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Short Sequence-Paper

Cloning and characterization of a glutamate transporter cDNA from human brain and pancreas ☆

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Abstract

L-Glutamate is the major excitatory neurotransmitter in the brain. Sufficient removal from the synaptic cleft after neurotransmission by the L-glutamate transport system is essential to prevent excitotoxicity and neurotoxicity. We isolated mRNA from human brain and pancreatic islet cells and screened for sequences of high homology to a previously characterized rat brain glutamate transporter. An isolated sequence (GLTR) shows a 87.5% and a 92.5% sequence similarity at the nucleotide and amino acid level, respectively, with a rat brain specific L-glutamate transporter but only a 65% homology to the recently cloned human glutamate/aspartate transporter. The human mRNA is differentially expressed in brain and to a lesser degree in pancreas and in fetal liver. The gene encoding for the newly identified cDNA is located on chromosome 5.

Keywords: L-Glutamate transporter; cDNA; Brain; Pancreatic islet cell; Sequence; (Human)

In most vertebrates the cellular uptake of amino acids into presynaptic nerve terminals or neuroglia is mediated by multiple independent transport systems. Several different classical amino acid transport systems have now been cloned and asigned to distinct gene families. These include system Gly [1–3], the neutral amino acid transport system A [4], the basic amino acid transport system y^+ [5,6], and the acidic amino acid transport system X^-, the glutamate transporters. The sodium dependent high-affinity glutamate uptake system transports glutamate from the extracellular fluid. Persistent elevation of extracellular concentrations of glutamate can lead to neuronal cell death. Deficient neurotransmission at the synaptic cleft has been implicated in the etiopathology of neuronal ischemia and amyotropic lateral sclerosis (ALS). Imbalances in glutamate levels have also been reported to occur in Huntington's chorea [7,8] and epilepsy [7,9].

We report the cloning and expression in human tissues of a human gene (referred to here as GLTR) that is structually related to the glutamate transporter gene family and shows a high homology to the glutamate transporter recently cloned from rat brain [10]. Another closely related glutamate transporter has been cloned from rabbit small intestine [11]. In contrast expression of GLTR was not detected in human intestine.

Total RNA from human brain, human pancreatic islets, and rat brain was isolated by the method of Chomcynski and Sacchi [12]. Human mRNA from hippocampus, hypothalamus, and pitiuitary gland was obtained from Clontech, CA. The highly purified pancreatic islet cells were a gift of C. Ricordi (Diabetes Research Institute, Miami, FL). Reverse transcription (RT) was done by using a Perkin Elmer RT-PCR Kit. cDNA was then amplified by the polymerase chain reaction (PCR) using Taq DNA polymerase (Perkin Elmer). The rat brain cDNA was amplified using the first set of primers (GLTRA 5'-TCACTGACTGTGTTTGGTGT, GLTRB 5'-GAGGAGCATGGTGACCAACC) corresponding to the first and eighth transmembrane region of a rat glutamate transporter [8].

☆ The sequence data reported in this paper have been submitted to the EMBL/GenBank Data Libraries under the accession number Z 32517.

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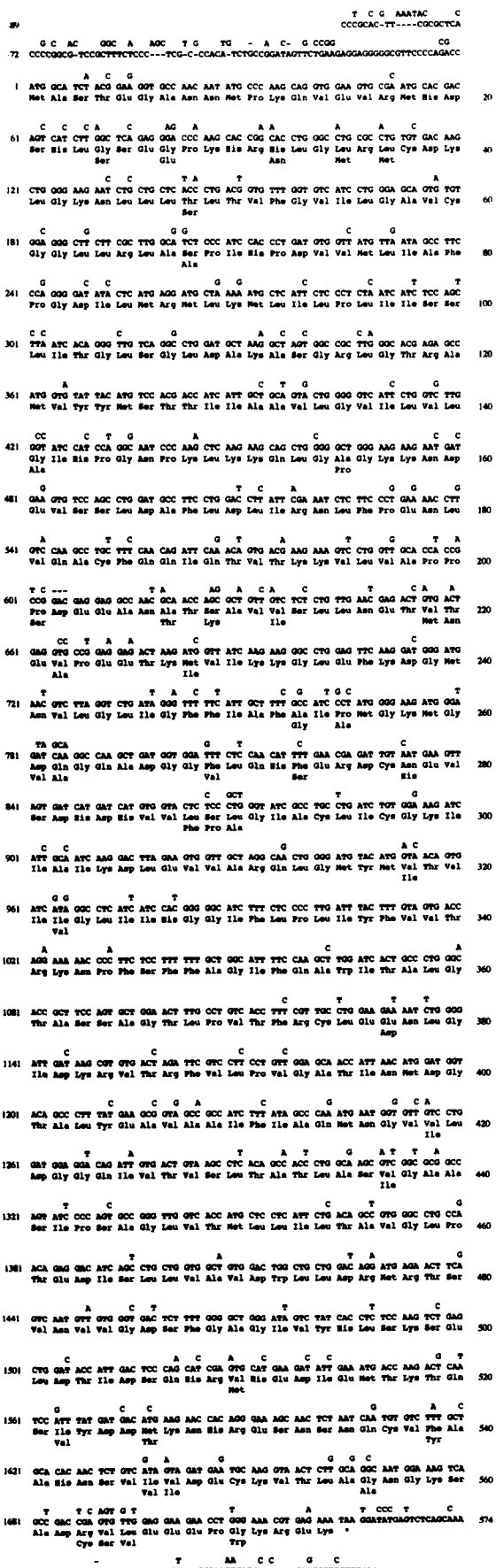

Amplification was carried out during 30 cycles on a Themocycler (Perkin Elmer, Model 9600) as follows: denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. The human brain cDNA was amplified under relaxed reaction conditions (annealing at 48°C for 30 s) with the same primer pair.

Sequencing of the DNA was carried out by using an automated DNA Sequencer (Applied Biosystems, Model 373A). For sequence confirmation, both strands of the DNA were sequenced using the dideoxy chain termination method with Taq- (cycle sequencing) and T7-DNA Polymerase (Sequenase), respectively. Human sequence specific primers were generated on a Oligonucleotide Synthesizer (Applied Biosystems) to isolate and clone the 5'- and 3'-portions of the human sequence from brain and pancreas using a 5'-RACE and 3'-RACE Kit (Gibco BRL). No differences between cDNA derived from brain and islet cells were detected. The nucleotide sequence and the deduced amino acid sequence are shown in Fig. 1.

In order to obtain a full length cDNA fragment, a pDNA1 cDNA library derived from human brain (Clontech Laboratories, Palo Alto) was screened according to standard protocols [13] using the PCR generated human sequence as probe. Hybridizations were performed at 65°C in 6 × SSPE, and the final wash was done at 65°C in 0.1 × SSC, 0.1% SDS.

Human tissue Northern blots were obtained from Clontech Laboratories. A 500bp PCR-generated coding sequence of GLTR was radiolabeled with [α-^{32}P]dCTP (Amersham) by the random priming method (Amersham). Filters were hybridized overnight at 65°C with this cDNA probe (10^6 cpm/ml) according to standard protocols [13]. Autoradiography was performed after a final wash in 0.1 × SSC, 0.1% SDS. Filters were subsequently reprobed with a radiolabeled human β-actin probe (Clontech Laboratories) as quality control of RNA(not shown).

Northern blot analysis of 16 different human tissues revealed that the isolated sequence was expressed in

Fig. 1. Nucleotide and deduced amino acid sequence of the human glutamate transporter cDNA (GLTR). Nucleotides of the homologous rat glutamate transporter [10] differing to the the human cDNA sequence are given above the sequence. Insertions and deletions are indicated by dashes. The nucleotide number is indicated on the left and the amino acid number at the right of the sequences. Positive nucleotide numbering begins at the putative translation initiation codon ATG: The stop codon is indicated with an asterix. The sequence shows a 87.5% and a 92.5% sequence similarity at the nucleotide and amino acid level, respectively, with a rat brain specific L-glutamate transporter but only a 65% homology to the recently cloned human glutamate/aspartate transporter [14].

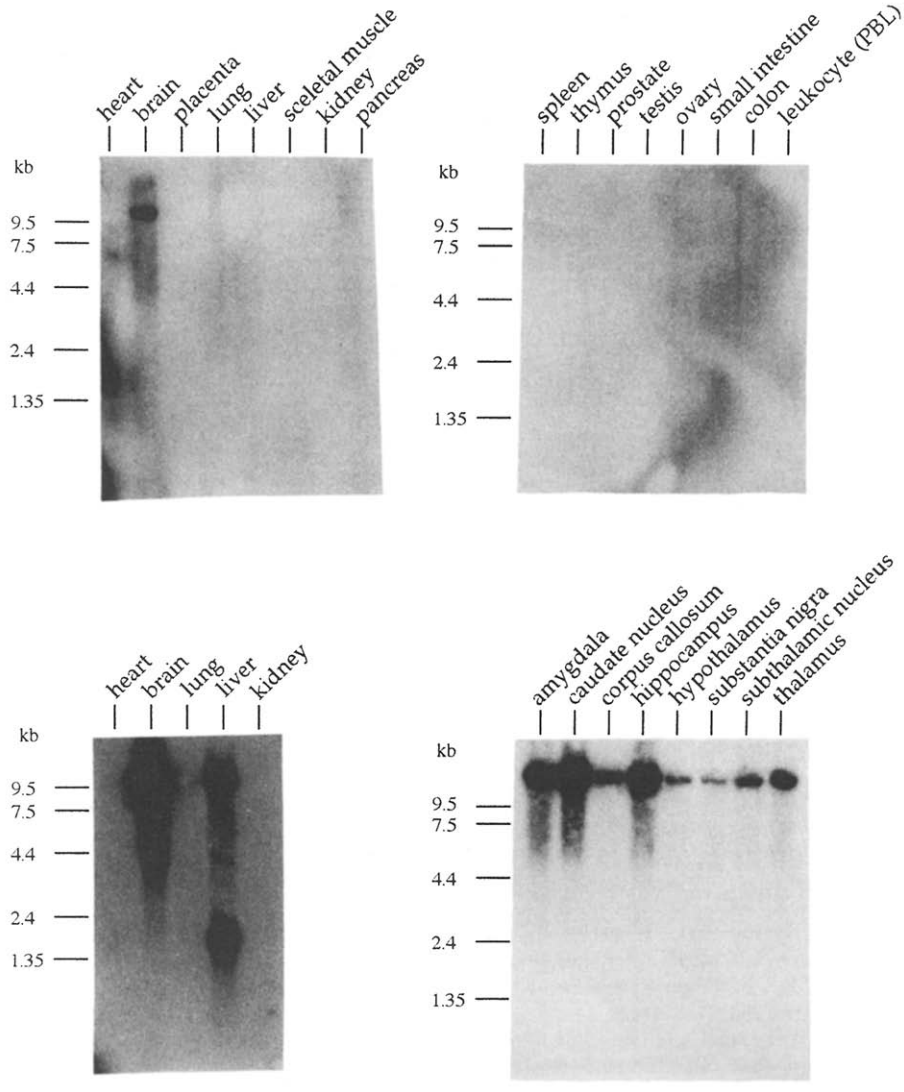

Fig. 2. Northern blot hybridization using a GLTR probe against mRNA from multiple human tissues.

brain, liver, and to a lesser extend in pancreas, while all other tissues gave a non-detectable signal (Fig. 2).

The chromosomal localization was performed using a somatic cell hybrid DNA panel (provided by the Imperial Cancer Research Fund, London). Only genomic DNA corresponding to the GLTR sequence was found on chromosome 5 (data not shown).

The sequence homology to the previously cloned L-glutamate transporters from rat and rabbit is indicated by a multiple sequence alignment (Fig. 3).

With this study we show a weak expression of a newly identified human L-glutamate transporter in human pancreas. Numerous neuron-specific proteins were found to be expressed in pancreatic β-cells, but little is known about their role in β-cell physiology. Further studies have to address the question, if glutamate is actively transported across the cell membrane of cells within the islets of Langerhans.

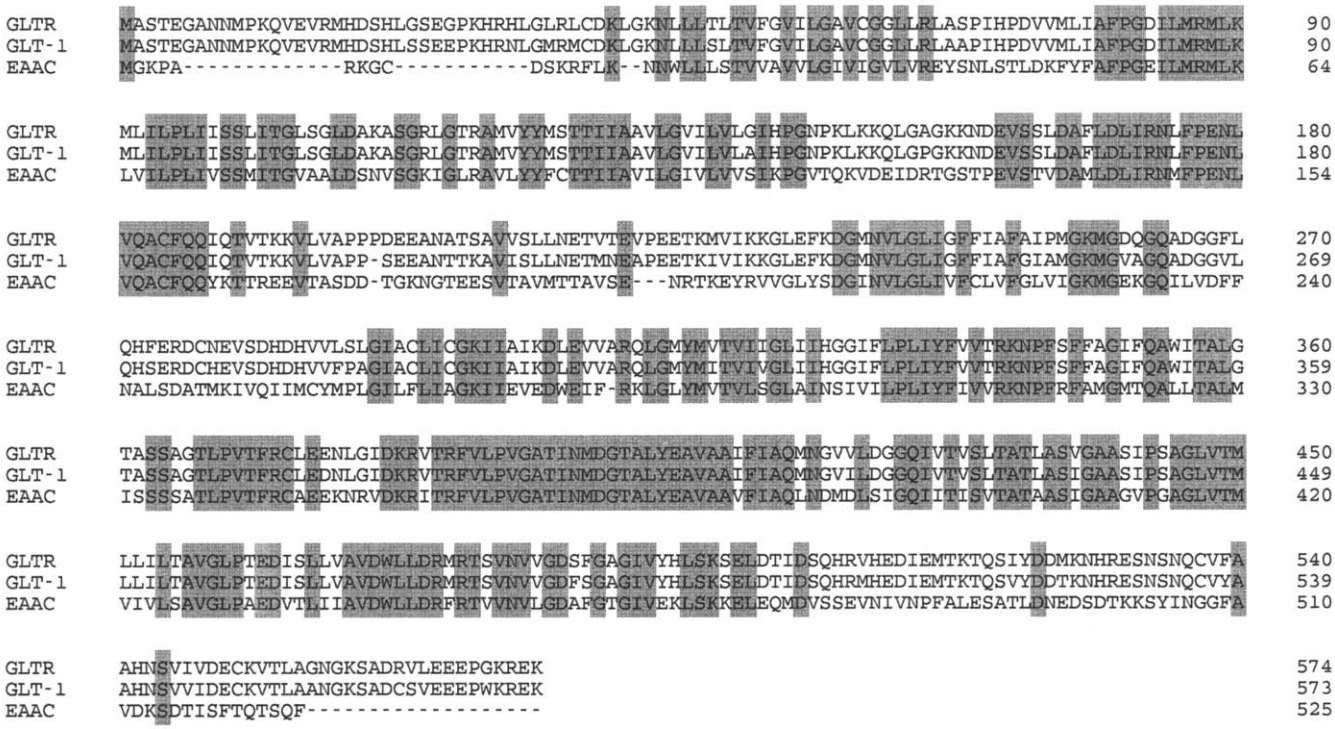

Fig. 3. Multiple sequence alignment of the human glutamate transporter (GLTR) with the homologous rat (GLT-1) and rabbit (EAAC) glutamate transporters. Shaded residues are conserved across all three proteins.

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