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Cytokines in the nervous system with emphasis on interleukin-1 receptor-mediated activity

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ABSTRACT

Interleukin-1 (IL-1) is one of the most important pro-inflammatory cytokines and plays a pivotal role in the neuroinflammation associated with certain forms of neurodegeneration, such as epilepsy, cerebral ischemia, trauma, and Alzheimer's disease (AD). Also, it was suggested that IL-1 is involved in physiological processes, namely modulation of sleep, glucose and lipid metabolism, and neuroendocrine regulation, as well as modulation of learning and memory. The objectives of the present work were to study the role of the IL-1 system in the pathogenesis of neurodegenerative disorders such as epilepsy and cerebral ischemia, as well as the influence of central IL-1 signalling on brain morphology, expression of AD-related proteins, and inflammatory markers, and on different aspects of behaviour, activation of hypothalamo-pituitary-adrenal (HPA)-axis, and brain monoamines.

It is well known that hypothermia protects against neurodegeneration induced by excitotoxic brain damage, while hyperthermia has an aggravating effect. In **Paper I and II**, experimental excitotoxicity was induced by peripheral administration of the glutamate analogue kainic acid (KA) in rats, a well known model for human temporal lobe epilepsy, which leads to epileptic seizures, temperature changes, and specific brain damage characterized by neuronal cell death and glial activation, as well as induction of inflammatory cytokines. The aims were to study the mechanisms underlying KA-induced core temperature changes, *i.e.* initial hypothermia followed by a longer hyperthermic effect, with regard to the subtypes of glutamate receptors and the involvement of IL-1, as well as interleukin-6 (IL-6) and IL-1 receptor antagonist (IL-1ra). We found that KA-induced changes in core temperature are not dependent upon NMDA-receptors activation and are unlikely to be a result of increased motor activity since dizocilpine, a non-competitive antagonist of NMDA-receptors, blocked KA-induced behavioural, but not temperature changes **(Paper I)**. The levels of IL-1β, IL-6 and IL-1ra in the hypothalamus did not seem to correlate with KA-induced changes in core temperature, except for the increase in IL-1β in the hypothalamus, at 2 h after KA-injection. The ratios between IL-1β and IL-1ra or IL-6 were increased at the time points when the core temperatures were modified, suggesting that imbalances between cytokine levels may contribute to KA-induced core temperature changes (**Paper II**).

The role of IL-1 receptor-mediated activity in the brain, upon focal cerebral ischemia and under normal conditions, was investigated in a previously developed transgenic mouse strain with central blockade of IL-1 signalling, due to the brain-directed overexpression of human soluble IL-1ra (Tg hsIL-1ra) (**Paper III-V**). A model of permanent focal cerebral ischemia was established in the Tg hsIL-1ra mice and analysis of neurological scores, lesion size, and inflammatory markers showed similar results in Tg hsIL-1ra (heterozygotic) and wild type mice, indicating that the brain-directed blockade of IL-1 signalling was not neuroprotective in this model of permanent focal cerebral ischemia in mice (**Paper III**).

Analysis of the phenotype of the Tg hsIL-ra mice indicated that there were no significant compensatory changes of IL-1β, IL-6, or tumour necrosis factor-alpha (TNF-α) in the brain. However, the marked reduction in area density and brain volume in the Tg hsIL-1ra (homozygotic) mice indicates that IL-1 signalling in the brain affects CNS development. Furthermore, the expression of amyloid precursor protein (APP) was lower in heterozygotic, but not homozygotic male Tg hsIL-1ra mice (**Paper IV**). In addition, we observed modulation of different aspects of behaviour, and of the expression of dopamine (DA), serotonin (5-HT) and their metabolites in the brain of Tg hsIL-1ra mice, but no effect on the activation of the HPA-axis, reflected in the serum levels of corticosterone (**Paper V**). Studies on the behavioural phenotype revealed higher locomotor activity, decreased habituation for locomotion, and lower anxiety levels in the Tg hsIL-1ra mice. The levels of DA, 5-HT, and their metabolites were decreased in certain brain regions of the Tg hsIL-1ra mice.

IL-1 signalling in the brain seems to have important roles, both in the basic functionality of the CNS, but also in the pathogenesis of excitototoxic neurodegeneration, suggesting that the manipulation of IL-1 system can have promising therapeutic implications.

LIST OF PUBLICATIONS

The thesis is based on the following publications and manuscripts, which are referred to in the text by their roman numerals.

I. Ahlenius S, **Oprica M**, Eriksson C, Winblad B, Schultzberg M
Effects of kainic acid on rat body temperature: unmasking by dizocilpine
Neuropharmacology, 2002, 43 28-35

II. **Oprica M**, Spulber S, Forslin-Aronsson Å, Post C, Winblad B, Schultzberg M
The influence of kainic acid on core temperature and cytokine levels in the brain *(Submitted)*

III. **Oprica M**, Van Dam A-M, Lundkvist J, Iverfeldt K, Winblad B, Bartfai T, Schultzberg M
Effects of chronic overexpression of interleukin-1 receptor antagonist in a model of permanent focal cerebral ischemia in mouse
Acta Neuropathologica, 2004, 108, 69-80

IV. **Oprica M**, Hjorth E, Popescu B, Spulber S, Ankarcrona, M, Winblad B, Schultzberg M
The influence of chronic overexpression of interleukin-1 receptor antagonist on brain morphology, cytokine levels, and Alzheimer's disease-related proteins *(Manuscript)*

V. **Oprica M**, Zhu S, Goiny M, Pham TM, Mohammed AH, Winblad B, Bartfai T, Schultzberg M
Transgenic overexpression of interleukin-1 receptor antagonist in the CNS influences behaviour, serum corticosterone and brain monoamines
Brain, Behavior, and Immunity *(in press)*

CONTENTS

LIST OF ABBREVIATIONS

5-HIAA	5-hydroxy-3-indole acetic acid
5-HT	5-hydroxytryptamine
ACTH	adrenocorticotropic hormone
AD	Alzheimer's disease
AICD	APP-intracellular domain
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxalolepropionate
APP	amyloid precursor protein
CBF	cerebral blood flow
CCA	common carotid artery
CNTF	ciliary neurotrophic factor
CST	corticosterone
DA	dopamine
DOPAC	dihydroxyphenylacetic acid
ECA	external carotid artery
ELISA	enzyme-linked immunosorbent assay
EPM	elevated plus-maze
GABA	gamma-aminobutyric acid
HPA	hypothalamo-pituitary-adrenal
i.c.v.	intracerebroventricular
i.p.	intraperitoneal
ICA	internal carotid artery
ICE	interleukin-1β-converting enzyme
IFN-γ	interferon-gamma
IL	interleukin
IL-1ra	interleukin-1 receptor antagonist
IL-1RAcP	interleukin-1 receptor accesory protein
IL-1RI	interleukin-1 receptor type I
IL-1RII	interleukin-1 receptor type II
iNOS	inducible nitric oxide synthase
IRAK	interleukin-1 receptor-activated kinase
KA	kainic acid
KO	knockout
LAF	lymphocyte-activating factor
LPS	lipopolysaccharide
LTH	long-term habituation
LTP	long-term potentiation
MCA	middle cerebral artery
myD88	myeloid differentiation factor 88
NGF	nerve growth factor
NF-κB	nuclear factor-κB
NMDA	N-methyl-D-aspartate
NO	nitric oxide
OF	open-field
RIA	radioimmunoassay

STH	short-term habituation
Tg	transgenic
TGF	transforming growth factor
TH	tyrosine hydroxylase
TNF-α	tumour necrosis factor-alpha
TRAF	TNF-receptor associated factor
WT	wild type

1 INTRODUCTION

One of the most important findings in modern biology is the crosstalk between the nervous and the immune systems through a common biochemical language which enables a bidirectional communication involving neurotransmitters, neuroendocrine hormones, cytokines, and their respective receptors.

The immune system seems to contain a large number of proteins specific to vertebrates or mammals, and the degree of homology between related protein molecules is low, which suggests a rapid and ongoing evolution of immune functions (Huising et al., 2004).

1.1 CYTOKINES – OVERVIEW

Cytokines are soluble, secreted, low molecular weight proteins that interact with specific receptors that are located in the cell membrane or are soluble proteins. Cytokines represent a major class of intercellular signalling molecules besides neurotransmitters, hormones and autacoids. They can be regarded as humoral regulators that act non-enzymatically at pico- to nanomolar concentrations and modulate the functional activities of their target cells, under normal and pathological conditions. The main characteristics of cytokines are the pleiotropism, multifunctionality, and the ability of different cytokines to carry out the same function, known as redundancy. Some cytokines are antagonistic, while others are synergistic.

Cytokines are traditionally associated with the innate immunity and adaptive host responses, as regulators of the intensity and duration of the immune and inflammatory responses by modulating the activation, proliferation and/or differentiation of various cells, and by regulating the secretion of antibodies or other cytokines.

Functionally, cytokines can be classified into three major groups (see table 1).

1.2 INTERLEUKIN-1 FAMILY

The interleukin-1 (IL-1) family consists of three known ligands for the IL-1 receptors type I (IL-1RI) and II (IL-1RII), *i.e.* the agonists IL-1α and IL-1β, and the IL-1 receptor antagonist (IL-1ra) (Dinarello, 1991). IL-1RI is the signalling receptor, while the binding of the agonists to IL-1RII does not transduce an intracellular response (see below).

Besides IL-1α, IL-1β and IL-1ra, the IL-1 family also includes a fourth structural member IL-18, previously known as interferon-γ (IFN-γ)-inducing factor due to its property of inducing the synthesis of IFN-γ by T-cells (Okamura et al., 1995). IL-18 is also a pro-inflammatory cytokine, but its actions are not mediated via IL-1RI, but through a related receptor, IL-18RI (Torigoe et al., 1997).

Six novel homologues of the IL-1 gene family, IL-1F5 – IL-1F10, have been described recently (Nicklin et al., 2002). These proteins are classified as IL-1 family members on the basis of amino acid sequence similarity, identity of gene structure and similarities in the tertiary structure (Sims et al., 2001).

A. Cytokines that regulate the innate immune response - produced in response to the molecular patterns of the pathogen - sources: macrophages and dendritic cells, T-lymphocytes, NK cells - act mostly on leucocytes and endothelial cells - involved in the control of early inflammatory responses - include interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α), IL-6, IL-10, IL-12, IL-15, IL-18, type I interferons (IFN), chemokines
B. Cytokines that regulate adaptative immune responses - produced primarily by T-lymphocytes after recognition of a specific antigen - have role in the proliferation and differentiation of B- and T-lymphocytes after antigen recognition and the activation of effector cells - include IL-2, IL-4, IL-5, IL-13, IFN-γ, transforming growth factor-β (TGF-β), lymphotoxin
C. Cytokines that stimulate hematopoiesis - produced by bone-marrow stromal cells - stimulate the growth and differentiation of immature leukocytes - include colony-stimulating factor, stem cell factor, IL-3, IL-7

Table 1. Functional classification of cytokines

1.2.1 Synthesis and release of IL-1α and IL-1β

The production of IL-1 is induced by nearly all microbes and microbial products, as well as by many other non-microbial stimuli, *i.e.* stress factors, neuroactive and inflammatory substances, clotting factors, lipids, other cytokines, etc. (Dinarello, 1996). A dissociation between synthesis of IL-1 mRNA and translation do exist, as shown for stimulants such as complement, hypoxia, clotting factors, which induce large amounts of IL-1β mRNA in monocytes without significant translation into protein (Dinarello, 1996). The translation can be augmented by adding bacterial endotoxin or IL-1 itself to cells with high levels of IL-1β mRNA (Schindler et al., 1990).

IL-1α and IL-1β are synthesised as 31 kDa precursors and are cleaved to their 17 kDa mature forms by specific enzymes. ProIL-1β is inactive and it is cleaved at specific aspartic residues by the intracellular IL-1β-converting enzyme (ICE), now known as caspase-1, a cysteine protease with apoptotic properties (Black et al., 1988) (Fig. 1). In addition to caspase-1, several other enzymes are able to cleave proIL-1β and generate biologically active molecules (Fantuzzi et al., 1997a).

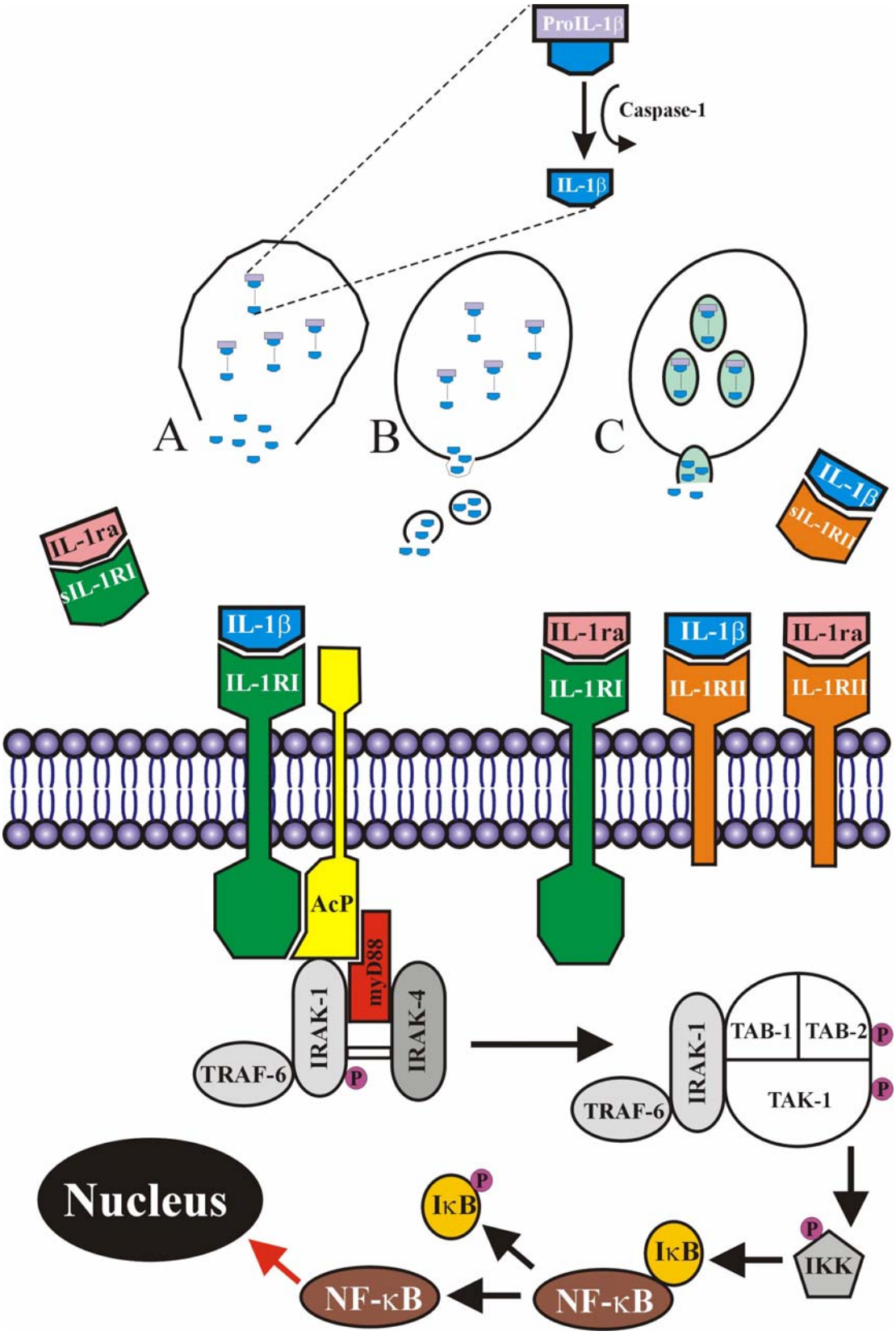

Figure 1. Synthesis, release, and signal transduction of IL-1β. Caspase-1 cleaves the precursor, proIL-1β and generates the mature, fully active IL-1β. There are several mechanisms proposed for the release of IL-1β after cleavage, namely cell membrane rupture (A), membrane blebbing (B), and lysosome exocytosis (C). Binding of IL-1β to IL-1RI is followed by the association of IL-IRAcP with the intracellular part of IL-1RI, which will initiate a series of intracellular events leading to the activation of the transcription factor, NF-κB (see text for further details on the intracellular signals).

ProIL-1α, the precursor for IL-1α, has biological activity (Mosley et al., 1987) and the mature form results from cleavage by the calcium-activated enzyme calpain (Kobayashi et al., 1990). However, most of the proIL-1α synthesised in monocytes and other cells remain intracellularly, in this state. ProIL-1α exists also as a membrane-bound biologically active form on the surface of monocytes and B-lymphocytes after stimulation *in vitro* (Dinarello, 1996). Upon cell death, proIL-1α is released from the cell and can be cleaved by extracellular proteases.

The mature IL-1β protein is secreted after the cleavage by caspase-1. The process of proIL-1β cleavage and secretion of the mature form has been extensively studied and interesting insights into the intricate mechanisms have been recently provided. Exogenous ATP and a decrease in intracellular potassium are crucial events for the release of IL-1β (Perregaux and Gabel, 1994). The mechanism of ATP-induced release of IL-1β is still controversial and several models have been proposed (see Fig. 1), *e.g.* an ATP-triggered cell death and rupture (Hogquist et al., 1991), release of microvesicles via membrane blebbing (MacKenzie et al., 2001), and exocytosis via secretory lysosomes (Andrei et al., 2004).

1.2.2 Signal transduction of IL-1α and IL-1β

The signalling pathway of IL-1α and IL-1β involves the activation of the nuclear factor-κB (NF-κB) (Fig. 1). The initiation of the intracellular signalling starts with the binding of the agonist to the signalling receptor, IL-1RI. This is followed by the association of the cytoplasmic domain of IL-1RI with an accessory protein (IL-1RAcP), which belongs to the IgG superfamily and has some homology to both IL-1RI and IL-1RII (Greenfeder et al., 1995; Guo et al., 1995). IL-1RAcP is essential for signalling to occur, *e.g.* shown by the lack of response to IL-1 in cell lines devoided of IL-1RAcP (Wesche et al., 1997). The conformational changes induced by the binding of the IL-1 agonists, but not of IL-1ra, to the receptors, allow the interaction of the receptor-ligand complex with IL-1RAcP. Thus, the lack of intrinsic activity of IL-1ra is related to the lack of the association of IL-1RAcP with the cytoplasmic domain of IL-1RI. The association IL-1RAcP with the agonist-receptor complex produces a fivefold increase in the affinity of IL-1 to the receptor, although IL-1RAcP does not bind the agonist itself (Greenfeder et al., 1995).

The signalling can be hampered at the stage of agonist-receptor binding due to the characteristics of IL-1RII and the existence of soluble forms of the receptors. The extracellular portion of IL-1RII has been shown to be highly homologous to that of IL-1RI, but it has a short cytoplasmic tail that was found not to bind the IL-1RAcP and, therefore, not to result in intracellular signalling (Stylianou et al., 1992). In this respect, IL-1RII acts as a ‘decoy’ receptor, since the binding of the agonists is not followed by

an intracellular response (Colotta et al., 1993). IL-1RI and IL-1RII also exist in soluble forms (sIL-1RI and sIL-1RII) which, through the binding of the active agonists or IL-1ra, can influence their biological activities. It has been shown that sIL-1RI has a much higher affinity for IL-1ra and can bind the antagonist produced *in vivo*, thereby limiting its anti-inflammatory properties (Arend et al., 1994; Burger et al., 1995). In contrast, sIL-1RII preferentially binds IL-1β (Arend et al., 1994; Symons et al., 1995), and can act as an inhibitor of IL-1-mediated actions in *vivo*, by preventing the binding of the agonist to the signalling membrane-bound receptor.

The signalling pathway activated by IL-1, resulting in activation of NF-κB, involves several components and steps. IL-1R-activated kinase (IRAK) type 1 (IRAK-1) and an adaptor protein, myeloid differentiation factor 88 (myD88) are recruited to the agonist-receptor complex through the association with IL-1RAcP (Huang et al., 1997; Muzio et al., 1997). IRAK-1 is stabilised into the complex by the interaction with myD88. The association of IRAK type 4 (IRAK-4) with myD88 is followed by the phosphorylation of IRAK-1, leading to the dissociation from myD88 of the complex formed by TNF receptor (TNFR) associated factor-6 (TRAF-6) and IRAK-1. The IRAK-1-TRAF-6 complex binds to a complex formed by transforming growth factor β (TGF-β)-activated kinase-1 (TAK-1), TAK-binding protein-1 (TAB-1) and -2 (TAB-2). IRAK-1 is further degraded by ubiquitination and active TAK-1 phosphorylates and activates inhibitory κB kinase (IKK). NF-κB is kept inactive in the cytoplasm by binding to an inhibitory protein (IκB). Activated IKK catalyses the phosphorylation and degradation of IκB, releasing NF-κB which will then bind to the target genes and induce their expression (Dower and Qwarnstrom, 2003). (Fig. 1)

IL-1 signalling is highly efficient. Normally, there are less than 200 molecules of IL-1RI on the cell surface, and less than 10% of these need to be occupied in order to generate a biological response (Bankers-Fulbright et al., 1996). Furthermore, 100-fold higher amounts of IL-1ra than those of the agonists are needed to reduce some of the IL-1-induced biological activities with 50% (Arend et al., 1990).

1.3 THE ROLES OF IL-1 AGONISTS IN BIOLOGY

IL-1 is one of the most important pro-inflammatory cytokines involved in the host response against infection or injury. The main distinction between the two agonists is that IL-1β is secreted after the cleavage by caspase-1, while IL-1α is primarily a cell-associated molecule (Dinarello, 1996) (see below). Except during severe diseases, when the release of proIL-1α into the circulation probably occurs upon cell death followed by extracellular protease cleavage to the mature protein, IL-1α is not commonly found in the circulation or in the body fluids (Wakabayashi et al., 1991).

IL-1β was described for its role in fever, as the most potent endogenous pyrogen and inducer of the "acute phase response" (Dinarello, 1991; Merriman et al., 1977), but

IL-1β was first identified as the lymphocyte-activating factor (LAF), due to its capacity to act as a co-activator of lymphocyte proliferation (Gery and Waksman, 1972). The biological activities of IL-1α and IL-1β are similar, although studies performed on transgenic mouse strains have shown differences between the two agonists. The study of differences between the activities of IL-1α and IL-1β was facilitated by the ability of the mouse cells to produce and release IL-1α, as opposed to the human cells (Dinarello, 1996). Thus, IL-1β, but not IL-1α, was shown to mediate the systemic response to local inflammation, since IL-1β-deficient mice had a marked reduction in fever and other indicators of systemic response to inflammation (Fantuzzi et al., 1997b; Fantuzzi et al., 1996), while the same responses were not affected in IL-1α-deficient mice (Horai et al., 1998). It is believed that IL-1β has more powerful effects in the CNS, than IL-1α (Katsuura et al., 1989).

The primary sources of IL-1 in blood are activated monocytes and macrophages. However, human monocytes do not constitutively express IL-1β in healthy subjects (Mileno et al., 1995). Keratinocytes constitutively express IL-1α and produce IL-1β upon stimulation. It has been suggested that intracellular IL-1α regulates normal cell differentiation in epithelial and ectodermal cells (Dinarello, 1996). Other sources for IL-1β are B-lymphocytes and natural killer (NK) cells.

There is evidence that IL-1 may have important regulatory functions in certain physiological processes such as modulation of sleep (Krueger et al., 1984), glucose (Endo et al., 1985) and lipid (Matsuki et al., 2003) metabolism, cardiovascular parameters (Watanabe et al., 1996), bodyweight homeostasis (Matsuki et al., 2003), as well as neuroendocrine regulation (Uehara et al., 1987). IL-1β has been shown to modulate the communication between the maternal endometrium and embryo, *i.e.* by influencing the implantation of the embryo into the endometrium (Fazleabas et al., 2004).

There are indications of a possible role for the newly described IL-1 homologues, IL-1F5 – IL-1F10 in immunity and inflammation. Most were found to be expressed in monocytes, macrophages and/or dendritic cells, and high levels of IL-1F5 and IL-1F9 were observed in keratinocytes. The expression of IL-1F9 was induced in skin upon contact hypersensitivity (Dunn et al., 2001).

1.4 THE ROLES OF IL-1RA IN BIOLOGY

IL-1ra is the first described specific endogenous receptor antagonist of any cytokine or hormone-like molecule (Liao et al., 1984). The identification of different isoforms of IL-1ra suggests that this cytokine may play complex roles in biology (Arend et al., 1998). Three structural variants of IL-1ra have been described: a 17-kDa secretory form (sIL-1ra) that is secreted from monocytes, macrophages, neutrophils, etc. (Eisenberg et al., 1990), an 18-kDa intracellular form (icIL-1raI) that remains in the

cytoplasm of *e.g.* keratinocytes, monocytes and hepatic cells (Haskill et al., 1991), and a 16-kDa intracellular form (icIL-1raII) that occurs in neutrophils, monocytes and hepatic cells (Malyak et al., 1998). A 25-kDa intracellular isoform of IL-1ra has been reported (Muzio et al., 1995), but further studies failed to confirm it (see (Arend et al., 1998).

IL-1ra is produced by hepatocytes as an acute-phase protein (Gabay et al., 1997). The circulating blood levels of sIL-1ra are low in normal conditions, but elevated levels were described in several human infectious, inflammatory and autoimmune disorders, including sepsis, chronic rheumatic diseases, and surgical trauma (Arend et al., 1998). The finding that IL-1ra knockout (KO) mice display growth retardation after weaning and an increased susceptibility to endotoxin-induced fever (Horai et al., 1998) and collagen-induced arthritis (Ma et al., 1998) further suggested that IL-1ra plays important roles in both health and pathological conditions.

The classical view was that the secretory form of IL-1ra binds to the signalling IL-1RI with the same affinity as the agonists, without inducing any intracellular response and acts as a selective, competitive antagonist of IL-1-induced actions (Dinarello, 1991) (see Fig. 1). However, recently published results showing that IL-1ra has intrinsic action in the hippocampus independent of IL-1RI, that mimics the effects of IL-1β, challenged this classical view (Loscher et al., 2003).

1.5 IL-1 EFFECTS ON THE BRAIN

The constitutive expression in the brain of the agonists, IL-1α and IL-1β, as well as of IL-1ra, IL-1RI and IL-1RII has been demonstrated for both mRNA and proteins, most of the data available being from studies on mice, rats and humans (Vitkovic et al., 2000). In humans, the constitutive expression of IL-1β protein has been described for the first time in the hypothalamus, by means of immunohistochemistry (Breder et al., 1988). Further immunohistochemical studies demonstrated the constitutive expression for both IL-1 isoforms in cerebral cortex in glial cells, but not in neurons (da Cunha et al., 1993). IL-1α was detected in neurons in the rat hypothalamus (Rettori et al., 1994). The constitutive expression of the IL-1 in the brain, albeit at low levels, suggests that this cytokine may have important functions in the CNS.

The "sickness syndrome" is a highly organised defence strategy of the organism, consisting of a coordinated set of changes observed during the course of infections that include fever, physiological and behavioural changes, *i.e.* reduced food and water intake, weakness, depression, inability to concentrate, learning and memory impairment (Dantzer, 2001). It has been demonstrated that the metabolical, physiological and behavioural components of the systemic response to infection is dependent upon the action of IL-1 and other pro-inflammatory cytokines, such as TNF-α and IL-6 in the

brain. The same pro-inflammatory cytokines are also responsible for the inflammation in the periphery.

For understanding the role of IL-1 in centrally mediated functions, the main approach has been to manipulate the IL-1 system by direct administration of the IL-1 agonists, and/or the antagonist IL-1ra, as well as by mimicking a peripheral inflammatory process. Injection of lipopolysaccharide (LPS), a major pro-inflammatory constituent of the outer membrane of Gram-negative bacteria, into rodents is a widely used method for studying the involvement of cytokines in the mechanisms underlying the "sickness syndrome". Using these approaches, the behavioural components of the sickness syndrome have been replicated in animal models. Thus, studies based on systemic or CNS-administration of IL-1 have shown suppression of feeding (McCarthy et al., 1985), modulation of drinking behaviour (Otterness et al., 1988; Shimommura et al., 1990), and reduction of exploratory behaviour in rats (Otterness et al., 1988) and mice (Dunn et al., 1991; Lacosta et al., 1998). Administration of IL-1 was also shown to produce decreased social exploration in mice (Crestani et al., 1991) and reduction in time spent in contact with novel stimuli (Spadaro and Dunn, 1990). Administration of IL-1ra has been shown to block the depressive effects of IL-1 on social exploration in rats exposed to a juvenile rat (Kent et al., 1992)**.**

When discussing the effects of IL-1 brain functions it is important to determine the pathways through which these actions are mediated. A peripheral inflammatory process is followed by the local synthesis of IL-1 and other pro-inflammatory cytokines which exert their actions on the brain in different ways, *i.e.* by means of neural and humoral pathways (Dantzer, 2001). Transmission of peripheral immune signals to the brain occurs via a fast transmission pathway through afferent nerve fibres with terminals at the site of inflammation, as well as via a slower transmission pathway through synthesis of cytokines within the brain. Peripheral administration of LPS in rodents has underlined the important role of vagal nerves in transmission of peripheral immune signals to the brain (Goehler et al., 1999). Thus, IL-1β immunoreactivity was detected in dendritic cells and macrophages within connective tissue around abdominal endings of the vagus nerve (Goehler et al., 1999). A peripheral inflammatory process can also influence brain functions through the direct action of pro-inflammatory cytokines synthesised within the brain. Indeed, IL-1β, IL-6, and TNF-α mRNA is induced in the brain by peripheral administration of LPS at doses that do not disrupt the blood-brain barrier (Pitossi et al., 1997). The main cellular sources of IL-1 after LPS administration were identified as microglial cells, perivascular cells, and macrophages in the meninges and choroid plexus (Konsman et al., 1999; van Dam et al., 1992). Brain IL-1β immmunoreactivity after peripheral endotoxin challenge was shown to appear first in the choroid plexus and circumventricular organs. It was suggested that IL-1β may activate local neurons via projections to the ventromedial preoptic area and the nucleus

of the solitary tract, in order to produce fever and neuroendocrine activation (Konsman et al., 1999).

A fundamental aspect of the communication between the immune and nervous systems is the activation of hypothalamo-pituitary-adrenal (HPA)-axis by pro-inflammatory cytokines, including IL-1, TNF-α and IL-6, resulting in the release of adrenocorticotrophic hormone (ACTH) and corticosterone (CST) (Besedovsky et al., 1986; Dunn, 2000). It has been shown that IL-1 produces indirect secretion of ACTH, via stimulating the release of corticotropin-releasing factor (CRF) from the paraventricular hypothalamic nucleus, both after peripheral (Dunn et al., 1991) and intrahypothalamic administration (Barbanel et al., 1990). CST has, in turn, a negative feedback regulatory effect on pro-inflammatory cytokines (see Turnbull and Rivier, 1999).

IL-1β appears to be involved in superior cognitive functions, *e.g.* learning and memory, and to play a neuromodulatory role in the hippocampus, since IL-1β gene expression is significantly increased in this brain region during long-term potentiation (LTP), both *in vivo* and *in vitro* (Schneider et al., 1998). Blockade of IL-1 receptors by addition of IL-1ra was observed to impair the maintenance of LTP without influencing its induction (Schneider et al., 1998). Furthermore, the generation of LTP was inhibited in IL-1RI KO mice (Avital et al., 2003). The pharmacological effect of IL-1β on LTP seems to follow a bell-shaped curve, in view of the findings by Katsuki et al. (Katsuki et al., 1990) and Bellinger et al. (Bellinger et al., 1993), showing that addition of IL-1β to hippocampal slices from mouse and rat inhibits the generation of LTP.

1.6 IL-1 SYSTEM AND BRAIN DEVELOPMENT

IL-1 and other cytokines, previously thought to be specific only for the immune system, are now known to directly or indirectly influence brain development and different aspects of CNS functionality. The expression of IL-1β and IL-1RI in developing neural circuits in the frog (Jelaso et al., 1998), and the immunohistochemical detection of IL-1β in the developing sheep neocortex (Dziegielewska et al., 2000), supports the idea that IL-1 is a highly conserved protein, with a potentially important role in CNS development. IL-1 was also detected in the developing rat brain, the source being identified as the amoeboid microglia (Giulian et al., 1986). In cell cultures, IL-1β has been shown to be produced both by activated ameboid microglia and by activated astrocytes, and to be one of the most powerful inducers of reactive astrogliosis, which consists of proliferation, de-differentiation and altered gene expression of the astrocytes (Giulian et al., 1988). Astrocytes are considered the effector cells that mediate most of the activities of IL-1β in the brain. The importance of IL-1β in the initiation of reactive astrogliosis was demonstrated in IL-1β KO mice in which the induction of glial fibrillary acidic protein (GFAP), an

astrogliosis marker, was absent after corticectomy (Herx and Yong, 2001). Also, IL-1β was detected in the mouse brain during the period of gliogenesis (Mizuno et al., 1994). The function of IL-1β as a growth factor was further suggested by the stimulatory effect on the differentiation of mesencephalic progenitor cells to dopaminergic neurons (Ling et al., 1998), while IL-1α was found to increase neuronal survival in dissociated spinal cord – dorsal root ganglion cultures (Brenneman et al., 1992). Furthermore, it was suggested that IL-1 may influence the developmental processes in the brain through the synthesis of certain neurotrophic factors, *i.e.* nerve growth factor (NGF) (Friedman et al., 1996), and ciliary neurotrophic factor (CNTF) (Herx et al., 2000).

Other cytokines were also found to be involved in developmental processes in the brain. Thus, high expression of IL-6 mRNA was described in the rat cerebral cortex during the prenatal period (Pousset, 1994). Proliferation and maturation of oligodendrocytes were shown to be prolonged by interleukin-2 (Benveniste and Merrill, 1986), while TNF-α and IFN-γ were shown to influence the growth and fate of neural precursor cells in the rat, by inhibition of proliferation and facilitation of their migration (Ben-Hur et al., 2003).

1.7 IL-1 SYSTEM AND NEURODEGENERATION

Inflammatory processes have been implicated in neurodegeneration and cytokines are involved as modulators and mediators of different forms of acute, subacute, and chronic neurodegenerative disorders. IL-1 is one of the most studied cytokines with regard to the inflammatory mechanisms involved in neurodegeneration.

Most of the excitatory neurotransmission in the mammalian CNS is mediated by glutamate, which exerts its action through two classes of receptors, ionotropic and metabotropic (for review see (Ozawa et al., 1998). The ionotropic receptors are further divided into three subtypes named after their preferred ligands: kainate, α-amino-3-hydroxy-5-methyl-4-isoxalolepropionate (AMPA) and N-methyl-D-aspartate (NMDA). The kainate and AMPA-receptors represent the non-NMDA group of glutamate receptors. Excessive stimulation of the glutamatergic receptors in the brain has been associated with the cellular death in both acute and chronic neurodegenerative disorders, *i.e.* cerebral ischemia and brain trauma (Arundine and Tymianski, 2004), and epilepsy, Alzheimer's disease (AD) (Hynd et al., 2004) and Huntington's diseases (Li et al., 2004).

It seems that there is a bidirectional relationship between IL-1 expression and activation of glutamatergic neurotransmission, suggesting the development of a positive feedback. Thus, activation of glutamatergic receptors has been associated with the synthesis of IL-1β in the brain, *e.g.* after kainic acid (KA) administration in rat (Eriksson et al., 2000a; Eriksson et al., 2000b; Eriksson et al., 1999; Vezzani et al., 1999), and it has been recently shown that microglial cells, probably the most important

source of IL-1 in the brain, possess functional AMPA-kainate receptors (Noda et al., 2000). IL-1 has been shown to potentiate the neurotoxic effects of NMDA and AMPA, without having a neurotoxic effect when administered alone, intracerebrally (Lawrence et al., 1998). Furthermore, recent studies on primary cultures of rat hippocampal neurons showed that IL-1β stimulates NMDA-receptor-mediated activity through the activation of tyrosine kinases and phosphorylation of the NMDA-receptor subunits 2A and 2B (Viviani et al., 2003).

1.7.1 Kainic acid – experimental model of temporal lobe epilepsy

Epilepsy is a neurodegenerative disorder produced by an excessive excitatory synaptic stimulation in the brain, clinically characterized by the occurrence of seizures, and pathologically by specific neurodegenerative changes in certain brain areas.

KA, isolated from *Digenea simplex,* is an analogue of glutamic acid. Central or peripheral administration of KA in rats is the most commonly used animal model for studying the pathogenic aspects of human temporal lobe epilepsy (Ben-Ari et al., 1980; Nadler, 1981). Administration of KA produces a sequence of behavioural and electroencephalographic abnormalities, accompanied by temperature changes, and followed by a specific pattern of neurodegeneration (Ben-Ari, 1985; Ben-Ari et al., 1980; Oprica et al., 2002; Turski and Kleinrok, 1980; Zagrean et al., 1993).

The discrepancies encountered among studies using the KA-model can be due to the different experimental designs, such as peripheral or central delivery of KA, or the differences in susceptibility to seizures between the strains of rats used. Thus, Wistar rats were demonstrated to be more susceptible to the convulsant effect of KA than Sprague-Dawley rats (Golden et al., 1991).

Systemic delivery of KA is followed by variations of the response and sometimes an "all or nothing" response can be seen. Previous studies have shown that systemic administration of 10 mg/kg KA in rats results in a complete seizure syndrome in 60-80% of the animals (Sperk et al., 1985). However, due to its convenience, the systemic administration of KA is widely used in epilepsy research and the variable responses among animals are verified using standardized seizure scales (Racine, 1972).

Behavioural changes

Peripheral administration of 8-12 mg/kg KA induces a specific sequence of motor and autonomic events (for review see (Sperk, 1994). The behavioural changes develop gradually and a rat responding to KA can be recognised within 5 min after the administration of the excitotoxin, due to an excessive immobility and a flat posture of the body. This stage has duration of about 5-15 min and is followed by the occurrence of masticatory movements and myoclonic twitches of the head. Specific KA-induced comportamental changes are the "wet dog shakes", which begin about 30 min after KA-administration and resemble ordinary chills. The behavioural pattern is completed

in the next 2-4 h by generalized tonic, clonic or tonico-clonic seizures, which can progress to status epilepticus. Rearing, loss of postural control and salivation can occur during this stage. After 2-4 h the number and intensity of the seizures decline to the complete disappearance. The number of the "wet dog shakes" and seizures are dependent upon the dose of KA.

Temperature changes

Administration of KA in the rat has been shown to induce specific temperature changes, *i.e.* hypothermia in the first hour after administration, followed by a longer-lasting hyperthermic effect when given peripherally (Oprica et al., 2002), or intracerebroventricularly (i.c.v.) (Turski and Kleinrok, 1980). It has been shown previously that dopamine (DA) is involved in the initial hypothermia, while serotonin (5-hydroxytryptamine, 5-HT) is necessary for the following hyperthermic effect (Turski and Kleinrok, 1980). Thus, the early hypothermic effect was abolished by the depletion of DA with 6-hydroxydopamine, whereas the electrolytic lesions of the raphe nuclei inhibited the ensuing longer-lasting hyperthermia (Turski and Kleinrok, 1980). The involvement of DA and 5-HT in thermoregulation has been demonstrated previously, predominantly hypothermic effects being reported for DA, and hyperthermic for 5-HT. DA has been shown to induce hypothermic effects in cats (Ruwe and Myers, 1978), rats (Brezenoff and Cohen, 1973), and mice (Meller et al., 1989), while depletion of serotonin in the preoptic area of the hypothalamus was shown to induce hypothermia (Huttunen et al., 1988). Administration of 5-HT agonists (Hayashi et al., 2004) and stimulation of serotonin release in the CNS have been shown to produce hyperthermia (Gordon et al., 1991). The DA-induced hypothermic effect was associated with activation of dopaminergic D_2-receptors (Meller et al., 1989), while stimulation of 5-HT_{2C} subtype of serotoninergic receptors mediated hyperthermia (Hayashi et al., 2004).

Studies on the mechanisms involved in KA-induced temperature changes is of particular interest, since hypothermia has been shown to be protective against KA-induced neurotoxicity (Liu et al., 1993b; Maeda et al., 1999), while hyperthermia had an aggravating effect (Liu et al., 1993b).

Neurodegeneration

Systemic, i.c.v., intrahippocampal, or intraamygdalian administration of KA induces a reproducible and typical pattern of neurodegeneration, if the tonico-clonic seizures are maintained for a certain critical period (Ben-Ari, 1985; Tanaka et al., 1992; Tremblay et al., 1983). The typical pattern of the KA-induced neurodegenerative changes is represented by the selective vulnerability of neurons with the highest degree in the CA3 region of the hippocampus, followed by the hilus of the dentate gyrus and the CA1 hippocampal region (Ben-Ari, 1985; Schwob et al., 1980; Sperk et al., 1985;

Tauck and Nadler, 1985). The pyramidal neurons in the CA2 region of the hippocampus and the granular neurons in the dentate gyrus are particularly resistant. KA-induced neurodegeneration has been demonstrated to occur both through necrotic and apoptotic mechanisms (Ferrer et al., 1995; Popescu et al., 2002; Simonian et al., 1996). The behavioural changes and neuronal damage produced by systemic administration of KA are believed to be initiated by activation of KA-receptors in the CA3 region of the hippocampus (Krnjevic et al., 1980), followed by release of endogenous excitatory amino acids, glutamate and aspartate (Ferkany et al., 1982; Köhler et al., 1978), that will further activate all types of glutamate receptors, including NMDA-receptors.

1.7.2 IL-1 system and kainic acid-induced brain damage

It has been suggested that immune mechanisms are responsible for certain types of primary or secondary forms of epilepsy (for review see (Aarli, 2000). The findings of increased levels of cytokines such as IL-1β (Haspolat et al., 2002), interleukin-6 (IL-6) (Peltola et al., 1998), and IL-1ra (Peltola et al., 2000) in the cerebrospinal fluid from patients with epilepsy, as well as the aggravating effect of IL-1β in experimental models of epilepsy (Vezzani et al., 1999; Vezzani et al., 2002), suggest that the study of inflammatory aspects involved in the pathogenesis of epileptic disorders can bring new insights into the molecular mechanisms and etiology of epilepsy.

IL-1β

Induction of IL-1β in the brain was observed after peripheral (Eriksson et al., 1999; Eriksson et al., 1998; Minami et al., 1991; Minami et al., 1990; Rizzi et al., 2003; Yabuuchi et al., 1993) and central (Vezzani et al., 1999) administration of KA. One of the most important advantages of the peripheral administration of KA is the possibility to study, in the same time, the effects of the excitotoxin on different brain regions. Upon peripheral administration of KA, *in situ* hybridization histochemistry demonstrated a transient biphasic expression of IL-1β mRNA, with the first peak in the hippocampus, cerebral cortex, thalamus and hypothalamus, at 1 – 3.5 h after KA administration (Eriksson et al., 2000b; Minami et al., 1990). The second peak of high expression for IL-1β mRNA was observed in the cortex, amygdala, thalamus and hippocampus at 12 h (Eriksson et al., 2000b). At 24 h, IL-1β mRNA was detected only in the hippocampus and amygdala (Eriksson et al., 2000b; Minami et al., 1990). No expression was found in the midbrain, pons, medulla and cerebellum, while a weak expression was observed in the striatum (Minami et al., 1990). IL-1β mRNA has been demonstrated to be followed by increased expression of IL-1β protein, both after peripheral (Eriksson et al., 1999) and central administration of KA (Vezzani et al., 1999). Immunohistochemical studies also showed that the source of IL-1β after KA

administration is activated microglia, at least in the first hours after injection (Eriksson et al., 1999; Vezzani et al., 1999).

The expression of IL-1β mRNA in the rat brain following KA-administration was decreased and/or blocked in a dose-dependent manner by the peripheral administration of a non-competitive (dizocilpine) and a competitive ([R]-CPP) NMDA-receptor antagonist (Eriksson et al., 2000c). This suggests that KA-induced cytokine synthesis is mediated via release of endogenous excitatory amino acids. Further support of an indirect effect of KA is the finding that diazepam, a GABA-receptor agonist, blocked the KA-induced IL-1β mRNA expression (Minami et al., 1990). However, a direct effect is suggested by the demonstration of functional AMPA-kainate receptors on microglial cells (Noda et al., 2000).

Both neurotoxic and neurorotective effects have been proposed in association with the KA-induced expression of IL-1β in the brain. Intrahippocampal delivery of human recombinant IL-1β significantly increased the duration of electroencephalographic seizures produced by local application of KA and this proconvulsant effect was blocked by IL-1ra given intrahippocampally (Vezzani et al., 1999), suggesting a receptor-mediated action of exogenously administered IL-1β. The proconvulsant effect of IL-1β was blocked also by [R]-CPP, demonstrating an excessive activation of NMDA-receptors (Vezzani et al., 2002).

Some of the neurotoxic effects of IL-1β may be explained by influence on glutamate levels available for glutamatergic neurotransmission. In vitro studies have shown that IL-1β impairs astrocytic glutamate uptake (Hu et al., 2000; Ye and Sontheimer, 1996), a mechanism that maintains low extracellular levels of glutamate and promotes efficient inter-neuronal signalling in the normal brain. The mechanism consists of a dose-dependent inhibition of mRNA expression for the glutamate transporter protein through a NO-dependent mechanism (Hu et al., 2000; Ye and Sontheimer, 1996), and the effect was blocked by IL-1ra (Hu et al., 2000). It is conceivable that the observed proconvulsant effect of IL-1β (Vezzani et al., 1999) is mediated by an increase in glutamate available for the activation of NMDA- and non-NMDA-receptors, due to inhibition of glutamate uptake by astrocytes, as well as by the direct stimulatory effect of IL-1β on NMDA-receptors (Viviani et al., 2003) (see above).

In vitro experiments demonstrated that astrocytes have a down-regulatory action on microglial synthesis of inducible nitric oxide synthase (iNOS) through the release of transforming growth factor-beta (TGF-β), and probably other yet undefined mechanisms (Vincent et al., 1997). This has been suggested to be an important endogenous protective mechanism against oxidative stress secondary to the increased peroxynitrite production. Interestingly, the inhibitory action on microglial iNOS production was found to be dependent upon the astrocytic differentiation state. Forced to undergo secondary activation due to the action of IL-1 released from activated

microglia, astrocytes lose their ability to inhibit the production of reactive oxygen species (ROS) by microglia (Schubert et al., 2000). Furthermore, stimulation of astrocytes by IL-1β resulted in increased NMDA-induced toxicity through the secretion of iNOS (Hewett et al., 1994).

There are some lines of evidence suggesting that KA-induced expression of IL-1β can also be considered neuroprotective. IL-1β itself acts as a growth factor, particularly in the developing brain, where it is involved in the differentiation, proliferation and survival of astrocytes and neurons (Giulian et al., 1988; Ling et al., 1998). Some of the potential neuroprotective roles of IL-1β could be related to the stimulation of the production of certain neurotrophic factors in the brain. The KA-induced expression of NGF mRNA (Gall et al., 1991) and protein (Strauss et al., 1994) in the rat brain seems to be mediated by IL-1β. Thus, IL-1β was shown to stimulate the expression of NGF mRNA in astrocytes in primary cultures and after i.c.v. administration in rats (Spranger et al., 1990), while IL-1ra inhibited the expression of NGF in a model of traumatic brain injury (DeKosky et al., 1996).

The balance between the presumably neurotoxic and neuroprotective effects of IL-1β seems to depend upon the dose and the duration of exposure. Pre-treatment of primary cortical neuronal cultures with 0.5 μg/ml human recombinant IL-1β (hrIL-1β) for 24 h was shown to provide protection against neuronal cell death induced by KA, glutamate, NMDA or AMPA, an effect that seemed to be mediated by NGF (Strijbos and Rothwell, 1995). In contrast, exposure to 100 μg/ml hrIL-1β for 72 h resulted in neurotoxicity that was blocked by co-application of IL-1ra (Strijbos and Rothwell, 1995). Furthermore, ciliary neurotrophic factor (CNTF), was absent in mice deficient in IL-1β (Herx et al., 2000), suggesting that its synthesis is also stimulated by IL-1β.

IL-1ra

Peripheral administration of KA, results in induction of IL-1ra mRNA expression in several regions of the rat brain (Eriksson et al., 1998), in relation to the extent and distribution of neurodegeneration and to the expression of IL-1β mRNA. IL-1ra mRNA was first detected 5 h after KA-administration and the strongest signal was seen after 24 h. The same temporal relationship between the brain mRNA expression of IL-1β and IL-1ra has also been described after direct limbic stimulation of rats (Vezzani et al., 2002). KA-induced expression of IL-1ra protein was delayed with several hours, but occurred in the same brain regions as IL-1β (Eriksson et al., 1999). This delay may have a functional significance, with regard to the potential neuroprotective mechanisms of IL-1β (see above). Like IL-1β, the main site of IL-1ra production upon systemic KA-injection occurred in microglial cells, but could also be seen in a few neurons (Eriksson et al., 1999; Eriksson et al., 1998).

IL-1ra has been proven to have anticonvulsant properties (Vezzani et al., 1999; Vezzani et al., 2002) and to inhibit neurodegeneration induced by KA (Panegyres and

Hughes, 1998). Intrahippocampal delivery of hIL-1ra inhibited the proconvulsant effect of IL-1β upon KA-administration (Vezzani et al., 1999), and the i.c.v. administration of 0.1 μg hIL-1ra 10 min before and 10 min after KA-injection had a powerful anticonvulsant effect (Vezzani et al., 2002). I.c.v administration of 10 – 20 μg hIL-ra selectively protected the neurons in the CA1 and CA3 regions of the hippocampus and in the dorsal thalamic nuclei, against KA-induced neurodegeneration (Panegyres and Hughes, 1998). Interestingly, the highest dose of the antagonist (40 μg) did not provide neuroprotection (Panegyres and Hughes, 1998).

Similar to IL-1β, the KA-induced expression of IL-1ra in the rat brain was dose-dependently blocked by competitive and non-competitive NMDA-receptor antagonists, suggesting that microglial synthesis of IL-1ra is regulated by NMDA-receptor activation (Eriksson et al., 2000c). However, a stimulatory effect of IL-1β on the synthesis of its own antagonist cannot be disregarded (Xiao et al., 1999).

1.7.3 IL-1 system and cerebral ischemia

Despite promising results obtained in experimental models of stroke for a multitude of neuroprotective substances, controlled hypothermia is the only therapeutic strategy that proved, so far, to be neuroprotective in clinical settings (Schwab et al., 1998). The discrepancy between the results obtained in clinical trials and those derived from rodent models of cerebral ischemia, the most commonly used in experimental stroke research may have several explanations. The delivery of a potentially therapeutic drug before or immediately after the ischemic event has begun is not realistic in clinical settings. The occurrence of other concomitant pathologies further complicates the situation and the outcome becomes unpredictable for patients challenged by a sudden decrease in cerebral flow as opposed to the situation in experimental animals which are homogeneous and previously healthy. Furthermore, the fact that physiological parameters of laboratory animals are well controlled during the ischemic event may reduce the clinical applicability of the conclusions drawn from animal models.

A large body of evidence regarding the involvement of inflammatory mechanisms in the pathogenesis of cerebral damage following cerebral ischemia has been accumulated in the latest years. In this respect, the IL-1 system received considerable attention. Most of the findings supporting the involvement of the IL-1RI-mediated activity in ischemic brain damage are derived from rodent models of stroke. Several studies have reported increased expression of IL-1β mRNA (Buttini et al., 1994; Hill et al., 1999; Liu et al., 1993a; Minami et al., 1992; Sairanen et al., 1997; Wang et al., 1994; Wiessner et al., 1993) and protein (Davies et al., 1999; Hillhouse et al., 1998; Legos et al., 2000; Zhang et al., 1998) after experimental cerebral ischemia. A common finding in most of these studies is the early expression of IL-1β, sometimes within 1 h after the induction of ischemia, and its persistence during the development of the

ischemic lesion. Although most of the research was focussed on IL-1β, IL-1α was also found to be involved in the pathogenesis of cerebral ischemia. mRNA (Hill et al., 1999) and protein (Legos et al., 2000) expression were increased in the brain upon cerebral ischemia. Furthermore, IL-1α gene polymorphisms have been associated with an increased risk for cerebral infarction (Um et al., 2003). Mice lacking both IL-1α and IL-1β were significantly protected against ischemic cell death following 30 min MCAO, in contrast to mice lacking either IL-1α or IL-1β alone (Boutin et al., 2001).

The mRNA levels of IL-1ra and IL-1Rs have also been shown to increase upon cerebral ischemia. The involvement of IL-1R-mediated activity in the pathogenesis of cerebral ischemia was further demonstrated by the neuroprotective effects of IL-1ra administration upon the peripheral (Garcia et al., 1995; Martin et al., 1994; Relton et al., 1996), intrastriatal (Stroemer and Rothwell, 1997), or i.c.v. (Relton and Rothwell, 1992) routes in models of focal cerebral ischemia. Endogenous IL-1ra was shown to provide neuroprotection, since passive immunoneutralisation of IL-1ra, enhanced cerebral damage in permanent focal ischemia in rats (Loddick et al., 1997). Several studies showed neuroprotection in cerebral ischemia by delivery of IL-1ra into the brain by adenoviral transfection (Arend et al., 1990; Betz et al., 1995; Tsai et al., 2003; Yang et al., 1998a; Yang et al., 1999). Promising results have been recently obtained with regard to the neuroprotective effect of delayed administration of IL-1ra, even at 3 h after the start of the ischemic event, by diminishing the size of the focal ischemic region in rats subjected to 60 min MCAO (Mulcahy et al., 2003).

1.7.4 Alzheimer's disease and IL-1 system

Alzheimer's disease (AD) is a neurodegenerative disorder with a complex etiology, existing in both a common, sporadic (idiopathic) form and in early- and late-onset familial forms. AD is characterised clinically by dementia with insidious onset and inexorable progression. The neuropathology profile of AD consists of 1) extracellular amyloid deposits, comprised mainly of β-amyloid (aβ) peptide derived by endoproteolysis of the amyloid precursor protein (APP), 2) intracellular neurofibrillary tangles (NFT), comprised of hyperphosphorylated forms of microtubule-associated protein tau, 3) reduced synaptic density, and 4) the loss of cholinergic neurons in the basal forebrain (for review see (Selkoe, 1991). Inflammatory processes in the brain seem to have important roles in the pathogenesis of AD, although the intimate mechanisms have not been yet elucidated. The inflammatory process in the brain of AD patients, as in other neurodegenerative disorders, generally consists of astroglial proliferation and microglial activation, with the potential of induced production of several inflammatory proteins, including acute phase reactants, proteolytic enzymes, pro-inflammatory cytokines and chemokines, as well as free radicals, nitric oxide (NO) and arachidonic acid. The microglial cell, the source of most of these factors, appears to

be an important participant in the inflammatory process (Streit, 2004).

Mutations in three genes associated with familial AD (FAD) have been described so far, *i.e.* APP in chromosome 21 (St George-Hyslop et al., 1987), presenilin 1 (PS1) in chromosome 14 (St George-Hyslop et al., 1992) and presenilin 2 (PS2) in chromosome 1 (Levy-Lahad et al., 1995; Rogaev et al., 1995; St George-Hyslop et al., 1992). The ε4 allele of the apolipoprotein E (apoE) gene is located on chromosome 1 (Saunders et al., 1993), and the amount of apoE4 is a risk factor for late-onset AD (Corder et al., 1993).

APP is a membrane protein widely distributed in the CNS. Normally, APP is cleaved proteolytically by α-secretase to a secreted form (α-APPs), while the remaining carboxy-terminal fragment (C83) undergoes proteolysis by γ-secretase to a small peptide, p3, and the APP-intracellular domain (AICD) (LaFerla, 2002) (Fig. 2). The abnormal cleavage of APP by β-secretase, leading to the soluble β-APPs, followed by γ-secretase cleavage of the remaining carboxy-terminal fragment (C99) to the aβ peptide and AICD, is the base for the "amyloid cascade hypothesis" of AD (Hardy, 1997). A new cleavage site in APP, termed ε-cleavage site, has recently been described (Weidemann et al., 2002) (Fig. 2).

Several lines of evidence suggest the involvement of inflammatory cytokines, such as IL-1, in the pathogenesis of AD. One of first studies supporting this demonstrated an increased expression of IL-1 in the AD brain (Griffin et al., 1989). The distribution pattern of the IL-1-expressing microglia was later found to be correlated with the distribution of the amyloid plaques (Sheng et al., 1995). Specific polymorphisms in the IL-1 genes have been shown to increase the risk for AD (Nicoll et al., 2000), further supporting a role of IL-1 in AD pathogenesis. Genetic polymorphisms in the IL-1 genes were found to be associated with an increase in the IL-1α levels *in vivo*, and with increased secretion of IL-1β *in vitro* (for review see (Mrak and Griffin, 2001), suggesting that the increased risk of AD in these patients may be related to increased levels of IL-1 in the brain.

The reciprocal influence between APP and IL-1 is supported by several findings. Thus, transgenic mice with AD plaque pathology have an increased expression of pro-inflammatory cytokines, including IL-1 and IL-6 (Apelt and Schliebs, 2001; Tehranian et al., 2001), and APP or aβ peptides stimulate increased secretion of IL-1 and IL-6 from microglia *in vitro* (Barger and Harmon, 1997; Del Bo et al., 1995; Lindberg et al., in press). Furthermore, IL-1β is known to stimulate the synthesis and processing of APP in neurons and glia (Buxbaum et al., 1992; Forloni et al., 1992). Recently, it was shown that IL-1β and TNF-α influenced the metabolism of APP by stimulating γ-secretase cleavage (Liao et al., 2004a).

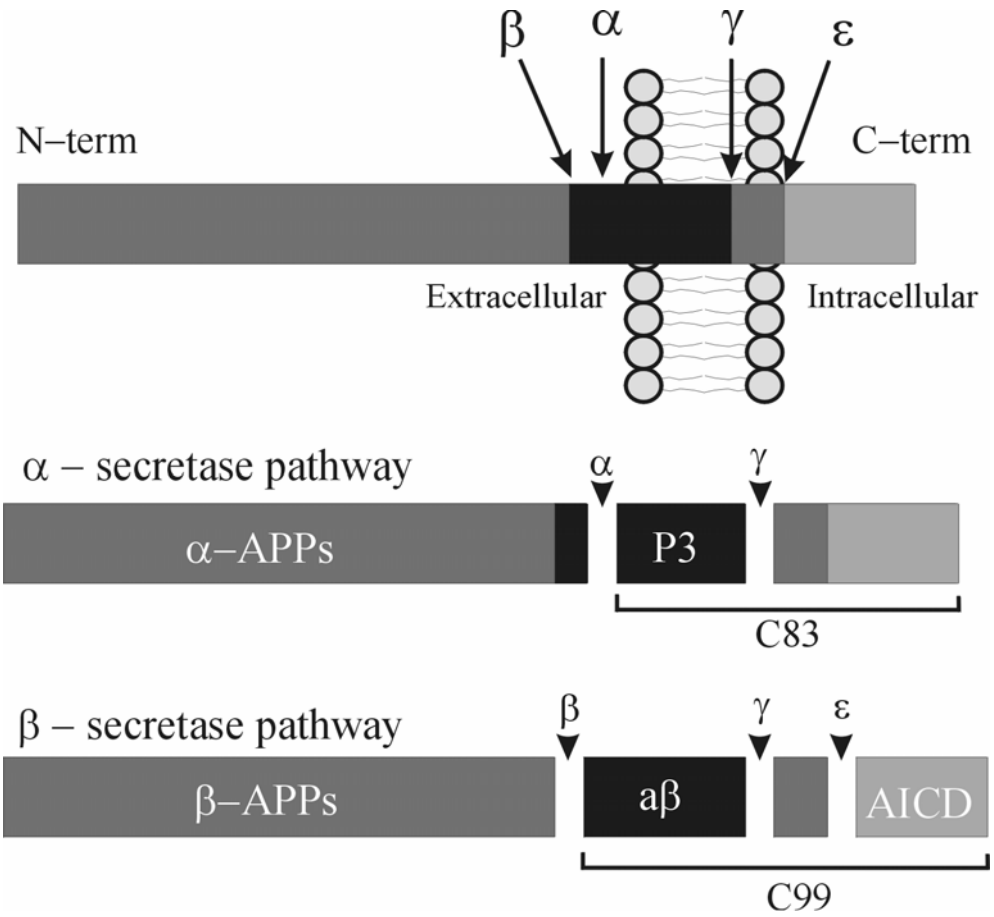

Figure 2. β-amyloid precursor protein (APP) is a transmembrane protein with the carboxy terminal portion embedded in the cell membrane. APP can undergo two proteolytic cleavages that either prevent (α-secretase pathway) or determine (β-secretase pathway) the production of aβ peptide. Normally, APP is cleaved proteolytically by α-secretase to a secreted form (α-APPs), while the remaining carboxy-terminal fragment (C83) undergoes proteolysis by γ-secretase to a small peptide, p3, and the APP-intracellular domain (AICD). The abnormal cleavage of APP by β-secretase, leading to the soluble β-APPs, followed by γ-secretase cleavage of the remaining carboxy-terminal fragment (C99) to the aβ peptide and AICD, is the base for the "amyloid cascade hypothesis" of AD.

The theory of immunopathogenesis of AD has clear therapeutic implication, in that anti-inflammatory drugs taken at critical stages in the disease process may protect against the development or progression of the disease. Anti-inflammatory therapy has been shown to reduce the plaque pathology and inflammation in a mouse model of AD (Lim et al., 2000)**.** Epidemiological studies have shown a decreased prevalence of AD and a decreased rate of progression of AD in elderly patients with history of long-term anti-inflammatory therapy (Rich et al., 1995). A lower prevalence of AD was found among rheumatoid arthritis patients with at least 3 years of chronic use of anti-inflammatory steroids, in comparison with the incidence of AD in the general population (Aisen and Davis, 1994).

2 AIMS

IL-1 is one of the most important pro-inflammatory cytokines involved in the initiation of the early stages of inflammation that is critical for the host defence against infections and injuries. IL-1 has been implicated in neuroinflammation associated with several neurodegenerative disorders, *i.e.* epilepsy, cerebral ischemia, trauma and AD. Furthermore, the IL-1 system may have important roles in physiological conditions as suggested by the expression, albeit at all levels, of all the members of IL-1 system in the brain.

The general aim of the studies was to investigate the involvement of IL-1R-mediated activity in different aspects of CNS function.

The specific aims were to study:

- the involvement of IL-1β, IL-6 and IL-1ra in temperature modifications associated with epilepsy
- the contribution of IL-1R-mediated activity to the pathophysiology of focal cerebral ischemia
- the effects of chronic, brain-directed blockade of IL-1R-mediated activity on the expression of the pro-inflammatory cytokines in the brain
- the role of IL-1R-mediated activity in brain morphology
- the role of IL-1R-mediated activity in the expression of APP, an AD-associated protein
- the role of IL-1R-mediated activity in different aspects of behaviour
- the role of IL-1R-mediated activity in regulation of the HPA-axis, and on brain levels of DA, 5-HT and their metabolites

3 METHODS

3.1 ANIMALS

The animal experimental procedures included in this project were approved by the Stockholm South local committée on ethics of animal experiments: S92-99 (Paper I), S14-04 (Paper II), S31-02 (Paper III), S30-02 (Paper IV), and S164-02 (Paper V).

In Paper I and II we used male Sprague-Dawley rats purchased from B&K Universal AB, Sollentuna, Sweden. In Paper III – V we have used a recently created transgenic mouse strain that was obtained using C57B6/CBA as the background strain, with overexpression of human soluble (hs) IL-1ra (hsIL-1ra) under the GFAP promoter (Lundkvist et al., 1999), in order to limit the expression of hsIL-1ra to the CNS. The mice were bred in the animal facility at Karolinska University Hospital, Huddinge. In addition to the wild type (WT) littermates, C57B6/CBA WT mice were purchased form the same provider (Charles River, Germany) as the mice used as background strain for the transgenic expression.

3.2 ANIMAL GENOTYPING

Animal genotyping was performed on DNA extracted and purified from round pieces of the ear (2-3 mm ∅) obtained when marking the animals (Paper IV). The concentration of DNA in each sample was determined in a spectrophotometer. Polymerase chain reaction (PCR) was performed using specific primers for hIL-1ra (sense: 5'-CGACCCTCTGGGAGAAAATC-3', anti-sense: 5'-CTCATCACCAGACTTGACAC-3'). PCR with primers specific for mouse β-actin (sense: 5'-AGGGAAATCGTGCGTGACAT-3', anti-sense: 5'-CATCTGCTGCAAGGTGGACA-3') to normalise the levels of hIL-1ra to a mouse house-keeping protein. Briefly, the DNA-samples were normalised according to DNA-concentration and the primers were added in equimolar amounts. Amplification was performed in an Eppendorf Thermocycler and the PCR-product was analysed by electrophoresis agarose gel. The gels were stained with ethidium bromide and scanned in ultraviolet light. The signal of the band for hIL-1ra was related to the signal for β-actin for each transgenic animal in order to differentiate between heterozygotic and homozygotic animals. There was no band at the size of hIL-1ra in samples from WT mice.

3.3 ANIMAL EXPERIMENTS, DRUG TREATMENTS – KA MODEL IN RATS (Paper I and II)

Administration of KA in rodents is a widely used model for studying different aspects of human temporal lobe epilepsy (Ben-Ari, 1985; Clifford et al., 1990; Sperk et

al., 1985; Turski and Kleinrok, 1980; Zagrean et al., 1993). Intraperitoneal (i.p.) injection of 10 mg/kg bodyweight KA in rats was used to investigate the mechanisms of the core temperature changes following the administration of the neurotoxic agent, in relation to the activation of different subtypes of glutamate receptors (Paper I) and to the induction of brain cytokines (Paper II). In order to study the involvement of different subtypes of glutamate receptors in the KA-induced temperature changes, we used dizocilpine (MK-801), a non-competitive NMDA-receptor antagonist. Dizocilpine was administered i.p. 1 h prior to the KA, at the doses of 3.0 and 5.0 mg/kg. These doses were previously shown to result in dose-dependent inhibition of the behavioural manifestations and the expression of brain IL-1β and IL-1ra induced by peripheral administration of KA (Eriksson et al., 2000c). In order to study the potential involvement of cytokines in KA-induced temperature changes we measured the core temperature at different time points together with the determination of cytokines (Paper II).

The systemic route of administration is an advantage due to the more uniform distribution of the excitotoxic agent that makes the model valuable for studying the consequences on several brain regions. Furthermore, a non-invasive induction of neurodegeneration in the CNS is an advantage when studying inflammatory responses in connection with the neuronal cell death, and not caused by a disruption of the blood brain barrier. The limitations of the model are represented by the approximate 20% of non-responders.

3.4 ANIMAL EXPERIMENTS – FOCAL CEREBRAL ISCHEMIA MODEL (Paper III)

In order to study the effects of transgenic brain-directed overexpression of hsIL-1ra on cerebral ischemia, we used a model of permanent focal cerebral ischemia in mouse (Paper III). The induction of permanent focal cerebral ischemia was based upon a previously described stroke model in mouse (Clark et al., 1997). The occlusion of the initial segment of the middle cerebral artery (MCA) was obtained by insertion of a nylon monofilament into the internal carotid artery (ICA), after the ipsilateral common carotid artery (CCA) had been permanently ligated. The filament was left in the ICA for 24 h. The body temperature of the animals was maintained at 37.0^{o}C - 37.5^{o}C with a thermostatically controlled heating pad during surgery and with homeothermic blankets, during 3 h following the surgery. The animals were then returned to their cages where they were housed alone and monitored for the presence of epileptic seizures. The following three exclusion criteria were used in: a) residual blood flow after ischemia > 15%; b) subarachnoid haemorrhage observed by visual inspection at the moment of brain dissection; c) presence of epileptic seizures.

3.5 NEUROLOGICAL EXAMINATION (Paper I-III)

The evaluation of the neurological status of the animals was examined in rats upon administration of KA (Paper I and II), and in mice after the induction of focal cerebral ischemia (Paper III).

The Racine scale (Racine, 1972): 0 = immobility, 1 = facial automatisms, 2 = head nodding, 3 = unilateral forelimb clonus/bilateral forelimb clonus, 4 = bilateral forelimb clonus and rearing, and 5 = rearing, falling, and generalized convulsions, was used for neurological examination after KA-administration in rats (Paper I and II).

A 5-point (Yang et al., 1994) and 24-point scale (Clark et al., 1997) were used for neurological examination in mice with permanent focal cerebral ischemia (Paper III).

3.6 MEASUREMENTS OF CEREBRAL BLOOD FLOW (Paper III)

Laser Doppler flowmetry (Perimed, Sweden) was used to assess cerebral blood flow (CBF), 15 min before, and 15 min and 24 h after the ischemia. A small incision was made in the skin overlying the temporal bone and the laser Doppler probe was positioned and secured with glue, 6 mm lateral and 2 mm posterior to bregma, which corresponds to the centre of the ischemic region.

3.7 TEMPERATURE MEASUREMENTS (Paper I and II)

In order to study the temperature changes induced by i.p. administration of KA in correlation with the expression of cytokines in the brain, the core temperature was measured by means of a digital termistor thermometer inserted into the rectum at a constant depth, until a stable temperature was reached.

3.8 BEHAVIOUR TESTS (Paper V)

A battery of behavioural tests consisting of open-field (OF) test, elevated plus-maze (EPM), and rotarod were performed in order to assess the effects of chronic, brain-directed overexpression of hsIL-1ra in homozygotic and WT mice.

3.8.1 Open-field test

The OF test was employed to evaluate the consequences of chronic brain-directed overexpression of hsIL-1ra on locomotor and rearing activity, upon exposure to a new environment. The test apparatus consisted of four Plexiglas arenas in which the horisontal (locomotion) and vertical (rearing) activity of four mice were detected automatically and simultaneously. The locomotion and rearing activities were measured for 60 min, divided in four continuous 15-min intervals. Each mouse was tested three times, three days apart, in the same box, and at the same time of the day.

3.8.2 Elevated plus-maze

The performance in the EPM test allows the detection of anxiety-like behaviour. The EPM consisted of two opposite open arms and two opposite closed arms surrounded by Plexiglas walls (presumably secure). The time spent, and the numbers of entries into the open and closed arms, respectively, were recorded for each animal during a 5-min period. An arm entry was defined as the entry of all four paws into the arm. All sessions were recorded using a video-camera linked to a video-recorder and a monitor.

3.8.3 Rotarod test

Motor coordination was investigated using the rotarod test. The animals were placed on a rotating cylinder, suspended 15 cm above an automated stop/start platform. Each mouse was subjected to an initial habituation trial at the speed of 4 rotations per minute (rpm), followed by three test trials at accelerating speed, for three consecutive days. Each trial started at 4 rpm and the speed was accelerated continuously until the animal fell down, or up to a maximum of 40 rpm for 300 s. The latency for the animals to fall down onto the platform and the corresponding speed were recorded.

3.9 TISSUE PROCESSING FOR ANALYSIS OF CYTOKINES AND AD-RELATED PROTEINS (Paper II and IV)

Enzyme-linked immunosorbent assay (ELISA) was used for studies on the effects of KA-administration on cytokine expression in the rat brain (Paper II), and the effects of chronic brain-directed transgenic overexpression of hsIL-1ra on the levels of cytokines in the mouse brain (Paper IV). The mouse brain samples were also analysed by Western blots for the AD-related protein APP (see 3.13 below). Briefly, the animals were sacrificed by decapitation, the trunk blood was collected and the brain regions of interest were rapidly dissected out on ice, frozen in dry ice, and stored at -80^{o}C until further processing. Each brain tissue sample (Paper II) was weighed and homogenised on ice with a tissue homogeniser (Tamro Medlab AB, Sweden) in 0.01 M phosphate buffered saline (PBS), pH 7.4, (1:10 vol/weight) supplemented with 1% protease inhibitors cocktail (Sigma-Aldrich, Inc., St. Louis, MO, USA). The brain tissue samples from mice (Paper IV) were immersed in ice-cold lysis buffer containing 20 mM Tris-HCl, 137 mM NaCl, 2% Nonidet P-40, 2% Triton X, and 1% protease inhibitors cocktail (Sigma-Aldrich, Inc., St. Louis, MO, USA), followed by sonication and centrifugation. The supernatants were collected and stored at -80^{o}C (Paper II and IV) until further processing. The sandwich ELISA experiments were performed according to the manufacturer's instructions, *i.e.* R&D Systems, UK (Paper II and IV), and Biosource International, California, USA (Paper II).

3.10 TISSUE PROCESSING FOR MORPHOLOGICAL ANALYSIS AND IMMUNOHISTOCHEMISTRY (Paper III and IV)

Morphological analysis was used to investigate the effects of chronic, brain-directed overexpression of hsIL-1ra in the transgenic mice on permanent focal cerebral ischemia (Paper III) and during control conditions (Paper IV). Immunohistochemistry was used to determine the effects on the expression of inflammatory markers in the brain upon focal permanent cerebral ischemia (Paper III). All morphological analyses, except for the immersion method (3.11.2 below), were performed on brain tissues fixed by intracardial perfusion with 4% paraformaldehyde (PF).

3.11 VOLUME ESTIMATION METHODS

3.11.1 Evaluation of area density (Paper III and IV)

Assessment of area density (cross-sectional area) was performed, on serial coronal brain sections stained with cresyl violet, by point-counting at 2x magnification with a grid positioned randomly on the section. The values obtained for the area density were used for the estimation of the infarct volume, according to the principle of Cavalieri (Gundersen et al., 1988) (Paper III). The estimation method is based on calculation of the cross-sectional area of the desired region in a series of equally spaced sections (Fig. 3), followed by integration of the partial volumes obtained from the cross-sectional areas and the distance between two consecutive sections.

3.11.2 Immersion method (Paper IV)

The immersion method and the principle of communicating vessels were used for the analysis of brain volume. Briefly, the brain was placed in a vertical 10 ml syringe filled with PBS, serving as a container. A 1 ml syringe, connected through a plastic tubing with the 10 ml syringe, was positioned horisontally and used for reading the volume dislocated by the immersion of the brain.

3.12 IMMUNOHISTOCHEMISTRY (Paper III)

The analysis of inflammatory markers following permanent focal cerebral ischemia was performed by immunohistochemical methods on brains sections from ischemic and sham-operated heterozygotic mice with brain-directed overexpression of hsIL-1ra and WT mice. The brains were sectioned in the coronal plane on a cryostat and the sections were collected at two levels, 0.02 mm anterior and 1.82 mm posterior to bregma (Frankllin and Paxinos, 1997). These levels included the different anatomical brain regions observed in the ischemic lesion. The sections were processed for immunohistochemistry with hIL-1ra, GFAP, IL-1β, and caspase-1 p10 and the immunoreactive structures were detected by incubation with secondary antibodies

conjugated with Alexa Fluor (dilution 1:800; Molecular Probes Europe BV, Leiden, The Netherlands) and analysed in a Nikon microscope (Eclipse E800). (For details on the primary antibodies see Paper III).

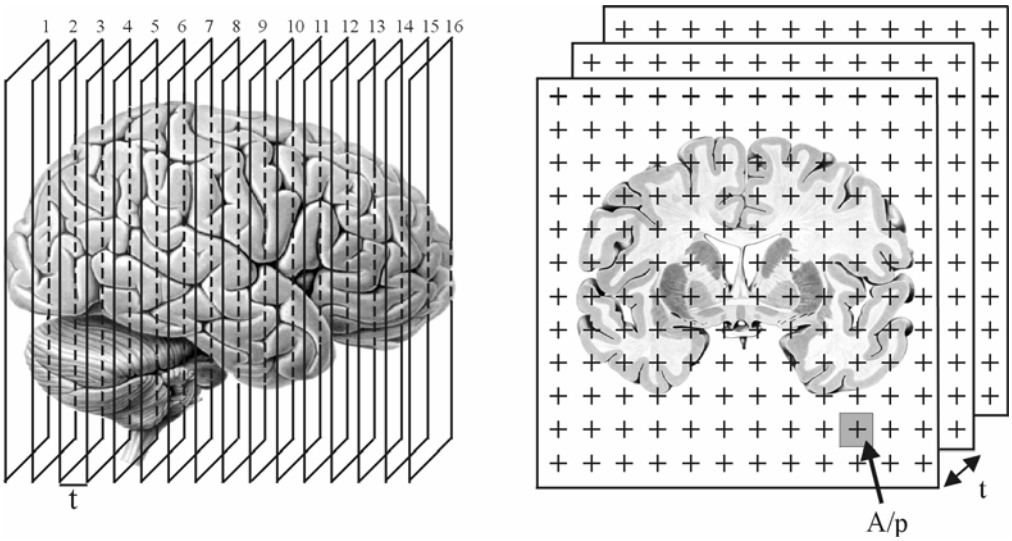

Figure 3. Calculation of area density and volum estimation (Cavalieri principle). Assessment of area density (cross-sectional area) is performed on stained sections by point-counting, with a grid positioned randomly on the section. The area density is calculated by multiplying the total number of points that hit the region of interest with the area per point (A/p). The values obtained for the area density are used for the estimation of the volume of the region of interest, by summimg up all the cross-sectional areas calculated from parallel sections separated by a known distance, and multiplying the result with the distance between the sections.

3.13 WESTERN BLOT ANALYSIS (Paper IV)

Western blot analysis was used for studies of the expression of APP in response to chronic brain-directed blockade of IL-1 signalling. The analysis was performed on regional brain homogenates from transgenic mice with brain-directed overexpression of hsIL-1ra. The preparation of the tissue extracts and the protein determination method is described above (Section 3.9). Equivalent amounts of protein were loaded on 10% polyacrylamide gels for separation. To ensure loading of equal amounts, the membranes were stained with Ponceau S solution (Sigma-Aldrich, St. Louis, MO, USA). The proteins were transferred onto Hybond ECL nitrocellulose membranes (Amersham Biosciences, UK Ltd.) and incubated with two different primary antibodies against APP, 22C11 and 6E10. After incubation with horseradish peroxidase (HRP)-linked anti-mouse IgG, the detection of bound antibodies was performed by the enhanced chemiluminiscence (ECL) method (Amersham Biosciences, UK).

3.14 RADIOIMMUNOASSAY (Paper V)

Serum CST levels were measured by radioimmunoassay (RIA) in samples from Tg hsIL-1ra and WT mice. The serum samples were obtained from trunk blood after decapitation and the levels of CST were analysed in duplicates using a ^{125}I RIA kit, according to the manufacturer's indications (ICN biomedicals Inc., CA, U.S.A.).

3.15 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) (Paper V)

The influence of central IL-1R-mediated activity on the levels of DA and 5-HT and their metabolites DOPAC and 5-HIAA, respectively, was studied by high-performance liquid chromatography (HPLC)-analysis in male transgenic mice with brain-directed overexpression of hsIL-1ra. The caudate, hypothalamus, hippocampus and prefrontal cortex were dissected out from each mouse brain and stored at -80^{o}C until preparation. Briefly, the tissues were weighed, homogenised and extracted with perchloric acid. After centrifugation, the supernatants were loaded on a 200 x 2.1 mm ODS Hypersil (5 μm particles) column (Hewlett Packard). The elution was achieved with a 0.1 M phosphate buffer containing 10% methanol, 2.4% EDTA, and 0.65 mM 1-octanesulfonic acid (as ion-pairing reagent). The flow rate was kept constant and the eluted peaks were detected by an amperometric detector (BAS LC-4B, USA) that was set at a voltage of 0.7 V. The concentrations were calculated as the ratio between peak height of each sample and that of freshly prepared standard solution by an HPLC integrator (SP 4600, Spectra-Physics, USA).

4 RESULTS AND DISCUSSION

4.1 KA-INDUCED CORE TEMPERATURE CHANGES (Paper I and II)

We found that systemic administration of KA produced a biphasic effect on core temperature in freely moving rats, *i.e.* an initial hypothermia in the first hour after injection followed by a longer lasting hyperthermic effect (Paper I and II). A more detailed analysis of the core temperature changes showed that the maximal hypothermic and hyperthermic effects occurred at 30 min and 4 h, respectively, after the administration of KA (Paper II). Turski at al. (Turski and Kleinrok, 1980) showed the same pattern of temperature changes after i.c.v. administration of KA, suggesting that, with regard to the effect on core temperature, 0.1 μg KA administered i.c.v. could be considered equivalent to the 10 mg/kg injected i.p. (Paper I and II).

It is of particular interest to study the mechanisms of KA-induced temperature changes, since controlled hypothermia has been shown to improve the clinical neurological outcome, after several acute neurodegenerative conditions involving excessive stimulation of glutamate receptors, including cerebral ischemia (Connolly et al., 1962), and severe head injury (Clifton et al., 1993). The neuroprotective effect of hypothermia in animal models of neurodegeneration has been shown in models of epilepsy, such as KA-induced brain damage (Liu et al., 1993b; Maeda et al., 1999), cerebral ischemia (Busto et al., 1989), and in traumatic brain injury (Clifton et al., 1991; Prusiner and Wolfson, 1968). In contrast, hyperthermia had an aggravating and/or precipitating effect on KA-induced and febrile seizures (Liu et al., 1993b; McCaughran and Schechter, 1982; Stensman and Ursing, 1971). Hyperthermia was demonstrated to increase the release of glutamate in the rat cortex in correlation with increased body temperature and the onset of seizures (Morimoto et al., 1993). Due to the major influence that temperature changes seem to have on the outcome in several neurodegenerative conditions, including animal models of epilepsy, we have investigated the role of the NMDA-subtype of glutamate receptors (Paper I) and the expression of cytokines in the hypothalamus (Paper II), in connection with the KA-induced changes in core temperature. Furthermore, the expression of different cytokines was examined in various brain regions (Paper II).

4.1.1 KA-induced core temperature changes – the role of NMDA-receptors (Paper I)

Dizocilpine is a non-competitive NMDA-receptor antagonist, known to diminish the neurodegeneration and dose-dependently block the epileptic seizures due to administration of KA in rats (Berg et al., 1993; Clifford et al., 1990; Eriksson et al.,

2000c). Dizocilpine was used to analyse the involvement of NMDA-receptor-mediated activity in the KA-induced temperature changes.

As in previous studies (Berg et al., 1993; Clifford et al., 1990; Eriksson et al., 2000c), the pretreatment with dizocilpine dose-dependently blocked the KA-induced convulsive syndrome. The KA-induced hypothermia was potentiated by dizocilpine, but the ensuing hyperthermia was not influenced, indicating that KA-induced changes in body temperature do not necessarily involve the activation of NMDA-receptors, as opposed to KA-induced behavioural changes. It is also concluded that KA-induced motor activity is not the causative factor for the KA-induced hyperthermia, since the higher dose of dizocilpine (5.0 mg/kg) completely blocked the seizures, but did not influence the KA-induced temperature changes.

The involvement of NMDA-receptors in KA-induced changes in body temperature cannot be ruled out since the initial hypothermic effect of KA was enhanced by dizocilpine. Dizocilpine has been shown to influence the activity of DA and 5-HT in the brain, *e.g.* stimulating their release in the nucleus accumbens upon systemic administration (Yan et al., 1997) and potentiating the serotonergic function (Dall'Olio et al., 1999). It seems possible that NMDA-receptor blockade may unmask KA-induced hypothermia, possibly through the effects of dizocilpine on the brain activity of DA and 5-HT.

The involvement of NMDA-receptors subtype in thermoregulation has been suggested by earlier findings that dizocilpine given alone increases core temperature in rats (Pechnick et al., 1989; Pucilowski et al., 1991). In Paper I we showed that dizocilpine alone produced an early hyperthermia at 30 min after *i.p.* administration. Alterations in the concentrations of calcium (Ca^{2+}) in the brain have been shown to affect thermoregulation (Feldberg and Saxena, 1970; Myers and Veale, 1970). The i.c.v. administration of verapamil, a blocker of Ca^{2+}-channels, was followed by a hyperthermic effect (Palmi and Sgaragli, 1989; Samardzic and Beleslin, 1984), while local delivery of verapamil into the hypothalamus produced opposite region-dependent effects on the core temperature in cats, *i.e.* hypothermia when administered into the hypothalamus/preoptic area (AH/POA), and hyperthermia upon infusion into the posterior hypothalamus (Beleslin et al., 1985; Rezvani et al., 1986). Dizocilpine is known to reduce the entry of Ca^{2+} into cells and the observed hyperthermic effect after *i.p.* administration may be the net effect of differential responses in hypothalamic subregions.

Changes in extracellular brain Ca^{2+}-levels have been suggested to mediate hyperthermia produced by i.c.v. delivery of IL-1β in rabbits, since this effect of IL-1β was blocked by a calcium chelator (Palmi et al., 1994). Our results showing that dizocilpine does not counteract KA-induced temperature changes, together with those demonstrating that dizocilpine blocks KA-induced mRNA expression of IL-1β and IL-1ra in the brain, were suggesting that the involvement of IL-1β in KA-induced

temperature changes is unlikely. However, mRNA synthesis is not always followed by protein synthesis (Dinarello, 1996) and, therefore, the uncertainty remained regarding the involvement of IL-1 in KA-induced temperature changes.

4.1.2 KA-induced core temperature changes and neurodegeneration – the roles of IL-1β, IL-6, and IL-1ra (Paper II)

In order to further study the potential involvement of IL-1β, as well as IL-6 and IL-1ra, in KA-induced temperature changes we analysed these cytokines in the hypothalamus, in correlation with KA-induced temperature changes, including the time points when the maximum hypothermia (30 min) and hyperthermia (4 h) were observed. Furthermore, these cytokines were measured at the same time points in three other brain regions, *i.e.* the hippocampus, cerebellum and frontal cortex.

Increased levels of IL-1β at 2 h after KA-administration overlapped with the first part of the hyperthermic phase, supporting a role of IL-1β in the KA-induced hyperthermia, whereas neither IL-6 nor IL-1ra were significantly altered by the KA-injection. This was unexpected in view of previous studies showing a lack of fever response following i.p. or i.c.v. administration of LPS or IL-1β in IL-6 KO mice (Chai et al., 1996). However, we noted that the ratios between the hypothalamic concentrations of IL-1β and IL-6 were significantly increased at 2 and 4 h after KA-injection. The ratio between the concentrations of IL-1β and IL-1ra was significantly increased at 2 h after KA-administration. Although speculative, the changes in ratios over time seem to indicate that there was an imbalance between the levels of IL-1β and the levels of IL-6 and IL-1ra, respectively, at the time points when the body temperature was altered. These imbalances were not observed at 1 h after KA-administration, *i.e.* when the core temperature was similar to that recorded at baseline.

The levels of IL-1β protein were significantly increased in hippocampus, cerebellum and frontal cortex, in agreement with previous reports (Eriksson et al., 2000b; Eriksson et al., 1999; Lehtimaki et al., 2003; Minami et al., 1991; Minami et al., 1990; Vezzani et al., 2002). Similarly to the hypothalamus, there was a biphasic trend in the levels of IL-1β in these brain regions with a first peak at 30 min – 1 h, and a second peak between 2 and 8 h. In the cerebellum and frontal cortex, IL-1β levels had returned to basal levels at 24 h after KA-administration, whereas both IL-1β and IL-1ra levels in the hippocampus were increased at this time point, confirming previous studies (Eriksson et al., 2000b; Eriksson et al., 1999; Eriksson et al., 1998; Vezzani et al., 2002). No other significant changes in the levels of IL-1ra and IL-6 were observed in these three brain regions.

Hippocampus is of particular interest in epilepsy research, being one of the brain regions in which specific aspects of the KA-induced neurodegeneration can be studied (Ben-Ari, 1985; Ben-Ari et al., 1980). Our results suggest that the observed increases in

IL-1β and IL-1ra are closely related to KA-induced neurodegenerative changes. IL-1β has been shown to potentiate the neurodegenerative effect of excitotoxic agents, when administered intracerebrally, without having an excitotoxic effect by itself (Lawrence et al., 1998). However, IL-1β stimulates the differentiation, proliferation, and survival of astrocytes (Giulian et al., 1988), which are known to be involved in reparatory functions in the CNS. Furthermore, IL-1β stimulates various neurotrophic factors, including NGF (Gall et al., 1991; Strauss et al., 1994) and CNTF (Herx et al., 2000). Thus, the persistent increase in IL-1β in the hippocampus, following KA-injection, can have both negative and positive effects.

4.2 THE EFFECTS OF BRAIN IL-1R-MEDIATED ACTIVITY IN PERMANENT FOCAL CEREBRAL ISCHEMIA (Paper III)

In view of the evidence from many studies that IL-1 is involved in cerebral ischemia, such as the increase in IL-1β levels in experimental animal models (Buttini et al., 1994; Davies et al., 1999; Hill et al., 1999; Legos et al., 2000; Liu et al., 1993a; Minami et al., 1992; Sairanen et al., 1997; Wang et al., 1997; Wang et al., 1994; Zhang et al., 1998), and that intracerebral injection of IL-1β exacerbates the neurodegeneration in cerebral ischemia, we analysed the outcome of permanent focal cerebral ischemia in the mouse strain with brain-directed overexpression of hsIL-1ra (Tg hsIL-1ra). Unlike earlier studies in which IL-1ra was shown to be neuroprotective upon external delivery (Garcia et al., 1995; Martin et al., 1994; Relton and Rothwell, 1992; Stroemer and Rothwell, 1997) or secondary to adenoviral transfection in the brain (Betz et al., 1995; Tsai et al., 2003; Yang et al., 1998a; Yang et al., 1999), we did not detect any differences between the transgenic mice and WT controls with regard to the neurological scores, brain infarct volume, or oedema formation. The extent of inflammation in the brain, as indicated by the immunoreactivity to GFAP in astrocytes, and of IL-1β and caspase-1 in activated microglia, was similar between the two genotypes, upon ischemia.

An explanation of the lack of neuroprotective effect may be that the infarcted region included almost the entire territory of the MCA in most of the mice and, therefore, the potential neuroprotective capacity given by the levels of hsIL-1ra expression in transgenic heterozygotic mice was not sufficient for this extensive brain damage. Notably, there was a decrease in hsIL-1ra expression in astrocytes in the infarcted area as compared to non-ischemic regions and sham-operated animals, in contrast with previous studies showing that endogenous IL-1ra is induced in the brain in experimental cerebral ischemia (Hill et al., 1999; Legos et al., 2000; Loddick et al., 1997; Wang et al., 1997). If we consider the increased expression of IL-1β due to ischemia and the apparent decrease in transgenic expression of hsIL-1ra in the infarct area, it is conceivable that the levels of IL-1ra might have been insufficient to provide

neuroprotection, particularly in view of the efficiency of IL-1β signalling (Arend et al., 1990). Other explanations for our findings could be provided by actions of IL-1β that are not mediated via the IL-1RI, as recently suggested (Touzani et al., 2002). Thus, IL-1β was shown to exacerbate the ischemic lesion in mice lacking IL-1RI, an effect that was not blocked by IL-1ra (Touzani et al., 2002). Finally, we cannot exclude mechanisms compensating for the reduced or blocked IL-1 signalling that may have evolved in the brain of the Tg hsIL-1ra mice.

4.3 THE EFFECTS OF CENTRAL IL-1R-MEDIATED ACTIVITY ON THE EXPRESSION OF IL-1β, TNF-α AND IL-6 IN THE BRAIN (Paper IV)

The occurrence of compensatory mechanisms is a common phenomenon in the case of cytokines, as shown *e.g.* by the higher levels of TNF-α induced by peripheral administration of LPS in IL-6 KO mice, when compared with the WT mice (Fattori et al., 1994). However, there were no major differences in the brain expression of IL-1β, IL-6 or TNF-α between the transgenic heterozygotic and homozygotic mice with brain-directed overexpression of hsIL-1ra and the WT littermates. The only difference observed was the lower levels of TNF-α in the parietal cortex of the homozygotic as compared to WT mice. The previous finding of higher TNF-α levels in the cortex of heterozygotic as compared to WT mice (Tehranian et al., 2002) was not confirmed in our study, probably due to the differences in the size and position of the dissected cortex, or to the different procedures for tissue processing, *i.e.* homogenisation in the presence (Paper IV) or absence (Tehranian et al., 2002) of detergents. It is known that TNF-α exists in a 17 kDa soluble form and a 26 kDa membrane-bound form (Schottelius et al., 2004). Hypothetically, an increase in the cytosolic form, as suggested by the results obtained by Tehranian et al., combined with a decrease in the membrane-bound isoform, would explain the discrepancies between the two studies regarding the TNF-α levels.

In addition to analysis of pro-inflammatory cytokines, the levels of the transgene, hIL-1ra, were also examined. As expected, the levels of hIL-1ra were approximately double in homozygotic as compared to the heterozygotic mice. The levels of hIL-1ra increased with age in the parietal cortex, but not in the hippocampus and cerebellum.

Analysis of the pro-inflammatory cytokines in young (40 days) and older (12 – 14 months) mice revealed an age-dependent increase, the only exception being the levels of TNF-α in the hippocampus. These results are in agreement with previous studies reporting higher levels of pro-inflammatory cytokines in the brain of aging rodents as compared to younger animals (for review see (Bodles and Barger, 2004). The basal levels of the mRNA for IL-6 and TNF-α have been shown to be elevated in aged mice as compared to younger mice (Sharman et al., 2002), and increased IL-1β and TNF-α gene expression was demonstrated in old mice by gene profiling studies using DNA

microarrays (Terao et al., 2002). Increased levels of pro-inflammatory cyokines with age may reflect the age-dependent increase in number of astrocytes and microglia (Mouton et al., 2002). However, the age-dependent increase in the levels of pro-inflammatory cytokines (Paper IV) was not influenced by the hsIL-1ra genotype of the animals, suggesting that brain-directed blockade of IL-1 signalling was not involve in this phenomenon.

The levels of cytokines were similar in male and female Tg hsIL-1ra mice, except in the hippocampus, where the levels of IL-6 and hIL-1ra were higher in female than in male mice. This finding is in agreement with the previous report of higher numbers of astrocytes and microglia in the hippocampus of the female mice (Mouton et al., 2002).

4.4 THE EFFECTS OF CENTRAL IL-1R-MEDIATED ACTIVITY ON BRAIN MORPHOLOGY (Paper IV)

Morphological analysis of the brains of transgenic mice with brain-directed overexpression of hsIL-1ra demonstrated a significantly smaller brain volume in the homozygotic mice, whereas the heterozygotic and WT mice did not significantly differ. The age had no influence *per se* and did not modify the effect of genotype on the brain volume. The total brain volume was related to the amount of transgenic expression, suggesting that it was dependent on the degree of central blockade of IL-1 signalling. Analysis of the gross morphology of the brain revealed an enlarged ventricular system in both young and older transgenic mice, visible at the level of the frontal horn of the lateral ventricle and with a maximum at the level of the dorsal hippocampus. Analysis of the area density by point-counting yielded similar genotype-, but not age-dependent results, *i.e.* the area density was significantly lower in the homozygotic than in the WT mice, whereas no significant difference could be seen between the young and older mice.

These results seem to indicate that chronic brain-directed overexpression of hsIL-1ra influences the brain development, rather than causing significant postnatal effects. This finding is in agreement with previous studies demonstrating that IL-1β regulates different aspects of growth in the CNS during embryogenesis. Thus, IL-1 has been shown to stimulate astroglial proliferation during embryogenesis and to be involved in regulating the growth of the CNS during embryogenesis (Giulian et al., 1988). Furthermore, IL-1 has been shown to increase neuronal survival in dissociated spinal cord cultures derived from foetal mice (Brenneman et al., 1992), and to stimulate the differentiation of mesencephalic progenitor cells to dopaminergic neurons (Ling et al., 1998). Interestingly, the expression of IL-1β in the cerebral cortex of sheep was correlated with the embryological period of neurogenesis rather than that of gliogenesis

(Dziegielewska et al., 2000). The results obtained in the Tg hsIL-1ra mice indicate that they can be useful tools in studies of the involvement of IL-1 in CNS development.

4.5 THE EFFECTS OF CENTRAL IL-1R-MEDIATED ACTIVITY ON AMYLOID PRECURSOR PROTEIN IN THE BRAIN (Paper IV).

An increasing body of evidence suggesting the involvement of IL-1 in the pathogenesis and progression of AD is partly based on the suggested bidirectional relation between IL-1 and APP, one of the most important AD-related proteins (for review see (Griffin and Mrak, 2002). In order to address the suggested link between IL-1 signalling and the pathogenesis of AD, we investigated the effects of brain-directed overexpression of hsIL-1ra on the levels of APP in the cerebellum and hippocampus of Tg hsIL-1ra mice in comparison with WT littermates. We observed that heterozygotic mice had lower APP levels in the cerebellum as compared to the WT littermates. It has been shown that IL-1 stimulates the APP gene promoter and synthesis (Yang et al., 1998b) and a recent study showed that IL-1β and TNF-α influence the metabolism of APP by stimulating γ-secretase cleavage (Liao et al., 2004b). APP and its cleavage product, aβ, have been found to increase the secretion of IL-1 from microglia *in vitro* (Barger and Harmon, 1997; Del Bo et al., 1995; Lindberg et al., in press), and to induce glial expression of pro-inflammatory cytokines, including IL-1, in the brain of transgenic mice with AD plaque pathology (Apelt and Schliebs, 2001; Tehranian et al., 2001). Our results are in agreement with these previous studies, *i.e.* brain-directed blockade of IL-1 signalling resulting in diminished APP synthesis. Interestingly, homozygotic mice had similar levels of APP as the WT mice and higher levels than heterozygotic mice, albeit statistically nonsignificant, suggesting that the effects of IL-1 on APP expression are modulatory rather than stimulatory. Further studies are needed to elucidate the interrelations between IL-1 signalling in the brain and APP expression and processing.

There were also age- and gender-dependent differences in APP expression. APP levels increased with age in the hippocampus and cerebellum, while female mice had higher levels of APP in the cerebellum than the male mice. These results are in agreement with previous reports demonstrating increased APP levels with age in the rat (Kawarabayashi et al., 1993) and human brain (Nordstedt et al., 1991). Both pro-inflammatory cytokines and APP were higher in older than in young animals and higher in female than in male mice.

4.6 THE EFFECTS OF CENTRAL IL-1R-MEDIATED ACTIVITY ON BEHAVIOUR (Paper V)

Peripheral and central administration of IL-1 induces a constellation of behavioural manifestation in rodents, generally reproducing the components of the "sickness syndrome" (Dantzer, 2001) (see Introduction). The generation of transgenic mice with brain-directed overexpression of hsIL-1ra in the brain allows the investigation of the effects of central deficit of IL-1 signalling on different aspects of behaviour. Thus we have tested the homozygotic and age-matched WT mice in a battery of tests for the analysis of OF behaviours, anxiety, and motor performance.

The homozygotic mice had higher overall locomotor activity in the OF and habituated less during the subsequent days of testing (inter-trial activity decrement, long-term habituation (LTH)) than the WT mice. With regard to rearing activity, the homozygotic mice showed a lower LTH than the WT mice. The habituation for the 15-min intervals during the 1-h testing period (intra-trial activity decrement, short-term habituation (STH)) was similar for the two genotypes. The higher overall locomotor activity observed in the WT mice is in agreement with previous studies showing that administration of IL-1α in rat (Otterness et al., 1988) and IL-1β in mouse (Lacosta et al., 1998) reduce locomotor activity. These studies and our results suggest that locomotor activity is inversely related to IL-1R-mediated activity.

The OF test demonstrated that the LTH for locomotion was present only in the WT mice. LTH to a novel environment (*e.g.* OF) is a simple form of non-associative learning, which has been shown to depend on different aspects of hippocampal functionality. The integrity of glutamate receptors and calmodulin-kinase II in the CA1 region of the hippocampus and the activation of different hippocampal protein kinase signalling cascades were shown to be involved in LTH elicited by a brief (5 min), exposure to OF (Vianna et al., 2000). The long exposure to the OF used in Paper V (1 h) was meant to produce a level of LTH that was previously suggested to enable the detection of changes in LTH (for review see (Cerbone and Sadile, 1994). The precise mechanism responsible for the reduced LTH in the homozygotic mice is not immediately obvious, since negative effects on different memory tasks in rodents have been reported both in excesses (Gibertini et al., 1995; Oitzl et al., 1993) and deficits (Avital et al., 2003; Yirmiya et al., 2002) of IL-1 signalling in the brain. Furthermore, the recent study showing that IL-1ra may have intrinsic effects in the hippocampus, independent of IL-1RI, that mimic the effects of IL-1β with regard to the decrease in glutamate release, impairment of long-term potentiation, and phosphorylation of c-Jun N-terminal kinase (JNK) (Loscher et al., 2003), could also explain the results obtained in Paper V.

The EPM test is used for the detection of anxiety-like behaviour, as a result of innate fear that laboratory rodents have for open spaces and their drive to explore a new environment (Montgomery, 1955). In the present study, chronic brain-directed overexpression of hsIL-1ra seemed to have an anxiolytic effect on the performance in the EPM, since the homozygotic mice spent more time in the open and, consequently, less time in the closed arms, than the WT mice. The involvement of IL-1β in the modulation of anxiety has been demonstrated previously. Depending on the dose of IL-1β , both anxiogenic (Connor et al., 1998; Montkowski et al., 1997) and anxiolytic (Montkowski et al., 1997) effects were obtained. Thus, 0.1 ng IL-1β (i.c.v.) increased the exploration of the open arms in EPM (Montkowski et al., 1997), whereas doses of 20 ng or higher had an inhibitory effect on this behaviour (Connor et al., 1998; Montkowski et al., 1997). These results combined with those obtained in Paper V support a modulatory effect of IL-1β on anxiety, in which higher levels of IL-1β are anxiogenic, whereas low levels of IL-1β or a deficit in available IL-1Rs, seems to have anxiolytic effects.

We did not detect any difference between the two genotypes with regard to the motor performance in rotarod. A previous study on the homozygotic mice reported reduced performance in balancing tasks and narrow-beam walking (Tehranian et al., 2002). These results suggest that the previously observed motor deficits of homozygotic mice (Tehranian et al., 2002) cannot be detected with the less-demanding rotarod test, used in Paper V.

4.7 THE EFFECTS OF CENTRAL IL-1R-MEDIATED ACTIVITY ON THE HPA-AXIS (Paper V)

One of the most well-known features of the communication between the immune system and the CNS is the activation of the HPA-axis by pro-inflammatory cytokines, *i.e.* IL-1β, TNF-α and IL-6, including the elevation of ACTH and CST concentrations in the blood (Dunn, 2000). We have used a mild form of stress, previously shown to produce a moderate increase in rat plasma CST (Hennessy et al., 1979), in order to investigate the effects of chronic overexpression of hsIL-1ra on the activation of the HPA-axis. Upon exposure to the mild stress, the serum levels of CST increased significantly, but there was no significant difference between the two genotypes. These results indicate that the HPA-axis of the homozygotic mice was at least as active as for the WT mice, despite the overexpression of hsIL-1ra. However, the interpretation of our results is, to some extent, limited by the determination of serum CST at only one time point.

Interestingly, similar increases in blood CST levels upon stress in modified mice with deficiencies in IL-1 signalling and their WT controls have been previously reported (Goshen et al., 2003; Liege et al., 2000). Thus, similar increases in blood

CST levels were observed in caspase-1 KO and WT mice subjected to 60 min of restraint stress, or injected with IL-1β or LPS (Liege et al., 2000). Exposure of IL-1RI KO mice to a 60 min restraining stress or to a high dose of 2-deoxyglucose, also resulted in similar levels of serum CST levels as in WT mice (Goshen et al., 2003). However, when exposed to milder stress, such as auditory stress or a low dose of 2-deoxyglucose, the IL-1RI KO mice had a hypoactive HPA-axis, as compared to the WT controls (Goshen et al., 2003). The difference with regard to mild stressors, in the homozygotic mice (Paper V), may have several explanations, including difference in background strain, or different levels and/or mechanisms of compensation for the deficit in IL-1 signalling. The context in which exposure to the stressful situation was performed in the two experiments was different. Goshen et al. used naïve mice for the stress experiments, while in Paper V the mice were exposed to stress one week after the last behavioural test. The two mouse strains, Tg hsIL-1ra and IL-1RI KO mice, have in common that in conditions of interference with IL-1 signalling, their HPA-axis was indeed activated upon stress.

4.8 THE EFFECTS OF CENTRAL IL-1R-MEDIATED ACTIVITY ON BIOGENIC AMINES IN THE BRAIN (PAPER V)

There is an increasing body of evidence with regard to the functional connections between IL-1 and biogenic amines in the brain, as shown by the effects of central or peripheral administration of IL-1 in rodents. Studies in the rat showed that i.c.v. administration of IL-1β activates the hypothalamic 5-HT system (Gemma et al., 1991). Injection of IL-1β directly into the mediobasal hypothalamus was shown to induce DA, DOPAC (Mohankumar et al., 1991), and 5-HIAA release (Mohankumar et al., 1993), and the release of NA, DA and 5-HT release was stimulated by injection into the anterior hypothalamus (Shintani et al., 1993). Furthermore, IL-1β has been shown to stimulate tyrosine hydroxylase (TH) activity in the median eminence (Abreu et al., 1994). In Paper V, chronic, brain-directed transgenic overexpression of hsIL-1ra influenced the concentrations of DA, 5-HT, and their metabolites in the caudate, hypothalamus, prefrontal cortex and hypothalamus, both under basal conditions and upon exposure to a mild stress. The homozygotic mice had significantly lower levels of these monoamines and their metabolites than the WT mice in the caudate (DA and 5-HT), hypothalamus (DA, DOPAC and 5-HIAA in non-stressed animals), prefrontal cortex (5-HT and 5-HIAA) and hippocampus (DA). The only finding of an increase due to the transgenic hsIL-1ra expression consisted of significantly higher levels of DOPAC in the caudate of homozygotic mice. These findings suggest that IL-1R-mediated activity modulates the synthesis and/or the metabolism of brain monoamines, and that the main effect of antagonising IL-1 signalling in the brain, was reduction in the levels of DA, 5-HT and their metabolites.

These findings are in agreement with earlier studies on monoamine levels, based on exogenous administration of IL-1β. Our data indicate that the hypothalamus is particularly sensitive to blockade of IL-1 signalling, in view of the significantly lower concentrations of DOPAC and 5-HIAA in homozygotic mice as compared to WT mice, under basal conditions.

The mild form of stress used in our study, *i.e.* brief exposure to the OF test followed by isolation, produced changes in monoamine concentrations in stress-sensitive brain regions such as prefrontal cortex, hypothalamus and hippocampus. The regional monoamine concentrations were genotype-dependent only in the hypothalamus (for DOPAC, 5-HT, and 5-HIAA) and caudate (for 5-HIAA). Previous studies showed the involvement of IL-1 in the release of NA, DA and 5-HT from the rat hypothalamus during immobilisation stress, since intra-hypothalamic delivery of IL-1ra before the start of the stress blocked the release of monoamines (Shintani et al., 1995). The present data support the view that IL-1R-mediated activity has consequences on monoamine regulation, both during basal and stressed conditions, suggesting that changes in IL-1 signalling in the brain affect *e.g.* mood and behaviour.

5 CONCLUSIONS

1. KA-induced changes in body temperature are not mediated by NMDA-receptors, since NMDA-receptor blockade with dizocilpine inhibited KA-induced behavioural changes, but did not counteract KA-induced temperature changes. This finding has an important practical implication, *i.e.* measurements of the body temperature can be used to identify the non-responders after peripheral administration of KA when the behavioural changes are blocked, *e.g.* with dizocilpine
2. Hyperthermia induced by KA is not a result of the seizure-induced motor activity, since KA-induced seizures were blocked by dizocilpine, while the KA-induced hyperthermia was not influenced.
3. The changes in the IL-1β levels and the imbalance observed between the levels of IL-1β, IL-6 and IL-1ra may account for some of the temperature changes induced by peripheral administration of KA, as suggested by the increased IL-1β/IL-6 and IL-1β/IL-1ra ratios at time points when core temperature was modified.
4. IL-1ra may not be useful in extensive ischemic infarcts in the territory of the MCA in view of the lack of neuroprotection of transgenic mice overexpressing hsIL-1ra.
5. Chronic, brain-directed blockade of IL-1R-mediated activity does not appear to induce major compensatory changes in brain cytokine levels at least until 12 - 14 months of age.
6. Age and gender influence the brain levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α.
7. IL-1 signalling in the brain is important for normal development of the CNS.
8. The results obtained in mice with brain-directed overexpression of hsIL-1ra suggest a modulatory role of IL-1 signalling on different aspects of behaviour.
9. Interference of IL-1R-mediated activity in the brain does not seem to influence the activation of the HPA-axis upon exposure to mild stress.
10. IL-1 signalling in the brain is involved in the regulation of monomines during basal conditions and upon stress, as previously suggested by the stimulatory effects of IL-1β on brain biogenic amines.

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