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Paroxetine differentially modulates LPS-induced TNFα and IL-6 production in mouse macrophages

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ABSTRACT

Paroxetine is a selective serotonin reuptake inhibitor (SSRI) that is clinically used for the treatment of depression in human patients. Because of recent reports on the role of serotonin in modulating inflammation and the link between inflammation and depression, we sought to test the effect of paroxetine directly on macrophage response to an inflammatory stimulus. Lipopolysaccharide (LPS) treatment of mouse macrophages significantly enhanced TNFα and IL-6 production. Paroxetine treatment of macrophages, however, significantly inhibited LPS-induced IL-6 production. In contrast, paroxetine enhanced LPS-induced TNFα production in macrophages. These effects of paroxetine were mimicked by fluoxetine, another SSRI. To determine if the effects of paroxetine are mediated via modulation of the 5-HT system, we treated macrophages with 5-HT or 5-HT receptor antagonist (LY215840) in the presence of LPS and/or paroxetine. 5-HT treatment by itself did not affect LPS-induced cytokine production. LY215840, however, reversed paroxetine's effect on LPS-induced TNFα production but not IL-6. To understand the signaling mechanisms, we examined paroxetine's effect on MAPK and NFκB pathways. While paroxetine inhibited LPS-induced IκBα phosphorylation, MAPK pathways were mostly unaffected. Together these data demonstrate that paroxetine has critical but differential effects on IL-6 and TNFα production in macrophages and that it likely regulates these cytokines via distinct mechanisms.

1. Introduction

Paroxetine is an FDA approved drug for treating depression in humans. It belongs to the class of "*S*elective *S*erotonin *R*euptake *I*nhibitors" (SSRIs). In addition to paroxetine (Paxil, Pexeva), other members of the SSRIs include fluoxetine (Prozac), citalopram (Celexa), escitalopram (Lexapro), and sertraline (Zoloft) [1,2]. SSRIs are prescribed mainly for major depressive disorders but are also used in the treatment of anxiety, panic and eating disorders and occasionally for post-traumatic stress disorder [3]. Paroxetine and other members of the SSRI class were identified based on their ability to inhibit reuptake of serotonin by blocking serotonin transporters (SERTs) that are present on the cell surface of the pre-synaptic neuron [1]. Serotonin (5-hydroxy tryptamine, 5-HT) is a monoamine neurotransmitter primarily synthesized in the gastrointestinal (GI) tract and the central nervous system. In addition to its effects on the GI tract and the neuronal system, non-neuronal serotonin modulates other physiological processes including inflammation.

Recent studies have established neurogenic inflammation as the likely cause of depression in humans [4]. Studies have also shown that increased pro-inflammatory cytokine (IL-6, and TNFα) levels, both in periphery and in the brain, precipitate development of depression (reviewed in [5]). Although anti-depressants in clinical use are effective in ameliorating the symptoms, there is considerable interest in identifying novel antidepressants and understanding the mechanisms of action of existing antidepressants. In this regard, SSRIs have been reported to have anti-inflammatory properties not only in neuronal tissues but also in non-neuronal cells [6,7]. In animal models of disease, SSRIs are able to effectively modulate neuronal as well as non-neuronal inflammatory diseases [8–10]. Given that paroxetine and fluoxetine are already FDA approved, there is also interest in re-purposing these drugs possibly as anti-inflammatory therapeutics for diseases such as arthritis and colitis [11,12]. In spite of these studies in animal models, the role of paroxetine on lipopolysaccharide-induced IL-6 and TNFα production in macrophages and the mechanisms of regulation are not well known. Here we provide evidence that paroxetine significantly modulates LPS-induced IL-6 and TNFα in mouse macrophages and that its effects on these two cytokines are differentially regulated.

2. Materials and methods

2.1. Reagents

Paroxetine hydrochloride hemihydrate (MW 374.83) and fluoxetine hydrochloride (MW 345.79) were purchased from Sigma-Aldrich (St

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Louis, MO). Serotonin hydrochloride (M 217.18) and LY 215840 (MW 400.04) were obtained from Tocris Bioscience (Bristol, UK). RPMI 1640 (Rosewell Park Memorial Institute) media, fetal bovine serum (FBS), Pen-Strep (Penicillin Streptomycin mixtures contain 5000 units of penicillin and 5000 μg of streptomycin/ml in saline) and Versene (0.2 g EDTA/l of PBS) were purchased from Life Technologies (Carlsbad, CA). Ultrapure LPS (from 0111:B4 *Escherichia coli*) was obtained from InvivoGen (San Diego, CA).

2.2. Antibodies

Antibodies (P-IκBα, P-ERK, P-JNK, Pp38, Pp105, and tubulin) were purchased from Cell Signaling Technology Inc. (Danvers, MA). Antibodies (GRK2 and ERK2) were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Alexa fluor goat anti-rabbit antibody (Invitrogen, Carlsbad, CA) and anti-mouse IgG IRdye 800 conjugated antibody (Rockland Immunochemicals Inc., Gilbertsville, PA) were used with LICOR's Odyssey system. Peroxidase conjugated anti-mouse antibody (Vector Laboratories Inc., Burlingame, CA) developed with Immunocruz luminal reagent (Santa Cruz Biotechnology) was used for chemiluminescence.

2.3. Animals and collection of primary peritoneal macrophages

Mice (C57BL/6) were obtained from NCI and were housed in groups of 4 to 5 mice per cage in rooms maintained at 22–24 °C with 50% humidity with a 12-hour light/12-hour dark cycle. All animals had access to normal chow and water ad libitum. Experimental procedures involving mice were conducted in accordance to the protocol approved by the Institutional Animal Care and Use Committee at Michigan State University and conformed to the NIH Guidelines. For thioglycollate-induced peritoneal cell collection, mice were injected intraperitoneally with 1 ml of 4% Brewer's thioglycollate broth (Sigma-Aldrich, St Louis). Peritoneal cells from each mouse were collected 4 days post-injection, as previously described [13]. 10^6 cells/well were seeded in 12-well plates for the experiments. Cells were serum starved at least for 1 h prior to treatment. All treatments were done in serum free media.

2.4. Cell culture

Raw 264.7 cells were purchased from ATCC (Manassas, VA) and grown in RPMI media supplemented with 10% fetal bovine serum (FBS) along with 1% Pen-Strep and maintained in 5% CO_2 at 37 °C.

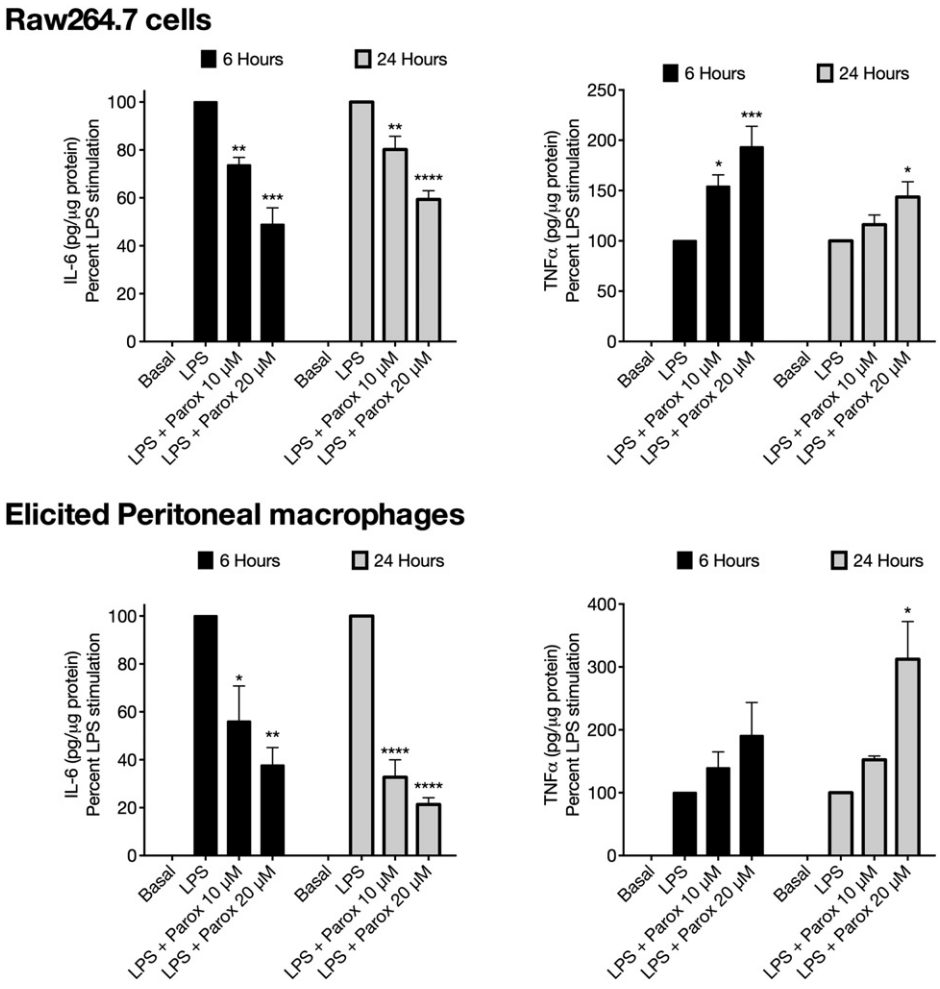

Fig. 1. Differential effect of paroxetine on LPS-induced IL-6 and TNFα production in mouse macrophages: RAW 264.7 (top panel) and thioglycollate-elicited peritoneal macrophages (bottom panel) were treated with or without LPS (1 μg/ml) and paroxetine hydrochloride (at the indicated concentrations) for 6 and 24 h, respectively. ELISA was used to determine IL-6 and TNFα levels in the culture supernatants. Cytokine levels were normalized to total cellular protein and were expressed as percent LPS stimulation (LPS stimulation = 100%). Note that cytokines were not detected in the basal group. LPS-induced IL-6 and TNFα levels were as follows: IL6-Raw 264.7: 1.9 ± 0.5 and 5.7 ± 2 ng/μg protein at 6 and 24 h, respectively; peritoneal macrophages: 252 ± 106 and 714 ± 146 pg/μg protein at 6 and 24 h, respectively. TNFα-Raw 264.7: 1.7 ± 0.2 and 2.2 ± 0.4 ng/μg protein at 6 and 24 h, respectively; peritoneal macrophages: 407 ± 145 and 287 ± 43 pg/μg protein at 6 and 24 h, respectively. N = 4–6; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.