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Bupropion pre-treatment of endotoxin-induced depressive symptoms

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ABSTRACT

Increased levels of inflammatory cytokines may play a role in depression. Depressive symptoms can be induced in humans with administration of low-dose lipopolysaccharide (LPS; endotoxin), which activates the innate immune system and causes release of inflammatory cytokines. We previously found that pre-treatment with the serotonin reuptake inhibitor citalopram reduced LPS-induced fatigue and anhedonia. This is a follow-up study to determine whether LPS-induced symptoms could be reduced by pre-treatment with bupropion, a norepinephrine and dopamine reuptake inhibitor. In this double-blind, randomized, placebo-controlled, cross-over study, 10 healthy subjects received intravenous LPS (0.8 ng/kg) after oral pre-treatment with bupropion (75 mg twice a day) or placebo for 7 days. The Montgomery-Åsberg Depression Rating Scale (MADRS), the Profile of Mood States (POMS), and a visual analog scale (VAS) were used to measure depressive symptoms. Serum levels of inflammatory cytokines and chemokines were measured with electrochemiluminescence assays. The results of this study, which must be considered preliminary, showed that LPS administration was associated with (1) increase in serum levels of all cytokines and chemokines assayed; (2) increase in total MADRS score, mostly due to items 7 (lassitude) and 8 (anhedonia); (3) increase in fatigue; (4) decrease in vigor; and (5) decrease in social interest. Bupropion pre-treatment had no statistically significant effect on the innate immune response to LPS or on LPS-induced behavioral changes, suggesting that 1-week pre-treatment with bupropion does not inhibit LPS-induced fatigue and anhedonia, contrary to what was found previously with citalopram.

1. Introduction

A large body of evidence from rodent and human studies has shown that inflammatory cytokines, such as tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6), have specific effects on the brain, including effects on mood and motivation (Reichenberg et al., 2001; Wright et al., 2005; Capuron et al., 2009; Eisenberger et al., 2009; DellaGioia and Hannestad, 2010). For example, experimentally elevating inflammatory cytokine levels with immune stimuli such as lipopolysaccharide (LPS) causes transient fatigue and anhedonia (Reichenberg et al., 2001; Eisenberger et al., 2009; DellaGioia and Hannestad, 2010) and changes in activity in specific brain regions (Eisenberger et al., 2010a,b; Inagaki et al., 2011). The dose of LPS used in these studies (0.8 ng/kg) causes smaller increases in TNFα and IL-6 levels than doses between 2 and 4 ng/kg, which induce a sepsis-like response (Michie et al., 1988; Suffredini et al., 1999). Depression is associated with increased levels of both TNFα and IL-6 (Dowlati et al., 2010); however, it is not known whether elevations in inflammatory cytokine levels contribute to depression, or whether they are simply a result of depression (Haroon et al., 2012). Interestingly, elevated levels of TNFα and IL-6 do not normalize with successful pharmacologic treatment of depression (Hannestad et al., 2011a). This suggests either (1) that these cytokines are not involved in the pathogenesis of depressive symptoms in depression, or (2) that antidepressants protect the brain from their "depressogenic" effect. Consistent with the latter hypothesis, we previously found that LPS-induced depressive symptoms could be reduced by 5-day pre-treatment with the serotonin reuptake inhibitor citalopram, even though this had no effect on the TNFα and IL-6 response to LPS (Hannestad et al., 2011b). The primary goal of this preliminary study was to explore whether pre-treatment with bupropion, which inhibits reuptake of norepinephrine and dopamine, could reduce depressive symptoms produced by acute administration of low-dose LPS. The secondary goal was to assess a broader set of cytokines and chemokines, to characterize their response to LPS administration *in vivo* in humans, and to determine whether the response to LPS of any of these cytokines or chemokines was influenced by pre-treatment with bupropion.

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2. Methods

2.1. Design

This was a double-blind, randomized-order, placebo-controlled, cross-over study in which each subject participated in three conditions: (1) oral placebo pre-treatment daily for 7 days followed by intravenous (IV) placebo administration (PBO–PBO condition), (2) oral placebo pre-treatment daily for 7 days followed by IV LPS administration (PBO–LPS condition), and (3) oral pre-treatment with bupropion daily for 7 days followed by IV LPS administration (BUP–LPS condition). Each IV administration day was separated by 7–14 days, and the order of the three conditions was randomized and blinded to everybody except the research pharmacist.

2.2. Subjects

Twenty-eight subjects were screened. Eight subjects were not eligible because of: positive urine drug screen ($n = 1$), body mass index > 30 ($n = 1$), positive HIV test ($n = 1$), elevated TSH ($n = 2$), positive pregnancy test ($n = 1$), above upper age limit ($n = 1$), and positive depression screen ($n = 1$). Seven subjects were eligible but were not able or willing to participate after the screening. Thirteen subjects started the study. Three did not return after the first study condition. One subject completed two study conditions, and 9 subjects completed all three conditions. Eligibility was based on medical and psychiatric history, review of systems, physical and neurologic exam, screening labs and electrocardiogram. All subjects provided written, informed consent, and the study had been approved by the Yale University Human Investigations Committee.

2.3. Bupropion pretreatment

Bupropion was chosen because the presumed antidepressant mechanism of action, inhibition of norepinephrine and dopamine, reuptake is different from that of citalopram, an inhibitor of serotonin reuptake. The dose, 150 mg/day, is a dose that has efficacy in the treatment of major depressive disorder. Subjects received 14 capsules of double-blind study medication (75 mg bupropion or placebo) which they took every morning and every evening starting 7 days before each IV administration day. Bupropion was given twice a day to reduce side-effects and thus the likelihood of unblinding. When subjects were asked to guess whether they had taken bupropion or placebo, they did not ascertain more than by chance. Each subject took bupropion for 7 days once (in the BUP–LPS condition) and placebo for 7 days twice (in the PBO–PBO and the PBO–LPS conditions). The last dose of each course was taken on the evening before each IV administration day to avoid acute effects of bupropion during the assessments. We chose a 7-day course because, with a half-life of 20 h, >95% of steady-state levels would be reached in <5 days.

2.4. LPS administration

Subjects fasted (except water) from midnight before each IV administration day. When they arrived at the Clinical Neuroscience Research Unit at 8 AM, an IV catheter was inserted and 500 ml of normal saline infused to ensure adequate hydration. Baseline ratings and blood draws were performed between 08:30 and 09:00 on each day. NIH Clinical Center Reference LPS was prepared by the research pharmacist the day before and stored at 4 °C overnight. At approximately 9 AM we administered LPS 0.8 ng/kg (in the PBO–LPS and BUP–LPS condition) or placebo (in the PBO–PBO condition) as an IV bolus, followed by normal saline to ensure complete delivery. After LPS/placebo administration heart rhythm was continuously monitored. Systolic and diastolic blood pressure (SBP and DBP, respectively) and heart rate (HR) were taken at 5, 10, 15, 20, and 30 min and then every 30 min thereafter. Body temperature was taken hourly. Behavioral ratings and blood samples for cytokine analysis were obtained at 60, 90, 120, 180, and 240 min after LPS/placebo administration on each day. Each LPS administration day was separated by at least 14 days to avoid tolerance to the effects of LPS (Biswas and Lopez-Collazo, 2009); in early experiments and in our previous study with citalopram we determined that no reduction in the LPS-induced cytokine response occurred if LPS administration days were >7 days apart.

2.5. Behavioral ratings

The primary outcome was the Montgomery-Åsberg Depression Rating Scale (MADRS), which was chosen over the Hamilton Depression Rating Scale because it is less oriented towards somatic symptoms (Demyttenaere and De Fruyt, 2003). Secondary outcomes included the Profile of Mood States (POMS) to measure fatigue and vigor (Norcross et al., 1984), and a visual-analog scale (VAS) to measure social interest (the extremes of the VAS stated "I want to be alone" vs "I want to be with other people") (Hannestad et al., 2011b).

2.6. Cytokine and chemokine assays

In addition to cytokines, which are commonly measured in human LPS administration studies, we also chose to include measurements of various chemokines. The cytokines and chemokines tested are listed in Supplementary Table 1. Electrochemiluminescence multi-array technology (Meso Scale Discovery, Gaithersburg, MD) was used to measure serum levels. Cytokine-specific capture assay are coated in arrays in each well of a 96-well carbon electrode plate surface. Serum (25 ml) was added to the wells in duplicate and incubated for 2 h at room temperature. After several washing steps SULFO-TAG® labeled secondary antibodies were added. A read buffer was then added to provide an appropriate chemical environment for electrochemiluminescence. The plates were read on the Sector Imager, which applies a voltage to the plate electrodes causing the bound labels to emit light. The intensity of emitted light is proportional to the amount of cytokine present in the sample. The lower limit of detection for each cytokine is listed in Supplementary Table 1.

2.7. Statistical analysis

Outcomes were summarized descriptively and assessed for normality prior to analysis using normal probability plots and Kolmogorov test statistics. The primary (MADRS-Total, MADRS-Lassitude, MADRS-Anhedonia) and secondary (each cytokine and chemokine, POMS-Fatigue, POMS-Vigor, and VAS-Social) outcomes were analyzed over time using a linear model, which included treatment (PBO–PBO, PBO–LPS, BUP–LPS) and time (0, 60, 90 120, 180, and 240 min; 0 and 120 for MADRS) as within-subject factors, and subject as a clustering factor. The best-fitting variance–covariance structure (unstructured, compound symmetry, heterogeneous compound symmetry, or first-order autoregressive) was chosen using information criteria. Significant main and interactive effects were followed by appropriate post hoc F-tests using the CONTRAST statement in SAS PROC MIXED. The post hoc tests were adjusted (denoted as "adj.") for the number of comparisons made within each outcome using the Bonferroni correction. Non-normal outcomes were analyzed with the same factors, using a nonparametric approach for repeated-measures data (Brunner et al., 2002), where the data were first ranked, and then fitted using a mixed-effects model with an unstructured