Chronic Unpredictable Stress and Riluzole Treatment Alters Glutamate Metabolism in Rat Prefrontal Cortex

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INTRODUCTION: Glutamate (Glu) and GABA are the major excitatory and inhibitory neurotransmitters in the brain. These amino acid neurotransmitters play vital roles in the regulation of several important CNS processes, and have been linked to the pathogenesis and pathophysiology of stress-related psychiatric disorders. Recent clinical studies suggest riluzole (RIL), an agent believed to modulate glutamatergic neurotransmission by alteration of glutamate release and facilitation of astrocytic uptake, possesses antidepressant and anxiolytic-like effects in patients with depression and anxiety disorders (1). In the present study, we assessed neuronal and glial metabolism from [1-^{13}C]glucose and [2-^{13}C]acetate in CUS treated rats with or without riluzole treatment using ^{1}H-[^{13}C] MRS ex vivo.

METHODS: Four treatment groups of Sprague-Dawley rats were studied: (1) saline-treated, n=10; (2) Riluzole (RIL,4mg/kg/day, i.p), n=10; (3) Chronic unpredictable stress (CUS) plus saline, n=10; (4) Chronic unpredictable stress (CUS) plus riluzole, n=10. Saline and RIL were administered once daily for 35 days. Stressors (CUS) were delivered twice daily for 35 days. The Chronic Unpredictable Stress (CUS) procedure was adapted from ref. (2). One day after the last administration of drug or saline, rats were anesthetized with urethane (1.5g/kg, i.p.) and infused intravenously (via tail vein) with either [1-^{13}C]glucose for 10 min or with [2-^{13}C]acetate for 15 min. The infusions yielded rapid and constant elevations of glucose or acetate concentrations and ^{13}C enrichments in the blood (3). At the designated times the anesthetized rats were euthanized using focused microwave irradiation (10 kW, <0.7s) allowing brain tissue to be removed without postmortem changes. Prefrontal cortex was removed and frozen with liquid N_2 and extracted using ethanol (3). The concentration and ^{13}C enrichment of amino acids in the extract were determined using ^{1}H-[^{13}C] MRS at 11.74T (Bruker Avance).

RESULTS: Cortical Glu, glutamine (Gln), GABA, succinate, alanine, lactate, creatine and NAA levels were similar (*P>0.2)* in control and riluzole-treated rats. ^{13}C enrichments of Glu, GABA and Gln from [1-^{13}C] glucose were greater after chronic riluzole treatment in control *(P<0.05)* and CUS-treated *(P<0.01)* rats, suggesting that neuronal TCA cycle flux and Glu/GABA-Gln cycling is enhanced by riluzole. Chronic stress (CUS) decreased ^{13}C labeling of Glu-C4, Gln-C4 and GABA-C2 from [2-^{13}C]acetate, but not from [1-^{13}C]glucose, suggesting that glial function (and possibly neurotransmitter clearance) was impaired. Unlike the controls where ^{13}C labeling from [2-^{13}C]acetate was unchanged by riluzole, ^{13}C labeling of GABA-C2 was significantly increased (*P<0.05)* in CUS-treated rats receiving riluzole.

CONCLUSIONS: Chronic riluzole treatment appears to increase neuronal TCA cycle flux and neurotransmitter cycling. Drugs which facilitate glutamate uptake, such as riluzole, may attenuate the effects of stress on glial neurotransmitter uptake and cycling, and reverse or attenuate the effects of stress in several rodent behavioral models.

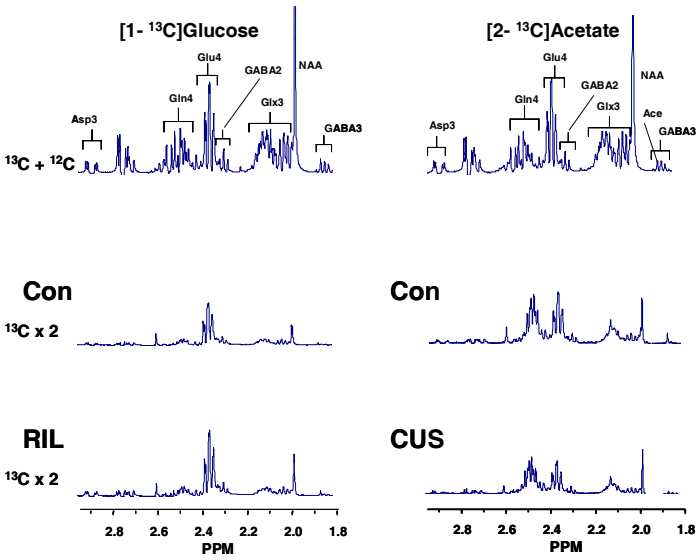

Figure: Representative *ex vivo* ^{1}H-[^{13}C]-NMR spectra of extracts of prefrontal cortex after intravenous infusion of [1,6-$^{13}C_2$]glucose (left column) and [2-^{13}C]acetate (right column). (Top) sum of ^{12}C and ^{13}C; (middle/bottom), ^{13}C labeled resonances only; Comparison of labeling from [1-^{13}C]glucose after RIL (bottom, left) with control (middle, left); Comparison of labeling from [2-^{13}C]acetate after CUS (bottom, right) with control (middle, right). Ace, Acetate-C2; Asp3, aspartate-C3; GABA, γ-aminobutyric acid; Gln4, glutamine-C4; Glu4, glutamate-C4; Glx3, (glutamate+glutamine)-C3.

ACKNOWLEDGEMENTS: This study was supported by NIH grants: MH25642, MH45481.

.**REFERENCES** : 1. Sanacora et al (2004)Am J Psychiatry 161:2132, 2. Ortiz et al (1996) Neuropsychopharmacology 14:443, 19961. 3. Patel et al. (2005) PNAS 102:5588.