RESEARCH ARTICLE

Antidepressant Compounds Can Be Both Pro- and Anti-Inflammatory in Human Hippocampal Cells

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Abstract

Background: The increasingly recognized role of inflammation in the pathogenesis and prognosis of depression has led to a renewed focus on the immunomodulatory properties of compounds with antidepressant action. Studies have, so far, explored such properties in human blood samples and in animal models.

Methods: Here we used the more relevant model of human hippocampal progenitor cells exposed to an inflammatory milieu, induced by treatment with IL-1β. This increased the levels of a series of cytokines and chemokines produced by the cells, including a dose- and time-dependent increase of IL-6. We investigated the immunomodulatory properties of four monoaminergic antidepressants (venlafaxine, sertraline, moclobemide, and agomelatine) and two omega-3 polyunsaturated fatty acids (n-3 PUFAs; eicosapentanoic acid [EPA] and docosahexanoic acid [DHA]).

Results: We found that venlafaxine and EPA were anti-inflammatory: venlafaxine decreased IL-6, with a trend for decreases of IL-8 and IP-10, while EPA decreased the levels of IL-6, IL-15, IL-1RA, and IP-10. These effects were associated with a corresponding decrease in NF-kB activity. Unexpectedly, sertraline and DHA had pro-inflammatory effects, with sertraline increasing IFN-α and IL-6 and DHA increasing IL-15, IL-1RA, IFN-α, and IL-6, though these changes were also associated with a decrease in NF-kB activity, suggesting distinct modes of action. Agomelatine and moclobemide had no effect on IL-6 secretion.

Conclusions: These observations indicate that monoaminergic antidepressants and n-3 PUFAs have distinctive effects on immune processes in human neural cells. Further characterization of these actions may enable more effective personalization of treatment based on the inflammatory status of patients.

Keywords: cytokines, depression, inflammation, neural stem cells, omega-3 fatty acids

Introduction

Given the central importance attributed to neuro-inflammation in the pathogenesis of depression, immunomodulation has emerged as a potential key pathway in the treatment of this disorder. In particular, evidence suggests that two groups of compounds with demonstrated antidepressant properties, monoaminergic antidepressants and omega-3 polyunsaturated fatty acids (n-3 PUFAs), could share modulation of the inflammatory response as a common pathway of action.

A number of studies have investigated the ability of these compounds to affect levels of inflammatory cytokines in animal brains or human blood samples. These studies have generated findings broadly indicating anti-inflammatory properties for both monoaminergic antidepressants (Xia et al., 1996; Kenis and Maes, 2002; Obuchowicz et al., 2006; Bielecka et al., 2010; Hannestad et al., 2011; Tynan and Weidenhofer, 2012) and n-3 PUFAs (Novak et al., 2003; Lu et al., 2010; Rangel-Huerta et al., 2012), though pro-inflammatory effects, or an absence of effect, have been reported as well (Kubera et al., 2005; Diamond et al., 2006; Maes et al., 2007; Jazayeri et al., 2010; Tynan and Weidenhofer, 2012). In addition to wide methodological variations amongst studies, complicating the drawing of overall conclusions, the major limitation so far has been a lack of studies in human brain cells, an issue to be addressed in the present study.

The regulation of Nuclear Factor-kB is a common mechanism of action proposed for the immunomodulatory properties of monoaminergic antidepressants and n-3 PUFAs. NF-kB is one of the primary transcription factors involved in the synthesis and release of pro-inflammatory cytokines (Tak and Firestein, 2001). It has been identified as a key mediator of the effects of stress on depressive behavior (Koo et al., 2010) and found to be upregulated in depressed patients (Pace et al., 2006). In particular, monoaminergic antidepressants have been shown to reduce NF-kB activity in rat glial cultures (Bielecka et al., 2010) and brains (Zhu et al., 2008). Similarly, n-3 PUFAs have also been shown to reduce NF-kB activity in murine macrophages (Novak et al., 2003) and microglia (Moon et al., 2007), as well as in human aortic endothelial cells (Wang et al., 2011).

Considering that the hippocampus is critically implicated in depression and antidepressant treatment (MacQueen and Frodl, 2011) and profoundly affected by inflammation (Koo and Duman, 2008), we have used our recently-validated human hippocampal progenitor cell *in vitro* model of depression to investigate this issue. This model is based on exposing human neural progenitor cells to a variety of depressogenic insults, leading to changes in cell phenotypes that resemble those described in the brains of depressed patients as well as in the brains of animals exposed to chronic stress (Anacker et al., 2011 2013a 2013b; Zunszain et al., 2012). To explore the possibility that immunomodulation may be a common pathway of antidepressant action, we chose to investigate a wide variety of antidepressant compounds—sertraline, a selective serotonin reuptake inhibitor (SSRI), venlafaxine, a serotonin-noradrenaline reuptake inhibitor (SNRI), moclobemide, a monoamine oxidase inhibitor (MAOI) and a novel melatonergic antidepressant (agomelatine)—as well as the two principal n-3 PUFAs—eicosapentanoic acid (EPA) and docosahexanoic acid (DHA)—all of which have demonstrated anti-inflammatory properties in previous studies (Novak et al., 2003; Vollmar et al., 2008; Bielecka et al., 2010; Lu et al., 2010; Tynan and Weidenhofer, 2012; Molteni et al., 2013). Interleukin-1 beta (IL-1β) was used as an inflammatory stimulus because it is increased in depressed patients (Howren et al., 2009) and is critical to the pathogenesis of depression (Koo and Duman, 2008). Moreover, we have previously shown that these neural progenitor cells are responsive to IL-1β (Zunszain et al., 2012). Indeed, IL-1β has been shown to induce chemokine and cytokine expression through the activation of NF-kB in differentiated human neural progenitor cells (Pugazhenthi et al., 2013). We hypothesize that these antidepressant compounds would be immunomodulatory through a mechanism involving NF-kB activity.

Methods

Cell Culture

All experiments were performed with the multipotent, human, hippocampal progenitor cell line HPC03A/07, provided by ReNeuron. Cells were grown in reduced modified media (RMM) consisting of Dulbecco's Modified Eagle's Media/F12 (Invitrogen) supplemented with 0.03% human albumin (Baxter Healthcare), 100 μg/mL human apo-transferrin, 16.2 μg/mL human putrescine DiHCl, 5μg/mL human recombinant insulin, 60ng/mL progesterone, 2mM L-glutamine, and 40ng/mL sodium selenite. To maintain proliferation, 10ng/mL human basic fibroblast growth factor (bFGF), 20ng/mL human epidermal growth factor (EGF), and 100nM 4-hydroxytamoxifen (4-OHT) were added. Cultures were grown in 75 cm² filtered, cap-cell culture flasks (Nunclon) at 37°C in 5% CO_2 and were regularly passaged at 60–80% confluence.

Assays with Antidepressant Compounds

To determine the effects of monoaminergic antidepressants from different classes and n-3 PUFAs on the inflammatory cascade, an inflammatory response was first induced in cells by treatment with IL-1β. The neural stem cells express the IL-1 receptor, as detected by polymerase chain reaction (PCR; data not shown). Cells were plated in 6 well plates (Nunclon) at a density of 300 000 cells per well in 2mL of RMM and allowed to firmly attach for 24 hours.

The optimal dose and duration of treatment of IL-1β required to provoke a robust inflammatory response was determined by examining multiple doses and treatment periods (see Results). Subsequently, cells were co-incubated with IL-1β (10ng/mL) either alone or in combination with one of the monoaminergic antidepressants (agomelatine, venlafaxine, moclobemide, or sertraline) or one of the n-3 PUFAs (EPA or DHA) for a further period of 24 hours. This paradigm was used for all experiments, unless otherwise stated. Treatment doses were informed by previous *in vitro* literature for antidepressants (Maes et al., 1999; Vollmar et al., 2008) and n-3 PUFAs (Lu et al., 2010), as well as therapeutic levels, where known (Schulz et al., 2012), before performing viability assays in our cells. All treatments had the same vehicle (including 0.01% dimethyl sulfoxide and 0.1% ethanol) to exclude the possibility of any differences observed being the consequence of differing concentrations of solvents. Supernatants were collected and stored at -80°C for subsequent measurement.

Cellular Viability

Cells grown and treated in 6 well plates were fixed with paraformaldehyde 4% for 20 minutes. After washing, the plates were allowed to air dry and were stained by addition of 750 uL of 10% crystal violet (Pro-lab) solution to each well. After 20 minutes of shaking, they were vigorously washed and allowed to air dry before being treated with 750 uL of 10% acetic acid. Absorbance, proportional to cell number, was measured at 595 nm on a DTX 880 Multimode Detector (Beckman-Coulter) and used to normalize relative levels of soluble proteins measured via ELISA and multiplex arrays.

Measurement of IL-6 Secreted Protein Levels

IL-6, secreted into the supernatant, was quantified using the human IL-6 Quantikine ELISA kit (R&D Systems). The procedure was performed according to the manufacturer's instructions. Absorbance was read at 450nm using a DTX 880 Multimode Detector (Beckman-Coulter). Data were analyzed using a four-parameter logistic algorithm to derive concentrations of samples from known standards using SoftMax Pro (Molecular Devices). All concentrations were then corrected by the number of cells as determined by crystal violet. At least three independent experiments were conducted on independent cultures, and each sample was tested in duplicate. Data is presented as

absolute concentrations when comparing vehicle and IL-1β treatment and as percentage changes when comparisons are made between IL-1β alone and co-incubation with IL-1β and antidepressant compounds.

RNA Isolation and cDNA Synthesis

RNA was isolated using the RNeasy Micro Kit (Qiagen) following the manufacturer's instructions, and samples were kept frozen at -80°C until further use. RNA quantity and quality were assessed by evaluation of the A260/280 and A260/230 ratios using a Nanodrop spectrometer (NanoDrop Technologies). Superscript III enzyme (Invitrogen) was used to reverse-transcribe 1 μg total RNA, as previously described (Anacker et al., 2011).

qPCR Analyses

Quantitative real-time PCR (qPCR) was performed using HOT FIREPol EvaGreen qPCR Mix (Solis BioDyne), according to the SYBR Green method and using a Chromo 4 DNA engine (BioRad). For each target primer set, a validation experiment was performed to demonstrate that PCR efficiencies were within the range of 90–100%. Relative expression of the target gene IL-6 was normalized to the arithmetic mean of expression levels of the housekeeping genes glyceraldehyde 3-phosphate dehydrogenase and beta-actin and expressed as fold change compared with controls using the Pfaffl method (Pfaffl, 2001). At least three independent experiments were conducted on independent cultures, and each sample was tested in duplicate.

NF-kB Transactivation

To determine whether NF-kB transactivation by IL-1β was modified by antidepressant or n-3 PUFA treatment, proliferating cells in 75 cm^2 flasks (Nunclon) at 90% confluence were treated for 24 hours. Nuclear extracts were obtained using a commercially-available nuclear extract kit (Active Motif), according to the manufacturer's instructions, and frozen at -80°C until further use. Protein concentrations were quantified with a bicinchoninic acid colorimetric assay system (Merck) using a bovine serum albumin standard curve. Protein samples (nuclear lysates) were incubated with the kit reaction mixture in a ratio of 1:10 for 30 min at 37°C and absorbance was measured at 595 nm with a DTX 880 Multimode Detector (Beckman Coulter). NF-kB transactivation was analyzed using the TransAM NF-kB p65 ELISA-based kit from Active Motif according to the manufacturer's protocol, using 4 μg nuclear protein extracts. Absorbance was read on a DTX 880 Multimode Detector (Beckman-Coulter) at 450 nm.

Multiplex Cytokine and Chemokine Measurements

Cell supernatants were run on the Human Cytokine Magnetic 25-Plex Panel (Invitrogen) according to the manufacturer's instructions. Data were analyzed with a four-parameter logistic algorithm to derive concentrations of the samples from known standards using SoftMax Pro (Molecular Devices), and are presented semi-quantitatively for comparisons between vehicle treatment and IL-1β, and as percentage changes for comparisons between IL-1β alone and co-incubation with IL-1β and antidepressant compounds. Levels of seven chemokines and cytokines are reported (Table 2); sixteen of them were below and one was above the detection limits of the assay, while values for IL-1β were considered confounded due to IL-1β being added to the supernatant. Values were normalized to cell numbers, determined by crystal violet.

Drugs and Reagents

All drugs and reagents were purchased from Sigma–Aldrich, unless otherwise stated. Growth factors EGF and bFGF were purchased from Peprotech. Sertraline hydrochloride, venlafaxine hydrochloride, moclobemide, and agomelatine were dissolved in 100% dimethyl sulfoxide; EPA (>98% pure, extracted and purified from fish) and DHA (>98% pure, extracted and purified from algal vegetable oil) were dissolved in 100% ethanol; and IL-1β was dissolved in RMM.

Statistical Analyses

Data are presented as mean ± standard error of the mean. All statistical analyses were performed using GraphPad Prism 4.03 (Graph Pad, Inc) on three or more independent biological replicates (indicated as n). One-way analyses of variance with Dunnett's post hoc test were used for multiple comparisons of treatment conditions to control. Student's t-tests were used to compare means of two independent treatment groups. P-values < 0.05 were considered significant.

Results

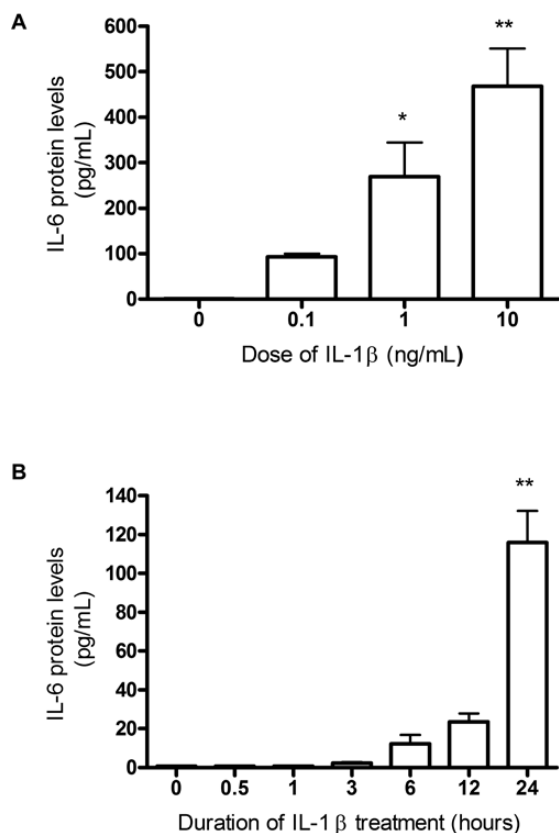### Human Hippocampal Progenitor Cells Respond to Immune Stimulation

We first sought to establish optimal conditions for inducing an inflammatory response in the progenitor cells by treating them with IL-1β. At baseline, IL-6 protein levels secreted in the supernatant were near to the detection limit of the ELISA assay (0.7 pg/mL). We observed a dose-dependent increase in IL-6 protein levels in response to incubation with IL-1β (Figure 1A). The greatest increase in IL-6 levels (674-fold, $p < 0.01$) was detected at a dose of 10 ng/mL of IL-1β (Figure 1A), with no impairment of cell viability; this dose was therefore selected for subsequent experiments. A time-dependent response was also demonstrated, with the greatest difference occurring after 24 hours of incubation (166-fold, $p < 0.01$, Figure 1B); this time point was therefore chosen for all subsequent experiments.

Monoaminergic Antidepressants and n-3 PUFAs can be Pro- and Anti-Inflammatory

To determine the immunomodulatory properties of the monoaminergic antidepressants, we co-incubated the cells with IL-1β (10 ng/mL) and one of the antidepressants (venlafaxine, agomelatine, sertraline, and moclobemide) at one of two concentrations (100 nM and 1 μM) for 24 hours. The presence of venlafaxine (1 μM) decreased IL-6 protein levels secreted in the supernatant by 14% compared to IL-1β alone ($p < 0.001$; Figure 2A), whereas sertraline (1 μM) showed the opposite effect, with a 27% increase in IL-6 levels ($p < 0.001$; Figure 2D). Agomelatine and moclobemide had no significant effect on the IL-6 levels at either of the doses tested (Figure 2A–D). We also sought to investigate the effects of the n-3 PUFAs, EPA and DHA, on immune processes. Upon co-incubation with IL-1β, EPA (10 μM) decreased IL-6 secretion by 8% ($p < 0.01$; Figure 2E), whereas DHA (10 μM) resulted in an increase of 12% ($p < 0.01$; Figure 2F). Smaller doses of EPA or DHA had no significant effect on the levels of IL-6 secreted.

Antidepressant Compounds that Affect IL-6 Protein Secretion have Differential Effects on IL-6 Gene Expression

In order to investigate whether the mechanisms underlying the changes detected in IL-6 secretion were due to differences in gene

Figure 1. Human hippocampal progenitor cells HPC03A/07 responded to immune stimulation. (A) Incubation of the cells for 24 hours led to a dose-dependent increase in IL-6, detected in the supernatant. (B) The cells also responded in a time-dependent manner to stimulation with IL-1β (10 ng/mL). Data are shown as mean ± standard error of the mean, $^*p < 0.05$, $^{**}p < 0.01$, compared with vehicle treatment. At least three independent experiments were conducted on at least three independent cultures (n = 3–4).

expression, we examined the effect of co-incubation with IL-1β and the four compounds shown to affect IL-6 (venlafaxine, sertraline, DHA, and EPA) on levels of IL-6 mRNA expression. On its own, IL-1β (10 ng/mL) stimulation time-dependently induced IL-6 mRNA levels, with the greatest effect occurring at 24 hours of incubation (36-fold increase, $p < 0.01$, Figure 3A). Co-incubation with sertraline (1 μM) for 24 hours increased IL-6 mRNA expression by 17% ($p < 0.05$, Figure 3B) compared with IL-1β alone, consistent with the direction and magnitude of change of levels of IL-6 protein at the same time point (Figure 2D, right-hand column). There was no significant effect of co-incubation of venlafaxine (1 μM) on IL-6 mRNA levels (Figure 3B). Co-incubation of EPA (10 μM) with IL-1β decreased IL-6 mRNA levels by 20% ($p < 0.01$, Figure 3B), in line with changes in IL-6 protein (Figure 2E, right-hand column). However, co-incubation of DHA (10 μM) along with IL-1β had no significant effect on IL-6 mRNA levels (Figure 3B).

Antidepressant Compounds Inhibit NF-kB Activity

In order to determine whether the antidepressant compounds modulated immune processes via mechanisms involving NF-kB, we studied NF-kB transactivation by quantifying activated NF-kB that binds specific DNA. IL-1β (10 ng/mL) induced NF-kB activation in a time-dependent manner, producing a 7-fold increase at 24 hours ($p < 0.001$, Figure 4A). Co-incubation with venlafaxine (1 μM) reduced NF-kB transactivation by 22% ($p < 0.05$, Figure 4B), consistent with changes to IL-6 protein secretion (Figure 2A, right-hand

column). Co-incubation with EPA (10 μM) also decreased NF-kB transactivation by 15% ($p < 0.001$, Figure 4B), again consistent with reduced IL-6 protein and mRNA levels (Figure 2E, right-hand column). However, in contrast with the increases of protein and/or mRNA levels of IL-6, co-incubation of IL-1β with either sertraline (1 μM) or DHA (10 μM) decreased NF-kB DNA binding (by 12% and 38%, respectively; $p < 0.001$, Figure 4B).

### Antidepressant Compounds Differentially Affect Inflammatory Cascades

Given some of the conflicting findings in our previous experiments of the effects of these antidepressant compounds on IL-6 protein secretion, IL-6 mRNA expression, and NF-kB activity, we sought to understand their effects on broader patterns of chemokine and cytokine secretion. We first characterized the cytokine and chemokine secretion profiles of the neural stem cells in unstimulated and stimulated conditions (Table 1). At baseline, IL-8, IL-15, and IL-1RA were detected at low levels (<40 pg/mL), with MCP-1 detected at high levels (>4,000 pg/mL). Stimulation with IL-1β (10 ng/mL) led to increases in the levels of the cytokines IFN-α, IL-6, IL-8, IL-15, the cytokine antagonist IL-1RA, and the chemokines monocyte chemoattractant protein-1 (MCP-1), interferon-gamma-inducible protein 10 (IP-10) and regulated on activation, normal T cell expressed and secreted (RANTES) (Table 1).

Comparisons were then made between the inflammatory profiles stimulated by IL-1β with and without each of the four immunomodulatory antidepressant compounds (Table 2). With regards to IL-6, the direction of changes detected in the multiplex array were identical to those detected using ELISA (in the experiments described above), and the magnitude was very similar; discrepancies in statistical significance were likely due to the superior accuracy of ELISA.

Co-incubation with sertraline (1 μM) increased levels of all cytokines and chemokines that were expressed in detectable levels (range +4% to 24%), showing the same direction of effect demonstrated on IL-6 levels via ELISA, reaching significance for IFN-α (+19%, $p < 0.05$), with the exception of the chemokine RANTES (-15%, n.s.). Co-incubation with EPA (10 μM) decreased the levels of all chemokines and cytokines measured, again consistent with the findings of secreted IL-6 protein via ELISA, reaching significance for IL-15 (-17%, $p < 0.01$), IL-1RA (-23%, $p < 0.01$), IP-10 (-16%, $p < 0.05$), and IL-6 (-14%, $p < 0.01$). Co-incubation with DHA (10 μM) increased the levels of all cytokines and chemokines, again consistent with changes in secreted IL-6 levels via ELISA, reaching significance for IL-1RA (+29%, $p < 0.01$), IFN-α (+16%, $p < 0.01$), IL-15 (+12%, $p < 0.05$), and IL-6 (+9.2%, $p < 0.01$), with the exception of RANTES (-14%, $p < 0.001$, Table 1B). Co-incubation with venlafaxine (1 μM) had mixed effects, with changes in both directions, though none reached significance.

Discussion

We report the novel finding that antidepressant compounds, commonly found to be anti-inflammatory in animal brains or human blood samples, diverge in their influence on immune processes in human hippocampal progenitor cells, an observation which may be pertinent to their clinical efficacy. In particular, we found that venlafaxine and EPA exerted an anti-inflammatory influence, with a corresponding decrease in NF-kB activity. In contrast, sertraline and DHA demonstrated pro-inflammatory effects, despite a reduction in NF-kB activity, suggesting alternative mechanisms underlying their immunomodulatory effects.
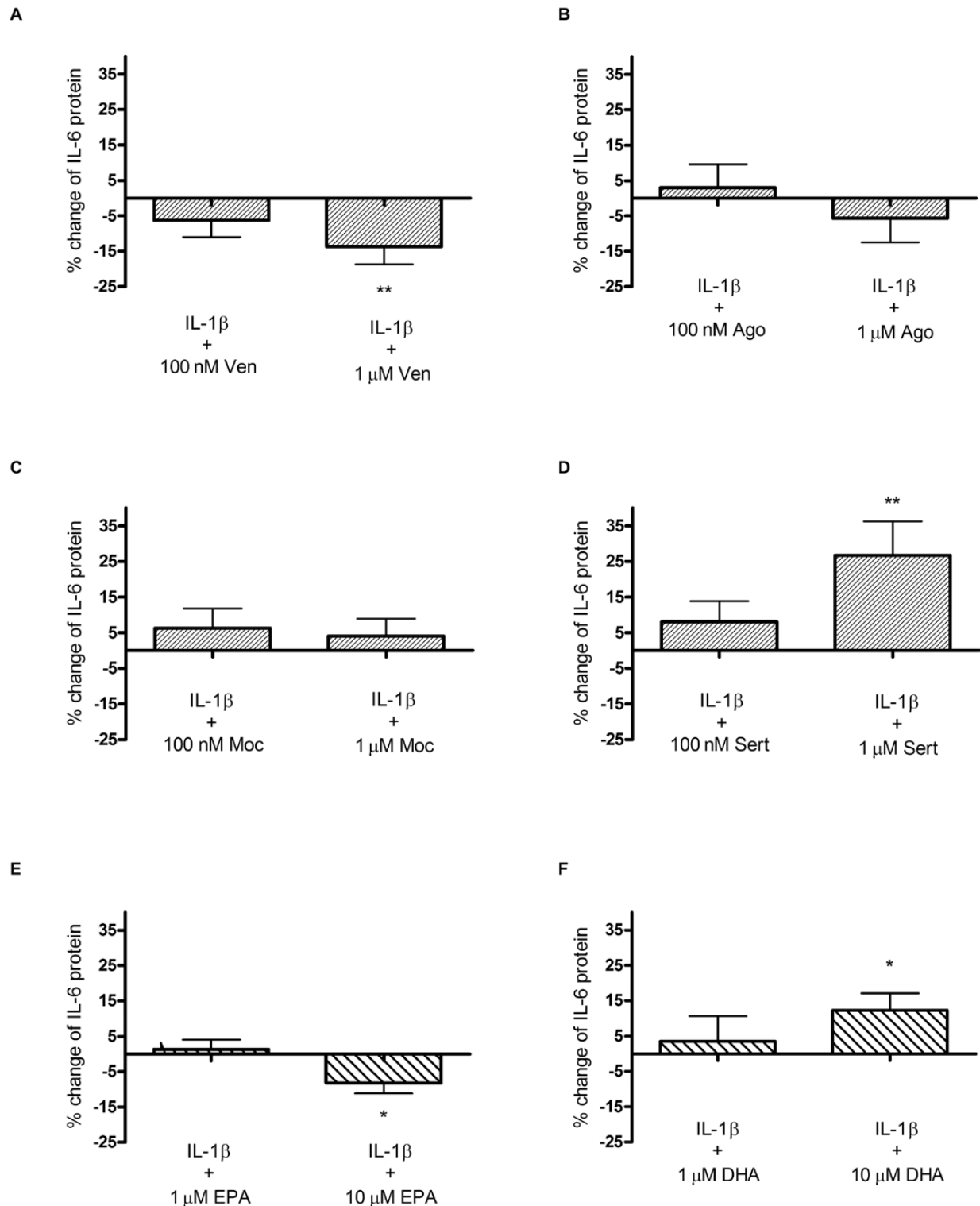
Figure 2. Modulation of IL-1β–induced IL-6 secretion in the human hippocampal progenitor cells by monoaminergic antidepressants or omega-3 polyunsaturated fatty acids. Cells were incubated with IL-1β (10 ng/mL) in the absence and presence of each drug for 24 hours. The levels of IL-6 secreted in the supernatant are presented as percentage changes from IL-1β treatment alone. (A–D) Venlafaxine (Ven, 1 μM) exerted anti-inflammatory effects, decreasing IL-6 secretion compared with IL-1β alone; sertraline (Sert, 1 μM) was pro-inflammatory, enhancing the inflammatory response to IL-1β; agomelatine (Ago) and moclobemide (Moc) had no significant immunomodulatory effects at the doses tested. Eight independent experiments were conducted (n = 8). (E and F) Eicosapentanoic acid (EPA) was anti-inflammatory, significantly reducing the inflammatory response to IL-1β, while docosahexanoic acid (DHA) had the opposite effect. At least six independent experiments were conducted (n = 6–8). Data are shown as mean ± standard error of the mean, $^*p < 0.01$, $^{**}p < 0.001$ compared with IL-1β treatment alone.

Venlafaxine and EPA both demonstrated anti-inflammatory effects, as measured by a decrease in secreted IL-6 levels in the context of IL-1β stimulation. This is consistent with previous studies that report venlafaxine to be anti-inflammatory in depressed patients (Lee and Kim, 2006), in an animal astroglia-microglia co-culture model (Vollmar et al., 2008), and in murine
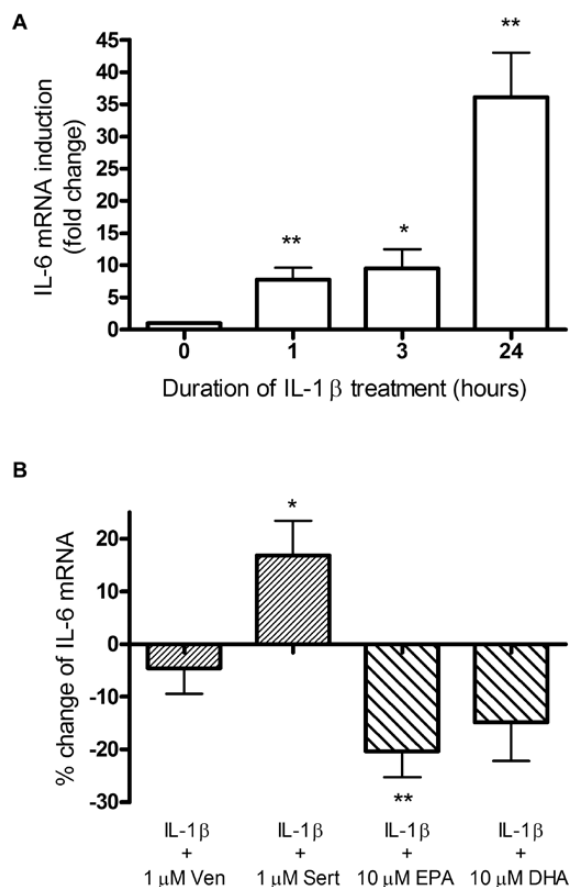
Figure 3. Antidepressant compounds have differential effects upon IL-6 mRNA expression. (A) The induction of IL-6 gene expression by IL-1β (10 ng/mL) was time-dependent. Results are expressed as fold change vs time of zero, and IL-6 levels were normalized using beta-actin and glyceraldehyde 3-phosphate dehydrogenase. At least four independent experiments were conducted (n = 4–6). (B) The four antidepressant compounds with modulatory effects on IL-6 protein levels were co-incubated with IL-1β (10 ng/mL) for 24 hours, and levels of IL-6 mRNA were measured. Results are presented as percentage changes from IL-1β incubation alone. (B) Venlafaxine (Ven; 1 μM) had no significant effect, while sertraline (Sert; 1 μM) increased IL-6 mRNA by 17% ($p < 0.05$). Co-incubation with eicosapentanoic acid (EPA; 10 μM) decreased IL-6 mRNA levels by 20% ($p < 0.01$), while docosahexanoic acid (DHA; 10 μM) had no significant effect. At least seven independent experiments were conducted (n = 7–12). Data are shown as mean ± standard error of the mean, *$p < 0.05$, **$p < 0.01$ compared with IL-1β treatment alone.

T-cell clones and peritoneal macrophages *in vitro* (Vollmar et al., 2009). Indeed, this matches reports finding that other noradrenaline reuptake inhibitors are also anti-inflammatory in rat primary cortical glial cells (O'Sullivan et al., 2009). EPA also reduced secretion of IL-15, IL-1RA, and IP-10, and downregulated IL-6 mRNA. This finding is in line with previous research showing that EPA exerts anti-inflammatory effects in stimulated murine macrophages (Novak et al., 2003), human macrophages (Hao et al., 2010), and rat microglia (Lu et al., 2010). Our study confirms and extends these previous findings to a clinically relevant model of human neural cells.

Contrary to our expectations, we found that both sertraline and DHA exerted pro-inflammatory effects in human hippocampal cells. Although sertraline and DHA have been reported to be anti-inflammatory in previous studies (Maes et al., 1999; Sutcigil et al., 2008; Lu et al., 2010; Antonietta Ajmone-Cat et al., 2012; Tynan and Weidenhofer, 2012), it is clear that their immunomodulatory effects are cell type–specific: for example,
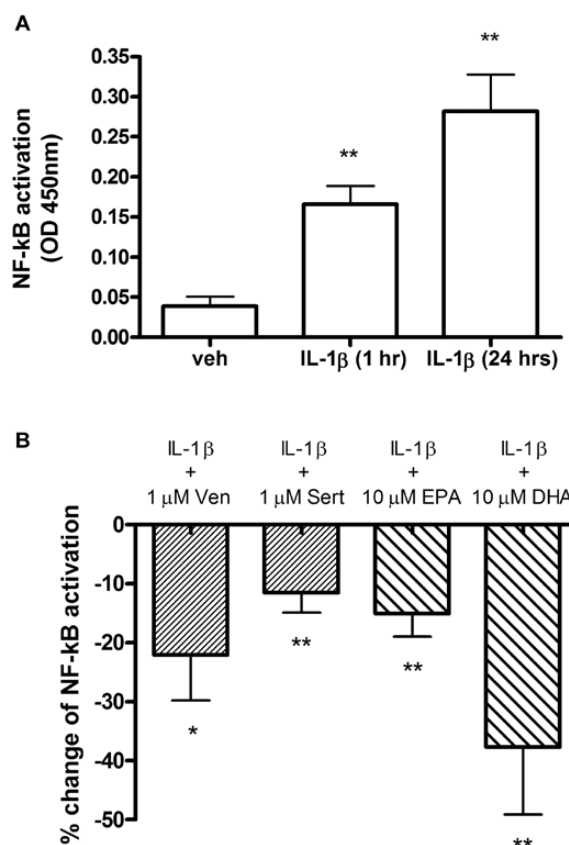
Figure 4. All four antidepressant compounds reduced NF-kB activity. (A) Stimulation of the neural stem cells with IL-1β (10 ng/mL) alone produced a time-dependent increase in NF-kB transactivation and DNA binding. Results are presented as fold change vs baseline levels of activation. At least 12 independent experiments were conducted (n = 12–14). (B) All four immunomodulatory antidepressant compounds reduced NF-kB activity when co-incubated with IL-1β (10 ng/mL). Results are presented as percentage changes of NF-kB activity from IL-1β alone. At least six independent experiments were conducted (n = 6–15). Data are shown as mean ± standard error of the mean, *$p < 0.05$, **$p < 0.001$ compared with IL-1β treatment alone. DHA, docosahexanoic acid; EPA, eicosapentanoic acid; Sert, sertraline; veh, vehicle; Ven, venlafaxine.

sertraline is anti-inflammatory in human PBMCs (Maes et al., 1999), while pro-inflammatory in rat neutrophils (Paschoal et al., 2013). Indeed, DHA has shown anti-inflammatory effects in murine microglia (Lu et al., 2010), while demonstrating pro-inflammatory effects, as measured by an increased IFN-γ/IL-10 ratio, in diluted whole blood (Maes et al., 2007). Another important determinant of immunomodulatory effects is dose of treatment. For example, at a dose of 2.5 μM, similar to the range employed in the present study, sertraline produced pro-inflammatory effects in murine microglia (Tynan and Weidenhofer, 2012), with anti-inflammatory effects only evident at concentrations above 10 μM, unlikely to correspond to clinically therapeutic levels (Schulz et al., 2012). Consistent with this notion, sertraline has been observed to increase the serum levels of cytokines in depressed patients (Marques-Deak et al., 2007). The same dose-related effects are recognized for DHA: in rat neutrophils, DHA increased TNF-α production at a dose of 25 μM, whereas it caused a reduction when used at 50 μM (Paschoal et al., 2013). We selected dosages of antidepressant compounds informed by both previous studies and likely therapeutic concentrations (Maes et al., 1999; Vollmar et al., 2008; Lu et al., 2010; Schulz et al., 2012), but we cannot exclude anti-inflammatory effects at concentrations larger than those employed here.

Table 1. Pattern of Chemokine and Cytokine Secretion in Unstimulated and IL-1β–Stimulated Hippocampal Progenitor Cells.

Protein	Unstimulated	Stimulated	Protein	Unstimulated	Stimulated
MCP-1	+++	++++	MIP-1α	-	-
IL-8	-	+++	GM-CSF	-	-
IL-15	+	++	MIP-1β	-	-
IL-1RA	+	++	IL-5	-	-
IP-10	-	+++	IFN-γ	-	-
IL-1β	-	++	IL-10	-	-
IL-6	-	+	IL-2	-	-
RANTES	-	+	IL-7	-	-
IFN-α	-	+	IL-2R	-	-
TNF-α	-	-	MIG	-	-
IL-13	-	-	IL-4	-	-
Eotaxin	-	-	IL-12	-	-
IL-17	-	-			

The pattern of chemokine and cytokine expression are indicated as: -, 0–40 pg/mL; +, 40–400 pg/mL; ++, 400–4 000 pg/mL; +++, 4 000–20 000 pg/mL; ++++, >40 000 pg/mL.

Table 2. Modulation of Cytokines and Chemokines by Antidepressant Compounds.

Protein	IL-1β + 1 µM Ven	IL-1β + 1 µM Sert	IL-1β + 10 µM DHA	IL-1β + 10 µM EPA
IL-8	-10.2 %	3.7 %	9.2 %	-13.4 %
IL-15	7.7 %	11.6 %	12.2 % *	-16.7 % **
IL-1RA	0.5 %	23.9 %	29.4 % **	-23.3 % **
IFN-α	1.4 %	18.6 % *	15.8 % **	-4.5 %
IP-10	-15.5 %	5.8 %	3.5 %	-16.1 % *
IL-6	-6.6 %	17.1 %	9.2 % **	-13.9 % **
RANTES	-2.5 %	-15.1 %	-13.9 % ***	-9.6 %

Modulation of cytokines and chemokines by antidepressant compounds is largely consistent with IL-6 changes. The effects of antidepressant compounds are shown as percentage changes in levels of the seven cytokines and chemokines in detectable levels in the supernatant compared with IL-1β (10 ng/mL) treatment alone. At least six independent experiments were conducted (n = 6–8). Data are presented as mean values, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared with IL-1β treatment alone. DHA, docosahexanoic acid; EPA, eicosapentanoic acid; Sert, sertraline; Ven, venlafaxine.

The contrast in the effects of sertraline (pro-inflammatory) and venlafaxine (anti-inflammatory), as well as in the effects of DHA (pro-inflammatory) and EPA (anti-inflammatory) are intriguing. These differences seem particularly pertinent in light of recent meta-analyses emphasising the importance of EPA over DHA in the antidepressant efficacy of fish oil preparations (Lin et al., 2010; Martins et al., 2012). While, to our knowledge, there are no direct comparisons of monoaminergic antidepressants in inflammation-induced depression, a meta-analysis found that venlafaxine is more effective than other SSRIs in treating depression (Smith, 2002), perhaps reflecting the anti-inflammatory properties identified in the present study. Moreover, a recent clinical trial has shown that EPA, but not DHA, reduces the incidence of depression in patients treated with IFN-α, a context in which pro-inflammatory cytokines are most clearly implicated in the development of depression (Su et al., 2014).

The finding that agomelatine and moclobemide did not demonstrate immunomodulatory effects in human neural stem cells as measured by IL-6 protein was somewhat distinct from findings in peripheral blood cells and animal models. Although there are no previous studies investigating the immunomodulatory effects of agomelatine *in vitro*, there have been studies in the periphery and brains of rats demonstrating an anti-inflammatory effect (Molteni et al., 2013). Moclobemide has also been found to be anti-inflammatory in primary rat mixed glial cell cultures (Bielecka et al., 2010) and in whole blood samples of healthy volunteers, as measured by IL-8 and TNF-α (but, interestingly, it had no effect on IL-6 levels; Lin et al., 2000). This suggests the possibility that either these are cell type-specific effects, or that moclobemide may have immunomodulatory

effects on cytokines other than IL-6 that may not have been identified in the present study.

Perhaps surprisingly, considering that venlafaxine, sertraline, EPA, and DHA demonstrated different effects on the expression of cytokines and chemokines, all four compounds reduced the activity of NF-kB. NF-kB has been implicated as a key target underlying the anti-inflammatory effects of many monoaminergic antidepressants (Zhu et al., 2008; Bielecka et al., 2010) but, to our knowledge, no previous study has examined the ability of venlafaxine to inhibit NF-kB activity, as demonstrated here. EPA has been previously observed to reduce endotoxin-induced activation of NF-kB in human macrophages (Novak et al., 2003) and in BV2 microglia (Moon et al., 2007), in line with our findings. Interestingly, both monoaminergic antidepressants and n-3 PUFAs may affect NF-kB activity through interactions with the neuronal cell membrane (Allen et al., 2007; Czysz and Rasenick, 2013). Specifically, these compounds have been thought to liberate G-protein alpha subunits from membrane-associated lipid rafts, leading to a cascade of intracellular events involving increases in adenylyl cyclase, cAMP, and PKA signalling, known to inhibit NF-kB activity (Parry and Mackman, 1997; Donati and Rasenick, 2005; Czysz and Rasenick, 2013).

The finding of decreased NF-kB activity in the context of pro-inflammatory effects with DHA is especially interesting. DHA has been observed to inhibit NF-kB in macrophages (Mullen et al., 2010), and to do so even more strongly than EPA (Weldon et al., 2007), as seen in the present study. However, DHA and EPA have distinct effects in a variety of cell types, in the pattern of cytokines affected, the time course of NF-kB inhibition (Mullen et al., 2010), and in the effects on cell membranes and

intracellular signalling pathways (Gorjão et al., 2009). Therefore, a different time-course of the effects of these compounds on NF-kB may explain the divergent effects on IL-6 levels. A further consideration is raised by recent work demonstrating anti-inflammatory effects of paroxetine in murine microglia without a significant effect on NF-kB; instead, the authors identified JNK1/2 and ERK1/2 pathways as more relevant (Liu et al., 2014).

In summary, we have demonstrated divergent effects of antidepressant compounds in a human, clinically-relevant, cellular model of depression, suggesting a possible explanation for the differences in efficacy seen in the different compositions of fish oil preparations, and perhaps in the putative variations of outcomes with monoaminergic antidepressants. An increased understanding of the molecular mechanisms underlying these effects may allow for more effective personalization of antidepressant choices based on the inflammatory status of depressed patients.

Acknowledgments

Statement of Interest

In the last three years, Dr Pariante has received fees as a speaker or as a member of an advisory board, as well as research funding, from pharmaceutical companies that commercialize or are developing antidepressants, such as Lilly, Servier, and Janssen. Dr Zunszain has received speaker fees from Servier.

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