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Taurine modulates expression of transporters in rat brain and heart

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Summary. In pro- and eucaryotic life, cellular and subcellular compartments are separated by membranes and the regulated and selective passage of specific molecules across these membranes is a basic and highly conserved principle.

We were interested whether taurine, a naturally occuring amino acid, would be able to induce or suppress expression of transporters with the Rationale that taurine was shown to detoxify a series of endogenous toxins and xenobiotics of various chemically non-related structures.

For this purpose we used a gene hunting technique, subtractive hybridization, subtracting mRNAs of taurine-treated rat brain and heart from untreated controls. Subtracted mRNAs were then converted to cDNAs, amplified, sequenced and identified by gene bank data.

We found five transporter transcripts, the phosphonate transport ATPase PHNC, multidrug transporter homolog MTH104, protein-export-membrane protein SECD, oligopeptide transporters oppA and oppD, in the brain and two: ABC-transporter BRAF-2 and cation-transport ATPase PACS, in the heart. Homologies of the sequences found were in any case >50% thus permitting the identification of transporters with high probability.

The biological meaning could be that a naturally occuring amino acid, taurine, modulates complex transport systems. The most prominent finding is the upregulation of a multidrug transporter transcript, explaining a mechanism for the nonselective detoxifying action of taurine.

Keywords: Amino acids – Taurine – Transporter – Rat – Brain – Heart

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Introduction

In procaryotes and eucaryotic life, cellular and subcellular compartments are separated by membranes. The regulated and selective passage of specific molecules across these membranes is a basic and highly conserved principle. The importance of membrane transport is exemplified by the fact that almost 20% of the E. coli genes are associated with transport functions (Bachmann, 1990). Transmembrane transport is mediated by specific proteins associated with the membrane and grouped into a number of families, and their members are related to each other in sequence, molecular mechanisms and evolutionary origin.

Transporters are still holding centre stage particularly in biological and medical research, with cystic fibrosis, drug and antibiotic resistance as persistent challenges.

We were interested whether taurine was able to induce or suppress membrane transport with the Rationale, that taurine's well-documented detoxifying action of endogenous toxins and xenobiotics of various and entirely unrelated chemical structure (Huxtable, 1992) may be due to activation of transport mechanisms pumping the noxae out of the cell; a mechanism described for multidrug resistance phenomena (Higgins, 1992).

For this purpose we selected the gene hunting principle of subtractive hybridization (SH), a method subtracting mRNAs in organs of taurine treated rats from mRNAs in organs of untreated rats and vice versa, thus forming a subtractive library.

Subtracted mRNAs are converted to cDNA by reverse transcription, amplified by PCR and resulting cDNAs are sequenced. The sequences from the SH are compared to gen bank sequences and thus identified.

Using this technique we found a series of up- or downregulated transporter transcripts in brain and heart of taurine treated rats. Our data suggest the modulation of several transport systems by taurine, may help to understand individual action mechanisms of taurine and may explain the detoxification activity, particularly by the upregulation of a multidrug transporter homolog. We are demonstrating a first cue to the understanding of taurine effects on transporters, challenging and providing the basis for further studies at the transcriptional, protein and functional level.

Methods

6 Sprague-Dawley rats, 12 weeks old, female, were fed orally 100 mg/kg body weight taurine (Sigma) for a period of three weeks and 6 animals served as controls (Institute of Animal Breeding, Himberg, Austria). They had free access to tap water and rat cake (Altromin^{R}) and were kept under day and night rhythm (Lubec et al., 1996). At the end of the feeding period they were sacrificed by neck dislocation, whole brain and the total ventricular tissue were taken and snap frozen in liquid nitrogen. Gene hunting was performed on pooled brains and hearts of each of the two groups using subtractive hybridization.

Subtractive hybridization protocol (Labudova and Lubec, 1998)

Rat brain and heart pools of taurine treated and untreated rats were taken into liquid nitrogen and ground for the isolation of mRNA.

Isolation of mRNA was performed using the Quick Prep Micro mRNA purification kit (Pharmacia Biotech Inc., Uppsala, Sweden, cat.27 92 55 01).

1 microgram of mRNA from each (of the two) preparation was quality – checked by cDNA cloning kit (Gibco, Life Technologies, Eggenstein, Germany, cat. 18248-013) using the incorporation of [alpha – ^{32}P] dATP (Amersham, Buckinghamshire, UK, cat AA0004)) with subsequent electrophoresis on 1% agarose followed by autoradiography. The Reflection film (Dupont NEF 496) was exposed to the gel for a period of two hours at room temperature.

Construction of the substractive library: 10 micrograms each of mRNA from brain of DS and control were biotinilated by UV irradiation at 360 nm according to the instructions supplied in the subtractor kit (Invitrogen, Leek, Netherlands, cat K4320-01). 1 microgram of mRNA – pools each from the brain sample was subject to reverse transcriptase reaction (subtractor kit, Invitrogen) and the cDNA – pools were hybridized with the corresponding biotinilated mRNAs from controls.

The substractive hybridization mixture was incubated with streptavidin according to the subtractor kit given above and thus the biotinilated molecules (non-induced biotinilated mRNAs and the hybrid [biotinilated mRNAs/cDNAs]) complexed. The streptavidine complexes were removed by repeated phenol-chloroform extraction and subtracted cDNAs were separated from the aquous phase by alcohol precipitation (subtractor kit).

In order to amplify and clone subtracted cDNAs, they were ligated with Not I – linkers followed by Not I – digestion. These Not I linked cDNAs were ligated to Not I site of sPORT 1 cloning vector (cDNA cloning kit, Gibco).

To enable visualization of subtracted cDNAs the cloned cDNAs were amplified using universal primers

I 5′-GTAAAACGACGGCCAGT-3′
II 5′-ACAGCTATGACCATG-3′

from multiple cloning site of the sPORT-1 vector (cDNA cloning kit, Gibco). Amplified cDNAs were analysed on 1% agarose electrophoresis.

Cloning of subtracted Not I – linked cDNAs

Not I linked cDNAs ligated with sPORT 1 vector were used for the transformation of highly competent INFalpha F′ E. coli cells (Invitrogen, Leek, Netherlands, cat C2020-03) and plated clones were analysed by plasmid isolation kit (Quiagen, Hilden, Germany, cat 12245) and digestion with Eco RI/Hind III. Recombinant clones were sequenced by K. Granderath, MWG – Biotech (Ebersberg, Germany).

Homologies were determined by computer assisted comparison of data from the genebank sequence library: fastA@ebi.ac.uk (GBALL, Gen Bank, EMBL, Heidelberg, Germany).

Subtractive hybridization was performed cross-wise i.e. taurine sample mRNA subtraction from control and vice versa at the 1:3 level (DSmRNA:control mRNA).

Results

Brain

All sequences obtained from the subtractive library were down- or upregulated at least threefold, as the level of mRNAs used for subtraction was set 1:3.

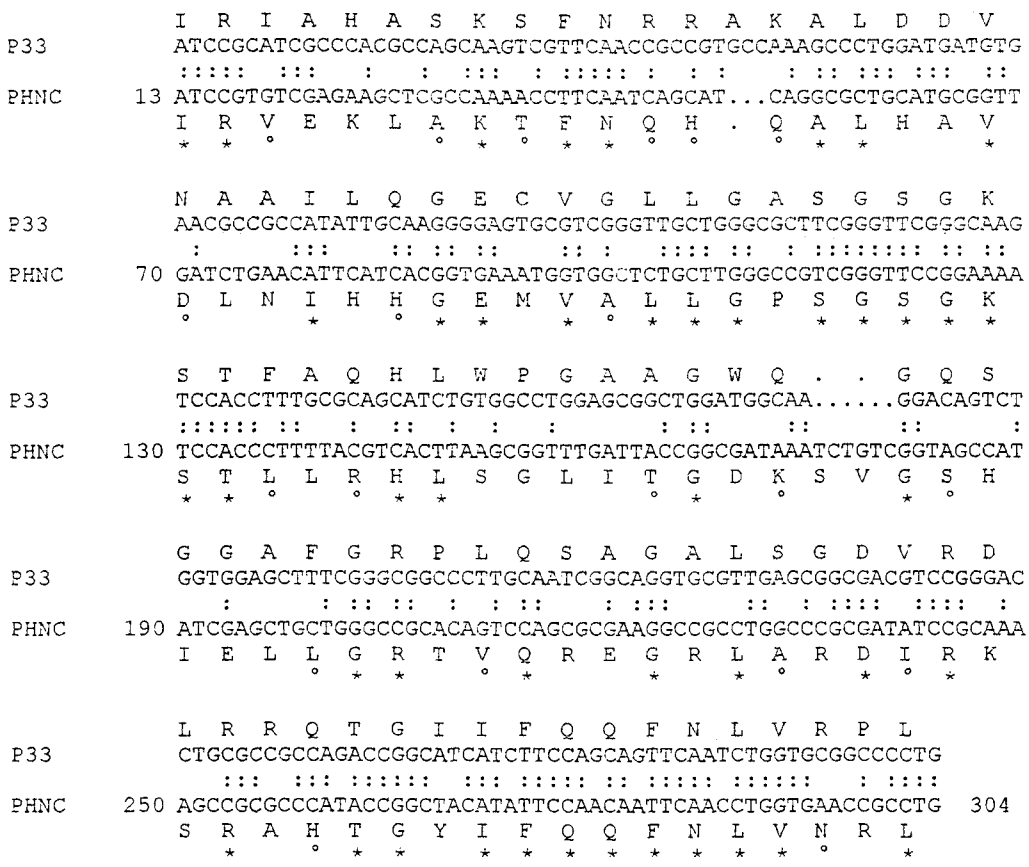

Fig. 1. The alignment of clone P33 from the taurine-subtractive library is aligned to the phosphonate transport ATPase PHNC-sequence from gene bank EMBL (P16677). : represents identical base pairs, * stands for identical amino acids and ° represents amino acids with similar function

1. The clone P33 from the subtractive library contained an upregulated sequence with high homology of 66.3% to the phosphonate transport ATPase PHNC of E. coli.

The length of the fragment was 262 bp and alignment of the clone with gene bank data are shown in Fig. 1.

2. The clone P29 from the subtractive library (SL) contained an upregulated sequence with high homology of 76.8% to the multidrug-transporter homolog MTH104 of Methanobacterium thermoautotrophicum.

The length of the fragment was 459 bp and the alignment of the clone with gene bank data are shown in Fig. 2.

3. The clone P21 from the subtractive library (SL) contained a downregulated sequence with high homology of 71.4% to the protein-export-membrane protein SECD of Haemophilus influenzae.

The length of the fragment was 616 bp and the alignment of the clone with gene bank data are shown in Fig. 3.

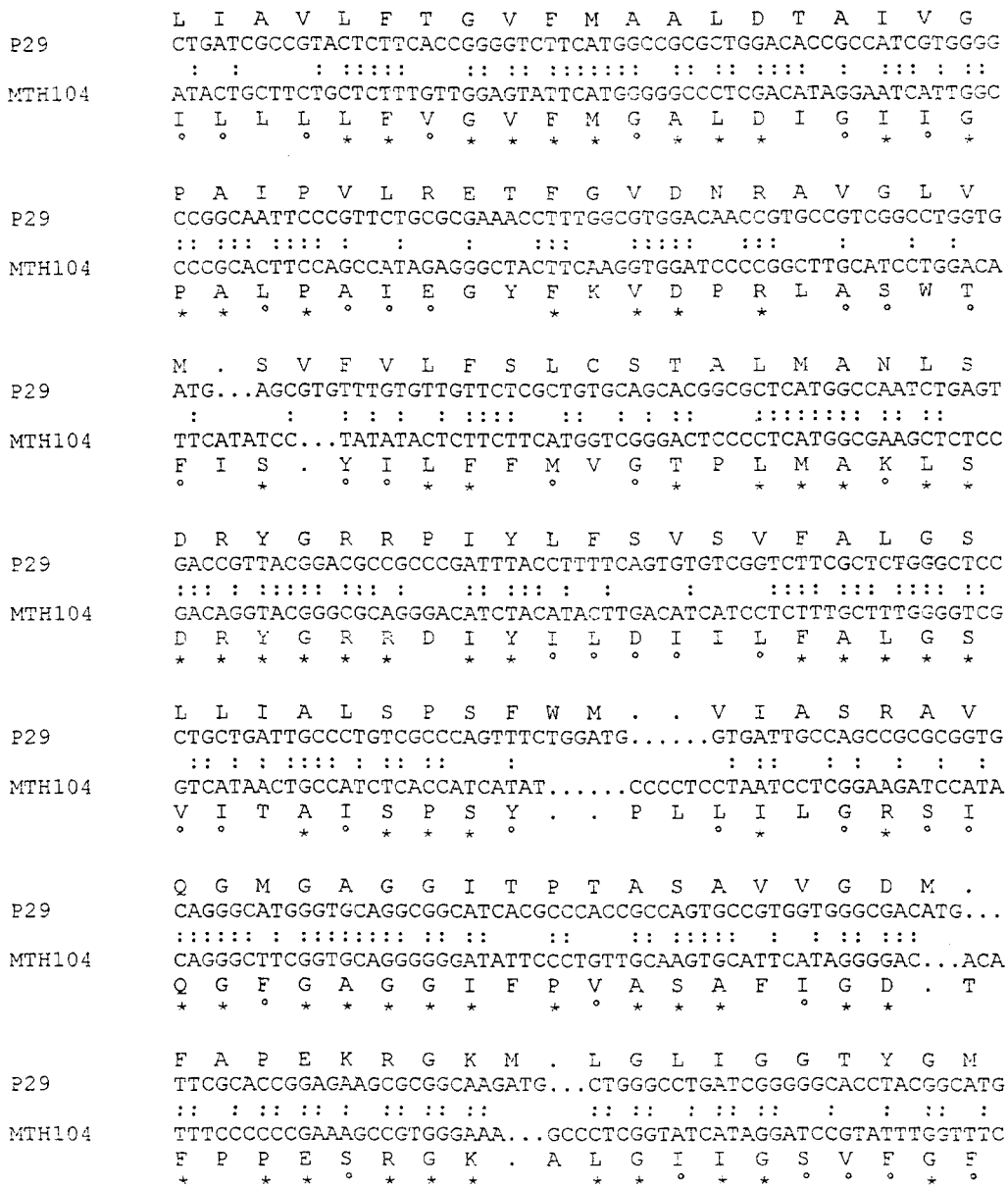

Fig. 2. The alignment of clone P29 from the taurine-SL is aligned to the multidrug-transporter homolog MTH104 of methanobacterium thermoautotrophicum-sequence (026207) from gene bank EMBL

4. The clone P23 from the subtractive library (SL) contained a downregulated sequence with high homology of 62.1% to the olkigopeptide transporter oppA of helicobacter pylori.

The length of the fragment was 594 bp and the alignment of the clone with gene bank data are shown in Fig. 4.

5. The clone D9 from the subtractive library (SL) contained a downregulated sequence with homology of 54.4% to the oligopeptide transporter oppD of rhodobacter sphaeroides.

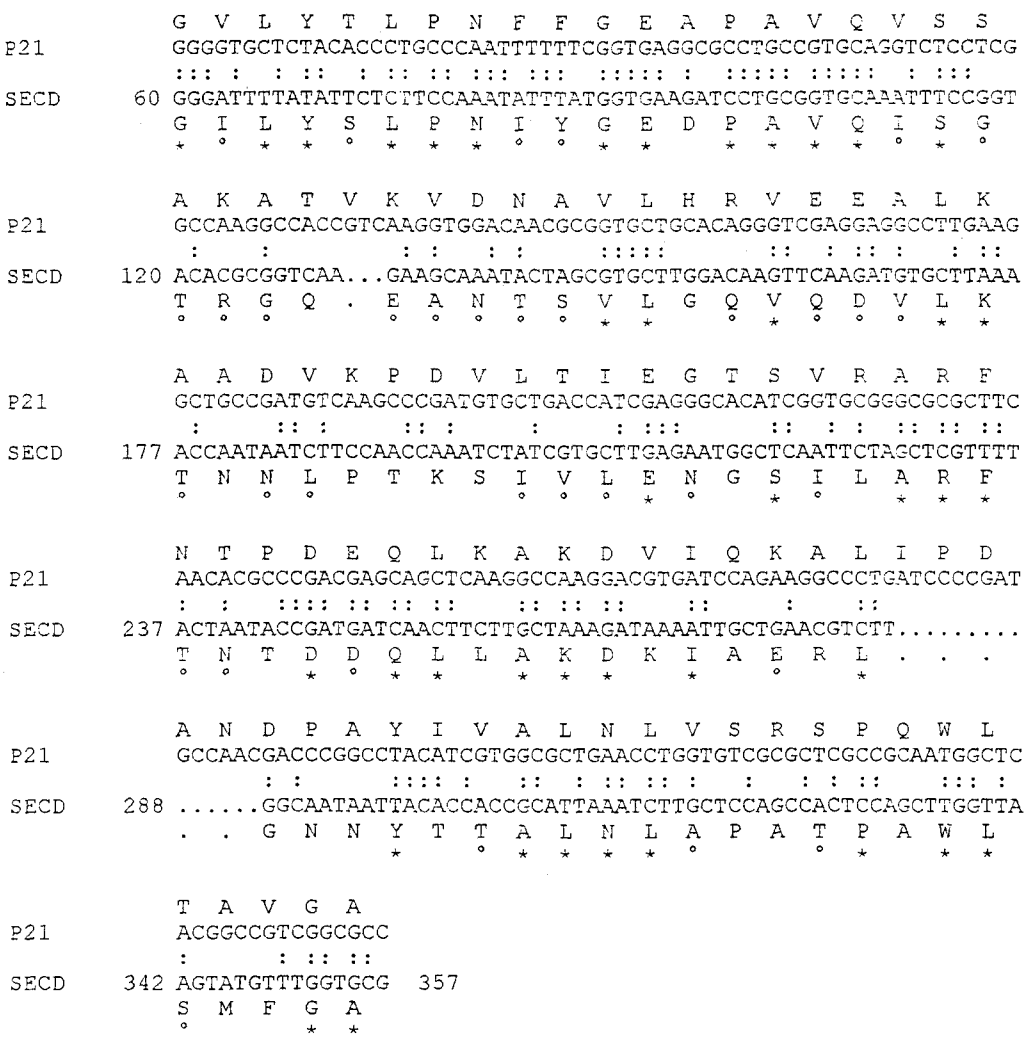

Fig. 3. The alignment of alone P21 from the taurine-SL is aligned to the protein export membrane protein SECD-sequence from gene bank EMBL (P44591)

The length of the fragment was 483 bp and the alignment of the clone with gene bank data are shown in Fig. 5.

Heart

6. The clone P14 from the subtractive library (SL) contained an upregulated sequence with high homology of 69.6% to the ABC-transporter BRAF-2 of archaebacterium fulgidus.

The length of the fragment was 238 bp and the alignment of the clone with gene bank data are shown in Fig. 6.

7. The clone P19 from the subtractive library (SL) contained an upregulated sequence with high homology of 68.1% to the cation-transport-ATPase PACS of Synechocystis sp.

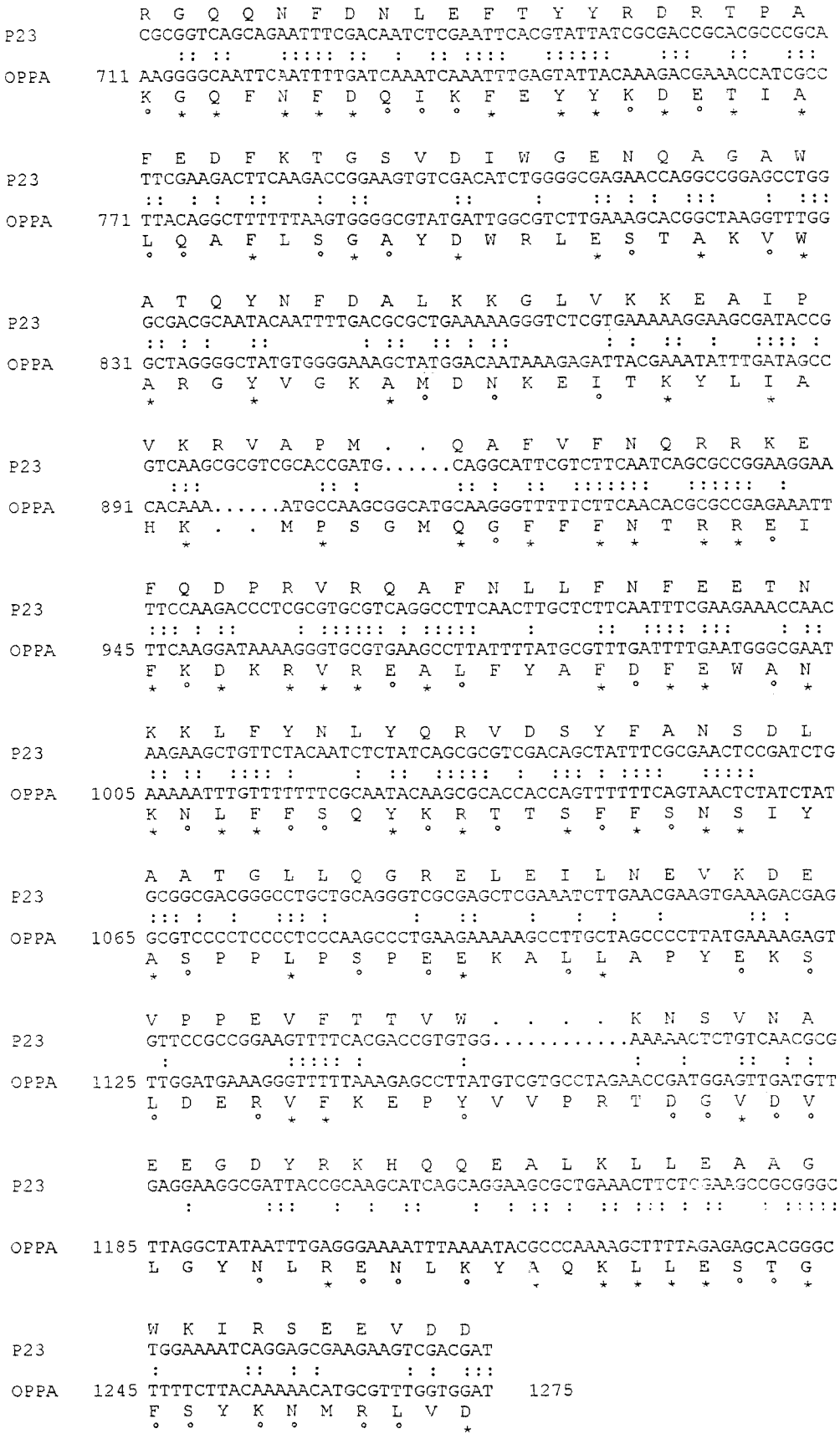

Fig. 4. The alignment of clone P23 from the taurine-SL is aligned to the oligopeptide transporter oppA-sequence from gene bank EMBL (025845)

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D9              TTGAGCGTGACGTGCAGGCCCGGCTTGTTGGGAATGAGCTTGGAGATGCCGTC..GC.AG
                :  : :       :   :    ::      ::    :::  :: :: :  :::  ::  :
RSB798    135   TCGACCAGTTGCCGGCCGATGCGCACCACCGGGTTCAGCGAGGTGAAGGGGTCTTGCGCG

D9              AAGGTGTAGCTCATGTCCAGCACGCGGCC..CACGTCGCGGATGCCGCGCGCGCGGCCAT
                : ::::  : :: :: ::  : ::: :::  : :  :::::: ::  :   ::: :::::
RSB798    195   ATGGTGGCGATCTTGGCC.CCCCGCAGCCGGCGCTGCGCGGAGGCATCCATCGCAGCCAT

D9              ...GGTGCCGAAGGTGGCGATCTGGCTGACGCGCCGCCCCCAATTGGGCGCCGCGCCGAC
                   :  ::::  :  ::  : : :::   :: : :::: ::    ::: ::   :: :::
RSB798    254   GTCGACGCCGTCGAAGGTCACCCGGCCCTCG.GTCGCCGCCTGCCGGG.GC..AGCAGAC

D9              CAGCCCCAGCATGCGCTGTCCATCGCCTACTGCAGCTTCTGTGTGGTGGCGACGGTGTTT
                : ::    ::  :::       ::: ::  ::: :  :: : :::: :::: : :    :
RSB798    310   CGGCGATGGC.CGCGGCAAGGCTCGACT..TGCCGGATCCG.GTGGCGGCGCCCGAAGCT

D9              TTCAG..CCAATTGTTCGAGTTCCGTCGCCATTGGATCTGGGCTGCGCACCTCTTCAGCG
                  :::  :   : :  ::    :: :::  :: ::  ::: : : :   : : ::: : :
RSB798    366   AGCAGCTCTTCTGGGACGGTCACCATCGANATCGGCACTGTGGT.CCTGCGTGTTCCGGG

D9              TGGTGGCCTGGCTGAA..TGCCATCGCACTGGGCCTGGCACTGTTGG.GTGGCTATG...
                 : :: :: ::: :::   ::   ::: ::::  : ::: :::  ::     : :::
RSB798    425   CGATGTCCCGGCCGAACGGGCGGCCGCGCTGGTGCGGGCGCTGCAGGANACACCATGATC

D9              ..CGCAGATC.TCGCGTGCCG.TGGTGCTCACGGCCCTG.GTGTCCACATCGTTCGGTGC
                  ::: :  :  ::  ::::: : ::: :  :: ::: :   ::: :: ::   :: :::
RSB798    485   GTCGCTGGCCAGCGGTTGCCGATCGTGGTGGCGACCCGGCCCGTCNACTTC..CCGCTGC

D9              GGTCTTCGTGCTGTACCTGCTGGTGGTCCGAAGGCAGTGGCAATACCTGCCGGGCGCGCT
                ::::  :  ::: :  : :::: : ::  : ::: :   ::   : ::      :::  :
RSB798    543   GGTCACCAAGCTCTGGC.GCTGATCGT.TGCAGG.ANCNGCTGAANCTCGATCCCGCATT

D9              GGCGTTTG.CGGTG.CCTTCCGCCCTGGGCATCCTGAACCTGCTGAGAACCCAGGGCCTG
                 : :  :: :::::   :::::: :   ::       ::  ::::: ::  : :: :  :
RSB798    600   CGGGCGTGACGGTGATTTTCCGCTCNAAGCCGGGGCGACAGGCTGAAAATTCTGGTCTGG
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Fig. 5. The alignment of clone D9 from the taurine-SL is aligned to the oligopeptide transporter oppD-sequence from gene bank EMBL (B07798)

The length of the fragment was 745 bp and the alignment of the clone with gene bank data are shown in Fig. 7.

Discussion

The oral administration of taurine led to the modification of transporter expression in brain and heart. As the level of subtraction was set at 1:3 allelic differences should not have been accounting for up- or downregulation of mRNA steady state levels.

The phosphonate transport ATPase PHNC (PHNC) as well as all other transporters observed in this study, have never been described in the mammalian system before, although the known highly conservation of

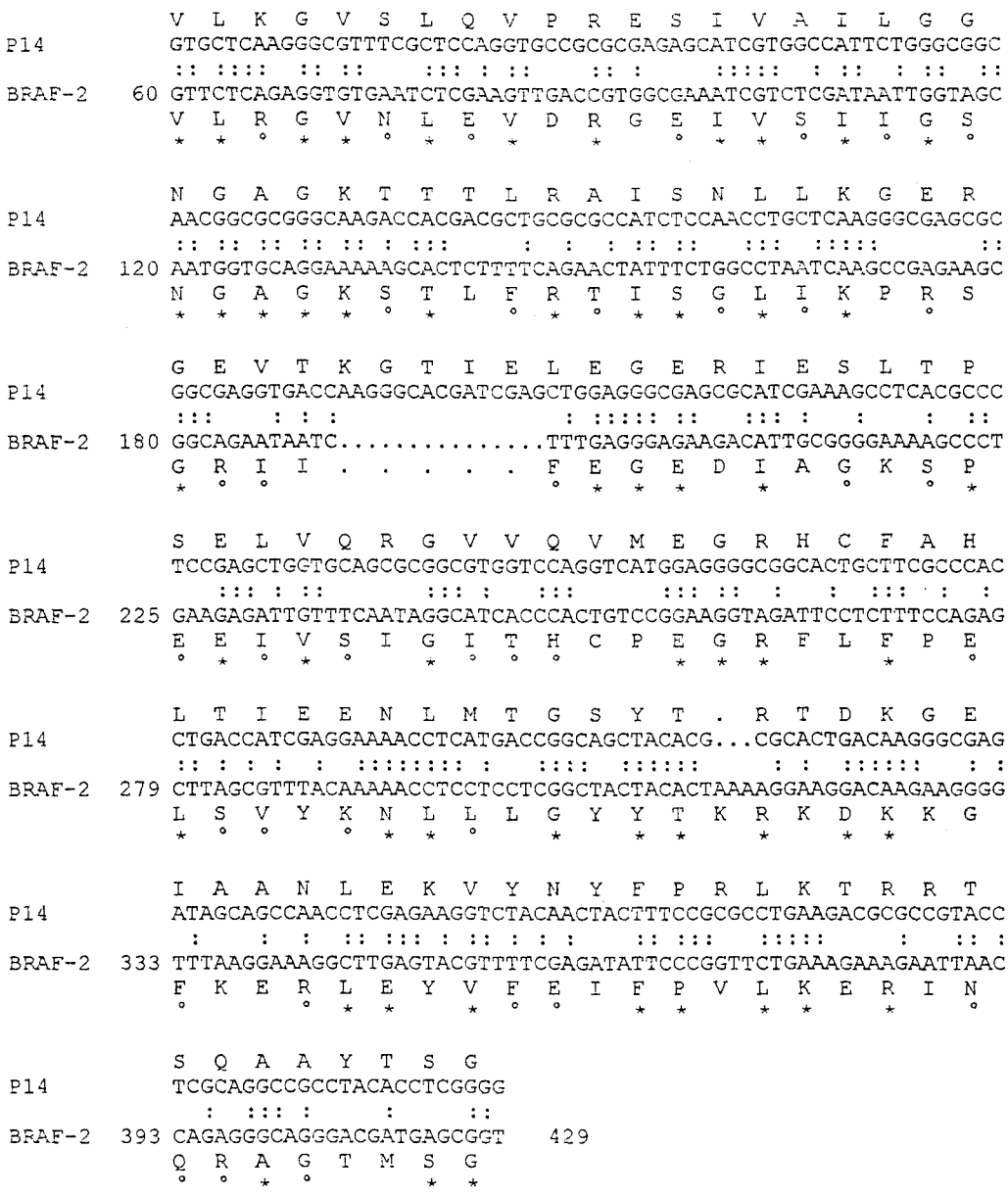

Fig. 6. The alignment of clone P14 from the taurine-SL is aligned to the ABC-transporter BRAF-2-sequence from gene bank EMBL (029436)

transport systems highly suggests the presence of these biomolecules in all species.

PHNC is a transport related protein necessary for phosphonate uptake and degradation in bacteria. Biochemically, it serves as a nucleotide-binding-protein of the binding protein-dependent phosphonate transport system (Chen et al., 1990). Metcalf and Wanner have extended knowledge by showing that PHNC along with other members of the PHN gene cluster, constitute a binding protein-dependent phosphonate transporter, which also transports

Fig. 7. The alignment of clone P19 from the taurine-SL is aligned to the cation-transport-ATPase PACS-sequence from gene bank EMBL (P73241)

phosphates, phosphate esters and inorganic phosphate (Metcalf and Wanner, 1997). The upregulation of a phosphate-phosphonate carrier is in line with the observations that taurine is involved in protein phosphorylation (Lombardini, 1996).

The overexpression of the multidrug transporter homology MTH104 in brain of taurine treated rats probably reflects the major finding of the study as it may link the detoxifying properties of taurine to the multidrug transport system.

The multidrug transporter system, represented by the multidrug resistance (MDR) system, plays a major role for multidrug resistance i.e. therapeutic failure, in chemotherapy of cancer and infection. Increased MDR expression of tumour cells leads to increased clearance of cytotoxic agents as chemotherapeutic drugs are pumped out from the cell (Higgins, 1992; Germann, 1993; Gottesmann et al., 1995). On the other hand, the MDR transport system under physiological conditions may be of utmost importance for detoxification processes, both, of endogenous and exogenous noxae and its overexpression may be necessary and beneficial in conditions with toxic overload. The non-specificity/non-selectivity of the transport process would

teleologically point to such a biological function and this would also explain the highly conserved nature of multidrug transporters (Van Veen et al., 1996; Neyfakh et al., 1991). The upregulation of the multidrug transporter homolog by taurine, which in turn detoxifies a multitude of chemically unrelated compounds, may well explain this taurine action.

The demonstration of the protein-export membrane protein SECD in rat brain is the first to show the presence of the bacterial integral membrane protein translocation factor sec for protein export in a mammalian organ. Gardel and coworkers showed that the secD locus of E. coli codes for two membrane proteins required for protein export (Gardel, 1990). Pogliano and Beckwith, however, could demonstrate that secD is not required but facilitating protein export in E. coli (Pogliani and Beckwith, 1994). We are not able to assign a functional implication to the finding of downregulation of the protein-export membrane protein SECD in rat brain.

The novel finding of the oppA and oppD ABC transporter-homologs in rat brain shows that this oligopeptide transporters are conserved in the mammalian system. This oligopeptide transport system consists of two ATP-binding proteins, oppD and oppF, two integral membrane proteins oppB and oppC and a substrate binding protein oppA. The role of the oppA in bacteria is not only the function as a peptide transporter (Tamme et al., 1994; Koide and Hoch, 1994; Tynkkynen et al., 1993) but also as a chaperone (Richarme and Caldas, 1997). The biological meaning of downregulated oppA and oppD by taurine in rat brain cannot be answered at present. There is, however, a clue from oppA deficient mutants which are not able to transport Leu-enkephalin (Tynkkynen et al., 1993). A general role for proteolytic and peptide recycling systems may be deduced.

In the heart two transport systems were found to be taurine-sensitive: The ABC transporter BRAF-2 may be the highly similar human homolog of the bacterial branched chain amino acid transport system, which is composed of five components: BraC, a periplasmic binding protein for branched chain amino acids; BraD and BraE, integral membrane proteins; BraF and BraG, putative nucleotide – binding proteins (Hoshino et al., 1992). Upregulation of BRAF-2 may be in agreement with increased branched chain amino acid transport in the heart and this in turn may be the consequence of taurine per se or by products resulting from its interaction with the carbohydrate-insulin-branched chain amino acid metabolic pathway (Lampson et al., 1983).

The second upregulated sequence was the copper transporting mammalian homolog of a copper – transporting P-type ATPase pacS found in the thylakoid membrane of synechococcus. This member of the large family of cation pumps play crucial roles in many organisms including bacteria, plants and mammals. PacS was found to possess a putative metal binding motif (gly-met-X-cys-X-X-Cys) in its N-terminal portion and indeed, metals specifically increase pacS-mRNA and there is evidence that pacS in involved in copper-homeostasis (Kanamura et al., 1994). It has been stressed that taurine is involved in the detoxification of metals/metallic cations (Howard and Nickless, 1977). The proposed detoxifying mechanism for metals was chelation and complexing of metals by taurine. Here we propose the

activation of the PACS-transport-machinery to compartmentalize metallic toxins.

We have described a series of bacterial transporter genes in mammalian brain and heart and their modulation by taurine. We are aware of the pitfalls and shortcomings of gene hunting methods but the high homology – sequences were assigned to a known data bank sequence when homology was >50% only – justifies the identifications. Modification of transporter gene expression may be of physiological relevance taken into account that taurine is not only a normal constituent of the body but also present in the diet.

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