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Fluoxetine and citalopram decrease microglial release of glutamate and D-serine to promote cortical neuronal viability following ischemic insult

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ABSTRACT

Depression is one of the most common disorders appearing following a stroke, and is also a major factor limiting recovery and rehabilitation in stroke patients. Antidepressants are the most common prescribed treatment for depression and have shown to have anti-inflammatory properties within the central nervous system (CNS). The major source of pro-inflammatory factors within the CNS is from activated microglia, the innate immune cells of the CNS. Antidepressants have been shown to promote midbrain and hippocampal neuronal survival following an ischemic insult and this survival is mediated through the anti-inflammatory effects on microglia, but the effects on cortical neuronal survival after this insult have yet to be investigated. The present study aimed to test and compare antidepressants from three distinct classes (tricyclics, monoamine oxidase inhibitors, and selective serotonin-reuptake inhibitors [SSRIs]) on the release of inflammatory factors and amino acids from activated microglia and whether altering this release could affect cortical neuronal viability after an ischemic insult. Primary microglia were treated with 1 μg/ml LPS and/or 10 μM antidepressants, and the various factors released into medium were assayed. Co-cultures consisting of microglia and primary cortical neurons were used to assess the effects of antidepressant-treated activated microglia on the viability of ischemic injured neurons. Of the antidepressants tested, most decreased the release of the proinflammatory factors nitric oxide, tumor necrosis factor-alpha, and interleukin 1-beta from activated microglia. Fluoxetine and citalopram, the SSRIs, decreased the release of the amino acids glutamate and D-serine from LPS-activated microglia. oxygen-glucose deprived (OGD) cortical neurons cocultured with LPS-activated microglia pre-treated with fluoxetine and citalopram showed greater survival compared to injured neurons co-cultured with untreated activated microglia. Microglial release of glutamate and D-serine was shown to be the most important factor mediating neuronal survival following antagonism studies. To our knowledge, our results demonstrate for the first time that fluoxetine and citalopram decrease the release of glutamate and D-serine from LPS-activated microglia and this causes an increase in the survival of OGD-injured cortical neurons after co-culture.

Introduction

Microglia are part of the immune defense in the central nervous system (CNS) of mammals. In the healthy adult brain, microglia are found in a 'resting' or ramified state, characterized by a rod shaped body and elongated processes, and primarily carry out maintenance, homeostatic, and surveillance roles (Davalos et al., 2005; Nimmerjahn et al., 2005; Streit, 2002). Microglial cells undergo morphological and functional changes in response to tissue damage, as in various neurodegenerative diseases and brain injury, or in response to foreign bodies, as in the case of viral or bacterial infection (Ling et al., 2001). Once activated, microglia acquire an amoeboid morphology, actively phagocytose, and secrete various cytokines and pro-inflammatory factors including interleukin 1-beta (IL-1β) (Kim et al., 2004; Lai and Todd, 2008), tumor necrosis factor-alpha (TNF-α) (Jung et al., 2007; Park et al., 2007; Perry, 2004), and nitric oxide (NO) (Bi et al., 2005; Nakamura, 2002; Wu et al., 2007).

Many hypotheses have been generated to elucidate the mechanisms underlying the onset and progression of depression; however, hypotheses focused on monoamines, the hypothalamic–pituitary–adrenal (HPA) axis, and neurogenesis are not sufficient to describe these mechanisms entirely. Recently, an alternative hypothesis has received considerable attention and attempts to associate the immune system and inflammation to the etiology of depression. For depression, the most common form of treatment is by the use of antidepressant drugs and only a few investigations have studied the effects of antidepressants on the inflammatory cells in the CNS, most notably microglia. For instance, antidepressants such as imipramine (a tricyclic antidepressant, TCA) and fluvoxamine (selective serotonin-reuptake inhibitor, SSRI) have been shown to significantly decrease NO release from stimulated

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microglia (Hashioka et al., 2007), whereas amitriptyline (a TCA) and its metabolite nortriptyline inhibit IL-1β and TNF-α release from lipopolysaccharide (LPS) activated microglial cell cultures (Obuchowicz et al., 2006). Furthermore, Tynan et al. (2012) have shown and compared the anti-inflammatory effects of various SSRI antidepressants on LPS stimulated microglia in vitro. In addition to the secretion of pro-inflammatory factors, microglia are capable of secreting amino acids after activation. After stimulation with LPS, cultured microglia have been shown to significantly increase glutamate release (Jin et al., 2007), which can lead to excitatory neurotoxicity primarily mediated by N-methyl-D-aspartic acid (NMDA) receptor signaling (Takeuchi et al., 2005). Microglia have similarly been shown to release D-serine (D-Ser) after amyloid β-peptide stimulation in vitro (Wu et al., 2004). D-Ser acts as a co-agonist with glutamate on NMDA receptors and shows approximately a three-fold greater potency than glycine at the same site (Matsui et al., 1995). The ability of stimulated microglia to release D-Ser and its demonstrated marked co-agonist potency at the NMDA receptor provide a basis for a major role of this amino acid during the progression of a disease, disorder, or an insult. However, to our knowledge, no investigation has compared the effects of the three distinct classes of antidepressants (TCAs, monoamine oxidase inhibitors [MAOIs], and SSRIs) on the inflammatory or amino acid release profile from microglia all within one study; here, we make these comparisons.

Depression is a common co-morbid disorder that accompanies diseases such as Parkinson's and Alzheimer's disease and insults such as stroke (Arbus et al., 2011; El Husseini et al., 2012; Even and Weintraub, 2010; Raskin and Durst, 2010; Tanaka et al., 2006; Weintraub et al., 2010). Depression is one of the most common disorders appearing following a stroke, and is also a major factor limiting recovery and rehabilitation in stroke patients (Li et al., 2012). Acute stroke causes an irreversibly damaged ischemic core and a region of salvageable surrounding tissue termed the penumbra, which is the pharmacological target for treatment of acute ischemic stroke (Liu et al., 2010). The goal in treating ischemic stroke is to salvage the penumbra as much and early as possible and it has been reported that roughly half of all acute ischemic patients show penumbra on MRI and are potentially treatable (Rivers et al., 2006). The primary treatment for depression before or after a stroke is SSRI antidepressants. These antidepressants have been shown to alleviate the depressed state and sleep disturbances in patients diagnosed with post-stroke depression (Sunami et al., 2012), in addition to reducing the levels of anhedonia and anxiety observed in rodents following a middle cerebral artery occlusion (MCAo), a model of ischemic stroke (Kronenberg et al., 2012). In addition to improving behavioral symptoms in rodents, SSRI antidepressants prevent the degeneration of specialized neurons following ischemic insult and there is evidence that this prevention is mediated by microglia. For instance, Kronenberg et al. (2012) showed that chronic SSRI treatment prevents the degeneration of DA midbrain neurons and striatal atrophy following MCAo in rodents. Furthermore, an in vitro study found that the SSRI fluoxetine protects mesencephalic dopaminergic and hippocampal neurons from damage by mediating the inflammatory properties of microglia in co-culture, whereas fluoxetine failed to protect these neuronal cultures from an insult in the absence of microglia (Chung et al., 2010, 2011; Zhang et al., 2012). However, depending on the location of a stroke, other neuronal subtypes are affected. For instance, ischemic insult in several regions of the brain affects cortical neurons. Cortical neurons are critical in mediating a number of factors including motor output, sensory input, cognition, and speech. In the literature, there is a lack of studies investigating effects of antidepressants on the survival and viability of weakened penumbra neurons in the cortex after ischemic insult. Therefore, our objective was to investigate if antidepressants can affect the neuronal viability of weakened cortical neurons following an ischemic insult and if any resulting effects are mediated through microglia.

Here we compare the effects of antidepressants from the TCA, MAOI, and SSRI classes in reducing the secretion of pro-inflammatory factors (TNF-a, IL-1B, and NO) and amino acids from cultured microglia after LPS stimulation. We present the novel finding that treatment with fluoxetine and citalopram (SSRIs) attenuates the release of the amino acids glutamate and D-Ser from LPS-stimulated microglia in vitro. Finally, as a means to mimic post-acute injury following a stroke and the effects of antidepressants on weakened cortical neurons after ischemia, we use a specialized neuronal and microglial co-culture system. We demonstrate that pre-treatment of microglia with the antidepressants fluoxetine and citalopram (SSRIs) improves the viability of weakened neurons after an in vitro model of ischemic insult and that this effect is mediated by the reduced release of glutamate and D-Ser from activated microglia.

Results

Antidepressants decreased NO, TNF-α, and IL-1β secretion from LPS-stimulated microglia

We measured the levels of nitrite (as an indicator of NO) released into the medium 24 h after addition of LPS to microglial cultures. Following a dose–response study (data not shown), treatment with 1 μg/ml LPS for 24 h was determined to be optimal at activating microglia, eliciting an increase in NO release of approximately 150% above control (Fig. 3A). Treatment concentrations of antidepressants were determined through a dose–response study and 10 μM was found to be optimal at reducing NO release from LPS-activated microglia (data not shown). These findings are consistent with previous reports (Diamond et al., 2006; Hashioka et al., 2007; Obuchowicz et al., 2006).

All antidepressants tested significantly reduced the release of NO from activated microglia. Antidepressants added to non-stimulated microglia had no significant effect on NO release compared to vehicle-treated controls (Fig. 3A). Similarly, we found that LPS-stimulated microglia significantly increased their release of TNF-α and IL-1β compared to vehicle-treated controls (Fig. 3B, C). Pretreatment with imipramine, TCP, fluoxetine, and citalopram significantly reduced the release of TNF-α from activated microglia, while TNF-α remained elevated after clomipramine and phenelzine treatment (Fig. 3B). All antidepressants significantly reduced release of IL-1β from activated microglia (Fig. 3C). No differences in TNF-α or IL-1β release, compared to vehicle-treated controls, were observed when antidepressants were added to cultured non-stimulated microglia. These results show that LPS stimulation increases microglial release of NO, TNF-α, and IL-1β and this increase is attenuated by most of the antidepressant drugs tested.

Fluoxetine and citalopram protected neurons from microglia-mediated toxicity

To determine the neuroprotective effects of antidepressants, we first examined whether antidepressants alone would directly affect the viability of normoxic or OGD-injured neurons. We found that under normoxic conditions, no antidepressant tested affected neuronal viability compared to vehicle-treated controls (Fig. 4A). Similarly, we found that a 30 min treatment with antidepressants prior to OGD insult did not affect neuronal viability compared to non-treated controls (Fig. 4A). Additionally, we sought to determine if the presence or absence of microglia would affect the viability of normoxic or OGD injured neurons. Under normoxic and OGD conditions, we found that co-culture of neurons with non-stimulated microglial media did not affect neuronal survival compared to neurons incubated in media devoid of microglia (Fig. 4B). In contrast, co-cultures of neurons with LPS-stimulated microglia resulted in a marked decrease in the viability of OGD-injured neurons (Fig. 4B). This decreased viability is proposed to be due to exchange of soluble factors between the two cultures, likely pro-inflammatory cytokines or reactive oxygen species released by the activated microglia (Lai and Todd, 2008).