Short communication

Inhibition of astrocyte TNFα expression by extracellular potassium

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Abstract

TNFα and IL-6 are cytokines of great interest, given the numerous biological activities and the documented expression in several central nervous system (CNS) pathologies. In this report, we have examined cultures of IL-1- or IL-1/IFNγ-activated human fetal astrocytes as a model to study mechanisms of cytokine regulation in the inflamed CNS. Since one of the major functions of astrocytes is spatial buffering of K⁺ ions, we examined the effect of high extracellular KCl on astrocyte cytokine expression by ribonuclease protection assay and ELISA. Results demonstrate that astrocyte TNFα production was potently inhibited by K with 44 and 89% inhibition at 25 and 55 mM K⁺, respectively. In contrast, astrocyte IL-6 inhibition required higher concentrations of K⁺ (≥75 mM). These results demonstrate a novel role for astrocyte potassium channel activity in modulation of glial cytokine production.

Theme: Endocrine and autonomic regulation

Topic: Neural-immune interactions

Keywords: Brain; Hyperkalemia; Cytokine; Inflammation

Astrocytes play an important role in the maintenance of an optimal microenvironment for neuronal growth and functional activity. For example, excess K⁺ ions excreted by overly active neurons into the extracellular space is shunted by astrocytes to an area of lesser K⁺-concentration, a process often referred to as ‘spatial buffering’ [15]. In this manner, astrocytes may modulate neuronal excitability and diminish spreading depression. This role may be of particular importance in pathological conditions such as ischemia, hypoxia or epileptic seizures, where the extracellular K⁺ concentration can rise to 80 mM from the 3.5 mM observed under resting conditions [16]. Recent studies have shown the presence of voltage-gated ion channels in astrocytes and their important role as modulators of astrocyte activation and signaling [1].

Mounting evidence has led to the emerging understanding of astrocytes as immunomodulatory cells. As an integral part of the blood–brain-barrier, astrocytes recognize inflammatory cells and cytokines and mediate their migration into the CNS parenchyma [2,8,9,14]. This is pertinent to inflammatory diseases of the CNS such as multiple sclerosis and HIV-1 encephalitis, in which astrocyte-activating cytokines such as IL-1, TNFα, and IFNγ have been shown to exist. Astrocytes are also important sources for cytokines such as TNFα and IL-6 in the CNS. Because one of the characteristics of diseased CNS is its change in ionic environment [4,6], we sought to determine if hyperkalemic conditions could modulate inflammatory activation of astrocytes. Our earlier study showed that potassium and potassium channel inhibitors could modulate the expression of inducible nitric oxide synthase (iNOS) in astrocytes [13]. In this report, we demonstrate that potassium is a potent inhibitor of astrocyte TNFα production.

Human fetal astrocytes were isolated and cultivated as described [10]. Briefly, cerebral tissue from second trimester fetal abortuses were dissociated by trituration and enzymatic digestion, and plated as mixed cultures comprising astrocytes, neurons and microglia [10]. At 2 weeks, culture media containing microglia were removed, and the remaining adherent cells were subjected to trypsinization and replating.

This procedure was repeated every 2 weeks until highly
enriched astrocytes (>99% glial fibrillary acidic protein: GFAP+) were obtained. Culture medium was DMEM (5 mM KCl, 110 mM NaCl, 44 mM NaHCO₃) with 4.5 g/l of glucose, 4 mM of L-glutamine, and 25 mM of HEPES buffer (from Life Technologies: Bethesda, MD: Catalogue # 11995) supplemented with 5% FCS (Gemini: Calabasas, CA), penicillin (100 U/ml)/streptomycin (100 μg/ml) and fungizone (0.25 μg/ml). High potassium medium with normal osmolarity was prepared by purchasing a custom-made DMEM identical to the astrocyte media described above, but without KCl or NaCl (Life Technologies) and then adding back desired amounts of KCl and NaCl [13]. The concentrations of K⁺ and Na⁺ in each medium were determined with ion-specific electrodes (Multichannel Analyzer: Roche Hitachi # 914) and the osmolarity by freezing point depression.

To determine the expression of cytokine mRNA by astrocytes, ribonuclease protection assay (RPA) was performed [7]. Highly enriched astrocytes were stimulated with 10 ng/ml each of IL-1β±IFNγ (PeproTech: Rocky Hill, NJ) for 16 h, then RNA was extracted by TRIZOL (Molecular Research Center, Cincinnati, OH). Ten (10) μg of total RNA was analyzed by RPA using a custom-made probe set from PharMingen following the manufacturer’s instructions. Densitometry was performed using Ambis Imagequant software. For ELISA, triplicate astrocyte cultures in 96 well plates were stimulated with cytokines for 16 h (for TNFα) or 48 h (for IL-6), then cytokine levels in culture supernatants were measured by commercial ELISA kits (Immunotech). The concentrations of stimulating cytokines and duration of stimulation were determined based on our previous studies [11].

To determine if high potassium media induced astrocyte toxicity, cell viability was determined by the lactate dehydrogenase assay. Mild cytotoxicity (~15%) was induced at 100 mM K⁺ but not at lower concentrations, as reported [13].

The results of cytokine assays are illustrated in Figs. 1 and 2. The results of nitrite assays are shown for comparison [13]. In human fetal astrocytes, maximal induction of cytokines and nitrite required stimulation with IL-1/IFNγ, as reported [3,11]. We tested whether high extracellular KCl could modulate IL-1/IFNγ-induced cytokine production in astrocytes. The results indicate that high extracellular K⁺ inhibited IL-1/IFNγ-induced astrocyte TNFα production in a dose-dependent manner, with approximately 40, 90, and 98% inhibition at 25, 55, and 75 mM K⁺ (Fig. 1B). IL-6 was also inhibited by K⁺, but only at 75 and 100 mM (Fig. 1C). Nitrite was inhibited by KCl at 55 mM or higher (Fig. 1A), as reported [13]. We further tested whether K⁺ exerted similar inhibitory effects on cytokine induction by IL-1β alone (Fig. 2).

Stimulation of astrocytes with IL-1β alone induced high nanogram amounts of IL-6 but picogram amounts of TNFα as reported [11], and these levels were regulated by K⁺. IL-1β-induced TNFα was inhibited dose-dependently at 25, 55, and 75 mM K⁺, similar to that observed with IL-1/IFNγ stimulation (Fig. 2B). In contrast, IL-1β-induced IL-6 levels were increased at 25, 55 and 75 mM K⁺ but reduced at 100 mM K⁺ (Fig. 2C). IL-1-induced nitrite levels were also increased at 25 and 55 mM K⁺, as reported [13]. Thus, both IL-1- or IL-1/IFNγ-induced astrocyte TNFα production was inhibited at physiological levels of hyperkalemic conditions, while IL-6 was not inhibited when stimulated with IL-1 alone, and inhibition...
TNFα mRNA expression (n=3), while inhibiting protein expression (Fig. 1).

In this report, we sought to determine the influence of neural molecules on astrocyte cytokine expression, to gain insight into how the neural environment may affect astrocyte activation in vivo. One of the important functions of astrocytes is spatial buffering of potassium. Excessive extracellular levels of potassium locally produced under physiological and pathological conditions can affect astrocyte function. We demonstrated that excess extracellular K⁺ in human fetal astrocyte cultures significantly inhibits IL-1β- or IL-1β/IFNγ-stimulated TNFα generation. The results suggest that TNFα production and function can become markedly limited in a high potassium CNS-environment. The results with IL-1 vs. IL-1/IFNγ on IL-6 production are interesting because they demonstrate that alterations in potassium levels can exert dual effects on astrocyte IL-6 expression, while consistently inhibiting TNFα. In this regard, regulation of IL-6 by K⁺ resembles that of iNOS, which also varied depending on the stimulation (IL-1 vs. IL-1/IFNγ) [13]. It is also noted that whereas 25 mM K⁺ significantly and consistently inhibited the level of TNFα protein production (n=3), no such inhibition was detected at the mRNA level. These data are consistent with the action of K⁺ at multiple steps of TNFα transcription and translation. Furthermore, IL-1/IFNγ-induced astrocyte ICAM mRNA was not altered by K⁺, even at extremely high doses, demonstrating that the expression of inflammatory molecules are differentially regulated by K⁺ in astrocytes.

The mechanism by which astrocytes involved in CNS injury regulate extracellular K⁺ depends on the composition of ion channels residing in the plasma membrane of these gliotic cells. MacFarlane and Sontheimer [12] have studied an in vitro glial scar model and shown that ‘scarring’ induces a rapid shift in potassium channel composition. A reduction in inwardly rectifying potassium channels (Kᵢ) was noted as well, as an increase in K⁺ conductance through delayed rectifiers (Kᵢ,). Shunting potassium out of astrocytes. These findings have implications for our study on astrocyte TNFα generation. Under normokalemic conditions, the increased shunting of K⁺ out of astrocytes through Kᵢ, channels would conceivably provide a condition favorable for TNFα induction. Yet various pathological conditions associated with elevated extracellular K⁺ would limit Kᵢ, activity, leading to intracellular K⁺ accumulation, and subsequent inhibition of TNFα induction. The fact that the extracellular concentration of K⁺ used to inhibit TNFα in our experiments was within the physiological norm implies that a subtle change in potassium concentrations may indeed affect astrocyte TNFα expression.

Cytokines TNFα and IL-6 are induced under a number of pathologic conditions of the CNS and are pivotal in the regulation of inflammatory and cytotoxic responses [2,5]. Whereas both neurotoxic and protective effects are ob-

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served with IL-6, TNFα appears to exert primarily toxic and proinflammatory responses in the CNS. Thus the differential regulation of TNFα and IL-6 by potassium found in our study may be a mechanism by which neural cells interact with immune system components to limit the extent of neural damage. These findings may have relevance to the pathogenesis of several CNS disorders, especially those in which altered K⁺ levels have been implicated.

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References