Expression of the Dopamine D₃ Receptor Protein in the Rat Kidney

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Abstract—The dopamine D₃ receptor subtype was identified in rat kidney using both light microscopic immunohistochemistry and electron microscopic immunocytochemistry. Antipeptide polyclonal antisera were directed to both extracellular and intracellular regions of the native D₃ receptor. Selectivity of the antipeptide antisera was validated by their ability to recognize native receptor protein expressed in permanently transfected mouse LTK² cells or Spodoptera frugiperda (Sf⁹) cell membranes. Light microscopic immunohistochemical staining for the D₃ receptor was observed only in the cortex. Specific staining was present in proximal and distal tubules, cortical collecting ducts, glomeruli, and renal vasculature. Immunostaining was observed predominantly in the apical portion of both the proximal and distal tubules. Renal arterial staining was prominent in the medial and adventitial layers. Electron microscopic immunocytochemistry revealed immunogold particles in arteriolar smooth muscle cells of the renal vasculature. In proximal and distal tubules and cortical collecting duct, immunogold staining was localized to apical portions of tubule cells. D₃ receptor immunogold staining in the glomeruli was clearly present in podocytes. Western blot analysis demonstrated a single D₃ receptor band in infected Sf⁹ cell membranes, in transfected LTK² cells, and in kidney and brain but not in noninfected Sf⁹ cell membranes or in D₂ or D₃ receptor transfected or nontransfected LTK² cells. The use of receptor subtype–selective antibodies allows for the tissue localization of specific dopamine receptors that are not distinguished by current pharmacological or ligand-binding technology. The rat kidney expresses the D₃ receptor at sites previously deemed to have D₂-like receptors. (Hypertension. 1998;32:886-895.)

Key Words: dopamine receptors, D₃ receptor, kidney immunocytochemistry glomerulus

The dopaminergic system depends on the interaction of dopamine with several specific receptors.¹ In the late 1970s, 2 dopamine receptors, D₁ and D₂, were identified in the brain.² These receptors were characterized pharmacologically with D₁-specific agonists and antagonists. Dopamine receptors subsequently were localized in peripheral tissues such as blood vessels, kidney, and adrenal cortex by ligand-binding studies and biochemical responses. Because structural similarities between central and peripheral dopamine receptors were indeterminate, a separate nomenclature for peripheral dopamine receptors was adopted: D₁-like and D₂-like.³ D₂-like receptors are located both presynaptically and postsynaptically, with the presynaptic receptor inhibiting catecholamine release from sympathetic neurons.³,⁴

In recent years, multiple dopamine receptor subtypes have been cloned and purified. Two D₁-like receptor subtypes have been identified (D₁A and D₁B) in the rat, or D₁ and D₃ in humans) and are coupled to the stimulation of adenylyl cyclase. The D₂ receptor has been found to exist in 2 isoforms (D₂L and D₂S), both coupled to the inhibition of adenylyl cyclase. The so-called D₃³ and D₃⁶ receptors are closely related to, but distinct from, the D₂ receptor. The pharmacological properties of D₃-like receptors resemble most closely those of the central nervous system D₁A and D₁B receptor subtypes, whereas those of the D₂-like receptors resemble the properties of the central nervous system D₂ receptor subclass. Molecular techniques have revealed that the peripheral D₁A and central D₁α receptors have identical mRNA nucleotide sequences.⁷,⁸

We have demonstrated that the D₁α dopamine receptor subtype, cloned from the rat brain, is expressed in rat kidney using antipeptide antisera directed to epitopes on the native receptor.⁹ Such receptor subtype–selective antibodies permit identification of specific receptors that remain indistinguishable when studied with agonist- and/or antagonist-based radioligand, biochemical, or functional studies. Indeed, the majority of D₂-like receptor ligands in use have high affinities for the D₂, D₃, and D₄ receptors, rendering specific identification impossible. Studies investigating the peripheral localization of D₂-like receptors with such ligands have revealed receptor(s) in renal vessels (both adventitial and endothelial cell layers), glomeruli, and cortical and medullary tissue.¹⁰–¹³ A novel D₂-like receptor has been described in the inner medullary collecting duct.¹³ In rabbit renal artery,¹⁴ rat glomeruli,¹² and rat renal cortex,¹⁵ D₂-like receptor activation

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results in inhibition of adenylyl cyclase activity. Of the cloned dopamine receptors, mRNA of the D1A, D2Long, and D3 receptor subtypes has been identified in the kidney. 5,16 –18

The present study used specific antibodies directed against synthetic peptides that correspond to epitopes in the putative amino acid sequence of the rat D3 receptor to characterize the regional distribution of this dopamine receptor subtype in the rat kidney. This characterization was achieved by a combination of light microscopic immunohistochemistry, electron microscopic immunocytochemistry, and Western blot analysis.

Methods

Antisera

Polyclonal antisera were raised against synthetic peptide sequences derived from the predicted rat dopamine D3 receptor amino acid sequence, corresponding to epitopes located on extracellular and intracellular portions of the receptor (Figure 1). The specific sequences were CHVSPELYR412 on the third extracellular loop and QPPSPQTHGLKR301 on the third intracellular loop. KLH was conjugated to the amino terminus, and prebled New Zealand White rabbits were repetitively immunized with the KLH-peptide immunogen until maximum titers of antibody were obtained. Antibody production was monitored by means of solid-phase enzyme-linked immunosorbent assays (ELISA), in which the ELISA plates were coated with peptide and demonstrated final titers of 10⁴ to 10⁶. The antibodies were affinity purified as follows. The D3 receptor synthetic peptide (Research Genetics) was immobilized to AminoLink coupling gel (Pierce Chemical Co) via sodium cyanoborohydride. Once the column had been prepared, crude D3 receptor antiserum was first IgG-purified via protein A column, as previously described.19 The IgG-rich fraction of D3 receptor antiserum was bound to the D3 receptor affinity column. The column was washed to remove all other IgGs. The D3 receptor affinity-purified antibody was then eluted with 0.1 mol/L glycine, pH 2.5. The protein concentration of the affinity-purified D3 receptor antibody was determined to be 0.38 mg/mL by Bradford protein assay (Bio-Rad).

The specificity of both polyclonal antipeptide antisera was verified by (1) attenuation of the ELISA response after preincubation of the purified antipeptide antibody with its immunization peptide and (2) the ability of the antipeptide antibody to recognize the native D3 receptor that had been stably transfected and expressed in a murine fibroblast LTK⁻ cell line or infected with a baculovirus expression.

Figure 1. Two-dimensional representation of the rat dopamine D3 receptor amino acid sequence. The presented results are those obtained using antibodies to an epitope on the third extracellular loop, outlined in black with an asterisk, except for those in which the designated peptide sequence on the third intracellular loop was used as the immunogen.
system into Spodoptera fragiperda (Sf9) insect cells. Figures were prepared from experiments using antibodies directed against the third extracellular loop, except for when the antibody to the third intracellular loop was used.

**Transfected or Infected Cells**

A murine fibroblast LTK<sup>−</sup> cell line was stably transfected with a full-length rat D<sub>2</sub> or D<sub>3</sub> receptor cDNA using a modified calcium phosphate method.<sup>20</sup> The cDNA was subcloned in the expression vector pRc/CMV (Invitrogen Corp) at the XbaI site. Both transfected and nontransfected LTK<sup>−</sup> cells had 10 mmol/L butyrate added to the medium 48 hours before the experiment to enhance D<sub>2</sub> or D<sub>3</sub> receptor gene expression.<sup>20</sup> Nontransfected LTK<sup>−</sup> cells were used as controls.

Parallel cell cultures were grown on poly-L-lysine–coated glass well slides and subsequently fixed for 30 minutes in 4% paraformaldehyde in PBS at room temperature. The cells then were permeabilized with 0.001% saponin and, following standard blocking procedures, were incubated with the purified antipeptide antibody diluted 1:3000 in Tris/saline/azide (TSA) for 16 hours at room temperature. Positive staining was visualized using an avidin-biotin immunoperoxidase reaction (Vectastain ABC Elite Kit, Vector Laboratory). Slides were lightly counterstained with hematoxylin. Controls included (1) omission of primary antibody, (2) replacement of primary antibody with preimmune serum and with normal rabbit IgG at the same protein concentration as the antibody, (3) preadsorption of primary antibody against the peptide-KLH conjugate, and (4) preadsorption of primary antibody against the pure peptide immunogen; in all but control 3, immunoreactivity was abolished.

**Electron Microscopic Immunocytochemistry**

Male Wistar-Kyoto rats (n=4) weighing between 250 to 500 g were anesthetized with sodium pentobarbital and transcardially perfused, as previously described.<sup>9</sup> The perfusion regimen consisted of a prefixative solution containing (per liter distilled water) 9 g NaCl, 25 g polyvinylpyrrolidone, 0.25 g heparin, and 5 g procaine (pH 7.35), followed by a fixative solution of either a combination of 0.1% glutaraldehyde and 2% paraformaldehyde or 4% paraformaldehyde alone in 0.05 mol/L sodium cacodylate buffer (pH 7.3). The kidneys were removed, decapsulated, hemisected, and subdivided into regions containing tissue from the cortex, juxtamedullary cortex, and medulla. Each region was minced into 1-mm<sup>3</sup> pieces and post-fixed for a further 2 to 3 hours. Tissue samples were dehydrated and then infiltrated in mixtures of absolute ethanol in Lowicryl K4 M resin. Subsequently, the tissue samples were embedded in pure Lowicryl K4 M using gelatin capsules and were polymerized under UV light (360 nm) for 30 to 48 hours at −20°C. Ultrathin silver-cream sections (60 to 70 nm) were cut and mounted on 200-mesh thin bar nickel grids. Throughout immunolabeling, the rinsing steps were performed by floating the grids on droplets on the covers of microtest plates and placing the covers on a magnetic stirrer.<sup>22</sup> Nonspecific binding in tissue sections was blocked for 15 minutes with a combination of 1% ovalbumin and 2% nonfat dry milk in Trizma, pH 7.2. The tissue then was incubated for 2 hours in one of the following, which were at a 1:100 dilution in 1% ovalbumin in Trizma, pH 8.3 (as previously used for similar experiments<sup>23</sup>): (1) primary antibody, (2) primary antibody preadsorbed with the peptide-KLH conjugate, (3) primary antibody preadsorbed with peptide (immunogen), or (4) preimmune serum or normal rabbit IgG. After a washing in 1% ovalbumin in Trizma, the grids were incubated in 10-nm protein-A gold containing cold-water fish gelatin for 30 minutes. After a wash sequence, the grids were dried, counterstained in saturated uranyl acetate (2 minutes) and lead acetate (30 seconds), and mounted on Formvar support. Sections were examined in a Zeiss 10 CA transmission electron microscope at 60 kV.

**Light Microscopic Immunohistochemistry**

Male Wistar-Kyoto rats (n=6) weighing between 250 to 500 g were anesthetized with sodium pentobarbital (50 mg/kg IP). The animals underwent a perfusion regimen as previously described.<sup>9</sup> Briefly, the animals were perfused through the left ventricle with a regimen of 0.15% NaCl, 0.5% glucose, 5 g procaine (pH 7.35), followed by a fixative solution of either a combination of 0.1% glutaraldehyde and 2% paraformaldehyde or 4% paraformaldehyde alone in 0.05 mol/L sodium cacodylate buffer (pH 7.3). The kidneys were removed, decapsulated, hemisected, and subdivided into regions containing tissue from the cortex, juxtamedullary cortex, and medulla. Each region was minced into 1-mm<sup>3</sup> pieces and post-fixed for a further 2 to 3 hours. Tissue samples were dehydrated and then infiltrated in mixtures of absolute ethanol in Lowicryl K4 M resin. Subsequently, the tissue samples were embedded in pure Lowicryl K4 M using gelatin capsules and were polymerized under UV light (360 nm) for 30 to 48 hours at −20°C. Ultrathin silver-cream sections (60 to 70 nm) were cut and mounted on 200-mesh thin bar nickel grids. Throughout immunolabeling, the rinsing steps were performed by floating the grids on droplets on the covers of microtest plates and placing the covers on a magnetic stirrer.<sup>22</sup> Nonspecific binding in tissue sections was blocked for 15 minutes with a combination of 1% ovalbumin and 2% nonfat dry milk in Trizma, pH 7.2. The tissue then was incubated for 2 hours in one of the following, which were at a 1:100 dilution in 1% ovalbumin in Trizma, pH 8.3 (as previously used for similar experiments<sup>23</sup>): (1) primary antibody, (2) primary antibody preadsorbed with the peptide-KLH conjugate, (3) primary antibody preadsorbed with peptide (immunogen), or (4) preimmune serum or normal rabbit IgG. After a washing in 1% ovalbumin in Trizma, the grids were incubated in 10-nm protein-A gold containing cold-water fish gelatin for 30 minutes. After a wash sequence, the grids were dried, counterstained in saturated uranyl acetate (2 minutes) and lead acetate (30 seconds), and mounted on Formvar support. Sections were examined in a Zeiss 10 CA transmission electron microscope at 60 kV.

**Figure 2.** Light photomicrographs of D<sub>3</sub> receptor transfected LTK<sup>−</sup> cells. A, Cells demonstrating positive staining with antiserum against the third extracellular epitope of the D<sub>3</sub> receptor (1:1000 dilution). B, Nontransfected parent cell line. Original magnification ×1600.
Western Blot Analysis of D3 Receptor Protein Expression

The kidneys and brains of adult Sprague-Dawley rats were dissected, minced, and homogenized with Tissuemizer (Tekmar Corp) in Buffer A (10% glycerol, 20 mmol/L Tris-HCl [pH 7.3], 100 mmol/L NaCl, 2 mmol/L PMSF, 2 mmol/L EDTA, 2 mmol/L EGTA, 10 mmol/L sodium orthovanadate, 10 μg/mL [~1.5 μmol/L] leupeptin, and 10 μg/mL [~1.5 μmol/L] aprotinin; Sigma). The homogenate was centrifuged at 30 000 g for 30 minutes at 4°C. The pellet was resuspended in Buffer B (Buffer A with 1% NP-40; Sigma), stirred for 1 hour at 4°C and centrifuged again at 30 000 g for 30 minutes at 4°C. The supernatant was used for analysis.

For control samples, D3 receptor infected or noninfected Sf9 cell membranes or D2 or D3 receptor transfected or nontransfected LTK2 cells were processed in the same way. The samples were analyzed with SDS–polyacrylamide gel electrophoresis (5% acrylamide stacking gel and 8% running gel) in a standard protocol as described.24 The resolved proteins were transferred by elecroblotting (15 V for 20 minutes, Trans Blot SD DNA, Bio-Rad) onto a nitrocellulose sheet (BA-S 83, Schleicher and Schull). The nitrocellulose sheet then was soaked in 5% nonfat dry milk in Tween 20 solution (0.05% Tween 20, 20 mmol/L Tris-HCl [pH 7.2], 250 mmol/L NaCl) for 1 hour, incubated with the antisera against the third extracellular epitope of the D3 receptor (1:5000 dilution in Tween 20 solution) for 1 hour, and reacted with a peroxidase-labeled secondary antibody (1:10 000 dilution) for 1 hour. Specific bands were visualized with chemiluminescence (ECL Western Blotting Detecting Kit, Amersham).

D3 Receptor Binding Studies

Transfected and nontransfected LTK2 cell membranes were prepared by lysis of cell monolayers in 1 mmol/L Tris-Cl, pH 7.5, for 15 minutes. The cell lysates then were scraped from the dish and centrifuged at 40 000g for 15 minutes. The pellet was resuspended in TME buffer (75 mmol/L Tris, pH 7.5, 12.5 mmol/L MgCl2, 1.5 mmol/L EDTA). Radioligand binding activity of the transfected or nontransfected LTK2 cell membranes and infected or noninfected Sf9 cell membranes was measured by specific binding of [125I]iodosipiperone.25 Quinpirole (1 μmol/L) was used to determine specific binding.

Results

The immunohistochemical detection of receptor-specific protein allows the anatomic localization of this receptor to be defined precisely. The synthetic peptides that were used as immunogens in the production of antisera to the D3 receptor were specifically chosen because of their predicted antigenicity and uniqueness in terms of absence of sequence homology with other known proteins, including all receptors in any species (Sequence Similarity Search, National Center for Biotechnology Information, National Institutes of Health).26 The antibodies, which were directed to epitopes on the both the putative third extracellular and intracellular loops of the rat D3 receptor, produced specific renal staining.

Specificity of the antisera to D3 receptor protein was validated by light microscopic immunohistochemistry studies of the LTK2 cells permanently transfected with the full-
length cDNA of the D₂ or D₃ receptor, as previously described. ¹⁷ D₃ receptor immunoreactivity was detected in the cytoplasm and cell membranes of the transfected cells (Figure 2A). Immunostaining was absent in the nontransfected LTK⁻ parent cell line (Figure 2B), in D₃ receptor transfected cells treated with preimmune serum, and in LTK⁻ cells transfected with the D₂ receptor. These results confirm that antipeptide antiserum to the D₃ receptor specifically recognizes the appropriate peptide antigen expressed in both experimental tissue and transfected cells. Our controls confirm that the staining reaction is not an artifact created by the manner of tissue processing or false-positive reactions produced in the rabbit sera before the inoculation with KLH-conjugated peptide.

Figure 3 shows a Western blot analysis of the D₃ receptor protein in D₃ receptor infected and noninfected Sf9 cell membranes, D₂ or D₃ receptor transfected and nontransfected LTK⁻ cells, and adult rat kidney and brain. A band of the appropriate predicted size (≈57 kDa) for the D₃ receptor was detected in D₃ receptor infected Sf9 cell membranes (lane 4) and in D₃ receptor transfected LTK⁻ cells (lane 7), but not in noninfected cell membranes (lane 3) or in nontransfected LTK⁻ cells (lane 6). A specific band of the same molecular weight was detected in the rat kidney (lane 2) and brain (lane 1). No band was detected in D₂ receptor transfected LTK⁻ cells (lane 5). For the infected Sf9 cell membranes, D₃ receptor binding density was 7.7 pmol/mg protein and for the transfected LTK⁻ cells, the receptor density was 1.4 pmol/mg protein.

Light microscopic immunohistochemical staining for the D₃ receptor was pronounced in the renal cortex. Staining was present in the proximal tubules (PT) and distal tubules (DT) (Figure 4A), the cortical collecting ducts (CCD), glomeruli, and renal parenchymal vasculature (Figure 4A). Staining was present in the cortex, but there was a line of demarcation with absence of staining in the medulla (Figure 4C). In PT and DT (Figure 5A and 5C) epithelia, immunostaining was observed in discrete tubular cells exclusively in the apical portion of these cells. Renal arterial staining was prominent in the medial and adventitial layers (Figure 6A). Consecutive sec-

Figure 5. A, Immunostaining in proximal tubules (arrows) and distal tubules (open arrows) was confined to the apical portions of the tubular cells. B, Preadsorption control showed absence of staining. C, High-power view of immunostaining in proximal tubules. The intensity of staining was localized to the brush border of tubular cells. D, Preadsorption control showed absence of staining. G indicates glomeruli. Original magnification: A and B, ×320; C and D, ×640.
tions processed with preimmune serum, normal rabbit serum at the same dilution as the anti-D₃ receptor serum, or immune serum preabsorbed against the pure peptide immunogen did not produce renal staining, whereas preabsorption of the antibody against the conjugated carrier protein KLH did not alter the immunohistochemical staining patterns described previously. Figure 4B and 4D and Figure 5B and 5D are typical examples of the renal cortex exposed to preabsorbed antisera.

The distribution of D₃ antisera immunostaining also was examined in rat brain sections processed for light microscopic immunohistochemistry. Positive staining was obtained in the nucleus accumbens, islands of Calleja, and olfactory tubercle. These regions of the brain are known to express the D₃ receptor. Figure 6B shows an example of discrete positive immunostaining in the nucleus accumbens.

Figure 7 shows heavy D₃ receptor immunostaining in the CCD. Figure 8 depicts D₃ receptor immunostaining in the glomerulus. Figure 8A and 8B depicts the heavy staining pattern of the glomerulus, in which glomerular podocytes are clearly labeled. Figure 8C demonstrates light staining in the macula densa and absence of staining in the juxtaglomerular cells of the afferent arteriole; Figure 8D is a preabsorption control and shows no D₃ receptor labeling.

Electron microscopic immunocytochemistry, as demonstrated by presence of 10-nm protein-A gold particles, further defined the intracellular sites of expression of renal D₃ receptor protein. Glomerular D₃ receptor immunoreactivity was confined to glomerular podocytes (Figure 9A). Staining was present not only on the membranes of podocytes but also on vesicles budding off these membranes, in the intracellular space, and at the attachment of the podocytes to the foot processes. In the PT, staining was membrane-bound in the apical region of the PT cells (Figure 10A). Control sections from identical cortical regions incubated with serum preabsorbed with D₃ receptor immunogen demonstrated minimal background staining (Figures 9B and 10B). In the CCD, staining was present on the apical membranes of intercalated cells (Figure 10C). Control sections stained with preimmune serum, or immune serum preabsorbed with immunogen, had no such immunogold labeling (Figure 10D). In the renal artery, electron microscopic immunocytochemistry revealed immunogold particles located predominantly in arteriolar smooth muscle cells (Figure 9C). In the DT, D₃ receptor signal also was present on the apical membranes (data not shown). In contrast with the D₁A receptor, D₃ receptor immunoreactivity was not evident in the juxtaglomerular cells (Figure 9D).

A renal staining pattern similar to that with the D₃ receptor antibody directed toward the third extracellular loop of the
receptor was obtained with antibody directed to the third intracellular domain of the receptor (data not shown).

**Discussion**

The dopamine D$_3$ receptor was the second receptor of the D$_2$-like family to be identified and cloned. Using probes derived from the D$_2$ receptor sequence, the rat D$_3$ receptor was isolated by screening cDNA and genomic libraries with a combination of reverse transcription and polymerase chain reaction (RT-PCR). An amino acid sequence of 446 amino acids was deduced from the open reading frame of the clone. The cellular expression of the mRNA encoding the D$_3$ receptor has been mapped in the rat brain by in situ hybridization histochemistry. Highest levels of expression are observed in the caudate putamen, nucleus accumbens, islands of Calleja, hypothalamus, and olfactory tubercle. Although these central areas contain both D$_3$ and D$_2$ receptor mRNA, there is no overlap of D$_3$ receptor with D$_2$ receptor mRNA at the cellular level, suggesting discrete receptor expression. This pattern of distribution has led to speculation that the D$_3$ receptor subtype may mediate dopaminergic control of cognitive and emotional function in the brain. In this regard, it is interesting to note that pharmacological studies have demonstrated dopamine as having a 20-fold higher affinity at the D$_3$ than the D$_2$ receptor.

Peripheral mapping of the D$_3$ receptor has been very limited. Sokoloff et al reported low copy expression of D$_3$ receptor mRNA in kidney as revealed by Northern blotting. More recently, Gao et al demonstrated kidney D$_3$ receptor mRNA expression using RT-PCR from isolated mRNA. As these authors pointed out, however, this technique precludes precise anatomic localization and may also suffer from cross-contamination of mRNA from different nephron elements. Barili et al localized a putative D$_3$ receptor to renal glomeruli and PT and DT by 7-OH-DPAT binding. However, 7-OH-DPAT also interacts with the D$_2$ receptors, albeit at higher concentrations than required for D$_3$ receptor binding.

The present study is the first to our knowledge to define the site-specific localization of D$_3$ receptor protein in whole kidney sections and corroborates and extends the previous reports that have suggested the renal presence of the D$_3$ receptor. Moreover, the sites of D$_3$ receptor protein as

**Figure 8.** A and B, Light photomicrographs of a glomerulus in the rat renal cortex showing heavy immunostaining for the D$_3$ receptor. Panel B shows uniform staining in a podocyte (arrowhead). C, High-power magnification of the juxtaglomerular apparatus, which demonstrated absence of staining in the juxtaglomerular cells (arrows) and light staining in the macula densa (MD). D, Preadsorption control showing absence of staining. Original magnification: A, ×640; B through D, ×1600.
demonstrated in the present study correspond closely to renal D_2-like receptor binding sites, including glomeruli, PT, and renal arteries, as previously defined by pharmacological binding, biochemical, and functional studies. Use of these studies to define the localization of receptor subtypes is made difficult by the relative inability of available D_2-like receptor ligands to distinguish between the D_2-like receptor subtypes. Such difficulties are avoided by the use of receptor-specific antibodies.

One significant difference between our results and those in the literature is the cellular distribution of the D_3 receptor. A radioligand-binding study showed the presence of D_2-like receptors in both brush border and basolateral membranes of the canine cortical tubules. Our study shows only apical binding in these cells. Also, Huo et al described a D_2-like (DA_2K) receptor in the rat renal medulla, whereas in our study the D_3 receptor was absent in the medulla. The identity of the DA_2K receptor and its relationship to the D_3 receptor are unclear.

The specificity of the antibody staining demonstrated in the present study was confirmed by the use of appropriate controls (including preadsorption with the pure peptide immunogen) in the immunostaining procedures and by the results of the D_2 and D_3 receptor transfection/infection studies in which only the D_3 receptor transfected cells or the infected cell membranes were labeled by immunocytochemistry and/or Western blot analysis. For Western blot analysis, a band of appropriate predicted size (~57 kDa) was identified in the D_3 receptor infected SF9 cell membranes and in D_3 receptor transfected cells. This band was similar to that reported by Boundy et al. The staining patterns were identical for the 2 different antisera (directed against both extracellular and intracellular domains) used in the studies of both native tissue and transfected cells, thereby excluding a nonspecific or spurious signal. In the kidney, a single band of approximately 57 kDa was detected by Western blot. D_3 receptor immunostaining in the nucleus accumbens of the brain and a specific D_3 receptor band on Western blot from the brain served as positive controls.

Quinpirole (LY 171555) is a dopamine-2 receptor agonist that has been used in radioligand and functional studies. Quinpirole has a KᵢD/KᵢD ratio of 113 in the rat and thus is relatively selective for the D_3 receptor. Previous experiments conducted by our research group in a conscious...
experimental animal model have demonstrated that intrarenal infusion of quinpirole produced antidiuresis, antinatriuresis, and a decrease in renal hemodynamic function. Other studies using quinpirole have revealed changes in renal blood flow and superficial nephron glomerular filtration rate. The present study confirms the presence of the D3 receptor in renal tubular, glomerular, and vascular sites, supporting the possible role of this dopamine receptor in modulating renal function.

The distribution of the D3 receptor differs significantly from the distribution of the D1A receptor as previously reported by our group. In the glomerulus, strong D3 receptor immunoreactivity was demonstrated and localized to podocytes. No staining was observed in mesangial cells. In the case of the D1A receptor, no glomerular immunoreactivity was detected. Whereas the D1A receptor had a strong immunoreactive signal in the juxtaglomerular cells, the D3 receptor has no detectable signal in these cells in situ. We have previously reported the presence of D3 receptor mRNA in rat juxtaglomerular cells in primary culture. The reasons for the discrepancy between the present results and our previous study are unclear. One possibility is that the primary juxtaglomerular cell culture has up to 5% contamination with renal tubular cells, and we may have been detecting D3 receptor mRNA in these contaminating cells. Another possibility is that our immunohistochemical technique is not sensitive enough to detect the D3 receptor signal, which is present in low copy, while PCR can detect the message. A third possibility is that the expression of the D3 receptor increases with culture.

With respect to the subcellular distribution of the D3 receptor, while the D1A receptor had both an apical and a basolateral localization in PT and DT cells, the D3 receptor labeling was confined to the apical region of these tubular cells. Whereas the D1A receptor labeling in the CCD was relatively weak, CCD labeling of the D3 receptor is comparatively strong. Immunoreactivity for both of these subtypes was evident in vascular smooth muscle cells in different orders of renal vessels. These differences conform to the respective differences in the distribution of D1- and D2-like receptors using older classification systems. Furthermore, the presence of both D1A and D3 receptors in similar locations provides morphological support for recent experimental evidence of an obligatory synergistic role of D1-like and D2-like
receptor agonism in the inhibition of Na\(^+/\)K\(^{-}\)-ATPase activity in the proximal tubule.\(^1\)

As described previously for the D\(_{1A}\) receptor in heart and kidney,\(^3,7\) the present study identified the D\(_{3}\) receptor in cell membranes, vesicles budding off of membranes, and in the cytoplasm of renal cells. The function of intracellular receptors is not clear, but their presence there may signify a receptor cycling mechanism in which receptors may be internalized and subsequently reinserted into the cell membrane. Further work is required to elucidate this possibility.

In conclusion, the dopamine D\(_{3}\) receptor protein, previously localized only to the central nervous system, is present in the rat kidney in sites previously labeled as D\(_{2}\)-like. The distribution of the D\(_{3}\) receptor is distinctively different from that of the D\(_{1A}\) receptor. The results suggest that at least some of the peripheral dopamine D\(_{3}\)-like receptors correspond structurally to the central dopamine D\(_{3}\) receptor.

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References
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