European Journal of Pharmacology 626 (2010) 219–228

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and Analgesia

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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ARTICLE INFO

Article history:
Received 6 February 2009
Received in revised form 18 September 2009
Accepted 28 September 2009
Available online 8 October 2009

Keywords:
IL-1β
IL-1ra
IL-1RII
Noradrenaline
β-adrenoceptor
Neuroprotection
Glial cell

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.

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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doi:10.1016/j.ejphar.2009.09.054

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