PERSPECTIVE

Regulation of β_2-adrenoceptors in brain glia: implications for neuroinflammatory and degenerative disorders

Noradrenaline: Within the central nervous system (CNS), the primary source of the catecholamine neurotransmitter noradrenaline is the locus coeruleus (LC) in the pontine tegmentum, with LC neurons projecting to almost all regions of the brain and spinal cord. Following its release from LC neurons, noradrenaline has wide ranging effects. For example, noradrenaline is the endogenous agonist for G-coupled α- and β-adrenoceptors that are expressed on many cell types, including neurons and glia, in both the peripheral nervous system and CNS. It is via these receptors that noradrenaline exerts its anti-inflammatory and neurotrophic effects in the brain. Noradrenaline additionally has adrenoceptor-independent neuroprotective actions, and as such plays a role in free radical scavenging and reducing oxidative stress (Feinstein et al., 2016).

Noradrenaline and neurodegenerative disease: Owing to the numerous functions of noradrenaline, dysregulation of the LC and noradrenergic signaling can have broad ranging effects and has been suggested to contribute to neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis (MS) (Feinstein et al., 2016). These conditions are generally characterized by an unresolved inflammatory response with chronic activation of glia (Marien et al., 2004) and sustained production of pro-inflammatory and potentially neurotoxic cytokines. Notably, studies have shown that noradrenaline can suppress the proliferation of microglia and limit the expression of inflammatory markers in glia *in vitro* (Feinstein et al., 2016). *In vivo*, depletion of noradrenaline increases the inflammatory response to the pathogenic protein amyloid-β_{1-42} and exacerbates the severity of experimental autoimmune encephalomyelitis, an animal analog of MS, while increasing noradrenergic tone has been shown to attenuate inflammation in these disease models. Drugs that target the noradrenergic system to enhance extrasynaptic concentrations of the transmitter are neuroprotective in animal models of neurodegenerative disease, with effects mediated by suppression of the expression of inflammatory cytokines, chemokines, and cell adhesion molecules following central and systemic inflammatory challenge, in addition to improving functional recovery after ischemia, and promoting neuronal survival *in vivo* (O'Neill and Harkin, 2018).

Noradrenaline and glial adrenoceptors: It is now widely accepted that within the CNS the neuroprotective effects of noradrenaline are primarily orchestrated by its endogenous anti-inflammatory and neurotrophic properties, mediated predominantly through its actions at glial β_2-adrenoceptors (**Figure 1A**). Direct stimulation of glial β_2-adrenoceptors *in vitro* with noradrenaline or β_2-adrenoceptor agonists suppresses nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), induces the expression of anti-inflammatory mediators and negative regulators of the interleukin (IL)-1 system (IL-1RA and IL-1RII), and protects against IL-1β-induced neurotoxicity, while stimulation of central β_2-adrenoceptors *in vivo* increases the expression of the broad spectrum anti-inflammatory cytokine IL-10 and its downstream mediator Suppressor of cytokine signaling 3 (O'Neill and Harkin, 2018). Moreover, stimulation of β_2-adrenoceptors induces neurotrophin expression, including brain derived neurotrophic factor, nerve growth factor beta, glial derived neurotrophic factor (GDNF), and basic fibroblast growth factor *in vitro* and *in vivo* (Culmsee et al., 1999; Day et al., 2014), and induces neurite outgrowth (Day et al., 2014). The increase in GDNF following β_2-adrenoceptor stimulation may be of particular importance from a neuroprotection standpoint since central infusion of GDNF can prevent dopaminergic neurodegeneration in a rat model of Parkinson's disease and increase dopamine storage and improve motor function in patients with this disease. Thus, noradrenaline has been proposed to have a bi-modal neuroprotective role through its effects on the downregulation of microglial pro-inflammatory mediator expression and the enhancement of astrocytic growth factor production and the promotion of neurotrophic effects (O' Neill and Harkin, 2018).

It has been proposed that the demyelination associated with the progression of MS may be due to a lack of β_2-adrenoceptors on astrocytes since it has been reported that β_2-adrenoceptors are absent from astrocytes in both normal appearing white matter and astrogliotic plaques in the white matter of patients with MS, though no association has been found between polymorphisms of the β_2-adrenoceptor gene and the incidence of MS (De Keyser et al., 2004). Such a phenomenon suggests that endogenous noradrenaline may fail to elicit anti-inflammatory effects in the CNS, thus contributing to the dysregulation of inflammatory markers characteristically observed in MS.

Regulation of the β_2-adrenoceptor by inflammatory stimuli: We recently investigated the effects of an immune stimulus comprising bacterial endotoxin lipopolysaccharide (LPS) and interferon-gamma (IFN-γ) on β_2-adrenoceptors in mixed and enriched astrocytic and microglial cultures in a bid to determine if induction of inflammation could contribute to a loss of the receptor. Previous studies have shown that inflammation can reduce β-adrenoceptor expression and responsiveness in the respiratory system, including tracheal smooth muscle, lung tissue, and airway smooth muscle cells (Ryan et al., 2019). Primary rat mixed glial cells were treated with LPS and IFN-γ to mimic the neuroinflammatory environment of MS, since LPS drives expression of the pro-inflammatory cytokines IL-1β and tumor necrosis factor-α and combined with the T-cell (Th1) cytokine IFN-γ is representative of the inflammatory milieu reported in MS brain lesions. Glial β_2-adrenoceptors were downregulated at both the mRNA and protein levels following exposure of mixed glial cultures (70% astrocytes, 30% microglia) to LPS + IFN-γ (**Figure 1B**; Ryan et al., 2019). Furthermore, exposure of mixed glia to LPS + IFN-γ decreased β_2-agonist-stimulated production of the intracellular second messenger cyclic adenosine monophosphate (cAMP). Notably, LPS + IFN-γ did not impact on intracellular cAMP accumulation in response to forskolin, a direct activator of adenylate cyclase. Thus, the reduction in cAMP induced by LPS + IFN-γ is an event specific to the β_2-adrenoceptor itself and not due to an effect on intracellular signaling where regulation of the β_2-adrenoceptor is independent of effects on adenylate cyclase. Moreover, the effects of LPS + IFN-γ on reducing expression appear specific to the β_2-adrenoceptor and do not generalize to other G-coupled receptors such as the adenosine A_{2A} receptor or the β_1-adrenoceptor, expression of which were increased by LPS + IFN-γ. Pre-treatment with the glucocorticoid dexamethasone, a drug commonly used in the treatment of MS, suppressed the LPS + IFN-γ-induced inflammatory response and blocked the decrease in β_2-adrenoceptor mRNA (**Figure 1B**).

Mechanisms of inflammatory associated β_2-adrenoceptor downregulation: The decrease in receptor function observed in mixed glial cultures following LPS + IFN-γ exposure is suggestive of receptor internalization, decreased translation

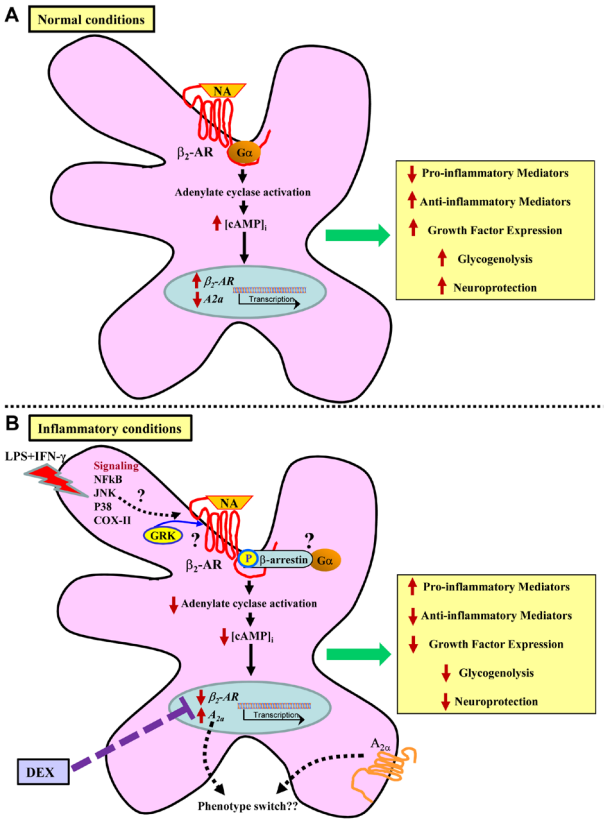

Figure 1 Glial β_2-adrenoceptor function in normal and inflammatory conditions.
(A) Normal conditions. Noradrenaline promotes an anti-inflammatory and neuroprotective phenotype through β_2-AR activation on glial cells, which results in adenylate cyclase activation and an increase in the accumulation of intracellular cAMP. (B) Inflammatory conditions. LPS + IFN-γ reduces β_2-AR mRNA expression, which in turn may promote a pro-inflammatory environment and a reduction in the neuroprotective effects of noradrenaline. LPS + IFN-γ may increase the recruitment of GRKs and β-arrestin to the receptor, which are responsible for the phosphorylation of and subsequent internalization and desensitization of the β_2-AR, respectively. LPS + IFN-γ may activate signal transduction pathways such as NFκB, JNK, P38, or COX-II to induce downregulation of β_2-AR function. LPS + IFN-γ may upregulate adenosine A_{2a} receptor expression and induce a phenotypic switch in glia. Dexamethasone (DEX) prevents LPS + IFN-γ-induced suppression of β_2-AR mRNA expression (purple dashed line). A_{2a}: Adenosine A_{2a} receptor; β_2-AR: beta2-adrenoceptor; cAMP, cyclic adenosine monophosphate; COX-II: cyclooxygenase-2; GRK: G-protein receptor kinase; IFN-γ: interferon gamma; JNK: c-Jun N-terminal kinase; LPS: lipopolysaccharide; NA: noradrenaline; NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells; P38: P38 mitogen-activated protein kinase.

of mRNA to receptor protein, or both. The simplest explanation for this is that a decline in transcription would lead to diminished translation of β_2-adrenoceptor protein, and this in turn would lead to reduced receptor cell surface expression and function as observed. However, treatment with dexamethasone restores β_2-adrenoceptor mRNA levels but fails to restore function of the receptor. It is possible that the time period required for dexamethasone to potentially restore the receptor to the cell surface and for it to become functional is longer than the time required to revert its effects on receptor mRNA levels. However, it may also be the case that the inflammatory-induced decrease in β_2-adrenoceptor mRNA and function are mediated by different mechanisms. Thus, there are several other potential explanations for reduced cell surface expression and function of the β_2-adrenoceptor (**Figure 1B**). One such possibility is that LPS + IFN-γ increases the recruitment of G-protein receptor kinases and β-arrestin to the receptor, which are responsible for the phosphorylation of and subsequent internalization and desensitization of the β_2-adrenoceptor (Pitcher et al., 1999; Luttrell and Lefkowitz, 2002), respectively. Another possibility is that signal transduction pathways activated by LPS play a role. While the effects of pharmacological inhibitors of p38, c-Jun N-terminal kinase, NFκB, and cyclooxygenase-2 on β_2-adrenoceptor expression were assessed individually, to no effect (Ryan et al., 2019), it may be the case that a combination of these signaling pathways is required to mediate downregulation of the receptor. microRNAs, small non-coding RNAs that function in post-transcriptional regulation, have also been demonstrated to mediate β_2-adrenoceptor regulation (Wang et al., 2011); thus, their role in inflammation-induced β_2-adrenoceptor downregulation warrants further investigation. Overall, the precise mechanisms leading to a decrease in β_2-adrenoceptor mRNA and cell surface expression in mixed glial cultures following exposure to an inflammatory stimulus remain to be fully elucidated. Moreover, it remains to be determined whether incubation of cells with LPS + IFN-γ leads to a reduced ability of noradrenaline or β_2-agonists to dampen the inflammatory response in glia and if this can be blocked by dexamethasone.

Implications of inflammatory associated β_2-adrenoceptor downregulation: An inflammatory-related decrease in β_2-adrenoceptor expression and function could result in an inability of endogenous noradrenaline to elicit anti-inflammatory and neuroprotective effects within the CNS. As such, downregulation of the receptor may contribute to neurodegeneration owing to a number of consequences. First, the transcription of trophic factors in astrocytes is dependent on cAMP signaling, mostly via the β_2-adrenoceptor. Hence, loss of the receptor and the associated decrease in cAMP would likely result in a loss of neurotrophic support. Second, production of pro-inflammatory molecules could become dysregulated in the absence of noradrenergic actions at β_2-adrenoceptors leading to damage of neurons and oligodendrocytes. Third, most of the brain's energy source is stored in astrocytes in the form of glycogen. Glycogenolysis is dependent on stimulation of astrocytic β_2-adrenoceptors and cAMP production; therefore, decreased production of intracellular cAMP would lead to reduced glycogenolysis and further impact on energy supply to glia and neurons in the CNS. Excessive Ca^{2+} influx due to failure of the Na^+/K^+ pump, which relies on ATP to function, and increased activity of Ca^{2+}-dependent degradative enzymes, such as proteases and phospholipases, are liable to induce neuronal damage.

A decrease in β_2-adrenoceptor expression may also result in a phenotypic switch of glia. It has been shown that under resting conditions microglia express β_2-adrenoceptor mRNA, though following exposure to an inflammatory stimulus β_2-adrenoceptor expression is reduced and there is a switch towards expression of α_{2A}-adrenoceptors. Notably, noradrenaline mediates microglial process retraction and modulates microglial motility under resting and activation states via β_2-adrenoceptors and α_{2A}-adrenoceptors, respectively. Moreover, upregulation of the adenosine A_{2A} receptor can result in a switch in the chemotactic activity of microglial cells from being attracted to ATP released at a site of injury to assuming an amoeboid shape and being repelled from ATP in the presence of LPS. This switch ultimately results in decreased phagocytic ability of microglia. LPS + IFN-γ increased adenosine A_{2A} receptor expression (**Figure 1B;** Ryan et al., 2019). In certain neurodegenerative conditions, notably Alzheimer's disease, adenosine A_{2A} receptors are upregulated in neurons and astrocytes. A_{2A} overexpression promotes transcriptional changes affecting inflammatory markers, including IL-1β and other genes related to immune response, angiogenesis, and cell activation, in primary astrocytic cultures (Paiva et al., 2019).

It is tempting to speculate that suppression of inflammatory associated β_2-adrenoceptor downregulation by dexamethasone, a drug commonly used in the treatment of MS, may contribute to its therapeutic efficacy. Downregulation of glial β_2-adrenoceptors, following exposure to inflammatory stimuli as described, may ultimately be detrimental in the CNS given that the anti-inflammatory action of endogenous noradrenaline may be impaired.

***In vivo* context:** The β_2-mediated effects of noradrenaline clearly represent a useful therapeutic strategy for the treatment of neuroinflammatory and neurodegenerative disease. Pharmacological targeting of β_2-adrenoceptors has been shown to be neuroprotective following acute exposure to LPS (4 hours) in the inflammatory rat model of Parkinson's disease (O'Neill et al., 2019) and treatment with the noradrenaline re-uptake inhibitor atomoxetine alone and in combination with the α_2-adrenoceptor antagonist idazoxan attenuates loss of dopamine and associated motor deficits in the acute (4 hours) LPS inflammatory rat model of Parkinson's disease (Yssel et al., 2018). However, it remains to be determined if prolonged *in vivo* exposure to LPS, for example 24 hours to replicate our *in vitro* model or longer, results in the downregulation of either β_2-adrenoceptor expression or function in the brain. If this phenomenon identified *in vitro* also occurs in the *in vivo* context then it will be of importance to elucidate strategies to prevent β_2-adrenoceptor downregulation in order to preserve the endogenous anti-inflammatory and neuroprotective actions of noradrenaline. Moreover, additional studies are required to determine if noradrenaline or noradrenergic agonists can combat neuroinflammatory or neurodegenerative processes in conditions of chronic inflammation.

Conclusion: Further work is required to fully elucidate the mechanisms involved in inflammation-induced glial β_2-adrenoceptor downregulation. Better understanding of glial β_2-adrenoceptor regulation may be of value for the development of treatments for neuroinflammatory disorders such as MS and chronic neurodegenerative conditions including Parkinson's and Alzheimer's diseases.

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