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Human Histamine *N*-Methyltransferase Pharmacogenetics: Cloning and Expression of Kidney cDNA

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SUMMARY

Histamine *N*-methyltransferase (HNMT) catalyzes the N^{τ}-methylation of histamine. The level of HNMT activity in human red blood cells is controlled by a common genetic polymorphism. We set out to clone and express a cDNA for HNMT from human tissue as a first step toward a determination of the molecular basis for this genetic polymorphism. The cloning strategy was based on possible sequence homology between rat and human kidney HNMT. Human kidney cDNA libraries were screened with the 885-nucleotide open reading frame of rat kidney HNMT cDNA. A 1.4-kilobase cDNA clone was isolated that contained two potential translation initiation codons, both in the same reading frame. The longest open reading frame of the human kidney cDNA clone contained 876 nucleotides and encoded a protein 292 amino acids in length. The amino acid sequence of this protein was 84% identical to that of rat kidney HNMT. The human kidney cDNA clone was transcribed *in vitro* and translated in a rabbit reticulocyte lysate system to yield a protein with an apparent molecular mass of 33 kDa, as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The human kidney cDNA was also subcloned into the eukaryotic expression vector p91023(B). Partially purified HNMT isolated from the cytosol of COS-1 cells transfected with this expression construct had biochemical properties similar to those of human kidney HNMT. Human renal cortical HNMT, partially purified human renal cortical HNMT, and partially purified transfected COS-1 cell HNMT had K_m values for histamine and *S*-adenosyl-L-methionine, the two cosubstrates for the enzyme reaction, of 20, 13, and 14 μM and 2.0, 3.0, and 6.2 μM, respectively. IC_{50} values for the HNMT inhibitor amodiaquine were 0.50, 0.48, and 0.40 μM, respectively, for enzyme from these same three sources. Northern blot analyses performed with poly(A)$^+$ RNA from a series of human tissues including kidney demonstrated three transcripts, approximately 1.3, 3.8, and 4.0 kilobases in length. Cloning of a cDNA for HNMT may now make it possible to determine the molecular basis for the HNMT genetic polymorphism in humans.

HNMT (EC 2.1.1.8) is a cytosolic enzyme that catalyzes the N^{τ}-methylation of histamine (1). Ado-Met is the methyl donor for the reaction. Histamine plays an important role in allergy and in the regulation of gastric acid secretion (2, 3). In addition, it is a neurotransmitter in the CNS (4, 5). There are two major metabolic pathways for histamine, N^{τ}-methylation catalyzed by HNMT and oxidative deamination catalyzed by diamine oxidase (EC 1.4.3.6) (5–7). N^{τ}-methylation is the major process responsible for termination of the neurotransmitter actions of histamine in the mammalian brain (8, 9) because diamine oxidase activity is not present in the CNS (8, 10) and there is no indication that a high affinity uptake system for histamine exists (5, 11). Therefore, individual variations in HNMT activity might contribute to individual differences in the neurotransmitter function of histamine and to its role in the pathophysiology of human disease.

HNMT activity in the human RBC varies over a 5-fold range, and this variation is controlled by a common genetic polymorphism (12, 13). We set out to clone a cDNA for HNMT from human tissue as a first step in determination of the molecular mechanism responsible for inherited variations of HNMT activity in humans. The cloning strategy was based on possible homology between the nucleotide sequences of rat and human HNMT cDNAs. The PCR was used to amplify the ORF of rat kidney HNMT cDNA (14), and the PCR amplification product was used to screen human kidney cDNA libraries. Use of this approach made it possible to isolate a cDNA clone that encoded a protein 84% identical in sequence to rat kidney HNMT. When this human kidney cDNA was expressed in COS-1 cells, the encoded protein catalyzed the HNMT enzymatic reaction and displayed biochemical properties very similar to those of human kidney HNMT. Cloning of a cDNA for human kidney HNMT may make it possible to determine the molecular mech-

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ABBREVIATIONS: HNMT, histamine *N*-methyltransferase; Ado-Met, *S*-adenosyl-L-methionine; HSS, high-speed supernatant; CNS, central nervous system; PCR, polymerase chain reaction; ORF, open reading frame; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; UTR, untranslated region; DMEM, Dulbecco's modified Eagle's medium; kb, kilobase(s); RBC, red blood cell(s).

anism responsible for the HNMT genetic polymorphism in humans.

Materials and Methods

Tissue acquisition and preparation. Renal tissue was obtained during autopsy, under guidelines approved by the Mayo Clinic Institutional Review Board. This tissue was stored at −80°. Frozen renal cortical tissue was weighed, minced with scissors, and homogenized in 4 volumes (w/v) of 5 mM potassium phosphate buffer, pH 7.5, with a Polytron homogenizer, and a 100,000 × *g* HSS was prepared as described previously (15). HSS pooled from five individual kidney samples was used as an enzyme source to partially purify HNMT for use in biochemical studies.

HNMT assay. HNMT activity was measured with a modification of the method of Van Loon *et al.* (16). This assay is based on the conversion of histamine to radioactively labeled N^{τ}-methylhistamine, with [*methyl*-^{14}C]Ado-Met as the methyl donor. Unless otherwise indicated, the final concentrations of histamine and Ado-Met were 37.5 and 12.5 μM, respectively. Pargyline was omitted from the assay procedure described by Van Loon *et al.* (16) because this compound was subsequently reported to be capable of inhibiting HNMT (17). Blanks were samples that did not contain histamine. One unit of HNMT activity represented the formation of 1 nmol of N^{τ}-methylhistamine per hr of incubation at 37°.

Protein assay. Protein concentrations were measured by the method of Bradford (18), with bovine serum albumin as a standard.

Partial purification of HNMT. Human kidney HNMT was partially purified by the use of ion exchange chromatography. Five milliliters of pooled renal cortical HSS or transfected COS-1 cell HSS was applied to a 0.9- × 15-cm DEAE-Sepharose CL-6B column that had been equilibrated with 5 mM potassium phosphate buffer, pH 7.5, containing 0.25 mM dithiothreitol and 0.05 mM EDTA (buffer A). The column was eluted with 5 ml of 100 mM NaCl dissolved in buffer A, followed by a 100-ml linear gradient that ranged from 100 to 200 mM NaCl in buffer A. HNMT activities and protein concentrations were measured in each fraction, and fractions with highest enzyme activities were pooled.

Preparation of RNA and PCR. Total RNA was isolated from male Sprague-Dawley rat renal tissue by extraction with guanidine HCl, followed by centrifugation through a cushion of CsCl (19). First-strand cDNA for use as a template for the PCR was synthesized with an oligo(dT) primer and murine reverse transcriptase. Oligonucleotide primers for the PCR were designed on the basis of the nucleotide sequence of rat kidney HNMT cDNA (14). The primers used were 5′-ATGGCATCTTTCATGAGGAGCTTA-3′ and 5′-TACATTTGCCTCAACCACTATAAA-3′. The PCR reaction was performed in a 100-μl reaction volume (10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 50 μM concentrations of each of the four deoxynucleoside triphosphates, and 1 unit of *Thermus aquaticus* polymerase), in a Perkin Elmer Cetus DNA thermal cycler (Emeryville, CA). After initial denaturation for 10 min at 95°, the conditions used were 35 cycles of 1 min at 94°, 2 min at 52°, and 3 min at 72°, followed by a final 10-min incubation at 72°. PCR products were applied to a 1% agarose gel and were visualized by staining with ethidium bromide. Bands of the expected size (approximately 900 nucleotides) were excised from the gel, and DNA was isolated with the GeneClean kit (BI0 101 Inc., La Jolla, CA).

Cloning of PCR product and screening of cDNA libraries. The ends of the PCR product were filled in with the Klenow fragment of DNA polymerase. The amplification product was then ligated into the *Sma*I site of pBluescript (Stratagene, La Jolla, CA), and the plasmid was used to transform *Escherichia coli* DH5α made competent by the method of Hanahan (20). One positive clone was isolated and the insert was partially sequenced by the dideoxy method of Sanger *et al.* (21) with the ^{35}S-sequencing protocol of the Sequenase kit version 2.0 (United States Biochemical Corp., Cleveland, OH). The sequence obtained was identical to that reported previously for rat kidney HNMT cDNA (14). The cloned PCR amplification product was radioactively labeled with [α-^{32}P]dCTP by random priming (22) and was used to screen approximately 10^6 plaques from a Stratagene human kidney cDNA library in Uni-Zap XR and 6 × 10^5 plaques from a Clontech (Palo Alto, CA) human kidney cDNA library in λgt11. The Stratagene cDNA library was prepared with renal tissue obtained from a 32-year-old man, and the Clontech cDNA library was prepared with renal tissue obtained from a 20-year-old white woman. Four clones from the λgt11 library remained positive through secondary and tertiary screening, and the longest of the clones was characterized completely. The λgt11 DNA insert was isolated by use of the PCR. PCR primers were designed on the basis of λgt11 nucleotide sequences located on both sides of the *Eco*RI cloning site. The sequences of the primers were 5′-GCCCGTCAGTATCG-3′ and 5′-CGACCGGCGCTCAG-3′. The PCR was performed as described for rat kidney cDNA, except that 2.5 units of *Pyrococcus furiosus* DNA polymerase were used. The 1.4-kb PCR product was separated on a 1% agarose gel and was isolated with the GeneClean kit. The ends of the PCR amplification product were filled in, and it was subcloned into the *Sma*I site of pBluescript. The resulting clone was sequenced completely on both strands.

***In vitro* transcription and translation.** The human kidney cDNA clone was linearized with *Hind*III, and T7 RNA transcripts were synthesized using the mCAP capping kit (Stratagene). These RNA samples were translated *in vitro* with a rabbit reticulocyte lysate system (23). Translated proteins were analyzed by SDS-PAGE.

Expression in COS-1 cells. To express the human kidney cDNA in COS-1 cells, the insert had to be ligated into the *Eco*RI site of the expression vector p91023(B). Because of the existence of an *Eco*RI site within the ORF of the clone, it was not possible to use *Eco*RI itself for this purpose. Therefore, we took advantage of the compatibility of *Mun*I and *Eco*RI digestion products to prepare the expression construct. Specifically, the 5′ UTR, the ORF, and approximately 500 nucleotides of the 3′ UTR of the human kidney clone were amplified with the PCR by using the following primers that contained restriction sites for *Mun*I: 5′-AGTCCAATTGGCTTCCTGCTCTGTCTT-3′ and 5′-AGTCCAATTGTTCTAAAGGTGCTTACT-3′. The PCR was performed in a 100-μl reaction volume with 2.5 units of *P. furiosus* DNA polymerase. Reaction conditions were 35 cycles of 1 min at 94°, 2 min at 52°, and 3 min at 72°, followed by a final 10-min incubation at 72°. The PCR mixture was then applied to a 1% agarose gel, bands of the expected size were excised, and DNA was isolated with the GeneClean kit. The ends of the PCR amplification product were filled in with the Klenow fragment of DNA polymerase, the product was digested with *Mun*I, and it was ligated into the *Eco*RI site of the eukaryotic expression vector p91023(B) (24, 25). Positive clones were isolated by colony screening (26) performed with PCR amplification product that had been radioactively labeled with [α-^{32}P]dCTP using random primers (22). The orientation of inserts was determined by partial DNA sequencing.

COS-1 cells were plated at a density of 1.5–1.8 × 10^6 cells/plate in DMEM with 10% fetal calf serum, in 100-mm plates, and were allowed to grow overnight. Three plates of cells were used for each transfection. Purified plasmid (0.6 μg) was mixed with DEAE-dextran and DMEM, and this mixture was added to the cell culture plates (27, 28). Control cells were treated with expression vector only or with constructs that contained the cDNA clone in the antisense orientation. After 1 hr, the DNA-DEAE-dextran solution was replaced for 2 min with DMEM that contained 10% dimethylsulfoxide, followed by incubation for 2 hr with 0.1 mM chloroquine in DMEM (29). Cells were then grown for 40–42 hr in DMEM with 10% fetal calf serum. The cells from each transfection were harvested, cell pellets were washed with 5 ml of phosphate-buffered saline, and the pellets were homogenized for 30 sec in 2 ml of 5 mM potassium phosphate buffer, pH 7.5. Homogenates were centrifuged at 15,000 × *g* for 15 min at 4°, supernatants from this step were centrifuged at 100,000 × *g* for 1 hr at 4°, and 25 μl of the 100,000 × *g* supernatant were assayed for HNMT activity.

Northern and Southern blot analyses. The probe used for hybridization with Northern and Southern blots consisted of the 5′ UTR, the ORF, and 14 nucleotides of 3′ UTR of the human kidney cDNA.

This probe was prepared by PCR amplification of the cDNA performed with the following primers: 5′-AGTCCAATTGGCTTCCTGCTCTGT CTT-3′ and 5′-TTTGTGATTGATAGTTAT-3′. The PCR was performed in a 100-μl reaction volume with 1 unit of *T. aquaticus* DNA polymerase. The amplification conditions were 35 cycles of 1 min at 94°, 2 min at 52°, and 3 min at 72°, followed by a final 10-min incubation at 72°. The PCR mixture was then applied to a 1% agarose gel, bands of the expected size were excised from the gel, and DNA was isolated with the GeneClean kit.

A Multiple Tissue Northern blot obtained from Clontech was used for Northern blot analysis. Each lane contained approximately 2 μg of purified poly(A)$^+$ RNA from a series of human tissues. Southern blot analysis was performed with lymphocyte genomic DNA obtained from Promega (Madison, WI). Aliquots of 5 μg of genomic DNA were digested with excess amounts of *Bam*HI, *Eco*RI, *Hind*III, and *Pst*I, followed by electrophoresis performed with a 0.8% agarose gel. The DNA was then transferred to a nylon membrane. The probe used for hybridization was labeled with [α-^{32}P]dCTP by the use of random primers (22).

Data analysis. The University of Wisconsin Genetics Computer Group software package (30) was used to analyze sequence information and to make comparisons between the amino acid sequence of the protein encoded by the human kidney HNMT cDNA and sequences of other cloned proteins. The GenBank Genetics Data Bank, the EMBL Nucleotide Sequence Database, and the Swiss-Prot Protein Sequence Database were used to search for nucleotide and amino acid sequence homologies. IC_{50} values were calculated with the GraphPAD Inplot curve-fitting program (GraphPAD InPlot Software, San Diego, CA). K_m values were calculated by the method of Wilkinson (31), with a computer program written by Cleland (32).

Materials. [*methyl*-^{14}C]Ado-Met (58 μCi/μmol) was obtained from DuPont-NEN (Boston, MA). [α-^{35}S]dATP (>1000 Ci/mmol) and [α-^{32}P]dCTP (>3000 Ci/mmol) were purchased from Amersham Corp. (Arlington Heights, IL). DNA ligase and murine reverse transcriptase were obtained from GIBCO BRL (Gaithersburg, MD). Restriction enzymes except for *Mun*I were purchased from GIBCO BRL and Boehringer Mannheim Corp. (Indianapolis, IN). *Mun*I was obtained from New England Biolabs (Beverly, MA). DEAE-Sepharose CL-6B and DEAE-dextran were purchased from Pharmacia LKB Biotechnology, Inc. (Piscataway, NJ). Bio-Rad Protein Assay Dye Reagent was obtained from Bio-Rad Laboratories (Richmond, CA). Bovine serum albumin, dithiothreitol, *S*-adenosyl-L-homocysteine HCl, Ado-Met HCl, and salmon testis DNA were purchased from Sigma Chemical Co. (St. Louis, MO). 3,4-Dimethoxy-5-hydroxybenzoic acid was obtained from ICN Pharmaceuticals, Inc. (Plainview, NY). A low molecular weight marker kit was obtained from Diversified Biotech (Newton Center, MA). Amodiaquine HCl was donated by Warner Lambert Co. (Ann Arbor, MI).

Results

Screening of human kidney cDNA libraries. The cloning strategy used was based on possible sequence homology between human and rat kidney HNMT cDNA. Therefore, the first step involved use of the PCR to amplify the ORF of rat kidney HNMT cDNA with primers designed on the basis of the nucleotide sequence of rat kidney cDNA (14). The PCR amplification product was sequenced for 156 nucleotides from the 5′ terminus and for 186 nucleotides from the 3′ terminus to verify that it was rat kidney HNMT cDNA. The sequence obtained was identical to that reported for rat kidney HNMT cDNA (14), with the exception of three nucleotides that could not be identified. We then screened human kidney cDNA libraries constructed in two different vectors, Uni-Zap XR and λgt11. The 885-nucleotide ORF of rat HNMT cDNA was used as a probe. No positive clones were isolated from the Uni-Zap XR human kidney cDNA library. However, initial screening of the cDNA library constructed in λgt11 yielded eight positive clones, four of which remained positive through secondary and tertiary screening. The longest of these clones was sequenced completely on both strands by using a total of 18 sequencing primers. This clone consisted of 1455 nucleotides, with two possible initial ATG codons separated by nine nucleotides (Fig. 1). The environment of the first of these ATG codons closely approximated the consensus sequence for translation initiation in higher eukaryotes (33). When it was assumed that this initial ATG triplet was the site at which translation was initiated, the ORF of the cDNA consisted of 876 nucleotides and encoded a 292-amino acid protein with a predicted molecular mass of 33.3 kDa. The 5′ UTR consisted of 39 nucleotides, and the 3′ UTR was 540 nucleotides in length and terminated in a poly(A) tract. An ATTAAA polyadenylation signal (34) was present 22 nucleotides upstream of the poly(A) tract (Fig. 1). The next step was to determine whether the cDNA could be translated *in vitro* and whether the protein that it encoded could catalyze the HNMT enzymatic reaction.

Expression of HNMT cDNA. The coding region of the human kidney cDNA clone was transcribed *in vitro*, and the RNA obtained was translated in a rabbit reticulocyte lysate system (23). The major translation product had an apparent molecular mass of 33 kDa, as estimated by SDS-PAGE (Fig. 2). The next step in the evaluation of the properties of the protein encoded by the human kidney HNMT cDNA clone was expression in COS-1 cells.

The human kidney cDNA clone was transiently expressed in COS-1 cells after subcloning, in both orientations, into the eukaryotic expression vector p91023(B). After transient expression, HNMT activity was measured in HSS prepared from

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AACCTTGCTTCCTGCTCTGTCTTTCTCAGAAAACCAAATATGGCATCTTCCATGAGGAGC      21
                                          M  A  S  S  M  R  S
TTGTTTTCTGACCACGGGAAATATGTTGAATCTTTCCGGAGGTTTCTCAACCATTCCACG      81
L  F  S  D  H  G  K  Y  V  E  S  F  R  R  F  L  N  H  S  T
GAACACCAGTGCATGCAGGAATTCATGGACAAGAAGCTGCCAGGCATAATAGGAAGGATT     141
E  H  Q  C  M  Q  E  F  M  D  K  K  L  P  G  I  I  G  R  I
GGAGACACAAAATCAGAAATTAAGATTCTAAGCATAGGCGGAGGTGCAGGTGAAATTGAT     201
G  D  T  K  S  E  I  K  I  L  S  I  G  G  G  A  G  E  I  D
CTTCAAATTCTCTCCAAAGTTCAGGCTCAATACCCAGGAGTTTGTATCAACAATGAAGTT     261
L  Q  I  L  S  K  V  Q  A  Q  Y  P  G  V  C  I  N  N  E  V
GTTGAGCCAAGTGCTGAACAAATTGCCAAATACAAAGAGCTTGGTAGCCAAGATATCGAAC     321
V  E  P  S  A  E  Q  I  A  K  Y  K  E  L  V  A  K  I  S  N
CTCGAGAACGTAAAGTTTGCTTGGCATAAGGAGACATCATCTGAATACCAAAGTAGAATG     381
L  E  N  V  K  F  A  W  H  K  E  T  S  S  E  Y  Q  S  R  M
TTGGAGAAAAAGGAGCTTCAAAAGTGGGACTTTATTCATATGATTCAAATGCTGTATTAT     441
L  E  K  K  E  L  Q  K  W  D  F  I  H  M  I  Q  M  L  Y  Y
GTAAAAGACATCCCAGCTACCCTGAAATTCTTCCATAGTCTCTTAGGTACCAATGCTAAG     501
V  K  D  I  P  A  T  L  K  F  F  H  S  L  L  G  T  N  A  K
ATGCTCATTATTGTTGTGTCAGGAAGCAGTGGCTGGGACAAGCTGTGGAAAAAGTACGGA     561
M  L  I  I  V  V  S  G  S  S  G  W  D  K  L  W  K  K  Y  G
TCACGCTTTCCCCAGGATGACCTCTGCCAGTATATCACATCAGATGACCTCACTCAGATG     621
S  R  F  P  Q  D  D  L  C  Q  Y  I  T  S  D  D  L  T  Q  M
CTGGACAACCTAGGGCTTAAGTATGAGTGCTATGACCTTTTGTCCACCATGGATATATCT     681
L  D  N  L  G  L  K  Y  E  C  Y  D  L  L  S  T  M  D  I  S
GACTGCTTTATTGATGGTAATGAAAATGGAGACCTGCTTTGGGATTTTTTGACTGAAACC     741
D  C  F  I  D  G  N  E  N  G  D  L  L  W  D  F  L  T  E  T
TGCAACTTTAATGCCACAGCACCACCTGATCTCAGAGCAGAGCTTGGGAAAGATCTACAA     801
C  N  F  N  A  T  A  P  P  D  L  R  A  E  L  G  K  D  L  Q
GAGCCTGAATTTAGTGCTAAGAAAGAGGGGAAGGTTCTTTTTAATAATACTCTGAGTTTC     861
E  P  E  F  S  A  K  K  E  G  K  V  L  F  N  N  T  L  S  F
ATAGTGATTGAGGCATAACTATCAATCACAAAAGTATATTCAAAAATTATATTTTGAACA     921
I  V  I  E  A  *
ACTCGAATCACTCATTTGTTTCCATATTAAAATCACAAACTCATCCATTAATGTAGATAA     981
AGCACTGTTTGGATATGAGATGTAGCAAATTCCAATACATTATTGGACTTCCATTTGGAA    1041
TCATATGGGATACTGCTGGTCTTATCCTGTCCCTCCTCCAGGTAGAGAGACCACATGCAG    1101
GCTCAACATAACATAAGCTAGAAAAATTAGATGACTGAATTTCTATGGCATATTGATAAT    1161
AAAATTCATTCCATTTGCTGATTGTCTGAAATTTTCTAGAATACTAATAAAATACATACT    1221
ATAGATTCTTTATTAGTGAAGTATGCACTAATCAATACTTTGAACACAAAGCCTGTGTTA    1281
CTGATTTGGCCGTTTTGTGAAGAAACATTTATCTTTGTACGTTCTTCTATTGTGCTTTCT    1341
ATCTAATTTTTATTAATTTGTAAGAGTAAGCACCTTTAGAATATTAAAAATTAATTCTTT    1401
ATC(A)12
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Fig. 1. Human kidney HNMT cDNA clone nucleotide sequence. The *underlined* sequence is a polyadenylation signal (34).

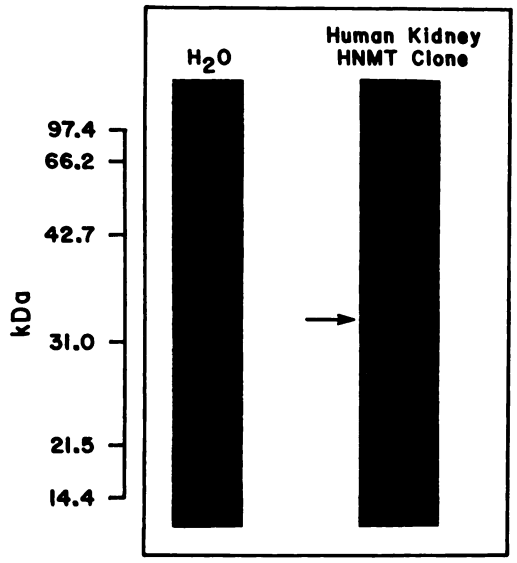

Fig. 2. Human kidney HNMT cDNA clone translation in a rabbit reticulocyte lysate system. SDS-PAGE of [^{35}S]methionine-labeled protein obtained by translation of the human kidney HNMT cDNA clone in a rabbit reticulocyte lysate system is shown. The clone encoded a protein with an approximate molecular mass of 33 kDa (*arrow*). Results obtained with a control sample in which only water was added to the rabbit reticulocyte lysate system (H_2O) are also shown (see text for details).

these cells, under optimal conditions for the assay of human kidney HNMT activity. When COS-1 cells were transfected with the human kidney cDNA clone in the sense orientation, HNMT enzymatic activity was 119, 188, and 93 units/mg of protein in three separate plates, whereas no HNMT activity was detected in cells transfected with only vector or with vector that contained insert in the antisense orientation.

Biochemical properties of expressed HNMT. Substrate kinetic and enzyme inhibition experiments were then performed to compare the biochemical properties of human renal cortical HNMT with those of enzyme expressed in COS-1 cells. To eliminate possible artifacts due to side reactions, HNMT in COS-1 cell preparations and in renal preparations was partially purified by ion exchange chromatography. The biochemical properties of this enzyme were compared with those of HNMT in human renal cortical preparations and with those of human kidney enzyme that had been partially purified by ion exchange chromatography. Apparent K_m values for histamine were determined for all three preparations in the presence of 12.5 μM Ado-Met and a total of seven different concentrations of histamine, ranging from 2.3 to 150 μM. Apparent K_m values for Ado-Met were determined in the presence of the "optimal" histamine concentration for each enzyme preparation, i.e., 37.5 μM for renal cortical HSS and partially purified renal cortical HNMT and 75 μM for partially purified COS-1 cell HNMT. These experiments were conducted in the presence of five different concentrations of Ado-Met that varied from 0.8 to 12.5 μM. Double-inverse plots of the data for the substrate kinetic experiments are shown in Fig. 3, and apparent K_m values estimated from these plots are listed in Table 1. Apparent K_m values were similar for all three enzyme preparations.

The response of partially purified COS-1 cell HNMT to a panel of methyltransferase inhibitors and ions was also compared with that of partially purified human renal cortical enzyme (Table 2). Both activities were inhibited by a known HNMT inhibitor, amodiaquine (35), and by *S*-adenosyl-L-homocysteine, an inhibitor of all Ado-Met-dependent methyltransferases (36). However, neither was inhibited by the catechol *O*-methyltransferase inhibitors $CaCl_2$ (37) and 3,4-dimethoxy-5-hydroxybenzoic acid (38). 3,4-Dimethoxy-5-hydroxybenzoic acid also inhibits thiopurine methyltransferase activity (39). Finally, human kidney HNMT and enzymes partially purified from either human renal cortex or transfected COS-1 cells were used to estimate IC_{50} values for amodiaquine. IC_{50} values were calculated on the basis of data obtained with seven different concentrations of amodiaquine that ranged from 0.001 to 10 μM (Fig. 4). Those values were also similar for HNMT in human renal cortical HSS, partially purified human renal cortical HNMT, and partially purified transfected COS-1 cell HNMT (Table 1).

Northern and Southern blot analyses. Northern blots were used to analyze patterns and abundance of HNMT mRNA in human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. The probe consisted of the ORF of human kidney HNMT cDNA plus approximately 50 nucleotides of the 5′ UTR and 14 nucleotides of the 3′ UTR. HNMT mRNA was detectable in all tissues studied (Fig. 5). Three transcripts, approximately 1.3, 3.8, and 4.0 kb in length, were observed (Fig. 5). The 1.3-kb transcript, approximately the size of our 1.4-kb cDNA clone, was the most abundant species in all tissues. Of the tissues studied, skeletal muscle, pancreas, and heart displayed the least HNMT mRNA and lung, liver, and kidney the most (Fig. 5). When the same membranes were probed with the ORF of cyclophyllin as a control (data not shown), a very different tissue distribution pattern was seen, with heart, brain, lung, and liver showing the highest cyclophyllin mRNA concentrations.

Human lymphocyte genomic DNA that had been digested with four different restriction enzymes was used for Southern blot analysis (Fig. 6). A restriction site for only one of these enzymes, *Eco*RI, was present within the ORF of the human kidney HNMT cDNA. The probe was the same as that used to perform the Northern blots. The patterns shown on these Southern blots could be useful in future attempts to detect restriction fragment length polymorphisms that cosegregate with the genetic polymorphism that regulates RBC HNMT activity levels.

Comparison of human kidney HNMT sequence with the sequences of other methyltransferases. The nucleotide sequence within the ORF and the deduced amino acid sequence of the protein encoded by the human kidney HNMT cDNA clone were compared with sequences in the GenBank Genetics Sequence Data Bank, the EMBL Nucleotide Sequence Database, and the Swiss-Prot Protein Sequence Database. No highly homologous sequences other than rat kidney HNMT were found. The deduced amino acid sequence of the protein encoded by the human kidney HNMT cDNA clone was then compared with those of 15 other mammalian cytosolic Ado-Met-dependent methyltransferase enzymes by use of the GAP program (40). The methyltransferase sequences that were compared included human and rat kidney HNMT (14), rat liver guanidinoacetate methyltransferase (EC 2.1.1.2) (41), rat liver glycine methyltransferase (EC 2.1.1.20) (42), rat liver and human placenta catechol *O*-methyltransferase (EC 2.1.1.6) (43, 44), mouse testis, rat brain, and human RBC protein carboxyl methyltransferase (EC 2.1.1.77) (45–47), rat adrenal medulla, bovine adrenal medulla, and human pheochromocytoma phenylethanolamine *N*-methyltransferase (EC 2.1.1.28) (48–

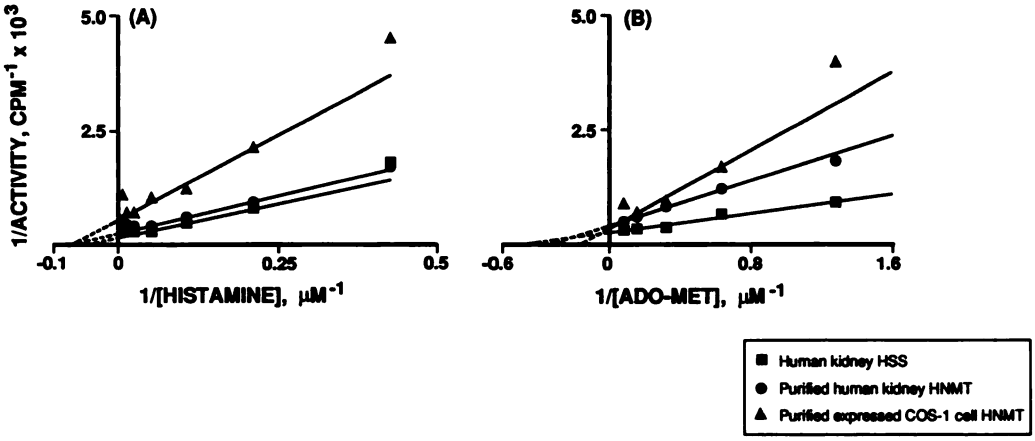

Fig. 3. HNMT substrate kinetics. Double-inverse plots of the relationship between HNMT activity of renal cortical HSS, HNMT partially purified from renal cortical HSS, and HNMT partially purified from COS-1 cells transfected with human kidney HNMT cDNA plotted versus histamine concentration (A) or Ado-Met concentration (B) are shown. Each *point* is the average of three determinations.

TABLE 1

Biochemical characteristics of HNMT in human renal cortical HSS, enzyme partially purified from renal cortical HSS, and enzyme partially purified from COS-1 cells transfected with cDNA ligated into p91023(B) in the sense orientation

All data used to make these calculations were assayed in triplicate.

	Apparent K_m values		IC_{50} values for amodiaquine
	Histamine	Ado-Met	
	μM		μM
Human renal cortical HSS	20	2.0	0.50
Partially purified human renal cortical HNMT	13	3.0	0.48
Partially purified COS-1 cell HNMT	14	6.2	0.40

TABLE 2

Effect of methyltransferase inhibitors and ions on HNMT activity in enzyme partially purified from human renal cortical HSS and enzyme partially purified from COS-1 cells transfected with cDNA ligated into p91023(B) in the sense orientation

Compound	Concentration	Activity remaining	
		Partially purified human renal cortical HNMT	Partially purified COS-1 cell HNMT
	mM	%	
Amodiaquine	1.0	1	2
Ado-Hcy[a]	0.1	46	22
$CaCl_2$	1.0	107	123
$MgCl_2$	1.0	110	104
DMHBA[a]	1.0	116	96

[a] Ado-Hcy, *S*-adenosyl-L-homocysteine; DMHBA, 3,4-dimethoxy-5-hydroxybenzoic acid.

50), mouse liver thioether *S*-methyltransferase (51), bovine pineal gland and human pineal gland hydroxyindole *O*-methyltransferase (EC 2.1.1.4) (52–54), and T84 human colon carcinoma cell thiopurine methyltransferase (EC 2.1.1.67) (55). HNMT displayed little homology with any of these enzymes other than rat kidney HNMT. The degree of sequence identity ranged from 14% when human kidney HNMT was compared with human pheochromocytoma and rat adrenal medulla phenylethanolamine *N*-methyltransferase, to 20% when the comparison was made with the sequence of human T84 colon carcinoma cell thiopurine methyltransferase.

Ingrosso *et al.* (47) have reported that enzymes that utilize Ado-Met as a cosubstrate share three regions of sequence

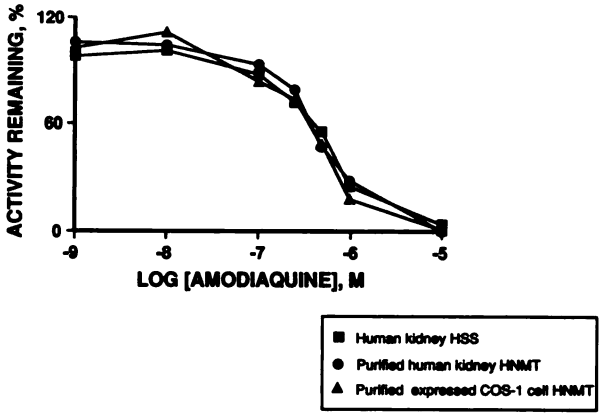

Fig. 4. Amodiaquine inhibition of HNMT activity in renal cortical HSS, HNMT partially purified from renal cortical HSS, and HNMT purified from COS-1 cells transfected with human kidney HNMT cDNA. Each *point* is the average of three determinations.

homology, regions that those authors designated I, II, and III. These "signature" sequences were further characterized by Wu *et al.* (56) and by Gomi *et al.* (57), authors who emphasized the importance of regions I and III. When we examined the amino acid sequence encoded by human kidney HNMT cDNA, a sequence comparable to that in region I was located between amino acids 57 and 64 and a sequence comparable to that in region III was located between amino acids 171 and 177 (Fig. 7). We found no sequence comparable to that which Ingrosso *et al.* (47) designated region II.

Discussion

Histamine is a neurotransmitter in the CNS (4, 5). It also plays an important role in allergic reactions and in the regulation of gastric acidity (2, 3). H_1 histamine receptor antagonists are mainstays in the treatment of allergy (58), and H_2 antihistamines are widely used in the therapy of peptic ulcer disease (59). Therefore, individual differences in the metabolism of histamine might have implications for the pathophysiology and therapy of several common disease processes. A major pathway in histamine metabolism is N^{τ}-methylation catalyzed by HNMT (8, 9). Wide individual variations in HNMT activity in human RBC are controlled by a common genetic polymorphism (12, 13). Understanding the molecular basis for this

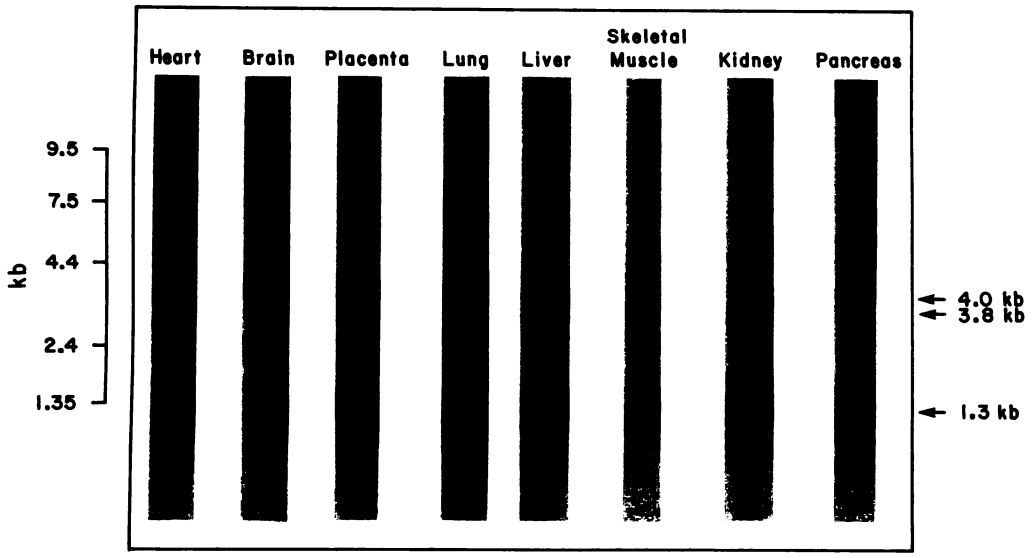

Fig. 5. Northern blot analysis of human HNMT. Each *lane* contained approximately 2 μg of poly(A)$^+$ RNA from (*left* to *right*) human heart, brain, placenta, lung, liver, skeletal muscle, kidney, or pancreas. The probe used was the ORF of human kidney HNMT cDNA.

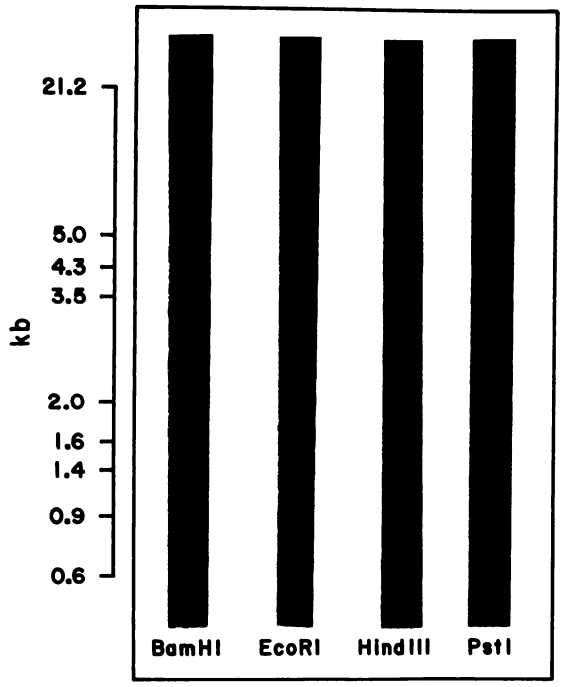

Fig. 6. Southern blot analysis of human HNMT. Five micrograms of human lymphocyte genomic DNA were exhaustively digested with restriction enzymes, resolved by electrophoresis in a 0.8% agarose gel, transferred to a nylon membrane, and probed with the ORF of human kidney HNMT cDNA (see text for details).

genetic polymorphism not only would extend our knowledge of mechanisms responsible for pharmacogenetic variation in the metabolism of endogenous compounds but also might provide insights into the role of histamine in the pathophysiology of human disease. Therefore, we set out to clone a cDNA for HNMT from human tissue as a first step toward understanding the molecular mechanism responsible for the HNMT genetic polymorphism. Obviously, it cannot be assumed that the polymorphism necessarily involves the structural gene for HNMT.

Our cloning strategy was based on possible sequence homology between rat and human kidney HNMT. We used the PCR to amplify 885 nucleotides of the ORF of rat kidney HNMT (14), and this amplification product was used as a probe to screen human kidney cDNA libraries. A cDNA 1455 nucleotides in length, with two potential translation initiation codons, was cloned by use of this approach. The longest of the possible ORFs contained 876 nucleotides and encoded a 292-amino acid protein with a sequence 84% identical to that of rat kidney HNMT. The first of the potential initiation codons was in a more favorable sequence "environment" than was the second (33), but we cannot exclude the possibility that both ATG codons might play a role in the initiation of translation. The cDNA clone contained a polyadenylation signal located 22 nucleotides upstream of a poly(A) tract. Translation of mRNA transcribed from the ORF of the human kidney cDNA clone in a rabbit reticulocyte lysate system yielded a protein with an apparent molecular mass of 33 kDa. The protein encoded by the rat kidney HNMT cDNA cloned by Takemura *et al.* (14) had a molecular mass of 33.9 kDa by SDS-PAGE. When the human kidney cDNA clone was expressed in COS-1 cells, the expressed enzyme had biochemical properties similar to those of human renal cortical HNMT. Northern blot analysis showed that mRNA for HNMT was present in all tissues studied. The most abundant transcript was 1.3 kb in length, similar in size to our human kidney cDNA clone.

HNMT is only one in a series of genetically polymorphic Ado-Met-dependent methylating enzymes (60). Thiopurine methyltransferase (61), catechol *O*-methyltransferase (62), and thiol methyltransferase (63) are also controlled by genetic polymorphisms in humans. When other "families" of drug-metabolizing enzymes such as the cytochromes P450 or the sulfotransferases have been studied, a high degree of homology has been found among related enzymes, both within and among species (64, 65). It is striking that a similar degree of sequence homology was not present among the 15 mammalian cytoplasmic methyltransferase enzymes that we compared with human kidney HNMT. These observations raise intriguing questions with regard to the evolutionary history of these proteins. However, there were two relatively short regions of sequence homology, signature sequences that have been speculated to be related to the binding of Ado-Met, which is the methyl donor for the reactions catalyzed by these enzymes (47,

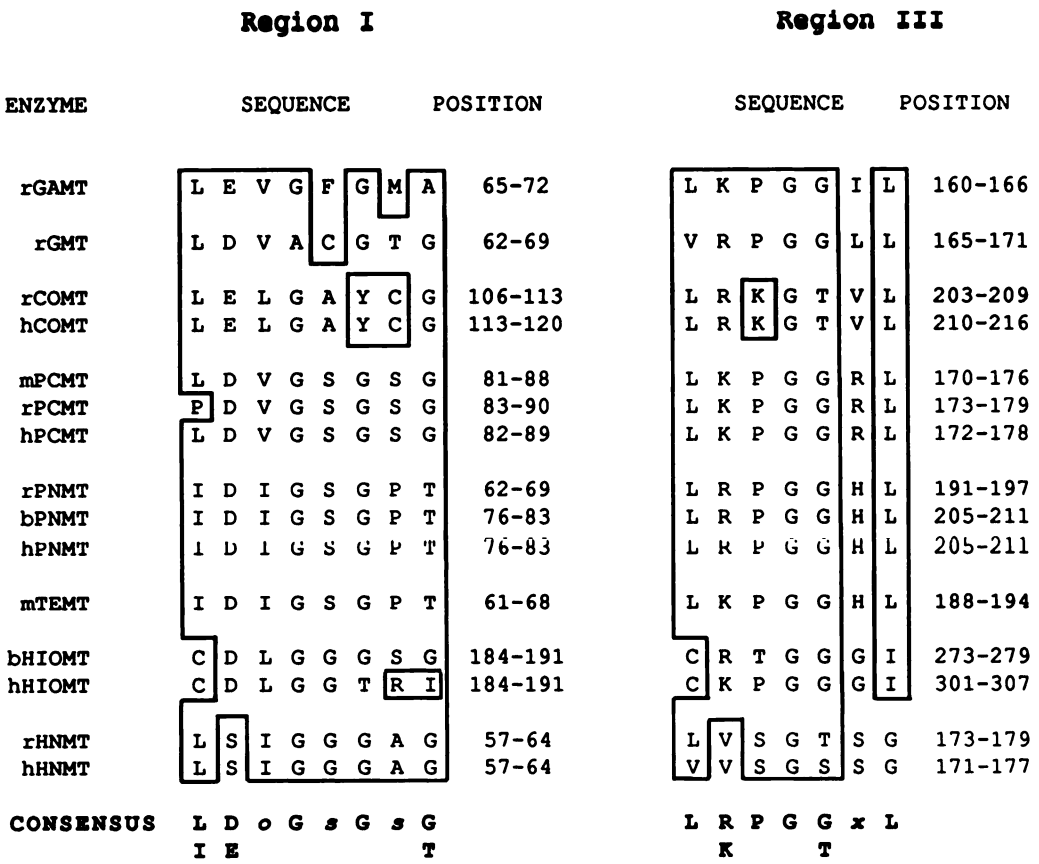

Fig. 7. Amino acid sequence alignments for Ado-Met-dependent mammalian cytosolic methyltransferase enzymes, based on the signature sequences designated region I and region III by Ingrosso *et al.* (47). Amino acids were separated into six groups as defined by Dayhoff *et al.* (66), as follows: alanine, glycine, proline, serine, and threonine; aspartate, glutamate, asparagine, and glutamine; histidine, lysine, and arginine; isoleucine, leucine, methionine, and valine; phenylalanine, tyrosine, and tryptophan; and cysteine. When residues belonging to the same group of amino acids were present in the aligned sequences of at least eight of the 15 enzymes, the amino acids within the group were *boxed*. If two different residues within a group were represented at a position, the least frequent had to be present in at least three of the 15 proteins to be listed in the consensus sequence line shown at the *bottom*. *o* and *s* in the consensus sequence line represent hydrophobic and small neutral amino acids, respectively; and *x* represents any amino acid. *hHNMT*, human kidney HNMT; *rHNMT*, rat kidney HNMT; *rGAMT*, rat liver guanidinoacetate methyltransferase; *rGMT*, rat liver glycine methyltransferase; *rCOMT*, rat liver catechol *O*-methyltransferase; *hCOMT*, human placental catechol *O*-methyltransferase; *mPCMT*, mouse testis protein carboxyl methyltransferase; *rPCMT*, rat brain protein carboxyl methyltransferase; *hPCMT*, human erythrocyte protein carboxyl methyltransferase; *rPNMT*, rat adrenal medullary phenylethanolamine *N*-methyltransferase; *bPNMT* bovine adrenal medullary phenylethanolamine *N*-methyltransferase; *hPNMT*, human pheochromocytoma phenylethanolamine *N*-methyltransferase; *mTEMT*, mouse liver thioether *S*-methyltransferase; *bHIOMT*, bovine pineal hydroxyindole *O*-methyltransferase; *hHIOMT*, human pineal hydroxyindole *O*-methyltransferase.

56, 57). That speculation must ultimately be tested experimentally. Whatever the evolutionary history of cytosolic methyltransferase enzymes, the cloning and expression of a cDNA for HNMT from human tissue represent potentially important steps toward determining the molecular basis for the pharmacogenetic regulation of individual differences in levels of this enzyme activity in humans.

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