Glial glutamine release and neuronal reuptake by SAT1 studied in vivo by N-15 NMR and microdialysis

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Introduction & Aim: The in vivo rates of glial glutamine (GLN) release to the extracellular fluid (ECF) and of its uptake into neuron, steps integral to the glutamate neurotransmitter cycle (Fig.1), are unknown Recent in vitro studies suggest that, in addition to SAT1, SAT2 (1) and ASCT2 (2,3) may contribute to neuronal GLN_{ECF} uptake. This study measures the rate of SN1-mediated glial GLN release in vivo in mildly hyperammonemic rat brain, and examines possible contributions of SAT2 and ASCT2 to neuronal GLN_{ECF} uptake, by analyses of the time courses of GLN_{ECF} and its ^{15}N enrichment in the absence and presence of transport inhibitors.

Methods: Rats were given i.v. $^{15}NH_4Ac$ infusion (2.3 mmol/h/g) to achieve steady-state brain [^{14}N+^{15}N]GLN conc. (8.5 ± 0.5 micromol/g). The time-course of intracellular [5-^{15}N]GLN was measured in vivo by NMR at 4.7 T (4). The time-course of extracellular GLN, collected by microdialysis, was analyzed by HPLC ([^{14}N + ^{15}N]GLN) or by gradient heteronuclear single-quantum correlation (gHSQC) NMR ([5-^{15}N]GLN). MeAIB (αmethylamino-isobutyrate; a SAT inhibitor in vivo (5) and D-threonine, an ASCT2- specific competitive inhibitor of GLN uptake (6) were perfused through the dialysis probe.

Results & Discussion: Fig. 2 shows that intracellular [^{14}N+^{15}N]GLN reaches steady-state after 2.5 h of $^{15}NH_4Ac$ infusion. Fig. 3 shows the time-course of (A) extracellular [^{14}N + ^{15}N]GLN, (B) extracellular [5-^{15}N]GLN (HSQC spectrum in the inset) and (C) the ^{15}N-enrichment of GLN_{ECF}. After t = 2.5 h, GLN_{ECF} continues to increase up to 4.9 h (Fig.3A). Hence, the rate of glial GLN release is faster than the rate of neuronal GLN_{ECF} uptake during this period. At $t \geq 4.9$ h, GLN_{ECF} levels off. Possible causes are (a) partial suppression of SN1-mediated glial GLN release (7), or (b) continued release combined with initiation of GLN_{ECF} uptake by the low-affinity SAT2 (K_m = 1.65 mM in vitro). Because the ^{15}N enrichment of GLN_{ECF} (which is expected to continue increasing in the latter case) levels off after 4.1 h (Fig. 3C), the latter possibility is unlikely. The most reasonable explanation for the plateau in [GLN_{ECF}] at $t > 4.9$ h is partial suppression of GLN release.

Accordingly, the rate of glial GLN release without partial suppression was measured during t = 2.4 – 4.1 h when glial [GLN] was at steady state. Fig. 4 shows the time-course of increase in GLN_{ECF} when its uptake into neuron mediated by SAT, was inhibited by MeAIB perfusion (duration shown by T) starting at t = 2.5 h of NH_4Ac infusion. In parallel experiments, MeAIB in ECF was found to reach maximum conc. at T = 15 min. During T = 15 - 27 min, GLN_{ECF} increased linearly at the rate of 0.058 mM/min, corresponding to 2.8 micromol/g/h. This represents a reasonable estimate for the minimum rate of glial GLN release into ECF. Our previous study (4) showed that, under identical experimental condition, the rate of glutamine synthesis in vivo was 3.3 ± 0.3 micromol/g/h. At steady-state, this is equal to the rate of glial glutamine release to ECF. Comparison of the two rates strongly suggests that at least 85% (= [2.8/3.3] x 100%) of neuronal GLN_{ECF} uptake is mediated by SAT. Possible contribution of ASCT2 to neuronal uptake of GLN_{ECF} was examined by the effect of D-threonine perfusion; no significant change in GLN_{ECF} was observed. The result is consistent with the dominant role of SAT1 in GLN_{ECF} uptake into neuron in vivo.

Conclusions: 1) The minimum rate of SN1-mediated glial GLN release to ECF in vivo is 2.8 micromol/g/h at steady-state brain GLN concentration of 8.5 micromol/g. 2) Transporter SAT1 accounts for at least 85% of neuronal uptake of GLN_{ECF} (3) Microdialysis, combined with ^{15}N NMR, contributes to a clearer understanding of the glutamate-glutamine cycle in vivo.

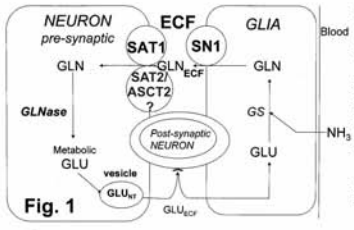

Fig. 1

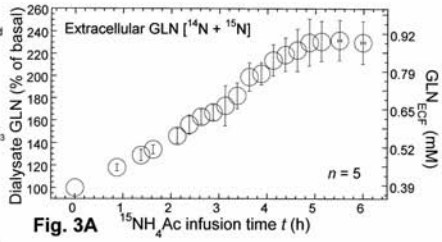

Fig. 3A

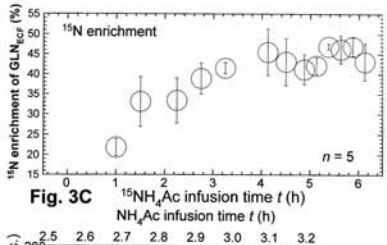

Fig. 3C

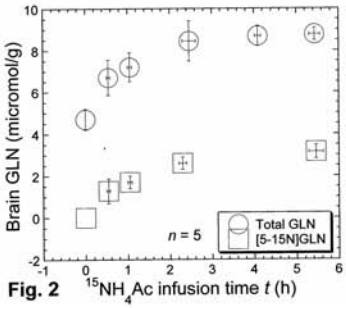

Fig. 2

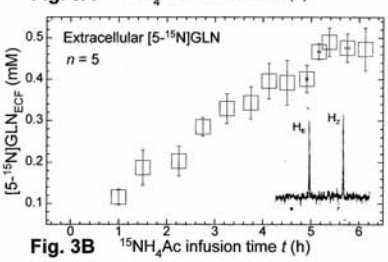

Fig. 3B

Inset: The amide protons of [5-^{15}N]GLN selectively detected by gradient ^{1}H-^{15}N HSQC

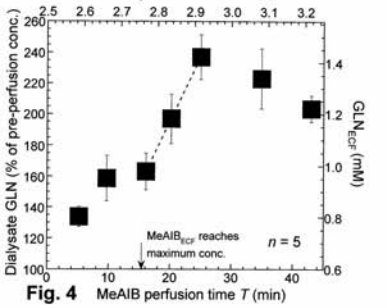

Fig. 4

References:
1) Yao *et al.* (2000) *JBC* **275**, 22790-97. 2) Dolinsky *et al* (2004) *Neurochem. Int.* **44**, 75-81. 3) Su *et al.* (1997) *Brain Res.* **757**, 69-78. 4) Kanamori *et al.* (1995) *Biochem. J.* **311**. 681-688. 5) Kanamori & Ross (2004) *J. Neurochem.* **90**, 203-210. 6) Utsunomiya-Tate *et al* (1996) *JBC* **271**. 14883-90. 7) Kanamori & Ross (2005) *J. Neurochem.* **94**. 74-85.

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