Understanding your inhibitions. A functional metabolomic approach to the GABAergic system

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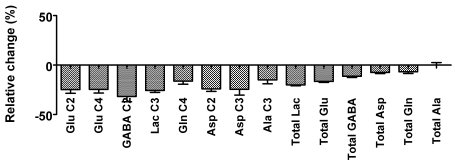
Introduction

The GABAergic system is complex; GABA may act at multiple subtypes of ionotropic and metabotropic GABA receptors, at GABA transporters and it may be metabolized in distinct metabolic pools of GABA. Discriminating this plethora of activities is problematic. Here, we describe a novel approach where the system is perturbed by specific GABA receptor ligands with known (or partially known) properties, the response of the system as a whole is evaluated and inferences made based on an objective overview of multiple effects of such perturbations.

Methods

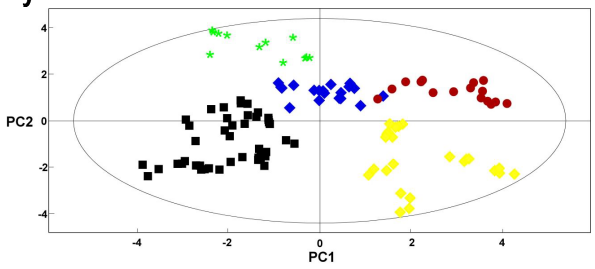
We used a Guinea pig cortical tissue slice model, incubating the slices with [3-^{13}C]pyruvate (control) and one of at least two concentrations of a range of ligands active at GABAergic sites. After one hour, metabolic activity was stopped and the slices extracted for analysis by {^{13}C-decoupled}^{1}H and {^{1}H-decoupled}^{13}C NMR spectroscopy (600 MHz, Bruker AVANCE system). A metabolic fingerprint unique for each ligand was generated consisting of 14 variables comprising both net flux and pool size information (e.g. Fig. 1, 1.0 μM Baclofen). These fingerprints were then subjected to principal components analysis and analysed for clustering based on similarity in metabolic outcomes, using SIMCA P+ (V 11.5).

Fig. 1. Example "fingerprint" Shows metabolic changes relative to the control expt.



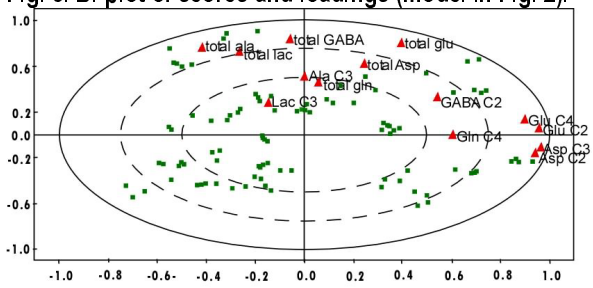
Results and Discussion

Fig. 2. Five group "footprint" of the GABAergic system.



The data were determined to cluster into five separate groups which corresponded not to receptor pharmacology but clustered around profiles generated by concentrations of exogenous GABA (Fig. 2). Black squares are patterns typifying mainstream GABAergic synaptic activity, responding to ~10 μM GABA, green stars α5-containing receptors (4.0 μM GABA), red circles extrasynaptic high affinity receptors (0.1 – 1.0 μM GABA), blue diamonds peri-synaptic activity responding to synaptic spillover (40 μM GABA) and yellow diamonds correspond to an as yet unclassified group related to the $GABA_C$ receptor. These groups are statistically meaningful ($P < 0.05$) [1]. This pattern approach can also be used to gauge the effect of activity on individual metabolites. The data can be plotted using a Bi plot showing the scores and the loadings (e.g. Fig. 3). This plot shows, for example, that the metabolic pool of GABA is orthogonal to principal component 1 (has minimal loading on PC1). PC1 is loaded most heavily by net flux into Krebs cycle intermediates, and represents metabolic work. This suggests that the metabolic pool of GABA (as opposed to net flux of 13C into GABA C2, is independent of the amount of metabolic work undertaken, as measured in pools accessible to label from [3-^{13}C]pyruvate.

Fig. 3. Bi-plot of scores and loadings (model in Fig. 2).



References:

[1] Rae C, Nasrallah, FA, Griffin JL & Balcar VJ. Now I know my ABC. A systems neurochemistry and functional metabolomic approach to understanding the GABAergic system. Journal of Neurochemistry (In press)