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Mechanisms of interleukin-6 synthesis and release induced by interleukin-1 and cell depolarisation in neurones

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Cytokines are important mediators of the immune response to infection and injury and are produced mainly by lymphocytes or monocytes. Many aspects of the acute phase response are mediated by the actions of cytokines such as interleukin (IL)-1 and IL-6 within the brain. IL-1-induced IL-6 expression in neuronal cells has been described previously, but the mechanisms of IL-6 transport and release remain unknown. We show here that IL-1 induces IL-6 gene and protein expression in mouse primary cortical neurones, but that the IL-6 protein is stored intracellularly in the perinuclear area. Depolarisation of IL-1-treated neurones caused the axonal transport and release of IL-6 into the extracellular compartment. The transport and release occurs via an active mechanism (blocked by colchicine) through the Golgi apparatus, but not secretogranin-II vesicles. These results reveal a neuronal-specific mechanism of IL-6 synthesis, transport and release in response to IL-1 and cell depolarisation.

Introduction

Pro-inflammatory cytokines, interleukin (IL)-1 and IL-6, are key mediators of local and systemic inflammation and immune response to tissue injury and infection. The actions of these cytokines in the brain regulate many aspects of the acute phase response to central and peripheral insults. These include induction of fever, hypophagia, slow-wave sleep, sickness behaviour, neuroendocrine changes, most notably via actions on the hypothalamic–pituitary–adrenal axis and the modulation of peripheral or central immune and inflammatory responses (for a review, see Rothwell and Hopkins, 1995; Rothwell and Luheshi, 2000).

IL-1 and IL-6 are produced in the periphery mainly by cells of the monocyte/lymphocyte lineage but can be expressed by many cell types. Their expression is increased dramatically during the acute phase response and activation of the peripheral immune response. As in the periphery, expression of these cytokines in the brain occurs mainly in cells associated with the central inflammatory response such as astrocytes and microglial cells (Pearson et al., 1999; Maeda et al., 1994). In particular, IL-1 is produced primarily by microglia, and its action on astrocytes to produce IL-6 has been documented widely (Aloisi et al., 1992; Parker et al., 2002). In addition to the known action of IL-1 on glia, this cytokine also has a number of reported actions on neurones, such as a rapid changes in membrane ion currents (Diem et al., 2003; Desson and Ferguson, 2003), activation of neurone-specific signalling pathways, including CREB (Srinivasan et al., 2004) and the sphingomyelinase/ceramide pathways (Davis et al., 2006). These signalling pathways are believed to trigger neuronal firing as well as the expression of specific genes. IL-1 induces IL-6 mRNA expression in neurones (Ringheim et al., 1995), suggesting that neurones can produce IL-6. A recent report showed that a neuronal-like cell line (PC12 cells) transfected with IL-6 gene, released IL-6 protein in response to growth factor exposure by an unknown mechanism (Moller et al., 2006). IL-6 has a leader sequence and is secreted by immune cells through the classical synthesis/secretory pathway (i.e. Golgi apparatus) that is normally used for all secreted cytokines (Rubartelli et al., 1990), but the mechanism of IL-6 synthesis, transport and release by neurones is unknown.

We report here for the first time that IL-1 induces in neurones the synthesis of IL-6 protein which remains in the intracellular compartment. Depolarisation of the cells triggers the axonal transport of IL-6 and subsequent release of the cytokine. These findings suggest that neuronal activity regulates IL-6 secretion and show for the first time axonal transport of an inflammatory cytokine.

Results

IL-1β induces IL-6 gene expression in primary cortical neuronal cultures

IL-6 gene expression was induced by IL-1β (30 U/ml) in wild-type, but not IL-1R1−/− neurones, with maximal induction detected 4 h after IL-1 treatment (Fig. 1A). Real-time PCR showed that IL-6 mRNA expression was upregulated significantly in response to IL-1β in wild-type neurones. No IL-6 gene expression was detected by real-time PCR in untreated IL-1R1−/− neurones (data not shown). In wild-type neurones, IL-6 expression was increased in response to different concentrations of IL-1β (0.30–30 U/ml) after

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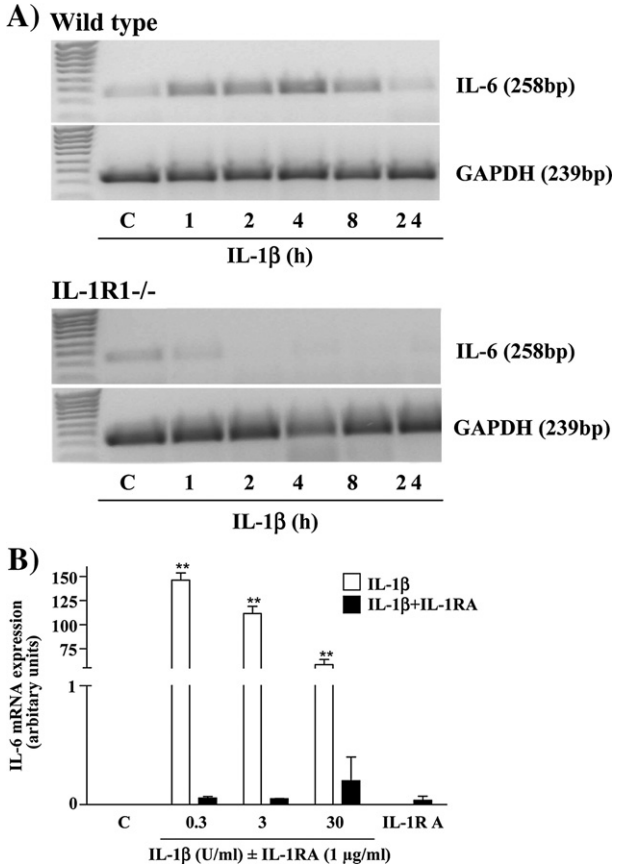

Fig. 1. IL-1β-induced IL-6 gene expression in primary cortical neurones. (A) RT-PCR for IL-6 in wild-type or IL-1R1−/− neuronal cultures treated with IL-1β (30 U/ml) for 1, 2, 4, 8 or 24 h. (B) Real-time PCR quantification of IL-6 mRNA expression after IL-1β (0.3–30 U/ml) treatment for 4 h, in the presence or absence of IL-1RA (1 μg/ml). Data are presented as mean± SD of three independent experiments on separate cultures, each performed in triplicate. $^{**}p<0.01$, treated cells versus control using one-way ANOVA and Dunnett's multiple comparison.

4 h of treatment (Fig. 1B). Effects of IL-1β were abolished by co-incubation with excess IL-1 receptor antagonist (IL-1RA), and IL-1R1−/− neurones showed no increase in IL-6 gene expression in response to any of the concentrations of IL-1β tested (data not shown).

Intracellular IL-6 protein is produced by neurones treated with IL-1β and is released by cell depolarisation

IL-1β evoked synthesis of IL-6 protein, which remained mainly intracellular (Fig. 2), and significant increases in intracellular IL-6 were detected in response to all concentrations of IL-1β tested. IL-1RA completely abolished the IL-1β-induced IL-6 production (data not shown). No significant IL-6 protein was detected in the medium in response to IL-1β, indicating that IL-6 is not released through a constitutive pathway. Depolarisation of neurones by exposure to KCl (45 mM, 3 h), after IL-1β treatment, induced significant release of IL-6 from the cell compartment into the supernatant (Fig. 2A). IL-1β-treated (0.3–30 U/ml) neuronal cultures exposed to NMDA (30 μM, 3 h) also released most of the intracellular IL-6 protein (Fig. 2B). After depolarisation by KCl or NMDA for 10 or 30 min, IL-6 protein remained intracellular, but after 1 or 2 h of depolarisation, the majority of IL-6 was released into the supernatants, showing that the optimal time point for NMDA- or KCl-induced IL-6 release was 1 h (Figs. 2C, D). Cell depolarisation induced a slight reduction in mitochondrial activity, as measured by MTT assays (Fig. 3A), but no necrosis (measured by LDH), and no significant apoptosis (measured by annexin V immunostaining) was detected (Figs. 3B, C), indicating that IL-6 is released through a regulated physiological mechanism rather than cell death.

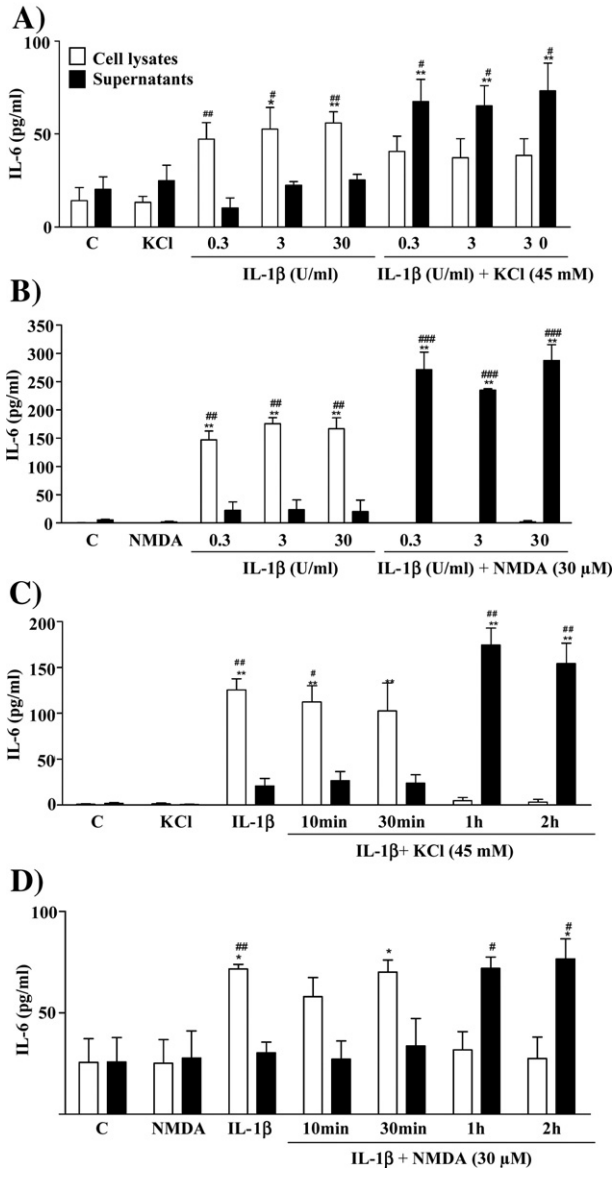

Fig. 2. IL-6 protein synthesis in IL-1β-treated primary cortical neurones and cytokine release induced by cell depolarisation. For A and B, neurones were treated with different concentrations of IL-1β (0.3–30 U/ml) for 7 h and then treated with KCl (45 mM) or NMDA (30 μM) for 3 h. For C and D, neurones were treated with IL-1β (3 U/ml) for 7 h and then treated with KCl (45 mM) or NMDA (30 μM) for 10 min, 30 min, 1 h or 2 h. Data are presented as mean ± SD of three independent experiments on separate cultures. $^{*}p<0.05$ and $^{**}p<0.01$ versus untreated cultures using one-way ANOVA and Dunnett's multiple comparison test. $^{\#}p<0.05$ and $^{\#\#}p<0.01$ cell lysates versus supernatants using unpaired Student's *t*-test.