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Methionine sulfoximine (MSO) inhibits glutamine synthetase and in sufficient doses causes seizures. We hypothesized that it would be possible to modulate the effects of MSO on cerebral metabolism by supplementation with exogenous glutamine. We measured the cerebral metabolic rate for glucose (CMR_{glc}) using 2-[6- ^{13}C]deoxyglucose (222-500 mg/kg) and ^{13}C -NMR spectroscopy 1 hour subsequent to an intravenous dose of MSO (100 mg/kg). Supplementing the [1- ^{13}C]glucose in the infusate with 4% (wt/vol) glutamine significantly increased CMR_{glc} ($p=0.0065$, t -test) from $1.202 \pm 0.896 \mu\text{mol/g-min}$ ($n=6$) to $4.230 \pm 0.786 \mu\text{mol/g-min}$ ($n=3$). Plasma glutamine can modulate the cerebral metabolic effects of MSO.

BACKGROUND. Methionine sulfoximine is a known inhibitor of glutamine synthetase (GS) and causes convulsions when given at sufficiently high doses (200 mg/kg). Since inhibition of GS will decrease the conversion of glutamate to glutamine as well as the trapping of ammonia, we hypothesized that at lower doses (100 mg/kg) plasma-borne glutamine might substitute for glutamine that would have been produced endogenously, and thereby reduce the effect of MSO on cerebral metabolism.

METHODS. All procedures were performed with the approval of the Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston. Male, Sprague-Dawley rats (approximately 300 g) were fasted for 24 h, then anesthetized with isoflurane (1-3%), intubated and mechanically ventilated. Femoral artery and tail vein catheters were inserted to monitor arterial blood pressure, pulse, hematocrit and plasma glucose concentration, as well as for infusion of [1- ^{13}C]glucose and/or glutamine. A catheter in the internal carotid artery was used to administer a bolus of 2-[6- ^{13}C]2-deoxyglucose.

Animals received a primed infusion containing [1- ^{13}C]glucose (25% wt/vol), and in half of the animals, glutamine (4% wt/vol, 0.274 M), at a flow rate of 1.62 ml/hr. Ten minutes after the start of the infusion, L-methionine sulfoximine (MSO) (100 mg/kg, Sigma) was given i.v. After another 50 minutes, an intravenous bolus of 2-[6- ^{13}C]deoxyglucose was given (222-500 mg/kg). Plasma samples were taken prior to injection of 2-[6- ^{13}C]deoxyglucose, centrifuged, and the supernatant stored at -80°C for further analysis.

The rats were placed in the 33-cm horizontal bore of a Varian Inova 4.7 T NMR spectrometer. Core temperature was continuously monitored with a rectal thermometer and regulated either by use of a jacket of circulating warm water or by warm air blown through the bore of the magnet. A 16 mm surface coil (Doty Scientific, Columbia, SC) dual tuned to ^1H and ^{13}C was placed on the head, and a vial containing dimethyl sulfoxide (DMSO) for use as an external standard rested on top of the coil. Scout images were taken to verify the position of the coil. Direct detection of ^{13}C was employed, with Waltz decoupling, acquisition time of 219 msec, recycle delay of 1.4 sec and spectral width of 13000 Hz. Transients were collected for periods of 5 minutes each for 50 minutes, at which time the accumulation of transients every 38.9 seconds began. These spectra were later summed in groups of 5 for data points representing 3.24 minutes of acquisition time. NMR spectra were processed using commercially available software (NMR Utility Transform Software, Acorn NMR, Inc, Livermore, CA) with a line broadening of 20-25 Hz, and a fifth-order polynomial to fit the baseline. Statistical calculations were performed using Minitab (State College, PA). Values are reported as mean $\pm$ SD.

Measurement of CMR_{glc} . During the 9.7 minutes following the bolus of 2-[6- ^{13}C]deoxyglucose, CMR_{glc} was estimated from rate of change $CMR_{2dg}(t)$ of 2-[6- ^{13}C]deoxyglucose-6-phosphate and the concentrations of glucose and 2-[6- ^{13}C]deoxyglucose, using ^{13}C -NMR spectroscopy:

$$CMR_{glc} = CMR_{2dg}(t) * [glucose] / (Kp * [2-[6-^{13}C]deoxyglucose])$$

where Kp is the phosphorylation coefficient for hexokinase with respect to glucose and 2-deoxyglucose and equals 0.22 in rat brain [1, 2, 3]. The ^{13}C signal from the DMSO standard was used to quantify the ^{13}C concentration, after calibration with a known amount of [1- ^{13}C]glucose and 2-[6- ^{13}C]deoxyglucose, and considering the effects of nuclear Overhauser enhancement, partial saturation, and coil loading. The concentration of cerebral glucose was obtained by dividing the concentration of [1- ^{13}C]glucose by the fractional enrichment of plasma, which had been determined in other experiments to be approximately 0.5 for overnight fasted rats.

RESULTS. The CMR_{glc} of rats that had been infused with [1- ^{13}C]glucose alone was $1.202 \pm 0.896 \mu\text{mol/g-min}$ ($n=6$) one hour subsequent to the injection of MSO. Rats whose infusate was supplemented with glutamine had a value of CMR_{glc} equal to $4.230 \pm 0.786 \mu\text{mol/g-min}$ ($n=3$), a statistically significant difference ($p=0.0065$).

DISCUSSION. Methionine sulfoximine (MSO) caused cerebral glucose utilization to increase by almost 100% during hyperglycemia induced by a 1-hour of infusion with [1- ^{13}C]glucose [3]. The presence of plasma glutamine further increased the metabolic rate by more than 200%. Although MSO is known to inhibit glutamine synthetase in the brain, we cannot offer a definitive explanation for the increase in CMR_{glc} afforded by the supply of glutamine. Inhibition of glutamine synthetase would inhibit the glutamate-glutamine substrate cycle between neurons and glia thereby interrupt the rate of chemical neurotransmission as well as oxidative metabolism. It is thought that glutamine is primarily taken up by glia, which contain glutaminase and can produce glutamate and ammonia from glutamine. One may conjecture that glutamate produced from exogenous glutamine can serve as substrate of alanine aminotransferase, converting pyruvate to alanine and glutamate to 2-oxoglutarate. This route would remove the glycolytic product and create a constant source of alanine. Alternatively, glutamate may be converted directly to 2-oxoglutarate by the actions of glutamate dehydrogenase, thereby providing a source of fuel for the glial citric acid cycle.

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