Rat Kidney Thromboxane Receptor: Molecular Cloning, Signal Transduction, and Intrarenal Expression Localization

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Abstract

Thromboxane (TX) plays important roles in control of renal hemodynamics and water and electrolyte metabolism, and is involved in the pathophysiology of many renal diseases. The aim of the present study is to isolate a rat kidney cDNA encoding functional TX receptor, and to reveal its intrarenal expression localization. A clone (rTXR2) was isolated from a rat kidney cDNA library by a homology screening approach. rTXR2 was shown to encode the amino acid sequence containing seven transmembrane spanning domains representing rat (r) TX receptor. The membrane from COS-7 cells transiently transfected with rTXR2 cDNA was shown to be specifically bound by a thromboxane receptor antagonist, SQ29548. Either in *Xenopus* oocyte expression or in transfected COS-7 cells, rTX receptor was shown to be linked with Ca^{2+} messenger system. TX receptor–mediated increase in cytosolic Ca^{2+} was also observed in cultured glomerular mesangial cells. In situ hybridization showed that rTX receptor mRNA was detected in renal glomeruli, smooth muscle cells in renal arterioles, and transitional cell epithelium of renal pelvis. Reverse transcription linked to PCR applied to microdissected nephron segments indicated the presence of rTX receptor mRNA exclusively in the glomerulus. In conclusion, we have cloned a functional rat kidney TX receptor, which is expressed specifically in renal glomerulus, arterial smooth muscle cells, and transitional cell epithelium of renal pelvis. The present study will provide important insights into the etiology and pathophysiology of renal diseases with relation to TX metabolism. (*J. Clin. Invest.* 1995. 96:657–664.) Key words: in situ hybridization • reverse transcription-PCR • calcium signal transduction • nephron segments • renal pelvis

Introduction

Thromboxane (TX)[1] A_2 is a metabolite of arachidonic acid, which evokes smooth muscle constriction and platelet aggregation (1). In the kidney, TXA_2 has been shown to decrease renal blood flow (2) and glomerular filtration rate (3), and to potentiate tubuloglomerular feedback (4), thus involved in the control of renal hemodynamics and water and electrolyte metabolism. TXA_2 is also implicated in the etiology or pathophysiology of many renal diseases such as nephritis (5–7), allograft transplantation rejection (8), and urinary tract obstruction (9). Blockade of TX synthase activity or TXA_2 receptor binding has been shown to prevent exacerbation of renal injury caused by these diseases (9–13).

Biological action of TX is exerted via its specific receptor present on the cell surface (1). Recently, TX receptor cDNA has been isolated from human placenta (14), human endothelial cells (15), or mouse lung (16). The receptor structure contains seven transmembrane domains characteristic of G protein–coupled receptors, and the functional study has indicated that this receptor is linked with inositol trisphosphate (IP_3)/Ca^{2+} signal transduction. The receptor mRNA is expressed mainly in the brain, thymus, heart, liver, spleen, uterus, and in the kidney. In isolated renal glomeruli (3, 17), cultured renal glomerular mesangial cells (18, 19), and epithelial cells of urinary bladder (20), TX has previously been shown to induce an increase in intracellular free calcium ($[Ca^{2+}]i$). These observations suggest that in the kidney there may be a functional TX receptor which is coupled to Ca^{2+} messenger system. The structure of kidney TX receptor, however, has not been revealed. We, therefore, decided to isolate kidney TX receptor cDNA and its possible isoform. Using this cloned cDNA, we examined signal transduction of the receptor, and expression of mRNA in the kidney either by in situ hybridization or by reverse transcription linked to PCR (RT-PCR) applied to microdissected nephron segments.

Methods

Materials. λZAPII phage vector and pBlueScript II were from Stratagene, Inc. (San Diego, CA). Thermus aquaticus (*Taq*) polymerase, T7 RNA polymerase and restriction enzymes were obtained from Takara Shuzo, Co., Ltd. (Kyoto, Japan). Hybon N^+ blotting membranes and β-Max film were from Amersham Corp. (Arlington Heights, IL). [^{3}H]-SQ29548 was from Dupont-New England Nuclear (Boston, MA). Oligonucleotides were synthesized by Sawady, Inc. (Tokyo, Japan). An expression vector, pcDNAI/Amp, was obtained from Invitrogen (San Diego, CA). NTB2, from Eastman Kodak Co. (Boston, MA). Fura-2/

Portions of this study were presented at the 26th Annual Meeting & Exposition for the American Society of Nephrology, 14–17 November 1993 in Boston, MA; at the 15th Biennial Scientific Meeting of the International Society of Hypertension, 20–24 March 1994 in Melbourne, Australia; and have appeared in abstract form (1993. *J. Am. Soc. Nephrol.* 4:481. and 1994. *J. Hypertens.* 12:S66.).

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Received for publication 27 June 1994 and accepted in revised form 13 April 1995.

J. Clin. Invest.

0021-9738/95/08/0657/08 $2.00
Volume 96, August 1995, 657–664

1. *Abbreviations used in this paper:* ang, angiotensin; $[Ca^{2+}]i$, intracellular-free calicium; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription PCR; rTX, rat thromboxane; TX, thromboxane.

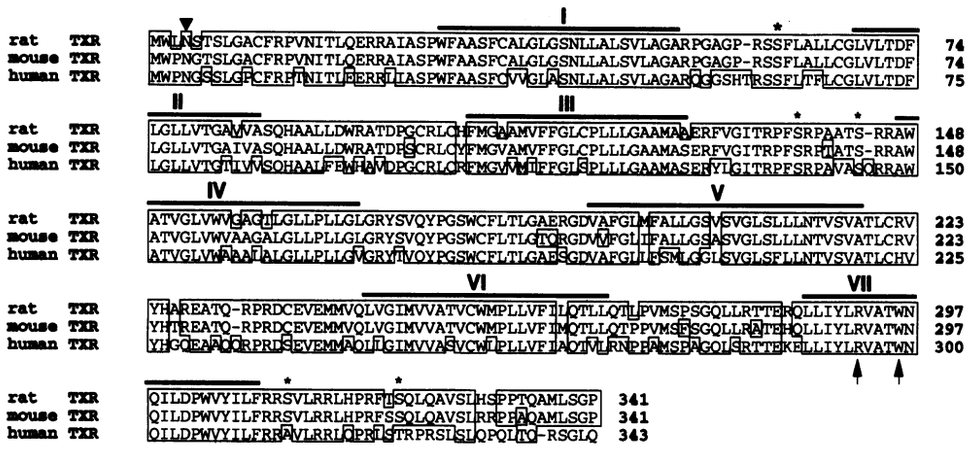

Figure 1. Amino acid sequence comparison of rat, mouse, and human thromboxane A_2 receptor (TXR). The three sequences are aligned with single letter notation by inserting gaps (−) to achieve maximum homology. The seven putative transmembrane segments (*I–VII*) were assigned on the basis of hydrophobicity analysis. The termini of these segments are tentatively defined. Other marks are as follows: triangle, potential *N*-glycosylation site; stars, possible phosphorylation sites. Arg-292 and Trp-296 are indicated by arrows. The nucleic acid sequence coding rat TXR has been deposited in the DDBJ/GenBank™/EMBL databases under accession number D21158.

AM was obtained from Dotite (Kumamoto, Japan). Ionomycin was from Calbiochem-Behring Corp. (San Diego, CA). U46619 and SQ29548 were from Cayman Chemical Co. Inc. (Ann Arbor, MI). Angiotensin (ang) II from Peptide Institute (Osaka, Japan). KW3635 (sodium(E)-11-[2-(5,6-dimethyl-1-benzimidazolyl)-etylidene]-6,11-dihydro-dibenz-[b,e]oxepin-2-carboxylate-mono-hydrate) (21, 22) was a gift from Kyowa Hakko Kogyo, Co., Ltd. (Tokyo, Japan). cDNA encoding mouse thromboxane receptor (15) was a gift from Dr. S. Narumiya, Kyoto University School of Medicine, Kyoto, Japan. cDNA (Ca18b) encoding rat angiotensin II AT_{1a} receptor (23) was provided by Dr. T.J. Murphy, Emory University School of Medicine, Atlanta, GA.

cDNA cloning. cDNA library was constructed from fractions containing more than 1-kb pair cDNAs with λZAPII vector as previously described (24). Three hybridization positive clones were finally isolated, and rescued into pBlueScript SK(−) with helper phage R408. The cDNA inserts of these clones showed an identical restriction enzyme digestion pattern, and one clone (rTXR2) was chosen for further sequence analysis. The sequence was determined in both directions by dideoxy chain-termination method.

Electrophysiological analysis in Xenopus oocytes. Electrophysiological analysis in *Xenopus* oocytes was performed by a previously reported method (24). Briefly, the prTXR2 clone was linearized with NotI. Capped mRNA (50 ng) was synthesized with T7 RNA polymerase, and injected into *Xenopus* oocytes. Whole cell currents were measured by a two microelectrode voltage clamp method.

Cell culture. COS-7 cells were cultured with DME supplemented with 10% FBS and penicillin–strepomycin (GIBCO-BRL, Gaithersburg, MD) in a humidified condition with 5% CO_2 in air at 37°C. Rat glomerular mesangial cells were obtained by a previously reported method (25, 26), and cultured with RPMI/L40 (GIBCO-BRL) with 10% FBS and penicillin–streptomycin. Passages six to nine were used for the experiments.

Transient expression in COS-7 cells. EcoRI cDNA fragment of rTXR2 (~ 1.8 kb) or rat vascular angiotensin II AT_{1a} receptor cDNA (~ 2.2 Kb) were subcloned into pcDNAI/Amp, designated pcDNAI-rTXR2, or pcDNAI-AT_{1a}, respectively. The plasmid for transfection was prepared by alkaline lysis of bacterial cultures, and the supercoil plasmid DNA was obtained by cesium chloride gradient. 25 μg of pcDNAI-rTX2 and 25 μg of pcDNAI-AT_{1a} was cotransfected into COS-7 cells cultured on coverglass by the calcium phosphate method as previously reported (27). pcDNAI-AT_{1a} is used as a positive control for transfection in analysis of calcium signal transduction.

Binding analysis. Receptor binding analysis was performed using the COS-7 cells transiently expressed with pcDNAI-rTXR2 (25 μg) by the previously reported method (14, 15, 28).

Measurement of intracellular calcium with fura-2/AM. Cytosolic-free calcium was measured with fura-2/AM in transfected COS-7 cells cultured on coverglass by a modification of previously reported methods (29–31) with a fluorometer (CAF-100; Nihon-Bunko Co., Ltd., Tokyo, Japan) at excitation wavelength of 340 nm and emission wavelength of 500 nm.

In situ hybridization. In situ hybridization was performed by a previously reported method (24, 28) with a 850-bp BglII–BglII fragment of radiolabeled cRNA. The hybridized sections were exposed to β-Max film for 28 d. For microscopic analysis, the sections were dipped into NTB2 diluted 1:1 with distilled water, developed after 12 w exposure, and then counterstained with hematoxylin and eosin.

RT-PCR using microdissected nephron segments. Microdissection (32) and RT-PCR with the microdissected nephron segments were performed by previously reported methods (33, 34). Briefly, nephron segments were from glomerulus, proximal tubule, cortical thick ascending limb of Henle's loop, and cortical collecting duct. To digest genomic DNA, mRNA samples from nephron segments were treated with RNase-free DNase I (Sigma Chemical Co., St. Louis, MO). To distinguish PCR amplification of contaminated genomic DNA, samples without reverse transcription were also prepared. To amplify the rat TX receptor cDNA fragment, the following oligonucleotides were used as primers: 5′-TGGACTGGCGTGCCACTGAT-3′ (sense primer, position 275–294) and 5′-AGCAAGGGCATCCAACACACCGTG-3′ (antisense primer, position 753–776). A fragment of rat cDNA for ubiquitous glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also amplified with the same samples simultaneously in different tubes with the following oligonucleotides (GAPDH primers): 5′-TCCCTCAAGATT-GTCAGCAA-3′ (from position 506 to 525 and 5′-AGATCCACA-ACGGATACATT-3′ (position 795–814) (35). Moreover, RT-PCR was also applied to examine mRNA expression of rat angiotensin II AT_{1a} receptor along nephron segments. The primers for AT_{1a} receptor cDNA amplification were: 5′-TCCTGTTCCACCCGATCACCG-ATCA-3′ (sense primer, position −230 to −206, located in the second exon) and 5′-CTTCAGCAGAAGAGTTAAGGGCCAT-3′ (antisense primer, position 1 to 25, located in the third exon) (23, 27). For PCR amplification, total PCR volume was 50 μl which contained 50 pmol of each primers, 1.25 mM deoxynucleotide mixture, 2.5 U *Taq* DNA polymerase, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (wt/vol) gelatine. PCR was carried out by the following protocol: 1 min of denaturation at 94°C, 2 min of annealing at 60°C, and 2

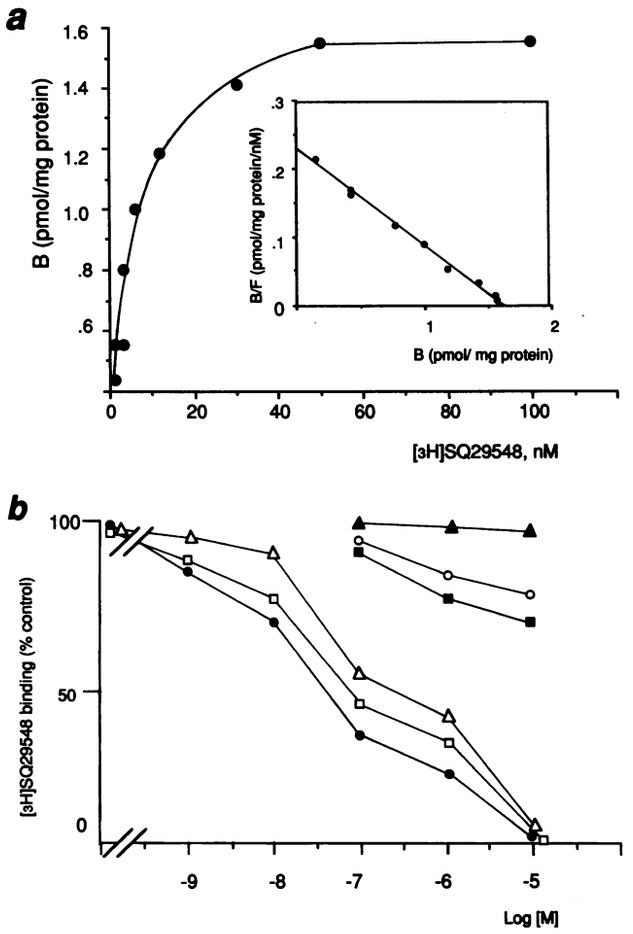

Figure 2. Binding characteristics of rTX receptor. *a* shows saturation of the specific binding of the TXA_2 antagonist [^{3}H]SQ29548 to membranes from COS-7 cells transiently transfected with pcDNA-rTXR2. Membrane (100 μg) of COS-7 cells were incubated with several concentrations of [^{3}H]SQ29548. *b* shows displacement of the binding of [^{3}H]-SQ29548 with other prostaglandins or TXA_2 mimetics. Displacement of [^{3}H]SQ29548 with unlabeled SQ29548, (●); U46619, (□); KW3635, (▲); PGE_2, (■); $PGF_{2\alpha}$, (○); PGD_2, (△). The results shown here are representative of three similar experiments with each point indicating the mean of four determinations.

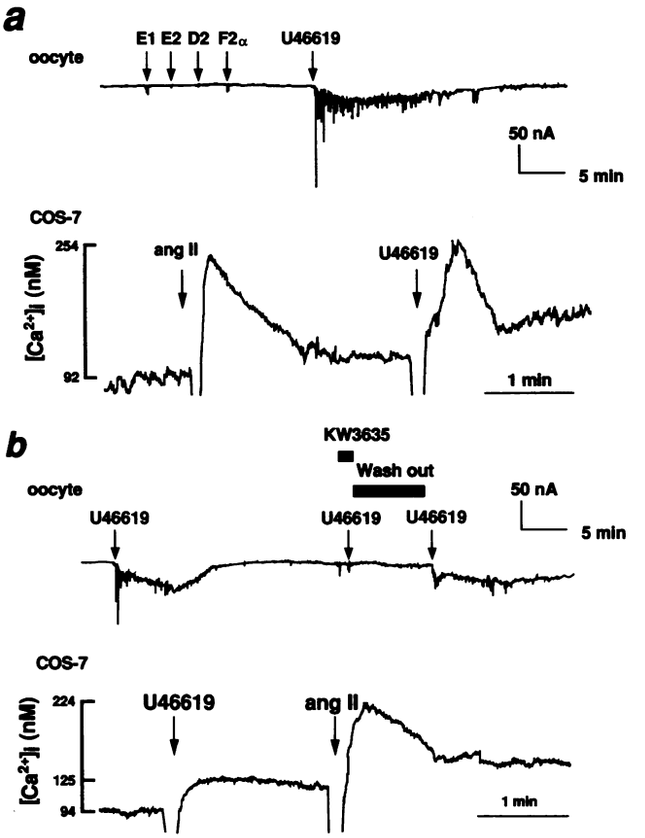

Figure 3. Functional expression of rTXR2 in COS-7 cells and *Xenopus* oocytes. Upper figures in Fig. 2, *a* or *b* show traces of currents recorded from oocytes injected with the in vitro synthesized mRNA from rTXR2. Response to U46619 (0.1 μM) was recorded under voltage clamp at −60 mV after 2 d incubation of the oocyte injected with ~ 20 ng of the rat rTXR2 mRNA. Downward deflection indicates inward currents. Lower figures represent changes in intracellular Ca^{2+} ($[Ca^{2+}]i$) measured by fura-2 method in monolayer of COS-7 cells which were co-transfected with rTXR2 and ang II AT_{1a} receptor cDNA. U46619 and ang II were sequentially added. Each result was representative of three reproduced observations. U46619, 1 μM; ang II, 10 nM; KW3635, 0.1 mM. The experiment was performed in the absence (*control*) (*a*) or in the presence of KW3635 (*b*) Maximum (F_{max}) and minimum fluorescence (F_{min}) were obtained by addition of 2 μM ionomycin and subsequent addition of 2.5 mM EGTA, respectively.

min of extension at 72°C, for 40 cycles. The PCR products were resolved in a 1.5% agarose gel, transferred to a Hybond N^+ nylon membrane, and the membrane was hybridized with radiolabeled rTXR2 cDNA in a solution containing 50% formamide, 5× SSC, 5× Denhardt's solution, 1% SDS at 42°C overnight. The membrane was then washed with a solution containing 0.1× SSC and 0.1% SDS at 65°C, and exposed to a film for 12 h.

Molecular biology procedures used in the present experiments were according to the standard protocols (36), unless otherwise indicated.

Results

Receptor structure. Fig. 1 shows the predicted amino acid sequence of rTXR2. The sequence consists of 341 amino acids with calculated molecular weight of 36925.71. Hydropathy analysis (37) has shown eight hydrophobic segments in the protein structure. The first one is located at the amino terminus, and may indicate a signal peptide (38). The following hydrophobic segments may form the structure of seven transmembrane domains characteristic of G protein–coupled receptors. The amino acid sequence of rTXR2 is highly homologous to human placenta (75.7% identity) (14) or mouse TX receptor (92.7% identity) (16) (Fig. 1), and the clone was considered to encode rat TX (rTX) receptor. In the rat TX receptor, Arg-292, which has been suggested to be important for ligand binding (14), and Trp-296, which has been shown to allow a definite discrimination between agonist and antagonist (39), are conserved. One potential *N*-glycosylation site (40), and five serine residues (Ser-61, -138, -144, -311, and -322) for possible phosphorylation in the cytoplasmic regions (41) are assigned.

Receptor binding assay. The membrane of transient transfectant revealed saturating [^{3}H]SQ29548 binding. Scatch-

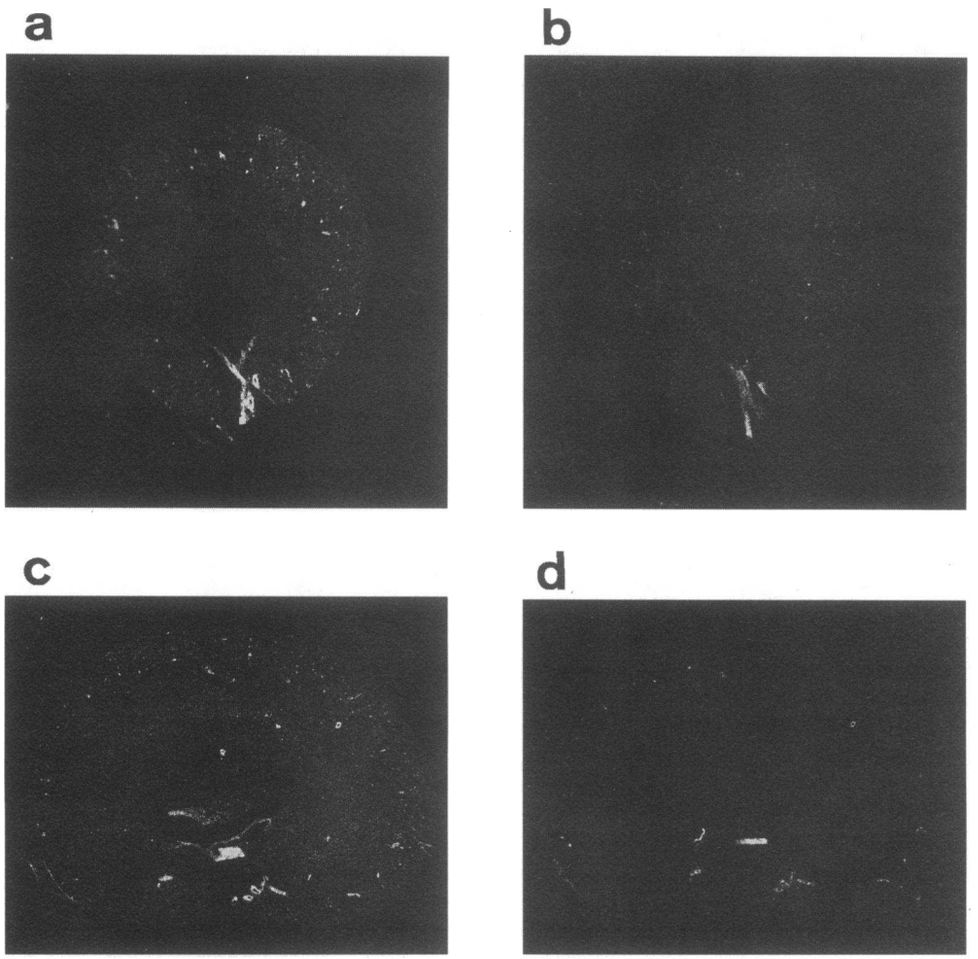

Figure 4. In situ hybridization of rat kidney TX receptor mRNA in the kidney. Negative film images of coronal sections of rat kidney *a* and *b* and sagittal sections *c* and *d* are shown. White regions indicate mRNA expression (*b* and *d*) displacement of radiolabeled cRNA by an excess of unlabeled cRNA.

ard analysis revealed a single class of binding site with B_{max} of 1.4 pmol/mg protein and K_d value of 1.1 nM (Fig. 2 *a*). The binding with [^{3}H]SQ29548 was displaced with an unlabeled SQ29548 (TX receptor antagonist), U-46619 (TX receptor agonist), or KW-3635 (TX receptor antagonist) with 50% inhibitory concentration of 0.48, 0.95, or 1.28 × 10^{-7} M (n = 4), respectively (Fig. 2 *b*). The binding, however, was not significantly inhibited by PGE_2, $PGF_{2\alpha}$, or PGD_2. These binding characteristics are in good agreement with those of human and mouse TX receptors (14, 16).

Signal transduction. Signal transduction of cloned rTX receptor was investigated by either *Xenopus* oocyte expression or transient expression in COS-7 cells. In the *Xenopus* oocyte–injected with rTX receptor mRNA, U46619 induced potent and long-lasting currents characteristic of the receptors linked with IP_3/Ca^{2+} signal transduction (42) (Fig. 3 *a, top*). Application of PGE_1, PGE_2, PGD_2 or $PGF_{2\alpha}$ (0.1 mM each) had no effect on the oocyte. Consistent with this observation, in COS-7 cells transfected with both rTX and AT_{1a} receptor cDNAs, U46619 (1 μM) induced an increase in cytosolic free calcium ([Ca^{2+}]i) (from 96±8 to 240±18 nM, n = 5), and ang II (10 nM) also induced an increase in [Ca^{2+}]i. (Fig. 3 *a, bottom*). Application of either U46619 (1 μM) or ang II (10 nM) did not change [Ca^{2+}]i in COS-7 cells without transfection (data not shown). We next examined the effect of a TX receptor antagonist, KW3635. As shown in Fig. 3 *b* (*top*), in the oocyte, KW3635 markedly inhibited U46619-induced currents. After washing out KW3635, currents were recovered. In COS-7 cells transfected with both rTX and AT_{1a} receptor cDNAs, pretreatment with KW3635 (0.1 mM) inhibited U46619 (1 μM)–induced increase in [Ca^{2+}]i, whereas the increase in [Ca^{2+}]i by ang II (10 nM) was not inhibited (Fig. 3 *b, bottom*). Thus, coupling of rTX receptor to calcium signal transduction system was confirmed.

In situ hybridization. As shown in Fig. 4, *a* and *c*, mRNAs for rTX receptor (*white dots*) were detected principally in the renal cortex. Interestingly, remarkable expression of mRNA was also observed in the renal pelvis. Specific hybridization was confirmed by displacement of signals with an excess of unlabeled cRNA (Fig. 4, *b* and *d*). Microscopic examination has shown that rTX receptor mRNA is present at glomeruli, and arterial walls (probably at smooth muscle cells) in the cortex region (Fig. 5, *a* and *b*). In the renal pelvis, rTX receptor mRNA was observed in the transitional cell epithelium (Fig. 5 *c*).

Localization of rTX receptor mRNA along nephron segments. In the experiments, mRNA samples was treated with RNase-free DNase I to digest genomic DNA which might possibly be contaminated. Moreover, specific RT-PCR amplification of mRNA was distinguished by the results obtained by treatment

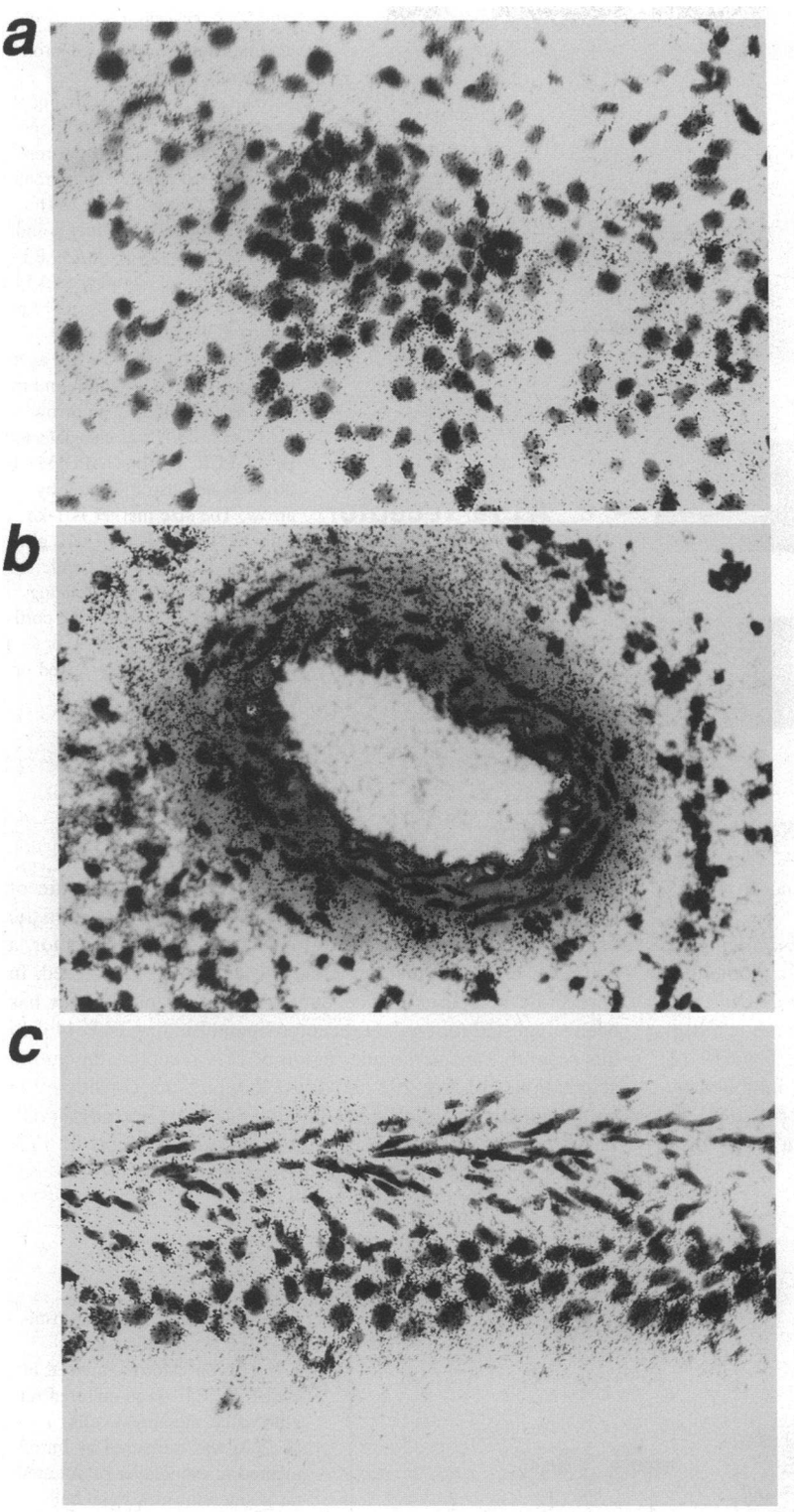

Figure 5. Cellular localization of rat TX receptor mRNA. Bright-field photographs of emulsified-dipped sections of the kidney in in situ hybridization. The sections were counterstained by hematoxylin and eosin. (*a*) glomerular region; (*b*) renal arteriole, (*c*) renal pelvis. Dotted black grains indicate mRNA expression.

or no treatment with reverse transcriptase. PCR product with expected size was only detected in glomerulus sample treated with RT (+), and this product was hybridized with a radiolabeled rTX receptor cDNA (Fig. 6 *a*). On the other hand, RT-PCR product was not detected either in samples without treatment with RT (−) or in RT (+) samples of proximal tubule, cortical thick ascending limb of Henle's loop, and cortical collecting duct. Ubiquitous GAPDH mRNA was expectedly detected in all RT (+) tubule samples (Fig. 6 *c*). Different from the expression of rTX receptor, PCR product for AT_{1a} receptor mRNA was detected in every samples with reverse transcription (Fig. 6 *b*). Samples without RT (−) gave no AT_{1a} receptor

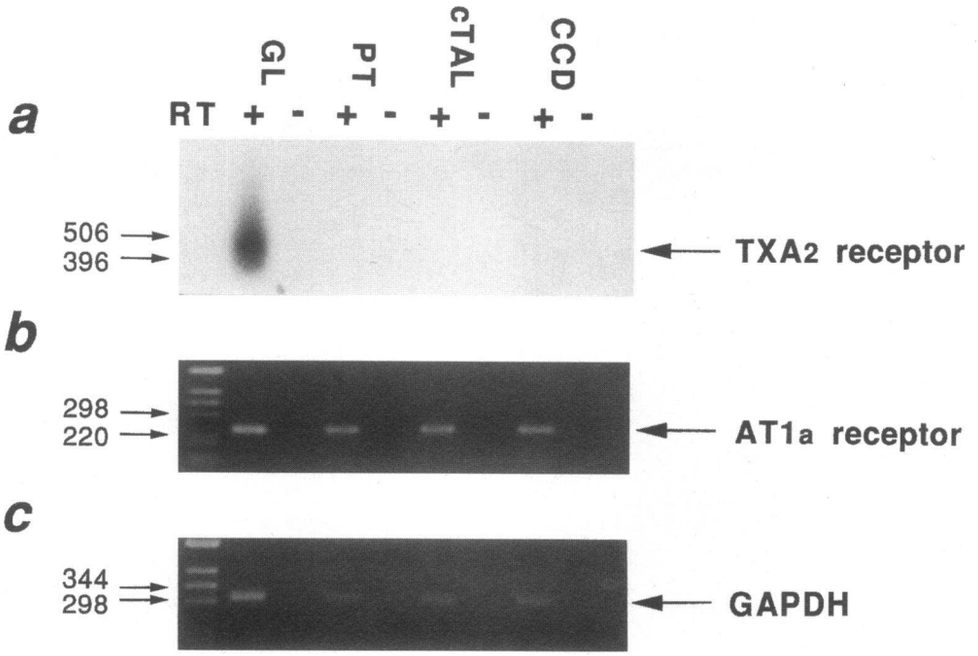

Figure 6. Distribution of rat TX receptor mRNA along nephron segments. RT-PCR was performed with microdissected nephron segments. (*a*) The 502-bp PCR product in a 1.5% agarose gel was analyzed by Southern blot with radiolabelled rTXR2. The TX receptor PCR product is indicated by an arrow. *b* shows RT-PCR analysis of angiotensin AT1a receptor mRNA. 255-bp AT1a receptor PCR products (*white bands*) are shown in a 1.5% agarose gel stained with ethidium bromide as indicated by an arrow. GAPDH mRNA was also detected by RT-PCR. 308-bp GAPDH PCR products are also indicated by an arrow. The size marker is 1-kb ladder (GIBCO-BRL). GL, glomerulus; PT, proximal tubule; cTAL, cortical thick ascending limb of Henle's loop; CCD, cortical collecting duct. RT (+) or (−) represents the samples treated or untreated with reverse transcriptase, respectively.

PCR product. Thus, the results indicate that rTX receptor is exclusively localized to the glomerulus, whereas AT_{1a} receptor is widely distributed along nephron segments distinct from rTX receptor expression.

Calcium signals in cultured rat glomerular mesangial cells. To confirm functional rTX receptor in the renal glomerular cells, rat glomerular mesangial cells were cultured and calcium signal was examined in the cells. Consistent with the result in functional expression with cloned rTX receptor, U46619 (1 μM) induced an increase in $[Ca^{2+}]i$, which was inhibited by KW3635 (0.1 mM) (Fig. 7, *a* and *b*). Ang II (10 nM) (a positive control) also induced an increase in $[Ca^{2+}]i$, which was not affected by KW3635 treatment.

Discussion

Rat kidney TX receptor is suggested to have the structure of seven transmembrane spanning domains, which is characteristic of G protein–linked receptors (Fig. 1). In this rTX receptor, a couple of characteristic amino acid structures are conserved. In adrenergic receptors, the seventh transmembrane domain has been suggested to regulate receptor-ligand binding (43, 44). In this seventh transmembrane region of rTX receptor, the amino acid residues of Arg-292 and Trp-296 which are considered to be crucial for specific ligand binding (14, 39) are conserved. The amino acid structure of third cytoplasmic loop of rTX receptor, which is suggested to be important for G protein cou-

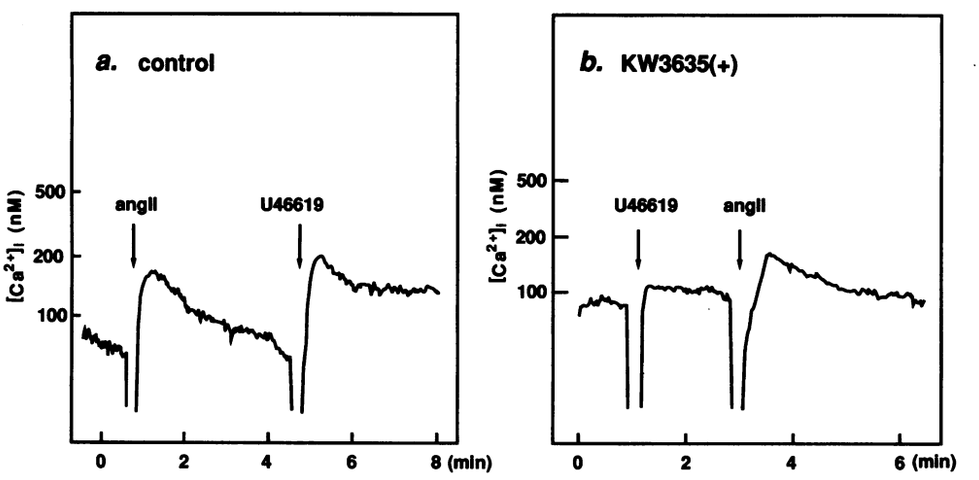

Figure 7. Effect of a nonprostanoic TX receptor antagonist KW3635 on U46619-induced increase in $[Ca^{2+}]i$ in cultured rat glomerular mesangial cells. $[Ca^{2+}]i$ was measured by fura-2 method in monolayer of cultured rat glomerular mesangial cells. U46619, 1 μM; KW-3635, 0.1 mM; ang II, 10 nM. Stimulants were sequentially added at the points indicated by arrows in the absence (*control*) (*a*) or presence of KW3635 (0.1 mM) (*b*), respectively. Each result was representative of three reproduced observations.

pling (45), is also highly conserved. These conserved amino acid residues or domains may determine ligand specificity and/or selective interaction with a G protein. In agreement with the previous report, the ligand binding study demonstrated the specific binding of the rat kidney TX receptor to TX mismatches as shown in Fig. 2.

In platelets, TX receptor has been shown to be linked to IP_3/Ca^{2+} signal transduction pathway (46). In the present study, oocytes injected with rTX receptor mRNA showed currents indicating Ca^{2+}-induced Cl^- currents (Fig. 3). Moreover, we have demonstrated an increase in $[Ca^{2+}]i$ by a TX mimetic U46619 in COS-7 cells transfected with cloned rTX receptor cDNA. Thus, consistent with the previous reports (14–16, 24, 46), rat kidney TX receptor is suggested to be linked with IP_3/Ca^{2+} signal transduction system.

TX receptor linked with Ca^{2+} signal transduction has been shown to be present in rat renal glomerular mesangial cells (17, 18). In the present study, we also confirmed the presence of functional receptor in rat mesangial cells (Fig. 7). Similar to the result in cultured rat vascular smooth muscle cells (47), KW3635 specifically inhibited U46619-induced increase in $[Ca^{2+}]i$. Moreover, consistent with the results of binding study, we have also shown that U46619-induced increase in $[Ca^{2+}]i$ is inhibited by KW3635 in COS-7 cells transfected with cloned rTX receptor cDNA (Fig. 3). Both in situ hybridization and RT-PCR applied to microdissected nephron segments have indicated the localized expression of rTX receptor mRNA to the glomerulus (Figs. 4–6). The presence of rTX receptor mRNA was also observed in smooth muscle cells of renal arterioles (Fig. 5). It is, therefore, suggested that the same TX receptor is expressed in mesangial cells as well as in vascular smooth muscle cells. Our observations, however, do not exclude a possible isoform in the glomerulus proposed by pharmacological studies (19), although we did not find a cDNA different from rTXR2.

By RT-PCR applied to microdissected nephron segments, we detected rTX receptor mRNA in the sample from glomeruli treated with reverse transcriptase, while no PCR product was detected in other nephron segments (Fig. 6 *a*). Using the same sample, we also detected a GAPDH PCR product consistently in every nephron segments only when treated with reverse transcriptase. This observation indicates that the targeted mRNA was specifically amplified, and contamination of genomic DNA was neglected. Moreover, we examined expression of mRNA for ang II AT_{1a} receptor. Since the primers used in the experiment are derived from separate exons (27), the PCR product of expected size indicates the product specific to AT_{1a} receptor mRNA. As shown in Fig. 6 *b*, AT_{1a} receptor mRNA is expressed in most nephron segments including glomerulus, consistent with other reports (34, 48). Thus, both rTX receptor and AT_{1a} receptor are shown to be expressed in the glomerulus, and these results have suggested that these vasoconstrictor hormone receptors mediate constriction of glomerular mesangial cells via Ca^{2+} messenger, and are involved in control of glomerular filtration rate by affecting ultrafiltration coefficient (K_f). In renal tubules, however, we did not detect rTX receptor mRNA expression by either in situ hybridization or RT-PCR (Figs. 4–6). So far, it has not been clarified that TX receptor with a biological action is present at renal tubules. We, however, do not absolutely deny the existence of rTX receptor in tubules because the sensitivity of our RT-PCR procedure might not be enough. Analyses using antibody against rTX receptor are required to further appraise the expression of rTX receptor along nephron segments.

Different from the mRNA expression of TX receptor, AT_{1a} receptor mRNA was detected in every nephron segments. Tubular function of AT_{1a} receptor is little known except the effect of ang II on sodium metabolism in proximal tubules (49). In other nephron segments, no conclusive study on tubular ang II response has been reported. As ang II has recently been shown to be a growth factor in the kidney (50), tubular AT_{1a} receptor may possibly be involved in cell growth of renal tubules or its related effects. In any events, we observed the differential expression between AT_{1a} and rTX receptor mRNA along nephron segments suggesting tissue (tubulus)–specific expression of vasoactive hormone receptors. Comparative analysis between 5′-flanking transcription regulatory region of AT_{1a} receptor gene (27) and that of rTX receptor gene (the structure analysis is under progress) may possibly clarify a molecular mechanism of the differential expression.

In the transitional cell epithelium of renal pelvis, we detected mRNA for TX receptor significantly (Figs. 4 and 5). It has been reported that there is a functional TX receptor linked with Ca^{2+} signal transduction pathway in epithelial cells of toad urinary bladder, and the TX action is shown to modulate the response to vasopressin (20, 51). The expression of TX receptor in the renal pelvis may possibly be involved in this function of TX receptor in the urinary bladder.

In summary, we have cloned the functional rat kidney TX receptor, which is linked with IP_3/Ca^{2+} signal transduction. In situ hybridization has shown that TX receptor mRNA is present in smooth muscle cells of renal arterioles, glomeruli, and transitional cell epithelium of the renal pelvis. RT-PCR with microdissected nephron segments has shown that rTX receptor mRNA is exclusively present in the glomerulus probably in mesangial cells. Distinct from TX receptor, AT_{1a} receptor is expressed in most of renal tubules, indicating differential tissue-specific expression of vasoactive receptors. The expression of rTX receptor in the transitional cell epithelium of renal pelvis may provide a new insight into the functional role of TX receptor in the kidney. The present observations are important for further examination of the role of TX receptor involved in the physiology or pathophysiology of kidney.

Acknowledgments

We are thankful to Dr. Kazuhiro Murakami, Department of Pathology I, Tohoku University School of Medicine, for technical assistance in in situ hybridization, and to Dr. Mitsuhiko Yanai, Department of Pharmacology I, Tohoku University School of Medicine, for advice in receptor binding assays.

This study is supported in part by a grant-in-aid (No. 06671124) from the Ministry of Education, Science, and Culture, Japan (K. Takeuchi), and a grant from the Takeda Foundation for Research on Metabolic Disorder, Japan (K. Takeuchi). T. Abe is a recipient of fellowship from the Japan Society for the Promotion of Science for Junior Scientists.

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