

# **<sup>1</sup>H-[<sup>13</sup>C]-NMR spectroscopy measures of chronic stress effects on TCA cycle flux and glutamate-glutamine cycling are attenuated by riluzole or ceftriaxone treatment in awake rat prefrontal cortex**

Golam M.I. Chowdhury<sup>1</sup>, Mounira Banas<sup>2</sup>, Kevin L Behar<sup>1</sup>, and Gerard Sanacora<sup>3</sup>

<sup>1</sup>Department of Psychiatry and Magnetic Resonance Research Center, Yale University School of Medicine, New Haven, Connecticut, United States, <sup>2</sup>Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut, United States, <sup>3</sup>Department of Psychiatry and the Ribicoff Research Facilities, Yale University School of Medicine, New Haven, Connecticut, United States

## **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 (1). Recent clinical studies suggest riluzole (RIL), an agent believed to modulate glutamatergic neurotransmission by alteration of glutamate release and facilitation of astrocytic uptake, and ceftriaxone (CEF), a  $\beta$ -lactam antibiotic known to elevate GLT1 expression, possesses antidepressant and anxiolytic-like effects in rodent models of depression and anxiety disorders (2). <sup>13</sup>C-labeled glucose is metabolized mainly in the neuronal TCA cycle and labels neuronal glutamate and GABA, which are released and taken up by astrocytes, followed by conversion (and labeling) of glutamine. <sup>13</sup>C-labeled acetate is metabolized by astrocytes labeling glutamine, which is released and taken up by neurons for synthesis of glutamate and GABA. Together <sup>13</sup>C-labeled glucose and acetate studies provide information on glutamate and GABA neurotransmitter cycling as well as neuronal and glial cell metabolism, reflecting neurotransmitter activity. In the present study, we assessed neuronal and glial metabolism from [<sup>1</sup>-<sup>13</sup>C]glucose and [2-<sup>13</sup>C]acetate in chronic unpredictable stress (CUS), a rodent model of depression, treated awake rats with or without riluzole or ceftriaxone treatment using <sup>1</sup>H-[<sup>13</sup>C]-MRS *ex vivo*.

## **METHODS:**

Six treatment groups of Sprague-Dawley rats were studied: (a) saline-treated, n=8; (b) Riluzole (RIL, 4mg/kg/day, i.p), n=8; (c) Ceftriaxone (CEF, 200mg/kg/day, i.p), (d) Chronic Unpredictable Stress (CUS) plus saline, n=8; (e) CUS plus riluzole, n=8; (f) CUS plus ceftriaxone, n=8. CUS procedure was adapted from ref. (3). Briefly the CUS animals were subjected to 12 stressors (2 per day for 35 days) and riluzole (4mg/kg) or ceftriaxone (200mg/kg) was administered once daily for the last 21 days. On day 35, sucrose preference was measured. Moreover, on day 36, animals used in the behavioral study described above were euthanized, the prefrontal cortex (PFC) dissected and tissue samples prepared for western blot analysis. For NMR study, one day after the last administration of drug or saline, rats were prepared with tail vein catheters under isoflurane anesthesia. Animals (8 rats/group) were allowed to recover from anesthesia for at least 30 min prior to receiving intravenous (i.v.) infusion of either [<sup>1</sup>-<sup>13</sup>C]glucose (99 atom%; Cambridge Isotopes, Andover, MA, USA) dissolved in water (0.75 mM per 200  $\mu$ g body wt.) for 15 min (4) or sodium [2-<sup>13</sup>C]acetate (99 atom%; Cambridge Isotopes) dissolved in water (2 M, pH 7) for 15 min (3). The infusions yielded rapid and constant elevations of glucose or acetate concentrations and <sup>13</sup>C enrichments in the blood (3). At the designated times rats were euthanized using focused microwave irradiation (5 kW, <1.4s) allowing brain tissue to be removed without postmortem changes. Prefrontal cortex was removed and frozen with liquid N<sub>2</sub> and extracted using ethanol with [2-<sup>13</sup>C]glycine added as a concentration standard (4). The concentration and <sup>13</sup>C enrichment of amino acids in the extract were determined using <sup>1</sup>H-[<sup>13</sup>C] MRS at 11.74T (Bruker AVANCE).

## **RESULTS:**

CUS induced significant reductions of the ratio of sucrose to water consumed (n=7-8/group, P<0.05). Chronic treatment with ceftriaxone or riluzole completely reversed CUS-induced anhedonia (P<0.05). GLT-1 protein levels were significantly decreased in rats subjected to CUS (~18%, n=7-8/group, P<0.05). CUS animals treated with riluzole and ceftriaxone were not significantly different from the saline control groups. Cortical Glu, glutamine (Gln), GABA, succinate, alanine, lactate, creatine and NAA levels were similar ( $P>0.2$ ) in control and riluzole or ceftriaxone-treated rats. Percent <sup>13</sup>C enrichments of Glu-C4 and GABA-C2 from [<sup>1</sup>-<sup>13</sup>C]glucose (Fig. 1A) were significantly lower in rats subjected to CUS (~11%, n=7-8/group, P<0.02) compared to saline rats although Gln-C4 enrichment trended lower but did not reach significance (P<0.07). Interestingly, chronic treatment with ceftriaxone or riluzole completely reversed CUS-induced <sup>13</sup>C enrichment of Glu-C4, Gln-C4 and GABA-C2, suggesting that neuronal TCA cycle flux and Glu/GABA-Gln cycling was enhanced by both riluzole and ceftriaxone. <sup>13</sup>C enrichments of Glu-C4 and Gln-C4 from [2-<sup>13</sup>C]acetate (Fig. 1B) were significantly lower in rats subjected to CUS (~23%, n=7-8/group, P<0.02) compared to saline rats, although GABA-C2 enrichment was not significantly (P<0.09) different. While, chronic treatment with ceftriaxone or riluzole completely reversed CUS-induced <sup>13</sup>C enrichment of Gln-C4, chronic treatment with riluzole but not ceftriaxone completely reversed CUS-induced <sup>13</sup>C enrichments of Glu-C4 and GABA-C2, although there was a tendency for increased enrichment. These data suggesting that glial TCA cycle flux and Glu/GABA-Gln cycling is enhanced by riluzole but not ceftriaxone.

## **CONCLUSIONS:**

Chronic riluzole treatment attenuates both the neuronal and glial TCA cycle flux and neurotransmitter cycling effects of chronic stress. However, chronic ceftriaxone treatment mainly attenuated the neuronal effects with less prominent effects on glial cell metabolism. These data suggest that 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.

## **ACKNOWLEDGEMENTS:**

This study was supported by NIH grants: MH25642, MH45481, NIMH R01 MH081211 (GS).

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