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Review

Inflammatory mechanisms associated with brain damage induced by kainic acid with special reference to the interleukin-1 system

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Abstract

The evidence of inflammatory processes in the clinical manifestations and neuropathological sequelae of epilepsy have accumulated in the last decade. Administration of kainic acid, an analogue of the excitatory amino acid glutamate, induces a characteristic behavioural syndrome and a reproducible pattern of neurodegeneration in several brain areas, closely resembling human temporal lobe epilepsy. Results from studies using the kainic acid model indicate that manipulation of pro- and anti-inflammatory cytokines can modify the outcome with regard to the behavioural syndrome as well as the neuropathological consequences. Interleukin-1 is one of the most important cytokines and has several actions in the brain that are critical for the host defense against injury and infection, and it is involved in the initiation of early stages of inflammation. It is believed that interleukin-1 plays a pivotal role in the neuroinflammation associated with certain forms of neurodegeneration, including cerebral ischemia, trauma and excitotoxic brain injury. In this review, we have summarized the experimental data available with regard to the involvement of the interleukin-1 system in kainic acid-induced changes in the brain and emphasized the modulatory role of interleukin-1β in this model of epilepsy.

Keywords: cytokines • excitotoxicity • epilepsy • neurodegeneration • interleukin-1 • kainic acid

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Introduction

The communication between the nervous and the immune system influences the development of the brain and regulates important physiological functions such as learning and memory [1], sleep [2] and neuroendocrine functions [3]. In addition, the immune system is involved in numerous neuropathological conditions: brain infections, demyelination, trauma, neurodegenerative disorders such as acute or chronic cerebral ischemia, dementing processes, epilepsy and mood disorders [4–12].

Epilepsy is a neurodegenerative disorder characterized by the occurrence of seizures and neurodegeneration in certain brain areas. Recent studies suggested that immunological aspects might be responsible for certain types of primary or secondary forms of epilepsy (for review see [13]). The findings of increased levels of cytokines such as interleukin-6 (IL-6) [14], interleukin-1β (IL-1β) [15] and interleukin-1 receptor antagonist (IL-1ra) [16] in cerebrospinal fluid from patients with epilepsy and the aggravating effect of IL-1β, one of the most important pro-inflammatory cytokines, in experimental models of epilepsy [8, 9], suggest that the study of inflammatory factors involved in the pathogenesis of epileptic disorders can bring new insights into the molecular mechanisms and etiology of epilepsy.

The most commonly used animal model of human temporal lobe epilepsy [17, 18] is the model of excitotoxicity in rats after systemic or intracerebral administration of the excitotoxic kainic acid (KA), an analogue of glutamic acid [19]. We have focussed this review on data concerning the inflammatory processes involved in KA-induced behavioural changes and neurodegeneration, centered on the activation of the IL-1 system.

Kainic acid as model of epilepsy

Glutamate receptors mediate most of the excitatory neurotransmission in the mammalian central nervous system (CNS), and two distinct classes, ionotropic and metabotropic receptors have been identified (for review [20]). 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)-receptors. The kainate and AMPA-receptors represent the non-NMDA group of glutamate receptors.

Administration of KA in rats is known to induce a sequence of behavioural events, electroencephalographic (EEG) and body temperature changes, which are followed by neurodegeneration in specific brain regions [17, 21–25]. The KA-induced behavioural changes develop in several stages, that start with staring and movement arrest 5-15 min after administration, and are followed by masticatory movements, head nodding, myoclonic twitches of the head and "wet dog" shakes that start 15-30 min after administration with a duration of about 30 min. The behavioural pattern is completed in the following 4-5 hours by recurrent generalised tonic-clonic seizures of varying number and duration, starting about 1 h after injection of KA. This stage is associated with rearing, loss of postural control and increased salivation (for review see [26]).

The behavioural changes are closely correlated with typical changes in EEG [17] and changes in body temperature, which consist of hypothermia in the first 30-60 minutes after KA administration followed by a hyperthermic effect, lasting for 5 to 8 h [23–25]. These events are followed by neurodegeneration particularly in the hippocampus, entorhinal and piriform cortex, thalamus and amygdala [19, 27, 28]. A typical pattern of neurodegeneration occurs in the hippocampus where the CA3 pyramidal cells and interneurons in the hilus of dentate gyrus [21, 29] are the most vulnerable, followed by CA1 pyramidal cells [28, 30]. The pyramidal cells in the CA2 region and the granule cells of the dentate gyrus are the most resistant. This reproducible pattern of neurodegeneration can be induced by systemic, intrahippocampal, intra-amygdaloid or intracerebroventricular (i.c.v.) administration of KA, if the generalized motor seizures are maintained for a certain critical period [21, 31, 32]. It has been demonstrated that KA-induced neurodegeneration occurs both through necrotic and apoptotic mechanisms [33–35]. 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 [36], followed by release of the endogenous exci-

tatory amino acids, glutamate and aspartate [37, 38], that will further activate all types of glutamate receptors.

Systemic delivery of KA, subcutaneous or intraperitoneal (i.p.) is followed by higher 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 [28]. 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 [39], allowing the identification of 'non-responders'.

Interleukin-1 family

Cytokines are multifunctional, pleiotropic, low molecular weight proteins that play important roles in many physiological and pathological processes in the brain. They interact with specific receptors located on the cell membrane or with soluble receptors and function in an autocrine or paracrine manner.

The IL-1 family consists of three known ligands for the IL-1 receptors, type I (IL-1RI) and type II (IL-1RII), the agonists IL-1alpha (IL-1α) and IL-1beta (IL-1β), and the IL-1 receptor antagonist (IL-1ra). The binding of IL-1α and IL-1β to the signaling receptor (IL-1RI) requires the association of the receptor with an accessory protein (IL-1AcP), whereas binding to IL-1RII does not induce an intracellular response and has been called a "decoy" receptor since it binds the IL-1 agonists and prevents their access to the signaling IL-1RI, thereby blocking their activity (see Fig. 1).

The 2 isoforms, IL-1α and IL-1β, are 17 kDa proteins in their active forms and exert similar actions. They are secreted as 31 kDa precursors and need to be cleaved by specific enzymes to their mature forms. ProIL-1α is active also as a precursor [40] and it is cleaved to the mature form by the membrane-associated cysteine protease calpain [41], while IL-1β is inactive in the precursor form and becomes activated after cleavage by the intracellular interleukin-1β converting enzyme (ICE), now known to be a cysteine protease, caspase-1, with apoptotic properties [42] (see below). IL-1α and IL-1β have similar actions in the periphery, but it is believed that IL-1β has more powerful effects in the CNS [43].

IL-1 is induced in the brain upon KA administration

The IL-1 system has physiological functions in the CNS, suggested by the constitutive expression of all the members of the IL-1 family, albeit at very low levels, in the normal brain (for review see [44]). The involvement of the IL-1 system in the CNS during pathophysiological conditions is extensively documented, both with regard to infections and neurodegenerative disorders. The KA model for epilepsy has given new information regarding the inflammatory process and involvement of the IL-1 system in the brain. In this section, the findings on induction and regulation of IL-1β in the KA system are summarized.

The peripheral administration of KA in rats was shown to induce a transient and biphasic expression for IL-1β mRNA in several brain regions [10, 11, 45–47] (see Fig. 2A). *In situ* hybridisation histochemistry (ISHH) demonstrated a strong induction of IL-1β mRNA in the cerebral cortex, thalamus and hypothalamus at 1.5 h, 2.5 h and 3.5 h, respectively, after KA administration [10], whereas a lower, but earlier (1 h after KA administration), expression was observed in the hippocampus [10, 11]. A second peak of high expression for IL-1β mRNA was observed in the cortex, amygdala, thalamus and, to a lesser extent, in the hippocampus at 12 h after KA administration [11] (Fig 2A) (Fig 3). No expression was detected in the midbrain, pons-medulla and cerebellum while a weak expression was observed in the striatum [10]. At 24 h, IL-1β mRNA was detected only in the hippocampus and amygdala [10, 11].

Several studies have shown that KA-induced synthesis of IL-1 mRNA is indeed followed by translation into protein. IL-1β protein was detected by immunohistochemistry at 5 h after i.p. administration of KA [47] and the highest expression was observed at 12 h in the thalamus and amygdala, and at 24 h in the CA1 pyramidal cell layer in hippocampus, perirhinal and piriform cortex [47] (see

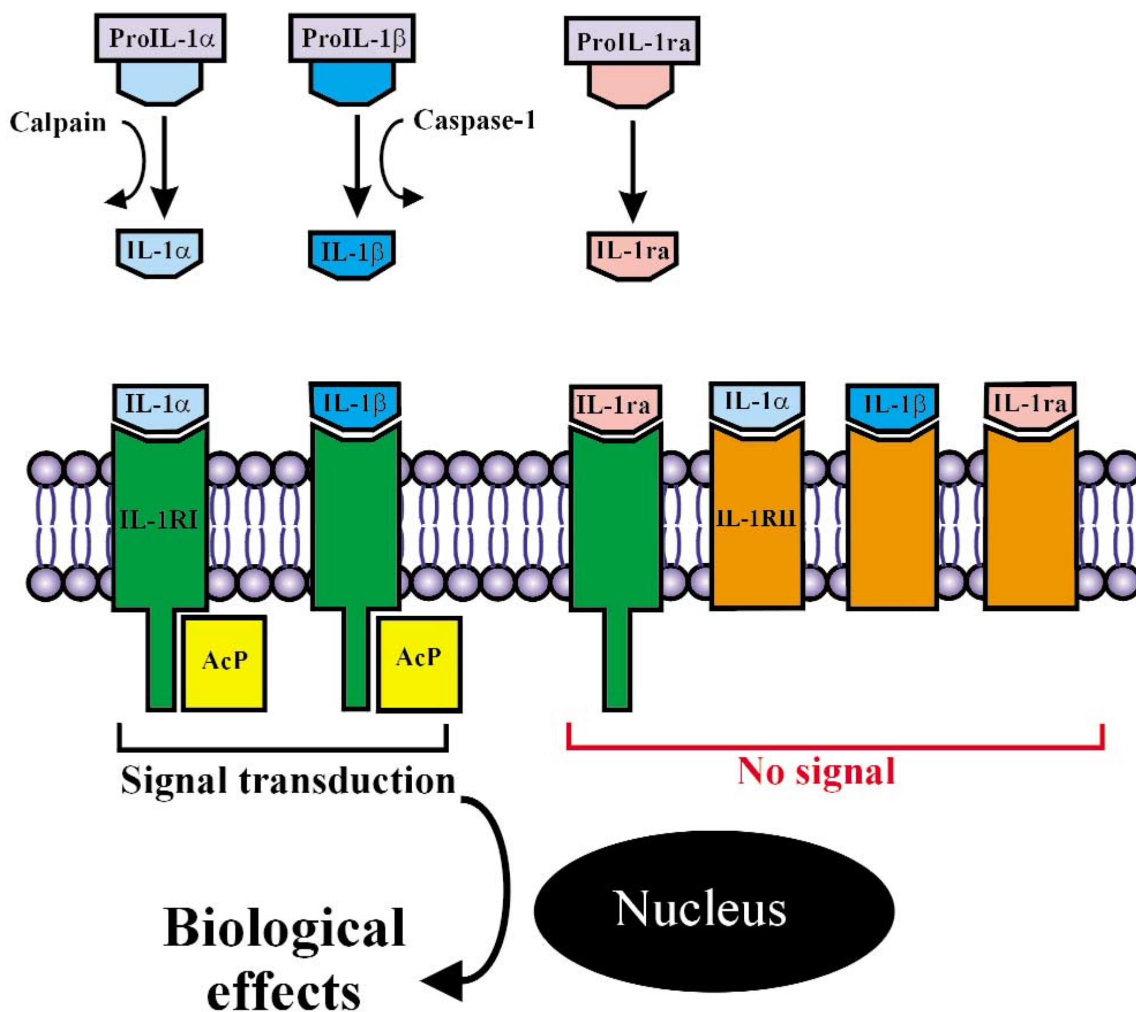

Fig. 1 Schematic drawing of signal transduction for the interleukin-1 system. The receptor agonists IL-1α and IL-1β are produced as inactive precursors. ProIL-1α is cleaved by calpain and proIL-1β is cleaved by caspase-1 to generate the mature bioactive forms. The binding of IL-1α and IL-1β to IL-1 receptor type I (IL-1RI) is followed by the attachment of IL-1R accessory protein (IL-1RAcP) to the receptor and this initiates the intracellular signal transduction. IL-1 receptor antagonist (IL-1ra) binds to IL-1RI without initiating an intracellular response and prevents the access of IL-1α and IL-1β to the receptor. IL-1RII lacks an intracellular domain and binding of either of the agonists or of IL-1ra fails to initiate an intracellular response.

Fig 2C). Intrahippocampal delivery of KA resulted in induction of IL-1β in the dentate gyrus after 3 h, while a more diffuse pattern of IL-1β-positive cells was observed one day later [9]. Using ELISA, a 16-fold increase in IL-1β was detected in the ipsilateral hippocampus after 24 h [9].

IL-1β has been shown in *in vitro* studies to be produced both by activated, amoeboid microglial cells and by activated astrocytes, and is one of the most powerful inducers of reactive astrogliosis, which consists of proliferation, de-differentiation and altered gene expression of the astrocytes [48]. In fact, astrocytes may be considered to be the effector cells that mediate many of the effects of IL-1β in the brain. The importance of IL-1β in the initiation of reactive astrogliosis was demonstrated in mice deficient in IL-1β in which the induction of glial fibrillary acidic protein (GFAP), an astrogliosis marker, did not occur upon corticotomy [49]. Immunohistochemical studies also showed that the source of IL-1β after KA administration is activated microglia, at least in the first hours after administration [9, 47].

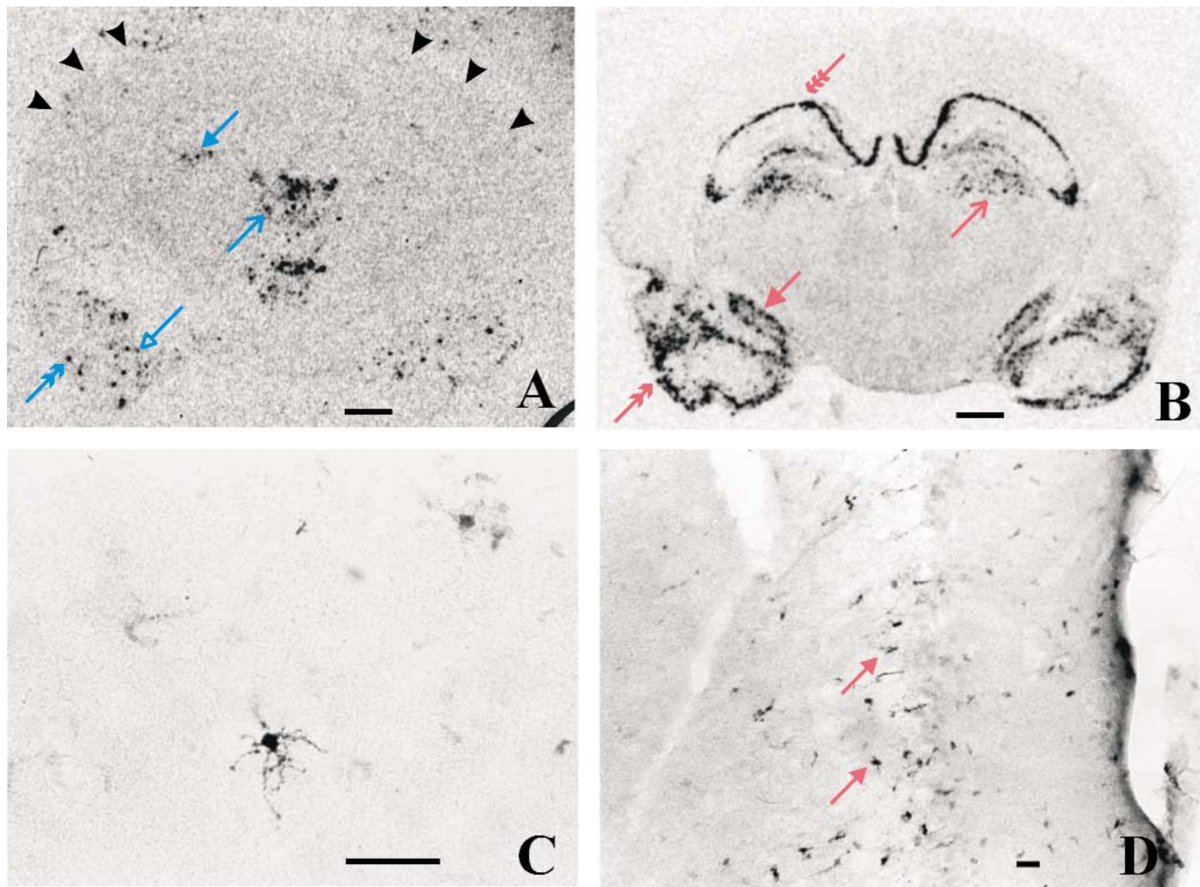

Fig. 2 Expression of IL-1β (A, C) and IL-1α (B, D) in the rat brain at 12 h (A) and 24 h (B – D), after intraperitoneal administration of kainic acid (KA). (A-B) Autoradiographs of sections of rat brain after *in situ* hybridisation histochemistry with radioactively labelled anti-sense cRNA probes for IL-1β (A) and IL-1ra (B). IL-1β (A) and IL-1ra (B) mRNA expression can be seen in the piriform cortex (double arrows, A-B), amygdala (unfilled arrow, A), thalamus (open arrows, A-B) the CA3 region of dorsal (filled arrow, A) and ventral hippocampus (filled arrow, B) and the CA1 region of hippocampus (triple arrow, B). The two groups of three arrows (A) indicate the limit of the cerebral cortex. Solid bars (A-B) indicate 1 mm. (C-D) Immunoperoxidase micrographs of sections of rat brain after incubation with antibodies to IL-1β (C) and IL-1ra (D), respectively. A couple of IL-1β immunoreactive microglia can be seen at high magnification (C), and many IL-1ra-positive microglial cells are observed in low magnification, in the piriform cortex (arrows on some cells in D). Solid bars (C-D) indicate 50 µm.

Several classes of drugs have been shown to decrease and/or block the KA-induced expression of IL-1β mRNA in the rat brain. Peripheral administration of dizocilpine and R-(CPP), a non-competitive and a competitive NMDA-receptor antagonist, respectively, blocked in a dose-dependent manner the expression of IL-1β in the rat brain, suggesting the involvement of this group of glutamate receptors in the initiation of cytokine synthesis in the brain [50]. Recently, it was confirmed that microglial cells have functional glutamate receptors of the AMPA-type [51], suggesting that the induction of IL-1β by KA is mainly indirect through release of endogenous glutamate [38]. Furthermore, induction of IL-1β mRNA by i.p. injection of KA was blocked by diazepam, a γ-amino butyric acid (GABA)-receptor agonist, as well as of dexamethasone [10]. However, in rats given intrahippocampal injection of KA, there was an increase in both mRNA and protein expression for the pro-inflammatory cytokines IL-1α, IL-1β and tumour necrosis factor-alpha (TNF-α), unlike the general anti-inflammatory roles of glucocorticoids in the periphery [52].

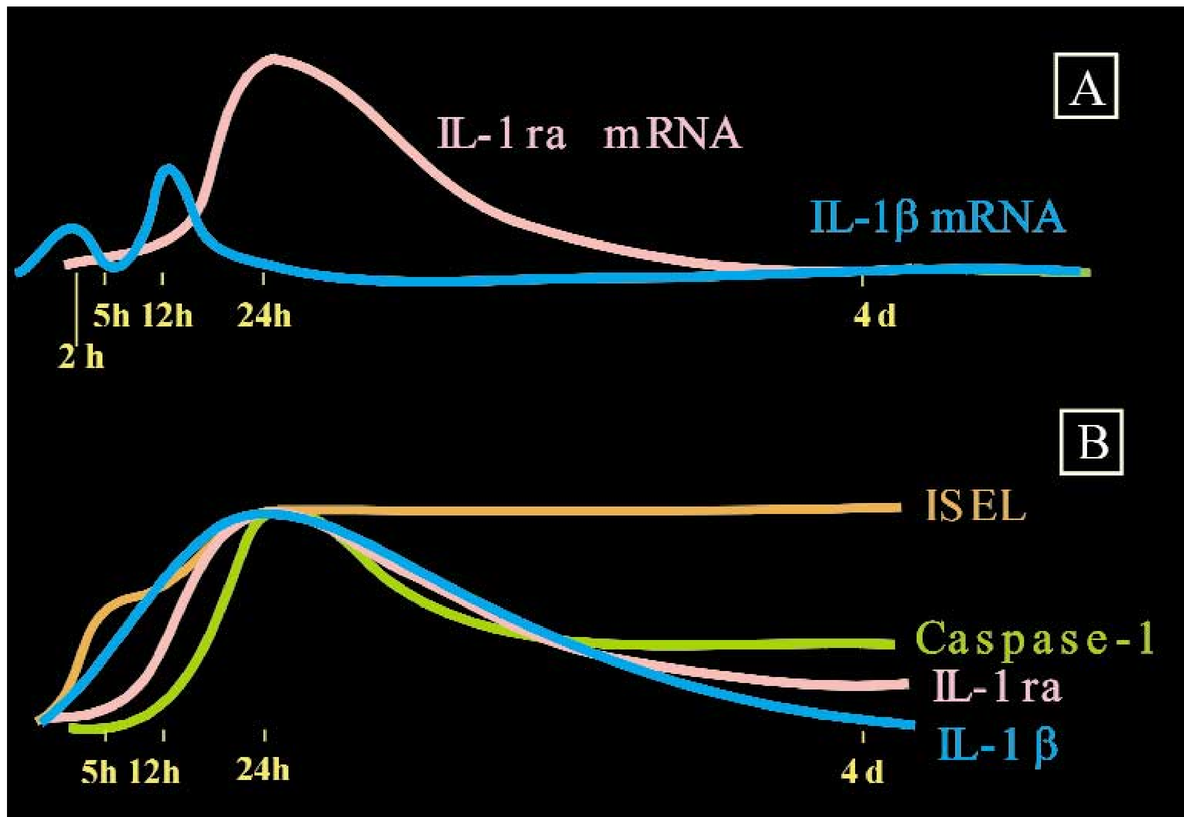

Fig. 3 Temporal expression of mRNA (A) for IL-1ß and IL-1ra and of the proteins (B) IL-1β, IL-1ra and caspase-1, in the piriform cortex after peripheral administration of 10 mg/kg kainic acid in rat. ISEL represents the time course for in situ end labelled neurons (see ref. [47]).

IL-1β has permissive effects on KA-induced neurodegeneration

Intrahippocampal injection of human recombinant (hr) IL-1β significantly increased the duration of electroencephalographic seizures produced by local application of KA, without modifying the time for the seizures onset or the number of seizures [9]. This proconvulsant effect was blocked both by IL-1ra, suggesting a receptor-mediated action of exogenously administered IL-1β, and by (R)-CPP demonstrating an excessive activation of NMDA-receptors [8, 9]. Glutamate uptake by the astrocytes is a well-known mechanism that maintains low extracellular levels of glutamate and promotes efficient inter-neuronal signalling in normal brain, and the same process is considered a neuroprotective mechanism during neuroinflammation. IL-1β impairs the astrocytic glutamate uptake both in primary cultures from rat postnatal hippocampus [53] and in human cortical astrocytes [54], producing a dose-dependent inhibition of mRNA expression for the glutamate transporter protein through a nitric oxide (NO)-dependent mechanism [53, 54]. This effect is observed also for TNF-α and is blocked by the anti-inflammatory cytokines interferon-β (IFN-β) and IL-1ra [54]. It is possible that the observed proconvulsant effect of IL-1β is the result of an increase in glutamate available for the activation of NMDA- and non-NMDA-receptors due to the inhibition of its uptake by astrocytes.

IL-1β has been shown to induce the activation of inducible NO synthase (iNOS), leading to the generation of NO and an increased release of glutamate and GABA *in vivo* [55, 56]. GABA, but not glutamate, release was found to be dependent on iNOS activation, and the iNOS-producing cells were identified as activated microglia [55] and astrocytes [56].

Resting astrocytes exert a down-regulatory action on microglial functions through the release of transforming growth factor-beta, probably serv-

ing as an important endogenous protective mechanism against excessive peroxynitrite production [57]. Astrocytes, forced to undergo secondary activation due to the action of IL-1 released from activated microglia, lose their ability to inhibit the production of reactive oxygen species by microglia [58]. Coupled with an increased iNOS-production in activated astrocytes, IL-1 may thus induce a significant increase in oxidative stress.

Hypothermia has been shown to mediate neuroprotection against KA-induced neurotoxicity [59, 60], and hyperthermia was shown to exacerbate the deleterious effects of KA [59]. IL-1β has a well-known pyrogenic effect reponsible for an increase in body temperature, and administration of IL-1β was shown to exacerbate excitotoxic brain damage [61]. In rats, KA has a biphasic effect on core temperature after i.c.v. [23] or i.p. administration [24], *i.e.* an early hypothermia in the first hour after injection, followed by a persistent hyperthermic effect lasting 3 to 6 h. The initial hypothermia is dependent on dopamine, while the hyperthermic effect requires an intact serotoninergic projection [23]. The NMDA-receptor antagonist dizocilpine did not inhibit KA-induced changes in body temperature [25]. Instead, the hypothermic effect of KA was enhanced, while the ensuing hyperthermia was not influenced by dizocilpine. Since dizocilpine was shown to cause a dose-dependent reduction in the KA-induced expression of IL-1β mRNA in the rat brain and a complete block by the highest dose, 5 mg/kg dizocilpine (i.p.) administered 1 h prior to KA [50], it seems less likely that IL-1β is involved in the KA-induced hyperthermia.

IL-1β has potential neuroprotective effects against KA-induced neurotoxicity

IL-1β can itself be regarded as a growth factor and evidence have been provided regarding its prenatal detection in the brain after the appearance of amoeboid microglia and its involvement in the differentiation, proliferation and survival of astrocytes and neurons during development of the mammalian brain [62, 63]. Some of the potential neuroprotective roles of IL-1β could be related to an ability to stimulate the production of certain neurotrophic factors, such as nerve growth factor (NGF).

The KA-induced expression of NGF mRNA [64] and protein [65] in the rat brain seems to be mediated by IL-1β. Thus, IL-1β stimulates the expression of NGF mRNA in astrocytes both *in vitro,* in primary cultures, and *in vivo,* after i.c.v. administration [66]. This effect may be due to a positive feedback mechanism, since i.c.v. injection of IL-1β stimulated the expression of both IL-1β and NGF mRNA in the hippocampus [66], and IL-1ra inhibits NGF expression in a model of traumatic brain injury [67].

It has been reported that TNF-α can release NGF from cultured astrocytes synergistically with IL-1β [68]. TNF-α is assumed to act synergistically with IL-1β in inducing NGF mRNA expression, since the mRNA for both cytokines was induced in several brain regions with comparable latencies after KA administration [45]. Pretreatment of primary cortical neuronal cultures with 0.5 μg/ml hrIL-1β for 24 h protected them against neuronal death induced by exposure to KA, glutamate, NMDA or AMPA, an effect that seemed to be mediated by NGF [69]. Exposure of the neuronal cultures to 100 μg/ml hrIL-1β for 72 h resulted in neurotoxicity that was blocked by co-application of IL-1ra [69]. These findings demonstrate that IL-1β may have both neuroprotective and neurotoxic effects, depending on the dosage, the duration of exposure and the presence of glial cells.

Another growth factor induced by IL-1β is ciliary neurotrophic factor (CNTF), a survival factor for both neurons and oligondendrocytes. The stimulation of CNTF synthesis was lacking in astrocytes from mice deficient in IL-1β [70].

It has been suggested that IL-1 modulates both inhibitory and excitatory neurotransmitter function in the brain in a neuroprotective manner, at least in view of the experimental results obtained *in vitro*. Thus, IL-1β was shown to reduce glutamate release in the hippocampus [71], enhance the effect of GABA in cortical synaptosomes and decrease calcium responses to NMDA and glycine in cultured chick cortical neurons [72]. These experiments suggest that IL-1β, at least in cell culture paradigms, can restrain the effects of an excessive activation of glutamate receptors.

Alpha-2-macroglobulin (α2M) is an acute-phase protein produced mainly in the liver during acute inflammation and contributing to the host defense

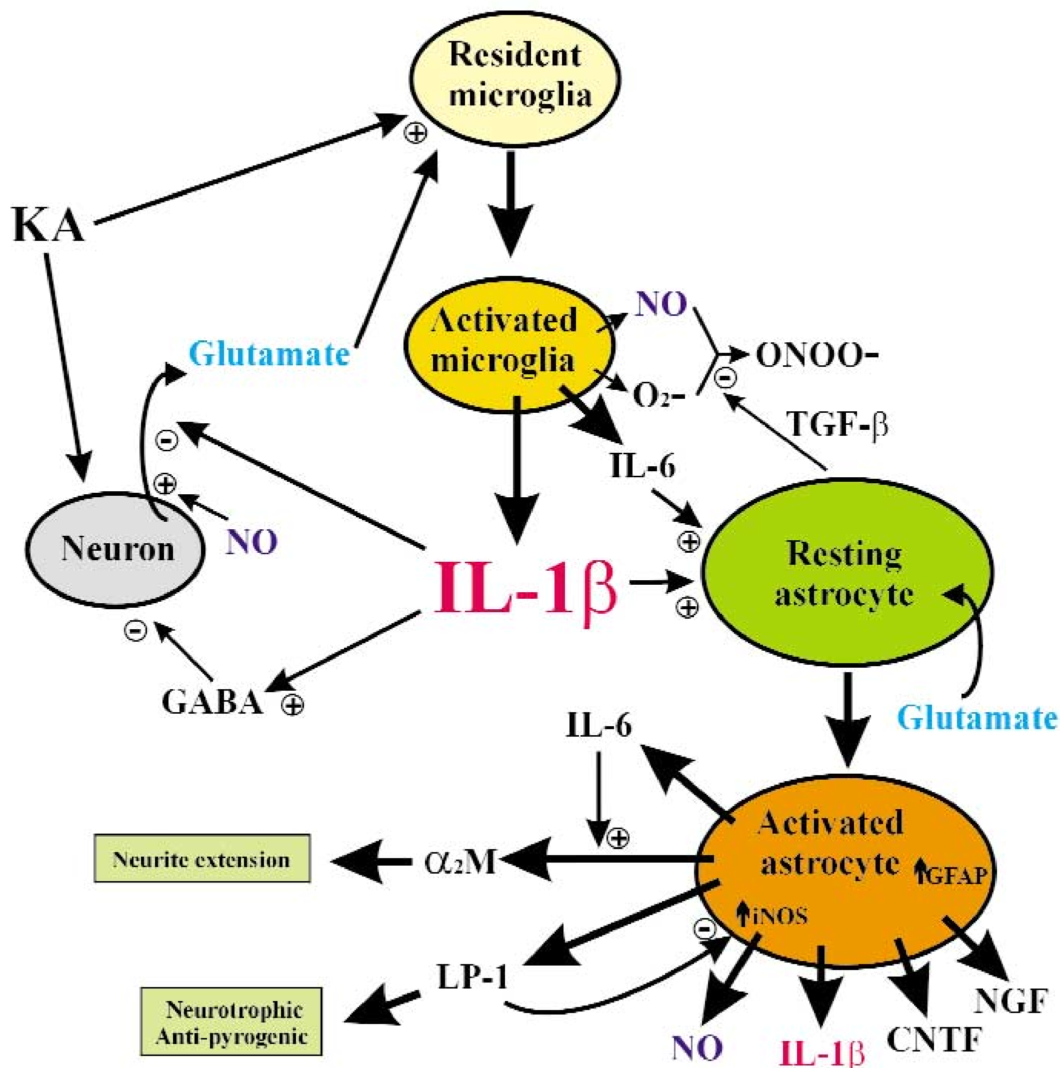

Fig. 4. Possible roles of IL-1β in KA-induced neurotoxicity. KA activates the neuronal receptors inducing a secondary release of glutamate that can bind to non-NMDA receptors on microglial cells and stimulate their activation including the induction of synthesis and release of IL-1β. It is possible that KA also activates microglial cells directly. IL-1β potently stimulates astroglial activation, which is characterised by the potentially neurotoxic effects: impaired glutamate uptake, reduction in the capacity to counteract the formation of peroxynitrite through a mechanism of negative feedback involving TGF-β, release of IL-1β, and increased synthesis and activity of iNOS, resulting in release of NO. Activated astrocytes also have potential neuroprotective effects through the synthesis of neurotrophic factors such as α_2-macroglobulin (α2M) through a IL-6 dependent mechanism, and lipocortin-1 (LP-1) (see text for details). IL-1β itself may be neuroprotective by inhibiting the release of glutamate and stimulating the release of GABA from neurons.

as a protease inhibitor [73]. α2M synthesis was also demonstrated to occur in cultured rat astrocytes [74] and to be controlled by IL-6 [75]. It was shown to promote neurite outgrowth [76]. Interestingly, α2M mRNA was induced in the rat brain upon peripheral KA administration with the highest expression observed after 3 days [77]. The induction of inflammatory factors upon peripheral administration of KA occurs in a sequential manner, starting with IL-1β, that is followed by IL-6 [10]. The finding that IL-6 is induced in cultured rat astrocytes [78] and in astrocytoma cell line [79] by

addition of IL-1β, suggests the involvement of IL-1β in a sequential activation of IL-6 upon KA administration. Furthermore, IL-1β may facilitate the synthesis of α2M through the synthesis of IL-6 and thereby indirectly have a neuroprotective role, since an impaired gliosis associated with increased oxidative stress and accentuated apoptotic neuronal death [49] was demonstrated in IL-6 KO mice injected with KA. This suggests that IL-1β acts synergistically with IL-6 in producing an adequate activation of glial cells during excitotoxic challenges, and that an impaired gliosis facilitates neuronal death.

Lipocortin-1 (LP-1) is an anti-inflammatory factor that diminishes prostaglandin synthesis secondary to the inhibition of phospholipase A_2 (PLA_2). LP-1 also has anti-pyrogenic and neurotrophic effects as well as inhibiting the synthesis of iNOS and NO [80]. The expression of LP-1 [81] was shown to be dose- and time-dependently increased in cultured rat astrocytes upon exposure to IL-1β. Inhibition of the mitogen-activated protein kinase (MAPK) pathway and of PLA_2 or protein synthesis significantly decreased this effect [81].

Interleukin-1 receptor antagonist

IL-1ra is the first described, naturally occurring receptor antagonist of any cytokine or hormone-like molecule [82] that binds to the signalling receptor (IL-1RI) with the same affinity as the agonists (IL-1α and β) without inducing any intracellular response, and acting as a selective, competitive antagonist of agonist (IL-1β)-induced actions [83].

Peripheral administration of KA in rats has been shown to induce a transient expression of IL-1ra mRNA in the hippocampus, thalamus and amygdala, the piriform, perirhinal and entorhinal cortex, and to a lesser extent in the hypothalamus and the parietal and temporal cortex [84]. The IL-1ra mRNA expression was closely related to the extent and distribution of neurodegeneration and the distribution of IL-1β mRNA. It was first detected 5 h after KA administration and the strongest signal was seen after 24 h (Fig 2B). Similarly to IL-1β mRNA, microglial cells are the major source of the KA-induced expression of IL-1ra mRNA [84]. Translation of the KA-induced expression of IL-1ra mRNA into functional protein has been demonstrated with immunohistochemical methods [47]. The IL-1ra protein expression (Fig. 2D) was delayed with several hours, but occurred in the same brain regions as IL-1β [11, 47]. The delay in IL-1ra synthesis with regard to IL-1β may have a functional significance. IL-1β has potential beneficial effects (see above) such as the induction of the NGF mRNA, which has been shown to occur several hours later than IL-1β mRNA after KA administration (Fig 3) [85]. If the action of IL-1β would have been blocked from the beginning, the potential neuroprotective effects would not occur. In this regard, it has been shown that the protective effects obtained by antagonising IL-1β-mediated activity were not the result of maximal inhibition [69, 86]

Neuroprotective effects of IL-1ra have been shown in different models of neurodegeneration. [4, 86–88]. In the case of excitotoxic models, i.c.v. administration of hrIL-1ra, given 10 min before and 10 min after the administration of KA (i.p.), selectively protected the neurons in the CA1 and CA3 regions of the hippocampus and in the dorsal thalamic nuclei, against KA-induced neurodegeneration. However, it did not have any effects on seizures nor on physiological parameters such as blood pressure, blood gases or body and hippocampal temperature [86]. The neuroprotective effect of the IL-1ra observed in this study can be explained as an increased resistance of the neurons to excitotoxic damage, since the seizure parameters were not modified. A reduced activation of astrocytes, assessed by GFAP-staining, was correlated with the neuroprotection. However, the highest dose of IL-1ra, 40 μg, did not reduce or modify the KA-induced neuronal cell death and astrocyte activation [86], suggesting that the neuroprotective effect of IL-1ra is not completely depending on blockade of IL-1 receptor mediated activity.

If we consider that binding of IL-1ra to the receptor does not transduce an intracellular response, the lack of an observable effect for the dose of 40 μg suggests that the neuroprotective effect of the lowest and most efficient dose (10 μg) was achieved with an incomplete blockade of IL-1. Indirectly, one might conclude that a complete blockade of IL-1 activity is not beneficial.

A lower dose of hrIL1-ra (0.1 μg) given i.c.v.10 min before and 10 min after intrahippocampal application of KA significantly decreased both the number and the duration of seizures [8]. This sug-

gests that an optimal level of IL-1β activity should be achieved in order to obtain a maximal neuroprotective effect, neither an excess nor a high blocking of IL-1β activity being of benefit [8, 86]. Other explanations for the differences encountered in these experiments with regard to the effect of IL-1ra on seizure activity could rely on the different experimental design, such as the i.p. [86] or intrahippocampal [8] delivery of KA or the differences in the susceptibility to seizures of the strain of rats used. It has been shown that Wistar rats, which were not protected against seizure activity [86], are more susceptible then Sprague-Dawley rats, with regard to the convulsant effect of KA [89].

The expression of IL-1ra in the rat brain after i.p. administration of 10 mg/kg KA was dose-dependently blocked by competitive and non-competitive NMDA-receptor antagonists, in a similar manner to the blockade of IL-1β, suggesting that microglial synthesis of cytokines is regulated by NMDA-receptor activation. The occurrence of functional AMPA-kainate subtypes of glutamate receptors has recently been demonstrated on cortical rat microglia [51]. KA-induced responses in rat microglial cells were predominantly mediated by AMPA-preferring receptors [51]. These findings suggest the induction of IL-1ra (as of IL-1β) by KA is indirect through endogenous release of glutamate acting on the non-NMDA-receptors. However, it is not yet clear whether the synthesis of IL-1ra is stimulated directly by glutamate receptor activation or via the induced synthesis of IL-1β, that in this way induces the synthesis of its own antagonist as seen in the peripheral immune response [90].

NMDA-receptor antagonists were able to dose-dependently block KA-induced seizures and neurodegeneration [47, 91, 92], whereas KA-induced changes in body temperature seem to be less dependent on NMDA-receptor activation [25].

IL-1 receptors

The signalling IL-1RI is expressed constitutively on neuronal cells in several brain regions in the murine normal brain including CA3 and CA4 pyramidal neurons, while the expression on non-neuronal cells was restricted to cells in the leptomeninges and in blood vessels such as perivascular microglia [93–95]. The production of IL-1RI mRNA and protein was stimulated by IL-1β and TNF-α in cultured astrocytes and hippocampal neurons and secondary to a brain insult *in vivo* [93]. The constitutive expression of IL-1RI on hippocampal neurons and its enhanced expression on glial cells suggests that IL-1β has important functional roles both in physiological (for review see [96]) and pathological conditions.

It is known that signal transduction for the IL-1 system is extremely efficient. Less than 10% of the approximate 200 IL-1RI molecules present on the cell surface have to be activated for inducing a biological response (for review see [97]). Doses of IL-1ra that are 10^2 to 10^3-fold higher than the concentration of IL-1β are required to block IL-1β activities [98]. A high density of IL-1RI has been demonstrated in granule cells of the dentate gyrus [99], *i.e.* cells that are known to be resistant to KA. The presence of IL-1RI on resistant cells would be quite efficient from a functional point of view, since this facilitates the persistence of the action of IL-1β in the brain for a long time following administration of KA.

KA stimulates the expression of IL-1RII mRNA in the rat brain, mostly in neurons [100]. The induction of IL-1RII mRNA can be detected 8 h in the amygdala and dentate gyrus after KA administration and after 12 and/or 24 h in the same and in other brain regions such as the hippocampus, piriform cortex, hypothalamus and thalamus. No positive cells were identified at 4 h or later then 48 h after KA administration. It has been suggested that IL-1RII, that lacks an intracellular domain, has a role as a "decoy" receptor, *i.e.* that binds IL-1 and blocks its activity by preventing it from reaching the signalling IL-1RI, rather then transducing an intracellular signal [101]. Since IL-1RII does not transduce an intracellular response, the induction of IL-1RII expression by KA may have a modulatory effect on IL-1β activity. The binding of IL-1β to IL-1RII reduces the availability of the cytokine for IL-1RI, the receptor that transduces the intracellular responses of IL-1β.

Caspase-1

Caspase-1 is an intracellular cysteine protease that cleaves the precursor protein proIL-1β at specific aspartic residues and generates the active form, IL-1β. Induction of caspase-1 in the rat brain after

peripheral administration of KA was not as extensive as that of IL-1β or IL-1ra [11, 47]. The highest expression for caspase-1 mRNA could be seen in the amygdala at 12 h after administration, while a modest non-significant increase was observed in hippocampus at 12 and 24 h [11]. The KA-induced expression of caspase-1 immunoreactivity was observed in microglial cells, particularly 24 h after the administration, when numerous positive cells were identified in the amygdala, the piriform, perirhinal and entorhinal cortex [47].

It can be observed that the induction of IL-1β mRNA occurred before the caspase-1 mRNA induction. It was suggested that the basal level of caspase-1 activity might be sufficient for the cleavage of IL-1β or that other enzymes may be responsible for the cleavage of proIL-1β during the first 24 h after KA administration [11].

Concluding remarks

All of the members of the IL-1 family are involved in different ways in the KA-induced changes in the brain. The effects of IL-1β can be regarded as modulatory since both neurotoxic and neuroprotective actions have been described for this cytokine, depending on the dosage and the experimental conditions. In the majority of cases, the neurotoxicity of IL-1β was demonstrated in *in vivo* experiments, in circumstances where an increased excitotoxicity was induced by the co-administration of IL-1β. A neuroprotective effect, especially against glutamate excitoxicity, was observed *in vitro* [72]. IL-1ra has been shown to have a neuroprotective role *in vivo*, where blocking IL-1R-mediated activity in a dose-dependent manner blocked the neurotoxic effects of IL-1β. *In vitro*, blocking IL-1R-mediated actions by IL-1ra had neuroprotective effects. The occurrence of 2 types of receptors for IL-1β with different functional properties, that are up-regulated by the cytokine itself in the context of KA-induced excitotoxicity, contribute to the complexity of the pathophysiological mechanisms involved in this model of neurodegeneration. This emphasizes the difficulty to interprete, in an integrative way, the complex effects of IL-1β in the brain, which would be necessary in order to manipulate its activity for obtaining a definite neuroprotective effect.

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References

1. **Schneider H., Pitossi F., Balschun D., Wagner A., del Rey A., Besedovsky H.O.,** A neuromodulatory role of interleukin-1beta in the hippocampus, *Proc. Natl. Acad. Sci.,* **95:** 7778-7783, 1998
2. **Opp M.R., Krueger J.M.,** Interleukin 1-receptor antagonist blocks interleukin 1-induced sleep and fever, *Am. J. Physiol.,* **260:** R453-457, 1991
3. **Berkenbosch F., van Oers J., del Rey A., Tilders F., Besedovsky H.,** Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1, *Science,* **238:** 524-526, 1987
4. **Tehranian R., Andell-Jonsson S., Beni S.M., Yatsiv I., Shohami E., Bartfai T., Lundkvist J., Iverfeldt K.,** Improved recovery and delayed cytokine induction after closed head injury in mice with central overexpression of the secreted isoform of the interleukin-1 receptor antagonist, *J. Neurotrauma,* **19:** 939-951, 2002
5. **Loddick S.A., Wong M.L., Bongiorno P.B., Gold P.W., Licinio J., Rothwell NJ.,** Endogenous interleukin-1 receptor antagonist is neuroprotective, *Biochem. Biophys. Res. Commun.,* **234:** 211-215, 1997
6. **Lawrence C.B., Allan S.M., Rothwell N.J.,** Interleukin-1beta and the interleukin-1 receptor antagonist act in the striatum to modify excitotoxic brain damage in the rat, *Eur. J. Neurosci.,* **10:** 1188-1195, 1998
7. **Holmes C., El-Okl M., Williams A.L., Cunningham C., Wilcockson D., Perry V.H.,** Systemic infection, interleukin 1beta, and cognitive decline in Alzheimer's disease, *J. Neurol. Neurosurg. Psychiatry,* **74:** 788-789, 2003
8. **Vezzani A., Moneta D., Richichi C., Aliprandi M., Burrows S.J., Ravizza T., Perego C., De Simoni M.G.,** Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis, *Epilepsia,* **43:** 30-35, 2002
9. **Vezzani A., Conti M., De Luigi A., Ravizza T., Moneta D., Marchesi F., De Simoni M.G.,** Interleukin-1beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: functional evidence for enhancement of electrographic seizures, *Neuroscience,* **19:** 5054-5065, 1999
10. **Minami M., Kuraishi Y., Yamaguchi T., Nakai S., Hirai Y., Satoh M.,** Convulsants induce interleukin-1 beta messenger RNA in rat brain, *Biochem. Biophys. Res. Commun.,* **171:** 832-837, 1990

11. **Eriksson C., Tehranian R., Iverfeldt K., Winblad B., Schultzberg M.,** Increased expression of mRNA encoding interleukin-1β and caspase-1, and the secreted isoform of interleukin-1 receptor antagonist in the rat brain following systemic kainic acid administration, *J. Neurosci. Res.,* **60:** 266-279, 2000
12. **Anisman H., Merali Z.,** Cytokines, stress, and depressive illness, *Brain Behav. Immun.,* **16:** 513-524, 2002
13. **Aarli J.A.,** Epilepsy and the immune system, *Arch. Neurol.,* **57:** 1689-1692, 2000
14. **Peltola J., Hurme M., Miettinen A., Keranen T.,** Elevated levels of interleukin-6 may occur in cerebrospinal fluid from patients with recent epileptic seizures, *Epilepsy Res.,* **31:** 129-133, 1998
15. **Haspolat S., Mihci E., Coskun M., Gumuslu S., Ozben T., Yegin O., Ozbenm T.,** Interleukin-1beta, tumor necrosis factor-alpha, and nitrite levels in febrile seizures, *J. Child Neurol.,* **17:** 749-751, 2002
16. **Peltola J., Palmio J., Korhonen L., Suhonen J., Miettinen A., Hurme M., Lindholm D., Keranen T.,** Interleukin-6 and interleukin-1 receptor antagonist in cerebrospinal fluid from patients with recent tonic-clonic seizures, *Epilepsy Res.,* **41:** 205-211, 2000
17. **Ben-Ari Y., Tremblay E., Ottersen O.P.,** Injections of KA into the amygdaloid complex of the rat: an electrographic, clinical and histological study in relation to the pathology of epilepsy, *Neuroscience,* **5:** 515-528, 1980
18. **Nadler J.V.,**. Kainic acid as a tool for the study of temporal lobe epilepsy, *Life Sci.,* **29:** 2031-2042, 1981
19. **Olney J.W., Rhee V., Ho O.L.,** Kainic acid: a powerful neurotoxic analogue of glutamate, *Brain Res.,* **177:** 507-512, 1974
20. **Ozawa S., Kamiya H., Tsuzuki K.,**. Glutamate receptors in the mammalian central nervous system, *Prog. Neurobiol.,* **54:** 581-618, 1998
21. **Ben-Ari Y.,** Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy, *Neuroscience,* **14:** 375-403, 1985
22. **Zagrean L., Varlas V., Oprica M., Munteanu A.M., Oltenschi C., Voicu T.,** EEG study of kainate-induced epilepsy in non-anaesthetized freely moving rats**,** *Rom. J. Physiol.,* **30:** 115-118, 1993
23. **Turski L., Kleinrok Z.,** Effects of kainic acid on body temperature of rats: role of catecholaminergic and serotonergic systems, *Psychopharmacology,* **71:** 35-39, 1980
24. **Oprica M., Forslin Aronsson Å., Post C., Eriksson C., Ahlenius S., Popescu L.M., Schultzberg M.,** Effects of alpha-MSH on kainic acid induced changes in core temperature in rats, *Peptides,* **23:** 143-149, 2002
25. **Ahlenius S., Oprica M., Eriksson C., Winblad B., Schultzberg M.,** Effects of kainic acid on rat body temperature: unmasking by dizocilpine, *Neuropharmacology,* **43:** 28-35, 2002
26. **Sperk G.,** Kainic acid seizures in the rat, *Prog. Neurobiol.,* **42:** 1-32, 1994
27. **Coyle J.T.,** Neurotoxic action of kainic acid, *J. Neurochem.,* **41:** 1-11, 1983
28. **Sperk G., Lassmann H., Baran H., Seitelberger F., Hornykiewicz O.,**. Kainic acid-induced seizures: dose-relationship of behavioral, neurochemical and histopathological changes, *Brain Res.,* **338:** 289-295, 1985
29. **Tauck D.L., Nadler J.V.,** Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats, *J. Neurosci.,* **5:** 1016-1022, 1985
30. **Phelps S., Mitchell J., Wheal H.V.,** Changes to synaptic ultrastructure in field CA1 of the rat hippocampus following intracerebroventricular injection of kainic acid, *Neuroscience,* **40:** 687-699, 1991
31. **Tremblay E., Ottersen O.P., Rovira C., Ben-Ari Y.,** Intra-amygdaloid injections of kainic acid: regional metabolic changes and their relation to the pathological alterations, *Neuroscience,* **8:** 299-315, 1983
32. **Tanaka T., Tanaka S., Fujita T., Takano K., Fukuda H., Sako K., Yonemasu Y.,** Experimental complex partial seizures induced by a microinjection of kainic acid into limbic structures, *Prog. Neurobiol.,* **38:** 317-334, 1992
33. **Ferrer I., Martin F., Serrano T., Reiriz J., Perez-Navarro E., Alberch J., Macaya A., Planas A.M.,** Both apoptosis and necrosis occur following intrastriatal administration of excitotoxins, *Acta Neuropathol.,* **90:** 504-510, 1995
34. **Simonian N.A., Getz R.L., Leveque J.C., Konradi C., Coyle J.T.,** Kainic acid induces apoptosis in neurons, *Neuroscience,* **75:** 1047-1055, 1996
35. **Popescu B.O., Oprica M., Sajin M., Stanciu C.L., Bajenaru O., Predescu A., Vidulescu C., Popescu L.M.,** Dantrolene protects neurons against kainic acid induced apoptosis in vitro and in vivo, *J. Cell. Mol. Med.,* **6:** 555-569, 2002
36. **Krnjevic K., Morris M.E., Reiffenstein R.J.,** Changes in extracellular Ca2+ and K+ activity accompanying hippocampal discharges, *Can. J. Physiol. Pharmacol.,* **58:** 579-582, 1980
37. **Ferkany J.W., Zaczek R., Coyle J.T.,** Kainic acid stimulates excitatory amino acid neurotransmitter release at presynaptic receptors, *Nature,* **298:** 757-759, 1982
38. **Köhler C., Schwarcz R., Fuxe K.,** Intrahippocampal injections of ibotenic acid provide histological evidence for a neurotoxic mechanism different from kainic acid, *Neurosci. Lett.,* **15:** 223-228, 1979
39. **Racine R.J.,** Modification of seizure activity by electrical stimulation. II. Motor seizure, *Electroencephalogr. Clin. Neurophysiol.,* **32:** 281-294, 1972
40. **Mosley B., Urdal D.L., Prickett K.S., Larsen A., Cosman D., Conlon P.J., Gillis S., Dower S.K.,** The interleukin-1 receptor binds the human interleukin-1 alpha precursor but not the interleukin-1 beta precursor, *J. Biol. Chem.,* **262:** 2941-2944, 1987
41. **Kobayashi Y., Yamamoto K., Saido T., Kawasaki H., Oppenheim J.J., Matsushima K.,** Identification of calcium-activated neutral protease as a processing enzyme of human interleukin-1 alpha, *Proc. Natl. Acad. Sci.,* **87:** 5548-5552, 1990
42. **Black R.A., Kronheim S.R., Cantrell M., Deeley M.C., March C.J., Prickett K.S., Wignall J., Conlon P.J., Cosman D., Hopp T.P.,** Generation of biologically active interleukin-1 beta by proteolytic cleavage of the inactive precursor, *J. Biol. Chem.,* **263:** 9437-9442, 1988
43. **Katsuura G., Gottschall P.E., Dahl R.R., Arimura A.,** Interleukin-1 beta increases prostaglandin E2 in rat astrocyte cultures: modulatory effect of neuropeptides, *Endocrinology,* **124:** 3125-3127, 1989

44. **Vitkovic L., Bockaert J., Jacque C.,** "Inflammatory" cytokines: neuromodulators in normal brain?, *J. Neurochem.,* **74:** 457-471, 2000
45. **Minami M., Kuraishi Y., Satoh M.,** Effects of kainic acid on messenger RNA levels of IL-1 beta, IL-6, TNF alpha and LIF in the rat brain, *Biochem. Biophys. Res. Commun.,* **176:** 593-598, 1991
46. **Yabuuchi K., Minami M., Katsumata S., Satoh M.,** In situ hybridization study of interleukin-1 beta mRNA induced by kainic acid in the rat brain, *Mol. Brain. Res.,* **20:** 153-161, 1993
47. **Eriksson C., Van Dam A-M., Lucassen P.J., Bol J.G.J.M., Winblad B., Schultzberg M.,** Immunohistochemical localization of interleukin-1β, interleukin-1 receptor antagonist and interleukin-1β converting enzyme/caspase-1 in the rat brain after peripheral administration of kainic acid, *Neuroscience,* **93:** 915-930, 1999
48. **Giulian D., Baker T.J., Shih L.C., Lachman L.B.,** Interleukin 1 of the central nervous system is produced by ameboid microglia, *J. Exp. Med.,* **164:** 594-604, 1986
49. **Herx L.M., Yong V.W.,** Interleukin-1 beta is required for the early evolution of reactive astrogliosis following CNS lesion, *J. Neuropathol. Exp. Neurol.,* **60:** 961-971, 2001
50. **Eriksson C., Zou L.P., Ahlenius S., Winblad B., Schultzberg M.,**. Inhibition of kainic acid induced expression of interleukin-1β and interleukin-1 receptor antagonist mRNA in the rat brain by NMDA receptor antagonists, *Mol. Brain Res.,* **85:** 103-113, 2000
51. **Noda M., Nakanishi H., Nabekura J., Akaike N.,** AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia, *J. Neurosci.,* **20:** 251-258, 2000
52. **Dinkel K., MacPherson A., Sapolsky R.M.,** Novel glucocorticoid effects on acute inflammation in the CNS, *J. Neurochem.,* **84:** 705-716, 2003
53. **Ye Z.C., Sontheimer H.,** Cytokine modulation of glial glutamate uptake: a possible involvement of nitric oxide, *Neuroreport,* **7:** 2181-2185, 1996
54. **Hu S., Sheng W.S., Ehrlich L.C., Peterson P.K., Chao C.C.,** Cytokine effects on glutamate uptake by human astrocytes, *Neuroimmunomodulation,* **7:** 153-159, 2000
55. **Casamenti F., Prosperi C., Scali C., Giovannelli L., Colivicchi M.A., Faussone-Pellegrini M.S., Pepeu G.,** Interleukin-1beta activates forebrain glial cells and increases nitric oxide production and cortical glutamate and GABA release in vivo: implications for Alzheimer's disease, *Neuroscience*, **91:** 831-842, 1999
56. **Hewett S.J., Csernansky C.A., Choi D.W.,** Selective potentiation of NMDA-induced neuronal injury following induction of astrocytic iNOS, *Neuron,* **13:** 487-494, 1994
57. **Vincent V.A., Tilders F.J., Van Dam A.M.,** Inhibition of endotoxin-induced nitric oxide synthase production in microglial cells by the presence of astroglial cells: a role for transforming growth factor beta, *Glia,* **19:** 190-198, 1997
58. **Schubert P., Morino T., Miyazaki H., Ogata T., Nakamura Y., Marchini C., Ferroni S.,** Cascading glia reactions: a common pathomechanism and its differentiated control by cyclic nucleotide signaling, *Ann. N. Y. Acad. Sci.,* **903:** 24-33, 2000
59. **Liu Z., Gatt A., Mikati M., Holmes G. L.,**. Effect of temperature on kainic acid-induced seizures, *Brain Res.,* **631:** 51-58, 1993
60. **Maeda T., Hashizume K., Tanaka T.,**. Effect of hypothermia on KA-induced limbic seizures: an electroencephalographic and 14C-deoxyglucose autoradiographic study, *Brain Res.,* **818:** 228-235, 1999
61. **Lawrence C.B., Allan S.M., Rothwell N.J.,** Interleukin-1beta and the interleukin-1 receptor antagonist act in the striatum to modify excitotoxic brain damage in the rat, *Eur. J. Neurosci.,* **10:** 1188-1195, 1998
62. **Giulian D., Young D.G., Woodward J., Brown D.C., Lachman L.B.,** Interleukin-1 is an astroglial growth factor in the developing brain, *J. Neurosci.,* **8:** 709-714, 1988
63. **Ling Z.D., Potter E.D., Lipton J.W., Carvey P.M.,** Differentiation of mesencephalic progenitor cells into dopaminergic neurons by cytokines, *Exp. Neurol.,* **149:** 411-423, 1998
64. **Gall C., Murray K., Isackson P.J.,** Kainic acid-induced seizures stimulate increased expression of nerve growth factor mRNA in rat hippocampus, *Mol. Brain Res.,* **9:** 113-123, 1991
65. **Strauss S., Otten U., Joggerst B., Pluss K., Volk B.,** Increased levels of nerve growth factor [NGF] protein and mRNA and reactive gliosis following kainic acid injection into the rat striatum, *Neurosci. Lett.,* **168:** 193-196, 1994
66. **Spranger M., Lindholm D., Bandtlow C., Heumann R., Gnahn H., Naher-Noe M., Thoenen H.,** Regulation of Nerve Growth Factor (NGF) Synthesis in the Rat Central Nervous System: Comparison between the Effects of Interleukin-1 and Various Growth Factors in Astrocyte Cultures and in vivo, *Eur. J. Neurosci.,* **2:** 69-76, 1990
67. **DeKosky S.T., Styren S.D., O'Malley M.E., Goss J.R., Kochanek P., Marion D., Evans C.H., Robbins P.D.,** Interleukin-1 receptor antagonist suppresses neurotrophin response in injured rat brain, *Ann. Neurol.,* **39:** 123-127, 1996
68. **Gadient R.A., Cron K.C., Otten U.,** Interleukin-1 beta and tumor necrosis factor-alpha synergistically stimulate nerve growth factor [NGF] release from cultured rat astrocytes, *Neurosci. Lett.,* **117:** 335-340, 1990
69. **Strijbos P.J., Rothwell N.J.,** Interleukin-1 beta attenuates excitatory amino acid-induced neurodegeneration in vitro: involvement of nerve growth factor, *J. Neurosci.,* **15:** 3468-3474, 1995
70. **Herx L.M., Rivest S., Yong V.W.,** Central nervous system-initiated inflammation and neurotrophism in trauma: IL-1 beta is required for the production of ciliary neurotrophic factor, *J. Immunol.,* **165:** 2232-2239, 2000
71. **Murray C.A., McGahon B., McBennett S., Lynch M.A.,** Interleukin-1 beta inhibits glutamate release in hippocampus of young, but not aged, rats, *Neurobiol. Aging.,* **18:** 343-348, 1997
72. **Miller L.G., Fahey J.M.,** Interleukin-1 modulates GABAergic and glutamatergic function in brain, *Ann. N. Y. Acad. Sci.,* **739:** 292-298, 1994
73. **Borth W.,** Alpha 2-macroglobulin, a multifunctional binding protein with targeting characteristics, *FASEB J.,* **6:** 3345-3353, 1992
74. **Gebicke-Haerter P.J., Bauer J., Brenner A., Gerok W.,** Alpha 2-macroglobulin synthesis in an astrocyte subpopulation, *J. Neurochem.,* **49:** 1139-1145, 1987

75. **Andus T., Geiger T., Hirano T., Kishimoto T., Tran-Thi TA., Decker K., Heinrich P.C.,** Regulation of synthesis and secretion of major rat acute-phase proteins by recombinant human interleukin-6 (BSF-2/IL-6) in hepatocyte primary cultures, *Eur. J. Biochem.,* **173:** 287-293, 1988
76. **Mori T., Iijima N., Kitabatake K., Kohsaka S.,** Alpha 2-macroglobulin is an astroglia-derived neurite-promoting factor for cultured neurons from rat central nervous system, *Brain Res.,* **527:** 55-61, 1990
77. **Higuchi M., Ito T., Imai Y., Iwaki T., Hattori M., Kohsaka S., Niho Y., Sakaki Y.,** Expression of the alpha 2-macroglobulin-encoding gene in rat brain and cultured astrocytes, *Gene,* **141:** 155-162, 1994
78. **Benveniste E.N., Sparacio S.M., Norris J.G., Grenett H.E., Fuller G.M.,** Induction and regulation of interleukin-6 gene expression in rat astrocytes, *J. Neuroimmunol.,* **30:** 201-212, 1990
79. **Yasukawa K., Hirano T., Watanabe Y., Muratani K., Matsuda T., Nakai S., Kishimoto T.,** Structure and expression of human B cell stimulatory factor-2 [BSF-2/IL-6] gene, *EMBO J.,* **6:** 2939-2945, 1987**Penkowa M., Molinero A., Carrasco J., Hidalgo J.,** Interleukin-6 deficiency reduces the brain inflammatory response and increases oxidative stress and neurodegeneration after kainic acid-induced seizures, *Neuroscience,* **102:** 805-818, 2001
80. **Minghetti L., Nicolini A., Polazzi E., Greco A., Perretti M., Parente L., Levi G.,** Down-regulation of microglial cyclo-oxygenase-2 and inducible nitric oxide synthase expression by lipocortin 1, *Br. J. Pharmacol.,* **126:** 1307-1314, 1999
81. **Miyachi T., Asai K., Tsuiki H., Mizuno H., Yamamoto N., Yokoi T., Aoyama M., Togari H., Wada Y., Miura Y., Kato T.,** Interleukin-1beta induces the expression of lipocortin 1 mRNA in cultured rat cortical astrocytes, *Neurosci. Res.,* **40:** 53-60, 2001
82. **Liao Z., Grimshaw R.S., Rosenstreich D.L.,** Identification of a specific interleukin 1 inhibitor in the urine of febrile patients, *J. Exp. Med.,* **159:** 126-136, 1984
83. **Dinarello C.A.,** Interleukin-1 and interleukin-1 antagonism, *Blood,* **77:** 1627-1652, 1991
84. **Eriksson C., Winblad B., Schultzberg M.,** Kainic acid induced expression of interleukin-1 receptor antagonist mRNA in the rat brain, *Mol. Brain Res.,* **58:** 195-208, 1998
85. **Zafra F., Lindholm D., Castren E., Hartikka J., Thoenen H.,** Regulation of brain-derived neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes, J. Neurosci., **12:** 4793-4799, 1992
86. **Panegyres P.K., Hughes J.,** The neuroprotective effects of the recombinant interleukin-1 receptor antagonist rhIL-1ra after excitotoxic stimulation with kainic acid and its relationship to the amyloid precursor protein gene, *J. Neurol. Sci.,* **154:** 123-132, 1998
87. **Loddick S.A., Wong M.L., Bongiorno P.B., Gold P.W., Licinio J., Rothwell NJ.,** Endogenous interleukin-1 receptor antagonist is neuroprotective, *Biochem. Biophys. Res. Commun.,* **234:** 211-215, 1997
88. **Yang G.Y., Zhao Y.J., Davidson B.L., Betz A.L.,** Overexpression of interleukin-1 receptor antagonist in the mouse brain reduces ischemic brain injury, *Brain Res.,* **751:** 181-188, 1997
89. **Golden G.T., Smith G.G., Ferraro T.N., Reyes P.F.,** Rat strain and age differences in kainic acid induced seizures, *Epilepsy Res.,* **20:** 151-159, 1995
90. **Xiao E., Xia L., Ferin M., Wardlaw S.L.,** Intracerebroventricular injection of interleukin-1 stimulates the release of high levels of interleukin-6 and interleukin-1 receptor antagonist into peripheral blood in the primate, *J. Neuroimmunol.,* **97:** 70-76, 1999
91. **Berg M., Bruhn T., Johansen F.F., Diemer N.H.,** Kainic acid-induced seizures and brain damage in the rat: different effects of NMDA- and AMPA-receptor antagonists, *Pharmacol. Toxicol.,* **73:** 262-268, 1993
92. **Clifford D.B., Olney J.W., Benz A.M., Fuller T.A., Zorumski C.F.,**. Ketamine, phencyclidine, and MK-801 protect against kainic acid-induced seizure-related brain damage, *Epilepsia,* **31:** 382-390, 1990
93. **Friedman W.J.,** Cytokines regulate expression of the type 1 interleukin-1 receptor in rat hippocampal neurons and glia, *Exp. Neurol.,* **168:** 23-31, 2001
94. **Yabuuchi K., Minami M., Katsumata S., Satoh M.,** Localization of type I interleukin-1 receptor mRNA in the rat brain, *Mol. Brain Res.,* **27:** 27-36, 1994
95. **Cunningham E.T., Wada. E., Carter D.B., Tracey D.E., Battey J.F., De Souza E.B.,** Localization of interleukin-1 receptor messenger RNA in murine hippocampus, *Endocrinology,* **128:** 2666-2668, 1991
96. **Lynch M.A.,** Interleukin-1 beta exerts a myriad of effects in the brain and in particular in the hippocampus: analysis of some of these actions, *Vitam. Horm.,* **64:** 185-219, 2002
97. **Bankers-Fulbright J.L., Kalli K.R., McKean D.J.,** Interleukin-1 signal transduction. *Life Sci.,* **59:** 61-83, 1996
98. **Arend W.P., Welgus H.G., Thompson R.C., Eisenberg S.P.,** Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist, *J. Clin. Invest.* **85:** 1694-1697, 1990
99. **Ban E., Milon G., Prudhomme N., Fillion G., Haour F.,** Receptors for interleukin-1 (alpha and beta) in mouse brain: mapping and neuronal localization in hippocampus, *Neuroscience,* **43:** 21-30, 1991
100. **Nishiyori A., Minami M., Takami S., Satoh M.,** Type 2 interleukin-1 receptor mRNA is induced by kainic acid in the rat brain, *Mol. Brain. Res.,* **50:** 237-245, 1997
101. **Colotta F., Re F., Muzio M., Bertini R., Polentarutti N., Sironi M., Giri J.G., Dower S.K., Sims J.E., Mantovani A.,** Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4, *Science,* **261:** 472-475, 1993