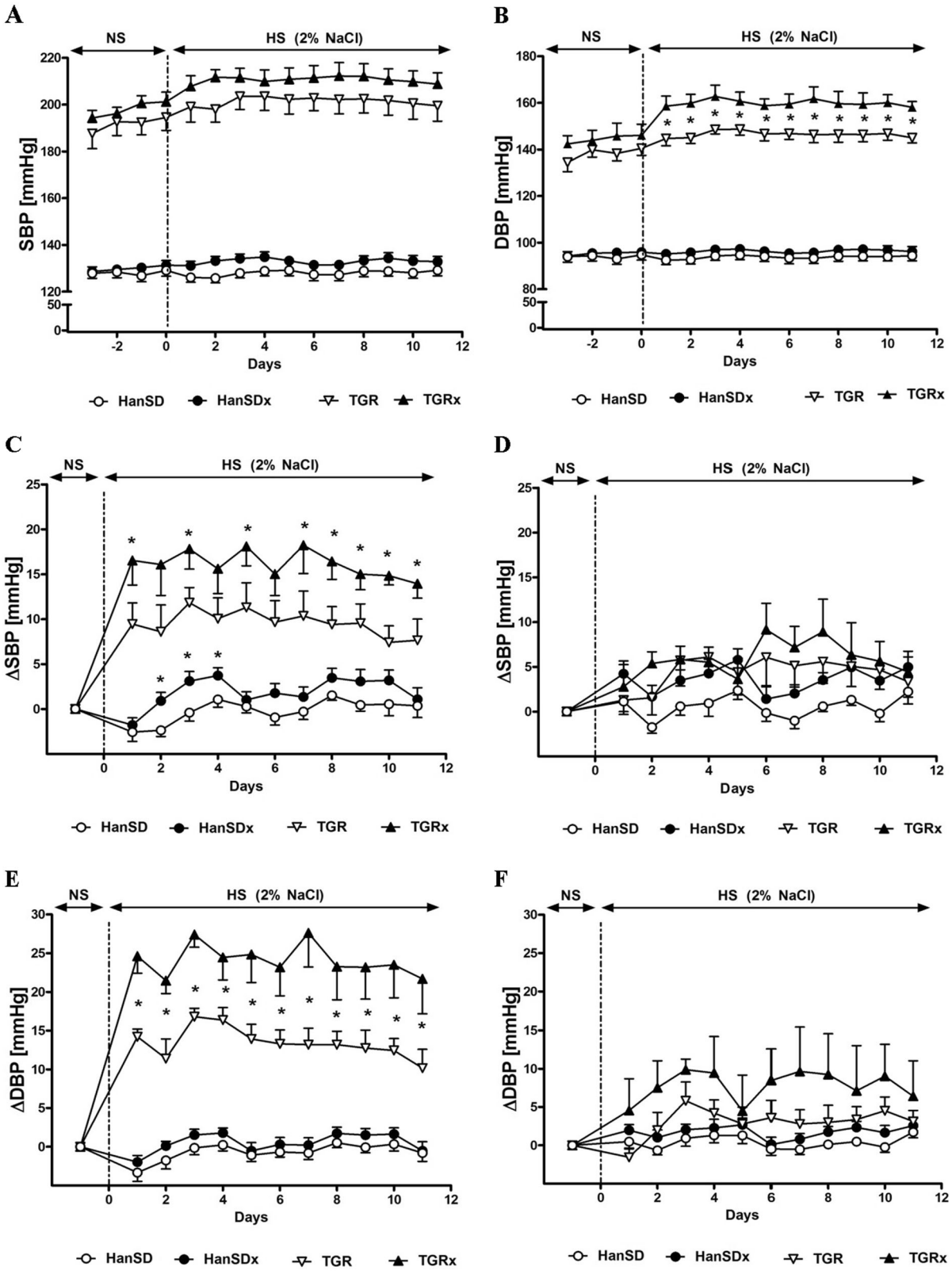
Na^+ and K^+ concentration were also analyzed in breast milk of lactating mothers under anesthesia. For induction of lactation, 0.5 UI of oxytocin (Zentiva, Czech Republic) was injected intraperitoneally. The mammary glands were punctured by needle, and the milk sample was aspirated into the syringe. Samples were diluted with deionized water (1:1), and ions were measured by flame photometry.

Western blotting analysis of mineralcorticoid receptor (MR) in the kidney

Tissues were homogenized and centrifuged at 13,000 g at 4 °C for 10 min. The total protein concentration was determined using bicinchoninic acid assay (BCA assay kit; Sigma-Aldrich, USA). Samples (15 µg) were subjected to 12% SDS–polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membrane was blocked during the night at temperature 4 °C using bovine serum albumin (Serva Electrophoresis, Heidelberg, Germany) to prevent nonspecific binding of antibodies. Afterwards, blots were incubated with anti-mineralcorticoid receptor antibody (Abcam, Cambridge, UK) followed by incubation with goat anti-rabbit HRP secondary antibody (Cell Signaling Technology, Danvers, MA, USA) at concentrations which were previously tested. For equal loading, membranes were re-probed with an antibody against GAPDH (Merck, Darmstadt, Germany). Proteins were visualized by the chemiluminescence ECL Substrate ClarityTM (BioRad, Hercules, CA, USA). Band densities were analyzed using Quantity One software (BioRad) and normalized for equal loading to GAPDH.

Statistical analysis

The normality of the data distribution was tested using the Kolmogorov–Smirnov test. To determine the effect of cross-fostering, we compared each strain separately (HanSD vs. HanSDx; TGR vs. TGRx) using a *t* test, one way ANOVA or ANOVA with repeated measurements in case of changes of cardiovascular parameters under high-salt conditions compared with normal salt conditions. Calculated data are expressed as means ± SEM.


A
NS
HS (2% NaCl)
SBP [mmHg]
Days
HanSD
HanSDx
TGR
TGRx
B
NS
HS (2% NaCl)
DBP [mmHg]
Days
HanSD
HanSDx
TGR
TGRx
C
NS
HS (2% NaCl)
ΔSBP [mmHg]
Days
HanSD
HanSDx
TGR
TGRx
D
NS
HS (2% NaCl)
ΔSBP [mmHg]
Days
HanSD
HanSDx
TGR
TGRx
E
NS
HS (2% NaCl)
ΔDBP [mmHg]
Days
HanSD
HanSDx
TGR
TGRx
F
NS
HS (2% NaCl)
ΔDBP [mmHg]
Days
HanSD
HanSDx
TGR
TGRx

◀ **Fig. 1** Time course in systolic blood pressure (SBP; **a**) and diastolic blood pressure (DBP; **b**) under normal salt (NS) and high salt (HS) conditions, changes in systolic blood pressure (ΔSBP) under high salt (HS) conditions during active (**c**) and passive phase (**d**) of the day and changes in diastolic blood pressure (ΔDBP) under high salt (HS) conditions during active (**e**) and passive phase (**f**) of the day in normotensive controls (HanSD), cross-fostered HanSDx, hypertensive control (TGR) and cross-fostered TGRx; ($n = 6$ per group). Data are presented as 24-h average with SEM; $^{*}p < 0.05$ vs. corresponding control group

Results

Cardiovascular parameters

Under normal salt conditions, SBP and DBP or any other cardiovascular parameters were not markedly different in cross-fostered TGRx and HanSDx compared with their controls (Fig. 1a, b). However, SBP responses to 2% NaCl intake for 11 days were stronger in TGRx males reared by HanSD mothers particularly during the active phase ($F_{(1,8)} = 9.90$, $p = 0.014$), as shown in Fig. 1c, without significant differences in the passive phase ($F_{(1,8)} = 0.054$, $p = 0.821$) when compared with the TGR control group (Fig. 1d). The responses in DBP were even more pronounced in TGRx (Fig. 1b), particularly in the active phase ($F_{(1,8)} = 9.05$, $p = 0.017$) without marked differences in the passive phase ($F_{(1,8)} = 0.92$, $p = 0.366$), as shown in Fig. 1e, f. In the HanSDx group fostered to TGR females, a smaller and transient SBP response to higher salt intake in comparison with controls was observed, especially during the active phase ($F_{(1,10)} = 6.00$, $p = 0.034$) as shown in Fig. 1c.

Excretory parameters

Twenty-four hours urine volume is shown in Table 1. Glomerular filtration rate assessed as creatinine clearance reached significantly lower values in the TGRx group fostered to HanSD mothers in comparison with other groups kept on a normal salt diet (Fig. 2a) and remained lower also after exposure to a higher salt diet (Fig. 2b). Analyses of albuminuria showed significantly higher level in TGR groups compared with HanSD strain under normal salt intake (Fig. 2c). However, TGRx displayed a significant increase in albuminuria after higher salt intake compared with TGR controls (Fig. 2d). Sodium excretion was similar in all groups under normal salt conditions (Fig. 3a). The increased sodium excretion reflected higher salt intake; however, TGRx exhibited lower sodium excretion compared with both HanSD groups (Fig. 3b). Urinary aldosterone was markedly higher in TGR compared with HanSD rats, as expected (Fig. 3c). However, the higher salt intake caused a significant increase in aldosterone concentration only in the TGRx group when compared with those in normal salt conditions (Fig. 3d).

Hormonal analyses

As expected, TGR groups exhibited markedly elevated PRA, plasma Ang I and plasma, and renal Ang II levels when compared with HanSD animals at age of 12 weeks (Table 2). Furthermore, the 2% NaCl diet significantly suppressed PRA and both angiotensin concentrations in all groups (Table 2), but no differences between cross-fostered animals and controls were recorded.

Histology

On the basis of GSI and CTI assessments, TGR groups exhibited significant signs of renal injury compared with HanSD animals (Fig. 4a, c). Moreover, CTI was higher in the TGRx group at week 12 (Fig. 4c; $Z = 2.81$, $p = 0.005$), and higher salt intake markedly accelerated glomerular damage in TGR groups, particularly in TGRx rats (Fig. 4b) without appreciable tendency in CTI (Fig. 4d). Representative histological kidney slices are given in Supplementary Figures 1 and 2.

Western blotting analysis of renal MR

We did not observe appreciable differences in MR protein expression among the groups. Although there is a tendency for higher MR expression in TGR (0.71 ± 0.14 relative intensity/GAPDH), this trend was diminished in TGRx (0.42 ± 0.13 relative intensity/GAPDH) to the level as measured in HanSD and HanSDx groups (0.38 ± 0.21 and 0.45 ± 0.29 relative intensity/GAPDH, respectively).

Reproductive parameters and milk ion compositions

The litter size in the TGR strain was significantly smaller than in the control HanSD strain ($t = -5.10$, $p < 0.001$). Birth weight of TGR offspring was also reduced ($t = 5.38$, $p < 0.001$) in contrast to HanSD. The male to female ratio did not differ between strains significantly ($t = 1.72$, $p = 0.090$). The hormonal background of the mothers affected the ion composition of their milk. The TGR dams had significantly lower concentrations of Na^+ ($t = 2.60$, $p = 0.025$) and higher concentrations of K^+ ($t = -3.26$, $p = 0.008$) in comparison with HanSD females.

Discussion

This is the first study evaluating effects of endogenously increased activity of RAAS on the development of an Ang

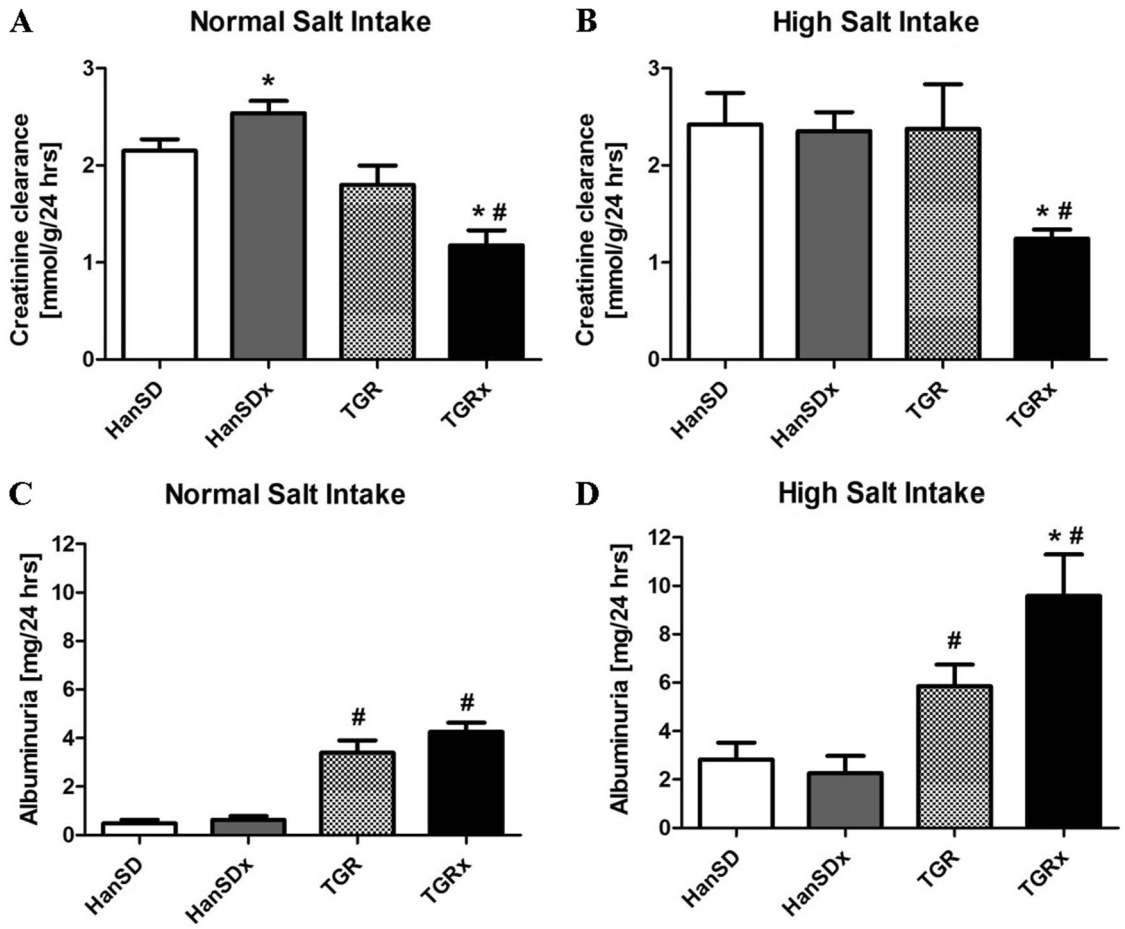


Fig. 2 Creatinine clearance under normal salt (**a**) and high salt (**b**) conditions and albuminuria under normal salt (**c**) and high salt (**d**) conditions in normotensive controls (HanSD), cross-fostered HanSDx, hypertensive control (TGR) and cross-fostered TGRx; ($n = 6$ per group). Data are presented as 24-h average with SEM; $^{*}p < 0.05$ vs. corresponding control group, $^{\#}P < 0.05$ vs. HanSD groups

II-dependent form of malignant hypertension and salt-sensitive response by cross-fostering of normotensive male offspring to hypertensive TGR mothers and vice versa. The major findings of our study were:

1. TGRx fostered to HanSD dams tended to have elevated BP in comparison with non-fostered TGR. Importantly, after exposure to higher salt intake, TGRx exhibited significantly elevated BP during the active phase of the day.
2. HanSDx rats reared by TGR mothers showed no significant differences in cardiovascular parameters under basal conditions, but also tended to elevate BP transiently on day 2, 3, and 4 after exposure to the higher salt diet during the active phase of the day.
3. Cross-fostering in our hypertensive model affected maturing kidneys, since TGRx offspring displayed a decreased glomerular filtration compared with non-fostered TGR and accelerated kidney damage.
4. Under higher salt conditions, TGRx displayed higher levels of urinary aldosterone and lower sodium excretion as compared with the non-fostered TGR rats.
5. The hormonal background of mothers affected the ion content of the breast milk; we found decreased sodium and increased potassium concentrations in milk from TGR, as compared with HanSD females.

Thus, our data indicate that cross-fostering in this model led to significant alterations in BP control and affected RAAS activity. These alterations affected renal function and increased sensitivity to higher salt intake.

Cross-fostering has been frequently used to evaluate effects of inadequate environment during lactation on postnatal development of the offspring. In several hypertensive strains of rats, such as SHR or Dahl salt-sensitive rats, fostering of hypertensive offspring to normotensive mother had long-term beneficial effects on the cardiovascular phenotype of the offspring [19, 20, 35]. In contrast, fostering of normotensive offspring to SHR mothers had no effect on cardiovascular characteristics in adulthood [36, 37]. Differences between those studies and our results

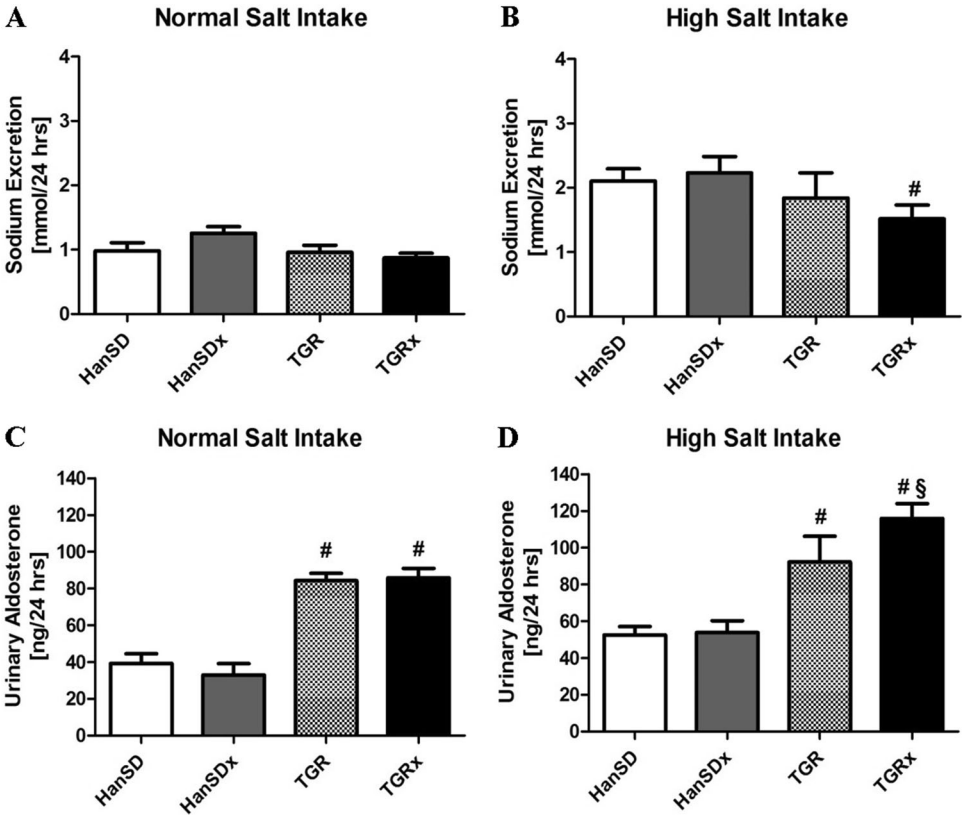


Fig. 3 Twenty-four hours sodium excretion under normal salt (**a**) and high salt (**b**) conditions and urinary aldosterone under normal salt (**c**) and high salt (**d**) conditions in normotensive controls (HanSD), cross-fostered HanSDx, hypertensive control (TGR), and cross-fostered TGRx; ($n = 6$ per group). Data are presented as 24-h average with SEM; $^{\#}p <$ 0.05 vs. HanSD groups, $^{\S}p <$ 0.05 vs. corresponding TGR NS group

Table 2 Strain differences between control and cross-fostered animas and the effect of high-salt intake on plasma renin activity (PRA) and plasma angiotensin I and II concentrations and renal Ang II concentration in controls and cross-fostered groups under normal salt (NS) and high-salt (HS) conditions

Group	PRA (ng/ml/h)	Plasma Ang I (fmol/ml)	Plasma Ang II (fmol/ml)	Renal Ang II (fmol/g)
HanSD + NS	6.5 ± 0.6	1529 ± 87	13.2 ± 0.7	43.8 ± 4.1
HanSDx + NS	6.1 ± 0.7	1507 ± 51	14.3 ± 0.9	36.7 ± 3.2
TGR + NS	14.9 ± 1.4*	3108 ± 145*	54.8 ± 6.4*	148.9 ± 9.8*
TGRx + NS	14.8 ± 0.9*	3214 ± 131*	47.4 ± 4.5*	150.6 ± 10.8*
HanSD + HS	3.2 ± 0.5$^{\#}$	1307 ± 68$^{\#}$	8.9 ± 0.5$^{\#}$	21.2 ± 3.2$^{\#}$
HanSDx + HS	3.6 ± 0.4$^{\#}$	1290 ± 49$^{\#}$	7.1 ± 0.3$^{\#}$	27.1 ± 1.7$^{\#}$
TGR + HS	5.7 ± 0.9*$^{\#}$	2153 ± 140*$^{\#}$	34.1 ± 5.2*$^{\#}$	101.4 ± 10.7*$^{\#}$
TGRx + HS	5.1 ± 0.8*$^{\#}$	1916 ± 129*$^{\#}$	21.8 ± 3.7*$^{\#}$	76.3 ± 10.9*$^{\#}$

Means ± SEM. *$p < 0.05$ vs. corresponding HanSD groups, $^{\#}p < 0.05$ vs. corresponding groups under normal salt conditions

probably reflect different etiology of hypertension in different strains of rats. In our study, we investigated for the first time effects of cross-fostering between homozygous transgenic Ren2 rats and their transgene-negative control strain. In contrast to SHR, in which the increased BP is explained by an upregulated sympathetic system [38], hypertension in TGR is clearly defined by upregulation of the RAAS with elevated levels of Ang II detectable in plasma and target tissues [32]. Therefore, Ren-2 transgenic rats represent a good model for evaluation effects of upregulated RAAS in lactating dams on postnatal development of the offspring. In our experiment, we found that the hypertensive TGR rats fostered to normotensive HanSD dams tended to have higher BP in the phase of established hypertension. This observation did not consent to previous studies using different models of hypertension, such as SHR, Dahl, or New Zealand genetically hypertensive rats [19, 20, 35, 39], and points to the important role of complex maternal effects in the process of developmental programming. Thus, an interaction between a genetic background and physiology and behavior of the mother represents an important factor in determination of postnatal development and susceptibility of offspring to cardiovascular diseases in adulthood.

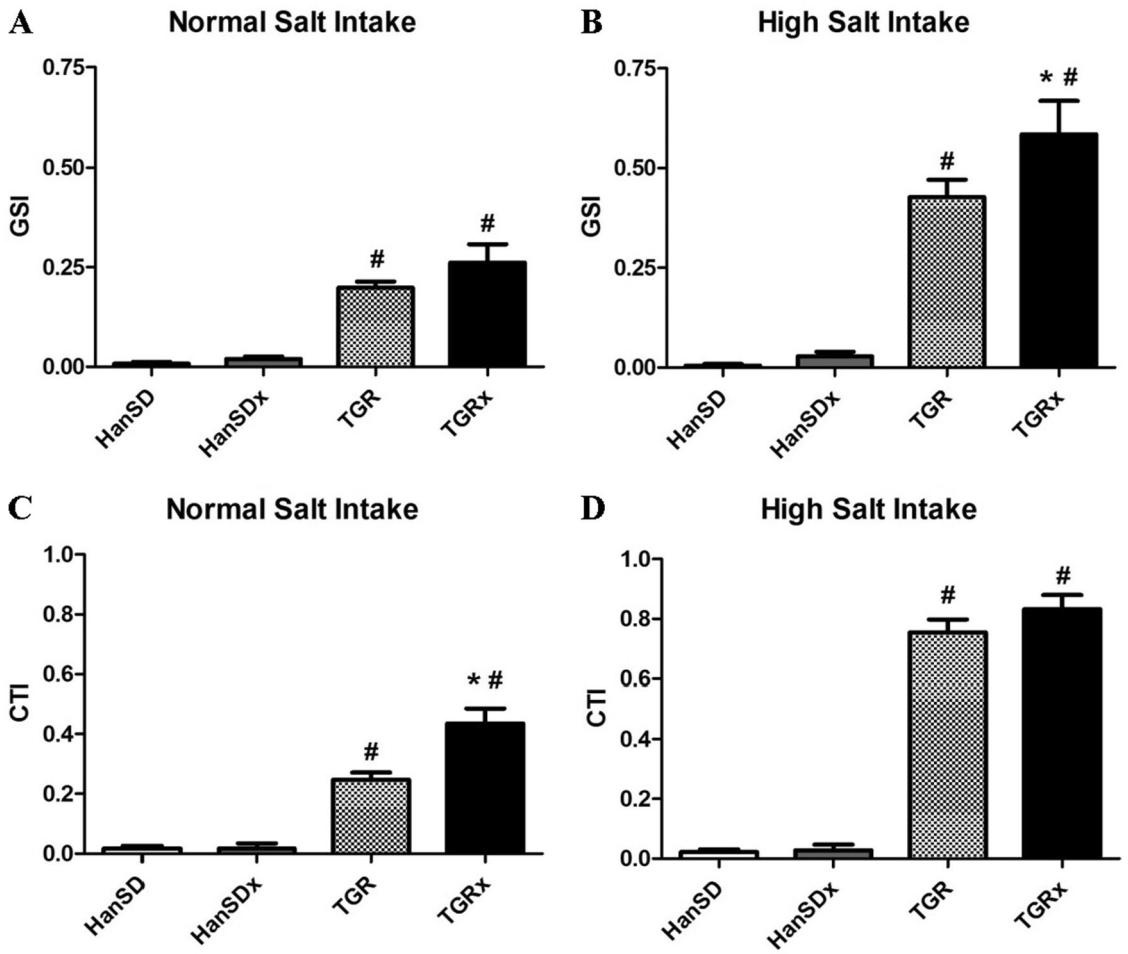


Fig. 4 Glomerulosclerosis index (GSI) under normal salt (**a**) and high salt (**b**) conditions and cortical tubulointerstitial injury (CTI) under normal salt (**c**) and high salt (**d**) conditions in normotensive controls (HanSD), cross-fostered HanSDx, hypertensive control (TGR), and cross-fostered TGRx; ($n = 6$ per group). Data are presented as 24-h average with SEM; $^{\#}p < 0.05$ vs. HanSD groups, $^{*}p < 0.05$ vs. corresponding control group

Moreover, fostered TGR rats manifested an increased sensitivity to the enhanced salt intake in monitored cardiovascular parameters, compared with control rats from the same strain. These differences were observed mainly during the active phase of the day. The TGR strain is known to exhibit salt sensitivity with a disrupted interaction between sodium homeostasis and the regulation of RAAS [31, 32]. Although, we observed diminishing effects of higher salt intake on PRA and concentrations of angiotensins, the increase of urinary aldosterone particularly in TGRx indicates an inappropriate control of local renal RAAS that may significantly modulate salt-sensitive response in this model. Moreover, it is likely that exaggerated RAAS activity plays a key role in the progression of renal damage in TGR [33]. Based on our analysis of renal damage, we observed that cross-fostering in this strain could accelerate kidney injury. To determine the effect of cross-fostering on function of offspring kidneys, we performed measurements of glomerular filtration assessed by creatinine clearance in adulthood. Cross-fostering had long-term effects on the matured kidney as the TGRx offspring had decreased glomerular filtration. On the other hand, HanSDx rats had a slightly increased glomerular filtration rate under normal salt intake. Therefore, the blunted ability to regulate normal kidney function can contribute to the BP increase in our experiment.

For a better understanding of maternal effects on offspring, we measured milk ion composition, which plays an important role in modulation of postnatal kidney development and maturation [35, 40]. In our experiment, lactating TGR dams exhibited lower sodium and higher potassium levels in their breast milk, which was significantly different on comparison with lactating HanSD dams. Therefore, it can be assumed the different milk composition and subsequent different sodium intake, as envisioned on the basis of genetic background, might significantly modulate RAAS

activity in both cross-fostered TGR and HanSD offspring. These alterations most likely affected renal morphology and function and also salt-sensitive response to higher salt intake in adult male rats. It was found, that increased sodium intake during early postnatal period led to the increase of BP in adulthood [13]. On the basis of our results, we suggest that a discrepancy between individual adaptation to environment programmed by the mother during prenatal development, and the environment into which its offspring is subsequently born has negative effects on morphology and function of organs involved in BP control. Our observations suggest a close relationship between the RAAS of lactating dams and activity of RAAS in their offspring.

In our study, the most pronounced effects of aldosterone on BP during the active phase may reflect the distinct circadian production of this mineralcorticoid, which is much higher at the end than at the beginning of the daytime in TGR as compared with HanSD rats [41]. A series of elegant studies [42] (see for a review) demonstrates a subtype of 3β-hydroxysteroid-dehadrogenase as the key enzyme in the control of circadian aldosterone biosynthesis. This enzyme is upregulated in salt-sensitive hypertension, exhibits circadian rhythmicity [43], and therefore may at least partially explain the increased aldosterone production and higher BP during the active phase in our study. We are aware that aldosterone could compromise renal function, induce renal damage particularly during high-salt intake as indicated in recent studies [44, 45]. However, we did not find the characteristic inverse ligand/receptor relationship between aldosterone concentrations and MR protein as was demonstrated in the heart [46]. Since MR protein expression in the kidney did not increase in TGRx, MR itself may not explain the observed negative effects of cross-fostering on BP regulation and increased salt sensitivity in our study. Further investigation related to the pathophysiology of aldosterone pathway particularly in this model of salt-sensitive hypertension is still needed to understand possible interactions.

In conclusion, our data indicate that cross-fostering of normotensive HanSD offspring to hypertensive TGR mothers with an Ang II-dependent form of hypertension and vice versa led to the significant alterations of BP regulation, particularly due to exaggerated salt sensitivity in TGR. Furthermore, postnatal modulation of RAAS activity in cross-fostered animals affected the renal morphology and function and, subsequently, the salt-sensitive response to higher salt intake in the phase of established Ang II-dependent form of hypertension. Our results suggest that a mismatch between a phenotype programmed by the mother in utero and un-matching postnatal environmental conditions may represent a risk factor for BP control and hypertension development in the adulthood.

Acknowledgements This study was supported by Ministry of Health, Czech Republic—conceptual development of research organization ("Institute for Clinical and Experimental Medicine - IN 00023001"). LK was partly supported by grant number 15-25396A, and MZ by grant VEGA 1/0557/15.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Barker DJ. In utero programming of chronic disease. Clin Sci. 1998;95:115–28.
2. Morton JS, Cooke CL, Davidge ST. In utero origins of hypertension: mechanisms and targets for therapy. Physiol Rev. 2016;96:549–603.
3. Gluckman PD, Hanson MA, Bateson P, Beedle AS, Law CM, Bhutta ZA, et al. Towards a new developmental synthesis: adaptive developmental plasticity and human disease. Lancet. 2009;373:1654–7.
4. Svitok P, Molcan L, Stebelova K, Vesela A, Sedlackova N, Ujhazy E, et al. Prenatal hypoxia in rats increased blood pressure and sympathetic drive of the adult offspring. Hypertens Res. 2016;39:501–5.
5. Shah A, Matsumura N, Quon A, Morton JS, Dyck JRB, Davidge ST. Cardiovascular susceptibility to in vivo ischemic myocardial injury in male and female rat offspring exposed to prenatal hypoxia. Clin Sci. 2017;131:2303–17.
6. Sheen JM, Yu HR, Tiao MM, Chen CC, Huang LT, Chang HY, et al. Prenatal dexamethasone-induced programmed hypertension and renal programming. Life Sci. 2015;132:41–48.
7. Koleganova N, Piecha G, Ritz E, Becker LE, Müller A, Weckbach M, et al. Both high and low maternal salt intake in pregnancy alter kidney development in the offspring. Am J Physiol Ren Physiol. 2011;301:F344–354.
8. Svitok P, Senko T, Panakova Z, Olexova L, Krskova L, Okuliarova M, et al. Prenatal exposure to angiotensin 2 increases blood pressure and decreases salt sensitivity in rats. Clin Exp Hypertens. 2017;39:489–94.
9. Nuyt AM, Alexander BT. Developmental programming and hypertension. Curr Opin Nephrol Hypertens. 2009;18:144–52.
10. Chong E, Yosypiv IV. Developmental programming of hypertension and kidney disease. Int J Nephrol. 2012;2012:760580.
11. Rice D, Barone S. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect. 2000;108:511–33.
12. Wood-Bradley RJ, Barrand S, Giot A, Armitage JA. Understanding the role of maternal diet on kidney development; an opportunity to improve cardiovascular and renal health for future generations. Nutrients. 2015;7:1881–905.
13. Holladay SD, Smialowicz RJ. Development of the murine and human immune system: differential effects of immunotoxicants depend on time of exposure. Environ Health Perspect. 2000;108:463–73.
14. Svitok P, Molcan L, Vesela A, Kruzliak P, Moravcik R, Zeman M. Increased salt intake during early ontogenesis lead to development of arterial hypertension in salt-resistant Wistar rats. Clin Exp Hypertens. 2015;37:142–7.
15. Siddique K, Guzman GL, Gattineni J, Baum M. Effecr of postnatal maternal protein intake on prenatal programming of hypertension. Reprod Sci. 2014;21:1499–507.

16. Chang HY, Tain YL. Postnatal dexamethasone-induced programmed hypertension is related to the regulation of melatonin and its receptors. Steroids. 2016;108:1–6.
17. Tare M, Parkington HC, Bubb KJ, Wlodek ME. Uteroplacental insufficiency and lactational environment separately influence arterial stiffness and vascular function in adult male rats. Hypertension. 2012;60:378–86.
18. Lozano G, Elmaghrabi A, Salley J, Siddique K, Gattineni J, Baum M. Effect of prenatal programming and postnatal rearing on glomerular filtration rate in adult rats. Am J Physiol Ren Physiol. 2015;308:F411–419.
19. Lee SK, Sirajudeen KN, Sundaram A, Zakaria R, Singh HJ. Effect of cross-fostering on renal anti-oxidant/oxidant status and development of hypertension in spontaneously hypertensive rats. Clin Exp Pharmacol Physiol. 2011;38:854–9.
20. Murphy CA, McCarty R. Maternal environment and development of high blood pressure in Dahl hypertensive rats. Am J Physiol. 1989;257:H1396–1401.
21. Atlas SA. The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. J Manag Care Pharm. 2007;13(8Suppl B):9–20.
22. Hilgers KF, Norwood VF, Gomez RA. Angiotensin's role in renal development. Semin Nephrol. 1997;17:492–501.
23. Yosypiv IV, El-Dahr SS. Role for the renin-angiotensin system in the development of the ureteric bud and renal collecting system. Pediatr Nephrol. 2005;20:1219–29.
24. Yamada H, Akishita M, Ito M, Tamura K, Daviet L, Lehtonen JY, et al. AT2 receptor and vascular smooth muscle cell differentiation in vascular development. Hypertension. 1999;33:1414–9.
25. Li JM, Mogi M, Tsukuda K, Tomochika H, Iwanami J, Min LJ, et al. Angiotensin II-induced neural differentiation via angiotensin II type 2 (AT2) receptor-MMS2 cascade involving interaction between AT2 receptor-interacting protein and Src homology 2 domain-containing protein-tyrosine phosphatase 1. Mol Endocrinol. 2007;21:499–511.
26. Lamparter S, Sun Y, Weber KT. Angiotensin II receptor blockade during gestation attenuates collagen formation in the developing rat heart. Cardiovasc Res. 1999;43:165–72.
27. Guan J, Mao C, Xu F, Geng C, Zhu L, Wang A, et al. Prenatal dehydration alters renin-angiotensin system associated with angiotensin-increased blood pressure in young offsping. Hypertens Res. 2009;32:1104–11.
28. Bullo M, Tschumi S, Bucher BS, Bianchetti MG, Simonetti GD. Pregnancy outcome following exposure to angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists: a systematic review. Hypertension. 2012;60:444–50.
29. Mullins JJ, Peters J, Ganten D. Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene. Nature. 1990;344:541–4.
30. Luft FC, Mervaala E, Muller DN, Gross V, Schmidt F, Park JK, et al. Hypertension-induced end-organ damage: a new transgenic approach to an old problem. Hypertension. 1999;33:212–8.
31. Dvorák P, Kramer HJ, Bäcker A, Malý J, Kopkan L, Vanecková I, et al. Blockade of endothelin receptors attenuates end-organ damage in homozygous hypertensive ren-2 transgenic rats. Kidney Blood Press Res. 2004;27:248–58.
32. Husková Z, Kramer HJ, Vanourková Z, Cervenka L. Effects of changes in sodium balance on plasma and kidney angiotensin II levels in anesthetized and conscious Ren-2 transgenic rats. J Hypertens. 2006;24:517–27.
33. Rakusan D, Kujal P, Kramer HJ, Husková Z, Vanourková Z, Vernerová Z, et al. Persistent antihypertensive effect of aliskiren is accompanied by reduced proteinuria and normalization of glomerular area in Ren-2 transgenic rats. Am J Physiol Ren Physiol. 2010;299:F758–766.
34. Kujal P, VČ Chábová, Vernerová, Walkowska Z, Kompanowska-Jezierska A, Sadowski E, et al. L. Similar renoprotection after renin-angiotensin-dependent and -independent antihypertensive therapy in 5/6-nephrectomized Ren-2 transgenic rats: are there blood pressure-independent effects? Clin Exp Pharmacol Physiol. 2010;37:1159–69.
35. Gouldsborough I, Ashton N. Effect of cross-fostering on neonatal sodium balance and adult blood pressure in the spontaneously hypertensive rat. Clin Exp Pharmacol Physiol. 1998;25:1024–31.
36. Azar S, Kabat V, Bingham C. Environmental factor(s) during suckling exert long-term effects upon blood pressure and body weight in spontaneously hypertensive and normotensive rats. J Hypertens. 1991;9:309–27.
37. Cierpial MA, Konarska M, McCarty R. Maternal influences on sympathetic-adrenal medullary system in spontaneously hypertensive rats. Am J Physiol. 1990;258:H1312–1316.
38. Lundin S, Ricksten SE, Thorén P. Renal sympathetic activity in spontaneously hypertensive rats and normotensive controls, as studied by three different methods. Acta Physiol Scand. 1984;120:265–72.
39. Ashton N, Kelly P, Ledingham JM. Effect of cross-fostering on blood pressure and renal function in the New Zealand genetically hypertensive rat. Clin Exp Pharmacol Physiol. 2003;30:820–6.
40. McCarty R, Tong H, Forsythe RC. Electrolyte content of milk differs in normotensive and spontaneously hypertensive rats. Psychobiology. 1992;20:307–10.
41. Zeman M, Petrák J, Stebelová K, Nagy G, Krizanova O, Herichová I, et al. Endocrine rhythms and expression of selected genes in the brain, stellate ganglia, and adrenals of hypertensive TGR rats. Ann N Y Acad Sci. 2008;1148:308–16.
42. Okamura H, Doi M, Goto K, Kojima R. Clock genes and salt-sensitive hypertension: a new type of aldosterone-synthesizing enzyme controlled by the circadian clock and angiotensin II. Hypertens Res. 2016;39:681–7.
43. Doi M, Takahashi Y, Komatsu R, Yamazaki F, Yamada H, Haraguchi S, et al. Salt-sensitive hypertension in circadian clock-deficient Cry-null mice involves dysregulated adrenal Hsd3b6. Nat Med. 2010;16:67–74.
44. Takahashi H, Nakagawa S, Wu Y, Kawabata Y, Numabe A, Yanagi Y, et al. A high-salt diet enhances leukocyte adhesion in association with kidney injury in young dahl salt-sensitive rats. Hypertens Res. 2017;40:912–20.
45. Hisamichi M, Kamijo-Ikemori A, Sugaya T, Hoshino S, Kimura K, Shibagaki Y. Role of bardoxolone methyl, a nuclear factor erythroid 2-related factor 2 activator, in aldosterone- and salt-induced renal injury. Hypertens Res. 2018;41:8–17.
46. Ricchiuti V, Lapointe N, Pojoga L, Yao T, Tran L, Williams GH. et al. Dietary sodium intake regulates angiotensin II type 1, mineralocorticoid receptor, and associated signaling proteins in heart. J Endocrinol. 2011;211:47–54.