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Age-related increases in basal ganglia glutamate are associated with TNF, reduced motivation and decreased psychomotor speed during IFN-alpha treatment: Preliminary findings

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ABSTRACT

Inflammation-induced alterations in central nervous system (CNS) metabolism have focused on glutamate. At excessive concentrations, glutamate is toxic to glia and neurons, and inflammatory cytokines have been shown to influence glutamate turnover by blocking glutamate reuptake and increasing glutamate release. Increased glutamate has also been found in depression, a disorder associated with increased inflammation. Data by our group have shown increased glutamate as measured by magnetic resonance spectroscopy (MRS) in basal ganglia and dorsal anterior cingulate cortex of patients administered the inflammatory cytokine interferon (IFN)-alpha. Given data that increasing age is associated with an exaggerated CNS inflammatory response, we examined whether older age (>55 years) would be associated with a greater IFN-alpha-induced increase in CNS glutamate. Using a longitudinal design, 31 patients with hepatitis C virus (HCV) underwent MRS, blood sampling for inflammatory markers, and behavioral assessments before (Visit 1) and after 4 weeks (Visit 2) of either IFN-alpha ($n = 17$) or no treatment ($n = 14$). Older patients treated with IFN-alpha exhibited a significantly greater increase in glutamate from Visit 1 to Visit 2 as reflected by the glutamate/creatine ratio (Glu/Cr) in left basal ganglia compared to older controls and younger IFN-alpha-treated and untreated subjects. In addition, increased Glu/Cr in older but not younger IFN-alpha-treated and untreated patients was associated with increased tumor necrosis factor, reduced motivation as measured by the Multidimensional Fatigue Inventory and increased choice movement time on the Cambridge Neuropsychological Test Automated Battery. Taken together, these preliminary data support the notion that older age may interact with inflammation to exaggerate the effects of inflammatory stimuli on CNS glutamate and behavior.

1. Introduction

Patients exposed to the inflammatory cytokine interferon (IFN)-alpha were found to exhibit increased glutamate in left basal ganglia and dorsal anterior cingulate cortex (dACC) as determined by single voxel magnetic resonance spectroscopy (MRS) (Haroon et al., 2014). Increased glutamate was in turn correlated with depressive symptoms. These data are consistent with increased CNS glutamate in bipolar and some unipolar depression (Yuksel and Ongur, 2010), both of which exhibit increased inflammatory markers (Miller et al., 2009).

Regarding mechanisms by which inflammation may increase CNS glutamate, inflammatory cytokines can decrease expression of glutamate transporters on astrocytes, resulting in decreased glutamate reuptake (Tilleux and Hermans, 2007), and stimulate astrocytic glutamate release (Malarkey and Parpura, 2008). Moreover, inflammatory cytokines can activate indoleamine 2,3 dioxygenase (IDO), an enzyme which catabolizes tryptophan into kynurenine that in turn can be converted into quinolinic acid (Miller et al., 2009). Quinolinic acid is an agonist at glutamate receptors and can also stimulate glutamate release while inhibiting astrocytic glutamate reuptake (Miller et al., 2009; Savitz et al., 2014; Tavares et al., 2002). At high concentrations, extracellular glutamate is toxic to both neurons and glia. However, it should be noted that current MRS methodology is limited in discriminating extra- versus

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intra-cellular glutamate, making it difficult to determine whether increases in CNS glutamate as determined by MRS represent increased extracellular glutamate concentrations or increased intracellular pools (Haroon et al., 2014).

One process that may interact with depression and the impact of inflammatory stimuli on CNS glutamate is aging. Advancing age is associated with chronic, low-grade central and peripheral inflammatory responses (Franceschi and Campisi, 2014; Norden and Godbout, 2013). As a consequence of aging, microglia have been shown in laboratory animals to exhibit a "primed" phenotype that is associated with an exaggerated and prolonged inflammatory response that correlates with increased depressive-like behavior and cognitive deficits (Norden and Godbout, 2013; Salminen et al., 2011). In addition, aging is associated with increased inflammation-induced CNS IDO activity in mice (Godbout et al., 2008). Moreover, expression of glutamate transporters appears to decline with age (Sheldon and Robinson, 2007). Finally, as indicated above, CNS glutamate is abnormally regulated in patients with mood disorders including those with late-life depression (Binesh et al., 2004). These data suggest that the intersection of aging, inflammation and depression in older patients may represent a "perfect storm" of glutamate pathology.

We therefore explored effects of aging on the glutamate response to IFN-alpha in patients with hepatitis C virus (HCV) and determined whether these effects were associated with alterations in inflammatory markers and behaviors previously shown to be altered in IFN-alpha-treated patients including tumor necrosis factor (TNF) and its soluble receptor sTNFR2, motivation, and motor activity (Capuron et al., 2012; Majer et al., 2008; Raison et al., 2010).

2. Materials and methods

2.1. Sample

Patients were recruited from the Emory University Division of Digestive Diseases and were serum positive for HCV–RNA. IFN-alpha treatment was determined by patient consultation with their gastroenterologist independent of study participation. Patients were excluded for advanced liver disease or liver disease due to any etiology other than HCV. Other exclusion criteria included unstable cardiovascular, endocrinologic, hematologic, renal or neurologic disease (based on medical history, physical exam and laboratory testing); a lifetime diagnosis of schizophrenia or bipolar disorder or current major depression based on DSM IV criteria determined by Structured Clinical Interview for DSM IV (SCID) (First et al., 2002); and substance abuse or dependence within the past year. Lifetime diagnosis of major depression was not exclusionary; however patients were required to be antidepressant-free for at least 1 year and off all other psychotropic medications for at least 1 month before participation. One patient was included after taking a sedative/hypnotic the night before scanning. Patients were free of contraindications to MRI including metallic implants and claustrophobia. The median age of the sample (55 years) was used to divide IFN-alpha-treated and untreated subjects into "older" (age >55 years) and "younger" (<55 years) subgroups. The study represents a secondary analysis of patients described elsewhere (Haroon et al., 2014).

2.2. Study design

A longitudinal, repeated measures design was employed. Individuals were administered MRS scans, behavioral ratings, neurocognitive testing, and blood sampling on the same day within 1 week before IFN-alpha treatment (Visit 1) and within 4–6 days following the 4th IFN-alpha dose (four doses given at weekly intervals) (Visit 2). The untreated control group underwent similar assessments at Visit 1 (baseline) and Visit 2 (~4 weeks later). The mean duration between Visit 1 and Visit 2 did not differ between groups (IFN-alpha: 37.1 ± 12.0 days versus controls: 31.6 ± 6.0 days) ($F[3,27] = 2.24$, $p = 0.11$). IFN-alpha was administered as either pegylated IFN-alfa-2b (Pegintron, Merck 1.5 mcg/kg/week SC) ($n = 1$) or pegylated IFN-alfa-2a (Pegasys, Roche 184 mcg/week/kg SC) ($n = 16$) plus ribavirin (800–1200 mg/d) ($n = 17$) in the presence or absence of telaprevir (2250 mg/d TID) ($n = 10$) or boceprevir (2400 mg/d) ($n = 1$) as determined by the treating physicians. All patients were asked to refrain from caffeine and tobacco the day of the study. Study procedures were approved *a priori* by Emory University Institutional Review Board, and all subjects provided written informed consent.

2.3. Behavioral assessments

Depressive symptoms were evaluated by the 17-item Hamilton Depression Rating Scale (Hamilton, 1960) and the Multidimensional Fatigue Inventory (MFI) (Smets et al., 1995). The MFI assesses five dimensions of fatigue commonly found among medically ill patients including general fatigue, mental fatigue, physical fatigue, reduced activity, and reduced motivation. Psychomotor speed was assessed using the reaction time task of the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Majer et al., 2008). The test includes simple and five-choice segments that provide distinction between reaction time (simple and choice reaction time) and movement time (simple and choice movement time) (Majer et al., 2008). Reaction time is the speed with which the subject releases the press pad in response to the onset of a stimulus. Movement time (motor speed) is the time taken to touch the stimulus on the computer screen after the press pad had been released (Majer et al., 2008).

2.4. Inflammatory markers

Plasma was isolated from centrifuged whole blood collected in chilled EDTA tubes at the same time of day (~2 pm) to reduce circadian variations. Samples were stored at −80°C for batched assay. Plasma TNF and sTNFR2 were measured in duplicate by quantitative enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN). Inter- and intra-assay variability were reliably less than 12% for TNF and 10% for sTNFR2. Lower limits of detection for TNF and sTNFR2 were 0.19 pg/ml and 2.3 pg/ml, respectively. Research staff was blinded to clinical status of subjects, and no samples were below detection limits.

2.5. MRI acquisition

In vivo levels of glutamate as reflected by the glutamate (Glu) to creatine (Cr) ratio (Glu/Cr) were obtained by ^{1}H MRS from 2 voxels each of $17 \times 30 \times 17$ mm^3 located on the right and left basal ganglia and one voxel sized $20 \times 30 \times 10$ mm^3 located on the dACC (BA24). Imaging was performed on a Siemens 3T MR system as described (Haroon et al., 2014). In brief, a standard point-resolved spectroscopy sequence (PRESS) was used for spatial localization, and chemical shift selective (CHESS) pulses were used for water suppression. The parameters used for spectral acquisition were TR = 3000 msec, TE = 30 msec, sampling size = 1024, 128 averages. Post processing was accomplished using LC Model software. Only Glu/Cr values whose metabolite variance ratios (Cramer-Rao lower bounds-CRLB) were <20% were used for analysis. MRS data from 2 left basal ganglia and 1 dACC voxels from older IFN-alpha treated individuals were excluded due to CRLB >20%. Water was used as the internal reference, and creatine (Cr) was used to scale the data to model spectra across subjects (per LC Model Manual) and to