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Noradrenaline induces IL-1ra and IL-1 type II receptor expression in primary glial cells and protects against IL-1β-induced neurotoxicity

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ABSTRACT

The pro-inflammatory cytokine interleukin-1β (IL-1β) plays a key role in initiating an immune response within the central nervous system (CNS), and is thought to be a significant contributor to the neurodegenerative process. The actions of IL-1β can be regulated by interleukin-1 receptor antagonist (IL-1ra), which prevents IL-1β from acting on the IL-1 type I receptor (IL-1RI). Another negative regulator of the IL-1 system is the IL-1 type II receptor (IL-1RII); a decoy receptor that serves to sequester IL-1. Consequently, pharmacological strategies that tip the balance in favour of IL-1ra and IL-1RII may be of therapeutic benefit. Evidence suggests that the neurotransmitter noradrenaline elicits anti-inflammatory actions in the CNS, and consequently may play an endogenous neuroprotective role. Here we report that noradrenaline induces production of IL-1ra and IL-1RII from primary rat mixed glial cells. In contrast, noradrenaline did not alter IL-1β expression, or expression of IL-1RI or the IL-1 type I receptor accessory protein (IL-1RAcp); both of which are required for IL-1 signalling. Our results demonstrate that the ability of noradrenaline to induce IL-1ra and IL-1RII is mediated via β-adrenoceptor activation and downstream activation of protein kinase A and extracellular signal-regulated kinase (ERK). In parallel with its ability to increase IL-1ra and IL-1RII, noradrenaline prevented neurotoxicity in cortical primary neurons induced by conditioned medium from IL-1β treated mixed glial cells. These data indicate that noradrenaline negatively regulates IL-1 system in glial cells and has neuroprotective properties in situations where IL-1 contributes to pathology.

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1. Introduction

Evidence suggests that inflammation contributes to pathology in neurodegenerative disease states such as Alzheimer's disease, Parkinson's disease, Stroke and Multiple Sclerosis (Allan et al., 2005; Block and Hong, 2005; Griffin and Mrak, 2002; Hauss-Wegrzyniak et al., 1998; Olsson et al., 2005), and also contributes to age-related neurodegeneration (Godbout and Johnson, 2006; Lynch and Lynch, 2002). The pro-inflammatory cytokine interleukin-1β (IL-1β) has been implicated as a key contributor to neuronal injury (see Allan et al., 2005; Basu et al., 2004). IL-1β signals via the IL-1 type I receptor (IL-1RI) and association of the IL-1RI with its accessory protein (IL-1RAcp) (Sims et al., 1988; Korherr et al., 1997), and this signalling system is present in the brain (Ericsson et al., 1995; Lynch and Lynch, 2002). The biological actions of IL-1β are regulated *in vivo* by IL-1 receptor antagonist (IL-1ra) (Carter et al., 1990); a molecule prevents IL-1β from binding to IL-1RI (Lundkvist et al., 1999; Gabay et al., 1997). *In vitro*, IL-1ra suppresses IL-1β-induced TNF-α production and iNOS expression in astrocytes (Liu et al., 1996), and protects against IL-1β and excitotoxin-induced neurotoxicity (Thornton et al., 2006). Furthermore, IL-1ra attenuates ischaemic and excitotoxic neuronal damage *in vivo* (Relton and Rothwell, 1992), and IL-1ra deficient mice exhibit increased neuronal injury following cerebral ischemia (Pinteaux et al., 2006).

The actions of IL-1 are regulated by binding to the IL-1 type II receptor (IL-1RII); a decoy receptor that sequesters IL-1β (Colotta et al., 1994). A number of studies have described IL-1RII expression in glial cells and in the intact brain (French et al., 1999; Parnet et al., 1994; Pinteaux et al., 2002). Furthermore, increased central expression of IL-1RII has been reported following insults such as cerebral ischemia (Wang et al., 1997), kainic acid administration (Nishiyori et al., 1997) and central administration of IL-1β (Docagne et al., 2005), most likely in an effort to limit the detrimental actions of IL-1β on neuronal function. Whilst there is no data available on its anti-inflammatory actions in the CNS, IL-1RII has been shown to inhibit the actions of IL-1 in the periphery in an arthritis model (Dawson et al., 1999). Considering the evidence, the balance between expression of IL-1β and the negative regulators IL-1ra and IL-1RII is likely to be of importance in combating neurodegeneration.

The monoamine neurotransmitter noradrenaline has anti-inflammatory properties, and plays an important role in maintaining the immunosuppressive environment within the brain (Feinstein et al., 2002; Marien et al., 2004). Noradrenergic innervation is widespread throughout the brain, and following its release noradrenaline can reach proximal glial cells (Aoki, 1992) and modulate glial cell function

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by activation of adrenoceptors (Mori et al., 2002). Consequently, here we examined the ability of noradrenaline to alter expression of IL-1β, IL-1ra and IL-1 receptors in rat primary mixed glial cells. Once we established that noradrenaline induced glial IL-1ra and IL-1RII expression, we investigated the signalling mechanisms involved. Finally we examined the ability of noradrenaline to protect neurons from neurotoxicity induced by conditioned media from IL-1β-stimulated glial cells.

2. Materials and methods

2.1. Materials

Noradrenaline, propranalol, phentolamine and dibutyryl adenosine 3′,5′-cyclic monophosphate (dbcAMP) were obtained from Sigma Chemical Co. (Poole, UK). Salmeterol and xamoterol were obtained from Tocris, UK, (9R,10S,12S)-2,3,9,10,11,12-Hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3′,2′,1′-kl]pyrrolo[3,4-i][1,6]benzodiazocine-10-carboxylic acid, hexyl ester (KT5720) was obtained from Calbiochem UK, and 1,4-Diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene (U0126) was obtained from Promega (UK). Antibodies and standards for interleukin (IL)-1β and IL-1ra ELISA reagents were obtained from R&D systems (UK) and Dr. Steve Poole, NIBSC (Potters Bar, UK) respectively. Rat IL-1RII antibody was purchased from Santa Cruz Biotechnology (USA) and antibodies for phosphorylated and total ERK were obtained from Cell Signalling Technologies (Ireland). Recombinant rat IL-1β was obtained from R&D systems (UK). Cytotox 96® Non-Radioactive Cytotoxicity Assay was purchased from Promega (UK). Gene expression assays for IL-1β, IL-1ra, IL-1RI, IL-1RII, IL-1RAcp, β-actin and Taqman master mix were obtained from Applied Biosystems. Cell culture reagents were obtained from Invitrogen (Ireland), and all other reagents were obtained from Sigma (UK) unless otherwise stated.

2.2. Primary cell culture

2.2.1. Preparation of cultured primary mixed glia

Primary mixed glial cultures were isolated and prepared from 1-day-old Wistar rats (BioResources Unit, Trinity College, Dublin 2, Ireland). Briefly, the rats were decapitated, the meninges were removed and the cortices were isolated and dissociated in sterile PBS. The cortices were incubated in Dulbecco's minimal essential media (DMEM:F12) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/ml penicillin and 100 IU/ml streptomycin (P/S) (complete DMEM:F12) for 20 min at 37 °C. The tissue was then triturated and gently filtered through a sterile mesh filter (40 µm). This cell suspension was collected by centrifugation for 2000 ×*g* for 3 min at 20 °C, and the pellet was re-suspended in warm complete DMEM:F12. Sterile 13-mm plastic coverslips in 24-well culture plates (Starstedt, Ireland) were pre-coated with poly-L-lysine (60 µg/ml) and viable cells were seeded at a density of 2×10^5 cells/ml. The mixed glial cells were cultured in a humidified atmosphere containing 5% CO_2:95% air at 37 °C and the medium was changed every 3 days. Experiments were performed when the mixed glial cells had been cultured for 12–14 days. This protocol yields primary mixed glial cultures containing astrocytes (70%) and microglia (30%) approximately, as seen by OX-42 (microglial stain) and GFAP (astrocyte stain) immunocytochemistry (Nolan et al., 2004). Mixed glial cultures were predominantly used in this study as they give a more appropriate representation of the *in vivo* environment than could be achieved using isolated microglia or astrocytes.

2.2.2. Preparation of cultured primary microglia and astrocytes

Enriched cultures of isolated microglia and astrocytes were prepared by seeding mixed glial cells on T25 cm^2 tissue culture flasks (Sarstedt, Ireland) at a density of 2×10^5 cells/ml in complete DMEM:F12 as before, supplemented with of GM-CSF (20 ng/ml) and M-CSF (5 ng/ml) (R&D systems). M-CSF and GM-CSF were included in the growth medium to stimulate microglial proliferation in order to facilitate harvesting a sufficient number of microglia for subsequent analyses. The media was changed after 7 days. On days 12–14 a loosely adherent layer of microglia was isolated by gentle agitation at 110 rpm for 2 h. The remaining monolayer of astrocytes was washed with sterile PBS and aspirated into solution using trypsin–EDTA. Isolated microglia and astrocytes were centrifuged at 2000 ×*g* for 3 min at 20 °C and viable cells were seeded on poly-L-lysine (60 µg/ml) coated 13-mm plastic coverslips at a density of 2×10^5 cells/ml, and incubated in a humidified atmosphere containing 5% CO_2:95% air at 37 °C. Enriched microglia and astrocyte cultures were grown for up to 3–4 days before treatment. This protocol yields enriched microglial and astrocyte cultures with greater than 95% purity as assessed using OX-42 (microglial stain) and GFAP (astrocyte stain) immunocytochemistry.

2.2.3. Preparation of cultured cortical neurons

Primary cortical neurons were isolated and prepared from 1-day-old Wistar rats (BioResources Unit, Trinity College, Dublin 2, Ireland). The rats were decapitated, the cerebral cortices were dissected, and the meninges were removed. The cortices were incubated in PBS with trypsin (0.25 µg/ml) for 25 min at 37 °C. The cortical tissue was then triturated in sterile PBS containing soy bean trypsin inhibitor (0.2 µg/ml) and DNase (0.2 mg/ml) and gently filtered through a sterile mesh filter (40 µm). The suspension was centrifuged at 2000 ×*g* for 3 min at 20 °C, and the pellet was re-suspended in warm neurobasal media (NBM), supplemented with heat-inactivated horse serum (10%), penicillin (100 units/ml), streptomycin (100 units/ml), and glutamax (2 mM). The suspended cells were plated at a density of 2×10^5 cells on circular 10-mm diameter glass coverslips, coated with poly-L-lysine (60 µg/ml), and incubated in a humidified atmosphere containing 5% CO_2:95% air at 37 °C. After 48 h, 5 ng/ml cytosine-arabinofuranoside was added to the culture medium to suppress the proliferation of non-neuronal cells. The media were changed for fresh media every 3 days, and the cells were grown in culture for up to 7 days before treatment. This protocol yields 97% pure cultures of primary neurons, as demonstrated by Neu-N immunocytochemistry (Minogue et al., 2003).

2.3. Cell culture treatments

All drugs were dissolved in complete DMEM:F12 (Gibco, Invitrogen), whereas control wells received complete DMEM:F12 alone. The cells were incubated with drugs at the doses and for the durations outlined in the various experiments below. A dose of 5 µM noradrenaline was used in most experiments based on pilot studies conducted in our laboratory, and published results from other laboratories studying the anti-inflammatory actions of noradrenaline. We chose equimolar concentrations of α and β-adrenoceptor antagonists to combat the actions of noradrenaline. Four to six replicates of each drug treatment were performed in each experiment. At the end of the incubation period cells were harvested for RNA isolation, and cell-free culture supernatants were removed and stored at −80 °C until cytokine production was determined by ELISA.

2.4. ELISA

IL-1ra and IL-1β concentrations were measured in cell-free supernatants collected after 6, 12, 24 and 48 h of culture using ELISA with cytokine specific antibodies and standards obtained from NIBSC, UK and R&D systems UK respectively. Assays were performed according to the manufacturer's instructions, and absorbance read at 450 nm using a microplate reader (Biotek instruments). Absorbance was then recalculated as a concentration (pg/ml) using a standard

curve derived using GraphPad Prism Software Version 4.00 (GraphPad software, Inc).

2.5. Real-time PCR

Total RNA was isolated from glial cells harvested following a 6 h culture period using a Total RNA isolation kit (Macherney Nagel). Any genomic DNA contamination was removed with the addition of DNase to the samples according to the manufacturer's instructions. The yield of the resulting purified RNA was determined by measurement of the absorbance at 260 nm in a spectrophotometer, and RNA samples were subsequently equalised. RNA samples were reverse transcribed into cDNA using a high capacity cDNA archive kit (Applied Biosystems) according to the manufacturer's protocol.

Real-time PCR was performed using an ABI Prism 7300 instrument (Applied Biosystems, Darmstadt, Germany) as previously described (Boyle and Connor, 2007). Taqman Gene Expression Assays (Applied Biosystems, Darmstadt, Germany) containing primers and a Taqman probe were used to quantify each gene of interest. Assay IDs for the genes examined were as follows: IL-1β (Rn00580432_m1), IL-1ra (Rn00573488_m1), IL-1RI (Rn00565482_m1), IL-1RII (Rn00588589_m1) and IL-1RAcp (Rn00492642_m1). PCR was performed in PCR plates in a 20 µl reaction volume (9 µl of diluted cDNA, 1 µl of Taqman Gene expression assay and 10 µl of Taqman® Universal PCR Master Mix) and PCR (40 cycles) was run in duplicate using ABIs universal cycling conditions. β-actin was used as endogenous control to normalize gene expression data, and an RQ value (2^{-DDCt}, where Ct is the threshold cycle) was calculated for each sample using Applied Biosystems RQ software (Applied Biosystems, UK). RQ values are presented as fold change in gene expression relative to the control group, which was normalized to 1.

2.6. Western immunoblotting

Serum-free cell cultures were lysed using ice-cold Hepes lysis buffer (Hepes (25 mM), $MgCl_2$ (5 mM), EDTA (5 mM) in d.d.H_2O) with the addition of DTT (5 mM) and 1% (v/v) of both protease/phosphatase inhibitors (Sigma, UK). The homogenized cell lysates were centrifuged at 13,000 rpm for 5 min at 4 °C to pellet the membrane fraction and insoluble debris. The resultant lysate containing the cytosolic fraction was assayed for protein content using a BCA protein assay (Pierce) and diluted to give equal protein concentrations. Samples were diluted 1:2 in sample buffer (0.5 mM Tris–HCl, pH 6.8, 10% glycerol, 10% SDS, 5% β-mercaptoethanol, and 0.05% w/v bromophenol blue) and boiled for 5 min. 20 µl aliquots were loaded onto 10% polyacrylamide gels. Proteins were separated by application of a constant voltage of 200 V for 35 min and then transferred onto nitrocellulose membranes at a constant voltage of 100 V for 1 h. After blocking in Tris-buffered saline (TBS)/Tween-20 (150 mM NaCl, 50 mM Tris–HCl, and 0.05% v/v Tween 20, pH 7.4) containing bovine serum albumin (BSA) (5% w/v), membranes were washed in TBS/Tween-20 and incubated in anti-phospho-extracellular signal-regulated kinase (ERK) [1:2000 in TBS/Tween-20 containing BSA (1% w/v); Cell Signalling Technology, USA] overnight at 4 °C. Membranes were washed and incubated in a horseradish peroxidase-conjugated anti-rabbit IgG [1:3000 in TBS/Tween-20 containing BSA (1% w/v); Amersham Biosciences, UK] for 1 h at room temperature before being reacted with Supersignal west dura chemiluminescent substrate (Pierce), exposed to CL-Xposure X-ray film (Pierce) for up to 20 s, and developed using an automated developer (Fugi). Initial control experiments determined the optimal time of exposure to film, which was maintained throughout the experimental procedure. Protein bands were quantified by densitometric analysis using Gelworks ID, Version 2.5.1. Gelworks provides a single value (in arbitrary units) representing the density of each blot.

To analyse total ERK content of the samples, membranes were stripped of antibody by incubating in reblot plus solution (Chemicon, Temecula, CA), washed, and blocked in TBS/Tween-20 containing BSA (5% v/v). No bands were detected in stripped blots incubated in secondary antibody alone, thus verifying the efficacy of the stripping protocol. Membranes were washed in TBS/Tween-20 and incubated with anti-ERK [1:2000 in TBS/Tween 20 containing BSA (1%); Cell Signalling Technology, MA, USA] overnight at 4 °C and then washed and incubated in a horseradish peroxidase-linked anti-rabbit IgG [1:3000 in TBS/Tween-20 containing BSA (1% w/v); Amersham Biosciences] before being reacted with Supersignal solution (Pierce). Blots were developed and protein bands quantified by densitometric analysis as outlined above. Phosphorylated ERK1 and ERK2 bands were normalized to the values of total ERK1 and ERK2 respectively.

Cellular membrane fractions to be analysed for membrane-bound proteins were incubated with 3-[(3-Cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) buffer (7 M Urea, 2 M thio Urea 2% (w/v) CHAPS, 65 mM DTT, distilledH_2O) and processed as above. As CHAPS buffer does not allow the use of a BCA protein assay for equalisation, a Biorad protein assay was performed instead. These membrane fractions were immunoblotted for IL-1RII protein using anti-IL-1RII [1:300 in TBS/Tween-20 containing BSA (1% w/v); Santa Cruz, USA] overnight at 4 °C, followed by incubation with anti-rabbit IgG [1:1000 in TBS/Tween-20 containing BSA (1% w/v); Amersham Biosciences, UK] for 1 h at room temperature before being reacted with Supersignal west dura chemiluminescent substrate (Pierce) and exposed to CL-Xposure X-ray film (Pierce) and bands were quantified by densometric analysis. Membranes were stripped of antibody and probed with a mouse monoclonal IgG antibody that targets rat β-actin (Sigma, 1:1000 dilution in TBS/Tween 20 containing 2% BSA) and immunoreactive bands detected using a peroxidase-linked anti-mouse IgG (1:2000 dilution in TBS/Tween 20 containing 2% BSA). Again, bands were quantified by densitometric analysis, and IL-1RII bands were normalized to the values of β-actin.

2.7. Quantification of neuronal cell death

A Cytotox 96® Non-Radioactive Cytotoxicity Assay (Promega, UK) was used to assess the levels of lactate dehydrogenase (LDH) release into the culture supernatant of treated cells according to the manufacturer's protocol. Briefly, 2 wells of each culture plate were combined per treatment group. Total cellular LDH was obtained from adherent primary neuronal cells by exposing them to 15 µl of lysis solution (9% (v/v) Triton-x-100 in distilled H_2O) per 100 µl of culture medium, followed by incubation at 37 ºC for 45 min. 50 µl of media supernatant or cellular lysate was aspirated from duplicate treatment and control groups and added to 50 µl of substrate mix (Kit) for 30 min at 37 ºC. Following this, 50 µl of stop solution was added to arrest the reaction and the absorbance is measured at 490 nm. Percentage cytotoxicity was obtained by expressing LDH absorbance in the supernatant (released)/LDH absorbance in the cell lysate (total cellular LDH). Data are expressed as fold-change of released LDH over media control.

2.8. Statistical analysis of data

All values are expressed as mean ± standard error of the mean (S.E.M.). Data were analysed using a Student *t*-test or a one- or two-way analysis of variance (ANOVA) followed, where appropriate, by a Newman–Keuls *post hoc* test (GB-Stat). A $P<0.05$ was considered statistically significant.

3. Results

3.1. Noradrenaline induces expression of IL-1ra and IL-1RII in primary mixed glial cells

Treatment of primary cortical glial cells with noradrenaline (5 µM) for 6 h significantly increased IL-1ra ($P<0.05$). Similarly, using ELISA it

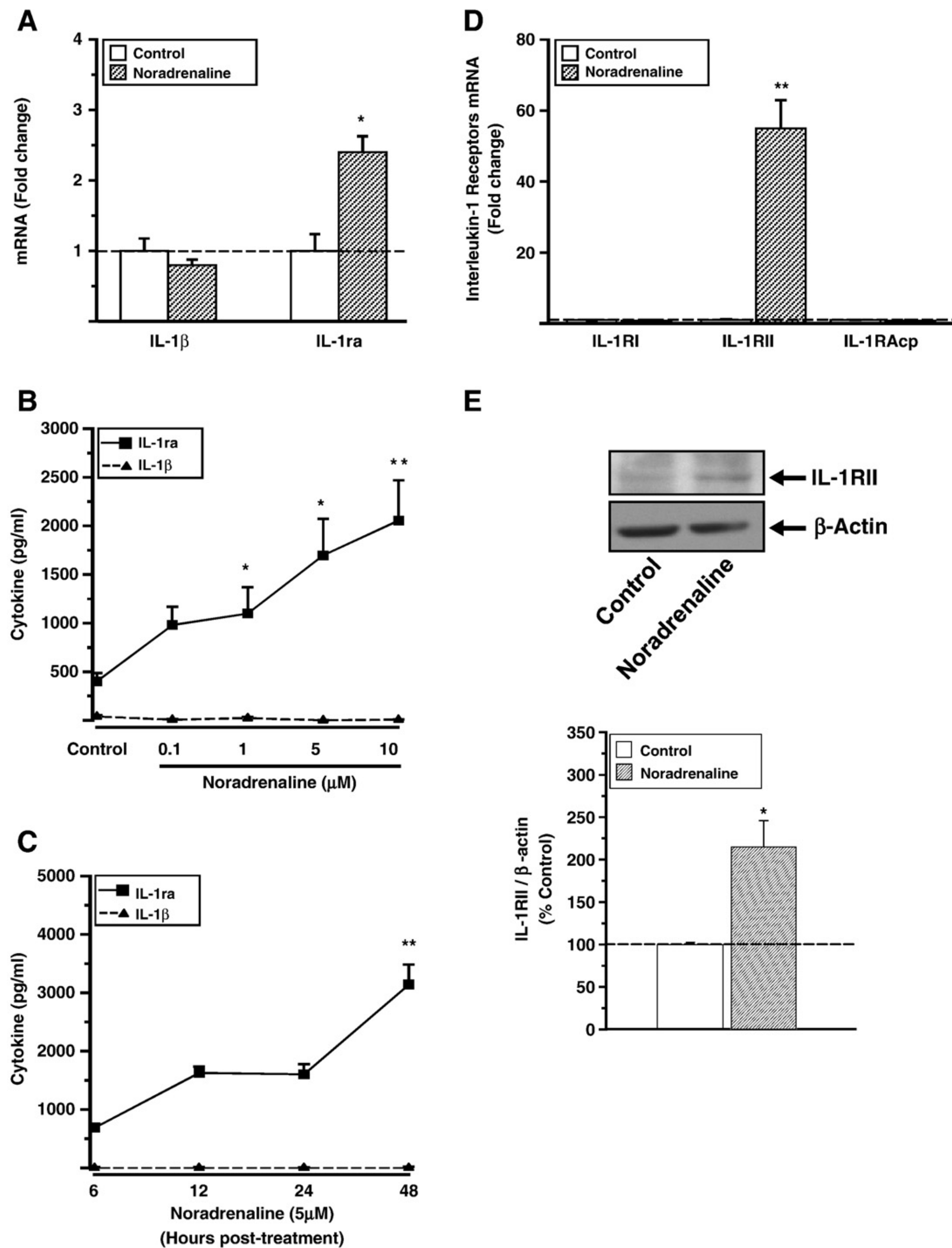

Fig. 1. Noradrenaline induced IL-1ra and IL-1RII expression in glial cells. Noradrenaline (5 μM) induces a significant increase in IL-1ra and IL-1RII mRNA expression in primary cortical mixed glial cells after a 6 h incubation and failed to alter expression of IL-1β, IL-1RI or IL-1RAcp (A and D). The noradrenaline-induced increase of IL-1ra was time and dose-dependent, with significant effects observed 48 h post-treatment in the 1–10 μM concentration range of noradrenand C). Noradrenaline (5 μM) also induced an increase in membrane-bound IL-1RII protein after a 24 h treatment (E). Data are expressed as mean ± S.E.M. ($n = 4$–6). (A, D and E) *$P < 0.05$, **$P < 0.01$ vs. control (Student *t*-test), (B and C) *$P < 0.05$, **$P < 0.01$ vs. control (Newman–Keuls test).

was demonstrated that noradrenaline increased IL-1ra protein production which was maximal 48 h following treatment (Fig. 1C), and this increase in IL-1ra production observed at 48 h occurred in a dose-dependent manner, with noradrenaline (1–10 μM) eliciting significant effects (Fig. 1B). Treatment of primary cortical glial cells with noradrenaline (5 μM) for 6 h significantly also increased IL-1RII ($P < 0.01$) mRNA expression. Similarly, using Western immunoblotting it was demonstrated that treatment with noradrenaline (5 μM) increased IL-1RII protein expression (Fig. 1E). In contrast, noradrenaline treatment failed to alter IL-1β expression/production or expression of IL-1RI or IL-1RAcp (Fig. 1A–D).

3.2. *β-adrenoceptors mediate the ability of noradrenaline to induce IL-1Ra and IL-1RII in primary mixed glial cells*

As previous literature has identified the β-adrenoceptor in mediating the anti-inflammatory properties of noradrenaline *in vitro* (Feinstein, 1998; Feinstein et al., 2002; Frohman et al., 1988; Galea

et al., 2003), the following studies were carried out to determine which adrenoceptor subtype mediated the ability of noradrenaline to induce IL-1ra and IL-1RII expression in glial cells. Pre-treatment of the primary glia with the α-adrenoceptor antagonist, phentolamine (5 μM) failed to attenuate the ability of noradrenaline to induce IL-1ra and IL-1RII expression (Fig. 2A–B). Conversely, pre-treatment with the β-adrenoceptor antagonist propranolol (5 μM) for 30 min significantly blocked noradrenaline-induced IL-1ra and IL-1RII expression ($P<0.01$) (Fig. 2C–D). Furthermore, treatment of glial cells with the selective β_2-adrenoceptor agonist salmeterol (0.1–10 μM) mimicked the ability of noradrenaline to induce IL-1ra ($P<0.01$) and IL-1RII ($P<0.01$) expression (Fig. 2E–F). Whilst treatment of glial cells with the selective β_1-adrenoceptor agonist, xamoterol (1–10 μM) increased IL-1ra mRNA expression ($P<0.01$) it failed to induce IL-1RII expression (Fig. 2E–F).

3.3. A role for the cAMP–PKA pathway and ERK activation in the ability of noradrenaline to induce IL-1Ra and IL-1RII in primary mixed glial cells

Consistent with the ability of β-adrenoceptors to activate the cAMP–PKA pathway, treatment of mixed glial cells with the lipophilic cAMP analogue, db-cAMP (100 μM) for 6 h, significantly induced expression of IL-1ra ($P<0.01$) and IL-1RII ($P<0.01$) (Fig. 3A–B). Conversely, a 30 min pre-treatment with the selective PKA antagonist, KT5720 (1 μM), attenuated the noradrenaline (5 μM)-induced increase in IL-1ra ($P<0.05$) and IL-1RII ($P<0.01$) expression (Fig. 3C–D).

Activation of the mitogen activated protein kinase (MAPK), extracellular response kinase (ERK), by either noradrenaline or cAMP-inducing agents has previously been shown to be neuroprotective (Chen et al., 2007; Troadec et al., 2002). Consequently, in this study the involvement of ERK in the noradrenaline-induced increase in IL-1ra and IL-1RII was assessed. Consistent with the previous literature, noradrenaline induced an increase in ERK phosphorylation in primary mixed glial cultures via β-adrenoceptor activation, as pre-treatment with the β-adrenoceptor antagonist, propranolol, inhibited this increase ($P<0.05$) (Fig. 4A). As MEK1/2 is directly upstream of ERK and induces ERK phosphorylation, we examined the ability of the selective MEK1/2 inhibitor U0126 to inhibit the induction of IL-1ra and IL-1RII induced by noradrenaline. Consistent with a role for ERK in the ability of noradrenaline to induce IL-1ra and IL-1RII, a 30 min pre-treatment of primary glial cells with U0126 (10 μM), inhibited noradrenaline-induced IL-1ra ($P<0.01$) and IL-1RII ($P<0.05$) mRNA expression (Fig. 4B–C).

3.4. Noradrenaline induces IL-1ra and IL-1RII expression in enriched microglial cultures but not in enriched astrocyte cultures

As the mixed preparation of primary glial cells used in these experiments best represents the glia cell ratio *in vivo*, it was proposed that this would be the optimum *in vitro* system to assay the basal expression of inflammatory cytokines and receptors, induced by noradrenaline. However, previous literature has identified microglia as being the predominant producer of IL-1 ligands and the sole producer of IL-1ra in the CNS (Liu et al., 1998; Pinteaux et al., 2006). To clarify the source of IL-1 ligands and receptors induced by noradenaline in mixed glia, primary cultures of enriched microglia and astrocytes were

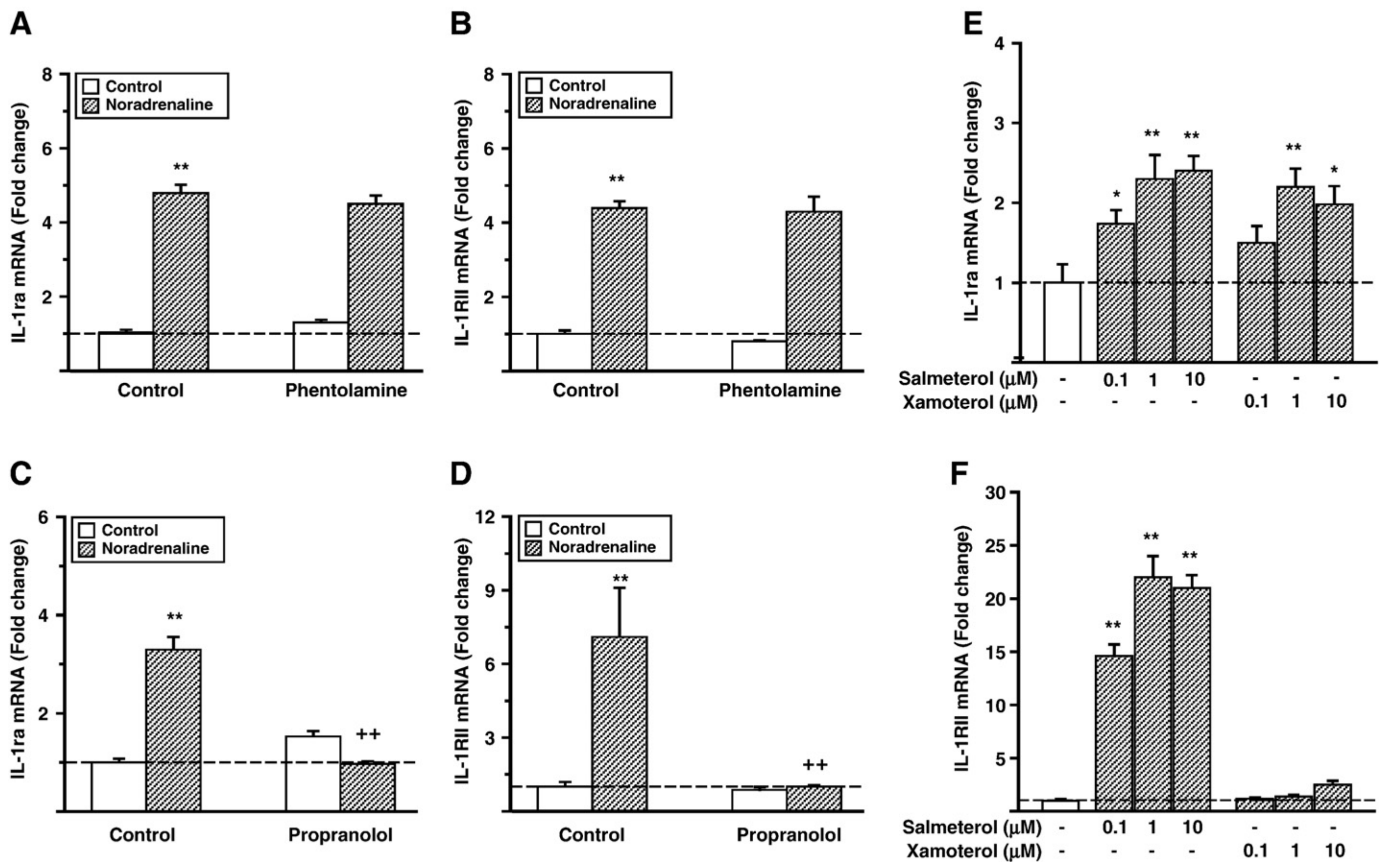

Fig. 2. β-adrenoceptors mediate the ability of noradrenaline to induce IL-1ra and IL-1RII expression in glial cells. Pre-treatment of primary mixed glial cells with the non-selective α-adrenoceptor antagonist, phentolamine (5 μM) failed to block noradrenaline (5 μM)-induced expression of IL-1ra and IL-1RII (A and B). Conversely, pre-treatment with the non-selective β-adrenoceptor antagonist, propranolol (5 μM) attenuated the noradrenaline-induced increase in IL-1ra and IL-1RII expression (C and D). The selective β_2-adrenoceptor agonist salmeterol (0.1–10 μM) induced a significant increase in IL-1ra and IL-1RII mRNA expression from primary mixed glial cells after a 6 h incubation (E and F). The selective β_1-adrenoceptor agonist xamoterol (1 μM and 10 μM) induced a significant increase in IL-1ra, but failed to alter IL-1RII mRNA expression (E and F). Data are expressed as mean ± S.E.M. as fold-change over media control ($n=6$). *$P<0.05$, **$P<0.01$ vs. control, ++$P<0.01$ vs. noradrenaline (5 μM) (Newman–Keuls test).

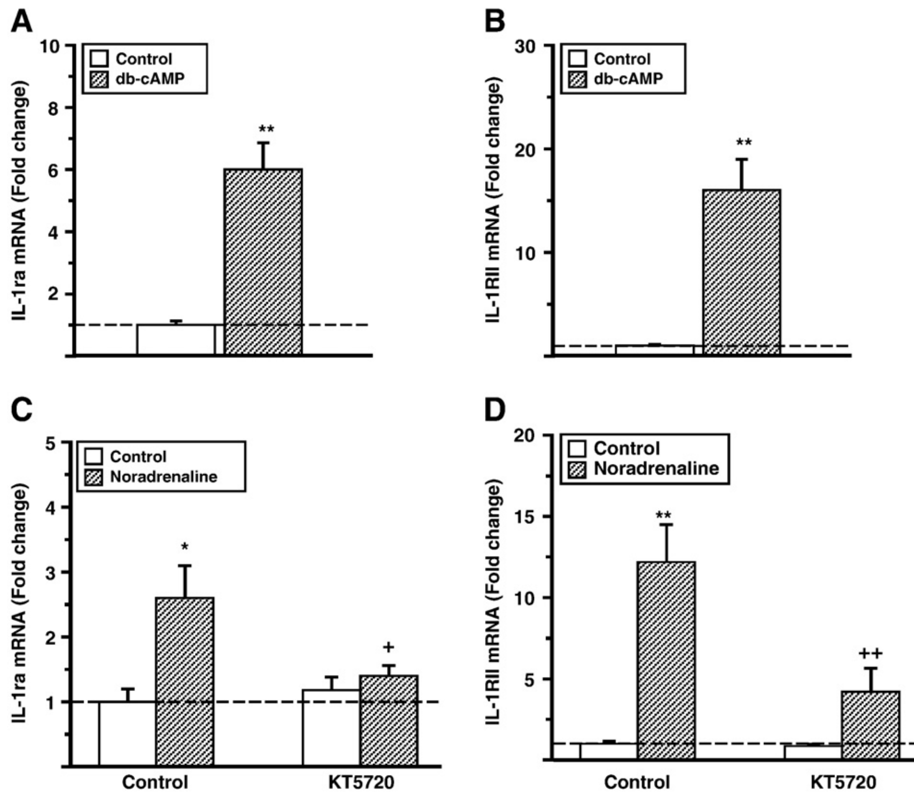

Fig. 3. Activation of the cAMP/protein kinase A pathway mediates the noradrenaline-induced increase in glial IL-1ra and IL-1RII expression. Treatment with the cAMP analogue dbcAMP (100 µM) induced a significant increase in IL-1ra and IL-1RII mRNA expression from primary mixed glial cells (A and B). Pre-treatment with selective PKA antagonist, KT5720 (1 µM) attenuated noradrenaline (5 µM)-induced IL-1ra and IL-1RII expression. Data are expressed as mean ± S.E.M. as fold-change over media control ($n = 6$). (A and B) $^{**}P < 0.01$ vs. media control (Student *t*-test). (C and D) $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control, $+P < 0.05$, $++P < 0.01$ vs. noradrenaline (Newman–Keuls test).

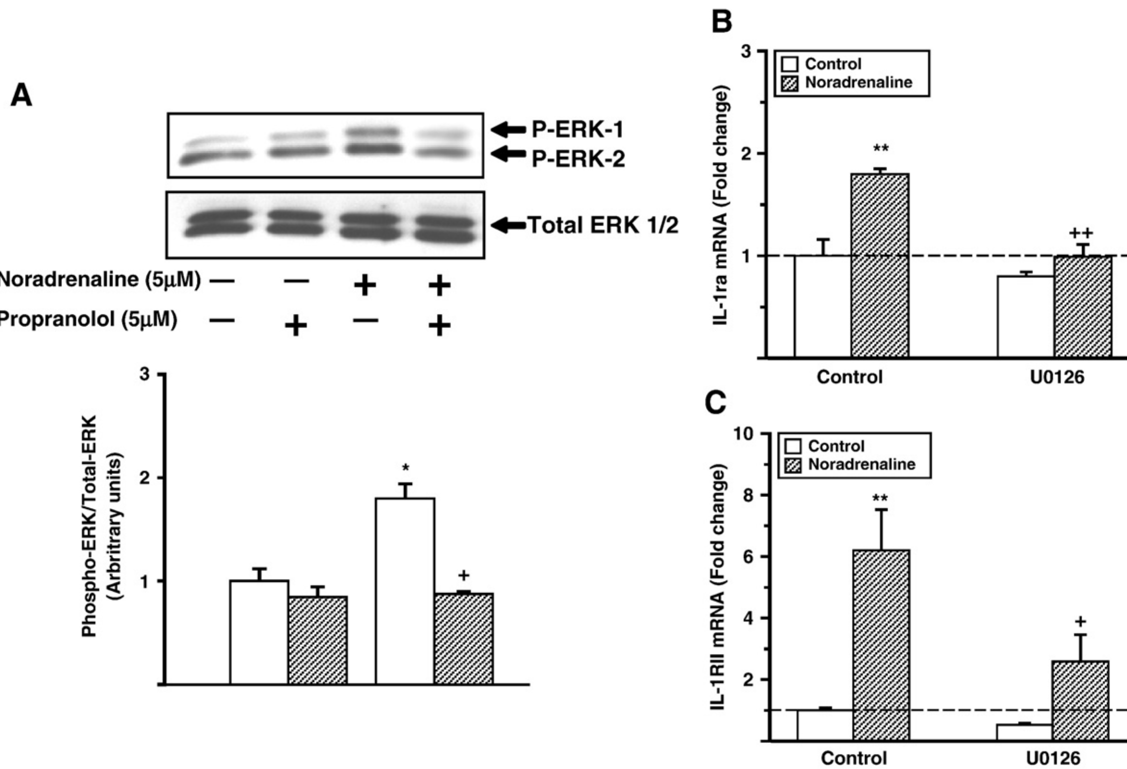

Fig. 4. A role for ERK in the noradrenaline-induced increase in IL-1ra and IL-1RII expression in glial cells. A 15 min treatment with noradrenaline (5 µM) increased ERK phosphorylation in glial cells, and this increase was blocked by pre-treatment with the β-adrenoceptor antagonist propranolol (5 µM) (A). In addition, pre-treatment with the selective Mek 1/2 antagonist, U0126 (10 µM) attenuated the noradrenaline-induced increase in IL-1ra and IL-1RII expressions (B and C). Data are expressed as mean + S.E.M. ($n = 5$–6). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control, $++P < 0.01$, $+P < 0.05$ vs. noradrenaline (Newman–Keuls test).

assessed for their ability to express both IL-1ra and IL-1RII mRNA expressions. The results demonstrated that noradrenaline (5 μM) significantly induced mRNA expression of IL-1ra ($P<0.05$) and IL-1RII ($P<0.05$) in enriched microglial cultures, but not in enriched astrocytes cultures (Fig. 5A–D).

3.5. Noradrenaline induces IL-1ra and IL-1RII expression in primary mixed glial cells in the presence of an inflammatory stimulus (IL-1β)

Under pathological conditions glial cells in the central nervous system may have already been exposed to IL-1β (Allan et al., 2005), thus it was important to determine if noradrenaline could induce expression of IL-1ra and IL-1RII in glial cells exposed to IL-1β. The results demonstrated that noradrenaline was equally as effective at inducing IL-1ra and IL-1RII in cells treated with IL-1β (5 ng/ml) as it was in unstimulated glial cells (Fig. 6A–B). In contrast, noradrenaline failed to alter the significant ($P<0.01$) induction of IL-1RI expression induced by IL-1β treatment (Fig. 6D), but significantly ($P<0.01$) attenuated IL-1β-induced IL-1β expression (Fig. 6C).

3.6. Noradrenaline inhibits IL-1β-induced neurotoxicity

It has been previously demonstrated that IL-1β is not directly neurotoxic to healthy neurons *in vitro,* but can induce neurotoxicity by increasing glial-derived neurotoxic agents such as reactive nitrogen species, free radicals, and inflammatory cytokines (Rothwell, 2003; Ma and Zhao, 2002; Thornton et al., 2006). As such, the following study was carried out to asses the ability of noradrenaline to limit neurotoxicity in primary cortical neurons treated with conditioned media from IL-1β stimulated glial cells. A dose of 5 ng/ml of IL-1β was employed based on previous studies which established the ability of IL-1β-stimulated glial cells to elicit neurotoxicity (Thornton et al., 2006). Primary cortical mixed glial cells were treated for 48 h with either a media vehicle or noradrenaline (5 μM) followed by a 24 h treatment with recombinant IL-1β (5 ng/ml). This glial-conditioned media (CM) was added to primary cortical neuronal cells for 48 h. As a control, primary neurons were also treated with IL-1β (5 ng/ml) to asses the direct effects of IL-1β on neuronal toxicity. IL-1β (5 ng/ml) had no significant direct affect on neuronal toxicity (as measured by LDH release) (Fig. 7). However, IL-1β (5 ng/ml)-conditioned glial media significantly induced neuronal cell death at a 48 h time-point ($P<0.01$) and this was attenuated by pre-treatment with noradrenaline (5 μM) ($P<0.01$) (Fig. 7). Treatment of neurons with noradrenaline (5 μM) alone did not alter neuronal viability (data not shown).

4. Discussion

The major finding of this study is that the monoamine neurotransmitter noradrenaline induced expression of two negative regulators of the IL-1 system (IL-1ra and IL-1RII) in primary mixed glial cell cultures. The importance of IL-1ra in regulating the actions of IL-1β has become apparent in recent years after it was shown that an endogenous imbalance between IL-1ra and IL-1β is of pathophysiological significance in peripheral inflammatory disorders including rheumatoid arthritis and inflammatory bowel disease (Palin et al., 2004). IL-1ra is generally found to be up-regulated in a dose-dependent manner with IL-1β to dampen down the inflammatory effects of IL-1β over-expression (Palin et al., 2004). However in this study it is noteworthy that the increase in glial IL-1ra production induced by noradrenaline does not occur secondary to IL-1β production, as IL-1β production was not altered by noradrenaline. Thus it appears that noradrenaline induces a selective upregulation of IL-1ra; the anti-inflammatory ligand in the IL-1 system. In conjunction with inducing IL-1ra, our results demonstrate that noradrenaline

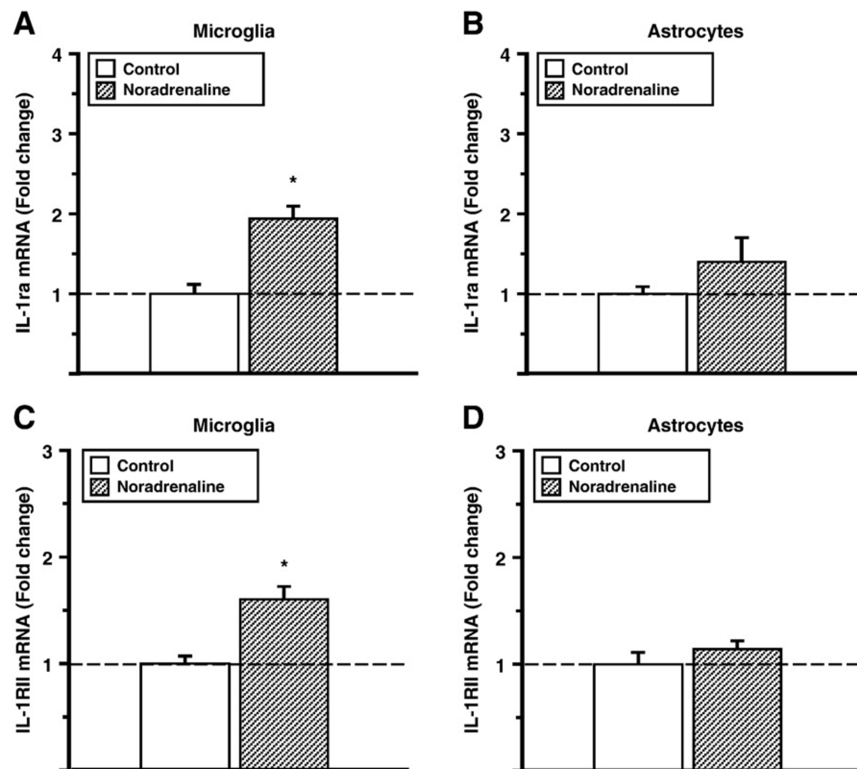

Fig. 5. Noradrenaline induces IL-1ra and IL-1RII expression in microglia but not astrocytes. Noradrenaline (5 μM) induced an increase in IL-1ra and IL-1RII mRNA in primary cultures of enriched cortical microglia 6 h post-treatment (A and C). Noradrenaline failed to alter IL-1ra or IL-1RII expression in primary cultures of enriched astrocytes (B and D). Data are expressed as mean ± S.E.M. ($n=4$). *$P<0.05$ vs. control (Student *t*-test).

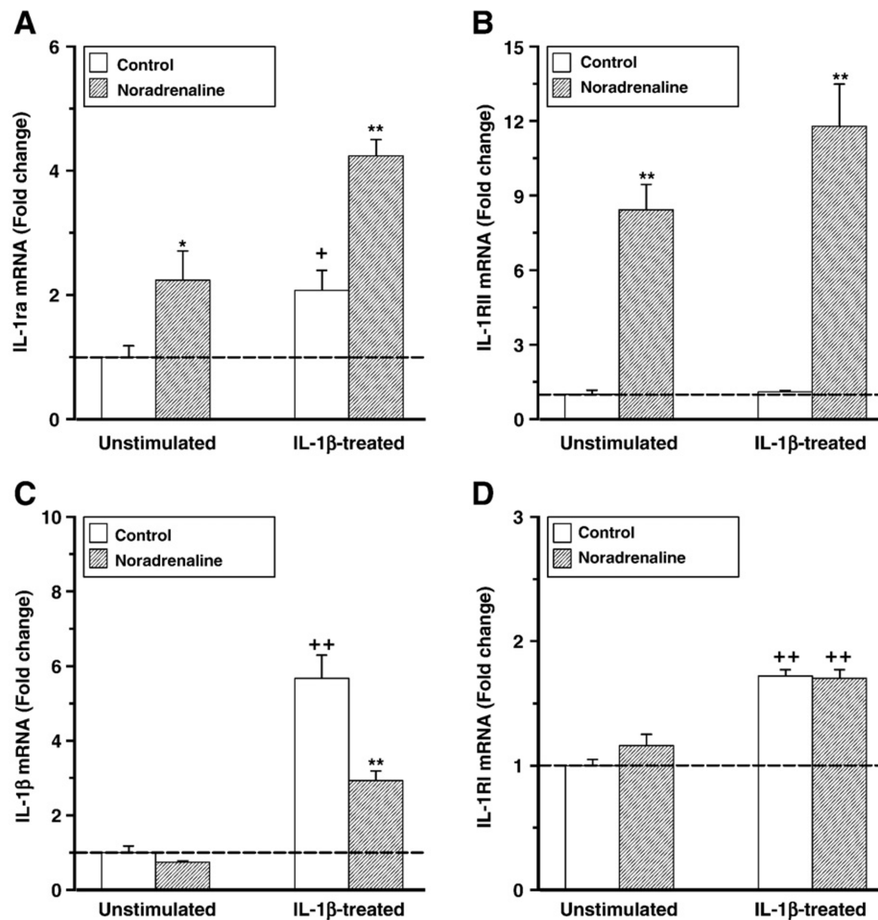

Fig. 6. Noradrenaline induces glial IL-1ra and IL-1RII expression in the presence of an inflammatory stimulus (IL-1β). Noradrenaline (5 μM) induced expression of IL-1ra and IL-1RII mRNA in both unstimulated glial cells, and glial cells treated with IL-1β (5 ng/ml) (A and B) for 6 h. Noradrenaline failed to alter the IL-1β-induced increase in IL-1RI expression (D), but attenuated IL-1β-induced IL-1β expression (C). Data are expressed as mean + S.E.M. ($n = 5$–6). *$P < 0.05$, **$P < 0.01$ vs. control counterparts, +$P < 0.05$, ++$P < 0.01$ vs. unstimulated counterparts (Newman–Keuls test).

induces a robust increase in IL-1RII expression in glial cells. This is significant, as IL-1RII is a decoy receptor that serves to sequester IL-1 and therefore like IL-1ra is a negative regulator of the IL-1 system. However, it must be noted that the observed increase in IL-1RII protein expression tends to be much lesser in magnitude than what we observe at the mRNA level. It is also noteworthy that noradrenaline is equally as effective at inducing IL-1ra and IL-1RII in the presence of an inflammatory stimulus, (IL-1β), as it is in unstimulated glial cells. This is an important finding, as under pathological conditions glial cells in the central nervous system can be exposed to IL-1β (Allan et al., 2005).

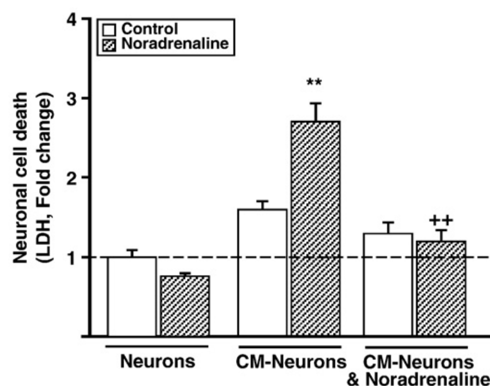

Fig. 7. Noradrenaline inhibits neurotoxicity in primary cortical neurons induced by conditioned media from IL-1β-stimulated glial cells. Treatment of neurons alone with IL-1β (5 ng/ml) did not induce neurotoxicity (LDH release). In contrast, treatment of neurons with conditioned media from IL-1β-treated mixed glial cells (CM) induced a neurotoxic response as measured by a significant increase in LDH release. This neurotoxic action of conditioned media from IL-1β treated glial cells was not evident if glial cells were pre-treated with noradrenaline (5 μM) prior to IL-1β exposure. Data are expressed as mean ± S.E.M. ($n = 4$). **$P < 0.01$ vs. control CM-neurons, ++$P < 0.01$ vs. IL-1β CM-neurons (Newman–Keuls test).

It is clear from our studies that induction of IL-1ra and IL-1RII by noradrenaline is mediated by activation of β-adrenoceptors. Specifically, induction of IL-1ra and IL-1RII by noradrenaline was completely blocked by the β-adrenoceptor antagonist propranolol. Furthermore, the selective β_2-adrenoceptor agonist salmeterol, mimicked the NA-induced increase IL-1ra and IL-1RII production. Whilst the β_1-adrenoceptor agonist, xamoterol, increased IL-1ra expression it failed to induce expression of IL-1RII. These data are consistent with the fact that both β_1- and β_2-adrenoceptors are expressed on glia, however, β_2-adrenoceptors are more abundantly expressed on microglia and produce greater amounts of cAMP upon stimulation with agonist than β_1-adrenoceptors (Mori et al., 2002). These data are also concordant with previous reports indicating that activation of β-adrenoceptors on glial cells provides protection from inflammatory insults (Junker et al., 2002) and decreases LPS-induced pro-inflammatory cytokine production (Dello Russo et al., 2004; Mori et al., 2002).

Consistent with the ability of β-adrenoceptors to activate adenylate cyclase and up-regulate intracellular cAMP, the lipophilic cAMP analogue, db-cAMP, increased both IL-1ra and IL-1RII expression. Furthermore, as protein kinase A (PKA) is a major intracellular target for cAMP, it is noteworthy that the selective PKA inhibitor, KT5720, blocked the noradrenaline-induced increase in IL-1ra and IL-

1RII expressions. It has been suggested that G-protein coupled receptors, such as the β-adrenoceptor, activate ERK-1 and -2, either downstream of second messengers like cAMP and Ca^{2+} or, that the Gα- and/or Gβγ-subunits may directly or indirectly activate the Ras/Raf pathway upstream of ERK through adapter proteins, tyrosine kinases (Faure et al., 1994; Kolch, 2005; Lopez-Ilasaca, 1998). Activation of ERK by either noradrenaline or cAMP-inducing agents has previously been shown to be neuroprotective by inducing brain-derived neurotrophic factor (BDNF) from hippocampal neurons as well as protecting dopaminergic neurons from oxidative stress-induced toxicity (Chen et al., 2007; Troadec et al., 2002). Consequently, the involvement of ERK in mediating the noradrenaline-induced increases in IL-1ra and IL-1RII was assessed. Consistent with the previous literature, noradrenaline induced an increase in ERK phosphorylation in primary mixed glial cultures via β-adrenoceptor activation, indicated by the fact that the β-adrenoceptor antagonist, propranolol, inhibited this increase. Moreover, treatment of glial cells with the MEK inhibitor, U0126, inhibited noradrenaline-induced IL-1ra and IL-1RII mRNA expressions. In all these data indicate that activation of the ERK pathway downstream of β-adrenoceptor activation mediates the induction of IL-1ra and IL-1RII induced by noradrenaline.

In an effort to identify the cellular source of the noradrenaline-induced IL-1ra and IL-1RII expressions from mixed glial *in vitro*, enriched cultures of both microglia and astrocytes were treated with noradrenaline under the same conditions. Our results indicate that microglia are the sole producer of IL-1ra and IL-1RII, as astrocytes failed to respond to treatment with noradrenaline. At least where IL-1ra is concerned, this is consistent with previous literature which has identified microglia as being the predominant producer of IL-1 ligands and the sole producer of IL-1ra in the CNS (Liu et al., 1998; Pinteaux et al., 2006). The magnitude of IL-1RII and IL-1ra expression induced by noradrenaline in enriched microglial cells is much less than observed in mixed glial cells, and this may be due to differences in experimental procedures. Specifically, the growth factors M-CSF and GM-CSF are used in the preparation of enriched microglia in order to yield a sufficient number of microglia for analysis. This growth factor cocktail induces a large reduction in β_2-adrenoceptor expression on microglia (Ryan et al., unpublished data), and we suggest that this reduction in β_2-adrenoceptors impairs the ability of noradrenaline to induce IL-1RII and IL-1ra expressions in enriched microglial cells relative to mixed glial cultures. It is also possible that induction of IL-1RII and IL-1ra expression by noradrenaline from microglial cells may require an as yet unidentified juxtacrine factor present in mixed glial cultures.

Previous literature demonstrates that IL-1β is not directly neurotoxic to healthy neurons *in vitro,* but rather, exacerbated neuronal damage by increasing glial-derived agents such as reactive nitrogen species, free radicals, and inflammatory cytokines (Rothwell, 2003; Ma and Zhao, 2002; Thornton et al., 2006). Considering the ability of noradrenaline to induce expression of IL-1ra and IL-1RII we examined the ability of noradrenaline to limit neuronal toxicity induced by conditioned media harvested from IL-1β treated mixed glial cells. In the present study, treatment of primary cortical neurons with IL-1β did not elicit neurotoxic effects as measured by LDH release. However, addition of conditioned media from IL-1β-stimulated mixed glial cells to neurons induced neurotoxicity indicated by an increase in LDH release. Treatment of glial cells with noradrenaline prior to addition of IL-1β, attenuated the neurotoxic effects of conditioned media from IL-1β-treated glial cells. These data are consistent with a previous study where pre-treatment with noradrenaline inhibited neurotoxicity in cultured cortical neurons induced by conditioned media from LPS-stimulated microglial cells (Madrigal et al., 2005).

The results presented here demonstrate that noradrenaline by activation glial β-adrenoceptors induces expression of two negative regulators of the IL-1 system (IL-1ra and IL-1RII) in glial cells. Furthermore, this expression of IL-1ra and IL-1RII is accompanied by protection against IL-1β-induced neurotoxicity. These results suggest that noradrenaline may elicit its endogenous anti-inflammatory and neuroprotective effects by increasing expression of IL-1ra and IL-1RII in the CNS. These data may partially explain why the loss of noradrenergic *Locus Ceruleus* neurons, and subsequent central noradrenergic tone, contributes to inflammatory activation in chronic inflammatory states such as Alzheimer's disease (Heneka et al., 2003). Of course a limitation of the present study is that is it performed entirely using cultured glial cells prepared from neonatal rats, and we don't yet know how these findings translate into the *in vivo* setting. Future studies will examine the impact of noradrenaline enhancing agents and β_2-adrenoceptor agonists on expression of IL-1ra and IL-1RII in glial cells in the intact CNS of adult animals.

While recombinant human IL-1ra is now a licensed subcutaneous treatment for rheumatoid arthritis (Cohen et al., 2002; Bresnihan et al., 1998) and is in a phase II clinical trials using intravenous infusions for stroke patients (Rothwell, 2003), at 17 kDa, IL-1ra is a large protein and is likely to have limited blood brain barrier permeability. Thus, pharmacological intervention that would increase the CNS expression levels of IL-1ra and/or IL-1RII may prove to be a beneficial alternative to peripheral administration of the recombinant protein. Considering the ability of physiologically relevant doses of noradrenaline to induce both IL-1ra and IL-1RII *in vitro*, we suggest that activation of the central noradrenergic system may be of therapeutic potential in neuroinflammatory conditions. In this regard, studies are ongoing to examine the ability of pharmacological agents that increase central noradrenergic tone to protect against inflammation-related neurodegeneration.

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References

Allan, S.M., Tyrrell, P.J., Rothwell, N.J., 2005. Interleukin-1 and neuronal injury. Nat. Rev. Immunol. 5, 629–640.
Aoki, C., 1992. Beta-adrenergic receptors: astrocytic localization in the adult visual cortex and their relation to catecholamine axon terminals as revealed by electron microscopic immunocytochemistry. J. Neurosci. 12, 781–792.
Basu, A., Krady, J.K., Levison, S.W., 2004. Interleukin-1: a master regulator of neuroinflammation. J. Neurosci. Res. 78, 151–156.
Block, M.L., Hong, J.S., 2005. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Prog. Neurobiol. 76, 77–98.
Boyle, N.T., Connor, T.J., 2007. MDMA ("Ecstasy") suppresses the innate IFN-gamma response in vivo: a critical role for the anti-inflammatory cytokine IL-10. Eur. J. Pharmacol. 572, 228–238.
Bresnihan, B., Alvaro-Gracia, J.M., Cobby, M., Doherty, M., Domljan, Z., Emery, P., Nuki, G., Pavelka, K., Rau, R., Rozman, B., Watt, I., Williams, B., Aitchison, R., McCabe, D., Musikic, P., 1998. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. Arthritis Rheum. 41, 2196–2204.
Carter, D.B., Deibel Jr., M.R., Dunn, C.J., et al., 1990. Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. Nature 344, 633–638.
Chen, M.J., Nguyen, T.V., Pike, C.J., Russo-Neustadt, A.A., 2007. Norepinephrine induces BDNF and activates the PI-3K and MAPK cascades in embryonic hippocampal neurons. Cell. Signal. 19, 114–128.
Cohen, S., Hurd, E., Cush, J., Schiff, M., Weinblatt, M.E., Moreland, L.W., Kremer, J., Bear, M.B., Rich, W.J., McCabe, D., 2002. Treatment of rheumatoid arthritis with anakinra, a recombinant human interleukin-1 receptor antagonist, in combination with methotrexate: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 46, 614–624.
Colotta, F., Dower, S.K., Sims, J.E., Mantovani, A., 1994. The type II 'decoy' receptor: a novel regulatory pathway for interleukin 1. Immunol. Today 15, 562–566.
Dawson, J., Engelhardt, P., Kastelic, T., Cheneval, D., MacKenzie, A., Ramage, P., 1999. Effects of soluble interleukin-1 type II receptor on rabbit antigen-induced arthritis: clinical, biochemical and histological assessment. Rheumatology (Oxford) 38, 401–406.
Dello Russo, C., Boullerne, A.I., Gavrilyuk, V., Feinstein, D., 2004. Inhibition of microglia inflammatory responses by norepinephrine: effects on nitric oxide and interleukin-1β production. J. Neuroinflammation 1, 9.
Docagne, F., Campbell, S.J., Bristow, A.F., Poole, S., Vigues, S., Guaza, C., Perry, V.H., Anthony, D.C., 2005. Differential regulation of type I and type II interleukin-1 receptors in focal brain inflammation. Eur. J. Neurosci. 21, 1205–1214.

Ericsson, A., Liu, C., Hart, R.P., Sawchenko, P.E., 1995. Type 1 interleukin-1 receptor in the rat brain: distribution, regulation, and relationship to sites of IL-1-induced cellular activation. J. Comp. Neurol. 361, 681–698.
Faure, M., Voyno-Yasenetskaya, T.A., Bourne, H.R., 1994. cAMP and beta gamma subunits of heterotrimeric G proteins stimulate the mitogen-activated protein kinase pathway in COS-7 cells. J. Biol. Chem. 269, 7851–7854.
Feinstein, D.L., 1998. Suppression of astroglial nitric oxide synthase expression by norepinephrine results from decreased NOS-2 promoter activity. J. Neurochem. 70, 1484–1496.
Feinstein, D.L., Heneka, M.T., Gavrilyuk, V., Dello Russo, C., Weinberg, G., Galea, E., 2002. Noradrenergic regulation of inflammatory gene expression in brain. Neurochem. Int. 41, 357–365.
French, R.A., VanHoy, R.W., Chizzonite, R., Zachary, J.F., Dantzer, R., Parnet, P., Bluthé, R.M., Kelley, K.W., 1999. Expression and localization of p80 and p68 interleukin-1 receptor proteins in the brain of adult mice. J. Neuroimmunol. 93, 194–202.
Frohman, E.M., Vayuvegula, B., Gupta, S., Van Den Noort, S., 1988. Norepinephrine inhibits γ interferon-induced major histocompatability class II (Ia) antigen expression on cultured astrocytes via β_2-adrenergic signal transduction mechanism. Proc. Natl. Acad. Sci. U.S.A. 85, 1292–1296.
Gabay, C., Smith, M.F., Eidlen, D., Arend, W.P., 1997. Interleukin 1 receptor antagonist (IL-1ra) is an acute-phase protein. J. Clin. Invest. 99, 2930–2940.
Galea, E., Heneka, M.T., Dello Russo, C., Feinstein, D.L., 2003. Intrinsic regulation of brain inflammatory responses. Cell. Mol. Neurobiol. 23, 625–635.
Godbout, J.P., Johnson, R.W., 2006. Age and neuroinflammation: a lifetime of psychoneuroimmune consequences. Neurol. Clin. 24, 521–538.
Griffin, W.S., Mrak, R.E., 2002. Interleukin-1 in the genesis and progression of and risk for development of neuronal degeneration in Alzheimer's disease. J. Leukoc. Biol. 72, 233–238.
Hauss-Wegrzyniak, B., Dobrzanski, P., Stoehr, J.D., Wenk, G.L., 1998. Chronic neuroinflammation in rats reproduces components of the neurobiology of Alzheimer's disease. Brain Res. 780, 294–303.
Heneka, M.T., Gavrilyuk, V., Landreth, G.E., O'Banion, M.K., Weinberg, G., Feinstein, D.L., 2003. Noradrenergic depletion increases inflammatory responses in brain: effects on IkappaB and HSP70 expression. J. Neurochem. 85, 387–398.
Junker, V., Becker, A., Huhne, R., Zembatov, M., Ravati, A., Culmsee, C., Krieglstein, J., 2002. Stimulation of beta-adrenoceptors activates astrocytes and provides neuroprotection. Eur. J. Pharmacol. 446, 25–36.
Kolch, W., 2005. Coordinating ERK/MAPK signalling through scaffolds and inhibitors. Nat. Rev. Mol. Cell. Biol. 6, 827–837.
Korherr, C., Hofmeister, R., Wesche, H., Falk, W., 1997. A critical role for interleukin-1 receptor accessory protein in interleukin-1 signaling. Eur. J. Immunol. 27, 262–267.
Liu, J., Zhao, M.L., Brosnan, C.F., Lee, S.C., 1996. Expression of type II nitric oxide synthase in primary human astrocytes and microglia: role of IL-1beta and IL-1 receptor antagonist. J. Immunol. 157, 3569–3576.
Liu, J.S., Amaral, T.D., Brosnan, C.F., Lee, S.C., 1998. IFNs are critical regulators of IL-1 receptor antagonist and IL-1 expression in human microglia. J. Immunol. 161, 1989–1996.
Lopez-Ilasaca, M., 1998. Signaling from G-protein-coupled receptors to mitogen-activated protein (MAP)-kinase cascades. Biochem. Pharmacol. 56, 269–277.
Lynch, A.M., Lynch, M.A., 2002. The age-related increase in IL-1 type I receptor in rat hippocampus is coupled with an increase in caspase-3 activation. Eur. J. Neurosci. 15, 1779–1788.
Lundkvist, J., Sundgren-Andersson, A.K., Tingsborg, S., Ostlund, P., Engfors, C., Alheim, K., Bartfai, T., Iverfeld, nK., Schultzberg, M., 1999. Acute phase responses in transgenic mice with overexpression of IL-1 receptor antagonist. Am. J. Physiol. 276, R644–R651.
Ma, J.Y., Zhao, Z.Q., 2002. The involvement of glia in long-term plasticity in the spinal dorsal horn of the rat. Neuroreport 13, 1781–1784.
Madrigal, J.L., Feinstein, D.L., Dello Russo, C., 2005. Norepinephrine protects cortical neurons against microglial-induced cell death. J. Neurosci. Res. 81, 390–396.
Marien, M.R., Colpaert, F.C., Rosenquist, A.C., 2004. Noradrenergic mechanisms in neurodegenerative diseases: a theory. Brain Res. Brain Res. Rev. 45, 38–78.
Minogue, A.M., Schmid, A.W., Fogarty, M.P., Moore, A.C., Campbell, V.A., Herron, C.E., Lynch, M.A., 2003. Activation of the c-Jun N-terminal kinase signaling cascade mediates the effect of amyloid-beta on long term potentiation and cell death in hippocampus: a role for interleukin-1beta? J. Biol. Chem. 278, 27971–27980.
Mori, K., Ozaki, E., Zhang, B., Yang, L., Yokoyama, A., Takeda, I., Maeda, N., Sakanaka, M., Tanaka, J., 2002. Effects of norepinephrine on rat cultured microglial cells that express alpha1, alpha2, beta1 and beta2 adrenergic receptors. Neuropharmacology 43, 1026–1034.
Nishiyori, A., Minami, M., Takami, S., Satoh, M., 1997. Type 2 interleukin-1 receptor mRNA is induced by kainic acid in the rat brain. Brain Res. Mol. Brain Res. 50, 237–245.
Nolan, Y., Martin, D., Campbell, V.A., Lynch, M.A., 2004. Evidence of a protective effect of phosphatidylserine-containing liposomes on lipopolysaccharide-induced impairment of long-term potentiation in the rat hippocampus. J. Neuroimmunol. 151, 12–23.
Olsson, T., Piehl, F., Swanberg, M., Lidman, O., 2005. Genetic dissection of neurodegeneration and CNS inflammation. J. Neurol. Sci. 233, 99–108.
Palin, K., Verrier, D., Tridon, V., Hurst, J., Perry, V.H., Dantzer, R., Lestage, J., 2004. Influence of the course of brain inflammation on the endogenous IL-1beta/IL-1Ra balance in the model of brain delayed-type hypersensitivity response to bacillus Calmette–Guerin in Lewis rats. J. Neuroimmunol. 149, 22–30.
Parnet, P., Amindari, S., Wu, C., Brunke-Reese, D., Goujon, E., Weyhenmeyer, J.A., Dantzer, R., Kelley, K.W., 1994. Expression of type I and type II interleukin-1 receptors in mouse brain. Brain Res. Mol. Brain Res. 27, 63–70.
Pinteaux, E., Parker, L.C., Rothwell, N.J., Luheshi, G.N., 2002. Expression of interleukin-1 receptors and their role in interleukin-1 actions in murine microglial cells. J. Neurochem. 83, 754–763.
Pinteaux, E., Rothwell, N.J., Boutin, H., 2006. Neuroprotective actions of endogenous interleukin-1 receptor antagonist (IL-1ra) are mediated by glia. Glia 53, 551–556.
Relton, J.K., Rothwell, N.J., 1992. Interleukin-1 receptor antagonist inhibits ischaemic and excitotoxic neuronal damage in the rat. Brain Res. Bull. 29, 243–246.
Rothwell, N., 2003. Interleukin-1 and neuronal injury: mechanisms, modification, and therapeutic potential. Brain Behav. Immun. 17, 152–157.
Sims, J.E., March, C.J., Cosman, D., et al., 1988. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science 241, 585–589.
Thornton, P., Pinteaux, E., Gibson, R.M., Allan, S.M., Rothwell, N.J., 2006. Interleukin-1-induced neurotoxicity is mediated by glia and requires caspase activation and free radical release. J. Neurochem. 98, 258–266.
Troadec, J.D., Marien, M., Mourlevat, S., Debeir, T., Ruberg, M., Colpaert, F., Michel, P.P., 2002. Activation of the mitogen-activated protein kinase (ERK1/2) signaling pathway by cyclic AMP potentiates the neuroprotective effect of the neurotransmitter noradrenaline on dopaminergic neurons. Mol. Pharmacol. 62, 1043–1052.
Wang, Y., Lin, S.Z., Chiou, A.L., Williams, L.R., Hoffer, B.J., 1997. Glial cell line-derived neurotrophic factor protects against ischemia-induced injury in the cerebral cortex. J. Neurosci. 17, 4341–4348.