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Differential regulation of renal prostaglandin receptor mRNAs by dietary salt intake in the rat

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Differential regulation of renal prostaglandin receptor mRNAs by dietary salt intake.

Background. In this study, we tested the hypothesis that prostaglandin (PG) receptor expression in the rat kidney is subject to physiological regulation by dietary salt intake.

Methods. Rats were fed diets with 0.02 or 4% NaCl for two weeks. PG receptor expression was assayed in kidney regions and cells by ribonuclease protection assay and reverse transcription-polymerase chain reaction analysis. Functional correlates were studied by measurement of PGE_2-induced cAMP formation and renin secretion in juxtaglomerular (JG) cells isolated from animals on various salt intakes.

Results. EP1 and EP3 receptors were predominantly expressed, and the EP2 receptor was exclusively expressed in the rat kidney medulla. The EP4 receptor was strongly expressed in glomeruli and in renin-secreting JG granular cells. IP receptor transcripts were found mainly in cortex. Maintaining rats on a low- or high-NaCl diet did not affect the expression of EP1 or IP receptors, whereas EP4 transcripts in glomeruli were increased twofold by salt deprivation. Consistent with this, we found that PGE_2-evoked cAMP production and renin secretion by JG cells from salt-deprived animals were significantly higher compared with cells obtained from salt-loaded animals. In the outer medulla, EP3 transcripts correlated directly with salt intake, and mRNA abundance was increased twofold by a high-NaCl diet.

Conclusions. Our results suggest that subtype-specific, regional changes in PG receptor expression are involved in the renal adaptation to changes in salt intake. The results are in accord with the general concept that renocortical PGE_2 stimulates renin secretion and maintains renal blood flow during low-salt states, whereas medullary PGE_2 promotes salt excretion in response to a high salt intake.

Under physiological conditions, the mammalian kidney synthesizes and releases prostaglandins (PGs), a heterogeneous group of biologically active C-20 fatty acid derivatives. The major PGs synthesized by the vascular and tubular structures of the kidney are PGI_2 and PGE_2, respectively [1]. Numerous studies have established that PGE_2 plays an important physiological role by promoting salt excretion by the renal medulla [1]. This mechanism becomes particularly important during pathological states of salt or volume excess. A significant heterogeneity of PGE_2 production exists among kidney regions. In contrast to the medulla, synthesis of PGE_2 is markedly enhanced in the kidney cortex during loss of salt or extracellular volume [2]. Under these conditions, the use of antagonists of PG formation reveals that PGs protect renal blood flow from excess vasoconstrictor action and stimulate renin secretion from granular cells of the juxtaglomerular (JG) apparatus [1, 3, 4]. The differential regulation of PGE_2 formation by dietary salt is accomplished, at least in part, through the compartmentalized activity of the separately regulated isoenzymes cyclooxygenase (COX) 1 and 2 [5–7], which are rate limiting for renal PG synthesis. In addition, a multiplicity of specific receptors on target cells in the kidney determines the final physiological response to PGs. Several of these receptors have recently been cloned, and their organ distribution and second messenger coupling have been elucidated [8–13].

The existence of specific receptors offers an additional regulatory capacity in addition to the regulation provided at the level of PG production. Indeed, earlier data show a positive correlation between the number of PGE_2 receptor-binding sites in the outer medulla and dietary salt intake and thus are consistent with this proposal [14]. We therefore found it relevant to examine this interplay in greater detail. Because several subtypes of PGE_2 receptors (EP receptors) and a single PGI_2 receptor (IP receptor) have been identified and there are not yet highly specific agonists and antagonists available for each receptor subtype, we chose a molecular biological approach. We developed mRNA assays for EP and IP

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receptor subtypes and determined mRNA abundance in kidney regions and cells. It was of particular interest to obtain information on the receptor subtypes associated with the renal medulla and with the renin-secreting JG granular cells because these cells and tissues are the major targets for salt-sensitive PGE_2 production in the kidney *in vivo* [1, 3]. We found that EP1 and IP receptors did not change with altered Na intake, whereas EP3 and EP4 subtypes showed region-specific regulation by dietary salt load. cAMP-coupled EP4 receptor expression and function increased in glomeruli in response to a low Na intake, whereas cAMP-inhibitory EP3 receptors increased in the outer medulla after a high Na intake.

METHODS

In vivo protocols

Male Sprague-Dawley rats (150 to 200 g) had free access to standard rodent diet (NaCl content 0.5% wt/wt; Altromin, Lage, Germany) and to tap water ($N = 6$). In one series, rats were kept on a high-salt (HS) diet (4% Na wt/wt, $N = 8$), and another group of rats received an NaCl-deficient (LS) diet (0.02% Na wt/wt; $N = 8$) for 10 days. Animals were killed by decapitation. Blood was sampled in ethylenediaminetetraacetic acid (EDTA)-coated vials, and organs were rapidly removed, weighed, frozen in liquid nitrogen, and stored at −80°C. Kidneys were separated into major regions by dissection under a stereomicroscope and then snap frozen in liquid nitrogen. The medullary rays were contained in the cortical tissue, and no attempt was made to separate the outer and inner stripes of the outer medulla.

Plasma renin activity measurements

Plasma samples were incubated for 1.5 hours at 37°C. The generated angiotensin I (Ang I) was determined by a radioimmunoassay kit (Sorin-Biomedica, Düsseldorf, Germany).

Isolation and primary culture of juxtaglomerular granular cells

Juxtaglomerular granular cells were isolated as previously described in detail [15]. In brief, kidney tissue from six- to eight-week-old mice (C57Bl; Bomholtgaard, Silkeborg, Denmark) was mechanically dissociated with scalpel blades and was further digested with a trypsin/collagenase mixture. Because it has been shown that PG receptors are subject to homologous desensitization *in vivo* [16], all solutions and culture media contained 10 μM indomethacin to inhibit endogenous COX activity. The resulting cell suspension was filtered (22.4 μm filter pores), washed twice (800 × *g* for 7 min), and centrifuged (27,000 × *g* for 30 min at 4°C) in a 30% isosmotic Percoll density gradient (Pharmacia, Uppsala, Sweden). The cellular layer with a density of 1.07 g/ml was used for cell culture [15]. These cells were washed once and suspended in RPMI 1640 (Biochrom, Berlin, Germany) containing 0.66 U/ml insulin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2% fetal calf serum (FCS). Cells were seeded in 1 ml aliquots for cAMP measurements and RNA extraction or in 100 μl aliquots for renin secretion studies. The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air.

Mesangial cells

Mesangial cells were obtained from male Sprague-Dawley rats (80 to 100 g) by outgrowth from isolated glomeruli as previously described [17]. Only cells in primary culture were used for the experiments. The cells were usually confluent after two to three weeks and were then harvested for RNA extraction. Mesangial cells were incubated for 16 hours in the absence of fetal calf serum with 0.1% bovine serum albumin (BSA) prior to the harvest of RNA.

Renin secretion studies

Experiments on renin secretion from mouse JG cells were performed during 20 hours of incubation. The medium was collected and centrifuged at 10,000 × *g* at room temperature to remove cellular debris. The renin content of the remaining cells was released by the addition of 100 μl phosphate-buffered saline (PBS) with 0.1% Triton X-100 to each culture well, followed by vigorous shaking for 45 minutes. Cell lysates were spun at 10,000 × *g*, and the supernatants were saved and stored at −20°C. Secretion rates for active renin were estimated by the appearance rate of renin in the culture medium. To minimize differences among different cell culture preparations, renin secretion rates were presented as renin released per 10^6 cells (Coulter counter). Renin concentration was determined by the ability of the samples to generate Ang I from the plasma of bilaterally nephrectomized rats. Ang I was measured by radioimmunoassay (Sorin Biomedica).

Extraction of RNA

Total RNA was extracted from cell cultures and from tissue samples basically according to the acid-guanidinium-phenol-chloroform extraction protocol of Chomczynski and Sacchi [18]. Final RNA pellets were dissolved in diethylpyrocarbonate-treated water, and the yield of RNA was quantitated by measuring the optical density (OD) at 260 nm.

Reverse transcription

One microgram of total RNA, 1.5 μg yeast tRNA, and 0.5 μg oligo dT primer (GIBCO, Paisley, Scotland, UK) were heated at 94°C for three minutes in a volume of 8 μl. Then samples were cooled on ice, and each of the following components (in μl) was added: 4 deoxyri-

bonucleotides (each 2.5 mmol/liter); 4 reverse transcription (RT) buffer (supplied with the RT kit), 2 dithiothreitol (100 mmol/liter), 0.5 RNase inhibitor (40 IU/μl; Promega, Rødovre, Denmark), 0.5 BSA (20 mg/ml), and 1 reverse transcriptase (200 U/μl; GIBCO, Paisley, Scotland). Samples were incubated for one hour at 37°C, and the reaction was stopped by heating to 95°C for two minutes.

Polymerase chain reactions

cDNA sequences coding for nonhomologous regions of the receptor proteins (EP1, EP2, EP4, IP) were used for amplification by polymerase chain reaction (PCR). EP3 primers were copied from Takeuchi et al [9]. All primer pairs amplify rat and mouse cDNA equally effectively. To facilitate cloning, the oligonucleotides were synthesized with adapters containing restriction sites for Bam HI and Eco RI (data not shown).

EP1. Sense: 5′AAC TGC TTC GCC TCC TAC C′3; antisense: 5′AAC TAC GCA GTG AAC TGG C′3, covering bases 1162 to 1260 (mouse EP1 cDNA), 98 bp [10].

EP2. Sense: 5′TTC GGA GCA AAA GAA GCC′3; antisense: 5′GAG CGC ATT AGT CTC AGG′3, covering bases 725 to 1025, 301 bp [13].

EP3. Sense: 5′ GCT GTC TGT GCT CGC CTT′3; antisense: 5′CCA TAA GCT GGA TAG′3, covering bases 474 to 784 of rat EP3 cDNA, 310 bp [9]. The sequence is common to the two known rat mRNA splice variants of the EP3 receptor, rEP_{3A}, and rEP_{3B}, which are colocalized in the kidney [19].

EP4. Sense: 5′GGA AGA CTG TGC TCA GTA′3; antisense: 5′GAA GCA AAT TCT TGC CTC′3, covering bases 1007 to 1246 of rat EP4 cDNA, 240 bp (formerly known as the "EP2" receptor) [12].

IP. Sense: 5′CTC ATG ACA GGC ATC ATG′3; antisense: 5′ACA GAA CAG CCA TCA CCA′3, covering bases 814 to 1199 of rat IP cDNA, 385 bp [11].

β-Actin and renin. These primers were copied from [20] and [21], respectively. The renin sense primer spanned the exon 6/7 border of the renin gene and the actin primers spanned an intron.

For PCR (Perkin Elmer Cetus 480), the cDNA was supplied with (in μl) one of each primer (10 pmol/μl), two deoxyribonucleotides (2.5 mmol/liter), two PCR buffer, and water to a final volume of 20 μl. The samples were denatured at 95°C for five minutes followed by annealing at 65°C for five minutes, during which 1 U of Taq polymerase (Boehringer, Mannheim, Germany) was added. PCR amplification of EP2 and EP4 required the addition of 1 μl of $MgCl_2$ (25 mmol/liter) to the reaction mixture. In the PCR protocols, initial annealing temperatures were set at 5°C below T_m and were then increased by 1°C or 2°C per cycle until 60°C, which were used for the remaining cycles. Products were amplified for 26 to 30 cycles.

Cloning of IP and EP subtype cDNA sequences

Polymerase chain reaction (PCR) products were ligated into Bam/Eco polylinker sites of vector pSP73 (Promega) for heat shock uptake into competent *Escherichia coli* (DH5α; GIBCO) by standard methods. Insert sequences were verified by sequencing in both directions by using SP6 and T7 polymerases (Sequiserve, Deisenhofen, Germany).

Ribonuclease protection analysis for EP, IP, and for GAPDH mRNAs

Messenger RNA levels for EP and IP receptor and for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were measured by the Ribonuclease Protection Assay basically as described [6]. In brief, after linearization with Hin dIII, the plasmids yielded radiolabeled antisense cRNA transcripts by incubation with SP6 polymerase (Promega) and (α-^{32}P)GTP (Amersham, Birkerød, Denmark) according to the Promega riboprobe *in vitro* transcription protocol. Five $\times$ 10^5 cpm of the cRNA probes were hybridized with samples of total RNA at 60°C overnight in a final volume of 50 μl. Sequential digestions were performed with a mixture of RNase A/T1 (Boehringer) and proteinase K (Boehringer). The fragments were separated on 8% polyacrylamide gels, and radioactivity in the protected probe was assayed in a Phosphoimager (Packard, Downers Grove, IL, USA). Autoradiography was performed at −80°C for one to three days. Assays were validated to be linear at least in the range of 10 to 80 μg kidney total RNA. Thus, hybridization of the labeled cRNA probes with whole kidney total RNA resulted in significant bands with sizes of the hybrids that corresponded to the expected values (Fig. 1).

Measurement of cAMP

Juxtaglomerular granular cell suspensions were diluted 1:2 and seeded in 24-well plates in 1 ml aliquots. The cells were allowed to adhere for one hour, and then the medium was removed, and 1 ml RPMI with 0.5 mmol/liter of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) was added together with the agonist to be tested. After 10 minutes, the cells were placed on ice, and cAMP was harvested. cAMP was measured by a radioimmunoassay kit (Amersham-Biotrak, Birkerød, Denmark) as previously described [22]. Each experiment represents the mean of duplicate culture wells.

Statistics

When several sets of data were compared at the same time (for example, data from the three salt-intake

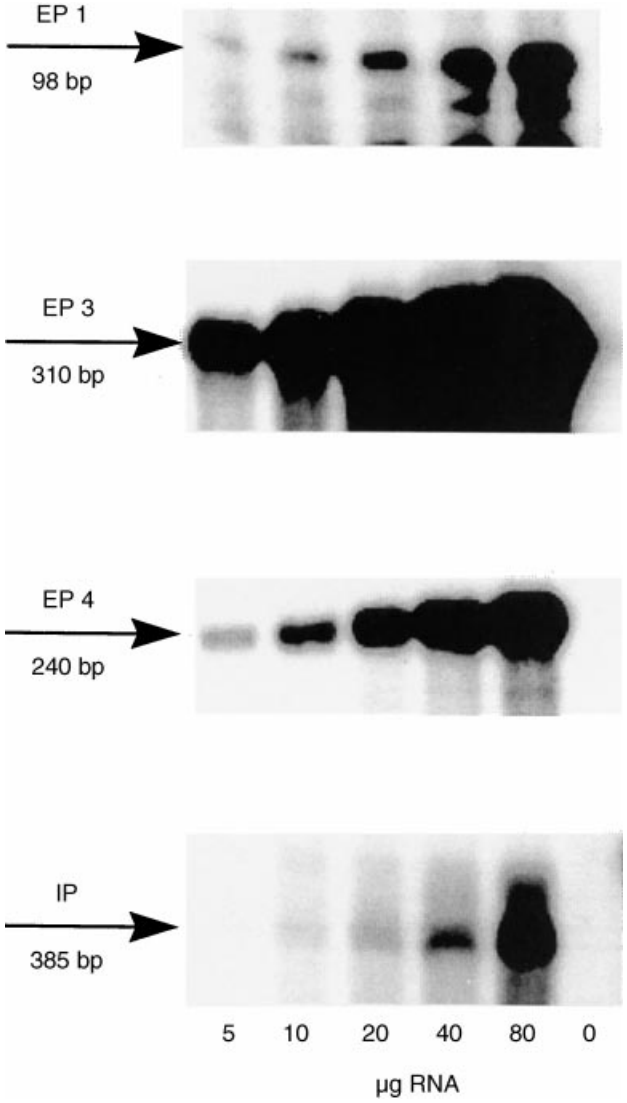


Fig. 1. Autoradiograph demonstrating protected cRNA probes for PGE_2-EP receptor subtypes and for the IP receptor. Kidney total RNA was hybridized with specific riboprobes. Hybridization was performed with 5, 10, 20, 40, and 80 μg total RNA, as indicated. The negative control was 20 μg yeast tRNA ("0"). The predicted size of each hybrid is indicated at the left.

groups), a one-way analysis of variance was used. If the one-way analysis of variance was significant at the 5% level, differences between data sets were established using 95% confidence intervals.

RESULTS

Control of salt diet

To confirm the efficiency of the salt diet, plasma renin activity (PRA) was determined. PRA changed inversely with salt intake as expected: In control rats, PRA was 8.6 ± 1.3 ng Ang I/ml × hr. In Na-loaded animals, it was 4.6 ± 0.5 ng Ang I/ml × hr, and in Na-depleted animals, PRA increased to 26.6 ± 0.9 Ang I/ml × hr. Values are mean ± SEM.

Table 1. Influence of salt intake on regional GAPDH mRNA abundance

NaCl%	Cortex	Outer medulla	Papilla
0.02	773 ± 70	925 ± 82	1057 ± 101
0.5	944 ± 46	905 ± 33	953 ± 113
4	925 ± 76	1028 ± 113	849 ± 208

All values are net counts per minute obtained with 2 μg total RNA; cortex $N = 10$, outer medulla and papilla $N = 5$. The control diet contains 0.5% NaCl. GAPDH mRNA abundance was not significantly altered by dietary salt content in any renal zone.

Distribution of EP and IP receptor mRNA expression in kidney regions

A/T1-ribonuclease protection assay and RT-PCR were used to determine the distribution of PG-receptor mRNAs (EP1, EP2, EP3, EP4, and IP) along the cortical-papillary axis of the rat kidney. The housekeeping gene GAPDH was not differentially expressed in kidney regions (control sodium diet; Table 1). GAPDH was therefore used as a standard internal control, and all comparisons between zones were made with GAPDH-normalized values. The abundance of the EP-subtype mRNAs exhibited great variation in the kidney. There was a marked cortical-papillary gradient for EP1 and EP3 receptor mRNAs. Thus, the ratio of cortex:outer medulla:papilla was 1:3:19 for EP1 mRNA and 1:7:0.7 for EP3 mRNA (Fig. 2A). Expression of the EP4 receptor was more evenly distributed with a ratio of 1:1.6:1.6 (Fig. 2A). Because of low abundance, the expression of the EP2 receptor was examined only by RT-PCR. A significant amplification product was obtained after 30 cycles when whole kidney RNA was used as a template in the RT-PCR (Fig. 2B). Notably, the amplification products for the EP2 receptor were localized in the outer and inner medulla, with no signals detected in the cortex (Fig. 2B). Actin was abundantly detected in all zones (Fig. 2B). The IP receptor was consistently detected in the kidney cortex with signals at the limit of detection in the renal medulla (Fig. 2A).

Isolated glomeruli expressed EP4 receptor mRNA at fourfold higher levels compared with kidney cortex (Fig. 3). EP1 receptors were also significantly expressed in glomeruli, whereas only weak signals for EP3 and IP receptors were observed (Fig. 3).

By correcting for the variable content of labeled nucleotide in the riboprobes, a rough estimate of the relative quantities of receptor mRNA in each region can be obtained. In kidney cortex and outer medulla, EP3 mRNA dominated and was 6- and 16-fold more strongly ex-

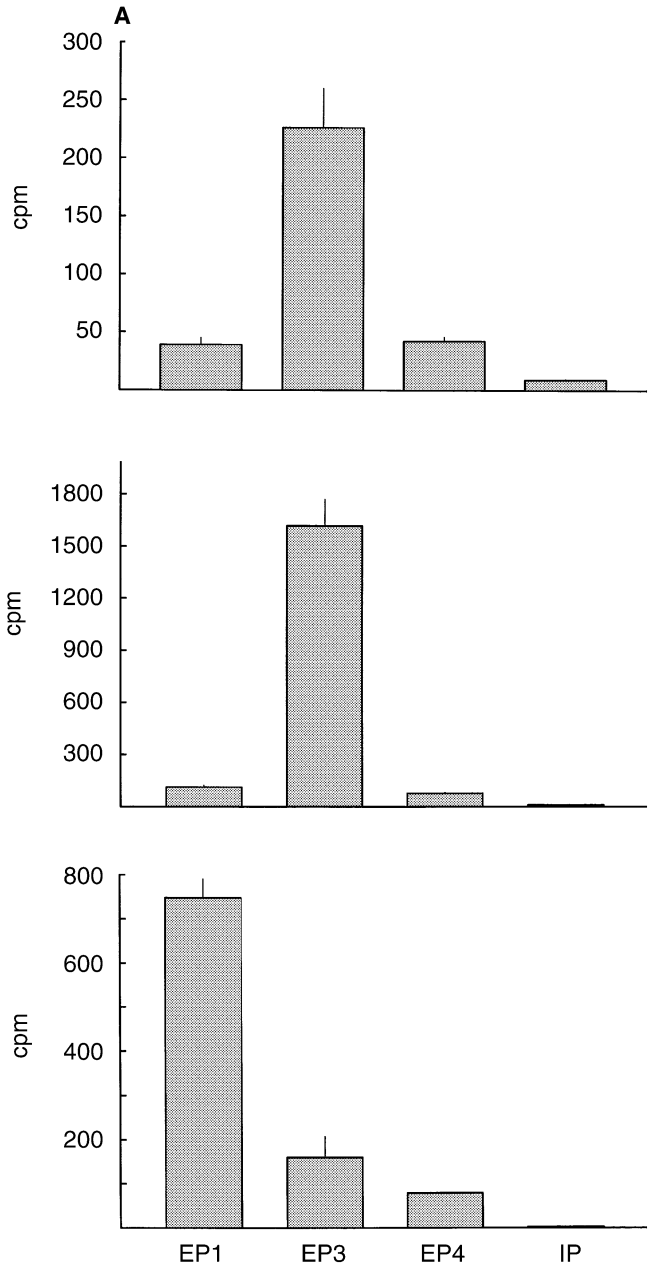
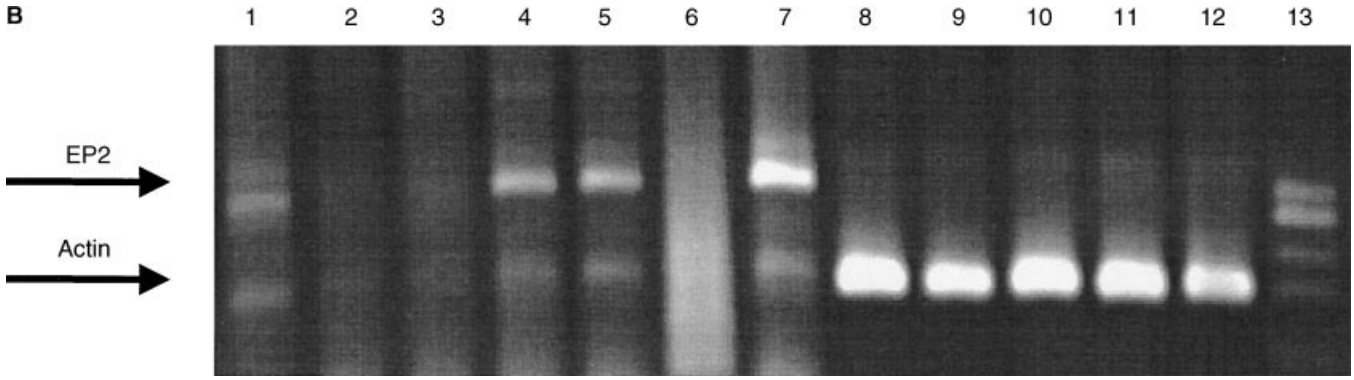


Fig. 2. Demonstration of IP and EP receptor mRNAs in kidney zones of Sprague-Dawley rats by ribonuclease-protection assay. (*A*) Comparison of EP1, EP3, EP4, and IP receptor mRNA abundance in renal cortex (upper), outer medulla (middle), and papilla (lower). Note the different scales on the *y* axes. Hybridization was performed with five separate RNA preparations. Values are mean ± SEM. (*B*) Expression profile of EP2 receptor mRNA in kidney regions as determined by RT-PCR. cDNA from the following tissues was amplified: Lane 1, whole kidney; lanes 2 and 3, kidney cortex; lanes 4 and 5, outer and inner medulla, respectively; lane 6, negative control, H_2O; lane 7, positive control, spleen; lanes 8 through 12, same template cDNAs as in lanes 1 through 5. β-actin was amplified; lane 13: molecular weight standard (ϕX174 RF DNA/*Hae* III). Ethidium bromide stained 2% agarose gel.



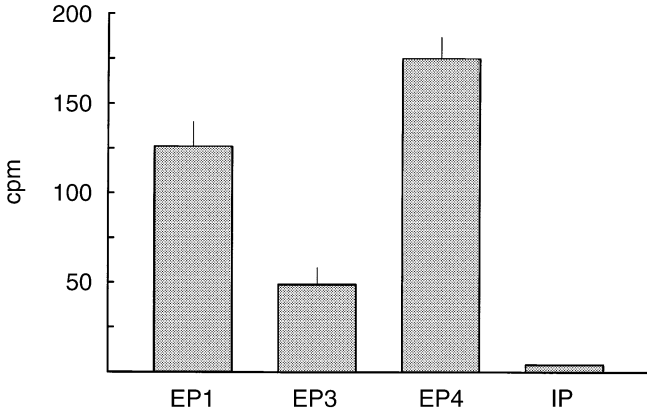


Fig. 3. Localization of EP and IP receptor mRNAs in rat glomeruli. Four separate preparations of glomeruli were used for isolation of RNA. Twenty micrograms of total RNA were used for each hybridization reaction. Values are averages ± SEM.

pressed, respectively, than EP1 and EP4, which were approximately equally abundant (Fig. 2A). In the papilla, EP1 was highly expressed at levels eightfold higher than EP4 and EP3. Thus, the renal medulla is dominated by EP1 and EP3 receptors, whereas the predominant receptor in the JG region is the cAMP-coupled EP4 subtype.

Expression of EP and IP receptor mRNAs in cultured renal cells

In order to investigate the EP receptor expression profile in glomerular cell types, total RNA was obtained from primary cultures of mouse JG granular cells and mesangial cells. EP and IP receptor expression was assessed by RT-PCR. In JG granular cells, cDNA amplification products for the EP1, EP3, and EP4 subtypes were observed, whereas no products for the EP2 or the IP receptor were found (Fig. 4). In comparison with kidney cortex, the signal for the EP3 receptor was much weaker in JG cells, whereas EP1 and particularly EP4 receptors appeared to be abundant. At a cDNA-dilution of 1:100, only EP4 transcripts were regularly observed, suggesting that this receptor subtype is the most abundant in JG granular cells. In primary cultures of mesangial cells, we most consistently detected EP4 receptor transcripts (data not shown).

Effect of dietary NaCl intake on EP-receptor mRNAs in kidney regions

Kidney regions from rats that received a high- or a low-NaCl diet were examined for changes in PG-receptor mRNA expression. Significant effects of the salt diet were detected in the outer medulla, where salt intake correlated directly with EP3 mRNA abundance (Fig. 5). Thus, the EP3 message increased twofold in the outer medulla following a high salt intake. In glomeruli, there

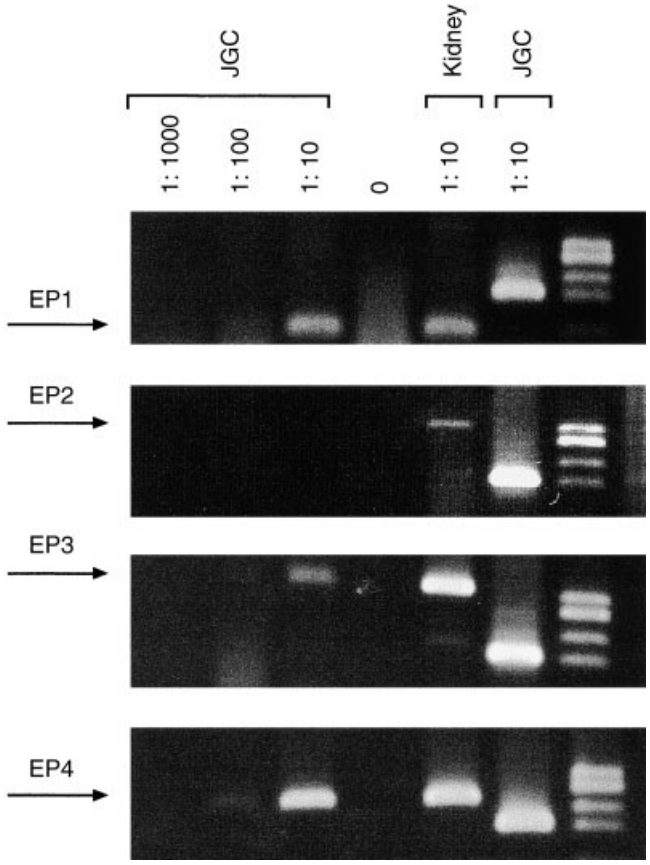


Fig. 4. Reverse transcription-polymerase chain reaction (RT-PCR) analysis demonstrating the presence of EP receptor mRNA in juxtaglomerular (JG) granular cells. RT-PCR was performed with three independent RNA preparations. "JGC" represents diluted cDNA from cultured JG granular cells. Negative control with water added instead of cDNA is seen in lane "0." Positive control (cDNA from whole kidney) is shown in lane "Kidney." Renin was amplified in every JG-cell cDNA sample tested. The band representing renin is seen in the last lane before the weight standard (ϕX174 RF DNA/*Hae* III). Ethidium bromide stained 2% agarose gels.

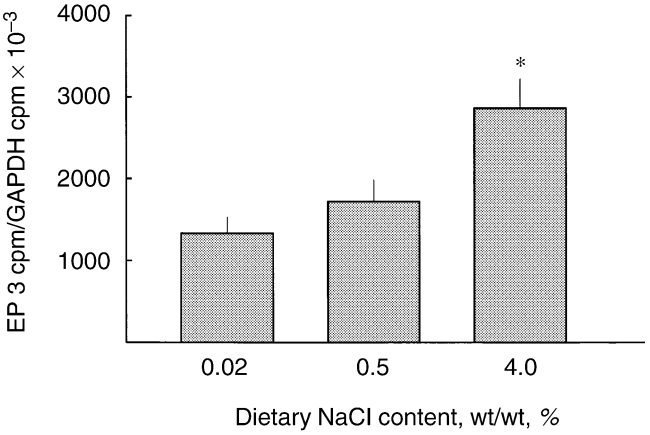


Fig. 5. Effect of salt intake on EP3 receptor mRNA in the rat kidney outer medulla. Values are means ± SEM of six separate mRNA determinations per condition. *Significant difference between high-salt diet and control estimated by 95% confidence interval.

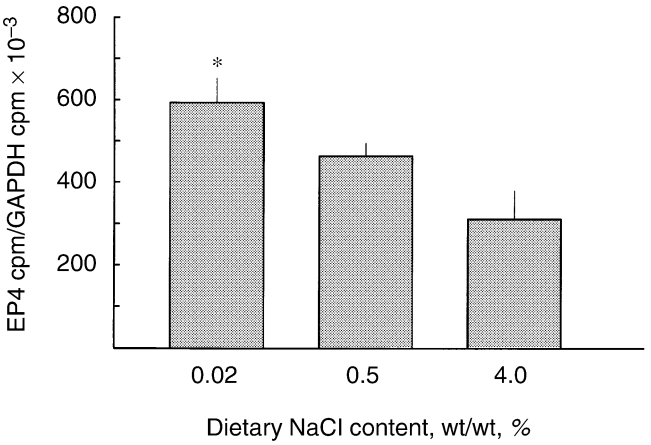


Fig. 6. Effect of salt intake on EP4 receptor mRNA in glomeruli isolated from rat kidney. Values are means ± SEM of six separate mRNA determinations per condition. *Significant difference between low- and high-salt diet groups, as estimated by the 95% confidence interval.

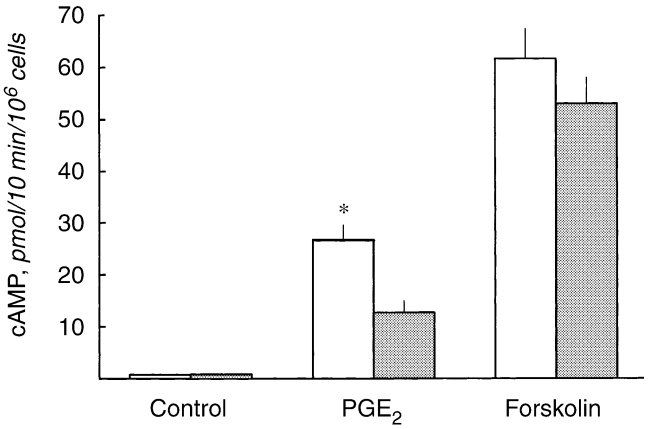


Fig. 7. Effect of prostaglandin E2 (PGE_2) and forskolin on cAMP-production in juxtaglomerular (JG) granular cells obtained from animals on a chronic high (■) or a chronic low (□) salt intake. Data are means ± SEM of three independent cell preparations, with two wells assigned per condition in one experiment. *Significant difference between low- and high-salt diet groups as estimated by a 95% confidence interval.

was an inverse relationship between salt intake and mRNA abundance of the dominant subtype EP4. Thus, EP4 expression was stimulated twofold by a low-sodium intake compared with a high-salt intake (Fig. 6). EP1 and IP receptor mRNAs were not influenced by salt load in any of the kidney regions tested. Dietary salt intake had no effect on mRNA levels for the housekeeping gene product GAPDH (Table 1). GAPDH mRNA was therefore used as an internal standard, and data are presented as receptor mRNA/GAPDH mRNA ratios. Thus, there was a subtype-selective localized regulation of mRNAs for EP receptors in kidney zones.

Effect of the salt diet on PGE_2-mediated responses in juxtaglomerular granular cells

To validate that the measured change in EP4-receptor mRNA in glomeruli was relevant at the functional level, we examined the impact of dietary salt intake on PGE_2-induced formation of cAMP in renin-secreting JG granular cells. Primary cultures of granular cells from sodium-loaded and sodium-depleted mice were established, and cAMP formation in response to PGE_2 was compared (Fig. 7). There was no significant difference in cell yield from the two experimental groups, but the cells obtained from the sodium-depleted animals contained 2.5-fold more active renin (676 ± 40 vs. 269 ± 23 ng Ang I/10^6 cells × hr × ml). Under control conditions (in the continuous presence of the phosphodiesterase inhibitor IBMX), there was no significant difference in basal cAMP levels between cells from animals on different Na intakes (Fig. 7). The formation of cAMP was markedly stimulated by the incubation with PGE_2 (10 μM) for 10 minutes in both groups of freshly isolated JG granular cells. However, in the low-Na^+ series, there was a significantly greater cAMP production (35-fold increase of basal cAMP) compared with cells from the high-Na^+ series, where PGE_2 increased cAMP 15-fold above control level. The response to 10 μM forskolin, which directly activates adenylyl cyclase, was not different between the two groups. This finding is consistent with the hypothesis of salt-induced down-regulation of glomerular and JG granular cell PGE_2-EP4 receptors.

The significance of these observations was evaluated at the level of renin secretion in parallel experiments with JG granular cells from salt-loaded and salt-deprived animals. In this series, we tested an array of cAMP-dependent agonists of renin secretion. The tested agonists significantly stimulated renin release compared with control in both the low- and high-salt series (Fig. 8). There was a significant increase in sensitivity of the JG granular cells to stimulation by PGE_2 under sodium-deficient conditions (2.7-fold stimulation above control) compared with sodium-loaded conditions (1.6-fold stimulation). The sensitivity of renin release to another receptor-dependent agonist, isoproterenol, was also markedly enhanced by a low-salt diet (twofold stimulation at high salt vs. 4.2-fold above control at low salt). There was a less marked, but significant, difference in the secretory responses to the receptor-independent agonists forskolin and IBMX. Thus, dietary salt seems to induce a general change in sensitivity of the cAMP-dependent pathway of renin secretion, which could involve more than simple changes in specific receptors.

DISCUSSION

The aim of this study was to investigate the distribution and regulation by dietary salt of PG receptor mRNAs

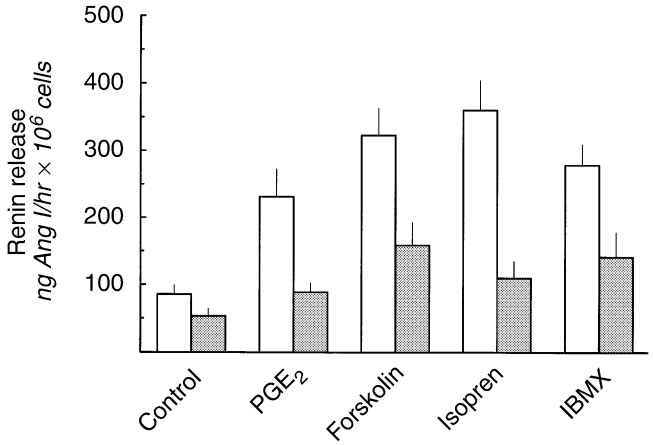


Fig. 8. Action of cAMP-dependent agonists on renin secretion from cultures of juxtaglomerular (JG) granular cells established from salt-depleted (□) and salt-loaded (■) animals. Data are means ± SEM of five independent cell preparations at each salt diet. Four wells were assigned per condition in one experiment. Abbreviations are: isopren, isoprenaline; IBMX, 3-isobutyl-1-methylxanthine.

in the rat kidney. We found significant expression in the kidney of all of the PG receptors examined (EP1–EP4 and IP), and there was a distinct spatial distribution of each receptor between kidney regions. Ca^{2+}-coupled EP1 receptors and cAMP inhibitory EP3 receptors were localized predominantly in the kidney medulla, and the EP2 receptor was exclusively expressed in this region. The cAMP-coupled EP4 receptor subtype was strongly expressed in glomeruli. This distribution is consistent with previous data on renal EP1, EP3, EP4, and IP receptor mRNAs obtained by *in situ* hybridization of kidney tissue from mouse [23, 24], rat [9], rabbit [25], and human [26]. On the contrary, EP2 receptor expression has not been detected in human [26], mouse [27], or rat [13] kidney by Northern blot or by *in situ* hybridization techniques. By more sensitive RT-PCR, we and Nemoto et al found a significant expression in the rat kidney of the EP2 receptor [28]. Our results suggest that EP2 expression is restricted to the kidney medulla. The precise localization of the cAMP-coupled EP2 and EP4 receptors in the medulla is not known, but it is interesting that these receptors are expressed in the medulla along with cAMP-inhibitory EP3 receptors. PGE_2 has been shown to dilate isolated vasa rectae significantly [29], so it is reasonable to speculate that these EP subtypes could play a relevant role for the control of medullary blood flow, whereas the EP3 receptor is confined mainly to the collecting ducts [9, 23, 26].

Among the receptors tested, only the EP3 and the EP4 isoforms were regulated by dietary salt intake. Transcripts for the EP4 receptor were detected in isolated glomeruli and in JG granular cells and mesangial cells. In agreement with this observation, PGE_2 potently stimulates cAMP formation in all of these structures [4, 22, 30, 31]. This presence of functional cAMP-coupled PGE_2 EP4 receptors in the glomerular area is physiologically important, particularly during conditions of volume contraction, where renal blood flow and renin secretion become increasingly PG dependent [1, 3]. Our data on glomerular EP4 mRNA suggest that there is a selective stimulation of this receptor subtype by dietary salt deprivation. Thus, in addition to the documented increase in the formation of PGE_2 in the JG area during salt deprivation [2, 32], an up-regulation of EP4 receptors is likely to contribute to the physiological adaptation of the JG apparatus to a chronic low-salt intake. Consistent with this idea, we found that short-term cAMP formation in response to PGE_2 was increased in JG granular cells harvested from salt-deprived animals compared with cells from salt-loaded animals. This finding appeared to have functional consequences because in experiments with cultured JG granular cells, there was a comparable enhancement in the sensitivity of renin secretion from these cells to PGE_2. Altogether, these findings are compatible with the hypothesis that an inverse relationship exists between dietary salt load and functional EP4 receptors on JG cells. In addition, cAMP-mediated renin release by receptor-independent agonists was similarly enhanced by a low-salt diet, so there seems to be a general increase in sensitivity of renin release to the cAMP pathway during sodium-deficient states. These findings are consistent with previous observations *in vitro* [33] and *in vivo* [34], where a low-sodium diet enhances the effect of sympathetic stimuli on renin release.

The EP3 receptor was the most abundant EP subtype mRNA in the kidney and was expressed in the outer medulla at levels that far exceeded those of other EP receptors. In agreement, PGE_2 binds predominantly to the outer medulla as assessed by autoradiography, and the maximal binding capacity of PGE_2 to isolated membranes from the outer medulla exceeds sevenfold to eightfold that of cortical membranes [35]. This ratio is similar to the present EP3 ratio between cortex and outer medulla at the level of mRNA. *In situ* hybridization experiments have localized EP3 transcripts in cortical and medullary parts of the thick ascending limb and in the collecting ducts in the outer medulla but not in the papilla [9, 23, 26]. We noted a weak expression of EP3 receptors in the papilla. However, only minute amounts of tissue from the outer medulla in the dissected tissue from the papilla could have caused this signal and could be a likely explanation for the discrepancy.

The maximal binding of PGE_2 to isolated membranes from outer medulla has previously been found to increase significantly in response to a chronic salt load [14]. We detected a quantitatively similar increase in EP3 subtype mRNA following a high-salt intake, which was limited to the outer medulla, whereas no other PG recep-

tor tested changed in response to dietary salt in this region of the kidney. It is, therefore, reasonable to assume that the receptor subtype involved in the NaCl-dependent increase of PGE_2 binding sites is EP3. Together, the data suggest that a localized regulation of the EP3 receptor is an integral part of the physiological adaptation that takes place in the kidney medulla to maintain salt balance during a dietary salt challenge. The adaptation further includes a stimulation of COX II mRNA, protein, and activity [2, 5–7]. At the nephron segments where EP3 receptors are expressed, it has been directly demonstrated that PGE_2 inhibits the production of cAMP [36]. This leads to an inhibition of salt and water reabsorption [37]. The resulting EP3-mediated natriuretic action of PGE_2 is particularly relevant in certain pathological states where inhibition of PG synthesis can result in edema formation and hypertension [38].

In summary, we found a region-specific regulation of PGE_2 EP3 and EP4 receptor expression by dietary salt intake in the rat kidney. cAMP-coupled EP4 receptors increased in glomeruli in response to a low-Na intake. This was reflected by an enhanced cAMP production and renin secretion in response to PGE_2 from JG granular cells harvested from salt-deficient animals compared with JG granular cells from salt-loaded animals. cAMP-inhibitory EP3 receptors increased in the outer medulla after a high-Na intake. We propose that zone-specific changes in PGE_2 receptor expression are involved in the renal adaptation to changes in dietary salt intake.

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