COX-2 activity determines the level of renin expression but is dispensable for acute upregulation of renin expression in rat kidneys

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Matzdorf C, Kurtz A, Höcherl K. COX-2 activity determines the level of renin expression but is dispensable for acute upregulation of renin expression in rat kidneys. Am J Physiol Renal Physiol 292: F1782–F1790, 2007. First published March 20, 2007; doi:10.1152/ajprenal.00513.2006.—The role of cyclooxygenase 2 (COX-2) in the control of renin is still a matter of debate, since studies with COX-2-deficient mice or with COX-2 inhibitors produced conflicting findings. Therefore, we studied the effect of the COX-2 inhibitor SC-58236 on the regulation of the renin system in adult rat kidneys. Renocortical tissue levels and urinary excretion of PGE2 were reduced to 65 and 40% of control values, respectively, after a single gavage of SC-58236 and did not further decrease on prolonged treatment. Plasma renin activity (PRA) and renin mRNA levels began to decrease after 3 days and reached a constant level of ~60% of control values after 5 days of treatment. Isoproterenol or left renal artery clipping for 2 days increased PRA and renin mRNA to similar levels in both vehicle- and SC-58236-treated rats after 2 days. Pretreatment with SC-58236 for 5 days, however, reduced the absolute increase in PRA and renin mRNA levels. Notably, the relative increases were not different between vehicle- and SC-58236-treated rats. Similar findings were observed for the stimulation of the renin system by angiotensin II inhibition and low salt intake. These findings indicate that COX-2 inhibition attenuates renin secretion and renin gene expression stimulated by a variety of parameters in proportion to the lowering of basal renin activity, while it does not interfere with the different stimulatory mechanisms per se. As a consequence, it appears as if COX-2 activity relevantly determines the set point of the activity of the renin system in rat kidneys.

SC-58236; renovascular hypertension; prostaglandins; cyclooxygenase; isoproterenol

THE CONVERSION OF ARACHIDONIC acid to prostaglandin (PG) H2 by cyclooxygenase (COX) is a key enzymatic step in the regulation of prostaglandin synthesis. Two isoforms of COX have been identified: a constitutive (COX-1) and an inducible (COX-2) form. COX-1 is the predominant isozymes in the mammalian kidney and has been localized to arteries, glomeruli, and collecting ducts. COX-2 has been localized to arteries, collecting ducts, interstitial cells, cortical thick ascending limb of Henle (cTALH) cells, and macula densa cells. Prostaglandins have a major impact on kidney function. They are involved in the control of renal blood flow, glomerular filtration, salt excretion, and in the secretion and expression of renin (22).

The renin-angiotensin system (RAS) plays a crucial role in the control of systemic blood pressure and in the control of salt and water balance (11). The formation and particularly the release of renin is the rate-limiting step within the RAS. Activation of the RAS increases and depression of the RAS lowers blood pressure and, as part of a negative feedback control, blood pressure in turn affects the synthesis and release of renin from the juxtaglomerular cells of the kidney. Until now, the functional mechanisms underlying this so-called renal baroreceptor mechanism has not been completely understood. Similarly, alterations of the NaCl concentration in the tubular fluid at the level of the macula densa cells also cause inverse changes in the release and expression of renin from the juxtaglomerular cells of the kidney, which lead to changes in plasma renin activity (PRA) and subsequently to an increased angiotensin II formation. Up until now, the precise signal for this so-called macula densa-dependent mechanism has been unknown (32, 34).

Several studies have suggested that the COX-derived products PGE2 and prostacyclin (PGI2) could be the origin for these signals. PGE2 and PGI2 have been shown to directly stimulate renin secretion and renin expression, and COX inhibitors were found to lower renin synthesis and renin secretion in response to typical renin-stimulatory maneuvers, like renal hypoperfusion, low salt intake, and angiotensin II inhibition (1, 7, 9, 21, 33).

Due to a parallel regulation of COX-2 and renin expression in the renal cortex in response to these stimuli, specifically COX-2-derived prostanoids have been implicated in the macula densa-mediated and renal baroreceptor-mediated regulation of renin release (5, 13, 25). However, studies with COX-2-deficient mice and with selective COX-2 inhibitors produced conflicting findings. It has been found that pharmacological inhibition of COX-2 with SC-58236 and SC-58125 as well as COX-2 deficiency attenuate the increase in renin release and renin expression due to renovascular hypertension, low salt intake, and angiotensin II inhibition (5, 6, 8, 37, 38), whereas commercially available COX-2 inhibitors, like rofecoxib and celecoxib, did not affect the stimulation of the renin system in response to these stimuli (14, 18, 19, 25, 28, 29). Recently, it has been reported that COX-2 deficiency reduces basal renin expression and renin release but does not attenuate the secretion of renin in response to acute stimuli (23). Therefore, the present study aimed to determine the impact of the duration of pharmacological COX-2 inhibition for its efficacy to interfere with the regulation of the renin system. To this end, we explored the time-dependent effects of the selective COX-2 inhibitor SC-58236 on the regulation of renin gene expression and renin secretion in response to typical stimuli. Since we found that the COX-2 inhibitor SC-58236 time dependently lowered basal PRA and renin mRNA expression, we further investigated the impact of COX-2-derived prostanoids on the
stimulation of the renin system by isoproterenol infusion, renovascular hypertension, low salt intake, furosemide infusion, and angiotensin II inhibition.

MATERIALS AND METHODS

Materials. Candesartan-cilexetil and ramipril were kind gifts from AstraZeneca (Möln达尔, Sweden). Isoproterenol, furosemide, and captopril were purchased from Sigma (Deisenhofen, Germany). SC-58236 was purchased from Axxora (Lörrach, Germany).

Animal experiments. All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and German laws relating to the protection of animals and were approved by the local ethics committee.

Male Sprague-Dawley rats (180–200 g, Charles River) were housed in cages in a temperature- and light-controlled environment. Rats were treated with SC-58236 (10 mg·kg⁻¹·day⁻¹; 5% gum arabic suspension) orally via a stomach tube. Captopril (100 mg·kg⁻¹·day⁻¹ for 7 days) and ramipril (10 mg·kg⁻¹·day⁻¹ for 7 days) were given via drinking water. Candesartan-cilexetil (15 mg·kg⁻¹·day⁻¹ for 7 days; 5% gum arabic suspension) was given orally via a stomach tube. Isoproterenol (160 µg·kg⁻¹·h⁻¹) was infused subcutaneously via osmotic minipumps (Model 2ML1, Alzet Osmotic Pumps, Durect, Cupertino, CA) for 2 days. Furosemide (12 mg/day) was infused subcutaneously via osmotic minipumps (Model 2ML1, Alzet Osmotic Pumps, Durect) for 2 days. Furosemide-treated animals and their respective control groups had free access to tap water and a solution containing 0.9% sodium chloride and 0.1% potassium chloride. Low-salt (0.02% NaCl, wt/wt, Sniff special diets, Soest, Germany), normal-salt (0.6% NaCl, wt/wt), and high-salt diets (8% NaCl, wt/wt) were given for 1 wk. Left renal artery stenosis was performed for 2 days as described previously (25). In brief, left renal arteries were clamped (0.2-mm ID silver clips, Degussa) under sevoflurane anesthesia. In addition, animals were treated with SC-58236 in combination with captopril, ramipril, candesartan-cilexetil, isoproterenol, low salt intake, high salt intake, and renal artery stenosis. Furthermore, subset groups of animals received SC-58236 for 7 days and isoproterenol infusion, furosemide infusion, or left renal artery clipping during the last 2 days. The number of animals was 8 rats/group. Vehicle-treated animals received a 5% gum arabic suspension orally via a stomach tube.

Systolic blood pressure and heart rate measurements were performed (tail-cuff method) before treatment and every day thereafter. At the end of the study period, the final doses were given 2 h before decapitation of rats during sevoflurane anesthesia (3%, vol/vol). Blood was collected into tubes containing EDTA. The kidneys were dissected with a scalpel blade under a stereomicroscope, frozen in liquid nitrogen, and stored at −80°C until extraction of total RNA.

RNA extraction and quantitative real-time PCR analysis of renin mRNA. Total RNA was extracted from renal cortex using TRIzol reagent (Invitrogen, Karlsruhe, Germany). Total RNA was reverse transcribed into cDNA according to standard protocols. In brief, cDNA was synthesized in a 20-µl reaction with 2 µg total RNA, 0.5 µg oligo(dT)₁₂–₁₈, 20 U Rnasin (Promega), 4 µl of 5× RT buffer, 0.5 mM dNTP, and 20 U Moloney murine leukemia virus RT enzyme (GIBCO Life Technologies).

Real-time RT-PCR was performed with the Light Cycler System (Roche, Mannheim, Germany). All PCR experiments were done using a Light Cycler-FastStart DNA Master SYBR Green I kit provided by Roche Molecular Biochemicals. Each reaction (20 µl) contained 2 µl cDNA, 3.0 mM MgCl₂, 1 pmol of each primer (renin sense: aagtcatcttgacaggc, antisense: ttcatacctgtggca; β-actin sense: ccgtcacctggagcactcgtttc, antisense: gctggggtgttgaaggtctcaaa), and 2 µl of Fast Starter Mix (containing buffer, dNTPs, Sybr Green dye, and Taq polymerase). The amplification program consisted of 1 cycle of 95°C with 10-min hold ("hot start") followed by 40 cycles of 15 s at 95°C, 5 s at 60°C, and 20 s at 72°C. Amplification was followed by melting curve analysis (increasing the temperature of the reaction mixtures up to 95°C, by 0.1°C/s, starting at 50°C for 15 s) to verify the correctness of the amplicon. The melting curves were converted to display the first negative derivative (-dF/dT) vs. the temperature. This is an indication of the purity of the products, in which one melting point (T_m) is indicative for one product, while more melting points indicate the presence of more amplicons, e.g., by nonspecific binding of one or both primers. This can be caused by annealing temperature and MgCl₂ concentrations. A negative control with water instead of cDNA was run with every PCR to assess specificity of the reaction. To verify the accuracy of the amplification, PCR products were further analyzed on ethidium bromide-stained 2% agarose gels. Analysis of data was performed using Light Cycler software, version 3.5.3 and LightCycler Relative Quantification (RelQuant) Software.

The expression of renin was quantified relative to cytoplasmatic β-actin. For this purpose, a standard calibration curve was made. The Roche software uses the second derivative maximum method to calculate the fractional cycle numbers where the fluorescence rises above background (crossing point; CP), that is, the point at which the rate of change of fluorescence is fastest. For the standard curve, CPs are plotted vs. log concentration for the standards. This standard curve is used to estimate the concentration of each sample. The standard curves for both renin and β-actin were saved in a coefficient file, which was used by the relative quantification software from Roche to calculate the renin levels relative to β-actin. This program also corrected for the differences in efficiency of the PCR reaction for each target. The efficiency was calculated after plotting the dilution series of renin and β-actin vs. the CPs defined by the LC program. The efficiency of the PCR was calculated using the formula

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E = 10^{-\frac{1}{Slope}}
\]

Determination of PRA. PRA was determined using a commercially available radioimmunoassay (Sorin Biomedical, Düsseldorf, Germany).

Determination of PGE₂. Renocortical tissue levels of PGE₂ and urinary excretion of PGE₂ were assayed as described previously (19). Urinary excretion and the renal cortical concentration of PGE₂ were assayed with a monoclonal EIA kit (Cayman Chemical, Ann Arbor, MI). In brief, renal cortices (100 mg) were homogenized in isotonic NaCl (1 ml) and centrifuged at 10,000 g for 15 min. The supernatant was assayed with a monoclonal EIA kit (Cayman Chemical, Ann Arbor, MI). In brief, renal cortices (100 mg) were homogenized in isotonic NaCl (1 ml) and centrifuged at 10,000 g for 15 min. The supernatant was assayed with a monoclonal EIA kit (Cayman Chemical, Ann Arbor, MI). In brief, renal cortices (100 mg) were homogenized in isotonic NaCl (1 ml) and centrifuged at 10,000 g for 15 min. The supernatant was assayed with a monoclonal EIA kit (Cayman Chemical, Ann Arbor, MI).

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

Time-dependent effects of SC-58236. Renocortical tissue levels of PGE₂ were reduced to ~65% of control values already 1 day after a single gavage of SC-58236 and did not decrease further up to 7 days of treatment (Fig. 1A). Similarly, urinary excretion of PGE₂ decreased to ~40% of control values 24 h after gavage of SC-58236 and did not further decrease during prolonged treatment (Fig. 1B).

PRA was not affected by SC-58236 1 and 3 days after the beginning of the treatment but clearly decreased to ~55% of control values after 5 and 7 days of treatment with SC-58236 (Fig. 1C). Renin mRNA abundance was not altered after 1 and 3 days of SC-58236 treatment but was decreased to ~60% after 5 and 7 days of treatment (Fig. 1D). Systolic blood pressure of control rats was 127 ± 4 mmHg. Treatment with SC-58236 did not significantly increase systolic blood pressure (135 ± 6 mmHg).
Effect of SC-58236 on isoproterenol-induced PRA and renin mRNA expression. Since we found a time-dependent effect of SC-58236 on basal PRA and renin expression, the effect of SC-58236 on the β-adrenergic stimulation of PRA and renin mRNA expression was investigated with treatment with SC-58236 for 2 or 7 days. In rats without SC-58236 treatment, isoproterenol infusion for 48 h increased PRA from 5.4 to 19.4 ng ANG I h⁻¹·ml⁻¹, and renin mRNA increased threefold. Treatment of rats with SC-58236 for 2 days did not change basal or isoproterenol-stimulated PRA values and mRNA levels (Fig. 2, A and B). When the animals were pretreated with SC-58236 for 5 days, both basal and isoproterenol-stimulated PRA values and renin mRNA levels were reduced compared with rats without treatment with SC-58236 (Fig. 2, A and B). Systolic blood pressure was not changed by any maneuver in this set of experiments. Isoproterenol infusion for 48 h did not change renocortical PGE₂ levels. SC-58236 treatment for 2 or 7 days clearly decreased renocortical PGE₂ levels in control rats and in rats treated with isoproterenol (Fig. 2C).

Effect of SC-58236 on PRA and renin mRNA expression induced by acute left renal artery clipping. The effect of SC-58236 on the stimulation of PRA and renin mRNA expression in response to left renal artery clipping was investigated with treatment with SC-58236 for 2 or 7 days. In rats without SC-58236 treatment, left renal artery clipping for 48 h increased PRA from 5.3 to 21.5 ng ANG I h⁻¹·ml⁻¹, and renin mRNA increased 2.3-fold. Treatment of rats with SC-58236 for 2 days did not change basal or clipping-induced PRA values and mRNA levels (Fig. 3, A and B). When the animals were pretreated with SC-58236 for 5 days, both basal and clipping-stimulated PRA values and renin mRNA levels were reduced compared with rats without treatment with SC-58236 (Fig. 3, A and B). Left renal artery clipping for 2 days increased renocortical PGE₂ levels 1.7-fold. Treatment of rats with SC-58236 for 2 or 7 days decreased renocortical PGE₂ levels in control rats and attenuated the increase in renocortical PGE₂ concentration due to left renal artery clipping (Fig. 3C).

Left renal artery clipping increased systolic blood pressure to 173 ± 8 mmHg. The increase was not affected in rats without pretreatment with SC-58236 but was clearly lower (140 ± 11 mmHg) in SC-58236-pretreated rats.

Effect of SC-58236 on the regulation of the renin system by salt intake. A high-salt diet (8% NaCl, wt/wt) for 1 wk decreased PRA to 49% and renin mRNA levels to 38% of the values for a normal-salt diet (0.6% NaCl, wt/wt), and a low-salt diet (0.02% NaCl, wt/wt) increased PRA 2.3-fold and renin mRNA expression 2.2-fold. SC-58236 did not alter PRA and renin mRNA levels during high salt intake but decreased PRA and renin mRNA expression during normal salt intake and low salt intake to ~60% of the respective control values (Fig. 4, A and B). A high-salt diet for 1 wk decreased renocortical PGE₂ levels to 70% of the values for a normal-salt diet, and a low-salt diet increased renocortical PGE₂ levels 1.5-fold. Treatment of rats with SC-58236 for 7 days decreased renocortical PGE₂ levels during the different salt intakes (Fig. 4C).

Systolic blood pressure was not altered by the different salt diets. SC-58236 did not change blood pressure during low or normal salt intake, but systolic blood pressure increased during high salt intake to 143 ± 4 mmHg.

Effect of SC-58236 on the stimulation of the renin system by angiotensin II inhibition. Captopril, ramipril, and candesartan treatment for 1 wk increased PRA to 54%, 80%, and 85%, respectively. The increases in PRA due to captopril, ramipril, and candesartan treatment were lower (26%, 46%, and 48%, respectively) in SC-58236-treated rats compared with vehicle-treated control rats (Fig. 5A). SC-58236 treatment lowered basal PRA and basal renin mRNA levels to ~56 and 57% of control values, respectively. Captopril, ramipril, and candesartan treatment for 1 wk increased renin mRNA levels 1.9-, 3.8-, and 5.6-fold, respectively. The increases in renin mRNA due to captopril, ramipril, and candesartan treatment were reduced (1.1-, 2.7-, and 3.2-fold, respectively) in SC-58236-treated rats compared with vehicle-treated control rats (Fig. 5B).
Captopril, ramipril, and candesartan treatment for 1 wk increased renocortical PGE2 levels 1.6-, 1.9-, and 2.3-fold, respectively. SC-58236 treatment lowered basal renocortical PGE2 levels and attenuated the increase in renocortical PGE2 concentration due to captopril, ramipril, and candesartan treatment (Fig. 5).

Captopril, ramipril, and candesartan treatment for 1 wk decreased systolic blood pressure to 109/110 mmHg, 99/110 mmHg, and 93/110 mmHg, respectively. SC-58236 moderately attenuated the decrease in systolic blood pressure due to captopril (116 mmHg), ramipril (109 mmHg), and candesartan (103 mmHg).

Relative changes in renin mRNA and PRA. Since SC-58236 lowered basal PRA and renin mRNA levels, we also calculated the relative changes in PRA and renin mRNA in response to the different stimuli. The relative changes in PRA and renin mRNA induced by isoproterenol infusion or left renal artery clipping for 48 h were not different in rats treated for 2 days with SC-58236 or with vehicle. The relative increase in PRA was 2.4-, 3.8-, 4.1-, 4.6-, 8.2-, and 8.5-fold in rats treated with SC-58236 for 7 days in combination with a low-salt diet, left renal artery clipping, isoproterenol, captopril, ramipril, and candesartan, respectively, and did not differ from vehicle-treated rats (Fig. 6A). The relative increase in renin mRNA levels was 2.2-, 2.3-, 3.0-, 1.9-, 3.8-, and 5.6-fold in rats treated with SC-58236 for 7 days in combination with a low-salt diet, renovascular hypertension, isoproterenol, captopril, ramipril, and candesartan, respectively, and did not differ from vehicle treated rats (Fig. 6B). In addition, there was a strong correlation between renin mRNA levels and PRA (Fig. 6C; \( r^2 = 0.86 \), slope \( P < 0.0001 \)).
Effect of SC-58236 on furosemide-induced PRA and renin mRNA expression. In rats without SC-58236 treatment, furosemide infusion for 48 h increased PRA from 5.4 to 31.0 ng ANG I/h/ml and renin mRNA 3.1-fold. Treatment of rats with SC-58236 for 2 days did not significantly change basal PRA and renin mRNA levels but decreased furosemide-induced PRA values to 14.0 ng ANG I/h/ml as well as renin mRNA abundance to 210% of control values (Fig. 7, A and B). When animals were pretreated with SC-58236 for 5 days, both basal and furosemide-stimulated PRA and renin mRNA values were reduced compared with rats without treatment with SC-58236 (Fig. 7, A and B). However, PRA and renin mRNA levels in animals treated with SC-58236 and furosemide were 2.8- and 2.1-fold higher, respectively, compared with animals treated only with SC-58236 (Fig. 7, C and D).

Furosemide infusion for 2 days increased renocortical PGE2 levels 2.0-fold. Treatment of rats with SC-58236 for 2 or 7 days decreased renocortical PGE2 levels in control rats and attenuated the increase in renocortical PGE2 concentration due to furosemide infusion (Fig. 7E). Systolic blood pressure was not significantly changed by any maneuver in this set of experiments.

DISCUSSION

The aim of our study was to investigate the contribution of COX-2-derived prostanoids in the secretion and synthesis of renin from the juxtaglomerular apparatus. Previous studies have shown that the COX-2 inhibitors SC-58236 and SC-58125, like COX-2 deficiency, but in contrast to rofecoxib,
celecoxib, or nimesulide, attenuate an increase in renin secretion and renin expression in response to renovascular hypertension (8, 14, 25, 28, 37), low salt intake (8, 16, 18, 29, 38), and angiotensin II inhibition (5, 6, 19). It has been suggested that this striking difference between the various COX-2 inhibitors may be due to a lower accessibility of rofecoxib or celecoxib at the level of macula densa cells and/or the juxtaglomerular apparatus, which may lead to insufficiently high enough drug levels for a complete inhibition of local prostaglandin production (23). Therefore, we now investigated the effects of the selective COX-2 inhibitor SC-58236 on the regulation of renin gene expression and renin secretion in adult rat kidneys.

Already 24 h after the start of treatment, basal renocortical PGE2 levels and urinary excretion of PGE2 were markedly lower in SC-58236-treated rats and did not decrease further on prolonged treatment. Since it has been suggested that the cells of the cTALH and macula densa represent the major site of renocortical COX-2 expression, and therefore for renocortical COX-2-derived prostaglandin synthesis (12), our data suggest that even a single dose of SC-58236 may effectively inhibit renal COX-2 activity.

We now found that SC-58236 also lowered basal PRA and renin mRNA levels after a treatment period of 5 days. This finding agrees with lower basal renin levels in COX-2-deficient mice and suggests that COX-2-derived prostanoids are involved in the control of basal renin activity (23, 39). PGE2 and PGI2 are well-known stimulators of renin secretion and renin synthesis. In contrast to changes in renin mRNA levels, the secretion of renin from the juxtaglomerular apparatus into the bloodstream is a rapid process. Although renocortical PGE2 levels as well as urinary PGE2 excretion were already lower after a single dose of SC-58236, PRA and renin mRNA levels decreased in parallel only after prolonged treatment with SC-58236. Therefore, the lag in lower PRA and renin mRNA levels during COX-2 inhibition seems to be rather due to a downregulation of renin expression, which takes place over periods of hours in vitro and may require a more prolonged time in vivo. In line with this are data obtained from isolated, perfused rat kidneys which demonstrate that acute inhibition of COX-2 does not influence basal secretion of renin (3). Thus it seems likely that inhibition of COX-2 decreases the activity of the basal renin system by suppression of renin gene expression.

Alternatively, the lag in lowering PRA and renin mRNA levels by COX-2 inhibition could be due to secondary mechanisms. It has been shown that inhibition of COX-2 increases blood pressure and leads to salt and water retention (16, 27, 30, 31), which in turn leads to hypervolemia. Both processes are known to inhibit renin secretion and renin expression (11). Since we found that systolic blood pressure was slightly increased in SC-58236-treated rats, we cannot exclude such a secondary mechanism in renin secretion and renin expression.

We were further interested in the contribution of COX-2-derived prostanoids to the increase in renin release and renin expression in response to acute stimuli, like isoproterenol infusion and left renal artery clipping, and chronic stimuli, like low salt intake and angiotensin II inhibition. As a result of the data obtained from basal renin secretion and renin release, we hypothesized rather that the basal activity of the renin system and not COX-2-derived prostanoids per se determines the magnitude of the stimulation of the renin system.

Therefore, we investigated the effect of SC-58236 on acute isoproterenol-induced renin secretion and synthesis, which is known to directly stimulate the renin system via activation of β-adrenoreceptors, independently of prostaglandin synthesis (15, 17, 24). In line with previous observations, we found that
infusion of isoproterenol for 2 days clearly increased renin synthesis and renin secretion (20) and that cotreatment with SC-58236 did not influence renin synthesis and renin secretion. However, if we used rats that had been pretreated with SC-58236 for 5 days, a time point with lower basal PRA and renin mRNA levels, the absolute increase in PRA and renin mRNA due to isoproterenol infusion was reduced, but the relative responses were not different in SC-58236-pretreated rats vs. vehicle-treated rats. This finding clearly demonstrates the importance of basal renin levels for the absolute stimulation of the renin system in a COX-2-independent manner. Moreover, our pharmacological investigation confirms similar findings that have been reported for COX-2-deficient mice (23).

To further investigate this issue, we used a second acute model, namely, left renal artery clipping, for the stimulation of the renin system. It has been suggested that the stimulation of the renin system by renal hypoperfusion depends on COX-2-derived prostanoids (8, 37). In line with previous reports, acute left renal artery clipping for 2 days increased renin synthesis and renin mRNA expression as well as systolic blood pressure (25). Similar to the prostanoid-independent isoproterenol-induced stimulation of the renin system, the absolute responses to left renal artery clipping were not attenuated by a parallel treatment with SC-58236 but were clearly lower if SC-58236-pretreated rats were used. However, the relative increases in PRA and renin mRNA levels were not different. These findings fit very well with results obtained by Wang et al. (37) using aortic banding as a model for renovascular hypertension. They found that cotreatment with SC-58236 for 1 wk attenuates the increase in PRA and renin mRNA expression in response to renal hypoperfusion. However, PRA and renin mRNA levels were still twofold higher than control values. Since these authors did not investigate the effects of SC-58236 on basal renin secretion and renin expression, one may conclude that COX-2-derived prostanoids activate the renin system. With regard to our present data, however, it seems more likely that the basal activity of the renin system is of major importance for the absolute increase. In addition, we found that SC-58236 pretreatment also attenuated the increase in systolic blood pressure, confirming the data of Wang et al. (37). Taken together, this model also strengthens the importance of basal renin levels for the absolute stimulation of the renin system. Moreover, these findings indicate that COX-2-derived prostanoids are not involved in the stimulation of renin synthesis and renin secretion during renal hypoperfusion. In addition, these data are in line with a previous report which indicates that the renal baroreceptor mechanism does not depend on prostaglandin formation (36).

It has also been suggested that COX-2-derived prostanoids may mediate the stimulation of the renin system in response to chronic angiotensin II inhibition, since cotreatment with SC-58236 for 1 wk attenuates the captopril-induced increase in PRA and renin mRNA expression as well as systolic blood pressure, confirming the data of Wang et al. (37). Taken together, this model also strengthens the importance of basal renin levels for the absolute stimulation of the renin system. Moreover, these findings indicate that COX-2-derived prostanoids are not involved in the stimulation of renin synthesis and renin secretion during renal hypoperfusion. In addition, these data are in line with a previous report which indicates that the renal baroreceptor mechanism does not depend on prostaglandin formation (36).
rather weak inhibitor of the angiotensin-converting enzyme (ACE), increased renin mRNA and PRA and that cotreatment with SC-58236 for 1 wk attenuated the captopril-induced increase. However, the relative response was still present. To obtain a more obvious regulation of the renin system by angiotensin II inhibition, we further investigated the effect of ramipril, a more long-lasting ACE inhibitor, and candesartan, an angiotensin II AT1-receptor antagonist. In line with previous reports, ramipril as well as candesartan strongly increased renin secretion and renin synthesis (2, 19). Moreover, we found that additional treatment with SC-58236 lowered absolute levels of PRA and renin mRNA but not the relative increase, indicating that COX-2-derived prostanoids are not essentially involved in the stimulation of renin synthesis and renin secretion during angiotensin II inhibition. This pharmacological finding is in good agreement with recent observations in COX-2-deficient mice with single genetic backgrounds and further shows the importance of basal renin activity for its absolute stimulation (23). Notably, SC-58236 also reduced the blood pressure-lowering effect of captopril, ramipril, and candesartan. Therefore, we cannot exclude a secondary effect of systemic blood pressure on the renin system.

COX-2-derived prostanoids have also been linked to the stimulation of the renin system in response to low salt intake (8, 38). In line with previous observations, we found that low salt intake increases renin release and renin synthesis (18). As already shown for COX-2-deficient mice and SC-58125-treated mice, cotreatment with SC-58236 inhibited the absolute values of renin secretion and renin synthesis. However, relative values were unchanged, indicating that COX-2-derived prostanoids are not essentially involved in the stimulation of renin synthesis and renin secretion during low salt intake. Again, this finding strengthens the impact of basal renin levels for the absolute stimulation of the renin system.

In line with previous observations, administration of furosemide increased PRA and renin mRNA levels in rats (4, 26). This stimulation is suggested to be prostaglandin dependent (10, 35). We now found that SC-58236 softens the increase in PRA and renin mRNA abundance already after 2 days of treatment. This finding would be in general agreement with the concept that COX-2-derived prostanoids are required for macula densa-triggered renin release. However, SC-58236 treatment did not completely inhibit the furosemide-induced rise in PRA and renin mRNA levels. Therefore, our data might suggest that COX-2-derived prostanoids are only in part mediating the stimulation of the renin system by furosemide and that additional stimulatory mechanisms are also required.

Our present data are also in congruence with previous findings from our own laboratory and from other investigators, where no effect of selective COX-2 inhibitors, like rofecoxib and celecoxib, on the stimulation of the renin system by renal hypoperfusion, low salt intake, or angiotensin II inhibition has been reported (14, 16, 18, 19, 25, 28). With regard to our new data, it seems as if these COX-2 inhibitors do not attenuate increases in PRA and renin mRNA expression, because these COX-2 inhibitors do not lower basal PRA and renin expression.

From our present findings, we cannot completely exclude the precise role of COX-2-derived prostanoids in mediating stimulus-induced renin synthesis. It is conceivable that COX-2 may mediate stimulus-induced renin synthesis under normal conditions, while under conditions when COX-2 is inhibited, its effect could be compensated by other mechanisms. Since we found that SC-58236 inhibits the increase of renocortical PGE2 levels in response to the different stimuli, a compensation via an induction of prostanoic synthases, for example, which may account for increased renin expression, seems to be unlikely.

In summary, these findings demonstrate that COX-2 inhibition decreases basal renin secretion and renin gene expression and therefore attenuates the stimulation of the renin system in response to a variety of factors. Since COX-2 inhibition does not attenuate relative responses, it seems unlikely that COX-2-derived prostanoids are involved in the signaling pathways for these stimulatory mechanisms per se. Therefore, it appears as if COX-2 activity essentially determines the set point of the activity of the renin system that can be stimulated by isoproterenol, a low-salt diet, renal hypoperfusion, or angiotensin II antagonists.

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GRANTS
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