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Treatment with the noradrenaline re-uptake inhibitor atomoxetine alone and in combination with the α2-adrenoceptor antagonist idazoxan attenuates loss of dopamine and associated motor deficits in the LPS inflammatory rat model of Parkinson's disease

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ABSTRACT

The impact of treatment with the noradrenaline (NA) re-uptake inhibitor atomoxetine and the α_2-adrenoceptor (AR) antagonist idazoxan in an animal model of Parkinson's disease (PD) was assessed. Concurrent systemic treatment with atomoxetine and idazoxan, a combination which serves to enhance the extra-synaptic availability of NA, exerts anti-inflammatory and neuroprotective effects following delivery of an inflammatory stimulus, the bacterial endotoxin, lipopolysaccharide (LPS) into the substantia nigra. Lesion-induced deficits in motor function (akinesia, forelimb-use asymmetry) and striatal dopamine (DA) loss were rescued to varying degrees depending on the treatment. Treatment with atomoxetine following LPS-induced lesion to the substantia nigra, yielded a robust anti-inflammatory effect, suppressing microglial activation and expression of the pro-inflammatory cytokine TNF-α whilst increasing the expression of neurotrophic factors. Furthermore atomoxetine treatment prevented loss of tyrosine hydroxylase (TH) positive nigral dopaminergic neurons and resulted in functional improvements in motor behaviours. Atomoxetine alone was sufficient to achieve most of the observed effects. In combination with idazoxan, an additional improvement in the impairment of contralateral limb use 7 days post lesion and a reduction in amphetamine-mediated rotational asymmetry 14 days post-lesion was observed, compared to atomoxetine or idazoxan treatments alone. The results indicate that increases in central NA tone has the propensity to regulate the neuroinflammatory phenotype *in vivo* and may act as an endogenous neuroprotective mechanism where inflammation contributes to the progression of DA loss. In accordance with this, the clinical use of agents such as NA re-uptake inhibitors and α_2-AR antagonists may prove useful in enhancing the endogenous neuroimmunomodulatory potential of NA in conditions associated with brain inflammation.

1. Introduction

Parkinson's disease is associated with progressive degeneration of dopaminergic (DAergic) neurons projecting from the substantia nigra (SN) to the putamen within the basal ganglia, culminating in motor dysfunction such as bradykinesia, hypokinesia, rigidity and resting tremor (Dickson, 2012). Inflammation-derived oxidative stress and toxicity caused by cytokine release from chronically activated microglia is considered to contribute to the degeneration of the nigro-striatal tract (Boka et al., 1994; Depino et al., 2003; Hirsch and Hunot, 2009; Long-Smith et al., 2009; Mogi et al., 1994; Tansey and Goldberg, 2010). Chronic activation of microglia is evident in Parkinson's disease (PD) (Gao and Hong, 2008). This creates a self-propelling inflammatory cycle termed reactive microgliosis, resulting in cell death and neurodegeneration (Collins et al., 2012; Gao and Hong, 2008). Inflammatory based models of PD simulate this process including intra-nigral administration of the bacterial endotoxin and immune stimulus lipopolysaccharide (LPS) which is used to provoke degeneration of DA neurons in the SN (Hoban et al., 2013).

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It has been reported that the Locus Coeruleus (LC), the primary source of NA cell bodies in the brain, is affected in PD (Baker et al., 1989; Chan-Palay and Asan, 1989; Greenfield and Bosanquet, 1953) and that LC cell numbers are reduced at autopsy in PD patients compared to healthy age-matched controls (Marien et al., 2004). Consequently, NA inputs to mid- and fore-brain structures are decreased and it has been suggested that loss of LC-NAergic neurons is a significant contributor to the progression of PD pathology and symptomology, including both motor and non-motor aspects of the disease (Del Tredici et al., 2002; Gesi et al., 2000). In support, many groups have reported a NA dependent enhancement of DA loss in various animal models of PD (Bing et al., 1994; Fornai et al., 1995; Marien et al., 1993; Mavridis et al., 1991), however the precise mechanism following LC lesion leading to potentiation of DAergic cell loss remains unknown (Szot et al., 2010).

A number of *in vitro* studies implicate a role for NA and β_2-ARs in promoting anti-inflammatory actions via communication with microglial, astrocytic and neuronal cell types (Ballestas and Benveniste, 1997; Braun et al., 2014; Feinstein, 1998; Frohman et al., 1988; Madrigal et al., 2005; McNamee et al., 2010a; Russo et al., 2004; and Willis and Nisen, 1995). Stimulation of astrocytic α_1 and/or β-adrenoceptors the subsequent increase in cAMP and protein kinase A, may also provide neuroprotection through secretion of neurotrophic factors including NGF (Furukawa et al., 1989), BDNF (Jurič et al., 2006) and NT-3 (Mele et al., 2010). Moreover, studies by Lindholm et al., 2007a,b) have demonstrated that intra-striatal delivery of the cerebral dopamine neurotrophic factor (CDNF) protects against 6-OHDA-induced degeneration of the nigrostriatal dopaminergic system and reduces amphetamine-induced ipsiversive rotations in response to the lesion. Delivery of CDNF in the striatum prevented striatal 6-OHDA-induced TH cell loss in the nigra but not the striatum, therefore it seems to have preferential efficacy for cell body populations (Voutilainen et al., 2009). To our knowledge, prior to conducting this study, the impact of NA related stimulation on midbrain CDNF levels has not been investigated to date in rodent models of Parkinson's disease.

Noradrenaline reuptake inhibitors (NRI's) are a class of drug that block the pre-synaptic NA transporter (NAT) thereby preventing the re-uptake of NA, resulting in an increase in extracellular NA concentration, which may promote signalling through adrenergic receptors. Additionally, these agents are currently used in the clinical setting for the treatment of attention deficit hyperactivity disorder (ADHD) and depression (Cipriani et al., 2005; Friedman and Kocsis, 1996; Nelson, 1999; Prince, 2006). Previous work has demonstrated an anti-inflammatory phenotype in rats following treatment with the NA-reuptake inhibitors desipramine and atomoxetine, resulting in the reduction of cortical pro-inflammatory and cytotoxic mediator expression (IL-1β, TNF-α, iNOS) and microglial activation markers (CD11b and CD40) following systemic LPS challenge (O'Sullivan et al., 2009). Additionally, co-treatment with the NA reuptake inhibitor reboxetine and α_2-AR receptor antagonist idazoxan induced cortical IL-1β, IL-1ra and IL-1RII expression (McNamee et al., 2010b).

Selective α_2-AR antagonists potentiate NA availability *in vivo* by blocking neuronal pre-synaptic α_2-AR's whose normal function is to regulate NA release (Veldhuizen et al., 1993; Invernizzi and Garattini, 2004; Swanson et al., 2006; Veldhuizen et al., 1993; Wortley et al., 1999). Treatment with the α_2-AR antagonist idazoxan has resulted in neuroprotective effects against various disease salient stimuli in rodents (Gustafson et al., 1990; Martel et al., 1998; Veyrac et al., 2005). In the 6-OHDA PD animal model, methoxy-idazoxan (2.5 mg/kg; b.i.d.; i.p. for 5 days) administration prior to medial forebrain bundle (MFB) lesion protects nigral DAergic neurons and reverses catalepsy and hypoactivity (Srinivasan and Schmidt, 2004). In addition, idazoxan and the selective α_2 adrenoceptor antagonist fipramezole reduced the number of abnormal involuntary movements in rodent models (Lundblad et al., 2002), primate models (Fox et al., 2001; Grondin et al., 2000) and human PD patients (LeWitt et al., 2012; Rascol et al., 2001) providing a rationale for the use of noradrenaline enhancing drugs as adjuvant treatments in PD.

Together these findings suggest that diminished NA input or signalling could exacerbate PD neuropathology, whilst mechanisms to enhance NA tone may provide neuroprotection. Surprisingly, given the wealth of evidence describing the ability of NA to elicit anti-inflammatory effects in the CNS, there has been little research on its potential as a neuroprotective agent. The current study sought to assess the impact of augmenting central noradrenergic tone on PD-related neuropathology and motor dysfunction in an animal model of PD. Specifically, the effect of treatment with the NRI atomoxetine, alone or in combination with an α_2-AR antagonist idazoxan, were assessed for amelioration of neuroinflammation, DA neuron loss and behavioural dysfunction following LPS-induced lesion to the substantia nigra.

2. Materials and methods

2.1. Animals

Male Wistar Han rats, aged 7–8 weeks (220–250 g) were obtained from the Comparative Medicine Unit (TCD) and housed in groups of 3 or 4 per cage in hard-bottomed polypropylene cages with stainless steel wire tops and wood shaving used as bedding. Animals were kept in climate-controlled rooms set to 21 °C (±2 °C) with relative humidity levels of 50%, on a 12:12 h light/dark cycle (lights on at 08:00). The experimental protocols involved were in compliance with the European directive 2010/63/EU on the protection of animals used for scientific purposes, approved by the Animal Research Ethics Committee in Trinity College Dublin and performed under licence granted by the Irish Medicines Board.

2.2. Experimental design

Rats (n = 48) were allowed to habituate to the animal unit for at least a week prior to behavioural training. This study involved eight treatment groups: (1) vehicle + vehicle, (2) vehicle + atomoxetine, (3) vehicle + idazoxan, (4) vehicle + atomoxetine/idazoxan, (5) LPS + vehicle, (6) LPS + atomoxetine, (7) LPS + idazoxan, (8) LPS + atomoxetine/idazoxan. Baseline testing in each behavioural paradigm was conducted 5 days prior to stereotactic surgery. Rats received a unilateral stereotaxic injection of LPS (10 μg/2 μl; Sigma-Aldrich, Ireland) into the substantia nigra. Control animals received a sham PBS injection. Four hours following surgery rats received an i.p. injection of the NRI atomoxetine (3 mg/kg; i.p.), the α_2-AR antagonist idazoxan (1 mg/kg; i.p.), a combination of both or saline vehicle (0.89% [w/v] NaCl). Dosing was then continued at twice daily intervals (b.i.d.) for the following 7 days (Morning drug treatment between 9 and 10 am; Evening drug treatment between 5 and 6 pm). Behavioural testing in the staircase, stepping and cylinder test was conducted (in that order) 7 days following surgery, between the hours of 9 am–1 pm. Behavioural testing was repeated 6 days later. At 14 days post-surgery animals were administered D-amphetamine (5 mg/kg; i.p.; Sigma-Aldrich, Ireland) placed in their home-cage and activity was recorded for 40 min. The following day rats were either euthanized by transcardial perfusion-fixation in preparation for immunohistochemistry or by decapitation whereby their brains were dissected freehand on dry ice in preparation for HPLC (see Fig. 1 for experimental timeline). Brain samples were stored at −80 °C prior to post-mortem assess-

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