Brief Communication

Endogenous Interleukin-1 Receptor Antagonist Mediates Anti-Inflammatory and Neuroprotective Actions of Cannabinoids in Neurons and Glia

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Interleukin-1 receptor antagonist (IL-1ra) is an important anti-inflammatory cytokine that blocks all known actions of IL-1 and markedly protects against experimentally induced ischemic, excitotoxic, and traumatic brain insults. Cannabinoids (CBs) also exert potent anti-inflammatory and neuroprotective effects, but the mechanisms of their actions are unknown. Here we tested the hypothesis that the actions of CBs are mediated by endogenous IL-1ra. We report for the first time that both CB1 and CB2 receptors modulate release of endogenous IL-1ra from primary cultured glial cells. Activation of CB1 or CB2 receptors increased lipopolysaccharide-induced IL-1ra release, and specific CB1 or CB2 antagonists blocked lipopolysaccharide-induced production of IL-1ra from glial cells. Comparison of neuronal cultures from wild-type mice and mice lacking IL-1ra (knock-out) indicates that endogenous IL-1ra is essential for the neuroprotective effects of CBs against excessive activation of glutamate receptors (excitotoxicity) in response to S-AMPA or NMDA. Similarly, analysis of mixed glial cultures from IL-1ra knock-out mice indicates that endogenous IL-1ra is required for the CB-induced inhibition of nitric oxide production in response to bacterial lipopolysaccharide. These data suggest a novel neuroprotective mechanism of action for CBs in response to inflammatory or excitotoxic insults that is mediated by both CB1 and CB2 receptor-dependent pathways.

Key words: cytokine; cannabinoid; neuroprotection; IL-1ra; excitotoxicity; nitric oxide

Introduction

Cannabinoids (CBs) exert diverse and potent actions in the brain (Howlett et al., 2002) and are under clinical evaluation as anti-inflammatory, neuroprotective, and analgesic agents (Mechoulam et al., 2002a,b). However, their mechanisms of action are primarily unknown. CBs modulate inflammatory and immune responses (Klein et al., 2000; Pertwee, 2002) and are neuroprotective in experimentally induced excitotoxicity (van der Stelt et al., 2001), ischemic (Nagayama et al., 1999), and traumatic brain injury in rodents (Panikashvili et al., 2001; Mechoulam et al., 2002b).

Exogenous and endogenous CBs exert neuroprotective and anti-inflammatory actions on glia and neurons through G-protein-coupled receptor-dependent pathways (Howlett et al., 2002). To date, two CB receptors have been identified: the CB receptor type 1 (CB1), which is expressed mainly in the brain, and the CB receptor type 2 (CB2), which is expressed primarily in the immune system, although recent data suggest that CB2 is present at low levels within the brain (Howlett et al., 2002), and additional receptors may exist. The neuroprotective actions of CBs are thought to be mediated through a variety of mechanisms, including antioxidative actions (Hampson et al., 1998), inhibition of NMDA-mediated calcium influx (Mackie and Hille, 1992; Nadler et al., 1993), and inhibition of glutamate release (Shen and Thayer, 1998). CBs act on glia and neurons to inhibit the release of proinflammatory molecules, including interleukin-1 (IL-1), tumor necrosis factor (TNF) α, and nitric oxide (NO) (Molina-Holgado et al., 1997, 2002; Shohami et al., 1997; Puffenbarger et al., 2000; Cabral et al., 2001), and enhance the release of the anti-inflammatory cytokines IL-4, IL-10 (Klein et al., 2000), and IL-6 (Molina-Holgado et al., 1998). IL-1 has been identified as an important mediator of diverse forms of experimentally induced brain damage and is expressed rapidly in response to many forms of experimental brain injury, initially by microglia and later by astrocytes (Davies et al., 1999). IL-1 receptor antagonist (IL-1ra) is a potent endogenous antagonist of all IL-1 actions in the brain (Dinarello and Thompson, 1991), protecting against ischemic, excitotoxic, and traumatic brain insults (Allan and Rothwell, 2001). Furthermore, inhibition or deletion of endogenous IL-1ra...
enhances ischemic brain injury (Loddick et al., 1997) and increases inflammatory responses (Josephs et al., 2000).

The aim of the present study was to test the hypothesis that the neuroprotective and anti-inflammatory effects of CBs are mediated by endogenous IL-1ra. Primary glial and neuronal cultures from C57BL/6 wild-type (WT) or IL-1ra knock-out (KO) mice (Horai et al., 1998) were exposed to inflammatory [bacterial lipopolysaccharide (LPS)] or neurotoxic (S-AMPA or NMDA) stimuli. We show here for the first time that the anti-inflammatory cytokine IL-1ra is an essential mediator of CB actions on neurons and glia and that both CB1 and CB2 receptors modulate the release of IL-1ra from primary cultured glial cells.

Materials and Methods

Reagents. All tissue culture reagents were obtained from Invitrogen (Paisley, UK). The antibody for the astrocytic marker, anti-glia fibrillary acidic protein (GFAP), was from Sigma-Aldrich (Poole, UK), and antibodies for the microglial marker MAC-1, respectively.

Primary mixed glial cultures. Primary mixed glial cultures were prepared from whole brains of 1-d-old mice (WT or IL-1ra KO mice) according to published protocols (Molina-Holgado et al., 2002) and grown in T150 flasks for at least 14 d in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin). The culture supernatants was assayed using a validated, mouse-specific sandwich ELISA (Safieh-Garabedian et al., 1995), supplied by Dr. Steven Poole (National Institute for Biological Standards and Control, London, UK). IL-1ra standards were assayed in triplicate and samples (100 μl) in duplicate. The assay detection limit for IL-1ra was 19 pg/ml.

Assessment of cell death. Cytotoxicity was measured by the release of cytosolic lactate dehydrogenase (LDH) by dead and dying cells using the CytoTox-96 LDH assay (Promega, Southampton, UK) according to the instructions of the manufacturer. Total LDH release was calculated by incubating untreated cells with 9% Triton X-100 for 45 min (37°C) to induce maximal cell lysis. Values for treated cells were then expressed as a percentage of the total LDH release. Background LDH release induced by media alone was subtracted from the experimental values.

Assay of nitric oxide synthase activity. NO synthase activity was assessed by measurement of nitrite (a breakdown product of NO) accumulation. Aliquots (100 μl) of culture supernatants were mixed with 100 μl of Griess reagent, a colorimetric indicator of the presence of nitrite (Green et al., 1982), and incubated in the dark at room temperature for 10 min. The absorbance was measured at 570 nm in a microplate reader. Fresh culture medium served as controls in all experiments.

Statistical analysis. Results are presented as the mean ± SEM of at least 4 independent experiments on separate cultures, each performed in triplicate.
Results

Cannabinoid agonists and antagonists modulate LPS-induced IL-1ra release from glial cells

Wild-type, primary, mixed glial cultures were used to test whether LPS-induced IL-1ra release is regulated by CB receptors, using specific and potent antagonists for the CB1 (SR-141617A) and CB2 (SR-144528) receptors (Howlett et al., 2002). Basal release of IL-1ra was not affected by incubation with CB1 or CB2 antagonists alone (data not shown). LPS increased release of IL-1ra by 15-fold (Fig. 1a), and this was almost completely abolished by cotreatment with either CB receptor antagonist (SR-141716A or SR-144528, by -12-fold and -13.6-fold, respectively). Thus, CB receptors regulate LPS-induced IL-1ra release. Moreover, the synthetic CB agonists HU-210 and CP-55940 had no effect on basal IL-1ra release (data not shown), but they enhanced LPS-induced IL-1ra release (by 28 and 15%, respectively) in WT mixed glial cultures (Fig. 1b,c).

Endogenous IL-1ra mediates the neuroprotective actions of cannabinoids in primary neuronal cultures

We next investigated whether endogenous IL-1ra contributes to the neuroprotective actions of CBs against excessive activation of glutamate receptors (excitotoxicity), induced by exposure of primary cerebrocortical neurons from WT and IL-1ra KO mice to S-AMPA or NMDA. In WT neuronal cells, both glutamatergic agonists increased cell death significantly (Fig. 2a,b), and the CB agonist HU-210 significantly inhibited the cell death induced by S-AMPA (38%) and NMDA (51%) (Fig. 2a,b). S-AMPA and NMDA were also cytotoxic in cells from IL-1ra KO mice, but the neuroprotective effects of HU-210 were completely abolished (Fig. 2c,d).

Endogenous IL-1ra mediates the anti-inflammatory actions of cannabinoids in glial cells

The contribution of endogenous IL-1ra to the actions of CBs was further analyzed by testing the effects of the synthetic cannabinoid agonists HU-210 and CP-55940 on LPS-induced NO synthase activity, because NO is an important mediator of neuroinflammation and neurodegeneration (Hobbs et al., 1999). HU-210 or CP-55940 significantly inhibited the release of NO induced by LPS in WT glia (-55 and -25%, respectively) (Fig. 3a,b). In contrast, HU-210 and CP-55940 failed to inhibit the LPS-induced release of NO in mixed glial cultures from IL-1ra KO mice (Fig. 3c,d). Similar effects were observed when mixed glial cultures from KO mice were coincubated with LPS and the synthetic CB cannabinoid anandamide or 11-OH-CBN (data not shown).

Discussion

Cannabinoids have potent anti-inflammatory and neuroprotective effects in the brain, but the underlying mechanisms are unclear. We and others have shown previously that CBs can modulate production of inflammatory mediators, including...
the cytokines TNFα and IL-6 (Molina-Holgado et al., 1998; Klein et al., 2000; Puffenbarger et al., 2000) and generation of Th1 and Th2 phenotypes (Yuan et al., 2002). The present report extends these studies and suggests for the first time that the neuroprotective and anti-inflammatory effects of CBs are mediated by CB-induced release of endogenous IL-1ra in primary neurons or glial cells in vitro. The synthetic CB agonists HU-210 and CP-55940, which have similar affinities for CB1 and CB2 receptors (Howlett et al., 2002), increased LPS-induced IL-1ra release from primary glial cells. Similar results were obtained with other cannabinoids, including 11-OH-CBN, and the endogenous cannabinoid anandamide (data not shown). The potent and specific antagonists for CB1 and CB2 receptors, SR-141716A and SR-144528, respectively, significantly abrogated the LPS-induced IL-1ra release, implicating both CB receptors in this effect. Because neither the CB agonists nor antagonists altered basal release, these results indicate that cannabinoids enhance release of IL-1ra specifically in response to proinflammatory stimuli. The exact participation of CB1, CB2, or new CB receptors in these neuroprotective actions remains to be elucidated. HU-210 and CP-55940 may promote LPS-induced IL-1ra expression and release directly, or they could modulate IL-1ra expression via an intermediate, such as IL-6. In support of the latter hypothesis, it has been reported that neutralization of endogenous IL-6 suppresses induction of IL-1ra (Jordan et al., 1995), and CB agonists modulate IL-6 production in glial cells by a CB receptor-mediated pathway (Molina-Holgado et al., 1998).

CBs are neuroprotective in a variety of in vitro and in vivo models of neuronal excitotoxicity. In the present study, low doses of HU-210 reduced cell death of WT neurons in response to S-AMPA or NMDA receptor activation. In contrast, HU-210 failed to protect against S-AMPA- or NMDA-induced death of neurons from IL-1ra KO mice. These results are consistent with previous observations that endogenous IL-1ra is neuroprotective (Loddick et al., 1997) and suggest that CB-induced neuroprotection is mediated, at least in part, by endogenous IL-1ra.

Many cell types are susceptible to excessive NO generation, and reduction of NO production markedly improves cell survival and is potentially beneficial in the treatment of a range of pathologies, including septic shock, neurodegeneration, and inflammation (Hobbs et al., 1999). CBs inhibit generation of NO by glial cells (Cabral et al., 2001; Molina-Holgado et al., 2002), and the present results demonstrate that reduction of LPS-induced NO generation by CBs in glial cells is dependent on endogenous IL-1ra.

In summary, the results presented here support our hypothesis that endogenous IL-1ra mediates the neuroprotective and anti-inflammatory actions of CBs in primary neurons and glia. These effects appear to be mediated by both CB1 and CB2 receptors. CB-induced IL-1ra release may negatively regulate IL-1β actions in the brain, via IL-1ra blocking the IL-1 receptor (IL-1RI), after inflammatory or excitotoxic insults. It is tempting to speculate therefore that the neuroprotective and anti-inflammatory actions of CBs depend in part on modification of the balance between proinflammatory and anti-inflammatory cytokines. These findings have important implications for our understanding of the mechanisms of action of CBs in diverse CNS disorders and for the development of new neuroprotective therapies.

References