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Coordinate Expression of Renin and Cyclooxygenase-2 in Rats with Two-kidney, One Clip and Deoxycorticosterone Acetate-Salt Hypertension

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The present study was aimed to examine whether the expression of renin is associated with that of cyclooxygenase-2 (COX-2) in the kidney. Male Sprague-Dawley rats were made two-kidney, one clip (2K1C) or deoxycorticosterone acetate (DOCA)-salt hypertensive, to stimulate or to inhibit the endogenous renin-angiotensin system, respectively. The expression of renin and COX-2 mRNA was determined in the cortex of the kidney by reverse transcription-polymerase chain reaction. 2K1C hypertensive rats showed an increased expression of renin as well as of COX-2 in the clipped kidney. The expression of renin was decreased in parallel with that of COX-2 in the contralateral non-clipped kidney. Removal of the renal arterial clip reversed the expression of both genes, along with the blood pressure, to the control level. On the other hand, DOCA-salt hypertension was associated with parallel decreases of renin and COX-2 expression. These results indicate that renin and COX-2 genes are coordinately expressed in the kidney.

Key Words: Renin, Cyclooxygenase-2, Two-kidney, One clip hypertension, Deoxycorticosterone acetate-salt hypertension

INTRODUCTION

The synthesis of prostaglandins from arachidonic acid is catalyzed by cyclooxygenase (COX). Between the two isoforms of COX, although COX-2 is generally considered as an inducible enzyme (Feng et al, 1993), it is also constitutively expressed in some organs including the kidney. It is predominantly localized in the macula densa and nearby cells in the cortical thick ascending limb of loop of Henle (Harris et al, 1994). Furthermore, its expression may be modified by several environmental conditions, such as salt intake (Harris et al, 1994; Harding et al, 1997; Yang et al, 1998), medullary tonicity (Yang et al, 1999), and adrenal steroids (Zhang et al, 1999), suggesting a role of COX-2 products in the regulation of salt, volume, and blood pressure homeostasis.

COX-2 products may directly act on juxtaglomerular cells to stimulate the synthesis and release of renin (Jensen et al, 1996). A selective blockade of COX-2 attenuates the increases of plasma and renal renin levels in response to inhibitors of angiotensin converting enzyme (Cheng et al, 1999), and diminishes the macula densa-mediated increase of renin production (Traynor et al, 1999). Furthermore, a blockade of COX-2 ameliorates the renovascular hypertension (Wang et al, 1999). It has been more recently observed that a genetic deletion of COX-2 prevents the increase of renin expression in response to angiotensin converting enzyme inhibition (Cheng et al, 2001). These findings specifically suggest a role of COX-2 products in the synthesis and release of renin.

Deoxycorticosterone acetate (DOCA)-salt hypertension has been associated with a diminished activity of renin-angiotensin system. High-salt intake results in a downregulation of cortical COX-2 mRNA levels

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(Yang et al, 1998). Therefore, the decrease of renin expression in DOCA-salt hypertension may be related with an attenuated expression of COX-2. However, previous studies only examined the expression of COX-2 in association with an enhanced renin synthesis, and a role of COX-2 in a pathophysiological state associated with a diminished renin synthesis has not been examined.

The present study was aimed to examine whether the expression of renin is associated with that of COX-2. Rats were made two-kidney, one clip (2K1C) or DOCA-salt hypertensive, to stimulate or to inhibit the renin synthesis, respectively. The expression of renin and COX-2 genes was determined in the cortex of the kidney by reverse transcription (RT)-polymerase chain reaction (PCR).

METHODS

Two-kidney, one clip hypertension

Male Sprague-Dawley rats weighing 160~190 g were used. They had free access to regular rat chow and tap water. To induce 2K1C hypertension, they were clipped at the left renal artery under anesthesia with ketamine (50 mg/kg, i.p.) with a silver clip having an inner gap of 0.25 mm. Sham-clipped rats served as control.

They were kept 4 weeks. On the experimental day, the systolic blood pressure was measured using an automated tail-cuff method. In clipped rats, the arterial clip was then removed or sham-removed under ketamine anesthesia. Twenty-four hours after the unclipping, the rats were again measured of the systolic blood pressure.

DOCA-salt hypertension

To develop DOCA-salt hypertension, rats were removed of their left kidneys under ketamine anesthesia (50 mg/kg, i.p.). One week after the unilateral nephrectomy, they were divided into two groups. One group was subcutaneously implanted with silastic strips impregnated with DOCA (200 mg/kg body weight), and supplied with 0.9% saline to drink. The other group was given 0.9% saline to drink without implantation of DOCA. They were used 4 weeks thereafter.

Reverse transcription-polymerase chain reaction

The rats were killed by decapitation under a conscious state to collect the trunk blood for determination of plasma renin concentration. Kidneys were then rapidly taken and frozen in liquid nitrogen. They were stored at −70°C until assayed. Total RNA was isolated according to the protocols of Ultraspec™ RNA isolation system (Biotecx Laboratories; Houston, TX, USA). RT followed by PCR was then applied.

For RT step, 1 μg total RNA was incubated with 200 U of reverse transcriptase (Gibco BRL; Grand Island, NY, USA), 10 mmol/L dNTP mix, 0.1 mol/L DTT, 25 mmol/L $MgCl_2$, and 0.5 μg/μL oligo (dT) in reaction buffer [200 mmol/L Tris-HCl (pH 8.4), 500 mmol/L KCl] at a final volume of 20 μL. PCR was performed in a thermal cycler (M. J. Research; Watertown, MA, USA) with the following profile: denaturation for 30 sec at 94°C, annealing for 60 sec at 60°C, and extension for 75 sec at 72°C for renin; denaturation for 90 sec at 94°C, annealing for 90 sec at 54°C, and extension for 90 sec at 72°C for COX-2; denaturation for 45 sec at 94°C, annealing for 45 sec at 56°C, and extension for 90 sec at 72°C for β-actin. The last cycle was ended with 5 min of elongation at 72°C. Renin primers were: sense 5'-TGCCACCTTGTTGTGTGAGG-3', and antisense 5'-ACCCGATGCGATTGTTATGCCG-3', allowing the amplification of 374 bp fragments. COX-2 primers were: sense 5'-ACACTCTATCACTGGCATCC-3', and antisense 5'-GAAGGGACACCCTTTCACAT-3', allowing the amplification of 584 bp fragments. β-Actin primers were: sense 5'-GACTACCTCATGAAGATCCTGACC-3', and antisense 5'-TGATCTTCATGGTGCTAGGAGCC-3', allowing the amplification of 423 bp fragments. The PCR contained 20 pmole of each primer, 250 μmol/L dNTP mix, 1.5 mmol/L $MgCl_2$, 40 mmol/L KCl reaction buffer [50 mmol/L Tris-HCl (pH 8.3)], and 1 U of Taq polymerase (Bioneer; Cheongwon, Korea) in a final volume of 20 μL.

The PCR products were size fractionated by 1.5% agarose gel electrophoresis, and visualized under ultraviolet light with ethidium bromide staining. They were quantified using IMAGER™ & 1 D MAIN (Bioneer; Cheongwon, Korea). The level of either renin or COX-2 cDNAs was normalized by comparison with that of β-actin cDNA.

Plasma renin concentration

Plasma renin concentration was determined as the rate of angiotensin I generated in the presence of excess homologous renin substrates using a radioimmunoassay kit (New England Nuclear; Delaware, NE, USA).

Statistical analysis

Results were expressed as means±SEM. The statistical significance of differences between the groups was determined using Student's t-test.

RESULTS

2K1C hypertension

Table 1 shows the blood pressure and plasma renin concentrations in 2K1C rats. The blood pressure was increased, along with plasma renin concentrations. Accordingly, the clipped kidney showed an increased expression, and the contralateral kidney showed a decreased expression of both renin and COX-2 (Figs. 1, 2). The removal of the clip reversed the blood pressure and plasma renin concentrations to the control level. It also reversed the expression of both genes to the control level not only in the clipped kidney but also in the contralateral kidney.

DOCA-salt hypertension

The blood pressure was also significantly increased in DOCA-salt rats compared with that in the control (182±7 vs 124±7 mmHg, n=6 each, $p<0.01$). The plasma renin concentration was more pronouncedly decreased in the hypertensive compared with that in the control (3.1±0.3 vs 7.1±0.5 ngAI/mL/h, n=7 each, $p<0.05$). Accordingly, the expression of renin

Table 1. Systolic blood pressure (SBP) and plasma renin concentrations (PRC) before and after removal of the arterial clip in 2K1C rats

	Control	2K1C	2K1C-UC
SBP (mm Hg)	125±3	162±4**	128±4
PRC (ngAI/mL/h)	36.9±7.0	74.1±6.1*	45.4±6.4#

2K1C and 2K1C-UC denote the groups with their arterial clips intact and removed, respectively. Number of rats in each group was 6. *$p<0.05$, **$p<0.01$: compared with control. #$p<0.05$: compared with 2K1C.

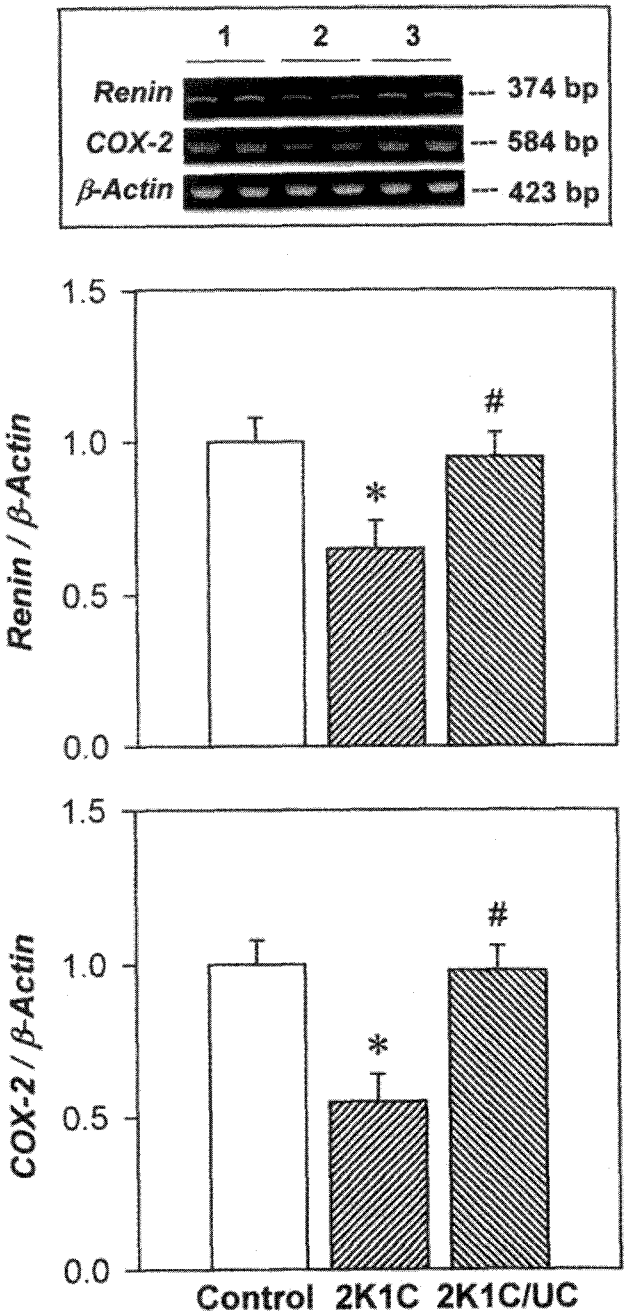

Fig. 1. The expression of renin and COX-2 mRNA in the clipped kidney in 2K1C rats. Fluorographs show ethidium bromide-stained agarose gels containing RT-PCR products. 1: control, 2: 2K1C (before unclipping), 3: 2K1C-UC (after unclipping). Each column shows mean±SEM of 6 experiments. *$p<0.05$, compared with control. #$p<0.05$, compared with 2K1C.

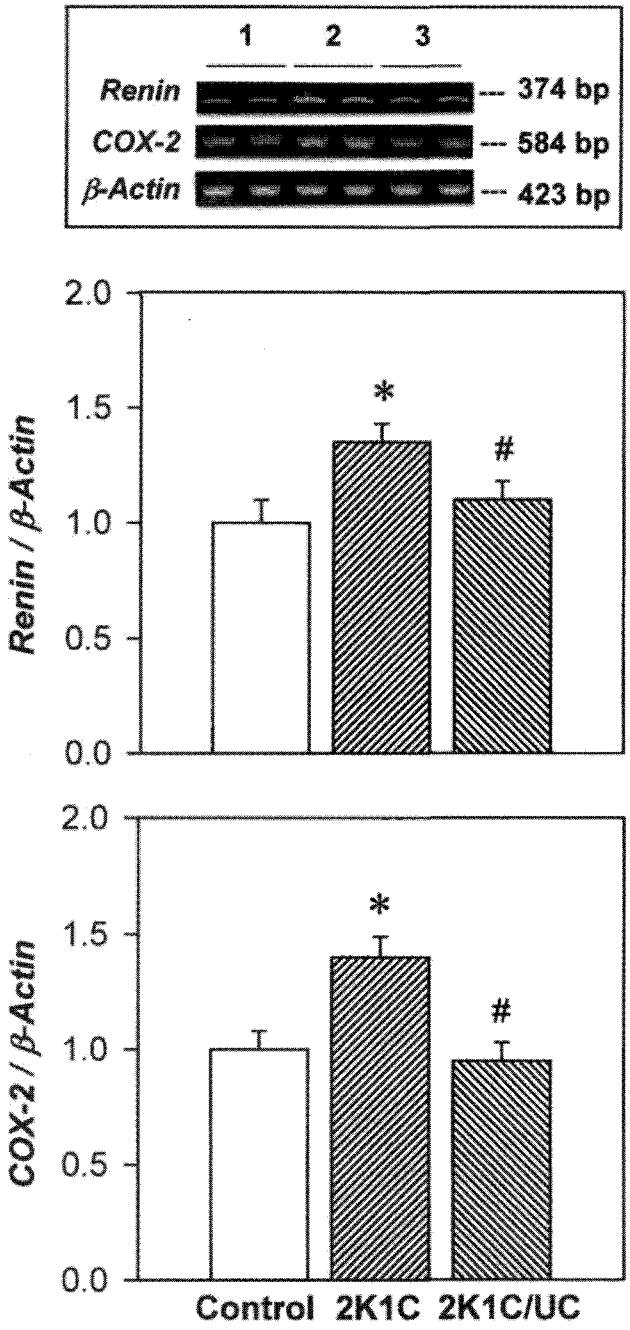

Fig. 2. The expression of renin and COX-2 mRNA in the contralateral non-clipped kidney in 2K1C rats. Legends as in Fig. 1.

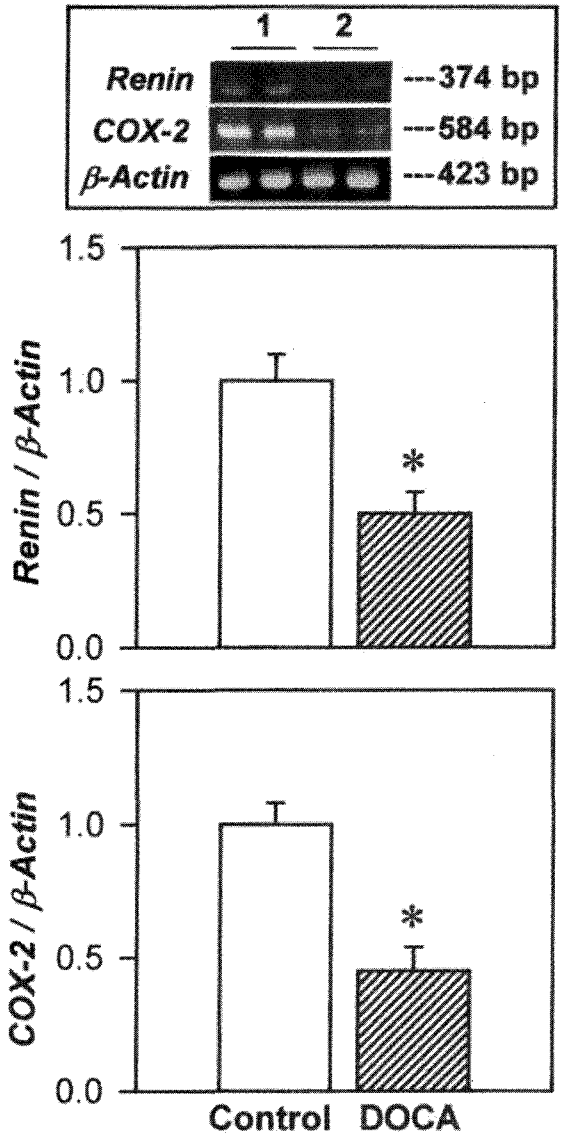

Fig. 3. The expression of renin and COX-2 mRNA in the kidney in DOCA-salt rats. 1, control; 2, DOCA-salt. Other legends as in Fig. 1.

as well as that of COX-2 was decreased (Fig. 3).

DISCUSSION

In 2K1C rats, the expression of renin and COX-2 genes was increased in the clipped kidney and decreased in the contralateral non-clipped kidney. Angiotensin II exerts an inhibitory effect on renin mRNA expression (Schricker et al, 1994). Accordingly, it is a negative regulator of cTALH/macula densa COX-2 expression via AT1 receptors (Cheng et al, 1999; Wolf et al, 1999; Harris et al, 2000). Taken together, an activation of renin-angiotensin system may feedback inhibit the COX-2 expression. The decreased COX-2 expression in the contralateral kidney may be a reflection of an increase of circulating angiotensin II levels. Because macula densa-derived prostaglandins are stimulators of renin synthesis and secretion,

inhibition of macula densa COX-2 by angiotensin II could form a novel indirect negative feedback control of the renin-angiotensin system.

The increase of COX-2 expression in parallel with renin expression in the clipped kidney is in line with a previous finding in which the number of juxtaglomerular apparatus positive for COX-2 increased in correlation with renin expression (Hartner et al, 1998). Unlike the contralateral non-clipped kidney, the increased COX-2 expression in the clipped kidney can not be related to angiotensin II. It has been shown that hypoxia increases the expression of COX-2 in human vascular endothelial cells in culture independent of other stimuli (Schmedtje et al, 1997). It has been further demonstrated that COX-2 is transcriptionally regulated by hypoxia in human vascular endothelium (Ji et al, 1998). During the early stage of experimental renovascular hypertension that is renin-dependent, COX inhibitors decrease plasma renin activity and ameliorate the hypertension (Jackson et al, 1981; Lin et al, 1991). It is likely that the acutely induced hypoxia in the clipped kidney increases transcription of COX-2 genes, and in turn renin genes. The speculation that COX-2-derived prostaglandins mediate the control of renin expression during hypoperfusion may be contradictory to one recent study, in which COX-2 blockers failed to affect the renin mRNA either in the clipped or in the contralateral kidney (Mann et al, 2001). The discrepancy between the studies remains to be explained.

A prolonged increase of angiotensin II may down-regulate the expression of COX-2 genes and eventually decrease the expression of renin also in the clipped kidney, resulting in an attenuation of the renin-dependency in the chronic phase of renovascular hypertension. Indeed, a blockade of renin-angiotensin system does not completely restore the blood pressure to the normal level, while removal of the clip further reduces the blood pressure in 2K1C hypertensive rats (Russell et al, 1982; Huang & Navar, 1983).

In the present study, the altered expression of renin and COX-2 was reversed, along with the blood pressure, to the control level by removal of the clip both in the clipped and contralateral non-clipped kidneys. The removal of the clip may primarily decrease the expression of COX-2 and as a consequence decrease the expression of renin. This is contradictory to a previous study that discarded a role for prostaglandins in the reversal of chronic renovascular hypertension (Russell et al, 1982). It is likely that there would be an altered interaction between COX-2 and renin in the chronic phase of 2K1C hypertension. The duration of hypertension may account for the discrepancy between the studies.

The present study also demonstrated that DOCA-salt hypertension was associated with a decreased expression of renin as well as that of COX-2. The cortical COX-2 mRNA levels decrease following a high-salt diet (Yang et al, 1998). Since the control rats were also on a high salt diet without DOCA, however, the altered expression of COX-2 and renin cannot be attributed to a high salt diet only, but rather to a volume expansion and hypertension.

In summary, it is suggested that renin and COX-2 genes are coordinately expressed in the kidney.

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