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Effects of Sodium Restriction and Cyclooxygenase-2 Inhibition on the Course of Hypertension, Proteinuria and Cardiac Hypertrophy in Ren-2 Transgenic Rats

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Summary

The present study was performed to evaluate the effects of sodium intake and of chronic cyclooxygenase-2 (COX-2) inhibition on systolic blood pressure (SBP) in heterozygous male transgenic rats harboring the mouse Ren-2 renin gene (TGR) and in transgene-negative normotensive Hannover Sprague-Dawley (HanSD). Twenty-eight days old TGR and HanSD were randomly assigned to groups fed either normal salt (NS) or low sodium (LS) diets. COX-2 blockade was achieved with NS-398 (1 $mg.kg^{-1}.day^{-1}$ in drinking water). During an experimental period of 26 days, SBP was repeatedly measured by tail plethysmography in conscious animals. We found that the LS diet prevented the development of hypertension in TGR and did not change SBP in HanSD. Low sodium intake also prevented proteinuria and cardiac hypertrophy in TGR. On the other hand, irrespective of sodium intake chronic COX-2 inhibition did not alter the course of SBP in either TGR or HanSD. The present data indicate that TGR exhibit an important salt-sensitive component in the developmental phase of hypertension. They also suggest that systemic COX-2-derived prostaglandins do not act as vasodilatory counterregulatory agents in TGR in which an exaggerated vascular responsiveness to angiotensin II is assumed as the pathophysiological mechanism in the development of hypertension.

Key words
Hypertension • Ren-2 transgenic rats • Cyclooxygenase-2 • Low sodium diet

Introduction

The rat strain transgenic for the mouse *Ren-2* renin gene [TGR; strain name TGR(mRen2)27] represents a monogenetic model of hypertension. The development of hypertension in this strain is the result of insertion of the mouse *Ren-2* renin gene into the rat genome (Mullins *et al.* 1990). Although hypertension that occurs in this model is clearly related to the insertion of the *Ren-2* renin gene, the exact pathophysiological mechanisms responsible for the development of hypertension remain unknown (for review see

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Langheinrich *et al.* 1996). Previous studies have shown that hypertension in TGR is angiotensin II (ANG II)-dependent and that activation of ANG II receptor subtype 1 (AT_1) substantially contributes to the development of hypertension in this model (Böhm *et al.* 1995, Gross *et al.* 1995, Mitchell and Mullins 1995, for review see Langheinrich *et al.* 1996). However, it has been reported that plasma and kidney ANG II levels are not elevated in TGR (Mitchell *et al.* 1997, Kopkan *et al.* 2004) and it has recently been found that plasma and kidney ANG II levels in hypertensive TGR are even lower than those in transgene-negative normotensive Hannover Sprague-Dawley (HanSD) rats. In addition, it has also been demonstrated that hypertension in TGR is not related to renin kinetics (Rong *et al.* 2003). Therefore, there is a general agreement that the development of hypertension in TGR cannot be simply explained on the basis of increased production of ANG II and/or overactivation of AT_1 receptors, but other mechanisms must also be considered.

Jacinto *et al.* (1999) reported that TGR exhibit exaggerated peripheral and renal vascular responsiveness to ANG II and they suggested that this contributes to the development and/or maintenance of hypertension in this model. However, the mechanisms responsible for the augmented vascular responsiveness to ANG II remain uncertain. It is well recognized that the vasodilator prostanoids, the products of the cyclooxygenase (COX) pathway of arachidonic acid metabolism, antagonize the renal vasoconstrictor actions of ANG II (Purdy and Arendshorst 1999, for review see Imig 2000). Although generally considered as an enzyme induced by inflammatory stimuli, cyclooxygenase-2 (COX-2) is also constitutively expressed in the thick ascending limb of the loop of Henle, medullary interstitial cells and in the macula densa cells (for review see Cheng and Harris 2004).

Several lines of evidence support a role of renal COX-2 in the pathophysiology of hypertension, especially because of the coordinate expression of renin and COX-2 in the macula densa under the conditions of high renin states (Hartner *et al.* 1998, 2003, Wang *et al.* 1999, Mann *et al.* 2001, Komers and Epstein 2002, Krämer *et al.* 2004, for review see Cheng and Harris 2004). In addition, it has been shown that renal COX-2-derived prostaglandins play an important protective role in the renal vasculature against the excessive renal vasoconstrictor influences of ANG II and norepinephrine (NE), such as in salt and volume depletion (Beierwaltes 2002, López *et al.* 2003a, for review see López *et al.* 2003b).

In contrast with these studies we recently found that despite the high level of COX-2 protein expression and tissue prostaglandin E_2 (PGE_2) concentrations in the renal cortex, COX-2-derived metabolites do not operate as compensatory vasodilatory substances in TGR (Vaněčková *et al.*, 2004). These results support the notion that the relative lack of COX-2-derived vasodilatory prostanoids might partially account for the enhanced renal vascular responsiveness to ANG II in TGR and may thus contribute to the maintenance of hypertension in this model.

However, it should be recognized that in the above study we employed adult TGR with established hypertension. Moreover, our experiments were performed in anesthetized, surgically stressed animals and therefore the results denote the acute effects of COX-2 inhibition on the renal hemodynamics and sodium excretory function. Taken together, these findings may not necessarily relate to the development of hypertension in TGR and may not reflect the role of COX-2-derived metabolites in the long-term blood pressure (BP) regulation in this model.

Much attention was given to the influence of dietary sodium intake on renal cyclooxygenase levels. Low-salt diet increases COX-2 mRNA levels (Jensen and Kurtz 1997) while high-salt diet decreases it. Moreover, there exists a difference in the expression along the tubule (Yang *et al.* 1998). Since the results were more conflicting with increasing amount of salt in the diet (0.03 g NaCl/100 g rat chow) and since we extended our previous studies, we used a diet with a very small amount of sodium (<0.01 g NaCl/100 g rat chow).

In the present study, the first aim was therefore to assess the effect of low sodium (LS) intake on the development of hypertension as well as on proteinuria and cardiac hypertrophy in TGR. Furthermore, we also tried to assess the effect of chronic COX-2 inhibition on blood pressure (BP) during the developmental phase of hypertension in TGR. Since we found in a recent study that LS diet caused markedly higher increases in COX-2 protein expression and renocortical tissue PGE_2 concentrations in TGR compared with HanSD (Vaněčková *et al.* 2004), the second aim of this study was to determine the effects of chronic COX-2 inhibition on BP of TGR under the conditions of normal and low sodium intake.

Methods

Animals

The present study was performed in accordance with guidelines and practices established by the Animal Care and Use Committee (Institute for Clinical and Experimental Medicine, Prague) and are in accordance with laws in the Czech Republic and Federal Republic of Germany. Experiments were performed in male heterozygous TGR of the hypertensive line TGR(mRen2)27 and on age-matched transgene-negative normotensive HanSD rats. All animals used were bred at the Center of Experimental Medicine of the Institute for Clinical and Experimental Medicine from stock animals supplied from the Max Delbrück Center for Molecular Medicine, Berlin, Germany.

Animals were fed either a normal salt (NS) diet (0.6 g NaCl/100 g rat chow, C 1000, Altromin, Lage, Germany) or a low sodium (LS) diet (<0.01 g NaCl/100 g rat chow, C 1036, Altromin) for 26 days. The NS-398 (Cayman Chemical Co., USA) was used as the specific COX-2 inhibitor. NS-398 was first dissolved in ethanol and then added to drinking water at a concentration adjusted (drinking volume was monitored twice a week) to achieve a daily dose of approximately 1 $mg.kg^{-1}.day^{-1}$. This dose of NS-398 was shown in previous chronic studies to effectively block COX-2 activity (Harding *et al.* 2000, Hartner *et al.* 2003).

Experimental design

Twenty-eight days old TGR and HanSD from several litters were randomly assigned to the following eight experimental groups provided that animals from a single litter did not prevail in any of the groups. TGR fed NS diet and drinking water with vehicle (n = 8); HanSD fed NS diet and drinking water with vehicle (n = 7); TGR fed NS diet and drinking water with NS-398 (n = 8); HanSD fed NS diet and drinking water with NS-398 (n = 7); TGR fed LS diet and drinking water with vehicle (n = 10); HanSD fed LS diet and drinking water with vehicle (n = 8); TGR fed LS diet and drinking water with NS-398 (n = 10); HanSD fed LS diet and drinking water with NS-398 (n = 7).

Systolic blood pressure (SBP) was measured in conscious animals by tail plethysmography at the age of 30, 32, 34, 37, 39, 41, 44, 46 and 51 days, each time as the mean of four measurements. This method was previously validated in our laboratory (Heller *et al.* 1993, Heller and Hellerová 1998) and a close correlation between measurements by tail plethysmography and direct BP measurements in conscious rats with an indwelling catheter was demonstrated (unpublished data). On day 52 of their age, rats were placed into individual metabolic cages (after appropriate accustoming training) and their 24-hour urine was collected for determination of protein and electrolyte output. At the end of the experiments (day 54 of the age), animals were anesthetized with thiopental sodium (50 $mg.kg^{-1}$ of body weight), and the right carotid artery was cannulated with PE-50 tubing for direct measurement of mean arterial pressure (MAP). The ratios of heart weight (mg)/body weight (g) and left kidney weight (mg)/body weight (g) were used as indices of cardiac hypertrophy (HW/BW) and kidney hypertrophy (KW/BW), respectively.

General analytic methods

Sodium and potassium concentrations were determined by flame photometry. Urinary protein concentrations were determined by biuret method using commercially available kit (Bio-Lachema-Test, Lachema, Brno, Czech Republic).

Statistical analysis

Data are expressed as means ± SEM. Statistical comparisons within groups were performed using ANOVA for repeated measurements, followed by the Newman-Keuls test. One-way ANOVA was used for comparisons between groups. Values exceeding the 95 % probability limits ($p<0.05$) were considered statistically significant.

Results

Effects of sodium restriction and COX-2 inhibition on systolic blood pressure and body weight

HanSD remained normotensive throughout the experiment and SBP was unaltered by sodium restriction or COX-2 inhibition with NS-398 (Fig. 1A). As shown in Figure 1B, administration of NS-398 did not change the pattern of hypertension development in TGR fed the NS diet. The final levels of SBP (208±7 vs. 211±6 mm Hg) and MAP (Table 1) were similar in NS-398 treated and untreated TGR. The final SBP level was also similar in NS-398-treated and untreated TGR. LS diet substantially attenuated the development of hypertension in TGR so that the final SBP was close to the normotension (149±5 mm Hg). Again NS-398 did not alter the course of SBP (Fig. 1B) and MAP (Table 1) in TGR fed LS diet.

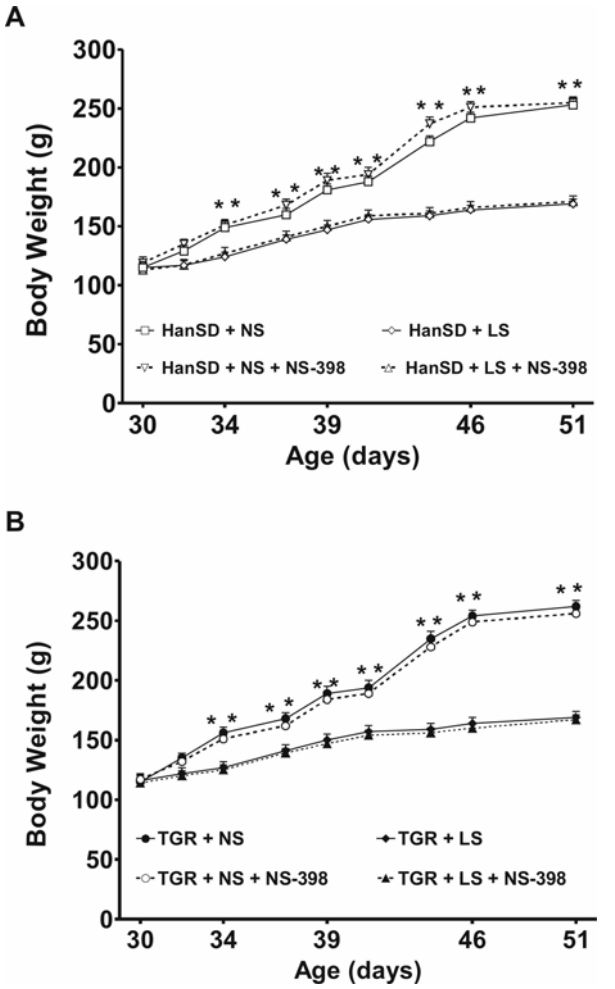

Fig. 1. Systolic blood pressure in HanSD (**A**) and TGR rats (**B**) fed either normal (NS) or low-sodium (LS) diet in the absence or presence of COX-2 inhibitor NS-398. * P<0.05 compared with HanSD and TGR rats on LS diet.

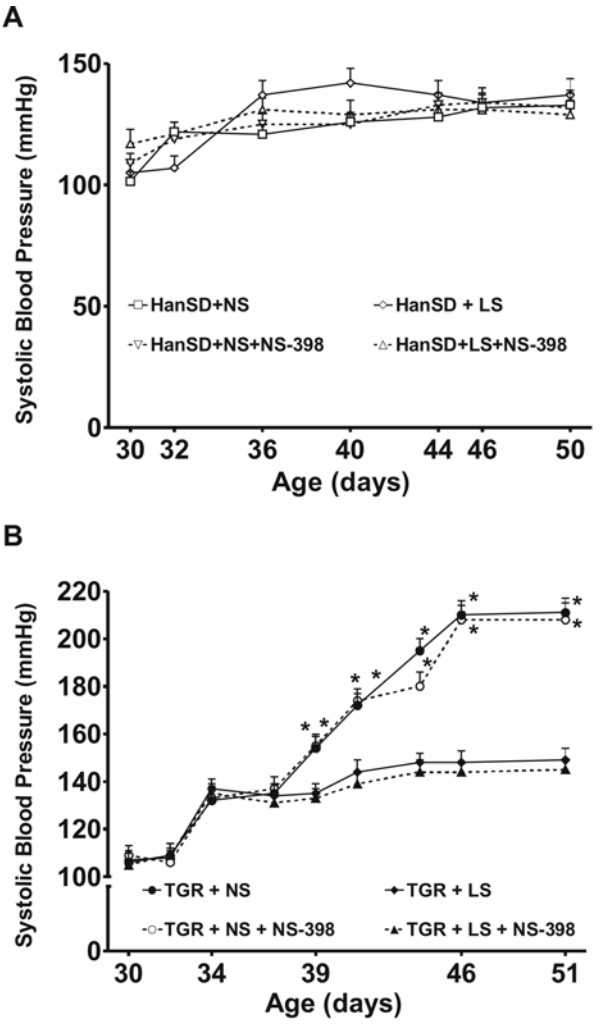

Fig. 2. Body weight in HanSD (**A**) and TGR rats (**B**) fed either normal (NS) or low-sodium (LS) diet in the absence or presence of COX-2 inhibitor NS-398. * P<0.05 compared with HanSD and TGR rats on LS diet.

As shown in Figures 2A and 2B, LS diet markedly attenuated body weight gain in HanSD as well as in TGR. Treatment with NS-398 did not modify the course of body weight gain in HanSD or TGR fed the NS or the LS diet (Figs 2A and 2B).

Effects of sodium restriction and chronic COX-2 inhibition on proteinuria, organ weights and urinary electrolyte excretion

As summarized in Table 1, MAP, proteinuria and index of HW/BW were significantly higher in TGR fed the NS diet compared with HanSD fed the NS diet. Treatment with NS-398 did not affect any of these parameters. LS diet markedly lowered proteinuria and the index of HW/BW to levels observed in HanSD. No significant differences in KW/BW were found in any of the groups. Urinary excretion of sodium and potassium reflected sodium intake and was not affected by NS-398 treatment (Table 1).

Discussion

The main finding of the present study concerns the fact that treatment with the LS diet prevents the development of hypertension in heterozygous TGR. This observation is in good agreement with our recent observation that treatment with LS diet substantially reduced BP in TGR almost to normotensive levels (Vaněčková *et al.* 2004). At present, conflicting data regarding the effect of sodium intake on the level of BP in TGR are available. In contrast to the results of several studies suggesting that TGR respond to sodium depletion by BP decrease and to salt loading by BP increase (Barrett and Mullins 1992, Callahan *et al.* 1996, Iyer *et al.* 2000, Opočenský *et al.* 2004), other authors reported no BP responses to high-salt intake in TGR (Chung *et al.* 1993, Li *et al.* 1998). These conflicting results might be explained by the use of animals of different age and sex

and also by employing different protocols of salt loading or salt restriction. However, it is important to note that in our study TGR were exposed to severe sodium restriction (<20 mmol Na^+/kg diet) immediately after weaning period. It has been shown previously that strict sodium restriction (17 or 9 mmol Na^+/kg diet) is associated with impaired growth and development of rats (Wilczynski and Leenen 1987, for review see Zicha and Kuneš 1999) and the relationship between body growth and BP has been well documented (Leenen and Toal 1989, Lever and Harrap 1992, Schork *et al.* 1994, for review see Zicha and Kuneš 1999). Since the treatment of TGR with the LS diet was associated with retardation of their body growth, this might be one of the underlying mechanism of the antihypertensive mechanisms of LS in this model. However, it should also be pointed out that in the present study we observed similar attenuation in body growth of HanSD rats without any change in BP.

Table 1. Mean arterial pressure, proteinuria, urinary sodium and potassium excretion and relative heart weight from individual experimental groups at the end of the experiment in Hannover Sprague-Dawley (HanSD) and in Ren-2 transgenic rats (TGR).

Group	n	MAP (mmHg)	Proteinuria (mg/24 h)	Sodium excretion (μmol/24 h)	Potassium excretion (μmol/24 h)	HW/BW (mg/g)
TGR + NS + vehicle	8	172 ± 5*	20.6 ± 1.1*	978 ± 38*	256 ± 23	4.24 ± 0.09*
TGR + NS + NS-398	8	170 ± 4*	20.3 ± 0.9*	943 ± 17*	227 ± 21	4.19 ± 0.06*
TGR + LS + vehicle	10	137 ± 4	7.9 ± 0.4	47 ± 9	589 ± 36*	3.81 ± 0.06
TGR + LS + NS-398	10	136 ± 4	7.6 ± 0.6	41 ± 7	544 ± 21*	3.79 ± 0.05
HanSD + NS + vehicle	7	123 ± 3	6.9 ± 0.4	1048 ± 32	212 ± 14	3.84 ± 0.07
HanSD + NS + NS-398	7	121 ± 3	6.4 ± 0.3	965 ± 41	240 ± 16	3.83 ± 0.07
HanSD + LS + vehicle	8	122 ± 3	6.3 ± 0.3	58 ± 11	592 ± 19*	3.69 ± 0.06
HanSD + LS + NS-398	7	119 ± 4	7.2 ± 0.4	42 ± 5	577 ± 13*	3.72 ± 0.07

NS, normal salt; LS, low sodium; HW, heart weight; BW, body weight; MAP, mean arterial pressure, * P<0.05 TGR vs. control HanSD rats.

The second aim of our study was to evaluate the effects of chronic COX-2 inhibition on BP during the developmental phase of hypertension in TGR. We found that the treatment with NS-398 did not alter the course of BP development in either TGR or HanSD, irrespective of sodium intake. The lack of BP responses to chronic COX-2 inhibition cannot be ascribed to insufficient COX-2 inhibition, because the dose of NS-398 employed in the present study is sufficient to substantially block COX-2 activity (Harding *et al.* 2000, Hartner *et al.* 2003).

Previous studies have explicitly showed that chronic COX-2 inhibition with a similar dose of the COX-2 inhibitor causes significant increases in BP both in normotensive Wistar-Kyoto (WKY) and in hypertensive rats (SHR). It indicates that COX-2-derived metabolites not only play a physiologically important role in normal BP regulation but also serve as important counterbalancing vasodilatory substances in the model of genetic hypertension (Höcherl *et al.* 2002, Muscará *et al.* 2000).

However, the effects of COX-2 inhibition on the BP in the ANG II-dependent model of hypertension remain controversial. It has been reported that COX-2 inhibition decreased BP in a model of 1K1C hypertension (Okumura *et al.* 2002), whereas no (Hartner *et al.* 2003) or significant BP reduction (Wang *et al.* 1999, Okumura *et al.* 2002) to COX-2 inhibitors was reported in two-kidney, one-clip (2K1C) hypertensive models. There is no satisfactory explanation for these controversial findings, but several possible explanations should be considered. First, the studies showing an antihypertensive effect of COX-2 blockade were performed 7 (Wang *et al.* 1999) or 13 days (Okumura *et al.* 2002) after clamping, whereas no effects of COX-2 inhibition on BP were reported in animals in which the experiment lasted four weeks (Hartner *et al.* 2003, for review see Krämer *et al.* 2004). Since plasma renin activity (PRA) is increased during the initial phase of renovascular hypertension, while in the maintenance phase PRA is normal (for review see Navar *et al.* 1998) and since COX-2-derived metabolites stimulate renin secretion (for review see Krämer *et al.* 2004), it is conceivable that COX-2

inhibition exhibits antihypertensive effects only in the states associated with high PRA. Second, the use of different COX-2 inhibitors should be taken into account. However, our previous results with two COX-2 inhibitors (namely DuP-697 and NS-398) revealed similar hemodynamic and excretory parameters together with substantial decrease of prostaglandin E_2 excretion induced by DuP-697 on both normal and low-salt diet (Vaněčková *et al.* 2004). Taken together, it seems reasonable to assume that differences in timing with respect to the course of hypertension may account for the divergent results obtained in above-mentioned studies.

Our observation that chronic COX-2 inhibition did not alter either the course of hypertension or the final blood pressure level in TGR is in good agreement with our recent study showing that despite high expression of renocortical COX-2 protein a selective intrarenal COX-2 inhibition did not influence renal function in TGR (Vaněčková *et al.* 2004). Moreover, Cheng *et al.* (2002) could not find any role for COX-2-derived prostaglandins in BP regulation of TGR with established hypertension. Therefore, these findings suggest that COX-2-derived metabolites do not act as vasodilatory counterregulatory agents in this model of hypertension.

In conclusion, our present study demonstrated that low salt intake prevented the development of hypertension in TGR, indicating that this model of hypertension exhibits an important salt-sensitive component during the developmental phase of hypertension. In addition, our results suggest that COX-2-derived prostaglandins do not particularly contribute to BP regulation in TGR.

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References

BARRETT GL, MULLINS JJ: Studies on blood pressure regulation in hypertensive ren-2 transgenic rats. *Kidney Int* **41** (Suppl 37): S125-S128, 1992.

BEIERWALTES WH: Cyclooxygenase-2 products compensate for inhibition of nitric oxide regulation of renal perfusion. *Am J Physiol* **283**: F68-F72, 2002.

BÖHM M, LEE MA, KREUTZ MA, KIM S, SCHNINKE M, DJAVIDANI B, WAGNER J, KALING M, WIENEN W, BADER M, GANTEN D: Angiotensin II receptor blockade in TGR(mRen2)27: effects on renin-angiotensin-system gene expression and cardiovascular functions. *J Hypertens* **13**: 891-899, 1995.

CALLAHAN MF, LI P, FERRARIO CM, GANTEN D, MORRIS M: Salt-sensitive hypertension in (mREN-2)27 transgenic rats. *Hypertension* **27**: 573-577, 1996.

CHENG HF, HARRIS RC: Cyclooxygenases, the kidney, and hypertension. *Hypertension* **43**: 1-6, 2004.

CHENG ZJ, TIKKANEN I, VAPAATALO H, MERVAALA EMA: Vascular effects of COX inhibition and AT_1 receptor blockade in transgenic rats harboring mouse renin-2 gene. *J Physiol Pharmacol* **53**: 597-613, 2002.

CHUNG O, SCHIPS T, ROHMEISS P, GRETZ N, STRAUCH M, UNGER T: Protein excretion and renal adaptation of transgenic mRen2 rats to changing oral sodium loads. *J Hypertens* **11** (Suppl 5): S188-S189, 1993.

GROSS V, LIPPOLDT A, SCHNEIDER W, LUFT FC: Effect of captopril and angiotensin II receptor blockade on pressure natriuresis in transgenic TGR(mRen2)27 rats. *Hypertension* **26**: 471-479, 1995.

HARDING P, CARRETERO OA, BEIERWALTES WH: Chronic cyclooxygenase-2 inhibition blunts low sodium-stimulated renin without changing renal haemodynamics. *J Hypertens* **18**: 1107-1113, 2000.

HARTNER A, GOPPELT-STRAUBE M, HILGERS KF: Coordinate expression of cyclooxygenase-2 and renin in the rat kidney in renovascular hypertension. *Hypertension* **31**: 201-205, 1998.

HARTNER A, CORDASIC N, GOPPELT-STRUEBE M, VEELKEN R, HILGERS KF: Role of macula densa cyclooxygenase-2 in renovascular hypertension. *Am J Physiol* **284**: F498-F502, 2003.

HELLER J, HELLEROVÁ S: Long-term effect on blood pressure of early brief treatment by different antihypertensive agents: a study in the Prague hypertensive rat. *Kidney Blood Press Res* **21**: 445-451, 1998.

HELLER J, HELLEROVÁ S, DOBEŠOVÁ Z, KUNEŠ J, ZICHA J: The Prague hypertensive rat: a new model of genetic hypertension. *Clin Exp Hypertens* **15**: 807-818, 1993.

HÖCHERL K, ENDEMANN D, KAMMERL MC, GROBECKER HF, KURTZ A: Cyclooxygenase-2 inhibition increases blood pressure in rats. *Br J Pharmacol* **136**: 1117-1126, 2002.

IMIG JD: Eicosanoid regulation of the renal vasculature. *Am J Physiol* **279**: F965-F981, 2000

IYER SN, AVERILL DB, CHAPPELL MC, YAMADA K, ALLRED AJ, FERRARIO CM: Contribution of angiotensin-(1-7) to blood pressure regulation in salt-depleted hypertensive rats. *Hypertension* **36**: 417-422, 2000.

JACINTO SM, MULLINS JJ, MITCHELL KD: Enhanced renal vascular responsiveness to angiotensin II in hypertensive ren-2 transgenic rats. *Am J Physiol* **276**: F315-F322, 1999.

JENSEN BL, KURTZ A: Differential regulation of renal cyclooxygenase mRNA by dietary salt intake. *Kidney Int* **52**: 1242-1249, 1997.

KOMERS R, EPSTEIN M: Cyclooxygenase-2 expression and function in renal pathophysiology. *J Hypertens* **20 (**Suppl 6): S11-S15, 2002.

KOPKAN L, KRAMER HJ, HUŠKOVÁ Z, VAŇOURKOVÁ Z, BÄCKER A, BADER M, GANTEN D, ČERVENKA L: Plasma and kidney angiotensin II levels and renal functional responses to AT_1 receptor blockade in hypertensive Ren-2 transgenic rats. *J Hypertens* **22***:* 819-825, 2004.

KRÄMER BK, KAMMERL MC, KÖMHOFF M: Renal cyclooxygenase-2 (COX-2). Physiological, pathophysiologicial, and clinical implications. *Kidney Blood Press Res* **27**: 43-62, 2004.

LANGHEINRICH M, LEE MA, BÖHM M, PINTO YM, GANTEN D, PAUL M: The hypertensive Ren-2 transgenic rat TGR(mRen2)27 in hypertension research. Characteristics and functional aspects. *Am J Hypertens* **9**: 506-512, 1996.

LEENEN HH, TOAL CB: Dietary sodium restriction and the renin-angiotensin system in young spontaneously hypertensive rats, *J Hypertens* **7**: 57-61, 1989.

LEVER AF, HARRAP SB: Essential hypertension: a disorder of growth with origins in childhood? *J Hypertens* **10**: 101-120, 1992.

LI P, MORRIS M, FERRARIO CM, BARRETT C, GANTEN D, CALLAHAN MF: Cardiovascular, endocrine, and body fluid-electrolyte responses to salt loading in mRen-2 transgenic rats. *Am J Physiol* **275**: H1130-H1137, 1998.

LÓPEZ R, LLINÁS MT, ROIG F, SALAZAR FJ: Role of nitric oxide and cyclooxygenase-2 in regulating the renal hemodynamic response to norepinephrine. *Am J Physiol* **284**: R488-R-493, 2003a.

LÓPEZ R, ROIG F, LLINÁS MT, SALAZAR FJ: Role of cyclooxygenase-2 in the control of renal haemodynamics and excretory function. *Acta Physiol Scand* **177**: 429-435, 2003b.

MANN B, HARTNER A, JENSEN BL, HILGERS KF, HÖCHERL K, KRÄMER BK, KURTZ A: Acute upregulation of COX-2 by renal artery stenosis. *Am J Physiol* **280**: F119-F125, 2001.

MITCHELL KD, MULLINS JJ: ANG II dependence of tubuloglomerular feedback responsiveness in hypertensive ren-2 transgenic rats. *Am J Physiol* **268**: F821-F828, 1995.

MITCHELL KD, JACINTO SM, MULLINS JJ: Proximal tubular fluid, kidney, and plasma levels of angiotensin II in hypertensive ren-2 transgenic rats. *Am J Physiol* **273**: F246-F253, 1997.

MULLINS JJ, PETERS J, GANTEN D: Fulminant hypertension in transgenic rats harboring the mouse Ren-2 gene. *Nature* **344**: 541-544, 1990.

MUSCARÁ MN, VERGNOLLE N, LOVREN F, TRIGGLE CR, ELLIOTT SN, ASFAHA S, WALLACE JL: Selective cyclooxygenase-2 inhibition with celecoxib elevates blood pressure and promotes leukocyte adherence. *Br J Pharmacol* **129**: 1423-1430, 2000.

NAVAR LG, ZOU L, VON THUN A, WANG T, IMIG JD, MITHCELL K: Unraveling the mystery of Goldblatt hypertension. *News Physiol Sci* **13**: 170-176, 1998.

OKUMURA T, HAYASHI I, IKEZAWA T, YAMANAKA M, TAKATA T, FUJITA Y, SAIGENJI K, YAMASHINA S, MAJIMA M: Cyclooxygenase-2 inhibitors attenuate increased blood pressure in renovascular hypertensive models, but not in deoxycorticosterone-salt hypertension. *Hypertens Res* **25**: 927-938, 2002.

OPOČENSKÝ M, DVOŘÁK P, MALÝ J, KRAMER HJ, BÄCKER A, KOPKAN L, VERNEROVÁ Z, TESAŘ V, ZIMA T, BADER M, GANTEN D, JANDA J, VANĚČKOVÁ I: Chronic endothelin receptor blockade reduces end-organ damage independently of blood pressure effects in salt-loaded heterozygous Ren-2 transgenic rats. *Physiol Res* **53**: 581-593, 2004.

PURDY KE, ARENDSHORST WJ: Prostaglandins buffer ANG II-mediated increases in cytosolic calcium in preglomerular VSMC. *Am J Physiol* **277**: F850-F858, 1999.

RONG P, CAMPBELL DJ, SKINNER SL: Hypertension in the (mRen2)27 rat is not explained by enhanced kinetics of transgenic Ren-2 renin. *Hypertension* **42**: 523-527, 2003.

SCHORK NJ, JOKELAINEN P, GRANT EJ, SCHORK MA, WEDER AB: Relationship of growth and blood pressure in inbred rats. *Am J Physiol* **266**: R702-R708, 1994.

VANĚČKOVÁ I, CAHOVÁ M, KRAMER HJ, HUSKOVÁ Z, ŠKAROUPKOVÁ P, KOMERS R, BADER M, GANTEN D, ČERVENKA L: Acute effects of cyclooxygenase-2 inhibition on renal function in heterozygous transgenic rats on normal or low sodium intake. *Kidney Blood Press Res* **27**: 203-210, 2004.

WILCZYNSKI EA, LEENEN HH: Dietary sodium intake and age in spontaneously hypertensive rats: effects on blood pressure and sympathetic activity. *Life Sci* **41**: 707-15, 1987.

WANG JL, CHENG HF, SHAPPELL S, HARRIS RC: Cyclooxygenase-2 inhibition decreases renin content and lowers blood pressure in a model of renovascular hypertension. *Hypertension* **34**: 96-101, 1999.

YANG T, SINGH I, PHAM H, SUN D, SMART A, SCHNERMANN JB, BRIGGS JP: Regulation of cyclooxygenase expression in the kidney by dietary salt intake, *Am J Physiol* **274**: F481-489, 1998.

ZICHA J, KUNEŠ J: Ontogenetic aspects of hypertension development: analysis in the rat. *Physiol Rev* **79**: 1227-1282, 1999.

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