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Differential effects of interleukin-1β on neurotoxicity, cytokine induction and glial reaction in specific brain regions

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Abstract

An appropriate inflammatory response is crucial for the maintenance of tissue homeostasis. The inflammatory responses seen in the brain parenchyma differ from those elicited in the periphery, ventricles and meninges. However, although an inflammatory component has been associated with many CNS diseases, the differences among parenchymal inflammatory responses in different brain regions have not yet been fully elucidated. Here, we performed a systematic comparison of the effects of a common pro-inflammatory stimulus, IL-1β, on the hippocampus, substantia nigra, striatum and cortex. We determined various responses, including cytokine mRNA induction, glial activation, immune cell infiltration and changes in neuronal integrity, in both injected and adjacent regions 1 and 6 days after the injection of IL-1β. We found that the mRNA for TGF-β was up-regulated in a region-specific manner after IL-1β administration. Contrary to its response in the periphery, IL-1α showed no detectable induction in the tested parenchymal regions. In addition, cytokine induction was also sometimes observed in regions distant from the site of injection. Interestingly, IL-1β-mediated neurodegeneration was observed in the dentate gyrus of the hippocampus, but not in the other tested regions. The cellular recruitment mediated by IL-1 β injection consisted mainly of polymorphonuclear cells (PMN), which correlated with IL-1βmRNA induction even in regions far from the injection site. These results indicate that various parenchymal regions show different inflammatory responses and neurodegeneration in response to IL-1β. Taken together, our results provide a more precise picture of regional inflammation in the brain and should provide a basis for differential interpretation of results based on regional inflammatory differences.

Keywords: Neurodegeneration; Cytokine network; Polymorphonuclear cells; Neuroinflammation; Distant effects

1. Introduction

Interleukin-1 (IL-1), a cytokine produced by a number of peripheral cell types including monocytes, macrophages, B cells, dendritic cells and endothelial cells, plays many roles in immune and inflammatory responses (reviewed in Burger and Dayer, 2000; Dinarello, 2000a,b). IL-1 action is regulated by a complex network of molecules that includes multiple ligands [IL-1α, IL-1β and the endogenous IL-1 receptor antagonist (IL-1ra)], several binding sites [IL-1 receptor (IL-1R) type I, IL-1R type II, IL-1 accessory protein and other soluble receptors], and a key regulatory enzyme (IL-1β converting enzyme). Most of these molecules have been identified in the brain under varying circumstances, both in neurons and glial cells (Loddick et al., 1998; Vitkovic et al., 2000).

IL-1 can elicit both adaptive and maladaptive responses in the brain. For example, low concentrations of IL-1β protected cultured cortical neurons against excitotoxicity, but high concentrations of IL-1β were neurotoxic (Strijbos and Rothwell, 1995). In vivo, infusion of hrIL-1β into the striatum or cortex caused no overt neuronal damage, but could exacerbate the damaged caused by excitotoxins (Lawrence et al., 1998). In vitro, IL-1β enhanced survival of 1-methyl-4-phenylpiridinium ion (MPP+) lesioned cultured fetal rat dopaminergic neurons (Akaneya et al., 1995), but neutralizing antibodies to IL-1β attenuated lipopoly-

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saccharide (LPS)-induced dopaminergic cell loss (Gayle et al., 2002). Another study showed that IL-1β had no effect on dopaminergic neuronal survival in vivo (Castaño et al., 2002).

Although the etiologies of CNS disorders vary, IL-1 appears to be a common link in many of the processes leading to neuronal death, and suppression of endogenous IL-1 activity has thus often yielded neuroprotective effects (reviewed in Hanisch, 2002; Rothwell et al., 1996). Stroke, head injury, cerebral or subarachnoid hemorrhage and ischemic brain damage all cause neuronal damage leading to neurodegeneration. In these cases, although the initial neuronal injury is acute, the subsequent neuronal loss can occur hours or days after the initial event (reviewed in Allan and Rothwell, 2001). This delayed cell damage results from endogenous factors that are released in response to the primary injury, among which IL-1 plays a crucial role (Rothwell, 2003).

A given cytokine (e.g. IL-1β) can have different effects on CNS cells, depending on the elicited glial and inflammatory responses. Regional differences in the expression of inflammatory mediators have been shown both for astrocytes (Denis-Donini et al., 1984) and microglia (Ren et al., 1999). In addition, the neuronal phenotype can be a crucial determinant of the inflammatory response. However, although inflammatory responses have been studied in a great number of neuronal types and brain regions, no previous study has systematically examined regional differences in responses to a given cytokine.

Here, we systematically compared the effects of IL-1β injection on neurons and glial cells in four different brain regions with highly differential characteristics: 1) the hippocampus, a highly laminar structure composed of glutaminergic neurons with large areas of neuronal processes and astrocytes; 2) the substantia nigra, which contains the highest concentration of microglia in the brain and a neuronal population highly susceptible to oxidative damage, i.e. the dopaminergic cells; 3) the striatum, a clustered region composed of neurons and myelinated axons, i.e. oligodendrocytes; and 4) the cortex, a laminar organization of neurons close to the pia matter. We used competitive semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) to determine the mRNA levels of IL-1α, IL-1β, IL-1ra and the anti-inflammatory cytokine, TGF-β, in injected and connected regions 1 and 6 days after injection of IL-1β. At the same time points, glial activation, immune cell infiltration and neuronal and myelin integrity were evaluated by immunohistochemistry. These regions are targets of different neurological diseases in which an inflammatory component is present but the functional relevance of inflammation is still not totally resolved. These diseases include diverse pathologies such as Alzheimer's disease (hippocampus and cortex), Parkinson's disease (substantia nigra and striatum), stroke (cortex) and epilepsy (hippocampus), among others (Aarli, 2003; Allan and Rothwell, 2001; Perry et al., 2003). Therefore, the elucidation of the effects of a key pro-inflammatory cytokine such as IL-1 in these regions, should give basic insights into the pathological involvement of this cytokine in these diseases.

2. Experimental procedures

2.1. Subjects

Adult male Wistar rats (250–300 g) were kept under controlled temperature (22 °C±2 °C) and artificially lit under a 12-h cycle period. Subjects were acclimated to the colony for a week before surgical procedures were performed. Standard rat chow and water were freely available. All animal procedures were performed according to the rules and standards of the European Communities Council Directive and the regulations for the use of laboratory animals of the National Institute of Health, USA, and approved by the ethical committee of the Leloir Institute.

2.2. Surgical procedures

For stereotaxic injections the animals were anaesthetised with ketamine chlorhydrate (80 mg/kg) and xylazine (8 mg/kg) and then injected unilaterally with 10 ng (1000 U) of human recombinant IL-1β (hrIL-1β) (Pharmingen) dissolved in 2 μl of 0.1 mg/ml of bovine serum albumin in endotoxin-free saline or vehicle only using a 10-μl Hamilton syringe. The stereotaxic coordinates were: 1) hippocampus: bregma, −3.8 mm, lateral, +1.5 mm, ventral, −3.5 mm; 2) substantia nigra: bregma, −5.8 mm, lateral, +2.0 mm, ventral, −8.0 mm; 3) striatum: bregma, +0.7 mm, lateral, +3.0 mm, ventral, −5.0 mm; 4) cortex: bregma, +0.7 mm, lateral, +2.7 mm, ventral, −2.0 mm. A group of non-treated animals was used to normalise the PCR values (naïve group).

We also injected 10 ng of denatured hrIL-1β (heated for 10 min at 70 °C) to control for unspecific effects of protein inoculation. In addition, we used a 50-μm-diameter capillary to reduce the traumatic effect of the needle.

2.3. Competitive RT-PCR

Animals were killed before noon 1 or 6 days after hrIL-1β or vehicle injection, the brain immediately removed and dissected. Naive animals were killed at the same moment for internal control for normalisation. Briefly, the brain was cut transversally around the needle tract and the structure injected and the contralateral one were identified and dissected. In addition, the cortex of the striatum-injected animals and the striatum of cortex-injected animals were extracted. Each group included 3–4 animals. Total RNAs from injected and contralateral structures were extracted as previously described (Alonso et al., 2002). Briefly, tissues