Interleukin-1 in the Brain

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CONTENTS

10

Regulation of the synthesis of nerve growth factor (NGF) by interleukin-1 (IL-1): facts and questions

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The two main goals of this chapter are: (a) to review the available information on the importance of IL-1 for the synthesis of NGF and (b) to outline the most important gaps in our understanding of the mechanisms of this regulation. Topics discussed in the context of this second goal, such as release and processing of IL-1, inhibitors and receptors (for brevity, activation of the IL-1 gene and its regulation will not be covered) are of general importance for the physiology and pathophysiology of IL-1 and thus overlap with other chapters of this volume is inevitable.

10.1. Introduction to the physiology and pathophysiology of NGF and related neurotrophic molecules

The neurotrophins form a family of proteins with established neurotrophic actions. At least three of its members have so far been identified and characterized in detail. NGF, which was discovered several decades ago (for a review of the history of this discovery, see Levi-Montalcini, 1987) is by far the best characterized of these molecules when compared to the more recently discovered brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) (for a short review, see Thoenen, 1991). All neurotrophins have a related primary structure, as exemplified by the conservation of all six cysteine residues. However, certain regions of the molecule display sufficient structural variability to explain the different spectrum of neuronal specificity of each of these proteins.

In terms of biochemistry and physiology, NGF is the most well-known of these proteins and in this chapter it will suffice to introduce in more detail only NGF (for details and literature on BDNF and NT-3, see Thoenen, 1991). Under physiological conditions the effects of NGF depend on the developmental stage of the responsive neurons (for extensive review of the physiology of NGF, see Levi-Montalcini and Angeletti, 1968; Thoenen and Barde, 1980; for an updated short review, see Barde, 1989). During the period of naturally occurring neuronal cell death, NGF regulates the extent of survival of specific populations of neurons (sympathetic and subpopulations of neural crest-derived primary sensory neurons)(see cited reviews; for a review on the survival of sensory neurons, see Johnson *et al.*, 1986) whereas in later developmental stages NGF mainly regulates specific neuronal properties; for example, NGF regulates the activity and synthesis of enzymes involved in neurotransmitter synthesis (i.e., tyrosine hydroxylase and choline acetyltransferase) and the synthesis of neuropeptides (for reviews, see Thoenen and Barde, 1980; Otten, 1984). Effects of NGF during the development of the CNS are less well-established. Administration of NGF prevents degeneration of cholinergic neurons of the basal forebrain after axotomy and improves the behavioural performance of memory impaired aged rats (for recent reviews, see Thoenen *et al.*, 1987; Whittemore and Seiger, 1987; Barde, 1989). NGF has also been shown to prevent neuronal degeneration after axotomy in the peripheral nervous system (Johnson *et al.*, 1985). Similar effects have been observed for the neurotrophins BDNF and NT-3 (see Thoenen, 1991).

10.2. Macrophage-derived IL-1 upregulates NGF mRNA after lesion of a peripheral nerve

After transsection of the rat sciatic nerve, a biphasic increase in NGF mRNA levels was observed in the nerve near the lesion site (Heumann *et al.*, 1987a). Directly distal and proximal to the lesion a first rapid increase of NGF mRNA occurred 6 hours after surgery attaining 25–30-fold augmented NGF mRNA levels. After a decrease to intermediate levels NGF mRNA reincreases to reach a second plateau phase 3-5 days after lesion. However, only the first rapid increase was observed in organ cultures of sciatic nerve. The addition of activated macrophages or medium conditioned by these cells to the organ cultures again results in an approximately 10-fold increase of NGF mRNA with maximum levels after 3-6 hours (Heumann *et al.*, 1987b). Of a variety of cytokines and growth factors which are synthesized by macrophages, recombinant human IL-1 (α or β) has the strongest effects on NGF mRNA levels in nerve organ culture. This increase reaches its peak 3-6 hours after IL-1 addition and is maximal at 10 U/ml IL-1β. A smaller effect is seen with PDGF. Furthermore, neutralizing antibodies to IL-1 abolishes most of the NGF mRNA-inducing activity of macrophage-conditioned media (Lindholm *et al.*, 1987). Taking into account the well-known invasion of macrophages to sites of peripheral nerve lesion (Beuche and Friede, 1986; Stoll and Müller, 1986; Perry *et al.*, 1987) these findings are compatible with the following interpretation. Two to five days after lesion monocytes from the blood accumulate at the site of injury, become activated and release IL-1. This

protein then plays a major role in the upregulation of NGF mRNA in non-neuronal cells of the nerve sheath. By *in situ* hybridization analysis of the distal and proximal nerve end, it has been shown that most non-neuronal cells of the nerve (including fibroblasts and Schwann cells) take part in the upregulation (Bandtlow *et al.*, 1987). Recently this interpretation received support from observations made in mouse mutants in which macrophage invasion to the distal parts of lesioned nerves is absent (Lunn *et al.*, 1989; Brown *et al.*, 1991). This lack of macrophage invasion is correlated with a lowered expression of IL-1β mRNA in the distal nerve stump (Heumann *et al.*, 1990). In these mice the initial increase of NGF mRNA after transsection occurs, but the second is substantially reduced (Brown *et al.*, 1991). Accordingly, electrophysiological and morphological data show a strongly reduced regeneration of sensory fibres. This is in contrast to motor fibres which regenerate at a normal rate and which (in contrast to sensory neurons) are not known to respond to NGF (Bisby and Chen, 1990; Brown *et al.*, 1991).

Meanwhile, data on the expression of a second neurotrophin, BDNF, in the lesioned peripheral nerve are available. Under physiological conditions BDNF mRNA is predominantly expressed in the CNS (for literature, see Thoenen, 1991). However, after sciatic nerve lesion, high levels of BDNF mRNA are detected near the lesion site. The time-course of BDNF mRNA induction is rather slow compared to the increase of NGF mRNA after nerve lesion (M. Meyer *et al.*, submitted). Additional evidence for a differential regulation of BDNF mRNA and NGF mRNA has been derived form nerve organ cultures, which under *in vitro* conditions express intermediate levels of BDNF mRNA. They fail, however, to respond to IL-1 by an increase in BDNF mRNA (M. Meyer *et al.*, submitted). Thus the expression of two members of a family of structurally closely related proteins is regulated in a differential way. This is interesting since neurons responsive to both proteins are present in dorsal root ganglia and extend their axons into the sciatic nerve. Therefore, it can be concluded that after nerve lesion, where a need for neurotrophic activity might be assumed, IL-1 does not act as a general inducer of all relevant neurotrophic proteins. In contrast, IL-1 seems to be a specific inducer of NGF mRNA.

Analysis of the cellular targets of IL-1 in the nerve reveal that IL-1 acts in a cell type-specific manner, at least *in vitro*. Primary fibroblasts cultured from the sciatic nerve respond to IL-1 by increasing NGF mRNA levels comparable in time-course and magnitude to the response described for nerve organ cultures (Lindholm *et al.*, 1988). However, primary Schwann cells fail to upregulate NGF mRNA in response to IL-1 (Matsuoka *et al.*, 1991). We have evidence that Schwann cells display IL-1 binding sites (unpublished observations), and effects of IL-1 on the release of cultured Schwann cells of plasminogen activator inhibitor have been published (Rogister *et al.*, 1990). Assuming that the interpretation of these data is correct, IL-1 can bind to Schwann cells and elicit effects different from induction of NGF mRNA. This apparent discrepancy leads to the following questions. First, other factors or cofactors (soluble and/or insoluble, such as cell-contact molecules) present in the lesioned nerve, but not in the culture, might be required to increase NGF mRNA in Schwann cells. Second, dissociation and culturing of Schwann cells (but not fibroblasts or nerve explants taken from

newborn or adult animals; Matsuoka *et al.*, 1991) might selectively uncouple the IL-1 receptor from part of its intracellular signal transduction machinery, and thus disable NGF mRNA induction while maintaining other responses. Alternatively, Schwann cells might express two different types of IL-1 receptor, one of them being functionally upregulated only after nerve lesion. The type(s) of IL-1 receptor expressed by Schwann cells has to await further analysis.

A question already addressed in the preceding paragraph is whether the IL-1 effects on NGF mRNA levels are mediated by other molecules. It has, for example, been shown that fibroblast proliferation observed after IL-1 application depends on IL-1 induced secretion of PDGF (Raines *et al.*, 1989). Furthermore, in the CNS some effects of IL-1 are apparently mediated by CRF secretion (see Rothwell, 1990). However, PDGF (AB or BB dimer) does not increase NGF mRNA levels in cultured Schwann cells (Matsuoka *et al.*, in press) although they express the appropriate PDGF receptor (Weinmaster and Lemke, 1990). Recently, a variety of growth factors and signal transduction pathways have been analysed for their influence on NGF mRNA levels in cultured Schwann cells (Matsuoka *et al*, 1991). Whereas all peptide growth factors tested so far failed to augment NGF mRNA levels, elevation of intracellular cAMP results in a rapid (maximum after 3–6 hours) elevation of NGF mRNA (6–8 fold) which can be further increased by coadministration of ionomycin and/or TPA. However, the extracellular signals which might trigger these pathways in pathophysiological situations are still elusive.

10.3. Possible role of IL-1 for the physiological regulation of NGF mRNA levels in the central nervous system

10.3.1. Localization and function of IL-1 in the brain

In view of the reports that cultured astrocytes (Fontana *et al.*, 1982) and microglial cells (Giulian *et al.*, 1988; Hetier *et al.*, 1988; Gebicke-Haerter *et al.*, 1989) can express IL-1 we questioned whether this protein is produced and might be involved in the regulation of NGF mRNA levels in the intact brain. Consequently, using *in situ* hybridization, the localization of NGF and IL-1β transcripts throughout the adult rat brain was analysed (Bandtlow *et al.*, 1990). Several interesting features of IL-1β mRNA expression in the brain were revealed. First, there is indeed a co-localization of NGF mRNA and IL-1β mRNA in most regions (periglomerular cell layer in the olfactory bulb, frontal cortex, pyramidal cell layer of the hippocampus, granule cell layer of the dentate gyrus), with some exceptions. IL-1β mRNA, but not NGF mRNA, was detected in the granule cell layer of the cerebellum, parts of the hypothalamus and the internal granular layer of the olfactory bulb. Limits in the sensitivity of the *in situ* analysis may explain some of these exceptions. Of course, this co-localization does not prove a causal relationship between IL-1 and NGF synthesis. Second, expression is mostly neuronal. Silver grains are found more or less exclusively over granule cells of dentate gyrus and cerebellum (but not over Purkinje cells of the cerebellum); periglomerular cells in the olfactory bulb; and neurons in the frontal cortex. Third,

the level of expression differs markedly between neurons in the same region (with one exception). This was not true for cerebellar granule cells which showed a rather homogenous labelling pattern. A similar pattern has been described, for example, for the expression in the brain of mRNAs encoding neurotrophins some of which have been shown to be regulated by neuronal activity (for literature, see Zafra *et al*, 1990). In contrast, other mRNAs, such as PDGF-A mRNA (Yeh *et al.*, 1991) show approximately equal levels of expression in all neurons of a specific brain region. This might suggest that neuronal activity also plays a role in the expression of IL-1β mRNA levels in contrast to, for example, PDGF A-chain mRNA levels. Direct evidence for this type of regulation has recently been provided. After kainic acid-induced convulsion a rapid upregulation of IL-1β mRNA has been observed in cerebral cortex, hypothalamus and hippocampus (but not in the cerebellum) (Minami *et al.*, 1991).

Our findings are in general agreement with data from other groups. Farrar *et al.*(1987) described the detection of IL-1β mRNA by *in situ* hybridization in the dentate gyrus and the pyramidal cell layer of the hippocampus. In contrast to their report, we did not see any signal over the choroid plexus. Lechan *et al.*(1990) found IL-1β immunoreactivity in processes of granule cells in the rat dentate gyrus. Breder *et al.*(1988) and Lechan *et al.*(1990) described IL-1 immunoreactivity in the hypothalamus of human and rat, respectively. Using polymerase chain reaction methods for the detection of IL-1β mRNA Higgins *et al.* (1991) confirmed the presence of very low levels of this mRNA in the intact rat brain. Using similar methods, Minami *et al.* (1991) detected low levels of IL-1β mRNA in a variety of brain regions, including cerebral cortex, hippocampus, hypothalamus and cerebellum.

All of these investigations have been performed in adult animals and data on the developmental expression of IL-1 in the brain, spinal cord and PNS are still lacking. A comparison of data on the expression of IL-1 and NGF during the development of the nervous system might further strengthen the hypothesis of a causal relationship between IL-1 and NGF synthesis.

Although substantial information on the regional distribution of IL-1 in the brain is available, little is known about the physiological function of endogenous IL-1. Intraventricular administration of IL-1β results in increased NGF mRNA levels in the surrounding brain tissue (Spranger *et al.*, 1990), but this does not necessarily reflect the effects of physiological concentrations of IL-1 in the non-lesioned brain. So far, there is no direct proof for a causal relationship between the synthesis of IL-1 and NGF. Furthermore, considering recent work on the influence of neuronal activity on the regulation of NGF mRNA and BDNF mRNA in the brain (see Zafra *et al.*, 1990) it is evident that IL-1 is only one regulator of NGF mRNA synthesis among others. The roles of these different regulatory mechanisms for physiological and pathophysiological processes remain to be investigated.

Outside the nervous system IL-1 is well-established as a mediator of pathological, mostly inflammatory reactions (for reviews see Martin and Resch, 1988; Dinarello and Endres, 1989; Schmidt and Tocci, 1990). What is its role (if any) in brain pathology?

Interleukin-1-like activity has been shown to accumulate in brain wounds (Nieto-Sampedro and Berman, 1987), but its source, in particular whether it is derived from within the brain parenchyma or produced by blood cells, has not been analysed. The presence of IL-1β mRNA and IL-1 immunoreactivity has been reported in the brain of Alzheimer patients (Griffin *et al.*, 1989; Rogers *et al.*, 1980; but see also Hofman *et al.*, 1986) and patients suffering from multiple sclerosis (Hofman *et al.*, 1986). Thus, IL-1 expression is modulated in pathological states of the brain. Whether this disease-induced IL-1 expression plays a role for restoration of brain function or, also possible, for maintenance of disease, remains to be elucidated.

In the following, some of the prerequisites for the action of IL-1 in the brain will be discussed. These include processes necessary for the generation of bioactive IL-1 (processing and release), the control of availability of IL-1 for target cells (inhibitors) and the regulation of IL-1 receptor expression.

10.3.2. Expression of the IL-1 receptor

The effects of IL-1 are mediated via cell surface receptors and a co-distribution of IL-1 and IL-1 receptor expression is a prerequisite of autocrine or paracrine IL-1 function in a particular brain region. Specific binding of iodinated IL-1 has been demonstrated in an autoradiographic study by Farrar *et al.*(1987) in the dentate gyrus, hippocampal pyramidal cell layer, hypothalamus and cerebellar granule cells. More recent autoradiographic studies by Haour *et al.* (1990 and this volume) and Takao *et al.* (1990) confirmed the binding in the dentate gyrus, but were unable to demonstrate further binding sites. Consistent with the autoradiographic data, Wada *et al.* (1990) in an *in situ* study, localized the mRNA coding for the type-1 IL-1 receptor in the dentate gyrus. Takao *et al.* (1990) demonstrated additional binding sites in membrane fractions derived from cerebral cortex and olfactory bulb. If this is interpreted as binding to neurons, the data on localization of IL-1 and IL-1 receptor expression suggest that IL-1 acts in an autocrine manner. However, considering the well-known endocrinological and metabolic effects of IL-1 (see the relevant chapters in this volume) it is still surprising that, besides Farrar *et al.* (1987), no other group has been able to reveal autoradiographically detectable binding sites for IL-1 in the hypothalamus. Either receptor density in this region (in contrast to the dentate gyrus of the hippocampus) is at the limits of sensitivity of the method or these effects of IL-1 are mediated via other brain regions.

Moreover, regulation of receptor expression and function might represent an important feature of the regulation of the response of the brain to IL-1 (see chapter by F. Haour, this volume). From the periphery (lymphocytes) it is known, for example, that glucorticoids upregulate the number of high-affinity IL-1 receptors (Akahoshi *et al.*, 1988). Since glucocorticoid receptors are widely expressed in the brain (see Arriza *et al.*, 1988) these regulatory mechanisms, if present in the brain, could be of relevance to the action of IL-1 in the normal and pathologic brain. In contrast to glucocorticoids, transforming growth factor β is a potent inhibitor of IL-1 receptor expression on lymphoid cells (Dubois *et al.*, 1990).

Synthesis of TGFβ mRNA has been demonstrated in the normal and lesioned brain (Nichols *et al.*, 1991; Lindholm *et al.*, unpublished).

10.3.3. **Processing and release of IL-1**

The release and processing of interleukin-1 might be another step where the action of IL-1 can be controlled. Both IL-1α and IL-1β are synthesized as precursor molecules without recognizable signal peptide (Lomedico *et al.*, 1984; Auron *et al.*, 1984; March *et al.*, 1985). In spite of these structural peculiarities, several studies on the kinetics of intracellular and extracellular accumulation of IL-1 have demonstrated that this protein can indeed be released from intact cells. This is particularly well-established for monocytes and macrophages (Giri *et al.*, 1985; Auron *et al.*, 1987; Hazuda *et al.*, 1988). Studies analysing the electron- or light-microscopic distribution of IL-1 in the cell, as well as those using subcellular fractionation techniques, localized the protein outside the compartments of the classical secretory pathway via endoplasmic reticulum and Golgi apparatus (Bayne *et al.*, 1986; Matsushima *et al.*, 1986; Conlon *et al.*, 1987; Bakouche *et al.*, 1987; Singer *et al.*, 1988; Baldari *et al.*, 1989; Beesley *et al.*, 1990; Rubartelli *et al.*, 1990). Furthermore, although human IL-1β contains N-glycosylation consensus sequences IL-1β released by human macrophages is not glycosylated. However, if human IL-1β is expressed with an aminoterminal extension coding for a known signal peptide it becomes N-glycosylated (Baldari *et al.*, 1987, Livi *et al.* 1991). This demonstrates again that IL-1β does not normally reach compartments where N-glycosylation takes place. For macrophages and monocyte cell lines it has recently been demonstrated that IL-1 might be released after uptake into a not yet characterized membrane-confined compartment (Rubartelli *et al.*, 1990). How this is achieved is not clear, but recent work suggests that also in eukaryotic cells, a special family of transporter proteins related to mammalian drug resistance gene products, helps to translocate proteins between different intracellular compartments. This is particularly well-established for yeast where the release of a small peptide, the pheromone a-factor, depends on the expression of a gene (STE6) coding for one of these putative transporter proteins (Kuchler *et al.*, 1989; McGrath *et al.*, 1989). A related translocation pathway apparently plays a role for the intracellular sorting of antigenic peptides (for an overview see, Parham, 1990). In contradiction to release mechanisms which allow survival of the IL-1 producing cells, recent work by Hogquist *et al.* (1991) demonstrates that IL-1 release from macrophages and its processing are correlated to apoptotic cell death.

Processing of IL-1 from the precursor to the mature form seems to occur simultaneously with its release. It has been demonstrated at least for human IL-1β that processing is necessary for full bioactivity and thus the regulation of processing might represent another post-translational control mechanism (Mosley *et al.*, 1987; Beuscher *et al.*, 1990). The enzymes required for processing are not ubiquitously expressed since fibroblasts transfected with cDNAs encoding the precursors of IL-1α or IL-1β (Fuhlbrigge *et al.*, 1988; Young *et al.*, 1988) failed to produce and release mature IL-1. Moreover, distinct proteases have been identified which can generate the mature forms of IL-1α (Kobayashi *et al.*, 1990) and IL-1β (Black *et al.*, 1989a; b; Kostura *et al.*, 1989, Howard *et al.*, 1991).

10.3.4. **Inhibitors of IL-1**

After release the action of IL-1 can be controlled by degradation and by IL-1 binding proteins and by IL-1 receptor antagonists. Whereas nothing is known about mechanisms of IL-1 degradation in peripheral organs or in the nervous system, a variety of IL-1 binding proteins and IL-1 inhibitors have been described (for a review on IL-1 inhibitors, see Larrick *et al.*, 1989). There is evidence that some of these binding proteins represent extracellular parts of the IL-1 receptors (Giri *et al.*, 1990; Eastgate *et al.*, 1990). Some of these binding proteins are specific for one form of IL-1 (in this case for IL-1β) and might regulate the differential availability of IL-1 isoforms. Surprising was the discovery of IL-1 receptor antagonists which are structurally related to IL-1 (but in contrast to IL-1 may contain an N-terminal signal peptide) and act as competitive antagonists of IL-1 at the type-I receptor (Bienkowski *et al.*, 1990; Eisenberg *et al.*, 1990; Hannum *et al.*, 1990; Carter *et al.*, 1990; Mazzei *et al.*, 1990; Haskill *et al.*, 1991). It remains to be investigated whether and where these inhibitors or receptor antagonists are expresssed in the nervous system.

10.4. **A second endogenous source of IL-1 might play a role during the first phase of NGF mRNA induction after peripheral nerve lesion**

As described above, we believe that the second phase of NGF mRNA induction after peripheral nerve lesion is due to IL-1 released from invading macrophages. But what are the signals and the mechanisms responsible for the first, rapid and transient, increase?

The time-course of first phase and its pharmacological characteristics (Lindholm *et al.*, 1988) together with the demonstration of IL-1 in neurons (see previous paragraph; Schultzberg *et al.*, 1987) suggested that this phase might also be caused by IL-1 synthesized in the nerve. Therefore we analysed the content of the sciatic nerve for NGF mRNA-inducing activity. Indeed, under experimental conditions which exclude protein synthesis, a strong NGF mRNA-inducing activity was detected. Furthermore, adsorption with polyclonal antibodies directed against the N-terminal end of rat IL-1β removed between 40-60% of this NGF mRNA-inducing activity (unpublished observations). These experiments show, first, that preformed factors present in the nerve might play a role for the upregulation of NGF mRNA after nerve lesion, and, second, that approximately half of this activity is IL-1β. Thus, in the peripheral nervous system IL-1β and some as yet unknown molecules act as lesion factors which are released after trauma. It remains to be seen where these lesion factors are stored in the intact nerve and whether they are stored in an active or inactive form which is processed during the degenerative process. We do not yet know whether IL-1α which has been detected in the peripheral nervous system (Schultzberg *et al.*, 1987 and this volume) plays a role in this context.

10.5. Conclusions

IL-1 is a potent regulator of many diverse processes in the central and peripheral nervous system including NGF mRNA upregulation in the lesioned peripheral nerve. Endogenous, mainly neuronal, expression of IL-1β mRNA and IL-1 activity has been discovered in the normal CNS. Data on the localization of NGF mRNA and IL-1b mRNA, and IL-1 receptor expression suggest that endogenous IL-1 might play a role in the physiological regulation of NGF expression in the CNS.

Many aspects of the regulation of IL-1 action in the nervous system have not yet been investigated. Analysis of the regulation of IL-1 mRNA expression in neural tissues and cells might be a rapid way to suggest possible roles for IL-1 in the normal brain and in brain pathology. However, due to the particular post-transcriptional and post-translational events necessary for the generation of active IL-1 (release, processing) attention has to be focused on the active protein. Furthermore, even after release of active protein many players can modulate the effects of IL-1 (degradation, binding proteins, receptor antagonists, receptor expression and coupling to signal transduction pathways).

Thus, IL-1, its function and the control of its action in the brain and in the PNS remain very attractive topics for future research.

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