## Closing the GATs. Metabolic consequences of blocking GABA uptake

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Reuptake of the inhibitory neurotransmitter GABA is largely accomplished via high affinity GABA uptake systems (GATs). The majority of uptake in the brain is *via* GAT1, with some contribution from GAT3. These transporters are located on both neurons and glia, although it is likely that GAT1 is found on neurons and glia, while GAT3 is mostly found on glial processes.

The contribution of GABA uptake to energy metabolism has been described as "minimal" and hence it has been speculated that GABA uptake is unlikely to make any meaningful contribution to metabolism. In order to discover what the metabolic consequences of blockade of GABA uptake actually were, we employed a Guinea pig cortical slice preparation, coupled with targeted neuropharmacology, $^{13}C$ NMR spectroscopy and multivariate statistics to determine the effect of inhibition of GATs.

In brief, slices (350 μM) were prepared from the Guinea pig cortex (excised from newly sacrificed animal) in the para-sagittal plane using a McIlwain tissue chopper. Slices were incubated in a modified, gassed (95% $O_2$, 5% $CO_2$) Krebs-Henseleit buffer with 10 mM glucose to allow metabolic recovery for 1 h, then washed and immediately incubated with [3-$^{13}C$]sodium pyruvate (control) and two different concentrations of the GAT inhibitor of choice. We used the broad spectrum inhibitor guvacine, the GAT1-specific inhibitors SKF899776a, tiagabine, NNC711 and CI-966, as well as the GAT3 specific inhibitor (*S*)-SNAP 5114. These were typically used at concentrations around the Ki, as well as 10 x this concentration (to allow for non-specific effects). Following 1h of incubation the experiment was stopped by rapid vacuum filtration and the slices extracted using chloroform/methanol, lyophilized and resuspended in $^2H_2O$ for $^1H/^{13}C$ NMR analysis at 14.1 T. The net flux into isotopomers of Glu, Gln, GABA, Asp, Ala and lactate was determined as well as the total metabolite pool size of lactate, Glu, GABA, Asp, Ala and Gln.

The broad spectrum inhibitor guvacine produced a metabolic profile that resembled that of the GABA-B agonist Baclofen, suggesting that the increased GABA concentration on GAT1/GAT3 inhibition is active at GABA-B receptors. The centrally active GAT1 inhibitor tiagabine produced a profile that was most similar to that of SKF899776a, although the two ligands could still be distinguished from one another using principal components analysis (PCA). CI-966 produced a unique metabolic profile, the nearest match to which from our library was that of the type II mGluR antagonist APICA, suggesting that CI-966 may act more on astrocytic GATs and/or interact with astrocytic mGluR. The GAT3 inhibitor SNAP 5114 was distinguishable from the other GAT ligands using PCA. The metabolic profiles generated by the inhibitors did not closely resemble that of any GABA-C agonist or that of any GABA-A agonist that we have studied to date. The response is the opposite of that seen at GABA-C suggesting that GATs and GABA-C receptors are not closely localized. Our ability to interpret the response to GABA-A ligands is hampered by lack of specific agonists for this receptor.

Taken together, these data suggest that GABA which builds up in the synapse is most likely to act upon GABA-B receptors, that the individual GAT inhibitors may well be acting on different subpopulations of GATs, that GAT inhibition produces a negative (decreased) impact on brain metabolism and that it is possible to distinguish between activity of the various ligands using this approach.