Antidepressant Regulation of Macrophage-derived TNF Production

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Abstract

The role of the cytokine tumor necrosis factor-α (TNF) has been well established in neuropathic pain and depression, two often comorbid conditions that are associated with chronic inflammation. Antidepressant drugs have been found to be effective in treating these conditions and in reducing brain-derived TNF, but the effect of these drugs elsewhere in the body is poorly characterized. This study seeks to determine the effects of antidepressant drugs on macrophage-derived TNF production and elucidate whether the macrophage inflammatory state influences these effects. This was studied using peritoneal macrophages isolated from Sprague-Dawley rats that were exposed to various concentrations of different classes of antidepressants, with inflammation simulated using lipopolysaccharide. It was found that antidepressants regulate macrophage-derived TNF production in a gender, inflammation, and dose dependent manner. These findings can have implications in elucidating an additional mechanism of antidepressant action and determining pharmacological differences between genders.

Introduction

- Tumor necrosis factor-α (TNF) is a pro-inflammatory cytokine that can be produced by a variety of different cell types.
- Macrophages (MΦ), a type of resident phagocyte, are modulated by many neurotransmitters and neuropeptides.
  - Norepinephrine (NE)- MΦ express both α_2-(promote TNF) and β_2-adrenergic (inhibit TNF) receptors.[1–3]
  - Dopamine – high extracellular concentrations suppress TNF production.[3]
  - Serotonin – associated with suppression of TNF production.[3]
- One mechanism of action of antidepressants is inhibition of reuptake mechanisms. This blockade prevents removal of neurotransmitters from extracellular space, prolonging the response to the corresponding neurotransmitter.
- Antidepressants have been found to have anti-inflammatory effects, which supports their use in depression and neuropathic pain, two conditions associated with chronic inflammation. [4–7]
- The link between antidepressant drugs and decrease in brain TNF has been established [8,9], along with modulation of the immune response.[10]

Purpose

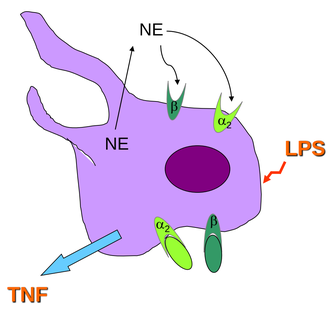
A gap in the literature exists for assessing the direct effect of antidepressants on MΦ production of TNF. This study will analyze the *in vitro* effects of several antidepressant drugs on MΦ-derived TNF production.

Antidepressant drugs for *in vitro* studies:

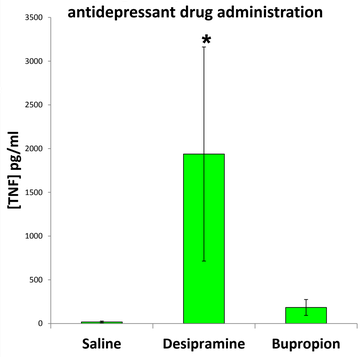
Desipramine - Selective NE reuptake blocker.

Amitriptyline – Mixed NE/serotonin reuptake blocker.

Bupropion (Wellbutrin) – Mixed NE/dopamine reuptake blocker.



Macrophage TNF production after *in vivo* antidepressant drug administration



Bkgd. MΦ production of TNF from male SD rats administered antidepressant drugs. Peritoneal MΦs were harvested from rats receiving either saline, desipramine (10 mg/kg, ip), or bupropion (10 mg/kg, ip) after 24 hrs and were plated with no LPS stimulation. Kruskal-Wallis one-way ANOVA followed by Dunn's method: * $p < 0.05$ vs saline. Note: When comparing MΦ groups with LPS stimulation, there were no significant differences from saline.[11]

Methods and Materials

- Male and female Sprague-Dawley rats (125-150 g, Envigo) were housed in the UB-Laboratory Animal Facilities.
- Animals were given food and water *ad libitum* and maintained on a 12 hour light/dark cycle.
- Peritoneal exudate cells were collected immediately after decapitation via peritoneal lavage and MΦs isolated via adhesion (2 hrs at 37°C, 95%O_2/5%CO_2).
- MΦs were exposed to antidepressants (desipramine, amitriptyline, bupropion) at 10^{-3}M, 10^{-5}M, and 10^{-7}M.
- Inflammation was simulated *in vitro* by the addition of Lipopolysaccharide (LPS; 30 ng/mL).
- Incomplete RPMI-1640 with glutamine was used for all incubations, drug, and LPS dilutions.
- Cell supernatants were collected after 4 hr-incubation with appropriate drugs and LPS.
- TNF levels were assessed with a spectrophotometric WEHI 164 subclone 13 fibroblast cytotoxicity assay.

Results

- Percent changes were calculated by comparing TNF levels of each sample with either a no LPS group (for non-inflammatory state) or an LPS only group (for inflammatory state).
- Male rat MΦs demonstrated a flipped response to antidepressants between non-inflammatory and inflammatory states. The relationship between inflammatory state and antidepressant response was not as clear in female rat MΦs.

Baseline TNF production by peritoneal MΦs

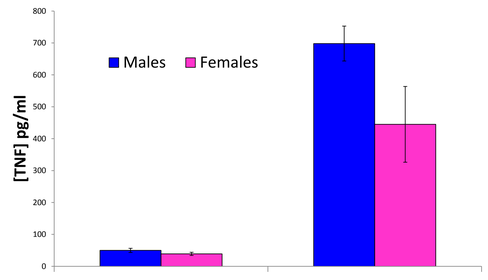


Figure 1. TNF production by peritoneal MΦs. Supernatants from MΦ cultures with and without LPS (30 ng/ml) stimulation (4 hrs) were assayed for levels of TNF using the WEHI bioassay.

Concentration-effect curves for MΦs from MALES.

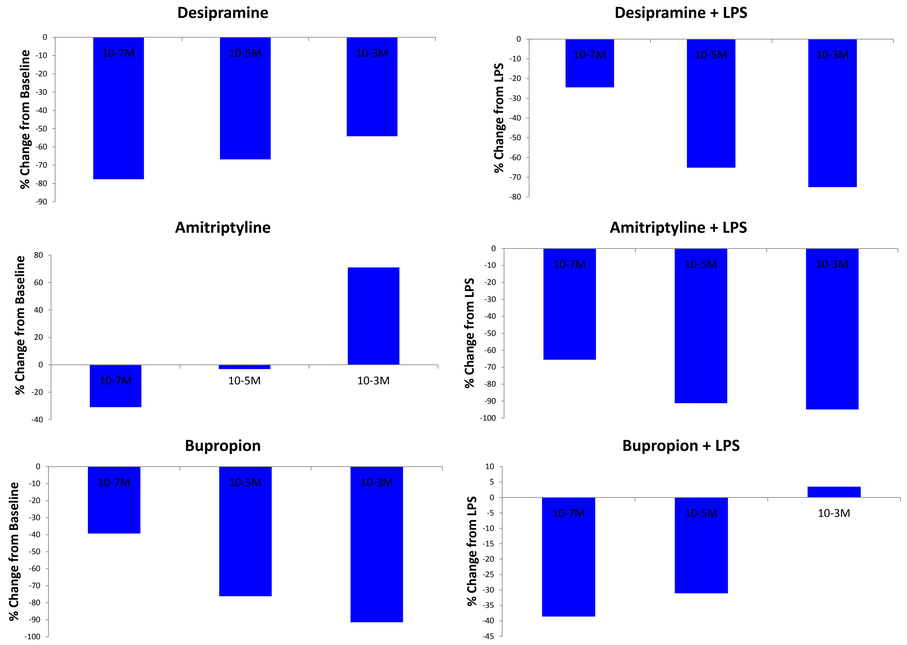


Figure 2. The effect of *in vitro* addition of antidepressant drugs on macrophage (MΦ) derived TNF production from male rats. Desipramine, amitriptyline, or bupropion (10^{-3}M, 10^{-5}M, 10^{-7}M) were added to macrophage, with and without LPS (30ng/mL), in culture and incubated for 4 hrs. Data are expressed as percent change from baseline TNF (no LPS) or as percent change from LPS alone. Data are represented as mean values (n=1).

Concentration-effect curves for MΦ from FEMALES

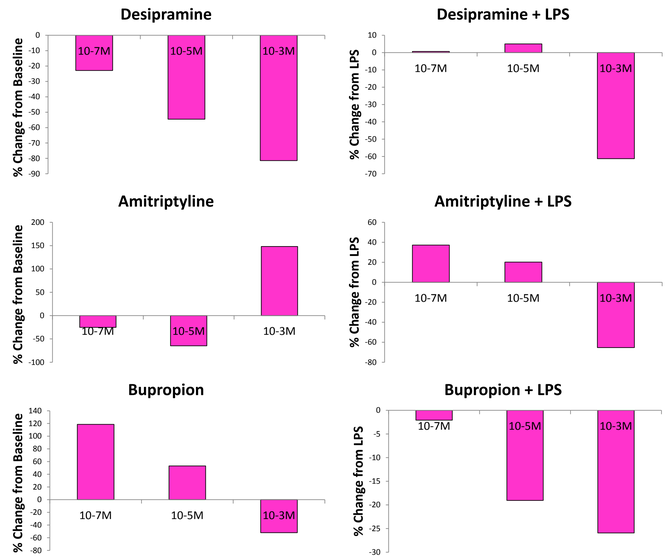


Figure 3. The effect of *in vitro* addition of antidepressant drugs on macrophage (MΦ) derived TNF production from female rats. Desipramine, amitriptyline, or bupropion (10^{-3}M, 10^{-5}M, 10^{-7}M) were added to macrophage, with and without LPS (30ng/mL), in culture and incubated for 4 hrs. Data are expressed as percent change from baseline TNF (no LPS) or as percent change from LPS alone. Data are represented as mean values (n=1).

Discussion

- The influence of antidepressants on MΦ-derived TNF production was found to be regulated differently depending on gender, inflammatory state, and drug concentration.
- These findings suggest an additional mechanism of action of antidepressant drugs. These drugs are used in both depression and neuropathic pain, both of which are connected to an enhanced systemic inflammatory state.
- The differential regulation based on inflammatory state may also suggest a possible unintended effect of antidepressant treatment: the inhibition of inflammation in non-inflammatory environments.
- The differential gender based regulation further supports the pharmacological differences between the genders, an important concept in drug design and testing.

Future Experiments

- A larger number of animals will be tested to perform statistical analysis of the data.
- The *in vitro* model of inflammation with LPS will be supported through the use of an *in vivo* model of systemic inflammation by using the Chronic Constriction Injury model of neuropathic pain in addition to LPS.
- A fourth group of antidepressants, the selective serotonin reuptake inhibitors, will be tested.

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