Dynamic measurement of cerebral perfusion using CASL: a tool for assessment of pharmacologic activity in the brain

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In recent years, modulation of the co-agonist glycine site of the NMDA receptor has gained recognition as a promising therapeutic target to enhance glutametergic and dopaminergic system function with no or limited excito-toxic side effects. Compounds that inhibit glycine uptake via blockade of glial and neuronal transporter GlyT1 have been shown to increase extracellular glycine levels in rodents, and have shown clinical efficacy in ameliorating schizophrenic symptoms(1). Several GlyT1 compounds are currently under development. The pharmacodynamic effects of novel therapeutics that work through indirect mechanisms, such as inhibition of GlyT1, are difficult to assess in vivo, requiring invasive techniques and/or terminal studies with large numbers of animals. A non-invasive method providing a physiologic biomarker for the pharmacodynamic activity of such compounds would be of great value for proof of concept, dose selection, etc. Here we explore the use of arterial spin labeling (ASL) to assess dynamic cerebral perfusion following administration of compounds that modulate the NMDA receptor function.

Methods: MR scanning was conducted on a Bruker 4.7T 40cm bore Biospec system. Sprague Dawley rats, 275-325g, were instrumented with venous and arterial catheters and mechanically ventilated. Body temperature and arterial pCO_2 were controlled and anesthesia was maintained with an alpha-chloralose infusion. Rats were placed in a 72mm Bruker transmit coil with a receive surface coil over the brain. Test groups were dosed with 50mpk iv D-serine (n=3), low dose (n=3) or high dose (n=3) GlyT1 inhibitor (GlyTi), 20 mpk iv of the glycine site antagonist L-701324 + low dose of GlyTi (n=3), 20 mpk iv L-701324 + high dose of GlyTi (n=3), 50 mpk iv D-serine + 20 mpk iv L-701324 (n=3), or vehicle (n=3). A 5 sec continuous labeling period, using adiabatic fast passage (2), located 2 cm from the detection plane was followed by a fast 2D gradient echo image (TR/TE 4.2 ms/3.1ms, matrix = 128x70, FOV=4 cm, SLTH=2 mm) from a coronal section centered on the forebrainInterleaved labeled and control images provided dynamic perfusion images at 1.5 minute intervals (NA=8). Perfusion was calculated over a cerebral ROI from intensities of labeled (M_L) and control (M_C) image pairs according to $f=(\lambda/2\alpha T_{1app})(M_C-M_L)/2M_C$, where λ= 0.9ml/g, T_{1obs} = of 1.6s were assumed.

Results: Figure 1 shows dynamic CBF measurements during administration of various agents. Administration of the specific NMDA glycine site agonist D-serine increased cerebral perfusion compared to baseline perfusion and vehicle controls (not shown). Blockade of the glycine site with the blocking agent L-701324 reduced this perfusion response. In contrast, administration of two doses of the GlyT1 inhibitor produced a robust decrease in cerebral perfusion compared to baseline perfusion and vehicle controls. Prior blockade of the glycine site with L-701324 attenuated this decrease. A structurally different GlyT1 inhibitor also produced a robust decrease in perfusion (data not shown) supporting that the CBF increase is connected with NMDA activity and not due to a systemic effect. Arterial CO_2 remained constant for all animals during the course of scanning. Blood pressure was not measured during scanning, but a separate bench experiments showed no increase in mean blood pressure after administration of the GlyTi compound under the same anesthetic regimen (data not shown).

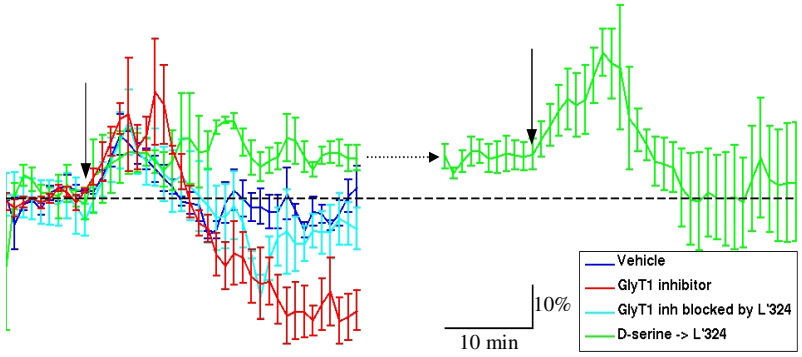

Figure 1 Temporal evolution of blood flow following administration of D-Serine and a GlyT1 inhibitor, with and without blockade of the NMDA glycine site via the known antagonist L-701324. Scale bars represent 10 min and 10% change in CBF with respect to baseline (dotted line). Solid arrows denote injections.

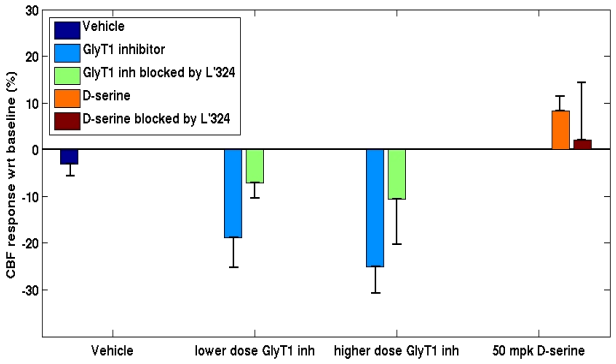

Figure 2 shows the magnitude of the change measured from the average blood flow during 10 minutes prior to injection and during the 10 minute period 20 min after injection of the agent.

Conclusion: We demonstrate increased cerebral perfusion following administration of the glycine site co-agonist D-serine, and decreased perfusion following GlyT1 inhibitor administration. Although not statistically significant, the data trends toward a dose-related effect on blood flow. Blockade of the NMDA glycine site with the antagonist L-701324 attenuated both the D-serine and GlyT1 inhibitor response, lending support to the idea that the changes in perfusion are related to NMDA activity.

To our knowledge, changes in cerebral blood flow have not previously been used to assess responses to pharmacologic challenges with GlyT1 inhibitors in the brain. However, using iron oxide contrast agent, Panizutti et al showed increased cerebral blood volume in the hippocampus and caudate putamen following D-serine administration, and subsequent reduction of the response after blockade with L-701324(3). While this group reported a more region-specific response at pre and post time points, their findings are consistent with our dynamic global perfusion measurements with D-serine before and after blockade. Given the positive response in perfusion elicited by D-serine, it is surprising that inhibition of GlyT1 produced a decrease in perfusion. Nevertheless, the NMDA receptor component of this response was demonstrated by the prior administration of the glycine site blocking agent L-701324. In addition, a similar perfusion decrease occurred after administration of a structurally different GlyT1 inhibitor, confirming this response is a result of GlyT1 inhibition and not off-target activity. Further investigation with other modulators of the NMDA receptor and GlyT1 transporter may provide insight as to why inhibition of this transporter results in decreased, rather than increased cerebral perfusion.

While further work will be needed to more fully characterize the effects of D-serine and the GlyT1 inhibitor on cerebral blood flow, these data suggest that ASL shows promise as a tool for non-invasive in vivo demonstration of target engagement, dose selection, and time-course studies that can be translated into the clinic. In conclusion, changes in cerebral perfusion, measured in vivo with non-invasive ASL, can provide a physiologic biomarker for assessment of the pharmacodynamic effects of novel psychoactive compounds.

References: 1. Lechner, SM, Cur Opin Pharm, Vol 6, pp 75-81, 2006.; 2. Williams,D et al., Radiology, 190(3), pp 813-18, 1994.; 3. Panizzutti, et al., Neuroscience Letters, Vol 380, pp111-115, 2005.