

Cyclo-oxygenase-2 inhibition increases blood pressure in rats

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1 It is known that nonselective cyclo-oxygenase (COX) inhibitors have small but significant effects on blood pressure (BP), most notably in hypertensive patients on antihypertensive medication. Whether selective COX-2 inhibitors also interfere with BP regulation is not well understood. Therefore, we aimed to examine the effect of chronic treatment with a selective COX-2 inhibitor (rofecoxib) on systolic blood pressure (sBP) in normotensive Wistar-Kyoto rats (WKY) and on the developmental changes of sBP in young spontaneously hypertensive rats (SHR). In addition, we investigated a possible influence of salt intake on the effects of COX-2 inhibition on BP in these two rat strains.

2 Rofecoxib dose dependently increased sBP and decreased plasma levels of 6-keto prostaglandin (PG)_{F_{1α}} in WKY rats fed a normal salt diet (0.6% NaCl, wt wt⁻¹), without affecting serum thromboxane (TX)_{B₂} levels.

3 Rofecoxib significantly elevated sBP in both rat strains fed normal salt or high salt diet (8% NaCl, wt wt⁻¹), but not in rats on low salt intake (0.02% NaCl, wt wt⁻¹).

4 Rofecoxib significantly decreased plasma levels of 6-keto PGF_{1α} in both rat strains fed normal or high salt diet, but not in rats during low salt intake.

5 Rofecoxib exerted no influence on the changes of body weight nor on water intake. Plasma renin activity (PRA) and renocortical renin mRNA abundance were not changed by rofecoxib, but plasma aldosterone concentration (PAC) was significantly reduced.

6 These results suggest that chronic inhibition of COX-2 causes an increase of blood pressure that depends on prostacyclin synthesis. Furthermore, this increase is independent on genetic predisposition and can be prevented by salt deprivation. Since water intake and body weight gain were not changed by rofecoxib, fluid retention appears not to be a major reason for the development of hypertension. Similarly, an activation of the renin-angiotensin-aldosterone axis appears to be an unlikely candidate mechanism.

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Abbreviations: ANG, angiotensin; BP, blood pressure; COX, cyclo-oxygenase; NSAID, non steroidal anti-inflammatory drug; PAC, plasma aldosterone concentration; PG, prostaglandin; PRA, plasma renin activity; RAAS, renin-angiotensin aldosterone system; sBP, systolic blood pressure; SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto rat

Introduction

Prostaglandins (PGs) are of relevance in physiological responses, inflammation and thrombosis (Narumiya *et al.*, 1999; Breyer & Breyer, 2000). They are formed from arachidonic acid by the catalytic activity of cyclo-oxygenases (COX) *via* the unstable endoperoxide PGH₂. Isomerization or reduction by cell-specific isomerases or reductases leads to the formation of the prostaglandins PGE₂, PGD₂, prostacyclin (PGI₂) and thromboxane (Tx) A₂ (Smith *et al.*, 1991). Cyclo-oxygenases exist at least in two isoforms (Vane *et al.*, 1998). COX-1 is constitutively expressed in all tissues, whereas COX-2 was thought to be an inducible isoform (Feng *et al.*, 1993), but recently found to be also constitutively expressed in some tissues such as the kidney, albeit to a much smaller extent as COX-1 (Feng *et al.*, 1993;

Harris *et al.*, 1994; Yang *et al.*, 1999). Prostacyclin (PGI₂) is known to be a potent vasodilator and an inhibitor of platelet aggregation. PGI₂ is produced mainly by the vascular endothelium (Bunting *et al.*, 1983) and its potent vasorelaxant properties contribute to the maintenance of normal vascular tone. Moreover, PGI₂ stimulates the release of renin from the kidney (Imig, 2000; Harris & Breyer, 2001). Thromboxane (Tx) A₂ is the major product of platelet COX-1 activity (Funk *et al.*, 1991), and lowering of serum TxB₂, its inactive hydrolysis product, reflects inhibition of this isoform. Furthermore, TxA₂ is known to be a potent vasoconstrictor and plays therefore also an important role in the regulation of vascular haemostasis (Bunting *et al.*, 1983). The balance between the formation of PGI₂ and of TxA₂ is thought to play also a role in the regulation of platelet aggregation and in vascular tone. Inhibition of prostaglandin synthesis is suggested to be responsible for both the therapeutic and adverse effects associated with the adminis-

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tration of conventional nonsteroidal anti-inflammatory drugs (NSAIDs) (Vane & Botting, 1995). Several studies in humans have demonstrated that NSAIDs attenuate the antihypertensive effect by different antihypertensive drugs, but the pathogenic mechanisms underlying being poorly understood (Chrysant *et al.*, 1980; Clive & Stoff, 1984; Patrono & Dunn, 1987; Muscara *et al.*, 1998). Since the cardiovascular, gastrointestinal and other side effects of nonselective NSAIDs have been mainly attributed to COX-1 inhibition, selective COX-2 inhibitors have been developed with the hope to avoid major adverse cardiovascular effects of NSAIDs. There is also emerging evidence for a physiological role of COX-2. Therefore, the possibility arises that selective COX-2 inhibition will result in adverse effects. In humans, it has been demonstrated that the selective COX-2 inhibitors celecoxib and rofecoxib markedly suppressed prostacyclin synthesis in healthy volunteers (McAdam *et al.*, 1999; Catella-Lawson *et al.*, 1999). Furthermore, it has been described that selective COX-2 inhibitors lower PGE₂ concentrations in the rat kidney cortex (Höcherl *et al.*, 2001) and decrease renal excretion of PGs as well (Kammerl *et al.*, 2001). Moreover, COX-2 inhibitors have been reported to decrease renal blood flow and to induce sodium retention (Rodriguez *et al.*, 2000; Rossat *et al.*, 1999). Whether and how selective COX-2 inhibitors interfere with BP regulation is yet not clear. In elderly humans, it has been reported that COX-2 inhibitors either do not change BP (Rossat *et al.*, 1999; Catella-Lawson *et al.*, 1999) or increase BP in a yet undefined subpopulation of patients suffering from osteoarthritis (Whelton *et al.*, 2001). In Wistar-Kyoto (WKY) rats, prolonged treatment with a COX-2 inhibitor increased BP (Muscara *et al.*, 2000), whilst in Sprague Dawley rats with renovascular hypertension COX-2 inhibition lowered BP (Wang *et al.*, 1999). Because of this diffuse situation we systematically investigated the effect of selective COX-2 inhibition on sBP in rats. In particular, we were further interested to learn more about the effects of COX-2 inhibition on BP in SHR during development of a genetically fixed hypertension, a period where SHR show a salt sensitivity for blood pressure regulation (Zicha & Kunes, 1999). For this purpose we subjected normotensive WKY rats and spontaneously hypertensive rats (SHRs) to different salt diets and treated them with the selective COX-2 inhibitor rofecoxib.

Methods

Selection of dose

To determine a dose of rofecoxib for further *in vivo* studies, which selectively inhibits COX-2 isoform, the air pouch model was performed. In brief, an air pouch was induced as described previously (Muscara *et al.*, 1998). Carrageenan (2 ml of a 1% wt vol⁻¹ solution in sterile isotonic saline; Sigma, Germany) was injected into the air pouch. Six hours later, the pouch was opened during sevoflurane anaesthesia (3% vol vol⁻¹) and the exudate was collected for PGE₂ measurement as an index for COX-2 activity using a commercially available EIA (Cayman Chemicals, Ann Arbor, U.S.A.). Blood was collected by cardiac puncture and incubated at 37°C for 60 min for measurement of whole

blood thromboxane synthesis (Cayman Chemicals, Ann Arbor, U.S.A.) as an index for COX-1 activity. After decapitation, the stomach was removed, cleaned and frozen in liquid nitrogen until determination of PGE₂. Rats were pre-treated orally with saline or rofecoxib with increasing doses (1, 3, 10 and 30 mg kg⁻¹ d⁻¹) 30 min before injection of carrageenan. A further control group received an injection of 2 ml saline and saline orally.

Animal treatment and blood pressure measurement

Male WKY and SHRs were obtained from Charles River (Sulzfeld, Germany) at an age of 21 ± 3 days. To determine dose dependent effects of rofecoxib on blood pressure, groups of eight rats each were orally treated each day with rofecoxib (1, 3, 10 or 30 mg kg⁻¹) or vehicle and received a normal salt diet (0.06% NaCl wt wt⁻¹) for 4 weeks. Rats were conscious during gavaging of the drug or vehicle. The volume of vehicle- or drug solution was about 0.2 ml at the beginning of the study and increased to about 1.0 ml at the end of the study.

For subsequent studies groups of eight rats each were treated by gavage with rofecoxib (10 mg kg⁻¹ d⁻¹) or vehicle and placed for 4 weeks on either a low salt diet (0.02% NaCl, wt wt⁻¹), a normal salt diet (0.6% NaCl, wt wt⁻¹) or a high salt diet (8% NaCl, wt wt⁻¹).

Body weight, water and food intake was determined daily before drug administration. Systolic blood pressure and heart rate measurements were performed (tail cuff method) prior to treatment and at 1-week intervals for a total of 4 weeks. Measurements were always performed 18–24 h after administration of rofecoxib or vehicle. At the end of the study period, the final doses were given 2 h prior to decapitation of rats during sevoflurane anaesthesia (3%, vol. vol⁻¹). Plasma and kidney cortex slices were frozen in liquid nitrogen and stored at –80°C until assay.

Determination of plasma renin activity (PRA), plasma aldosterone concentration (PAC) and plasma 6-keto PGF_{1α} levels

PRA and PAC were determined using commercially available radioimmunoassays (Sorin Biomedical, Düsseldorf, Germany). The reliable detection limit for the PAC RIA was approximately 8.0 pg. The lowest value for the standard curve was 6.25 pg. Concentrations of plasma 6-keto PGF_{1α} were determined by using an EIA kit (Cayman Chemical, Ann Arbor, U.S.A.).

Ribonuclease protection assay for renocortical renin mRNA and β-actin mRNA abundance

Renocortical renin mRNA and β-actin mRNA levels were measured by specific RNase protection assay basically as described previously (Mann *et al.*, 2001). In brief, 40 μg of total renocortical mRNA were used for the determination of renin mRNA and 1 μg for β-actin mRNA.

Determination of plasma potassium levels

Plasma potassium levels were determined in triplicate using Reflotron[®] (Boehringer Mannheim, Mannheim, Germany).

Statistical analysis

Level of significance was calculated by one-way ANOVA followed by student's *t*-test. A *P* value <0.05 was considered significant.

Results

Selection of dose of rofecoxib

Rofecoxib did not significantly affect COX-1 activity, as measured by whole blood thromboxane synthesis, at any dose tested (1–30 mg kg⁻¹) (Figure 1). Furthermore, rofecoxib, at any dose tested, exerted no influence on gastric PGE₂ concentration (Figure 1). However, rofecoxib dose-dependently inhibited inflammatory COX-2 activity. Thus, 1 mg kg⁻¹ reduced PGE₂ concentration in the pouch exudates to about 60% and 30 mg kg⁻¹ to about 25% compared to vehicle treated rats (Figure 1).

Dose-dependency of rofecoxib on systolic blood pressure in WKY rats during normal salt intake

In vehicle treated WKY rats sBP developmentally increased from 109 ± 3 mmHg at an age of 3 weeks to about 123 ± 2 mmHg at an age of 7 weeks. Daily oral treatment of rofecoxib at a dose of 10 or 30 mg kg⁻¹ d⁻¹ resulted in a significant increase in systolic blood pressure in normotensive WKY rats after 1 week as compared to vehicle treated rats. Rofecoxib at a dose of 3 mg kg⁻¹ d⁻¹ increased blood pressure after 2 weeks, whereas 1 mg kg⁻¹ d⁻¹ of rofecoxib increased systolic blood pressure after 3 weeks of the beginning with the treatment (Figure 2) as compared to

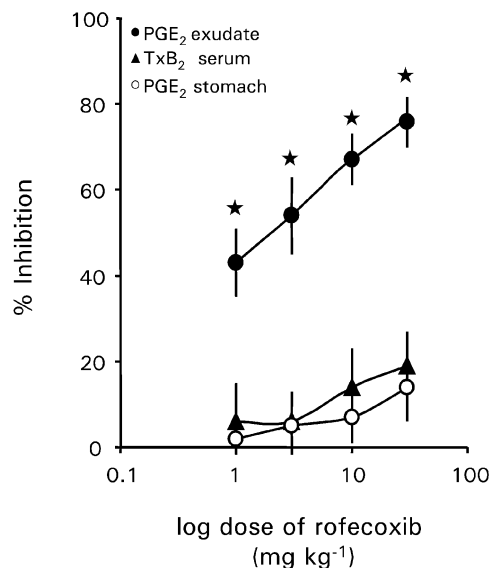


Figure 1 Effect of increasing doses of rofecoxib (0, 1, 3, 10 and 30 mg kg⁻¹ d⁻¹) on the release of PGE₂ into inflammatory exudate, on whole blood TxB₂ synthesis and gastric PGE₂. Rats were pre-treated with rofecoxib 30 min before injection of carrageenan (2 ml of a 1% wt vol⁻¹ solution in sterile saline) into the air-pouch. Data represent the mean ± s.e. mean of *n* = 4–5 rats. Asterisk indicates significance of control rats to rofecoxib treated rats.

vehicle treated rats. At the end of the treatment the mean sBP of rofecoxib treated rats was 15, 17, 21 and 23 mmHg higher than vehicle treated rats, respectively (Figure 3a).

Daily treatment with rofecoxib for 4 weeks had no significant effect on serum thromboxane B₂ levels. However, rofecoxib dose-dependently decreased plasma 6-keto PGF_{1α} levels. Thus, 30 mg kg⁻¹ d⁻¹ of rofecoxib reduced plasma levels of 6-keto PGF_{1α} to about 45% compared with vehicle treated rats (Figure 3c).

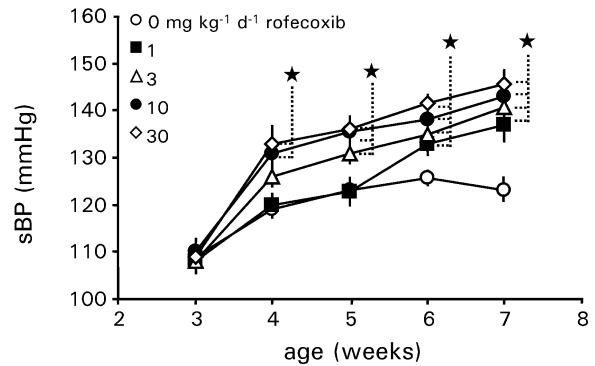


Figure 2 Development of systolic blood pressure of WKY rats treated with vehicle or rofecoxib at increasing doses (1, 3, 10 and 30 mg kg⁻¹ d⁻¹) for 4 weeks during normal salt intake (0.6%, wt wt⁻¹). Data represent the mean ± s.e. mean of *n* = 8 rats. Asterisk indicates significance of control rats to rofecoxib treated rats.

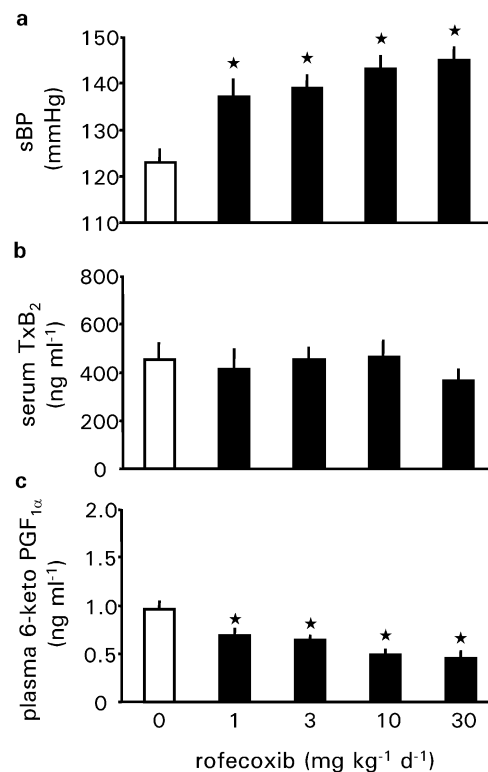


Figure 3 Systolic blood pressure, serum thromboxane B₂ concentration and plasma 6-keto prostaglandin F_{1α} concentration of WKY rats treated with vehicle or rofecoxib at increasing doses (1, 3, 10 and 30 mg kg⁻¹ d⁻¹) for 4 weeks during normal salt intake (0.6%, wt wt⁻¹). Data represent the mean ± s.e. mean of *n* = 8 rats. Asterisk indicates significance of control rats to rofecoxib treated rats.

Daily treatment with rofecoxib for 4 weeks had no significant effect on body weight, heart rate, plasma renin activity, renocortical renin mRNA abundance and plasma potassium levels (Table 1). Furthermore, rofecoxib did not affect the hematocrit (Table 1). However, rofecoxib treatment for 4 weeks dose-dependently decreased plasma aldosterone concentration to about 50% of control levels at a dose of 30 mg kg⁻¹ d⁻¹ (Table 1).

Since treatment with rofecoxib at a dose of 10 mg kg⁻¹ d⁻¹ resulted in similar results as a dose of 30 mg kg⁻¹ d⁻¹, subsequent experiments in spontaneously hypertensive rats compared to normotensive Wistar-Kyoto rats during low, normal and high salt intake for 4 weeks were performed by treating rats with rofecoxib with a dose of 10 mg kg⁻¹ d⁻¹.

Influence of rofecoxib on blood pressure and heart rate during salt intake

In vehicle treated WKY rats sBP developmentally increased from 110±4 mmHg at an age of 3 weeks to 124±4 mmHg at 7 weeks of age during normal salt intake. In WKY rats, different salt intake for 4 weeks did not alter sBP. The mean sBP of WKY rats was 119±5 mmHg during low salt intake and 131±4 mmHg during high salt intake. Administration of rofecoxib resulted in a significant increase in sBP during normal salt and high salt intake, whereas sBP was not influenced by rofecoxib during low salt intake. At an age of 7 weeks, the mean difference in sBP after a 4-week treatment was 23 mmHg during normal salt or 18 mmHg during high salt intake (Figure 4a–c) compared to vehicle treated rats, respectively.

Systolic BP was similar between SHR and WKY at 3 weeks of age. Blood pressure of SHR on normal salt intake increased gradually from age 3 weeks and was significantly higher at ages 4, 5, 6, and 7 weeks compared to those of WKY rats during normal salt intake.

SHRs showed a salt dependent time pattern for the development of hypertension. During normal salt diet sBP increased from 112±3 mmHg at an age of 3 weeks to 166±4 mmHg at an age of 7 weeks. During low salt intake sBP increased to 152±4 mmHg only ($P < 0.05$ vs normal salt intake) and to 180±4 mmHg during high salt intake ($P < 0.05$ vs normal salt intake). Rofecoxib treatment further increased sBP in SHRs fed normal salt diet into the range of 193±4 mmHg and in SHRs fed a high salt diet into the range of 205±5 mmHg, respectively. As for WKY rats,

rofecoxib exerted no influence on sBP in SHRs during low salt intake (Figure 4d–f).

Heart rate was not altered either by any salt diet or by rofecoxib treatment in WKY rats.

In SHRs, heart rate was significantly higher compared to WKY rats at any given salt diet. High salt intake further enhanced heart rate in SHRs compared to normal salt intake. Rofecoxib treatment did not change heart rate on any salt diet (Table 2).

Influence of rofecoxib on body weight, water intake and hematocrit during salt intake

To detect a possible effect on fluid retention induced by rofecoxib, we measured body weight gain as an indirect marker. Treatment with rofecoxib did not change age dependent body weight gain compared to vehicle-treated rats in any rat strain or for any salt diet (Table 2).

For WKY rats, daily water intake normalised to body weight was dependent on the salt content of the diet. During a normal salt intake, the ratio of water intake per day to body weight decreased slightly with increasing age. Low salt diet significantly reduced the values and a high salt diet enhanced it (Figure 5). Also at low or high salt intake the decline of water intake during development was observed. Treatment with rofecoxib did not alter water intake at any salt diet.

SHRs behaved very similar to WKY rats with regard to the water intake at any salt diet. Also in these animals, rofecoxib exerted no influence on the water intake at any salt diet (Figure 5). The hematocrit of WKY rats and SHRs was not dependent on salt intake or on rofecoxib treatment (Table 3).

Influence of rofecoxib treatment on plasma renin activity, plasma aldosterone concentration and renocortical renin mRNA during salt intake

The renin-angiotensin-aldosterone system (RAAS) plays an important role for the regulation of blood pressure and also for salt- and water-homeostasis. Its activity depends primarily on the activity of plasma renin. Therefore, we determined PRA which was found to be inversely related to the salt intake. Low salt diet increased PRA about 2.0 fold and high salt diet decreased PRA 2.5 fold compared with a normal salt intake in WKY-rats (Table 3). Similar data were obtained for SHRs. Treatment with the COX-2 inhibitor rofecoxib

Table 1 Body weight (g), heart rate (beats per minute), hematocrit (%), PRA (ng ANG I h⁻¹ ml⁻¹), PAC (pg ml⁻¹), renocortical renin mRNA abundance ((c.p.m. c.p.m.⁻¹) × 1000) and plasma potassium levels (mmol·l⁻¹) in 7 week old rats following a 4 week treatment period with rofecoxib at increasing doses (1, 3, 10 or 30 mg kg⁻¹ d⁻¹) or with vehicle (0)

Rofecoxib	0	1	3	10	30
Body weight	178.8±4.6	184.6±6.8	176.5±2.9	179.4±3.3	173.7±3.7
Heart rate	384±8	390±8	378±9	387±7	395±9
Hematocrit	37.8±0.3	36.8±1.7	34.2±2.4	35.4±2.0	35.2±1.6
PRA	9.23±1.20	11.13±0.92	11.29±1.11	9.39±1.19	10.25±2.47
PAC	74.6±8.3	51.1±4.6*	47.9±4.7*	43.4±9.4*	35.3±8.6*
Plasma potassium	5.97±0.15	6.09±0.23	6.08±0.17	6.16±0.16	5.59±0.21
Renin mRNA/β-actin mRNA	25.8±4.2	27.9±3.9	26.7±4.3	24.2±4.1	26.8±4.2

Data are means±s.e.m. of n=8 rats. * $P < 0.05$ vs vehicle treated rats.

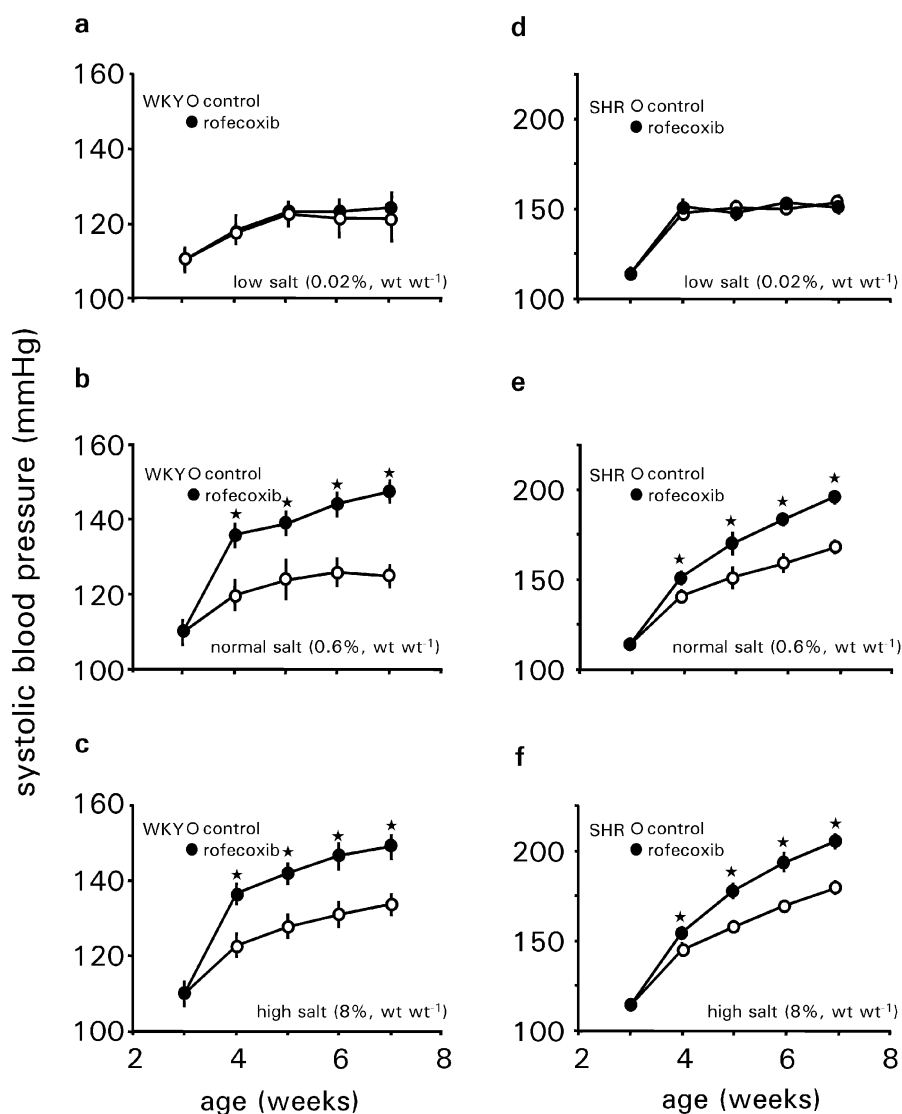


Figure 4 Development of systolic blood pressure of WKY rats (a–c) and SHRs (d–f) treated with vehicle or rofecoxib ($10 \text{ mg kg}^{-1} \text{ d}^{-1}$) for 4 weeks during low salt intake (0.02% , wt wt^{-1}) (a, d), normal salt intake (0.6% , wt wt^{-1}) (b, e) or high salt intake (8% , wt wt^{-1}) (c, f). Data represent the mean \pm s.e. mean of $n=8$ rats. Asterisk indicates significance of control rats to rofecoxib treated rats.

Table 2 Heart rate (beats per minute), hematocrit (%) and body weight (g) in 7 week old rats following a 4 week treatment period with rofecoxib or with vehicle; salt content of diet (% NaCl, wt wt^{-1})

Rat strain	Salt content of diet	Rofecoxib	HR	Hematocrit	Body weight
WKY	0.02	–	375 ± 8	39.2 ± 1.4	175.7 ± 6.8
	0.02	+	371 ± 14	37.8 ± 1.5	172.3 ± 4.4
	0.6	–	375 ± 12	38.5 ± 0.9	178.2 ± 3.7
	0.6	+	371 ± 10	37.7 ± 1.8	174.5 ± 5.3
	8	–	383 ± 10	36.2 ± 1.2	171.0 ± 4.4
	8	+	387 ± 11	36.6 ± 1.3	179.8 ± 3.3
SHR	0.02	–	$406 \pm 9^\dagger$	39.0 ± 0.9	$147.3 \pm 5.2^\dagger$
	0.02	+	408 ± 11	37.8 ± 0.8	147.8 ± 4.0
	0.6	–	$409 \pm 12^\dagger$	38.2 ± 0.7	$155.7 \pm 4.9^\dagger$
	0.6	+	413 ± 13	35.3 ± 1.5	149.3 ± 5.8
	8	–	$461 \pm 14^\dagger^*$	37.1 ± 1.3	$147.2 \pm 3.8^\dagger$
	8	+	465 ± 12	35.6 ± 1.1	147.5 ± 3.4

Data are means \pm s.e.m. of $n=8$ rats. * $P < 0.05$ vs SHR during a normal salt intake; $^\dagger P < 0.05$ vs WKY during the respective salt intake; WKY, Wistar Kyoto rat; SHR, spontaneously hypertensive rat; –, control rats; +, rofecoxib treated rats.

(10 mg kg⁻¹ d⁻¹) did not influence PRA at any salt diet and in any strain (Table 3).

In the same way as PRA, renin mRNA abundance changed inversely with the salt content of the diet. Thus in WKY rats, a low salt diet increased renin mRNA abundance about 2 fold and a high salt diet decreased renin mRNA abundance about 2.5 fold compared with a normal salt diet (Table 3). SHR rats behaved very similar to WKY rats, with the exception, that SHR rats had significantly lower renin mRNA

levels under all diets ($P < 0.05$). Rofecoxib treatment did not influence renocortical renin mRNA (Table 3).

Furthermore, we measured PAC, another important component of the RAAS. PAC was also inversely correlated to the salt intake. In WKY rats, low salt diet increased PAC about 12 fold and a high salt diet decreased PAC about 2.0 fold compared with animals during normal salt intake (Table 3). SHR rats showed a similar behaviour of PAC in response to the salt diet as WKY rats.

Treatment with rofecoxib reduced PAC to about the half of the values at any given diet and in both rat strains (Table 3).

Influence of rofecoxib treatment on plasma levels of 6-keto PGF_{1α} during salt intake

Plasma levels of 6-keto PGF_{1α} changed in both rat strains with the salt intake. SHR rats had increased levels during normal and high salt intake as compared to WKY rats. Additional treatment with rofecoxib did not affect plasma levels of 6-keto PGF_{1α} in both rat strains during low salt intake. However, during normal and high salt intake rofecoxib treatment decreased plasma levels of 6-keto PGF_{1α} in both rat strains to about the levels found for low salt intake (Figure 6).

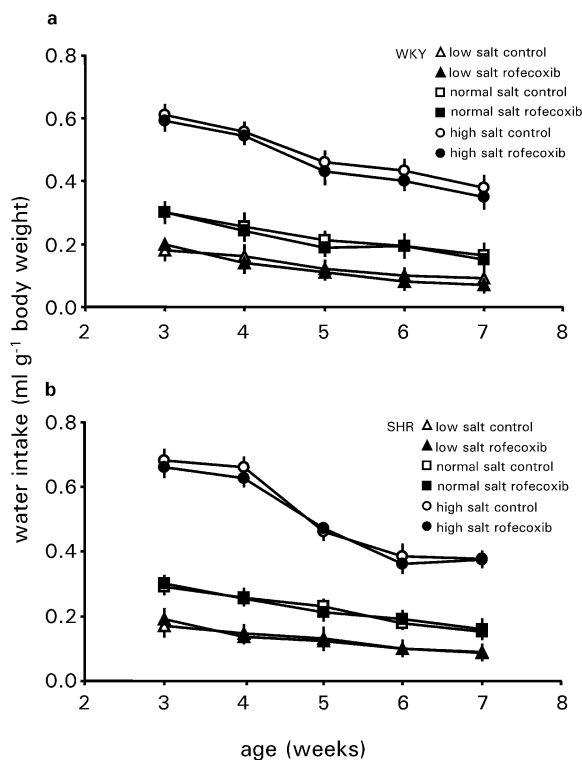


Figure 5 Development of water intake of WKY rats and SHR rats treated with vehicle or rofecoxib (10 mg kg⁻¹ d⁻¹) for 4 weeks during low salt intake (0.02%, wt wt⁻¹), normal salt intake (0.6%, wt wt⁻¹) or high salt intake (8%, wt wt⁻¹). Data represent the mean \pm s.e. mean of $n = 8$ rats.

Discussion

Our study aimed to investigate the influence of a prolonged inhibition of COX-2 on the salt dependent developmental regulation of sBP in young SHR rats compared with normotensive WKY rats, as genetic control.

In line with previous findings we found an increase in sBP in both rat strains during development (for review see Zicha & Kunes, 1999). This increase was more pronounced in SHR resulting in significant higher levels than at an age of 4 weeks. Furthermore, the increase of sBP in SHR is attenuated by sodium deficient diet and enhanced by high sodium diet, whereas different salt intake has no significant effect on sBP in WKY rats (for review see Zicha & Kunes, 1999). Confirming previous observations, heart rate of SHR rats was

Table 3 PRA (ng ANG I h⁻¹ ml⁻¹), PAC (pg ml⁻¹) and renocortical renin mRNA abundance ((c.p.m. c.p.m.⁻¹) \times 1000), after a treatment period of 4 weeks \pm rofecoxib at an age of 7 weeks; salt content of diet (% NaCl, wt wt⁻¹)

Rat strain	Salt content of diet	Rofecoxib	PRA	PAC	Renin mRNA/ β -actin mRNA
WKY	0.02	-	14.4 \pm 2.0 [†]	776 \pm 40 [†]	44.9 \pm 4.3 [†]
	0.02	+	13.0 \pm 1.5	484 \pm 3.0*	43.8 \pm 3.6
	0.6	-	8.2 \pm 2.1	64 \pm 15	26.1 \pm 3.7
	0.6	+	6.5 \pm 1.6	20 \pm 12*	24.3 \pm 3.6
	8	-	1.7 \pm 0.8 [†]	28 \pm 8 [†]	10.2 \pm 2.4 [†]
	8	+	1.8 \pm 0.7	10 \pm 7*	8.1 \pm 3.0
SHR	0.02	-	11.3 \pm 2.2 [†]	773 \pm 31 [†]	13.8 \pm 3.4 [†] ‡
	0.02	+	11.5 \pm 1.6	475 \pm 33*	14.0 \pm 3.3
	0.6	-	7.2 \pm 1.4	122 \pm 17	6.4 \pm 2.2 [‡]
	0.6	+	6.1 \pm 1.3	22 \pm 18*	6.1 \pm 2.0
	8	-	2.5 \pm 1.3 [†]	58 \pm 6 [†]	2.0 \pm 1.2 [†] ‡
	8	+	2.1 \pm 0.9	19 \pm 6*	1.9 \pm 1.3

Data are means \pm s.e.m. of $n = 8$ rats. * $P < 0.05$ vs vehicle treated rats during the normal salt intake; [†] $P < 0.05$ vs normal salt intake; [‡] $P < 0.05$ vs WKY, during the respective salt intake HR, heart rate; b.p.m., beats per minute; WKY Wistar Kyoto rat; SHR, spontaneously hypertensive rat; -, control rats; +, rofecoxib treated rats.

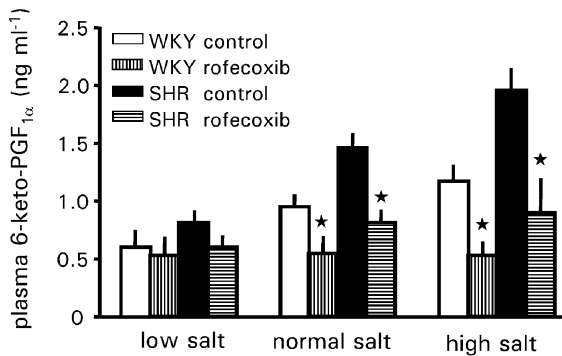


Figure 6 Plasma 6-keto PGF_{1α} levels of WKY-rats and SHRs treated with vehicle or rofecoxib (10 mg kg⁻¹ d⁻¹) for four weeks during low salt intake (0.02%, wt wt⁻¹), normal salt intake (0.6%, wt wt⁻¹) or high salt intake (8%, wt wt⁻¹). Data represent the mean ± s.e.mean of *n* = 8 rats. Asterisk indicates significance of control rats to rofecoxib treated rats.

increased during development at any time when compared to WKY (Dickhout & Lee, 1998). A chronic high salt diet further enhanced heart rate in young SHRs but not in WKY, suggesting enhanced activity of the sympatho-adrenal system in SHRs (Gradin *et al.*, 1986).

Our data now show that COX-2 inhibition for 4 weeks dose dependently increased sBP in WKY rats during normal salt intake, whereas plasma 6-keto PGF_{1α} concentration, a stable metabolite of prostacyclin, decreased in parallel. A low dose of rofecoxib, comparable to the recommended dose of rofecoxib for treatment of human inflammatory disease did increase sBP only after 3 weeks of treatment. This finding would be compatible with recent data obtained with the COX-2 inhibitor NS-398 using a dose of 1 mg kg⁻¹ d⁻¹, where no increase in sBP was found after acute or during a 2-weeks treatment (Harding *et al.*, 1997, 2000) and with nimesulide (Llinas *et al.*, 2000; Rodriguez *et al.*, 2000; Roig *et al.*, 2001), a less selective COX-2 inhibitor than rofecoxib (Warner *et al.*, 1999). At doses of 10 or 30 mg kg⁻¹ d⁻¹ blood pressure increase was achieved already after 1 week of treatment with rofecoxib and remained constantly elevated further on. In line with previous observations, we found that during low salt intake COX-2 inhibition did not increase sBP (Harding *et al.*, 1997, 2000; Rossat *et al.*, 1999). Further, a marked increase of salt intake did not enhance the hypertensive response. With regard to human studies, the VIGOR study has revealed that rofecoxib treatment is associated with an increase of systolic and diastolic blood pressure which is more pronounced than that observed with the non-selective COX-inhibitor naproxen (Mukherjee *et al.*, 2001). Furthermore, a trend towards a rise in sBP was observed by the administration of MK-966 after 14 days (Catella-Lawson *et al.*, 1999). However, it should be noted that in contrast to animal studies, humans respond less sensitive to increases in blood pressure by COX-2 inhibition.

The two prostanoids TxA₂ and PGI₂ play an essential role in the maintenance of vascular homeostasis. Prostacyclin is the main product of macrovascular endothelial cells and it is a potent vasodilator. Recent studies in healthy volunteers showed that treatment with selective COX-2 inhibitors decreases systemic prostacyclin production without affecting platelet derived TxA₂ synthesis (Catella-Lawson *et al.*, 1999;

McAdam *et al.*, 1999). This data suggest that during normal physiologic conditions, COX-2 is expressed in the vasculature and is the main source of prostacyclin production. However, the expression of COX-2 seemed to be barely in endothelial cells under static conditions *in vitro*, but it has been found that laminar shear stress is an inductor of COX-2 (Topper *et al.*, 1996). This finding suggests that COX-2 may be constitutively activated in arterial endothelium *in vivo*. In the meanwhile, other investigators have shown that COX-2 protein is constitutively expressed in the vasculature and aorta (Matrougui *et al.*, 2001; Arbabi *et al.*, 2000; Garcia-Cohen *et al.*, 2000).

Therefore, we determined plasma levels of 6-keto PGF_{1α} in SHRs and WKY rats during different salt intakes and found that these levels were increased during normal and high salt intake in comparison to low salt intake. This increase was clearly attenuated by COX-2 inhibition, suggesting that vascular COX-2 increases with enhanced salt intake. This conclusion is in good accordance with the recent observations that COX-2 protein in rat mesenteric arteries or in human umbilical vein endothelial cells increases in parallel with the NaCl content of the diet or of the media (Matrougui *et al.*, 2001; Arbabi *et al.*, 2000). Furthermore, the increased amount of 6-keto PGF_{1α} in SHR fits with the recent finding, that COX-2 protein is increased in aorta from SHRs (Garcia-Cohen *et al.*, 2000). Our data also confirm those studies, which report that the vascular endothelium produces increased amounts of the vasodilator prostanoids PGE₂ and PGI₂ as a compensatory anti-hypertensive mechanism in response to high salt intake and one may conclude that particular PGI₂ is of importance in vascular homeostasis during salt load (Falardeau & Martineau, 1983; Uehara *et al.*, 1987; Ishimitsu *et al.*, 1991) and those studies in humans, which suggest that COX-2 is a major source of vascular endothelial prostacyclin biosynthesis (McAdam *et al.*, 1999; Catella-Lawson *et al.*, 1999).

In summary, our data strongly suggest that blood pressure regulation depends on the balance of PGI₂ and TxA₂, since we did not observe an effect of rofecoxib on serum TxB₂ levels. Thus, direct inhibition of PGI₂ by COX-2 inhibitors may be a causal factor for the increase in blood pressure.

Our data further show that rofecoxib increased sBP on normal and high salt diet, but not on low salt diet. This pattern of response is therefore not typical for a primary salt induced hypertension. For SHRs we obtained very similar findings as with WKY rats, and it appeared as if the increase of sBP in response to COX-2 inhibition in WKY rats was almost additive to the known developmental changes of blood pressure in SHRs. Therefore, one may conclude that COX-2 inhibition neither mimics nor potentiates the mechanisms that cause high blood pressure in SHR.

Nevertheless, with regard to BP, similar data were previously obtained by Muscara *et al.* (2000), who reported that the COX-2 inhibitor celecoxib increased sBP in WKY rats on normal salt diet nearly to the same extent as we found in this study and who also reported the same additive increase of sBP in WKY rats made hypertensive by pre-treatment with the NO-synthase inhibitor L-NAME. These authors speculated from their findings that the increase of sBP might be causally related to volume retention. Although we cannot definitively rule out such a linkage, our data do not support such a mechanism for two reasons. Firstly, we

found no effect of the COX-2 inhibitor on fluid intake, or body weight gain, or on hematocrit suggesting that a possible volume expansion has to be rather minute. Secondly, we found no inhibitory effect of rofecoxib treatment on PRA or on renocortical renin expression, which are known to be sensitively suppressed by volume expansion.

It has been found that renal renin mRNA of SHR is, either elevated (Samani *et al.*, 1989), unchanged (Pratt *et al.*, 1989) or, in confirmation to our data, decreased (Yu & Di Nicolantonio, 1996) when compared to age matched WKY. These contrary findings may be caused by the genetic heterogeneity of SHR from different sources affecting gene expression (Nabika *et al.*, 1991; Zicha & Kunes, 1999). Similar findings as for renal renin mRNA have been observed for plasma renin activity (Yu & Di Nicolantonio, 1996; Samani *et al.*, 1989; Sen *et al.*, 1972). However, we could not observe a decrease in PRA in 7 weeks old SHR compared to age matched WKY. It should be noted, that our study takes place during a period of dramatic shifts in the endogenous renin-angiotensin system (Solomon *et al.*, 1977; Gomez *et al.*, 1988), which may mask strain related differences in PRA.

In contrast to our study, Wang *et al.* (1999) found that the COX-2 inhibitor SC-58236 lowers blood pressure in a model of renovascular hypertension. They found further that SC-58236 also decreases renin content and plasma renin activity in this study, which led to the suggestion that COX-2 derived prostanoids mediate the increase in the renin-angiotensin system and are also responsible for the increase in blood pressure. This finding and other investigations have led to the concept, that mainly COX-2 derived prostanoids are of relevance for the stimulation of the renin system during low salt intake (Harding *et al.*, 2000), by angiotensin-II antagonists (Cheng *et al.*, 1999) and by renal hypoperfusion (Wang *et al.*, 1999). With regard to the data obtained with the COX-2 selective antagonist NS-398 (Harding *et al.*, 2000) it seems that COX-2 is not an essential link in the dietary NaCl restriction response of PRA, because acute administration had no effect on PRA, and long term administration only partially inhibited renin secretion. Furthermore, the evidence that COX-2 derived prostanoids may be essential stimulators of the renin system was obtained under conditions of strong activation of the renin system (Cheng *et al.*, 1999; Wang *et al.*, 1999). However, our study was not performed during such a situation and it has also been previously shown that the degree of renin stimulation by low salt intake is maximal after 1 week, followed by a decline (Holmer *et al.*, 1993). This decline and the fact that our study takes place during a period of dramatic shifts in the endogenous renin-angiotensin system (Solomon *et al.*, 1977; Gomez *et al.*, 1988) may be explanations, why we could not

observe an effect of rofecoxib treatment on renin mRNA or on PRA in both rat strains. However, it should be noted that there are also studies questioning a causal involvement of COX-2 derived prostanoids in the regulation of the renin system by salt intake (Kammerl *et al.*, 2001; Rossat *et al.*, 1999). In summary, our data do not support a major role for COX-2 derived prostanoids in the stimulation of renin by low salt intake. However, this question needs to be further evaluated.

Conversely, it appears also unlikely that a stimulation of the renal renin-angiotensin-aldosterone system itself is the primary event by which COX-2 inhibition increases blood pressure, since renin was normal and aldosterone was even decreased under COX-2 inhibition. That NSAIDs can lower PAC has already previously reported (Miller *et al.*, 1980; Stokes *et al.*, 1991; Campbell & Gomez-Sanchez, 1985) and probably reflects a direct stimulatory effect of prostanoids on aldosterone production in the adrenal gland as previously suggested by *in vitro* (Csukas *et al.*, 1998) and *in vivo* (Golub *et al.*, 1976) observations. Since the typical inverse relationship between salt intake and renin was not changed, and the relationship between salt intake and plasma aldosterone was only attenuated but not blunted by the COX-2 inhibitor, we may infer that COX-2 mediated prostanoids are not essentially involved in the control of the RAAS by salt. This conclusion would be in harmony with the idea that the regulation of aldosterone production by salt intake is primarily mediated by angiotensin-II (Mulrow, 1999), which in turn is generated by the renin activity. In conclusion, our data do not support an impact of the RAAS on the increase in sBP. However, from the present data we cannot exclude an influence of rofecoxib on paracrine renin systems, e.g. in the adrenal gland, which are considered to be important in the development of cardiovascular diseases.

Taken together, our data suggest that prolonged treatment with selective COX-2 inhibitors results in an increase on blood pressure, which could be due to decreased prostacyclin levels. Furthermore, the rise in blood pressure induced by COX-2 inhibition can be prevented by sodium deprivation. In SHRs, which are considered as a human model for essential hypertension (Doggrell & Brown, 1998), COX-2 inhibition did not interfere with the typical temporal development of hypertension but produced a more constant additional increment of blood pressure increase.

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