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Unique properties of a renal sulfotransferase, St1d1 in dopamine metabolism

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d) Abbreviations
ST, St or SULT: sulfotransferase, MAO: monoamine oxidase, 4'-OH-PhIP: 2-Amino-1-methyl-6-(4-hydroxyphenyl)-imidazo[4,5-*b*]pyridine, IPTG: isopropyl-β-D-thiogalactopyranoside, DTT: dithiothreitol, PAPS: 3`-phosphoadenosine-5'-phosphosulfate, NTA : Ni-nitrilotriacetic acid agarose, SDS-PAGE: sodium dodecylsulfate-polyacrylamide gel electrophoresis, DOPA : 3,4-dihydroxyphenylalanine, DOPAC: 3,4-dihydroxyphenylacetic acid, DOMA: 3,4-dihydroxymandelic acid, DHPG: 3,4-dihydroxyphenylene glycol, HMPG: 4-hydroxy3-methoxyphenylethylene glycol, VMA: vanillylmandelic acid.

e) Absorption, Distribution, Metabolism, & Excretion

Abstract

Although catecholamine sulfation is higher in the kidney than in the liver of mice, no detectable amounts of previously reported sulfotransferases (STs) such as St1a, St1b, St1c and St1e, were expressed in mouse kidney cytosols.

A new sulfotransferase (St1d1) cDNA was isolated from kidney cDNA library of BALB/C strain by RT-PCR using information of EST database.

The cDNA sequence resembled that of cDNA reported previously (AA238910) (Sakakibara et al., 1998), but differed in two amino acids, ^{206}Q/K and ^{216}Y/F, in the deduced amino acid sequence. The St1d1 expressed had unique substrate specificities for catecholamine derivatives, which preferred their deaminated metabolites rather than their parent amines. St1d1 showed the highest activity towards 3,4-dihydroxyphenylacetic acid (230.2±2.69 nmol/mg/min) among the examined substrate. St1d1 protein was abundant in kidney, followed by liver, lung and uterus. Furthermore, an addition of anti-St1d1 serum in the cytosolic reaction mixture resulted in complete inhibition of the sulfotransferase activity, suggesting a major role of St1d1 on catecholamine sulfations. No human ST1D orthologue was detected at both mRNA and protein levels, although ST1A5 selectively catalyzing parent amine sulfation was detected in human kidney. These results indicate the functional basis of sulfation and the clear species difference on renal catecholamine metabolisms in mouse and human.

Introduction

Catecholamines such as dopamine and norepinephrine are enzymatically biotransformed through several different pathways (Kopin, 1985). Deaminated metabolites by monoamine oxidase (MAO) are further converted to their alcohols or carboxylic acid derivatives. Catecholamines also undergo the methylation to form O-methylated derivatives. Both deaminated and O-methylated metabolites are detected at 10-70 nM levels in plasma. Considerable portions of the metabolites are also excreted as the sulfates in urine in experimental animals and humans. Although the liver is considered to be a major site of the conjugation, sulfotransferase activity for dopamine and p-nitrophenol in mice was found to be higher in kidney than in liver in our preliminary experiments, as reported previously (Wong and Yeo, 1982).

These data suggest to us a possible role of the kidney for the biotransformation of phenolic chemicals into the readily excretable sulfates peripherally regulating catecholamine levels.

Cytosolic enzymes mediating sulfation of various endobiotics and xenobiotics have been identified and are now known to constitute a gene superfamily, ST (SULT) 1-5 (Nagata and Yamazoe, 2000). Phenol and estrogen sulfotransferases including the ST1 family are detected in livers of humans as well as rodents. Four different cDNAs of arylsulfotransferases have been isolated from mouse cDNA libraries of liver (St1a4 (Kong et al., 1993) and St1b3 (Saeki et al., 1998)), olfactory (St1c4 (Tamura et al., 1998)) and testis (St1e4 (Song et al., 1995)), although substrate specificities of these forms were not yet identified except for St1a4 (Honma et al., 2001). Furthermore, limited amounts of information are available on kidney sulfotransferase form(s). Therefore, kidney sulfotransferase has been investigated to assess the possible role for metabolism of catecholamines.

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In the present study, we have identified a sole expression of St1d1 among arylsulfotransferases in mouse kidney and characterized the unique substrate specificity.

Materials and Methods

Materials

Restriction endonucleases, DNA modifying enzymes and Takara Ex Taq were purchased from Takara Shuzo (Kyoto, Japan). Enterokinase was obtained from Biozyme Laboratories, Ltd. (Gwent, UK). Thermo Sequenase^{TM} cycle sequencing kit was obtained from Shimadzu Co. (Kyoto, Japan). 2-Amino-1-methyl-6-(4-hydroxyphenyl)-imidazo[4,5-*b*]pyridine (4'-OH-PhIP) was kindly donated by Dr. K. Wakabayashi (National Cancer Center Research Institute, Tokyo, Japan). Isopropyl-β-D-thiogalactopyranoside (IPTG) was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Dithiothreitol (DTT), alkalinephosphatase-conjugated goat anti-rabbit IgG, 5-bromo-4-chloro-3-indolylphosphate, nitro blue tetrazonium and phenolic chemicals used as substrates were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 3'-phosphoadenosine-[^{35}S]-5'-phosphosulfate ([^{35}S]-PAPS) (2,000 mCi/mmol) was from New England Nuclear (Boston, MA, USA). QIAexpress and Ni-nitrilotriacetic acid agarose (NTA) were obtained from Qiagen (Chatsworth, CA). Bio-Rad protein assay kit and sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) molecular weight standards (low range) were from Bio-Rad (Richmond, CA, USA). All other chemicals used were of the highest grade available.

Human kidney cytosols were obtained from BioChain Institute, Inc. (Hayward, CA, USA). Human livers were from the Department of Anatomic Pathology (School of Medicine, Tohoku University, Sendai). Experiments on human livers were approved by the Ethics Committee in Tohoku University.

BALB/C mice (8 weeks old) and Japanese white rabbits (female, 3.0 kg) were obtained from Japan SLC (Shizuoka, Japan).

Methods

Preparation of cytosols from mouse kidney and liver

Renal and hepatic cytosols were prepared from male and female BALB/C mice (8 week old). Kidneys and livers were rinsed with phosphate-buffered saline (PBS) and homogenized with a buffer containing 0.075 M potassium phosphate (pH 7.4), 0.075 M KCl and 1 mM DTT. The homogenates were centrifuged at 9,000 x g for 20 min and the supernatants were centrifuged at 105,000 x g for 60 min. The resultant supernatants were used as cytosols. The protein concentration was determined by the method of Bradford with bovine serum albumin (BSA) as the standard (Bradford, 1976).

Assays of sulfotransferase activities

Sulfotransferase activities were determined by the radioactivities of the metabolites using [^{35}S]-PAPS as a sulfate donor after thin layer chromatography. The incubation mixture (final volume of 10 μl) consisted of 100 mM Tris-HCl buffer (pH 7.4), 0.3 mM $MgCl_2$, 2 mM DTT, 1 mg/mL BSA, 125 μM [^{35}S]-PAPS, 10 or 100 μM substrate dissolved in 1% dimethyl sulfoxide and 2.5 μg of cytosolic protein from male mouse kidney or liver, or 50 ng of 6xHis St1d1 protein. The reaction was initiated by addition of [^{35}S]-PAPS, incubated at 37°C for 20 min and then terminated by addition of 5 μl of acetonitrile. Aliquots of the reaction mixture (10 μl) were applied to a thin layer plate (thin layer chromatography aluminum plate silica gel 60; Merck, Darmstadt, Germany; or chromatogram sheet 13255; Kodak, Rochester, NY). Metabolites on the chromatogram were developed with a solvent system of *n*-propanol/ 28% aqueous ammonia / water (6:3:1) (Fujita et al., 1999). The radioactive spots were analysed by FLA-3000 image analyzer (Fuji Film, Tokyo, Japan). The apparent kinetic parameters, K_m and V_{max}, were calculated from double-reciprocal Lineweaver-Burk plots using

linear regression analyses. The substrate concentrations used are ranging from 5 μM to 300 □M for DOPAC and 10 μM to 10 mM for dopamine.

Isolation of St1d1 cDNA

St1d1 cDNA fragment was obtained by the polymerase chain reaction (PCR) from mouse kidney or liver cDNA library using oligonucleotides as the primers (St1d1-5': CGGCATGCGATGACGATGACAAAATGGATAACAAACTGGATGTC, and St1d1-3': CGCAAGCTTCAACTCTCCCTAGATCTC) containing *Sph* I/*Hin* dIII restriction sites, respecitvely. A 5'-region from the initiation codon of St1d1 cDNA fragment contained nucleotides encoding seven additional amino acid residues (AlaCysAspAspAspAspLys) as enterokinase (EK) cleavage site. The PCR reaction mixture consisted of 1 μl of the template cDNA, 3 μl each of dATP, dCTP, dGTP and dTTP, 1.5 units of Takara Ex *Taq*, 20 pmol of St1d1-5' and St1d1-3' primers and the Ex *Taq* buffer in a final volume of 30 μl. After an initial denaturation at 94°C for 2 min, the amplification was performed for 30 cycles, with 1 min at 94°C for denaturation, 30 seconds at 55°C for annealing, 2 min at 72°C for extension, and a final extension period of 7 min at 72°C.

Sequencing

DNA sequence was determined by using fluorescent-labeled primers and DSQ2000-L DNA sequencer (Shimadzu Co., Kyoto, Japan) according to the dideoxy method (Shimada et al., 2002). The PCR reaction mixture (8 μl) contained 5 μg of the template DNA, 1.25 pmol of the fluorescent-labeled primer, 2 μl of the reagent containing ddATP, ddGTP, ddTTP or ddCTP. After an initial denaturation at 98°C for 5 min, the amplification was performed for 25 cycles, with 1 min at 98°C for denaturation, 45 sec at 50°C for annealing, 1.5 min at 72°C for extension, and a final extension period of 1

min at 72°C. After addition of 8 μl of the formamide loading dye for termination of the reaction, denaturation was performed at 98°C for 5 min. These samples were applied to the gel, and then electrophoresed for 24 hours.

Expression and purification of St1d1

The cDNA obtained (St1d1) was ligated into an expression vector, pQE30 (Qiagen, Tokyo). The constructed plasmid DNA was transformed into *Escherichia coli*, M15 [pREP4] strain. Recombinant St1d1 (6xHis- St1d1) was expressed and purified from bacterial cytosols by Ni-NTA acid affinity chromatography (Fujita et al., 1997). 6xHis-St1d1 was eluted with the buffer containing 300 mM NaCl, 10 % glycerol, 0.025 % Tween 20, 50 mM sodium phosphate (pH 6.0) and 200 mM imidazole. St1a4 and St1b3 were also expressed as histidine-tagged proteins.

Preparation of antibody

Japanese White rabbit (3.0 kg, female) was immunized intradermally with 100 μg of purified 6xHis St1d1 in Titer max GOLD (CytRx, Atlanta) and 4 weeks later, immunized intravenously with 100 μg of the protein. After one week, 40 mL of the blood was obtained and centrifuged. The resultant supernatant was used as the St1d1 antibody. In Western blotting anti-St1d1 antibody serum recognized only one band in kidney and liver cytosols, and did not immunocross with other known arylsulfotransferases examined.

Electrophoresis and Western blotting

Cytosolic proteins (10-100 μg/lane) from mouse kidney or liver were separated by SDS-PAGE using a 10.5% gel and electrophoretically transferred to a nitrocellulose membrane. STs on the membrane were reacted with the antibody (1:1,000 dilution)

and with alkalinephosphatase-conjugated goat anti-rabbit IgG (1:3,000 dilution), 5-bromo-4-chloro-3-indolylphosphate and nitro blue tetrazolium. The stained sheets were scanned with EPSON Calario GT-8700 and their intensities were measured by use of the NIH image (Version 1.59) software. The contents in each sample were determined using the corresponding recombinant proteins cleaved 6X His by enterokinase (Δ6xHis) as the standards.

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RESULTS

Sulfotransferase activities towards catecholamines in cytosols from mouse kidney and liver

Sulfotransferase activities towards catecholamines at 100 μM of the substrate concentration were compared between kidney and livers (Fig. 1). Renal and hepatic cytosols mediated dopamine sulfation at the rate of 745 ± 36.0 and 220 ± 10.4 pmol/mg/min, respectively. Three-fold higher sulfating rates were also observed in kidney than in liver towards norepinephrine and epinephrine.

Western blot analyses of arylsulfotransferase in mouse kidney and liver

To identify which form(s) of sulfotransferase mediates sulfation of catecholamines in mouse kidney, renal cytosols were subjected to Western blotting analyses as shown in Fig. 2. St1a4 and St1b3 were not detected in kidney, although both forms were detected in livers of male and female mice. Anti-ST1B1 sera recognized an unknown band at about 33 kDa, which is distinct from authentic St1b3 and St1a4 protein. In addition, neither St1c4 nor St1e5 proteins were detected in kidney and liver (data not shown).

Structure of a new renal ST cDNA

The cDNA prepared from BALB/C strain of mice was explored to identify the renal sulfotransferase mediating catecholamine sulfation. A possible sulfotransferase cDNA was isolated and the nucleotide and deduced amino acid sequences of a renal ST are shown in Fig. 3. The identified renal ST mRNA contained an open reading frame of 888 bp, which encoded 295 amino acids with a molecular weight of 35,081 Da. The renal sulfotransferase was closely related to sulfotransferase found in EST database (Accession no. AA238910), except for ^{206}Q/K (^{616}C/A) and ^{216}Y/F (^{629}A/T) in the

deduced amino acid sequences. Only one fragment of mRNA (AA238910) from EST database exactly matched to be a part of the previously reported sequence (Sakakibara et al., 1998). On the contrary, this newly isolated cDNA was exactly matched in three EST fragments (AA245085, 109895, 109782) and thus judged to correspond to St1d1 in BALB/C mice. These two closely related St1d1 forms are possible to exist in mouse liver and kidney cytosols.

Characterization of general substrate specificity of St1d1

To characterize the substrate specificity of the renal St1d1,

Stld1 was expressed in *E. coli* as a fused protein. As shown in Table 1, recombinant St1d1 catalyzed sulfation of dopamine, in addition to p-nitrophenol, triiodothyronine and estradiol among phenolic chemicals.

St1d1 also mediate sulfation of 2-methylbenzyl alcohol, but showed only marginal activities towards other alcohols including corticosterone, dehydroepiandrosterone, cholesterol, lithocholic acid and 1-pyrenemethanol. No sulfating activity towards amines such as aniline and desipramine was detected with recombinant St1d1.

Sulfotransferase activity of St1d1 for catecholamine and their metabolites

Due to the high activity towards dopamine, substrate specificity of St1d1 was investigated further on catecholamines and their metabolites at 10 μM substrate concentration (Fig 4). St1d1 had no sulfating activity on 3,4-dihydroxy-L-phenylalanine (L-DOPA), a precursor of catecholamine.

Other catecholamines such as norepinephrine (6.04 ± 1.59 nmol/mg/min) and epinephrine (1.40 ± 0.07 nmol/mg/min) are also sulfated, in spite of the high activity towards dopamine (22.8±3.14 nmol/mg/min) by St1d1. St1d1 had no sulfating activity with 3-methoxytyramine, normethanephrine and methanephrine, which were formed

from the parent amine by catechol *O*-methyltransferase. St1d1, however, showed markedly high activities towards 3,4-dihydroxyphenylacetic acid (DOPAC) (230.2 ± 9.21 nmol/mg/min), 3,4-dihydroxymandelic acid (DOMA) (20.3 ± 2.69 nmol/mg/min) and 3,4-dihydroxyphenylene glycol (DHPG) (209.3 ± 13.7 nmol/mg/min). These metabolites are formed from their parent amines by monoamine oxidase (MAO), followed by aldehyde oxidase (AO) or aldehyde reductase (AR). St1d1 also catalyzed sulfation of homovanilic acid (HVA) (116.9 ± 7.04 nmol/mg/min) and 4-hydroxy3-methoxyphenylethylene glycol (HMPG) (72.4 ± 7.00 nmol/mg/min) except for vanillylmandelic acid (VMA).

Kinetic parameters were examined for dopamine and DOPAC sulfations (Table 2). Apparent Km and Vmax value of St1d1 were 713.2±14.2 μM and 1474±9.03 nmol/mg protein/min, respectively, for dopamine sulfation, while were 35.02 ± 1.13 μM and 1678 ±159 nmol/mg protein/min for DOPAC sulfation. The values of Vmax/Km for dopamine and DOPAC sulfations were 2.07 and 47.92, respectively.

Distribution and quantification of cytosolic St1d1 protein

St1d1 protein was detected in kidney and liver at about 33 kDa, and comigrated with corresponding to Δ6xHis St1d1. The levels are higher in kidney than in liver, followed by lung and uterus (Fig. 5). The protein levels in lung and uterus were calculated about 10% of the liver level. Cytosolic contents of St1d1 in kidney and livers were determined. Mean contents of renal St1d1 were 5.1 and 3.4 μg/mg of cytosolic protein in three different male and female mice, respectively. On the contrary, hepatic St1d1 content was rather higher in female mouse (2.0 μg/mg of cytosolic protein) than in males (2.5 μg/mg of cytosolic protein). To know whether St1d1 is a major form in liver, we also determined the contents of St1a4 and St1b3. St1a4 content was 2.3 and 4.4 μg/mg of cytosolic protein in male and female livers, respectively.

St1b3 content was 0.5 and 0.4 μg/mg of hepatic cytosol from male and female, respectively.

Role of St1d1 on renal dopamine and DOPAC sulfations

To assess the role of St1d1 on renal catecholamine sulfation, anti-St1d1 antisera were added in the incubation mixture containing DOPAC and renal cytosols. To verify the form-specificity of anti-St1d1 antiserum, the antiserum was added to an incubation system containing recombinant St1d1 or St1a4 for p-nitrophenol sulfation (Fig. 6A). Addition of anti-St1d1 antisera (15 μg) resulted in the decreased St1d1-meditated *p*-nitrophenol sulfation to 17 % of the control, but not the St1a4-mediated sulfation. Addition of 15 μg of anti-St1d1 antisera caused the decrease of kidney cytosolic dopamine and DOPAC sulfations below the 5% level for the control values (addition of preimmunized serum) as similar to the St1d1-mediated sulfation (Fig. 6B and 6C).

Detection of human renal catecholamine sulfotransferase mRNA and protein

To ascertain the possible expression of human ST1D form, the specific mRNA was detected with RT-PCR using human ST1D primers generated from the ST1D genome sequence found in human genome database. As shown in Fig. 7A, neither a human ST1D mRNA containing open reading frame nor the fragment of 353 kb, was detected in human kidney cDNA library. In stead, a dopamine sulfotransferase, ST1A5 cDNA was isolated from human kidney cDNA library (Fig. 7B). The nucleotide sequence was identical with that of cDNA reported previously (L19956).

Further ST1A5 protein was detected in human kidney cytosols (Fig. 7C).

The amount was very low and estimated about l% of the level in liver cytosols.

Discussion

In the present study, St1d1 is shown to be a major arylsulfotransferase in mouse kidney and no other St1 forms like St1a4 and St1b3, were detected in livers in contrast to their high expression.

These data indicate the primary role of St1d1 for catecholamine sulfation in this tissue. Unique properties of St1d1, which showed higher activities towards the deaminated derivatives of catecholamine such as DOPAC, DOMA, and DHPG, rather than parent amines, are characterized by the use of the recombinant protein.

From kinetic parameters of St1d1, the value of Vmax/Km for
DOPAC is 20 fold higher than that for dopamine. These data suggest that DOPAC is a selective substrate for St1d1. The substrate specificity of St1d1 was distinct from those of a human catecholamine sulfotransferase, ST1A5 form (Honma et al., 2001). As shown in our previous report, ST1A5 have the higher activity towards parent catecholamines rather than the deaminated derivatives.

Chemicals containing acidic carboxyl groups are rather poor substrates for previously known sulfotransferases including in ST1 family. St1d1, however, catalyses sulfation of phenol-carboxylic acids such as DOPAC and HVA, except for VMA. The latter is consistent with reported data that VMA is excreted mainly in urine as unconjugated in rodents. Presence of methylether moieties in catecholamines seems to have complex influences on the St1d1-mediated sulfations. The substrate specificities are unlikely to be determined only by regioselectivity, chemico-physical properties such as acidity and/or hydrophobicity. The sole expression of St1d1 in kidney among ST1 forms was observed in the present study. This phenomenon is in contrast to the expression of multiple families of sulfotransferase in liver. Recently, another sulfotransferase form, which is a orthologue to human SULT1C2 (ST1C2) was detected in kidney (Sugimura

et al., 2002). The recombinant protein showed only p-nitrophenol sulfation, however, and the functional role of mouse SULT1C2 in kidney was not reported.

In rodents, plasma concentration of dopamine was ca. 5 μM (Tsunoda et al., 2001) and renal concentrations of dopamine and DOPAC were reported at 200 and 20 nM, respectively (Shoaf and Elchisak, 1983; Eldrup and Richter, 2000). These values were estimated to be 1/1000 ~1/100 of Km values for recombinant St1d1 in the presence of 125 μM PAPS as shown in Table 2. Any reason of this discrepancy remained obscure. These are few reports on urinary excretion of catecholamines in rodents. DOPAC sulfate is a major urinary excretion in rats. Renal St1d1, in our preliminary experiment, was stained immunohistochemically at proximal and distal tubule, and collecting duct. The presence of organic cation transporter (OCT) is reported in rats and humans (distal tubule) (Gorboulev et al., 1997). Combined with the information, dopamine and DOPAC may be concentrated through the nephron and uptaked form blood into the renal tubule by OCT1/2, and followed by St1d1-mediated sulfoconjugation and then excreted in urine as the sulfate ester through an organic anion transporter. These results suggest that renal St1d1 is also involved in excretion of catecholamines to maintain the homeostasis of the whole body.

During the preparation of this study, a recent report showed that the expression of canine SULT1D (ST1D) is expressed in kidney and shown the sulfotransferase activity towards dopamine (Tsoi et al., 2001). Rat ST1D2 cDNA is also isolated, although the substrate specificity has not yet been characterized.

Human SULT1D (ST1D) is suggested to be a pseudogene because of the existence of mutated splice donors/acceptors site and in-frame stop codons (Meinl and Glatt, 2001). These data, together with our present result, suggest that ST1D form is not expressed as a functional protein in human kidney. Thus, ST1A5, instead of ST1D form, has a primary role in controlling of catecholamine sulfation in human kidney.

In the present study, St1d1 (mouse) and ST1A5 (human) are firstly identified as renal neurotransmitter sulfotransferases and suggest the involvement in the excretion profile of the sulfoconjugated catecholamines in urine.

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Figure legends

Fig. 1 Cytosolic sulfotransferase activities of mouse kidneys and livers

Each value represents the mean ± S.D. of three different male mice. Sulfotransferase activities were assayed using 3'-phosphoadenosine[^{35}S]-5'-phosphosulfate ([^{35}S]-PAPS) as a sulfate donor. The assays were performed at pH 7.4 in the presence of 100 μM of the substrates and 2.5 μg of cytosolic protein from kidneys and livers of male mice.

Fig. 2 Western blot analysis using anti-ST1A or anti-ST1B antibody

Proteins were subjected to SDS-PAGE on a 10.5% gel and electrically transferred to a nitrocellulose membrane. Sulfotransferases on the membrane were reacted with anti-ST1A1 (rat) (A) or anti-ST1B1 (rat) (B) antisera as the first antibody. Lane: 6xHis St1a4 (A1), 6xHis St1b3 (B1), 6xHis / Δ(6xHis) St1a4 (A2), 6xHis /Δ(6xHis) St1b3 (B2), Liver (male) (3), Kidney (male) (4), Liver (female) (5), Kidney (female) (6). Lane (1) 20 ng and lane (2) 290 ng of protein. Lane (3-6) 20 μg of mouse cytosolic proteins. Δ(6xHis) form was prepared from 6xHis-tagged form after the cleavage of 6xHis-tag by enterokinase.

Both St1c4 and St1e5 were not detected in liver and kidney (data not shown).

Fig. 3. Nucleotide and deduced amino acid sequences of St1d1 cDNA

The initiation and termination codons are underlined and the restriction enzyme sites are boxed.

Fig. 4 Sulfotransferase activities towards catecholamines and their metabolites by recombinant St1d1

Each value represents the mean ± S.D. of at least three determinations. Data are shown as nmol/mg protein/min. The assays were performed at pH 7.4 in the presence of 10 μM substrate and 50 ng of recombinant St1d1 proteins. N.D.: Not detected. (< 0.15 nmol/mg protein/min). COMT: catechol-*O*-methyltransferase, MAO: monoamine oxidase, AO: aldehyde oxidase, AR: aldehyde reductase. DL-mixtures of normethanephrine , vanillylmandelic acid, metanephrine, or 3,4-dihydroxyphenyletylene glycol were used in this experiment. Other catecholamines and their metabolites were used as natural L-forms.

Fig. 5 Western blot analysis using anti-St1d1 antibody

Proteins are subjected to SDS-PAGE on a 10.5% gel and electrically transferred to a nitrocellulose membrane.

Sulfotransferase fomrs on the membrane were reacted with anti-St1d1 as the first antibody.

Lane: 6xHis /Δ(6xHis) St1d1 (1), male liver (2), female liver (3), male lung (4), female lung (5), testis (6), uterus (7), male brain (8), female brain (9), male kidney (10),female kidney (11). Lane (1) 200 ng of protein. Lane (2, 3, 10, 11) 10 μg, lane (4, 5, 6, 7, 8, 9) 100 μg of mouse cytosolic protein.

Fig. 6 Inhibitory effect of anti-St1d1 antiserum on St1d1-mediated p-nitrophenol, dopamine and DOPAC sulfation

A) Effects of anti-St1d1 antiserum on St1d1- or St1a4-mediated *p*-nitrophenol sulfation. Data are shown as the relative percent of the respective controls (no addition of serum). Anti-St1d1 antiserum (15 μg) was added to the incubation mixture containing 50 ng of recombinant protein and 5 μM *p*-nitrophenol.

B) Effects of addition of anti-St1d1 antiserum on St1d1- mediated (a) or renal cytosolic (b) catecholamine sulfation

Open circles and closed circles indicated the relative percent of the controls (no addition of serum) by the addition of preimmunized and anti-St1d1 antiserum , respectively. The incubation mixture contains 12.5 ng of recombinant St1d1 or 2.5 μg of renal cytosols, and 10 μM dopamine or DOPAC. Other experimental details are described in Materials and Methods.

Fig. 7 Detection of catecholamine sulfotransferase in human

A. Detection of St1a4 and St1d1 mRNA in mouse kidney by RT-PCR. First-strand cDNA, as a template for PCR, was synthesized from total RNA of mouse kidney, using Ready-to-Go You-prime First-Strand Beads (Pharmacia Bio Tech). After an initial denaturation at 94˚C for 2 min, the amplification was performed for 25 cycles, with 30 seconds at 94˚C for denaturation, 30 s at 58˚C for annealing, 90 s at 72˚C for extension and a final extension period of 7 min at 72˚C.
Lane (1): detection of St1a4 mRNA. Primers were St1a4-5': GCGGATCCGATGACAAAATGGCTCAGAACCCCAGC and St1a4-3': GCGTCGACCAGTGTTAGGACTGATGGC. Lane (2): detection of St1d1 mRNA. Primers were St1d1-5': CGGCATGCGATGACGATGACAA AATGGATAACAAACTGGATGTC and St1d1-3': CGCAAGCTTCAACTCTCCCTAGATCTC.

B. Detection of ST1A mRNA in human kidney by RT-PCR. Human kidney 5'-STRETCH PLUS cDNA, as a template for PCR, pooled from 8 male/female Caucasians (age 24-55) was purchased from CLONTECH. After an initial denaturation at 94˚C for 10 min, the amplification was performed for 35 cycles, with 30 s at 94˚C for

denaturation, 30 s at 55˚C for annealing, 90 s at 72˚C for extension and a final extension period of 7 min at 72˚C.

Lane (1): detection of ST1A mRNA. Primers were ST1A-5': GCGGATCCGATGACGATGACAAAATGGAGCTGATCCAGGAC and ST1A-3': GCAAGCTTCCCCTCTCACAGCTCAGAGCGG. Lane (2): detection of ST1D mRNA. Primers were ST1D-5': ATGGACAATGAGCAGCGTGTC and ST1D-3': AATTTTCCTTTACCAAACACAGG. Lane (3): detection of ST1D mRNA (338 bp). Primers were ST1D633-5': GGAGAAAGACAAGCCAGAAACAATT and ST1D-3'. M represents 1 kbp marker.

C. Western blot analysis using anti-ST1A5 antibody

Proteins were subjected to SDS-PAGE on a 10.5 % gel and electrically transferred to a nitrocellulose membrane. ST forms on the membrane were reacted with anti-ST1A5 antibody. Lane(1): 20 ng of each Δ(6xHis) ST1A3 and Δ(6xHis) ST1A5, (2) 200 μg of human kidney cytosolic protein (3) 20 μg of human liver cytosolic protein.

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Table 1 Substrate specificity of mouse recombinant St1d1

Each value represents the mean ± S.D. of at least three determinations. Sulfotransferase activities were assayed using 3'-phosphoadenosine-[^{35}S]-5'-phosphosulfate ([^{35}S]-PAPS) as a sulfate donor. The assays were performed at pH 7.4 in the presence of 100 μM of the substrate, except for *p*-nitrophenol (5 μM) and 4'-OH-PhIP (10 μM).

N.D.: Not detected (< 0.15 nmol/mg protein/min).

Compound	Sulfotransferase activity (nmol/mg protein/min)	Compound	Sulfotransferase activity (nmol/mg protein/min)
p-Nitrophenol	129.8 ± 6.5	Corticosterone	N.D.
Dopamine	130.7 ± 27.4	Dehydroepiandrosterone	N.D.
4'-OH-PhIP	73.0 ± 2.3	Cholesterol	N.D.
Estradiol	9.8 ± 6.8	Lithocholic acid	N.D.
L-Triiodothyronine	13.5 ± 7.5	1-Pyrenemethanol	N.D.
2-Methylbenzyl alcohol	37.2 ± 3.9	Aniline	N.D.
Minoxidil	3.2 ± 1.0	Desipramine	N.D.

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Table 2 Apparent kinetic parameters for sulfation of dopamine and DOPAC by recombinant St1d1

Each value represents the mean ± S.D. of at least three different determinations. Sulfotransferase activities were assayed using 3'-phosphoadenosine-[^{35}S]-5'-phosphosulfate([^{35}S]-PAPS) as a sulfate donor. The assays were performed at pH7.4 in the presence of 10 μM–10 mM dopamine or 5 μM–300 μM and 50 ng of recombinant St1d1.

Substrate	K_m (μM)	V_{max} (nmol/mg protein/min)	V_{max}/K_m
Dopamine	713.2 ± 14.2	1474 ± 9.03	2.07 (1)
DOPAC	35.0 ± 1.1	1678 ± 159	47.9 (23.14)

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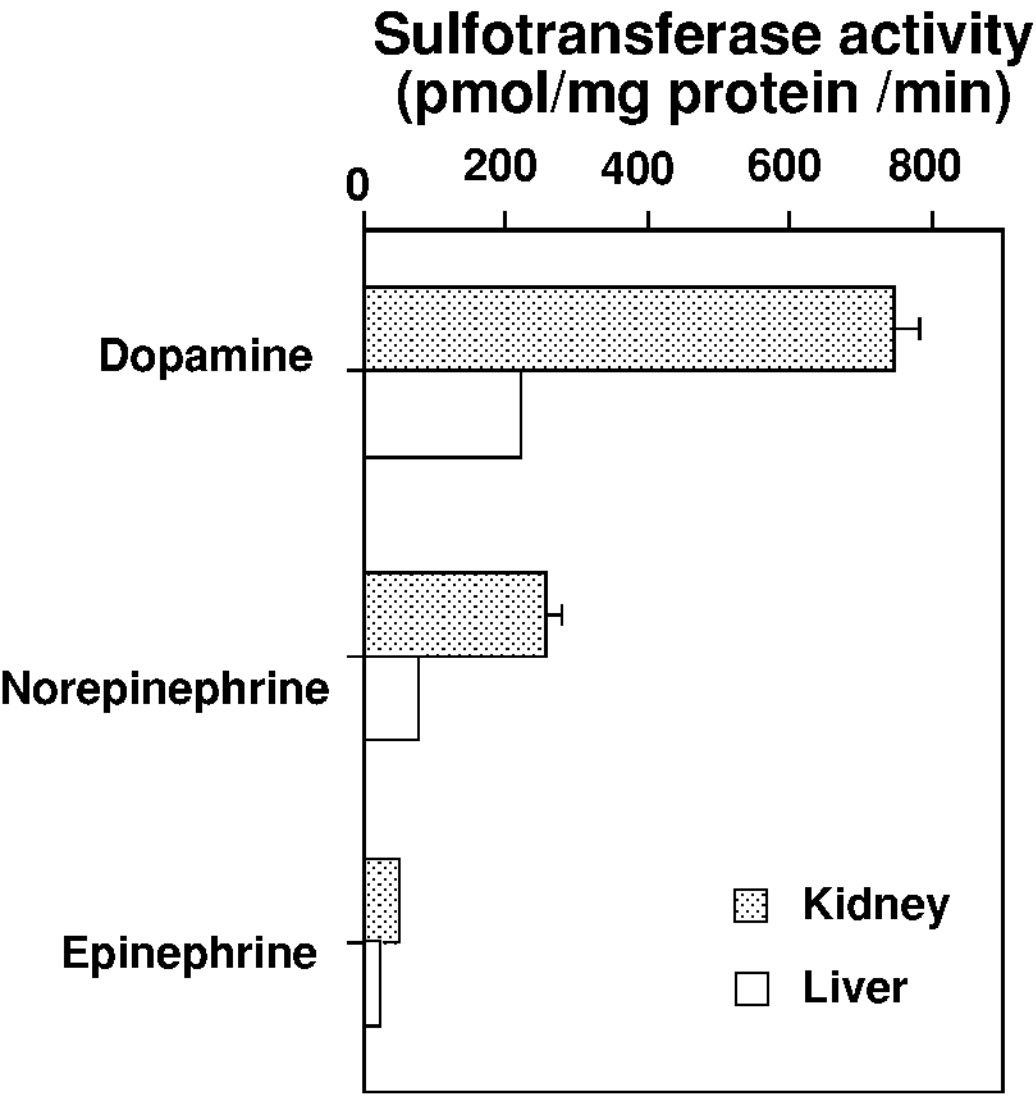

Figure 1, JPET#65532

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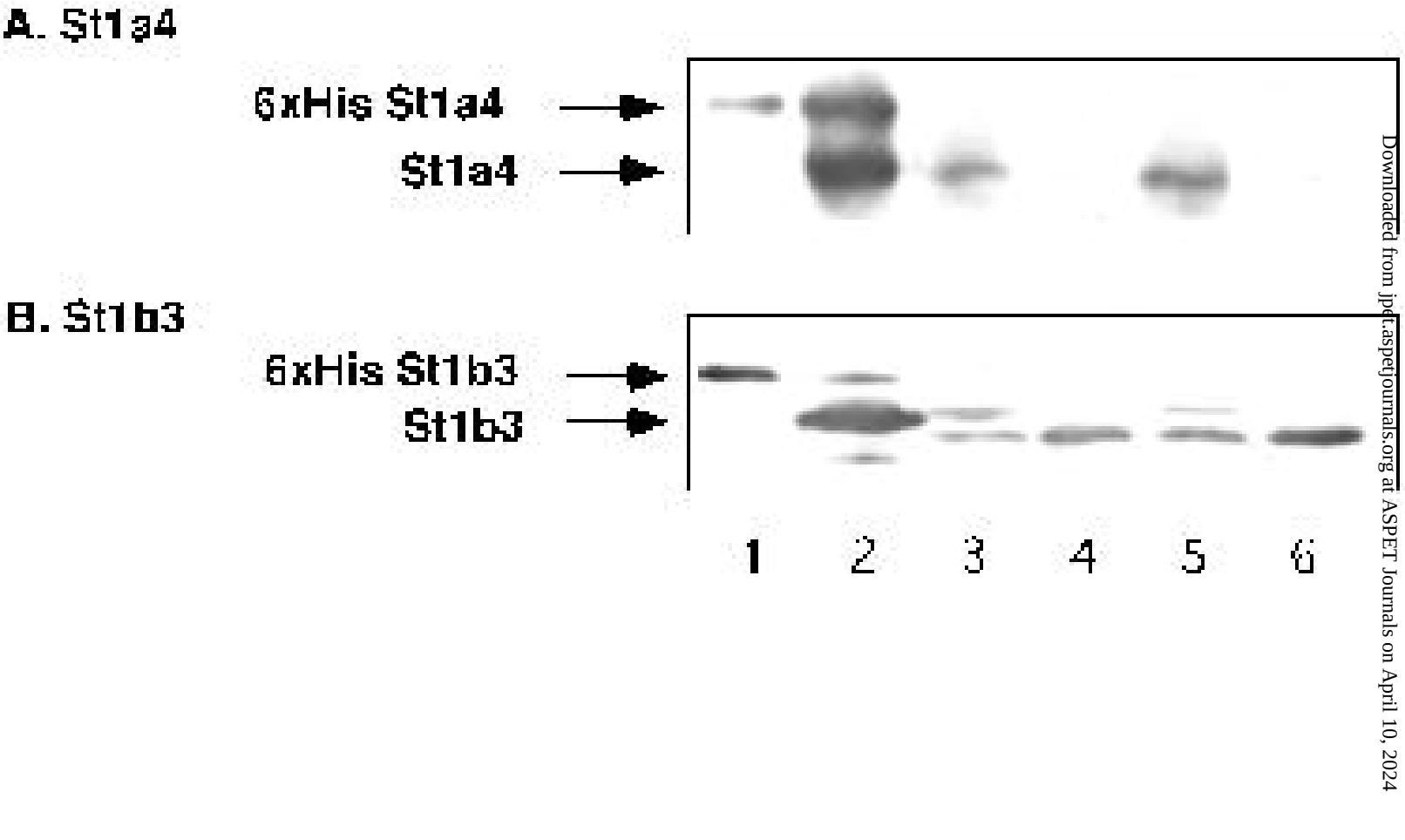

Figure 2, JPET#65532

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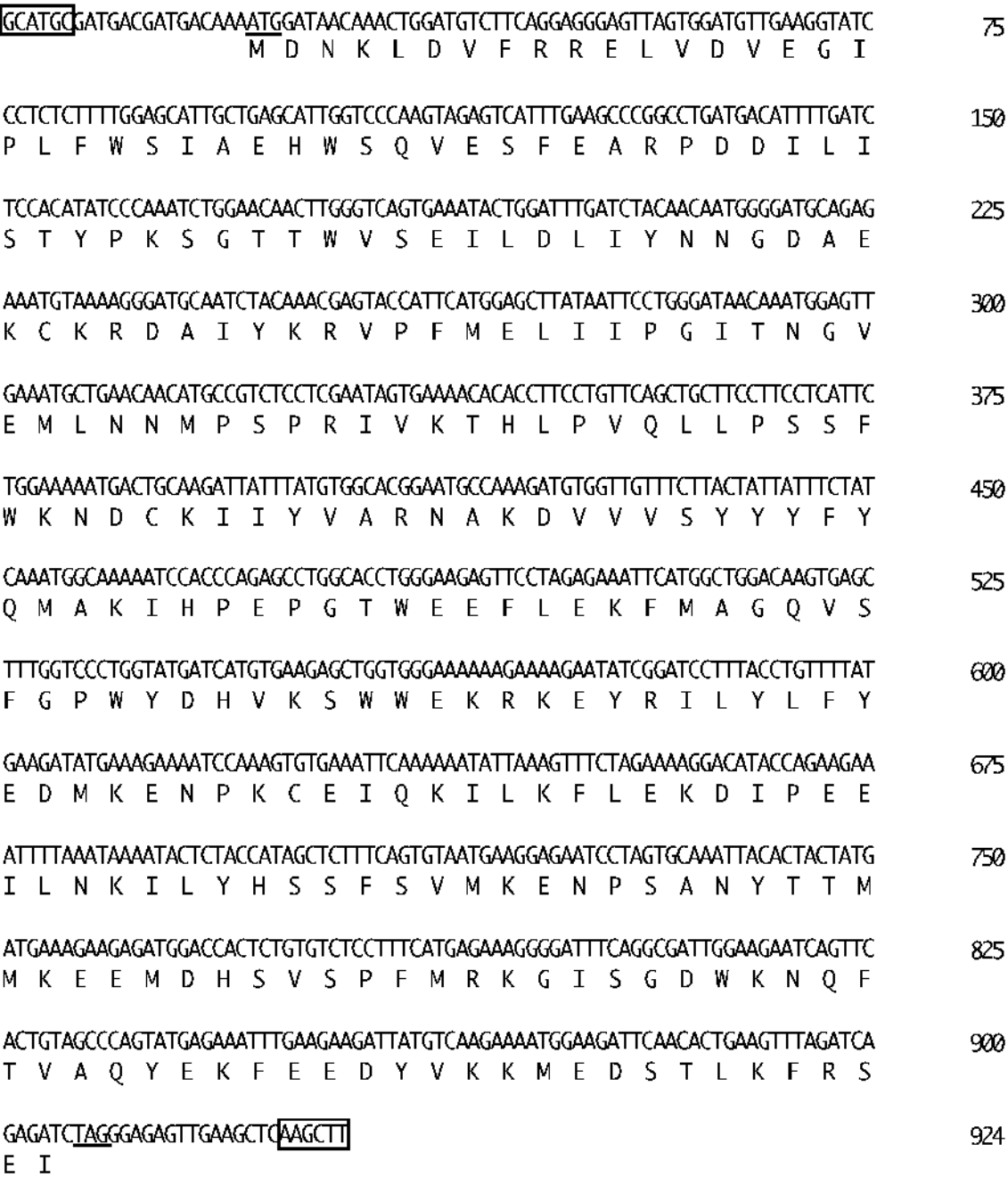

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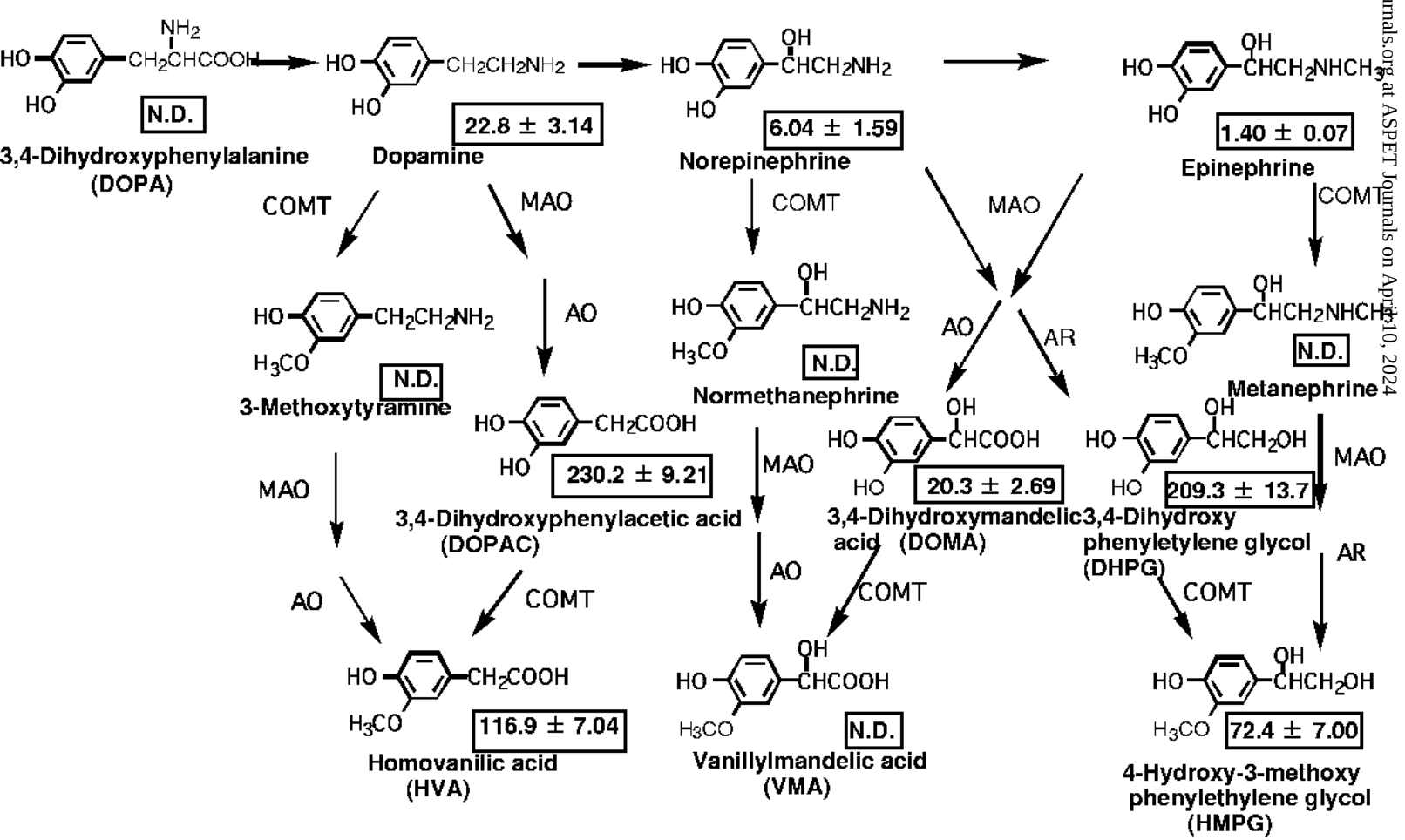

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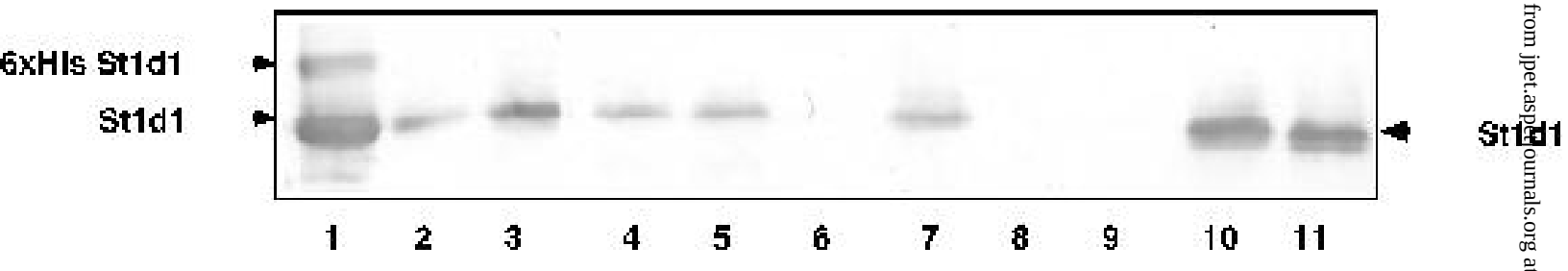

Figure 5, JPET#65532

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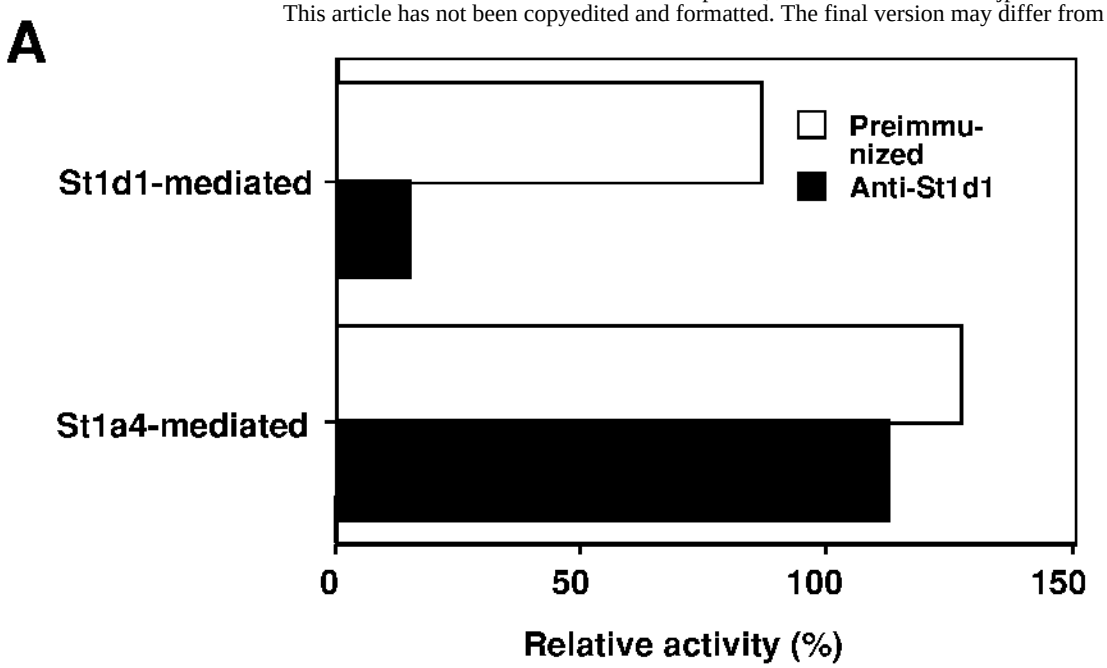

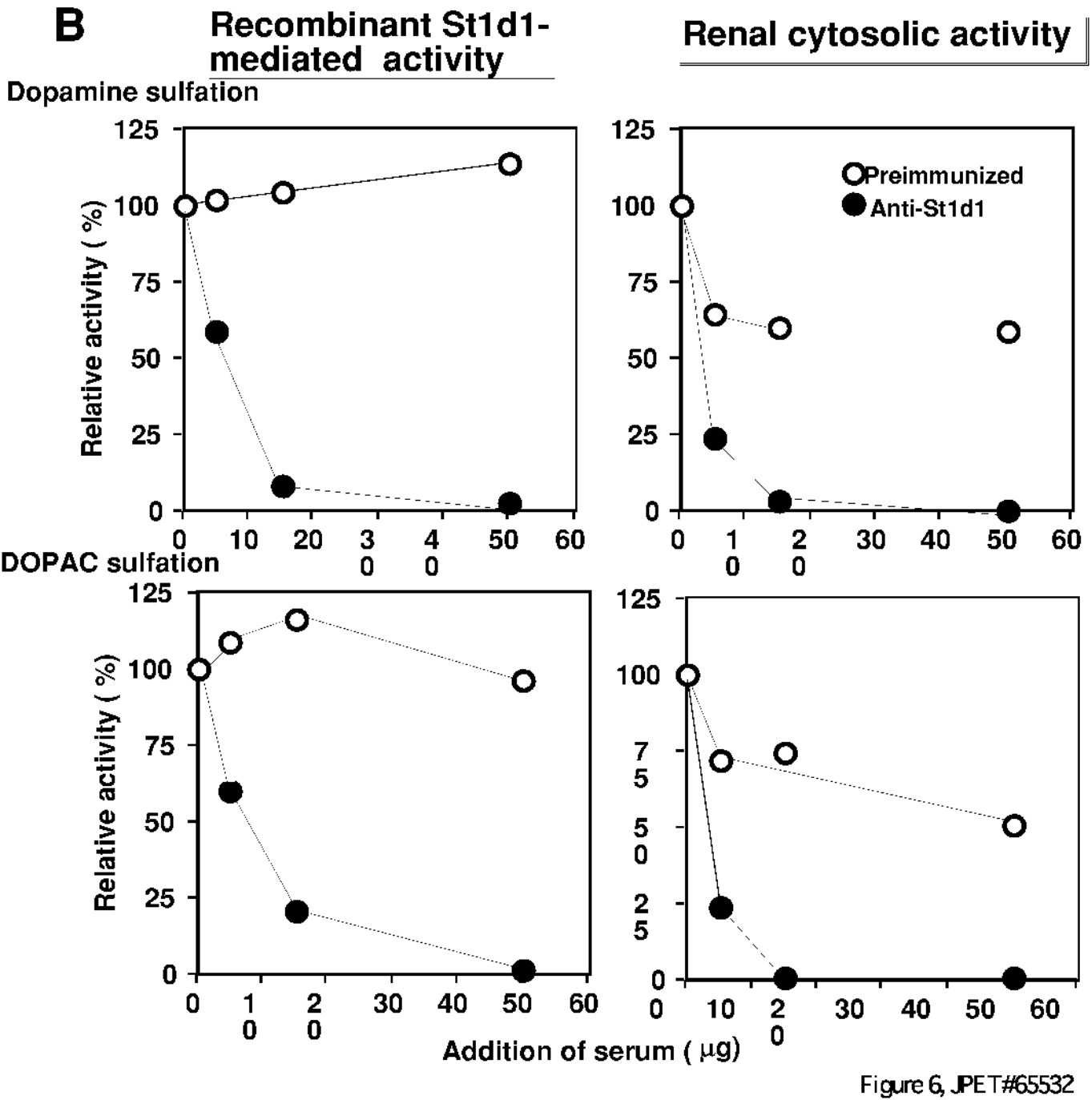

Figure 6, JPET#65532

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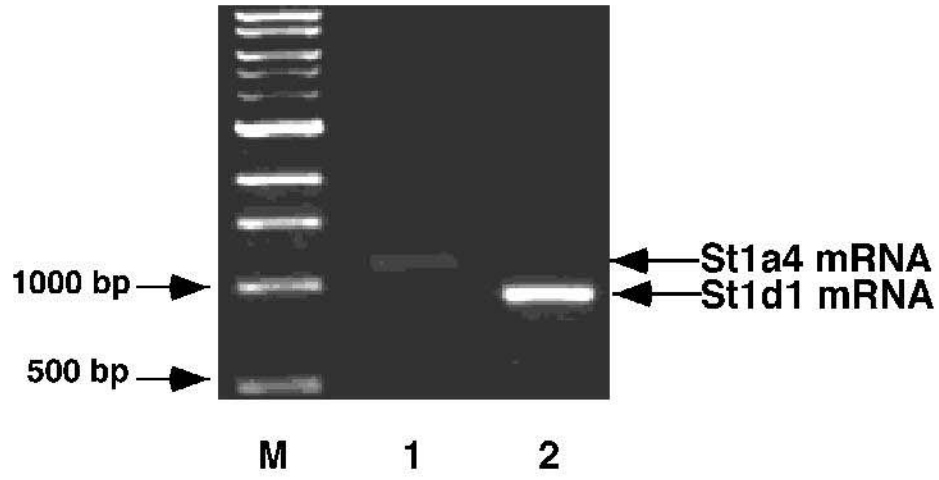

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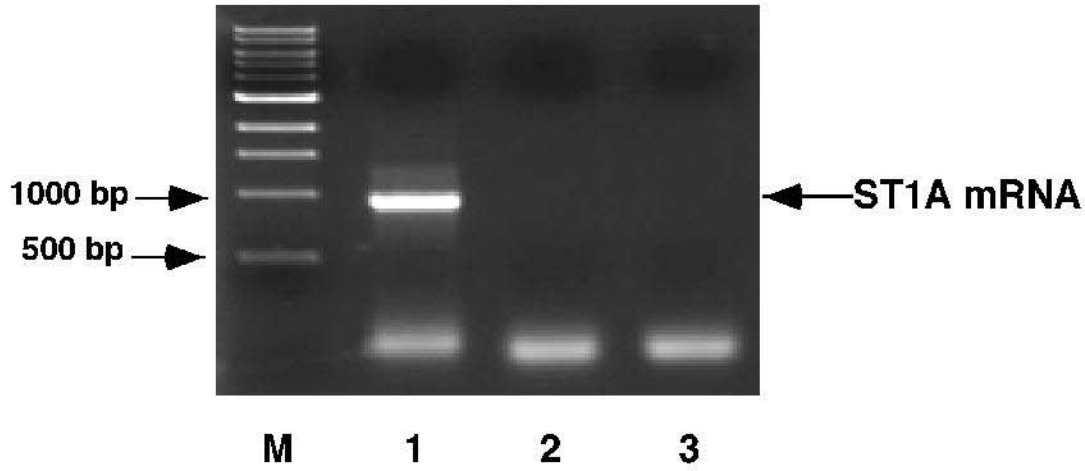

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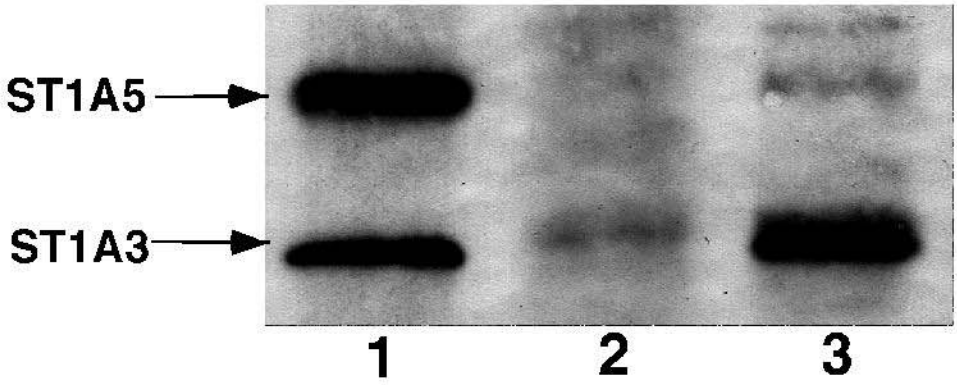

Figure 7, JPET#65532