Biochemical and Biophysical Research Communications **263,** 123–129 (1999)
Article ID bbrc.1999.0947, available online at http://www.idealibrary.com on IDEAL®

Identification of a Mammalian Brain Sulfate Transporter

Aven Lee, Laurent Beck, Richard J. Brown, and Daniel Markovich[1]
Department of Physiology and Pharmacology, University of Queensland, Brisbane, Queensland 4072, Australia

Received May 31, 1999

Sulfate is an essential anion involved in many biosynthetic and pharmacological reactions. Sulfate is an important constituent of myelin membranes in the brain; however, very little is known as to how sulfate enters brain cells. In this study, our aim was to determine whether the mammalian brain possesses a sulfate transporter. Injection of rat brain poly A$^+$ RNA into *Xenopus* oocytes led to an induction of Na$^+$-independent sulfate transport, which was inhibited by oxalate, probenecid, phenol red, thiosulfate and DIDS. Hybrid depletion using sat-1 antisense oligodeoxyribonucleotides led to a complete inhibition of brain mRNA-induced sulfate transport in *Xenopus* oocytes, suggesting the presence of a functional sat-1 transcript in the brain. By RT-PCR, sat-1 mRNA was detected throughout the rat brain and *in situ* hybridisation showed highest sat-1 expression in the hippocampus and cerebellum. This is the first study to identify and characterise a functional mammalian brain sulfate transporter. © 1999 Academic Press

Key Words: **sulfate uptake; *Xenopus* oocytes; sat-1 transporter.**

Sulfate is the fourth most abundant anion in plasma and plays a very important role in many physiological processes. One major role of sulfate is in sulfate conjugation reactions, also termed sulfation (1). Sulfation has been recognised as an important pathway for the biotransformation of a wide range of compounds including analgesics, anti-inflammatory agents and steroids, as well as adrenergic stimulants and blockers (2). In most cases, the sulfation of these compounds results in a change in biological activity and an increase in their urinary excretion (3). Sulfate conjugation is also an important pathway for the biosynthesis of macromolecules. In fact, many of the structural components of membranes and tissues are sulfate conjugates, e.g. sulfated glycosaminoglycans. In the central nervous system (CNS), sulfation plays an important role in the biosynthesis of sulfated proteoglycans which are involved in modulating cell interactions in developing nervous tissues (4). Neuronal heparan sulfate proteoglycans are involved in cell adhesion, neural crest migration and neurite extension (5, 6), whereas chondroitin sulfate proteoglycans (of astrocyte origin) have been shown to inhibit neurite growth (7). Despite the importance of sulfate in the brain, it is not clear whether cells of the brain require and/or possess a sulfate uptake system.

Several findings have proposed that the brain may possess a sulfate transport system. Ventriculocisternal perfusion studies have demonstrated that a carrier mechanism for sulfate transport exists in the brain, capable of mediating sulfate transport from the cerebrospinal fluid (CSF) to plasma (8, 9). This proposed mechanism of sulfate transport was shown to be saturable, with the addition of unlabelled sulfate to the perfusate leading to significantly reduced ^{35}S-sulfate clearance. In addition, sulfate transport was markedly reduced by the addition of thiosulfate, a sulfate analogue that competed for the same transport mechanism. Since sulfate transport in the brain is important for maintaining CSF sulfate concentration, it is likely that similar transport processes may also be required in the brain, where the import of sulfate into cells of the CNS would be needed.

Although the cellular mechanisms of sulfate uptake have been well characterised in several organs of the body (i.e. small intestine, kidney and liver), it is not yet known whether the brain does in fact contain a protein that facilitates sulfate uptake. Currently, two distinct classes of mammalian sulfate transporters have been cloned and characterised: [i] the rat NaSi-1 cDNA (10), encoding a Na$^+$-dependent sulfate-transporter; and [ii] three cDNAs sharing significant homologies at the amino acid level: the rat sat-1 cDNA (11), the human DTDST cDNA (12) and the mouse dra cDNA (13), each encoding Na$^+$-independent sulfate/anion exchangers,

Abbreviations used: sat-1, sulfate anion transporter-1; NaSi-1, Na$^+$/sulfate-cotransporter-1; RT-PCR, reverse transcriptase-polymerase chain reaction; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonate; BNPI, brain-specific Na$^+$-dependent phosphate transporter; MMLV-RT, murine Maloney leukaemia virus-reverse transcriptase; DTT, dithiothreitol.

[1] To whom correspondence should be addressed. Fax: +61 7 3365 1766. E-mail: danielm@plpk.uq.edu.au.

0006-291X/99 $30.00

but sharing no sequence homology with the NaSi-1 protein (10). By expression cloning, we isolated the Na^+/Sulphate inorganic transporter-1, NaSi-1, from a rat kidney cDNA library (10). By Northern blotting, we detected NaSi-1 signals only in the kidney and small intestine (10). Using a similar strategy, the sulphate anion transporter-1, sat-1, was isolated from a rat liver cDNA library (11). Sat-1 showed strong Northern blot hybridisation with rat liver and kidney mRNA, with weaker signals in muscle and brain (11). The human DTDST (diastrophic dysplasia sulphate transporter) gene was isolated by positional cloning, and its cDNA showed ubiquitous expression (12). The mouse dra (down regulated in adenomas) cDNA was cloned by subtractive hybridisation of intestinal cell lines and was shown to be primarily expressed in normal intestinal tissues, with its mRNA expression reduced in colonic adenomas and adenocarcinomas (13). To date, none of these cloned proteins have been suggested to be associated with sulfate transport in the mammalian brain. The fact that sulfation reactions play an important role in the CNS, suggest that proper sulfate transport would be essential in the brain (i.e. to maintain an optimal intracellular stores of sulfate). However, currently nothing is known with respect to the cellular mechanisms nor the molecular identity of proteins that govern sulfate transport in the brain.

Therefore, the aim of this study was to determine whether the brain does indeed contain a sulfate transporter by functionally characterising brain mRNA induced sulfate transport in *X. laevis* oocytes and by defining the localisation of this transport activity in the rat brain. In this study, we present the first evidence of a functional sulfate transporter in the mammalian brain, which is encoded by the cloned sulfate anion transporter sat-1.

MATERIALS AND METHODS

RNA Isolation and X. laevis Oocyte Uptake

Total RNA was isolated from Male Wistar rat brains (3 regions: forebrain, midbrain and hindbrain) and liver, using the total RNA isolation reagent and commercial protocol (Advanced Biotechnologies Ltd.). Poly A^+ RNA (mRNA) was purified through an oligo dT column as described previously (10, 14). Female *Xenopus laevis* frogs were obtained from the African *Xenopus* Facility C.C., Noordhoek, South Africa. Stage V-VI *X. laevis* oocytes were prepared as described previously (10, 14), maintained at 18°C in modified Barth's solution (MBS: 88 mM NaCl, 1 mM KCl, 0.82 mM $MgSO_4$, 0.4 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, 2.4 mM $NaHCO_3$, 10 mM HEPES/Tris pH 7.4, gentamicin sulfate 20 mg/l) and injected with either 50 nl water (control) or brain mRNA using a Nanoject automatic oocyte injector (Drummond Scientific Co., Broomall, PA). For hybrid-depletion experiments, rat brain mRNA (0.2 μg) was denatured (5 min 65°C), then incubated (15 min 58°C) in 50 mM NaCl solution containing 5 ng of one of the following oligodeoxyribonucleotides (ODNs): (a) sense ODN: 5′-ACTGCAGAGGAGCTGCTG-3′ [corresponding to sat-1 cDNA (10) nucleotide position: 2384–2403] and (b) antisense ODN: 5′-AACAGCAGCTCCTCTG-3′ [corresponding to sat-1 cDNA (10) nucleotide position: 2388–2403]. Oocytes were then injected directly either with the mRNA/ODN mixtures, water (control) or mRNA alone.

X. laevis oocyte ^{35}S-sulfate uptakes (10, 14) were performed on day 3 post injection as follows: 10 oocytes (individual data point) were washed at room temperature for 1–2 min in solution A (100 mM choline chloride, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES/Tris pH 7.5), then placed into 100 μl of solution B (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES/Tris pH 7.5) containing 0.1 mM K_2SO_4 and $^{35}SO_4$ 10–20 μCi/ml (New England Nuclear Radiochemicals). For Na^+-independent experiments, 100 mM NaCl was replaced with 100 mM choline chloride. The oocytes were washed 3 times with ice cold solution A, lysed with 1% SDS, dissolved in scintillant (Emulsifier Safe, Canberra Packard) and counted by liquid scintillation spectrometry.

RT-PCR, Cloning and DNA Sequencing

First strand synthesis was performed (90 min 37°C) with 1 μg total RNA from rat forebrain, midbrain, hindbrain, cerebellum and liver, using random hexamers (25 ng), dNTPs (0.5 mM), DTT (10 mM), MMLV-RT (25 U), in 1× first-strand buffer (50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$). PCR amplification was performed using the following primers: forward ODN: 5′-GGTGTCAGTAACAGAAGTC-3′ [corresponding to sat-1 cDNA (10) nucleotide position: 2135–2153] and reverse ODN: 5′-CAGAGTCAGCAGCCATCAGC-3′ [corresponding to sat-1 cDNA (10) nucleotide position: 2471–2490]. PCR mixtures contained: 1.5 mM $MgCl_2$, 25 ng cDNA, 0.2 μM of each primer, 0.2 mM of dNTPs and 2.5 units of Taq polymerase (Biotech Pty. Ltd.) in 1× PCR buffer (67 mM Tris-HCl pH 8.8, 17 mM ammonium sulfate, 0.45% Triton X-100, 0.2 mg/ml Gelatin). Amplification (35 cycles) was carried out by: 1 min 94°C (denaturation), 30 sec. 57°C (annealing) and 1 min 72°C (extension). The amplified product was separated through a 1.5% 1× TAE (40 mM Tris-acetate, 2 mM EDTA) agarose gel and visualised with ethidium bromide under UV light. All primers were obtained from Auspep Pty Ltd. (Melbourne, Australia).

Using the same RT-PCR protocol above (with the exception of annealing, performed at 59°C for 2 min), full length brain sat-1 cDNA (coding region) was amplified with the following primers: forward ODN: 5′-GTGACAGGATGGATGCTTCTC-3′ [corresponding to sat-1 cDNA (10) nucleotide position 360–380] and reverse ODN: 5′-CAGAGTCAGCAGCCATCAGC-3′ [corresponding to sat-1 cDNA (10) nucleotide position: 2471–2490]. PCR-amplified DNA fragments of expected length were subcloned into PCR2.1 vector using a TA cloning kit (Invitrogen Ltd.). Dye-termination-sequencing was performed using the *Big Dye* Termination kit (Perkin Elmer) and gel separation was performed at the Australian Genome Research Facility, The University of Queensland.

In Situ Hybridisation

Tissue preparation. Whole brains were removed from male Wistar rats (12–14 weeks old) that had been perfused with 4% paraformaldehyde in 1× PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4. 7 H_2O, 1.4 mM KH_2PO_4; at pH 7.3) and then placed in 15% sucrose overnight (or until the tissue sank). The tissue was then immersed in a series of solutions (for 12 h each at 4°C): 3 parts 15% sucrose, 1 part *O.C.T.* (Sakura Finetech Inc., Torrance); 2 parts 15% sucrose, 2 parts *O.C.T.;* 1 part 15% sucrose, 3 part *O.C.T.*, then in 100% *O.C.T.*. Rat brain was then dissected, placed in foil trays, covered with *O.C.T.*, frozen on dry ice and stored at −80°C. Frozen sections (10 μm) were prepared using a cryostat (Leica), mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh) and dried at room temperature.

Probe synthesis. The sat-1: pSPORT-1 plasmid was linearised at the 3′ end by Not I (for sense probe) and the 5′ end by Sal I (antisense probe). The digested DNA was *in vitro* transcribed at 37°C for 30 min

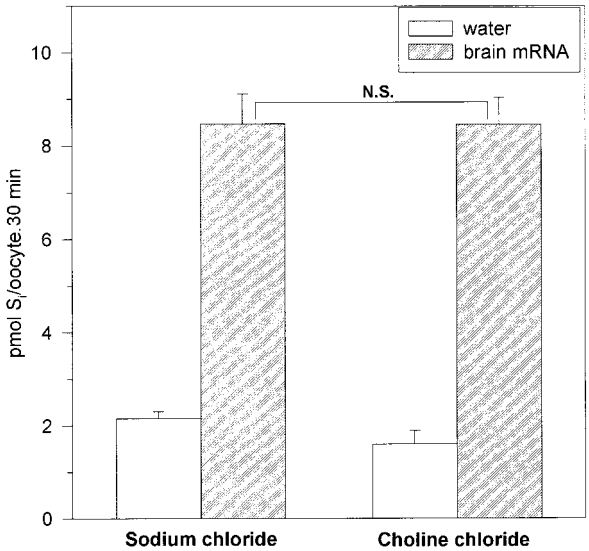

FIG. 1. Brain mRNA-induced sulfate transport in *X. laevis* oocytes. Oocytes were injected with either water (control) or rat brain mRNA (10 ng/oocyte). ^{35}S-sulfate uptake was performed 3 days post injection in either a Na^+-containing (sodium chloride, 100 mM) or Na^+-free (choline chloride, 100 mM) solution. Data represent the mean ± SE of 8–10 oocytes per condition and are representative of 3 similar experiments.

in the following mixture: 1× transcription buffer (80 mM Tris-HCl pH 7.9, 12 mM $MgCl_2$, 20 mM DTT, 20 mM NaCl, 2 mM spermidine), rATP (0.5 mM) rCTP (0.5 mM), rGTP (0.1 mM), rUTP (0.5 μM), 7-mG(5′)ppp(5′)G (0.5 mM), ^{35}S-rUTP (60 μCi), RNase inhibitor (10 U), T7 or T3 RNA polymerase (50 U), then RNase-free DNase I (10 U) at 37°C for 15 min Probes were hydrolysed (30 min 60°C) with 0.6 M Na_2CO_3 and 0.4 mM $NaHCO_3$, then stopped with glacial acetic acid.

Hybridisation. Sections were treated with 4% paraformaldehyde (20 min), washed in 1× PBS (5 min), placed in 0.2 M hydrochloric acid (5 min), then rinsed in MilliQ water (1 min) and 30 min (37°C) Proteinase K digestion (10 μg/ml in 0.1 M Tris, 50 mM EDTA, pH 8.0). Sections were rinsed in MilliQ water (1 minute) and placed in 0.1 M triethanolamine/0.25% acetic anhydride (10 min), then washed for 5 min in 2× SSC (with 1× SSC: 0.3 M NaCl, 30 mM Na_3citrate pH 7.0) and dehydrated through increasing ethanol (30%, 50%, 70%, 85%, 90%, 100%) washes and allowed to air dry (2 h). Slides were then covered with hybridisation buffer (50% formamide, 0.3 M NaCl, 20 mM sodium acetate pH 5.0, 5 mM EDTA, 10% dextran sulfate, 0.1 M DTT, 56 U Rnasin and 0.02% each of Ficoll, polyvinyl pyrrolidone and bovine serum albumin) containing the ^{35}S-labelled antisense or sense sat-1 cRNA probes, wrapped in Clingfilm and hybridised overnight at 50°C. Slides were then treated as follows: 4 washes (15 min room temperature) with 2× SSC/10 mM DTT; then 15 min 60°C in 50% formamide, 0.3 M NaCl, 20 mM sodium acetate (pH 5.0), 5 mM EDTA and 10 mM DTT; then 1 min in 2× SSC; then placed (30 min 37°C) in RNase A buffer (0.5 M NaCl, 10 mM Tris, 1 mM EDTA and RNase A 20 μg/ml); then 3 times (10 min 37°C) in RNase A buffer containing 10 mM β-mercaptoethanol; then 4 washes (15 min room temperature) in 2× SSC containing 10 mM β-mercaptoethanol; and finally washed (25 min 55°C) in 0.1× SSC containing 10 mM β-mercaptoethanol; and dehydrated by increasing ethanol (30%, 50%, 70%, 85%, 90%, 100%) washes, in 0.3 M ammonium acetate. Slides were air dried (2 h) and exposed to Kodak X-OMAT AR film (5 days, −80°C).

Data Presentation and Statistics

Each experiment was performed at least 3 times and error bars not visible on graphs are smaller than the symbol used for that point. Statistical significance was tested using the unpaired Student's t-test, with $p < 0.01$ considered significant (*) and anything above that considered non-significant (N.S.).

RESULTS

Injection of poly A^+ RNA (mRNA) from total rat brain into *Xenopus laevis* oocytes, led to a greater than 4-fold induction in sulfate uptake (Fig. 1). Similar uptake rates were observed in the presence and absence of sodium, suggesting that the brain mRNA induced sulfate transport was sodium independent. To further characterise the brain mRNA induced transport activity using *X. laevis* oocytes, known inhibitors of Na^+-independent sulfate transport were tested in competition studies. Strong inhibition was observed by the stilbene derivative 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS), as well as by oxalate, phenol red, probenecid and thiosulfate, whereas succinate and cholate had no effect, on the brain mRNA induced sulfate transport activity (Fig. 2). This pattern of inhibition was identical to that observed for the sulfate anion transporter, sat-1, cloned from rat liver (11). With the sat-1 transporter being a DIDS sensitive anion exchanger of sulfate/bicarbonate, the half-maximal inhibition concentration (IC_{50}) of DIDS on brain mRNA

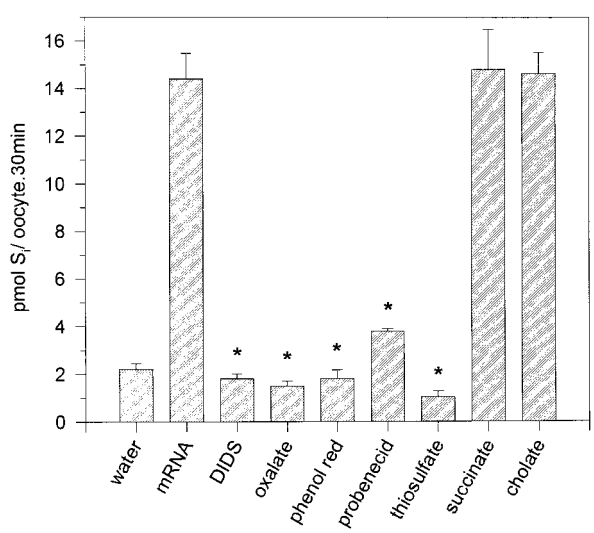

FIG. 2. Specificity of brain mRNA-induced sulfate transport in *X. laevis* oocytes. Oocytes were injected with either water or rat brain mRNA (10 ng/oocyte). ^{35}S-sulfate uptake was performed 3 days post injection in a Na^+-free (choline chloride 100 mM) solution, in the absence (mRNA control) or presence of various inhibitors: DIDS (1 mM), oxalate (5 mM), phenol red (1 mM), probenecid (1 mM), thiosulfate (5 mM), succinate (5 mM), or cholate (1 mM). Data represent the mean ± SE of 7–10 oocytes per condition and are representative of 3 similar experiments. Data are compared with the mRNA control group and considered significant (*) at 99% probability ($p < 0.01$).

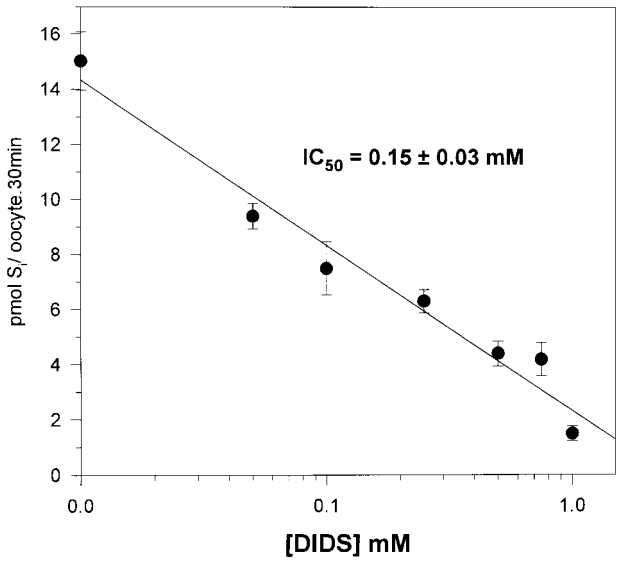

FIG. 3. DIDS inhibition of brain mRNA-induced sulfate uptake in *X. laevis* oocytes. Oocytes were injected with either water or rat brain mRNA (10 ng/oocyte). ^{35}S-sulfate uptake was performed 3 days post injection in a Na^+-free (choline chloride 100 mM) solution, in the presence of increasing concentrations of DIDS. Data are shown as net mRNA-induced sulfate uptake (water values subtracted from mRNA values), with the mean ± SE of 8–10 oocytes per condition and are representative of 3 similar experiments. The calculated half maximal inhibition concentration (IC_{50}) of DIDS on sulfate transport is shown.

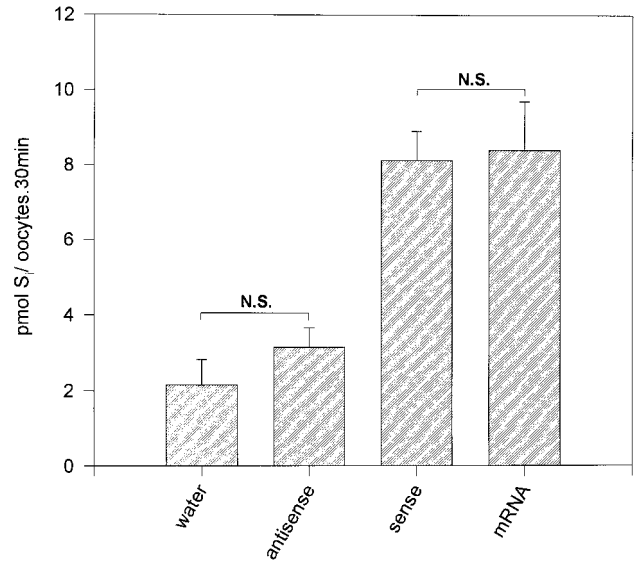

FIG. 4. Inhibition of brain mRNA-induced sulfate transport in *X. laevis* oocytes by sat-1 ODNs. Oocytes were injected with either water, rat brain mRNA (5 ng/oocyte) pre-incubated with antisense or sense sat-1 ODNs (see Materials and Methods), or rat brain mRNA (5 ng/oocyte) alone. ^{35}S-sulfate uptake was performed 3 days post injection in a Na^+-free (choline chloride 100 mM) solution. The data represent the mean ± SE of 7–10 oocytes per condition and are representative of 3 similar experiments. Non significant (N.S.) difference was observed between the water and antisense groups ($p < 0.01$), and the sense and mRNA groups ($p < 0.01$).

induced sulfate transport activity was determined in *X. laevis* oocytes (Fig. 3). The calculated $IC_{50\ DIDS}$ of brain mRNA induced sulfate transport was 0.15 ± 0.03 mM, which is comparable to the value obtained for the sat-1 transporter (11).

Due to the similarity in the expressed activity and inhibition pattern, we attempted to determine whether the sat-1 transporter may be responsible for the sulfate transport activity in the rat brain. Hybrid depletion experiments were performed with *X. laevis* oocytes using rat brain mRNA subjected to sat-1 sense and antisense oligodeoxyribonucleotides (ODNs) prior to injection. Using this approach, we have successfully identified the involvement of a protein responsible for the transport activity in another tissue, e.g. identification of the liver sat-1 protein responsible for the Na^+-independent sulfate transport activity in the kidney (14). Sat-1 antisense ODNs abolished the induced brain mRNA sulfate transport activity down to control levels (water injected oocytes), whereas the sense ODNs had no effect (Fig. 4). This would suggest that sat-1 specific antisense ODNs blocked the expression of a sat-1 homologous transcript in rat brain, thereby leading to a complete (antisense) inhibition or hybrid depletion of the brain mRNA induced sulfate transport activity.

To establish the presence of the sat-1 mRNA in rat brain, RT-PCR was performed using RNA from rat forebrain, midbrain, hindbrain, cerebellum and liver (positive control) with sat-1 (11) specific primers. Sat-1 specific fragments were amplified in all brain sections tested (Fig. 5). Using the same approach, numerous attempts (>10 pairs of primer combinations) were performed in order to determine if the NaSi-1 transporter (10) was present in the brain, however no fragments in

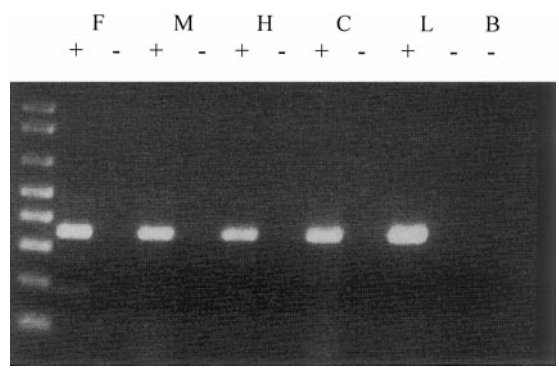

FIG. 5. Detection of sat-1 mRNA in rat brain by RT-PCR. Total RNA was isolated from rat forebrain (F), midbrain (M), hindbrain (H), cerebellum (C), and liver (L). RT-PCR was performed using sat-1 primers and amplified products were electrophoresed through a 1.5% agarose gel. sat-1-specific bands (344 bp) were visualised by ethidium bromide staining. A PCR blank (B) showed no visible band. RT reaction was performed in the presence (+) or absence (−) of MMLV-RT. Identical data were obtained in 3 similar experiments.

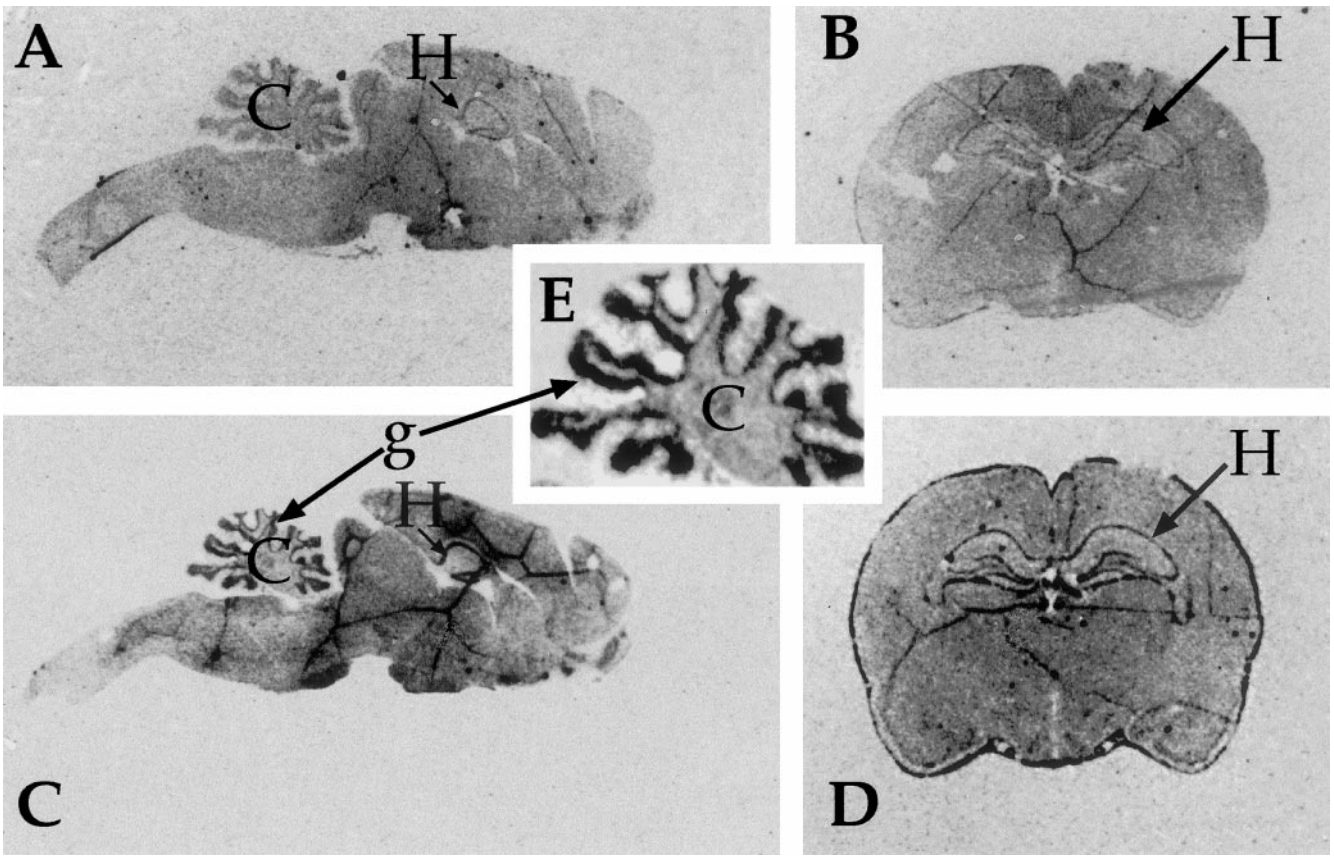

FIG. 6. *In situ* hybridisation of sat-1 mRNA in rat brain sections. Saggital (A and C) and coronal (B and D) sections of adult rat brains were hybridised with sat-1 sense (A and B) or antisense (C and D) cRNA ^{35}S-labelled probes. E is an enlargement of the cerebellum of C. Arrows point to hippocampus (H), cerebellum (C) and the granular cell layer (g). Autoradiographic signals were evenly distributed in control sections (A and B; background staining), with elevated sat-1 signals (C and D) in the granular cell layers of the cerebellum and hippocampus.

any brain section (although strong bands were observed in kidney controls) were amplified, suggesting its absence in brain tissue (data not shown). Since sat-1 fragments were successfully amplified from brain mRNA, sat-1 primers at cDNA ends, were used to amplify the entire coding region of the sat-1 cDNA by RT-PCR from brain mRNA (data not shown). The amplified fragments corresponding to the brain sat-1 cDNA were subcloned and both strands sequenced (data not shown). Not one single difference was observed in the nucleotide sequence of the cloned brain sat-1 cDNA when compared to the published sat-1 cDNA sequence (Genbank Accession No. B8.L23413; ref. 11), confirming that the functional sulfate transporter expressed in brain mRNA was in fact encoded by the sulfate anion transporter, sat-1.

To further identify a more precise localisation of sat-1 mRNA in the brain, *in situ* hybridisation was performed with sat-1 probes hybridised with brain sections (Fig. 6). The strongest hybridisation of sat-1 mRNA in the coronal section of the rat brain (antisense probe, Fig. 6A) was observed in the hippocampus (regions CA1, CA2, CA3 and dentate gyrus). The apparent labelling of the pial surface of the brain (Fig. 6A) is believed to be due to "chemography" and not true hybridisation, since it was variably present in both sense and antisense hybridisations (see also spinal cord region of Fig. 6B). The strongest hybridisation of sat-1 mRNA in the saggital section of the rat brain (antisense probe, Fig. 6B) was observed in the hippocampus and cerebellum (granular cell layer). Cellular localisation was confirmed by correlative histological analysis (light microscopy) of the same sections (data not shown). As a control, *in situ* hybridisation was performed with rat liver sections, which showed very strong sat-1 labelling throughout, with a stronger intensity than observed in brain slices (data not shown).

DISCUSSION

In contrast to renal, intestinal and hepatic sulfate transport, the transport of sulfate in the brain has not been previously examined at the molecular level. Early experiments have shown that a carrier mechanism for sulfate transport may exist in the brain, responsible for mediating sulfate transport from the cerebrospinal

fluid to plasma (8, 9). However, to date no studies have determined whether a sulfate transport mechanism in fact exists in the mammalian brain. In this study, we present the first evidence for the presence of a sulfate transporter in the rat brain, which shares structural and functional characteristics with the previously cloned sulfate transporter, sat-1 (11).

We have used the *Xenopus laevis* oocyte expression system to identify the presence and to characterise the activity of sulfate transport in the rat brain. Our experiments suggest that this transport activity is encoded by sat-1, a cDNA encoding a sulfate transporter, expressed in rat liver and kidney (11, 14). We provide the following functional evidence that sat-1 is responsible for the sulfate transport activity in rat brain: (i) the brain mRNA induced sulfate transport in *X. laevis* oocytes was Na^+-independent (Fig. 1), a distinguishing transport feature of the sat-1 (and not NaSi-1) transporter. (ii) The pattern of inhibition (by oxalate, probenecid, phenol red, thiosulfate and the stilbene derivative DIDS (IC_{50} = 0.15 ± 0.03 mM) was in strong agreement with sat-1 transport activity (Figs. 2 and 3). (iii) Hybrid depletion with sat-1 antisense ODNs, completely inhibited the expression of the brain mRNA induced sulfate transport (Fig. 4). Since sat-1 ODNs completely blocked all activity of the brain mRNA induced sulfate transport in *X. laevis* oocytes, this may imply that the contribution of other Na^+-independent sulfate transporters, i.e. DTDST (12) or dra (13), may not be significant to sulfate transport in the brain. However, further work is needed to clarify if DTDST or dra play a role in brain sulfate uptake.

In addition to the functional data, we also provide structural evidence for the involvement of sat-1 in the rat brain: (i) Using RT-PCR, we detected sat-1 mRNA in the forebrain, midbrain, hindbrain and cerebellum (Fig. 5). (ii) *In situ* hybridisation experiments showed strongest sat-1 mRNA expression in two regions of the rat brain, the hippocampus and cerebellum (Fig. 6). These latter findings confirm the presence and identify the localisation of the sat-1 transporter in brain tissues.

sat-1 mRNA levels were observed to be lower in brain than in liver (data not shown; 11). Northern blot analysis using brain total RNA (40 μg) failed to detect sat-1 signals in any section of the rat brain, whereas strong signals were observed in liver RNA (data not shown). Despite not having performed quantitative (competitive) RT-PCR, our RT-PCR studies showed reproducibly a more intense sat-1 signal in liver than in brain (Fig. 5 and data not shown). In addition, our *in situ* hybridisation experiments also showed moderate sat-1 mRNA expression in brain sections, whereas very strong hybridisation was observed in liver sections (data not shown). In agreement with the study by Bissig *et al.* (1994) in which Northern blot analysis using poly A^+ RNA (5 μg) showed a weak sat-1 mRNA signal in brain but very strong signals in liver and kidney (11), our studies suggest that sat-1 mRNA expression is less in the brain than in liver tissue. This may be due to the enormous amounts of different cell types present in the brain (as compared to the liver), or possibly due to the fact that sat-1 mRNA may be differentially expressed in different tissues.

Previously, a Na^+-dependent phosphate transporter (BNPI) was identified in rat brain (15) which shared significant homology to the renal Na^+-dependent phosphate transporter (NaPi-1; 16). Since it is well established that the renal Na^+/P_i-cotransporter mediates the transepithelial transport of phosphate from the renal tubule into the systemic circulation in order to maintain total body phosphate homeostasis (17, 18), BNPI was postulated to serve a similar physiological role in the brain (15). Available evidence suggests that BNPI could be involved in regulating intracellular concentrations of phosphate important for ATP synthesis in the brain (15, 19).

In an analogous manner, the brain sulfate transporter, sat-1, characterised in this study, could play an important role in regulating intracellular concentrations of sulfate required for sulfation reactions. It is well documented, that sulfation reactions play an important role in the CNS, in the synthesis of cerebroside sulfate and proteoglycans (20). The sulfation pathway which produces proteoglycans is largely dependent on the availability of sulfate and ATP (3). This is due to the fact that both sulfate and ATP are primary substrates for the biosynthesis of 3′-phosphoadenosine-5′-phosphosulfate (PAPS), the universal sulfate donor in all sulfation reactions. Several studies have reported that reducing the availability of sulfate inhibits the synthesis of PAPS (21, 22). Therefore, regulation of cellular sulfate uptake in the brain may serve to govern the intracellular pool of sulfate necessary for maintaining the sulfation potential of cells in the CNS. The requirement for both sulfate and ATP in neural tissues also suggests that the expression of a sulfate transporter, as well as a phosphate transporter, in these tissues may be crucial.

Our *in situ* hybridisation experiments indicate that, in addition to sat-1 mRNA signals in the granular cell layer of the cerebellum, sat-1 is also expressed in the hippocampal regions CA1, CA2, CA3 and dentate gyrus; signals were localised to layers rich in neuronal and glial cell bodies. This localisation may suggest that sat-1 could be important for neuronal and/or glial function. Since sat-1 and BNPI (19) are colocalised in the same cellular regions of the brain, we could speculate that they share a common functional relationship (i.e. ion transport) in the same cells of the brain (as observed in the renal proximal tubules; 14, 16).

In conclusion, we present the first evidence of the existence of a functional brain sulfate transporter in the mammalian brain. Using a structural and func-

tional approach, we have characterised the activity of this novel brain sulfate transporter and have identified it to be encoded by the liver and kidney expressed sat-1 transporter, located on canalicular membranes of hepatocytes and basolateral membranes of renal proximal tubular cells. Functional data suggests that the brain sat-1 transporter may play an important role in the uptake of sulfate into neurons and/or glial cells. This study will permit future investigations into determining the precise role(s) of sat-1 and the mechanisms that regulate sat-1 expression, in the mammalian central nervous system.

ACKNOWLEDGMENTS

This work was supported by grants from the Australian National Health and Medical Research Council and the University of Queensland Early Career Award (to D.M.). A.L. is a recipient of an Australian Postgraduate Award scholarship. L.B. is a recipient of the University of Queensland Postdoctoral fellowship. Many thanks to Dr. D. V. Pow for critical review of the paper, histological analysis of brain sections and for the preparation of Fig. 6. Thanks also to Dr. P. B. Osborne for help in sectioning of the rat brain. Portions of this work were presented at the Fourth Congress of the Federations of Asian and Oceanian Physiological Societies in Brisbane, Australia, 27 September to 1 October, 1998.

REFERENCES

1. Tallgren, L. G. (1980) *Acta Med. Scand. Suppl.* **640,** 1–100.
2. Mulder, G. J. (1981) *in* Sulfation of Drugs and Related Compounds (Mulder, G. J., Ed.), pp. 53–82. CRC Press, Boca Raton, FL.
3. Falany, C. N. (1997) *FASEB J.* **11,** 206–216.
4. Dow, K. E., and Riopelle, R. J. (1994) *Dev. Brain Res.* **80,** 175–182.
5. Nurcombe, V., Ford, M. D., Wildshut, J. A., and Bartlett, P. F. (1993) *Science* **260,** 103–106.
6. Dow, K. E., Mirski, S. E. L., Roder, J. C., and Riopelle, R. J. (1988) *J. Neurosci.* **8,** 3278–3289.
7. Guo, M., Dow, K. E., Kisilevsky, R., and Riopelle, R. J. (1993) *J. Chem. Neuroanat.* **6,** 239–245.
8. Cutler, R. W. P., Robinson, R. J., and Lorenzo, A. V. (1968) *Am. J. Physiol.* **214,** 448–454.
9. Lorenzo, A. V., Hammerstand, J. P., and Cutler, R. W. P. (1970) *J. Neurol. Sci.* **10,** 247–258.
10. Markovich, D., Forgo, J., Stange, G., Biber, J., and Murer, H. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8073–8077.
11. Bissig, M., Hagenbuch, B., Stieger, B., Koller, T., and Meier, P. J. (1994) *J. Biol. Chem.* **269,** 3022–3026.
12. Hastbacka, J., De la Chapelle, A., Mahtani, M., Clines, G., Reeve-Daly, M. P., Hamilton, B. A., Kusumi, K., Trivedi, B., Weaver, A., Coloma, A., Lovett, M., Buckler, A., Kaitila, I., and Lander, E. S. (1994) *Cell* **78,** 1073–1087.
13. Silberg, D. G., Wang, W., Moseley, R. H., and Traber, P. G. (1995) *J. Biol. Chem.* **270,** 11897–11902.
14. Markovich, D., Bissig, M., Sorribas, V., Meier, P. J., and Murer, H. (1994) *J. Biol. Chem.* **269,** 3022–3026.
15. Ni, B., Rosteck, P. R., Jr., Nadi, N. S., Paul, S. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5607–5611.
16. Werner, A., Moore, M. L., Mantei, N., Biber, J., Semenza, G., and Murer, H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9608–9612.
17. Dennis, V. W. (1991) *in* Handbook of Physiology (Schultz, S. G., Ed.), pp. 1785–1815. American Physiological Society Press, Bethesda, MD.
18. Murer, H., Manganel, M., and Roch-Ramel, F. (1992) *in* Handbook of Physiology (Windhager, E., Ed.), Renal Section 8. Vol II, Chap. 47, pp. 2165–2188 Oxford Univ. Press, Oxford, UK.
19. Ni, B., Wu, X., Yan, G., Wang, J., and Paul, S. M. (1995) *J. Neurosci.* **15,** 5789–5799.
20. Mulder, G. J. (1984) *Prog. Drug Metab.* **8,** 35–100.
21. Balasubramanian, A. S., and Bachhawat, B. K. (1961) *J. Sci. Ind. Res.* **20C,** 202–204.
22. Jansen, G. S. I. M., Van Eik, R., and Van Kempen, G. M. J. (1973) *J. Neurochem.* **20,** 9–12.