Alanine transport, metabolism and cycling in the brain.

C. Rae[1], J. T. Hansen[1], W. A. Bubb[1], S. Bröer[2], A. Bröer[2]

[1]Biochemistry, The University of Sydney, Sydney, NSW, Australia, [2]Biochemistry, The Australian National University, Canberra, ACT, Australia

Introduction As classically written, the glutamate/glutamine cycle results in net transfer of ammonia from astrocytes to neurons. It is apparent that this ammonia must be returned to the astrocyte and a number of carrier molecules have been proposed, including leucine [1] and alanine [2-5]. Alanine is known to be the substrate of a number of amino acid transporters including system A, ASC and L [6] although the role played by these transporters in alanine transport *in vivo* remains unknown. In this work, we wished to identify the main transporter responsible for alanine uptake and release in the brain and to determine whether alteration of alanine transport played any role in regulating glutamatergic neurotransmission.

Methods Guinea pig cortical tissue slices were prepared and maintained as in [3]. To determine the contribution of different transporters to alanine cycling slices were incubated with 10 mM [1-^{13}C]D-glucose, under depolarizing (40 mM K^+) conditions (to induce metabolic work and glutamate/glutamine cycling) and with 10 mM His (to block system N and L) or with 10 mM 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH, to block system L). Thirty min later the experiment was stopped by extraction in 6% perchloric acid.

The contribution of exogenous alanine to metabolism was examined by incubating slices with 0.4 mM [1-^{13}C]D-glucose (90 min), 8 mM [3-^{13}C]L-lactate (60 min), 2 mM [3-^{13}C]pyruvate (60 min) or 5 mM [2-^{13}C]acetate with 5 mM D-glucose (90 min) with and without 1 mM L-alanine. The experiment was stopped as above.

The neutralized extracts was lyophilized and resuspended in D_2O containing 2 mM Na[^{13}C]formate. ^{13}C[^{1}H-Decoupled] spectra (14,000-18,000 scans, T_R 4 s) were acquired at 9.4 T, and ^{1}H[^{13}C-decoupled] spectra (64 scans, T_R 30 s) at 14.1 T. After appropriate adjustment for saturation and nOe, net flux of ^{13}C into various isotopomers and total metabolite pool sizes were calculated as described previously [3].

Results Addition of BCH resulted in significant decreases in the net flux of ^{13}C into Ala C3 (Ctl 0.41 (0.10); His 0.31 (0.03); BCH 0.22 (0.03), μmol/100 mg protein) but had no effect on flux into any other isotopomer. Addition of His produced results similar to those seen previously [3] consistent with blockage of systems N and L. Addition of exogenous alanine to slices incubated with either hypoglycemic concentrations of glucose or acetate and glucose together had no effect on net flux of ^{13}C into any isotopomer measured. By contrast, alanine produced significant labeling alterations when added to [3-^{13}C]L-lactate (significant increases in net flux into Glu C2, C3 and C4, GABA C2 and Asp C3, with decreased pool sizes of Gln) or [3-^{13}C]pyruvate (significant decreases in flux into Glu C2, C4, Lac C3, Asp C2 and C3, with decreased pool sizes of Glu and Gln).

Discussion Addition of BCH to slices resulted in inhibition of flux into Ala C3 consistent with blockage of system L isoform, LAT2, suggesting that LAT2 is the transporter responsible for alanine transport as part of glutamate/glutamine cycling. The increase in net flux into Glu C2, C3 and C4, GABA C2 and Asp C3 when 1 mM exogenous alanine was added in the presence of 8 mM [3-^{13}C]lactate can be explained by *trans*-stimulation of the monocarboxylate transporter [7]. A fraction of the added alanine taken up by cells would have been converted to pyruvate via alanine transaminase and this pyruvate would exchange with the exogenous [3-^{13}C]lactate (or compete with [3-^{13}C]pyruvate where this was used as substrate) causing increased net uptake of ^{13}C label and hence increased net flux. However, given that metabolite pool sizes of glutamate, lactate, GABA and aspartate were not altered, it is likely that this stimulation of uptake was not reflected in actual stimulation of metabolism. The increased incorporation of ^{13}C label in this case likely represents simple exchange. It would therefore appear that exogenous alanine does not provide a serious contribution to energy metabolism, but takes part in ammonia homeostasis, via the LAT2 transporter.

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