Suppression of glial glutamine release to the ECF studied in vivo by N-15 NMR and microdialysis/HSQC

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Introduction: In the glutamate/glutamine (GLU/GLN) cycle, the transport of GLN from glia to neuron had been the missing link. Recently, GLN transporters, SAT and SN1 (Fig. 1), have been identified and their kinetic properties studied in vitro (1). Their transport properties in vivo remain unexplored. In vitro, SN1 mediates co-transport of GLN with Na^+ in exchange for H^+, with a stoichiometry of $[GLN]_i/[GLN]_0 = ([Na^+]_o/[Na^+]_i \times ([H^+]_i/[H^+]_o)$. The direction of transport (uptake or release) depends on the ratio (i/o:inside/outside) of these species. The effects of changes in $[Na^+]$ and pH have been studied in vitro, but the effect of a change in $[GLN]_i/[GLN]_o$ ratio on GLN release has not been examined in vitro or in vivo.

Aim: This study examines how a change in $[GLN]_i/[GLN]_o$ ratio affects GLN release from glia to the extracellular fluid (ECF) at physiological concentrations of $[Na^+]$ and $[H^+]$ in the intact brain of hyperammonemic rats.

Methods: Rats were given i.v. $^{14}NH_4Ac$ infusion (5.9 mmol/h/kg wt). At t = 3 h, the infusate was changed to $^{15}NH_4Ac$ to ^{15}N enrich brain GLN. Increase in intracellular [5-^{15}N]GLN was monitored by ^{15}N NMR (2). Extracellular GLN was collected by microdialysis. Dialysate [GLN] was converted to unperturbed [GLN] in ECF far from the dialysis probe, $[GLN]_{ECF}$, as described in our study (3). [5-^{15}N]GLN in ECF (in 15-min fractions containing 5-15 nmol) was measured by selective observation of its amide protons by gradient heteronuclear single-quantum correlation (gHSQC) NMR at 600 MHz.

Results: Fig.2A shows a gradual increase in $[GLN]_{ECF}$ during t = 0 – 4 h, a significant elevation at 4.5 ± 0.1h, followed by a decrease at 5 h (n = 5). The rapid elevation may be due to accumulation of GLN_{ECF} as a result of saturation of neuronal uptake by SAT (K_m = 0.49 mM). At t = 4.5 h, $[GLN]_i$ = 25 mM (2) and $[GLN]_o = [GLN]_{ECF}$ = 2.2 mM (Fig. 2A). Hence, $[GLN]_i/[GLN]_{ECF}$ ratio is ~ 11.4, a significant decrease from the ratio of ~20 (8 mM/0.385 mM) in the normal brain (3). Fig. 2B shows the time-course of $[GLN]_{ECF}$ at 15-min intervals and extended to t = 6 h (n = 5). The maximum elevation at t = 4.6 h followed by a significant decrease at 4.8 h is consistent with the result in Fig. 2A. Fig. 3 shows a typical ^{1}H gHSQC spectrum of the ECF. The amide protons, H_Z and H_E, of [5-^{15}N]GLN were selectively observed, and quantified. Fig. 4 compares the time-courses of (A) intracellular [5-^{15}N]GLN and (B) extracellular [5-^{15}N]GLN (n = 5) during T = 0.5 – 3.0 h of $^{15}NH_4Ac$ infusion (t = 3.5 – 6.0 h of total NH_4Ac infusion). The intracellular [5-^{15}N]GLN (~80% of which is in glia) increased progressively. By contrast, [5-^{15}N]GLN in ECF leveled off after T = 1.9 h. The result strongly suggests that release of [5-^{15}N]GLN from glia to ECF is partially suppressed in vivo when $[GLN]_i/[GLN]_{ECF}$ ratio falls to ~11. Similar perturbation of the ratio may occur in hepatic encephalopathy patients with elevated GLN in the brain and CSF. The suppression of release can prevent prolonged GLN efflux to ECF when neuronal uptake of GLN_{ECF} for recycling to GLU is saturated.

Conclusions: We show, for the first time, that (1) combination of ^{15}N NMR to monitor glial intracellular [5-^{15}N]GLN and microdialysis/^{1}H-^{15}N HSQC NMR to monitor [5-^{15}N]GLN_{ECF} is a novel and powerful method for studying the in situ operation of the GLN transporter; (2) release of GLN from glia to ECF by the bi-directional transporter SN1 is partially suppressed in vivo when elevation of $[GLN]_{ECF}$ causes a significant decrease in $[GLN]_i/[GLN]_{ECF}$ ratio. This provides a potential additional regulatory site for the GLN/GLU neurotransmitter cycle.

References: (1) Broer & Brookes (2001) J. *Neurochem.* **77**, 705-719. (2) Kanamori, Ross, Chung & Kuo (1996) *J. Neurochem.* **70**, 1304-1315. (3) Kanamori & Ross (2004) *J. Neurochem.* **90**, 203-210. **Acknowledgments:** (1) Dr. Scott Ross, manager of NMR facility at California Institute of Technology for his expertise on gHSQC. (2) funding from HMRI and Rudi Schulte Research Institute.

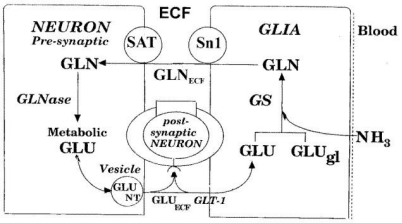


Fig.1. Sn1 in GLN transport

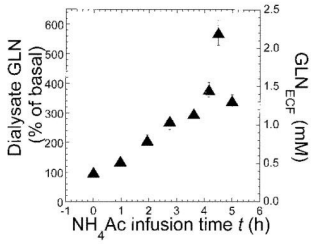
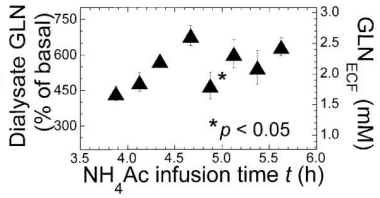


Fig. 2A GLN_{ECF}

Fig. 2B. GLN_{ECF} (3.5 - 6 h)



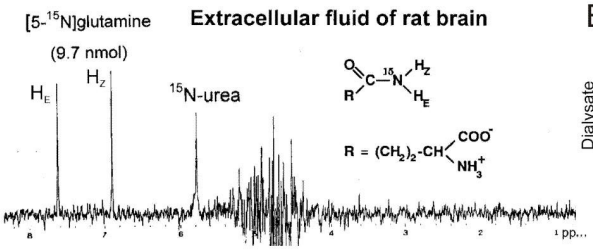


Fig. 3. ^{1}H gHSQC spectrum of ECF

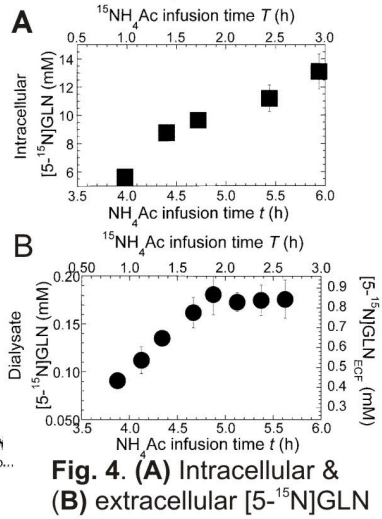


Fig. 4. (A) Intracellular & **(B)** extracellular [5-^{15}N]GLN