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Molecular cloning of the cDNA for an MDCK cell Na^+- and Cl^--dependent taurine transporter that is regulated by hypertonicity

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ABSTRACT **Cells in the hypertonic renal medulla maintain their intracellular ion concentration at isotonic levels, despite much higher concentrations of extracellular electrolytes, by accumulating high concentrations of nonperturbing small organic solutes termed osmolytes. Taurine has been identified as a nonperturbing osmolyte in the renal medulla and Madin–Darby canine kidney (MDCK) cells. In hypertonic medium, the increased accumulation of taurine in MDCK cells is the result of increased activity of a Na^+- and Cl^--dependent taurine transporter. We have isolated a cDNA encoding a Na^+- and Cl^--dependent taurine transporter, whose sequence corresponds to a protein of 655 amino acids with significant amino acid sequence similarity to previously cloned Na^+- and Cl^--dependent transporters, including the MDCK cell betaine/γ-aminobutyric acid transporter and several brain neurotransmitter transporters. Northern hybridization indicates that mRNA for the taurine transporter is present in renal cortex and medulla, ileal mucosa, brain, liver, and heart. The abundance of mRNA for the taurine transporter is increased in MDCK cells cultured in hypertonic medium, suggesting that regulation of transport activity by medium hypertonicity occurs at the level of mRNA accumulation.**

Taurine (2-aminoethanesulfonic acid) is a major intracellular amino acid in mammals (1). It is involved in a number of important physiological processes, including bile acid conjugation in hepatocytes, modulation of calcium flux and neural excitability, osmoregulation, detoxification, and membrane stabilization (1).

The cells of virtually all organisms respond to hypertonicity by the intracellular accumulation of high concentrations of small organic solutes (osmolytes) that, in contrast to high concentrations of electrolytes, do not perturb the function of macromolecules (2). The renal medulla is normally the only tissue in mammals that undergoes wide shifts in tonicity. Its hypertonicity when the kidney is excreting a concentrated urine is fundamental to water conservation. The taurine content of the renal medulla of rats infused with 5% NaCl is higher than that in controls (3), suggesting that taurine behaves as an osmolyte in the renal medulla. Taurine functions as an osmolyte in Madin–Darby canine kidney (MDCK) cells (4). When MDCK cells cultured in isotonic medium were switched to hypertonic medium, their content of taurine doubled (4). The accumulation of taurine in response to medium hypertonicity was completely dependent on taurine in the medium, suggesting that taurine was taken up from the medium. Taurine transport in MDCK cells is dependent on Na^+ and Cl^- and is localized primarily in the basolateral plasma membrane. Medium hypertonicity increased the maximum velocity (V_{max}) of the taurine transporter in the basolateral plasma membrane without change in K_m (4).

Taurine transport in renal brush border membranes has also been well characterized (5). The activity of the cotransporter in the brush border of the proximal tubule contributes to whole-body homeostasis of taurine; activity increases in animals fed diets deficient in taurine and in sulfur-containing amino acids (6). The addition of 50 μM taurine to taurine-free medium results in a decrease in the activity of the taurine transporter in MDCK cells (4, 7). However, the rate of taurine transport was up-regulated by hypertonicity to the same degree as in cells cultured in taurine-free medium, suggesting that the regulation of taurine transport by hypertonicity and the regulation by medium taurine are independent of each other (4).

In this report we describe the cloning of the cDNA for the MDCK cell taurine transporter. The sequence of the cDNA‡ indicates that the taurine transporter has considerable amino acid sequence similarity to the previously cloned Na^+- and Cl^--dependent transporters. Northern hybridizations indicate that the abundance of mRNA for the taurine transporter in MDCK cells is regulated by hypertonicity.

MATERIALS AND METHODS

Cell Culture. MDCK cells were purchased from the American Type Culture Collection and grown in defined medium (8). The medium was made hypertonic by adding raffinose to a total osmolality of 500 mosmol/kg of H_2O.

Reverse Transcription/Polymerase Chain Reaction (RT-PCR). The size fraction of poly(A)$^+$ RNA that had been identified as a peak fraction for taurine transport expression in *Xenopus* oocytes (9) was used for RT-PCR. The reverse transcription reaction was performed in 20 μl of 1× PCR buffer (50 mM KCl/10 mM Tris·HCl, pH 8.3/1.5 mM $MgCl_2$/0.01% gelatin) containing 1 mM dNTP, RNasin ribonuclease inhibitor (Promega) at 1 unit/μl, 100 pmol of random hexamer (Promega), 0.5 μg of size-fractionated mRNA, and 100 units of SuperScript reverse transcriptase (BRL) at 42°C for 40 min. After cDNA synthesis, the reaction was stopped by heating at 95°C for 5 min, then the tube was quickly chilled on ice. Degenerate primers and *Taq* DNA polymerase were added to the whole reverse transcription reaction mixture, and PCR was performed in 100 μl of 1× PCR buffer, using the following temperature profile: denaturation, 45 sec at 94°C; annealing, 1 min at 50°C; polymerization, 2 min (in the last cycle, 5 min) at 72°C. There were 35 cycles. The primers used

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Abbreviations: MDCK, Madin–Darby canine kidney; GABA, γ-amino-*n*-butyric acid; RT-PCR, reverse transcription/polymerase chain reaction.

‡The nucleotide sequence from which the amino acid sequence reported in this paper was deduced has been deposited in the GenBank data base (accession no. M95495).

were as follows: sense strand, CCGGATCCAAYGTNTGGAGRTTYCCNTAYCTNTG; anti-sense strand, CCGAATTCRAARAADATYTGNGTNSCNGCRTC (Y = C or T; N = G, A, T, or C; R = G or A; D = T, G, or A; and S = C or G). The sense strand primer contains a *Bam*HI site (underlined) and degenerate sequence corresponding to amino acids 58–66 of the MDCK cell betaine/γ-amino-*n*-butyric (GABA) transporter (BGT-1) (10), NVWRFPYLC, and the antisense strand primer contains an *Eco*RI site (underlined) and degenerate sequence corresponding to amino acids 286–293 of BGT-1 (10), DA(G/A)TQIFF (see Fig. 4).

Construction and Screening of cDNA Library. A directional cDNA library was constructed by using the same size-fractionated poly(A)$^+$ RNA as used for RT-PCR, using a commercial kit (SuperScript Plasmid System; BRL) with the vector pSPORT1 and following the supplier's instructions. First-strand synthesis was performed with a primer-adaptor that contains 15 dT residues and four restriction sites, including that for *Not* I. After the second-strand synthesis, *Sal* I adaptor was blunt-ligated to the cDNA, which was then cut with *Not* I and ligated into *Sal* I- and *Not* I-cut pSPORT1 vector.

DNA Sequencing. Dideoxy chain termination was used for sequencing with Sequenase T7 DNA polymerase (United States Biochemical). To read the sense strand, pNCT, the cloned taurine transporter cDNA, was digested with *Sph* I and *Bam*HI, and then nested deletion clones were prepared by exonuclease III digestion (Erase-a-Base system; Promega). The antisense strand was sequenced by using synthetic oligonucleotides as primers.

Northern Analysis. RNA from MDCK cells and dog tissues was isolated as described (9, 10). PCR products or the full-length pNCT insert was labeled by random priming (Promega) using [^{32}P]dCTP (Amersham). RNA was electrophoresed in a 1.2% agarose/2.2 M formaldehyde gel and transferred to GeneScreen*Plus* (DuPont/NEN). The blot was hybridized overnight at 42°C in 0.9 M NaCl/60 mM sodium phosphate, pH 7.4/6 mM EDTA/0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50% (vol/vol) formamide/1% SDS containing salmon sperm DNA at 100 μg/ml and then washed twice for 30 min at 50°C in 15 mM NaCl/1.5 mM sodium citrate, pH 7.0/1% SDS before autoradiography.

Oocyte Assays. Preparation of oocytes from *Xenopus laevis*, injection of mRNA and *in vitro* synthesized mRNA into oocytes, and measurement of [^{3}H]taurine uptake were performed as described (9, 10).

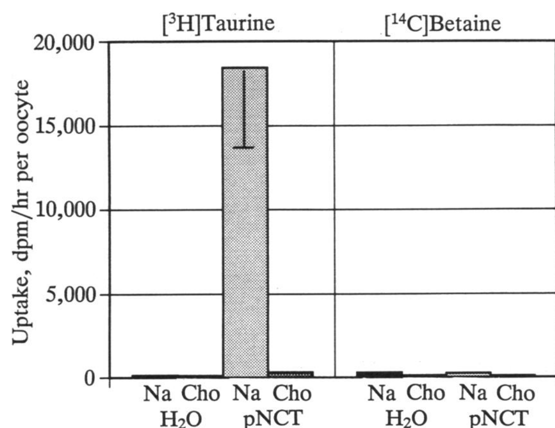

FIG. 1. Expression of the Na$^+$-dependent taurine transporter by *Xenopus* oocytes after the injection of mRNA synthesized *in vitro* from pNCT. Capped transcript was made by SP6 RNA polymerase using *Sal* I-cut pNCT as a template. Ten nanograms of transcript or 50 nl of H_2O was injected into each oocyte. Twenty-four hours later, uptake experiments were performed in Na$^+$-containing (Na) or Na$^+$-free (Cho; Na$^+$ replaced by choline) solution.

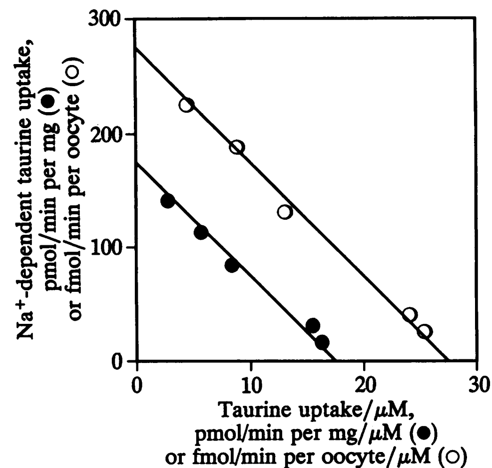

FIG. 2. Eadie–Hofstee plot of taurine uptake by oocytes and by MDCK cells on plastic dishes. Each oocyte was injected with 10 ng of capped NCT transcript, and 24 hr later, uptake experiments were performed with various concentrations of taurine in the uptake medium with or without Na$^+$. The uptake values are the Na$^+$-dependent portion of uptake. For oocytes (○), K_m = 12 μM and V_{max} = 278 fmol/min per oocyte. For MDCK cells (●), K_m = 9.1 μM and V_{max} = 167 pmol/min per mg of protein.

Materials. [^{3}H]Taurine (20.1 Ci/mmol; 1 Ci = 37 GBq) and [^{3}H]GABA (34.8 Ci/mmol) were purchased from DuPont/NEN. [^{14}C]Betaine was a kind gift from M. B. Burg (National Institutes of Health).

RESULTS AND DISCUSSION

Cloning and Identification of the Taurine Transporter cDNA. Since oocytes injected with poly(A)$^+$ RNA from hypertonic MDCK cells express a considerable amount of taurine transporter activity (9), we attempted to clone the

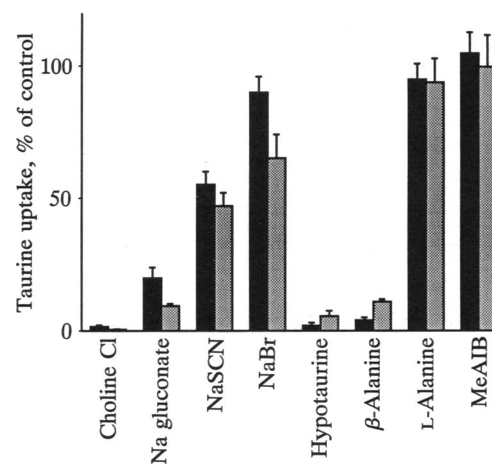

FIG. 3. Ion dependency and inhibitor sensitivity of taurine uptake in oocytes (black bars) and MDCK cells (stippled bars). Control uptake, measured in the presence of NaCl, was used to set 100% on the ordinate. Na$^+$-free solution (Choline Cl) was made by replacing NaCl with choline chloride. In NaBr, NaSCN, and Na gluconate solutions, only NaCl and KCl were replaced by the corresponding sodium and potassium salts. Amino acids as inhibitors were added to the assay medium in a concentration of 2 mM. MeAIB, *N*-methyl-α-aminoisobutyric acid. Twenty-four hours after injection of 10 ng of NCT transcript into oocytes, taurine uptake was assayed for 1 hr at room temperature in the presence of 10 μM [^{3}H]taurine. Uptake values in MDCK cells are from ref. 4. Each value is the mean ± SD of 5–10 oocytes.

MDCK cell taurine transporter, using expression in oocytes to screen a cDNA library. We were not successful. Our successful strategy was based on the assumption that the taurine transporter is a member of the same gene family as the MDCK cell betaine/GABA transporter (10) and the brain neurotransmitter transporters for GABA (11) and norepinephrine (12). The assumption was based on the observations that all of those transporters and the MDCK cell taurine transporter are dependent on Na^+ and Cl^- and that taurine transport in MDCK cells is inhibited by high concentrations of GABA, and GABA transport in MDCK cells is inhibited by high concentrations of taurine (unpublished observations). We designed degenerate PCR primers based on regions of highly conserved sequence in the three cloned cDNAs to probe the cDNA library prepared from hypertonic MDCK cells. Initially we used the primers to perform RT-PCR on the poly(A)$^+$ RNA size fraction prepared from hypertonic MDCK cells that was most enriched in mRNA for the taurine transporter. The RT-PCR product included cDNA that behaved as we anticipated for the product from mRNA

```
         1                                                    50
     Tau .......... .......... .......... .......... ..........
    Bgt1 .......... .......... .......... .......... ..........
    Gaba .......... .......... .......... .......... ..........
   Sert1 .......... .......... ....MVFYRR VSP.PQRTGQ SLAKYPMGTL
   Sert2 METTPLNSQK VLSECKDRED CQENGVLQKG VPTTADRAEP SQISNGYSAV
 Dopabov .......... .......... .......... .........M SEGRCSVAHM
 Doparat .......... .......... .......... .........M SKSKCSVGPM
      Ne .......... .......... .......... .......MLL ARMNPQVQPE

         51                                                  100
     Tau .....MATKE KLQCLKDFHK DILKPSPGKS PGTRP...ED EAEGKPPQRE
    Bgt1 .MDRKVAVPE DGPPVVSWLP E......... EGEKL...DQ EGEDQVKDRG
    Gaba .....MATDN SKVADGQIST EVSEAPVASD KPKTLVVKVQ KKAGDLPDRD
   Sert1 QSPGTSAGDE ASHSIPAATT TLVAE..... .......... ...IRQGERE
   Sert2 ..PSTSAGDE ASHSIPAATT TLVAE..... .......... ...IRQGERE
 Dopabov SSVVAPAKEA NAMGPKAVEL VLVKEQNGVQ LTNSTLLNPP QSPTEAQDRE
 Doparat SSVVAPAKES NAVGPREVEL ILVKEQNGVQ LTNSTLINPP QTPVEAQERE
      Ne NNGADTGPEQ PLRARKTAEL LVVKERNGVQ ....CLLAPR DG..DAQPRE
              *                                              **

         101          M1                                  M2 150
     Tau KWSSKIDFVL SVAGGFVGLG NVWRFPYLCY KNGGGAFLIP YFIFLFGGGL
    Bgt1 QWTNKMEFVL SVAGEIIGLG NVWRFPYLCY KNGGGAFFIP YFIFFFTCGI
    Gaba TWKGRFDFLM SCVGYAIGLG NVWRFPYLCG KNGGGAFLIP YFLTLIFAGV
   Sert1 TWGKKMDFLL SVIGYAVDLG NIWRFPYICY QNGGGAFLLP YTIMAIFGGI
   Sert2 TWGKKMDFLL SVIGYAVDLG NIWRFPYICY QNGGGAFLLP YTIMAIFGGI
 Dopabov TWSKKADFLL SVIGFAVDLA NVWRFPYLCY KNGGGAFLVP YLFFMVVAGV
 Doparat TWSKKIDFLL SVIGFAVDLA NVWRFPYLCY KNGGGAFLVP YLLFMVIAGM
      Ne TWGKKIDFLL SVVGFAVDLA NVWRFPYLCY KNGGGAFLIP YTLFLIIAGM
         **   * **** ** * ** *  ********** ******** * *      *

         151                                   M3            200
     Tau PVFFLEVIIG QYTSEGGITC WEKICPLFSG IGYASIVIVS LLNIYYVIIL
    Bgt1 PVFFLEVALG QYTSQGSVTA WRKICPLLQG IGLASVVIES YLNIYYIIIL
    Gaba PLFLLECSLG QYTSIGGLGV W.KLAPMFKG VGLAAAVLSF WLNIYYIVII
   Sert1 PLFYMELALG QYHRNGCISI WRKICPIFKG IGYAICIIAF YIASYYNTII
   Sert2 PLFYMELALG QYHRNGCISI WRKICPIFKG IGYAICIIAF YIASYYNTII
 Dopabov PLFYMELALG QFNREGAAGV W.KICPILRG VGYTAILISL YIGFFYNVII
 Doparat PLFYMELALG QFNREGAAGV W.KICPVLKG VGFTVILISF YVGFFYNVII
      Ne PLFYMELALG QYNREGAATV W.KICPFFKG VGYAVILIAL YVGFYYNVII
         ***  * *** **    *     * ****  * *   *   *    *  ** **

         201                                                 250
     Tau AWATYYLFQS FQSELPWAHC NHSWNTPQCM EDTMRKNKS. LWITLSTKNF
    Bgt1 AWALFYLFSS FTSELPWTTC TNTWNTEHCM D..FLNHSG. ARTATSSENF
    Gaba SWAIYYLYNS FTTTLPWKQC DNPWNTDRCF ........S. NYSLVNTTNM
   Sert1 AWALYYLISS LTDRLPWTSC TNSWNTGNCT NYFAQDNITW TLHS......
   Sert2 AWALYYLISS LTDRLPWTSC TNSWNTGNCT NYFAQDNITW TLHS......
 Dopabov AWALHYLLSS FTTELPWTHC NHSWNSPRCS DARAPNAS.. S..GPNGTSR
 Doparat AWALHYFFSS FTMDLPWIHC NNTWNSPNCS DAHASNSS.. DGLGLNDTFG
      Ne AWSLYYLFSS FTLNLPWTDC GHTWNSPNCT DPKLLNGSVL GNHTKYSKYK
         **** ** ** **  ***  *     **   *

         251                                          M4     300
     Tau TSPVTEFWER NVLSL..SSG IDDPGSLKWD LALCLLLVWL VCFFCIWKGV
    Bgt1 TSPVMEFWER RVLGI..TSG IHDLGALRWE LALCLLLAWL ICYFCIWKGV
    Gaba TSAVVEFWER NMHQM..TDG LDKPGQIRWP LAITLAIAWV LVYFCIWKGV
   Sert1 TSPAEEFYLR HVLQIHQSKG LQDLGTISWQ LTLCIVLIFT VIYFSIWKGV
   Sert2 TSPAEEFYLR HVLQIHQSKG LQDLGTISWQ LTLCIVLIFT VIYFSIWKGV
 Dopabov TTPAAEYFER GVLHLHESQG IDDLGPPRWQ LTSCLVLVIV LLYFSLWKGV
 Doparat TTPAAEYFER GVLHLHQSRG IDDLGPPRWQ LTACLVLVIV LLYFSLWKGV
      Ne FTPAAEFYER GVLHLHESSG IHDIGLPQWQ LLLCLMVVVI VLYFSLWKGV
         * *  ** ** **       *   *  *  *   * ** *       ** ****

         301           M5                                    350
     Tau KSTGKVVYFT ATFPFAMLLV LLVRGLTLPG AGAGIKFYLY PDISRLEDPQ
    Bgt1 KTTGKVVYFT ATFPYLMLVI LLIRGITLPG AYQGVIYYLK PDLLRLKDPQ
    Gaba GWTGKVVYFS ATYPYIMLII LFFRGVTLPG AKEGILFYIT PNFRKLSDSE
   Sert1 KTSGKVVWVT ATFPYIVLSV LLVRGATLPG AWRGVVFYLK PNWQKLLETG
   Sert2 KTSGKVVWVT ATFPYIVLSV LLVRGATLPG AWRGVVFYLK PNWQKLLETG
 Dopabov KTSGKVVWIT ATMPYVVLFA LLLRGITLPG AVDAIRAYLS VDFHRLCEAS
 Doparat KTSGKVVWIT ATMPYVVLTA LLLRGVTLPG AMDGIRAYLS VDFYRLCEAS
      Ne KTSGKVVWIT ATLPYFVLFV LLVHGVTLPG ASNGINAYLH IDFYRLKEAT
         ** ****   ** **  *   ** ** ****  *   *   **

         351           M6                                 M7 400
     Tau VWIDAGTQIF FSYAICLGAM TSLGSYNKYK YNSYRDCMLL GCLNSGTSFV
    Bgt1 VWMDAGTQIF FSFAICQGCL TALGSYNKYH NNCYRDSIAL CFLNSATSFA
    Gaba VWLDAATQIF FSYGLGLGSL IALGSYNSFH NNVYRDSIIV CCINSCTSMF
   Sert1 VWVDAAAQIF FSLGPGFGVL LAFASYNKFN NNCYQDALVT SVVNCMTSFV
   Sert2 VWVDAAAQIF FSLGPGFGVL LAFASYNKFN NNCYQDALVT SVVNCMTSFV
 Dopabov VWIDAAIQIC FSLGVGLGVL IAFSSYNKFT NNCYRDAIIT TSVNSLTSFS
 Doparat VWIDAATQVC FSLGVGFGVL IAFSSYNKFT NNCYRDAIIT TSINSLTSFS
      Ne VWIDAATQIF FSLGAGFGVL IAFASYNKFD NNCYRDALLT SSINCITSFV
         ** *** *** ** * * * *  *  ***** ******       *  ***

         401                                              M8 450
     Tau SGFAIFSILG FMAQEQGVDI ADVA.ESGPG LAFIAYPKAV TMMPLPTFWS
    Bgt1 AGFVVFSILG FMAQEQGLPI SEVA.ESGPG LAFIAFPKAV TMMPLSQLWS
    Gaba AGFVIFSIVG FMAHVTKRSI ADVA.ASGPG LAFLAYPEAV TQLPISPLWA
   Sert1 SGFVIFTVLG YMAEMRNEDV SEVAKDAGPS LLFITYAEAI ANMPASTFFA
   Sert2 SGFVIFTVLG YMAEMRNEDV SEVAKDAGPS LLFITYAEAI GNMPASTFFA
 Dopabov SGFVVFSFLG YMAQKHSVPI GDVAKD.GPG LIFIIYPEAL ATLPLSSVWA
 Doparat SGFVVFSFLG YMAQKHNVPI RDVATD.GPG LIFIIYPEAI ATLPLSSAWA
      Ne SGFAIFSILG YMAHEHKVNI EDVATE.GAG LVFILYPEAI STLSGSTFWA
          **** ** ** **        *   *  *** * ** ****     * *  **

         451                                              M9 500
     Tau ILFFIMLLLL GLDSQFVEVE GQVTSLVDLY PSFLRKGFRR EIFIAFMCSI
    Bgt1 CLFFIMLIFL GLDSQFVCVE CLVTASMDMF PSQLRKSGRR ELLILAIAVF
    Gaba ILFFSMLLML GIDSQFCTVE GFITALVDEY PRLLRN..RR ELFIAAVCIV
   Sert1 IIFFLMLITL GLDSTFAGLE GVITAVLDEF PHIWAK..RR EWFVLIVVIT
   Sert2 IIFFLMLITL GIDSTFAGLE GVITAVLDEF PHIWAK..RR EWFVLIVVIT
 Dopabov VVFFVMLLTL GIDSAMGGME SVITGLADEF .QLLHR..HR ELFTLLVVLA
 Doparat AVFFLMLLTL GIDSAMGGME SVITGLIDEF .QLLHR..HR ELFTLGIVLA
      Ne VVFFVMLLAL GLDSSMGGME AVITGLADDF .QVLKR..HR KLFTFGVTFS
           ** **  *  * * **      *    **  ***      *    * * *

         501                                 M10             550
     Tau SYLLGLSMVT EGGMYVFQLF DYYAASGVCL LWVAFFECFV IAWIYGSDNL
    Bgt1 CYLAGLFLVT EGGMYIFQLF DYYASSGICL LFLAMFEVIC ISWVYGADRF
    Gaba SYLIGLSNIT QGGIYVFKLF DYYSASGMSL LFLVFFECVS ISWFYGVNRF
   Sert1 CVLGSLLTLT SGGAYVVTLL EEYAT.GPAV LTVALIEAVA VSWFYGITQF
   Sert2 CVLGSLLTLT SGGAYVVTLL EEYAT.GPAV LTVALIEAVA VSWFYGITQF
 Dopabov TFLLSLFCVT NGGIYVFTLL DHFAA.GTSI LFGVLMEVIG VAWFYGVWQF
 Doparat TFLLSLFCVT NGGIYVFTLL DHFAA.GTSI LFGVLIEAIG VAWFYGVQQF
      Ne TFLLALFCIT KGGIYVLTLL DTFAA.GTSI LFAVLMEAIG VSWFYGVDRF
            *   *  * ** ** *    *   *   *    *    *      ****  *

         551                                 M11             600
     Tau YDGIEDMIGY RPGPWMKYSW AVVTPVLCVG CFIFSLVKYV PLTYNKVYVY
    Bgt1 YDNIEDMIGY RPWPLVKISW LFLTPGLCLA TFLFSLSQYT PLKYNNIYVY
    Gaba YDNIQEMVGS RPCIWWKLCW SFFTPIIVAG VFLFSAVQMT PLTMGS.YVF
   Sert1 CSDVKEMLGF SPGWFWRICW VAISPLFLLF IICSFLMSPP QLRLFQ.YNY
   Sert2 CSDVKEMLGF S.GMVWRICW VAISPLFLLF IICSFLMSPP QLRLFQ.YNY
 Dopabov SDDIKQMTGR RPSLYWRLCW KFVSPCFLLF VVVVSIATFR PPHYGA.YVF
 Doparat SDDIKQMTGQ RPNLYWRLCW KLVSPCFLLY VVVVSIVTFR PPHYGA.YIF
      Ne SNDIQQMMGF RPGLYWRLCW KFVSPAFLLF VVVVSIINFK PLTYDD.YIF
            *   *  *  *    *  **   *  *            *       *

         601           M12                                   650
     Tau PTWAIGLGWS LALSSMMCVP LVMVIRLCQT EGPFLVRLKY LLTPREPNRW
    Bgt1 PPWGYSIGWF LALSSMICVP LFVIITLLKT RGSFKKRLRQ LTTPDPS...
    Gaba PKWGQGVGWL MALSSMVLIP GYMAYMFLTL KGSLKQRLQV MI........
   Sert1 PHWSIVLGYC IGMSSVICIP TYIIYRLIST PGTLKERIIK SITPETPTEI
   Sert2 PHWSIVLGYC IGMSSVICIP TYIIYRLIST PGTLKERIIK SITPETPTEI
 Dopabov PEWATALGWA IAASSMSVVP IYAAYKLCSL PGSSREKLAY AITPETEHGR
 Doparat PDWANALGWI IATSSMAMVP IYATYKFCSL PGSFREKLAY AITPEKDHQL
      Ne PPWANWVGWG IALSSMVLVP IYVIYKFLST QGSLWERLAY GITPENEHHL
         * *    **   * ***   *   *   *        *   **    ***

         651                                                 700
     Tau AVEREGATLQ LPPGRERGSH ETDPHHSRDH DVSSWADGPL PCCLLTLDSH
    Bgt1 .......... LPQPKQHLYL DGGT..SQDC GPSPTKEG.. ....LIVGEK
    Gaba .......... ..QPSEDIVR PENGPEQPQA GSSASKEAYI ..........
   Sert1 PCGDIRMNAV .......... .......... .......... ..........
   Sert2 RVGHPHECCV THPGRGHLFP ATSLSSEKPT GLLL...... ..........
 Dopabov VDSGGGAPVH APPLARGVGR WRKRKSCWVP SRGPGRGGPP TPSPRLAGHT
 Doparat VDRG...... ......EVRQ FTLRHWLLL. .......... ..........
      Ne VAQ....... .....RDIRQ FQLQHWLAI. .......... ..........

         701                                     743
     Tau RTRFTELSIC TRIVFFF... .......... .......... ...
    Bgt1 ETHL...... .......... .......... .......... ...
    Gaba .......... .......... .......... .......... ...
   Sert1 .......... .......... .......... .......... ...
   Sert2 .......... .......... .......... .......... ...
 Dopabov RAFPWTGAPP VPRELTPPST CRCVPPLVCA HPAVESTGLC SVY
 Doparat .......... .......... .......... .......... ...
      Ne .......... .......... .......... .......... ...
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FIG. 4. Deduced amino acid sequence of the MDCK cell taurine transporter (Tau) compared with the deduced sequences of the MDCK cell betaine/GABA transporter [Bgt1 (10)] and the brain transporters for GABA [Gaba (11)], serotonin [Sert1 (13) and Sert2 (14)], dopamine [Dopabov (15) and Doparat (16, 17)], and norepinephrine [Ne (12)]. Gaps have been added to obtain good alignment. The proposed membrane-spanning domains of the taurine transporter are underlined and numbered M1 through M12. Two highly conserved regions used for generation of PCR primers (see *Materials and Methods*) are marked on Bgt1 by double underlining. Asterisks are placed below amino acids that are identical in six of the eight transporter sequences.

for the taurine transporter. We then used the same primers to perform PCR on pools of cDNA to identify a clone that yielded a PCR product that appeared to be appropriate for the cDNA of the taurine transporter. Finally, we confirmed the identity of the cloned cDNA by expressing the taurine transporter in oocytes injected with *in vitro* prepared transcripts from the cloned cDNA.

The major product of RT-PCR using the poly(A)$^+$ RNA fraction enriched for taurine mRNA (9) had the expected size, 720 base pairs (bp). The RT-PCR product hybridized with bands of 2.4 and 3.0 kilobases (kb), corresponding to the betaine/GABA transporter mRNA (10) and with a 7-kb band. The 7-kb band was most enriched in the mRNA size fraction (9) that elicited the peak taurine transport in *Xenopus* oocytes. These results indicated that the 720-bp RT-PCR product contained at least two different sequences: one from the betaine/GABA transporter mRNA and another from the 7-kb mRNA which might encode the taurine transporter. The RT-PCR products were inserted into pSPORTI, and a clone that recognized only the putative taurine transporter 7-kb mRNA was isolated. Sequencing the cloned insert revealed that, as expected, it had considerable sequence similarity to the betaine/GABA transporter but was not the same.

Screening of the cDNA library by hybridization using the PCR clone that hybridized with 7-kb mRNA as a probe was not successful because of high nonspecific hybridization. Therefore we used PCR in combination with Northern analysis to identify clones that hybridized to 7-kb mRNA. From 60 ng of cDNA ligated to the vector, 10 pools of about 500 colonies were prepared on separate dishes. The plasmid DNA of each pool was isolated separately. Using that DNA as template, we performed PCR under the same conditions used for RT-PCR. Eight of the 10 pools gave the expected size PCR product. Northern blotting of MDCK cell poly(A)$^+$ RNA with the PCR product of each pool as a probe revealed that only 1 pool out of the 8 yielded a PCR product that hybridized with 7-kb mRNA. The 500 clones of that pool were subdivided into smaller pools and PCR/Northern analysis was performed, using DNA from subpools as templates until a single clone (pNCT) was isolated.

The isolated clone had a 6.2-kb insert. Its sequence revealed a single long open reading frame (1965 bp; see below). Injection into oocytes of mRNA (10 ng) synthesized *in vitro* by using pNCT as a template elicited, after 24 hr, a 1400-fold increase of Na^+-dependent taurine uptake compared with water-injected oocytes but did not elicit betaine uptake (Fig. 1). We characterized further the cloned transporter expressed in oocytes. Na^+-dependent taurine transport activity encoded by pNCT was saturable, with a K_m for taurine of 12 μM, which is similar to that of MDCK cells grown on plastic tissue culture dishes (9) (Fig. 2). Taurine transport activity expressed in oocytes was similar to that in MDCK cells when Na^+ or Cl^- was replaced or inhibitors were added (Fig. 3). Transport was eliminated when choline was substituted for Na^+. Transport was inhibited when Cl^- was replaced by gluconate, SCN^-, or Br^- (in order of potency). Hypotaurine and β-alanine inhibited transport, whereas L-alanine and *N*-methyl-α-aminoisobutyric acid did not. Taken together, these results indicate that pNCT encodes the taurine transporter in MDCK cells.

Amino Acid Sequence. The 6257-nucleotide cDNA contains a single long open reading frame composed of nucleotides 59-2026, which corresponds to a polypeptide of 655 amino acids with a relative molecular mass of 73,925. As shown in Fig. 4, the protein exhibits highly significant sequence similarity to the MDCK cell betaine/GABA transporter (10) and the other cloned Na^+-, Cl^--dependent neurotransmitter transporters (11, 12, 14–17), suggesting that the taurine transporter is also a member of the gene family composed of the brain neurotransmitter transporters and the renal betaine/GABA transporter. There was 56% amino acid identity with the MDCK cell betaine/GABA transporter and 39–45% identity with the neurotransmitter transporters. The Kyte–Doolittle hydropathy profile (18) of the taurine transporter is very similar to the hydropathy profiles of the other Na^+-, Cl^--dependent transporters. A tentative two-dimensional structural model is drawn in Fig. 5. The taurine transporter has 12 hydrophobic domains that presumably are membrane spanning. There are two potential N-glycosylation sites (19) in the second extracellular loop, and one potential phosphorylation site (20) was found in the fourth cytosolic loop and four in the last cytosolic segment.

Tissue Distribution of Taurine Transporter mRNA. Northern blot analysis of poly(A)$^+$ RNA from various dog tissues

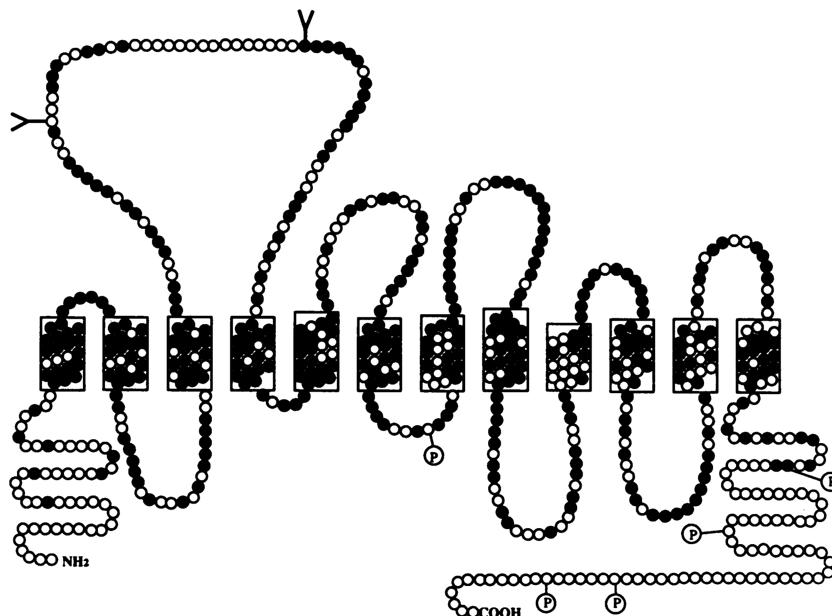

FIG. 5. Proposed membrane topology of the Na^+-, Cl^--dependent taurine transporter. Each amino acid is represented by a circle. ●, Amino acids identical with those in MDCK cell betaine/GABA transporter. Putative N-glycosylation sites (Y) and phosphorylation sites (Ⓟ) are indicated (amino acids 322, 581, and 637 for protein kinase C, 607 and 643 for cAMP-dependent protein kinase).

with full-length pNCT insert used as a probe revealed a major band of 7 kb and a few minor bands (2.5, 5.7, and 10 kb) (Fig. 6). The same pattern of hybridization was obtained when the cloned insert of the PCR product was used. These minor bands might be due to differences in polyadenylylation or to alternative splicing. The order of message abundance among tissues was kidney cortex ≈ kidney medulla > ileal mucosa > brain > liver > heart > epididymis. All of these organs have taurine transporters (1, 5, 21–23), but relative taurine transporter activity among these tissues has not been reported. Heart has the highest taurine content (1). The signal in Northern blots of heart RNA, however, was one of the lowest among the tissues tested. This discrepancy could be explained by taurine accumulation in heart resulting largely from synthesis, by the action of a taurine transporter whose mRNA is not recognized by pNCT, or by lower rates of taurine efflux from heart.

Regulation of Taurine Transporter mRNA Abundance by Medium Tonicity in MDCK Cells. In hypertonic medium, MDCK cells up-regulate taurine transporter activity and accumulate taurine (4). As shown in Fig. 6, hypertonicity increased the abundance of mRNA for the taurine transporter. The abundance of mRNA for β-actin is slightly decreased in hypertonic cells (data not shown). Thus the increase of taurine transporter mRNA was not part of a general increase in mRNA. The increase in abundance of mRNA for the taurine transporter confirms our earlier suggestion (9) that regulation of the taurine transporter by medium hypertonicity occurs at the level of mRNA abundance, as shown for the Na^+-dependent *myo*-inositol transporter and the Na^+-, Cl^--dependent betaine/GABA transporter in MDCK cells (10, 24). The other bands seen in the MDCK cell mRNA lanes appeared to be regulated in a similar fashion, suggesting that all of these bands might be derived from a single gene. Of course, there may be regulation at other levels, including posttranslational, in view of the multiple putative phosphorylation sites. Taurine transport in kidney-derived LLC-PK_1 cells (25) and in a human placental choriocarcinoma cell line (26) is inhibited by activators of protein kinase C.

The taurine transporter in MDCK cells that is regulated by medium hypertonicity is located in the basolateral plasma membrane of the epithelium (4), indicating that the cloned transporter is the basolateral taurine transporter. Na^+- and Cl^--dependent taurine transport has been demonstrated in the apical membrane of intestinal mucosa (27) and renal proximal tubule (5), where it would function in lumen-to-blood transport of taurine. The strong signals detected in Northern hybridizations with mRNA from ileal mucosa and kidney cortex may be the result of hybridization with mRNA for an apical membrane transporter.

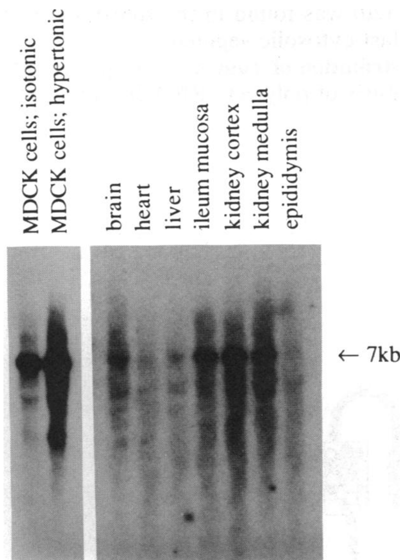

FIG. 6. Expression and regulation of Na^+-, Cl^--dependent taurine transporter mRNA. Northern analysis of poly(A)$^+$ RNA from MDCK cells cultured in isotonic medium or in hypertonic medium (24 hr after switching from isotonic to hypertonic medium) and from various canine tissues—brain, heart, liver, ileal mucosa, kidney cortex, kidney medulla, and epididymis. Each lane has 5 μg of poly(A)$^+$ RNA. The blot of MDCK mRNA was exposed for autoradiography for 24 hr, and that of canine tissues was exposed for 3 weeks.

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