Characterization of cysteine sulphinate transport by intact rat brain cerebrocortical synaptosome fractions

ANGUS GRIEVE, DENISE CAMERON and ROGER GRIFFITHS
Department of Biochemistry and Microbiology, University of St Andrews, St Andrews, Fife KY16 9AL, Scotland, U.K.

A number of endogenous sulphur-containing amino acids have been shown in electrophysiological studies to possess excitatory properties similar to those exhibited by L-cysteine sulphinate as substrate, with the inherent possibility that the aromatic sulphur-containing amino acids may function as putative excitatory neurotransmitters. The evidence is particularly strong for t-cysteine sulphinate.

Demonstration of the presence of a selective, Na+-dependent high-affinity transport carrier for physiological inactivation of neurotransmitters is regarded as one of several criteria required for fulfilling the requirement of transmitter candidacy [2]. A number of factors have contributed to the current re-evaluation of the transport characteristics of t-cysteine sulphinate, namely (a) previous transport kinetics studies were undertaken using radiolabelled L-cysteine sulphinate in nerve endings, as recently demonstrated [1]; (b) the substrate specificity of the cysteine sulphinate transporter has not been fully defined; and (c) the possible existence of a Cl--dependent transporter for t-cysteine sulphinate in nerve endings, as recently demonstrated [6]. These factors have been investigated using synaptosome fractions, prepared from the cerebral cortices of 220 male Wistar rats, by discontinuous Ficoll-density-gradient centrifugation as described by Nicholls [6]. The synaptosomes recovered from the gradient were maintained as a pellet at 0°C before resuspension (within 3 h) for use in uptake studies. For transport kinetic studies, synaptosomal pellets were resuspended to 8 mg of protein/ml of incubation medium [25 mM-NaCl, 3.5 mM-KCl, 1.2 mM-MgSO₄, 5 mM-NaHCO₃, 0.4 mM-KH₂PO₄, 1.3 mM-CaCl₂, 20 mM-Tris (sodium salt) and 10 mM-p-nitrophenol, pH 7.4]. Aliquots were preincubated for 5 min at 35°C before initiation of assays undertaken in the presence of varying concentrations (5-5000 μM) of t-cysteine sulphinate. For substrate specificity assays, synaptosomal fractions were incubated with a fixed concentration (200 μM) of each of a variety of potential transport inhibitors in the presence of either 2 μM-D-[3H]aspartate (0.3 μCi/ml of assay mixture), 2 μM-[3H]glutamate (0.5 μCi/ml of assay mixture), 3 μM-γ-[3H]aminobutyric acid (GABA) (1.0 μCi/ml of assay mixture), 11 μM-[3H]taurine (2.5 μCi/ml of assay mixture) or 50 μM-t-cysteine sulphinate (non-radiolabelled) as transport substrates. The extent of inhibition of t-cysteine sulphinate uptake by one inhibitor amino acid was assessed against the extent of inhibition of each of the remaining transport substrates by the same inhibitor. In determining the ion specificity and Cl--dependency of transport, the composition of the incubation medium was altered appropriately but always maintaining equal ionic strength in the individual solutions.

This report describes the use of an h.p.l.c. method [7] to measure net uptake of t-cysteine sulphinate (see Fig. 1). Uptake of [3H]-labelled neurotransmitters was undertaken as described elsewhere [8]. The net intrasynaptosomal accumulation of t-cysteine sulphinate as a function of the external t-cysteine sulphinate concentration exhibited a monophasic dependence when corrected for non-saturable diffusion. Quantification of the kinetic parameters of transport revealed the presence of a single, high-affinity (Kₘ = 36.7 ± 6.17 μM), relatively high capacity (Vₘₐₓ = 1.16 ± 0.13 nmol/min per mg) transport system. This is in contradiction to the results obtained with other brain preparations (e.g. slices) or crude synaptosomal fractions using radiolabelled t-cysteine sulphinate [9, 10] wherein both high- and low-affinity systems appear to be demonstrable.

The inhibition profile of t-cysteine sulphinate transport by a number of L-enantiomers of acidic excitatory amino acids exhibited a significant positive correlation (r = 0.95) when compared with the inhibition of high-affinity D-[3H]aspartate or L-[3H]glutamate transport by the same inhibitors, but a negative correlation when assessed against inhibition of either [3H]GABA or [3H]taurine transport. These observations indicate that t-cysteine sulphinate may share a common transporter with the dicarboxylic excitatory amino acids. At a fixed concentration of 50 μM-t-cysteine


References


Abbreviation used: GABA, γ-aminobutyric acid.
The functional significance of Cl- dependent transport of cysteine sulphinate uptake. These observations are also consistent with the presence of a Cl- dependent cysteine sulphinate transporter in synaptosomal plasma membranes; although further work is required to show this unequivocally.

There was a reduction in this value when other anions (e.g. CH3COO-) replaced Cl- in the incubation medium and a highly marked reduction in this value when Na+ was replaced by Li+.

The effect of Cl- on t-cysteine sulphinate transport was studied in the absence of Na+ in the incubation medium. Addition of ammonium chloride (0.25 mM) resulted in only a slight dose-dependent accumulation of t-cysteine sulphinate within synaptosomes (1.9-fold background fluorescence at t-cysteine sulphinate retention time).

While confirming the Na+ dependency of high-affinity t-cysteine sulphinate uptake, these observations are also consistent with the presence of a Cl- dependent t-cysteine sulphinate transporter in synaptosomal plasma membranes; although further work is required to show this unequivocally. The functional significance of Cl- dependent transport systems for the excitatory amino acids remains unclear.

This work is supported by the Wellcome Trust and the Scottish Home and Health Department.

Increased levels of a 230 kDa synaptic antigen after long-term potentiation

ANDREW SCHOLEY*, SARAH BULLOCK* and BERND LÖSSNER*
*Brain and Behaviour Research Group, Open University, Milton Keynes MK7 6AA, U.K. and Institute of Pharmacology and Toxicology Magdeburg, Magdeburg, G.D.R.

Long-term potentiation (LTP) refers to an enduring increase in the synaptic efficacy of specific brain nuclei after tetanic stimulation of their inputs [1]. The multiphasic character of hippocampal LTP [2], its long duration (days to weeks) [3], and the importance of the hippocampus in many forms of learning [4] have all lead to LTP being regarded as an important model in the study of the mechanisms of synaptic plasticity underlying learning and memory.

Previous work in our laboratories has found a post-tetanic increase in the titre of the monoclonal antibody 411B in the target, CA1/dentate area, of rat hippocampus [5]. 411B recognizes a 180 kDa antigen which is highly enriched in postsynaptic densities. In the study reported here, we

Received 22 November 1989