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Neuroimmune Regulation of Neurophysiology in the Cerebellum

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Abstract

Recent studies have established the existence of an innate immune system in the central nervous system (CNS) and implicated a critical role for this system in both normal and pathological processes. Astrocytes and microglia, normal components of the CNS, are the primary cell types that comprise the innate immune system of the CNS. Basic to their role during normal and adverse conditions is the production of neuroimmune factors such as cytokines and chemokines, which are signaling molecules that initiate or coordinate downstream cellular actions. During adverse conditions, cytokines and chemokines function in defensive and repair. However, if expression of these factors becomes dysregulated, abnormal CNS function can result. Both neurons and glial cells of the CNS express receptors for cytokines and chemokines, but the biological consequence of receptor activation has yet to be fully resolved. Our studies show that neuroadaptive changes are produced in primary cultures of rat cerebellar cells chronically treated with the cytokine interleukin-6 (IL-6) and in the cerebellum of transgenic mice that chronically express elevated levels of IL-6 in the CNS. In the cerebellum in culture and in vivo, the neuroadaptive changes included alterations in the level of expression of proteins involved in gene expression, signal transduction, and synaptic transmission. Associated with these changes were alterations in neuronal function. A comparison of results from the cultured cerebellar cells and cerebellum of the transgenic mice indicated that the effects of IL-6 can vary across neuronal types. However, alterations in mechanisms involved in Ca^{2+} homeostasis were observed in all cell types studied. These results indicate that modifications in cerebellar function are likely to occur in disorders associated with elevated levels of IL-6 in the cerebellum.

Keywords

Interleukin-6; Western blot; Ca^{2+} signaling; Transgenic mice; Culture

Introduction

Interleukin-6 (IL-6) is a 21- to 29-kDa glycoprotein (size depends on post-translational modification) that recent studies demonstrate is an important signaling molecule in the central nervous system (CNS). The primary source of IL-6 in the CNS is glial cells (astrocytes and microglia), although some neurons have been shown to express IL-6, including granule neurons and Purkinje neurons of the cerebellum [1]. The cellular effects of IL-6 are mediated by a receptor system that is also expressed by cerebellar neurons [1–3]. The IL-6 receptor system consists of the IL-6 receptor (IL-6R), which binds to IL-6 but does not have signaling capabilities, and a ubiquitous membrane protein called gp130 that has signaling capabilities. Through associations with gp130, IL-6R gains the ability to signal

through JAK-STAT, MAP kinase, and other signal transduction molecules and induce downstream alterations in cellular function and gene expression. In addition to the membrane-bound receptor, a soluble form of IL-6R is found in the CNS and will interact with gp130 in cells that do not express IL-6R, thus expanding the cellular targets and consequences of IL-6 production. Expression of IL-6 is generally low in the CNS under normal conditions. However, neuronal activity can result in increased production of IL-6 [4], which may be important in physiological processes. Increased glial production of IL-6 and other cytokines is a hallmark of CNS injury and disease. Under these adverse conditions, the elevated levels of IL-6 have been shown to function in a neuroprotective role or to contribute to pathological processes that compound the CNS damage.

The expression of IL-6 and IL-6R by cerebellar neurons and glial cells implicate a physiological role for IL-6 as a signaling molecule in the cerebellum. However, such a role for IL-6 has yet to be identified. Increased expression of IL-6 in the cerebellum has been documented in animal models of several disease states, suggesting that IL-6 also plays a role in pathological conditions. For example, IL-6 was reported to be increased in the cerebellum in animal models of Niemann–Pick disease [5], autism [6], global cell leukodystrophy [7], systemic lupus erythematosus [8], viral infection [9], chronic inflammation [10], and aging [11]. An understanding of effects of the increased levels of IL-6 on the function of cerebellar neurons is basic to an understanding of these and other diseases. To address this question, we are studying the effects of chronic exposure to elevated levels of IL-6 in cultures of cerebellar neurons and in the cerebellum of transgenic mice that express elevated levels of IL-6 in the CNS through enhanced astrocyte expression. Our results show that activation of the IL-6 signaling pathway can result in significantly altered neuronal physiology (Fig. 1a).

IL-6 Alters the Expression of Neuronal Proteins and Neuronal Physiology

Cerebellar granule neurons play a critical role in the transmission of information to the cortical region of the cerebellum. Consequently, the physiology and development of granule neurons has been extensively studied both in vivo and in culture. Cultured granule neurons have been an important model for elucidating mechanisms involved in neuron development. We have used this preparation to determine if exposure to IL-6 affects the physiology of developing neurons. The culture preparation enabled the study of granule neurons in relative isolation from other cerebellar cell types that also express IL-6R. The granule neuron cultures were prepared from 8-day postnatal rats, an early developmental stage, and treated with IL-6 (0.5–500 ng/ml) in the culture medium starting at 1 day in vitro (DIV). IL-6R and gp130 are expressed in the cultured granule neurons [2].

Western blot analysis of neuronal proteins showed that after 5 DIV, when the granule neurons are morphologically well developed, IL-6-treated cultures expressed significantly lower levels of several important neuronal proteins compared with sister control cultures that were not exposed to IL-6 [2, 12]. These proteins included proteins associated with synaptic transmission and Ca^{2+} signaling including the L-type voltage-gated Ca^{2+} channel (VGCCs), the plasma membrane Ca^{2+} pump, the metabotropic glutamate receptor 1 (mGluR1), PLC β , and the IP3 receptor (IP3R) [12]. Western blot analysis of cerebella from the IL-6 transgenic mice (1–2 months of age) also showed reduced expression of neuronal proteins including L-type VGCCs, mGluR1, PLC β , and IP3R [12]. In both the cultures and IL-6 cerebellum, the level of the structural protein β -action was not altered by IL-6 suggesting that the reduced levels of neuronal proteins were not due to neuronal loss.

Western blot analysis of the level of protein for the GluN1 subunit of *N*-methyl-D-aspartate receptors (NMDAR), which also play a key role in Ca^{2+} signaling, was significantly increased in the IL-6-treated granule neuron cultures compared with control cultures,

although a decrease was observed in the cerebella from the transgenic mice [12]. In parallel electrophysiological studies we examined NMDAR function in the cultured granule neurons to determine if the altered protein level for GluN1 was associated with functional changes. Both the current response and Ca^{2+} signal produced by brief application (1 s) of NMDA to the granule neurons were significantly larger in the IL-6-treated neurons compared with control granule neurons (Fig. 1b–d). Studies with blockers of Ca^{2+} release from intracellular stores showed that enhanced release of Ca^{2+} from intracellular stores contributed significantly to the enhanced Ca^{2+} signal observed with NMDAR activation [13]. Enhanced release from intracellular Ca^{2+} stores was also observed for Ca^{2+} signals evoked by activation of VGCCs by K^+ evoked membrane depolarization [14].

Chronic Exposure to IL-6 Enhances mGluR1-Activated Ca^{2+} Signaling

Cerebellar Purkinje neurons express an abundance of mGluR1, which plays an important role in synaptic transmission and plasticity. Activation of mGluR1 results in the production of IP3, activation of IP3R, and Ca^{2+} release from internal stores. Chronic exposure of cerebellar cultures prepared from the cortical region of the cerebellum from 20-day rat embryo resulted in reduced expression of mGluR1 in the Purkinje neurons as measured by immunohistochemical methods [15]. No effect of the IL-6 treatment was observed on the level of calbindin, a Ca^{2+} binding protein, or the IP3R in the Purkinje neurons in these immunohistochemical studies. Neuronal size and culture density were unaltered by the IL-6 treatment [3]. Measurement of the Ca^{2+} signal evoked by brief application (1 s) of the mGluR1 agonist DHPG showed that Purkinje neurons treated with IL-6 (5 ng/ml) during the culture period showed an enhanced Ca^{2+} signal to DHPG compared with Purkinje neurons in control cultures (Fig. 1b–d). The enhanced Ca^{2+} signal to DHPG was not evident in the chronic IL-6-treated Purkinje neurons after treatment of the Purkinje neurons with drugs that reduced Ca^{2+} release from intracellular Ca^{2+} stores [15]. In addition, IL-6 treatment (5 ng/ml) enhanced the Ca^{2+} signal evoked by brief application of AMPA to the Purkinje neurons to activate AMPA receptors [3]. The Ca^{2+} signal produced by AMPA primarily results from Ca^{2+} influx through VGCCs followed by Ca^{2+} release from intracellular stores.

Taken together, these studies demonstrate that chronic exposure to IL-6 significantly alters processes involved in Ca^{2+} signaling and homeostasis in cerebellar neurons. These effects are likely to play an important role in the altered cerebellar function and ataxia that has been reported for the IL-6 transgenic mice and in disease states where the innate immune system of the cerebellum is activated.

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Fig. 1. Elevated levels of IL-6 in the cerebellum alter neuronal function. **a** Diagram illustrating interactions between the innate immune system of the cerebellum and cerebellar neurons. **b** Rat cerebellar Purkinje neurons (*arrows*) in control and IL-6 treated cerebellar cultures. The neurons were loaded with the Ca^{2+} -sensitive dye fura-2 and used for Ca^{2+} imaging studies. **c** Representative recordings of Ca^{2+} signals produced by brief (1 s; at the *arrow*) application of the mGluR1 agonist DHPG to the Purkinje neurons in a control and IL-6 treated culture. **d** Mean values for the peak amplitude of the Ca^{2+} signals evoked in the cultured Purkinje neurons by DHPG. Numbers in the bar indicate the number of neurons studied. *Significantly different from control neurons (unpaired *t* test)