Excitatory Amino Acids

Metabolism and neuropathologic significance of quinolinic acid and kynurenic acid
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Neurologic symptoms and neurodegeneration are important causes of morbidity and mortality in man. Although many pathogenic mechanisms could account for neurologic dysfunction, considerable interest has focused on a group of neurotoxins known as the 'excitotoxins'. Quinolinic acid (QUIN) is an excitotoxic L-tryptophan and kynurenine pathway metabolite that is an agonist of N-methyl-D-aspartate (NMDA) receptors (Figure 1) [1-3]. The related metabolite, kynurenic acid (KYNA), is an antagonist of NMDA and other excitatory amino acid receptors that may protect against excitotoxins [4, 5]. KYNA also induces neurologic dysfunction by interfering with excitatory amino acid neurotransmission [6]. Therefore, disturbances in the concentrations of QUIN and KYNA within the brain may have neurologic and neuropathologic consequences.

An understanding of the sources of kynurenine pathway metabolites in blood and central nervous system (CNS) is incomplete. One contemporary model has been derived from studies in normal rats. The liver is viewed as the predominant source of kynurenine pathway metabolites [7], and has been suggested as the only source of QUIN in the body [8]. KYNA originates from L-kynurenine [9-11], which is synthesized in brain or enters the CNS from the blood [12-14]. QUIN is not synthesized from L-tryptophan or L-kynurenine in the normal brain [15], but originates from blood-derived precursors [14], particularly anthranilic acid [16]. QUIN is synthesized within the CNS from 3-hydroxyanthranilic acid via 3-hydroxyanthranilate-3,4-dioxygenase localized in astrocytes [17, 18].

A significantly different picture of how QUIN and KYNA are metabolized has emerged from studies of the consequences of immune activation. This review will summarize these responses.

Kynurenine pathway metabolism following immune activation in man
Two simple studies have essentially defined the direction of our research program since 1987 on the effects of immune stimulation on the synthesis of QUIN and KYNA. Because the activity of indoleamine-2,3-dioxygenase (IDO), the first enzyme of the kynurenine pathway in extrahepatic tissues, is increased following administration of immune stimuli [19, 20], we predicted that IDO induction should be associated with accumulation of QUIN. We found that systemic administration of either endotoxin or pokeweed mitogen to mice resulted in an accumulation of QUIN in both the CNS and blood [21, 22]. This observation led to the hypothesis that QUIN, as an NMDA receptor agonist, may be involved in the pathogenesis of seizures or neurodegeneration in inflammatory neurologic diseases [21]. To investigate whether immune stimulation in man was actually also associated with increases in QUIN, we first studied patients infected with the human immunodeficiency virus type-1 (HIV-1), an inflammatory neurologic disease [23]. The results showed that 10 non-demented acquired immunodeficiency syndrome (AIDS) patients had 2.9-fold elevations in cerebrospinal fluid (CSF) QUIN levels compared with nine control volunteers [24].

HIV-I infection and AIDS
After establishing the results described above, we discovered that CSF QUIN levels were elevated 3.5-fold in the very early stages of HIV-1 infection, whereas CSF KYNA levels were not increased significantly [25-27]. CSF QUIN levels could be correlated with the severity of motor deficits in a task sensitive to basal ganglia lesions [25]. In later-stage patients with the AIDS dementia complex, opportunistic CNS conditions or aseptic meningitis, the increases in CSF QUIN averaged over 20-fold [26] (Figure 2). Importantly, there were statistically significant correlations between the magnitude of the increases in CSF QUIN and quantitative measures of neuropsychologic deficits. Treatment with azidothymidine or anti-microbial therapy of opportunistic conditions appreciably reduced CSF QUIN levels, while neurologic status improved.

Abbreviations used: AIDS, acquired immunodeficiency syndrome; CSF, cerebrospinal fluid; CNS, central nervous system; HIV-I, human immunodeficiency virus type-1; IDO, indoleamine-2,3-dioxygenase; KYNA, kynurenic acid; NMDA, N-methyl-D-aspartate; QUIN, quinolinic acid.
CSF QUIN levels were also elevated in HIV-1-infected children and the concentrations of QUIN also correlated with the severity of neurologic impairments [28].

In the later-stage patients taken collectively, reductions in l-tryptophan accompanied proportional increases in 1-kynurenine and QUIN in both serum and CSF [27, 29]. Further, close inter-correlations were found between QUIN, KYNA and 1-kynurenine with both β₂-microglobulin and neopterin in CSF and serum [27, 29, 30]. These correlations support the hypotheses that the kynurenine pathway is activated in association with inflammation and is associated with induction of IDO. Notably, there were no relationships between the ratio of serum in CSF QUIN, 1-kynurenine or KYNA were not dependent on a breakdown of the blood-brain barrier [27, 29]. Further, although serum QUIN exceeded CSF QUIN levels by 20-fold in control subjects, we found that 14.4% of the later-stage HIV-1-infected patients had higher CSF than serum QUIN levels, an observation which supports intracerebral QUIN synthesis [26].

Other inflammatory neurologic diseases
Because IDO is induced by interferon-γ, tumour necrosis factor, endotoxin and pokeweed mitogen, we predicted in 1988 that accumulations of QUIN within the CSF would occur in a broad spectrum of inflammatory neurologic diseases. We have found markedly increased concentrations of QUIN in both lumbar CSF and post mortem brain tissue of patients with inflammatory diseases (bacterial, viral,
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Figure 2
Comparison of CSF QUIN concentrations in non-inflammatory neurologic disease with HIV-1-infected patients

Data from [26, 27, 29]. Abbreviations used: PSP, progressive supranuclear palsy; WR 1–2, Walter Reed 1–2.

fungal and parasitic infections, meningitis, autoimmune disease and septicemia), independent of breakdown of the blood-brain barrier [27, 31, 32]. Correlations between CSF QUIN, KYNA and l-kynurenine with markers of immune stimulation (neopterin, white blood cell counts and IgG levels) again indicate a relationship between accelerated kynurenine pathway metabolism and the degree of intracerebral immune stimulation. Proportional increases in CSF l-kynurenine and reduced l-tryptophan accompanied the increases in CSF QUIN and KYNA and are consistent with induction of IDO (Figure 3).

Studies in experimental animals have shown that KYNA can attenuate the excitotoxic effects of QUIN when present in higher molar concentrations [5]. The concentrations of QUIN in the CNS normally exceed those of KYNA. Further, we have found consistently that, following immune activation in man, non-human primates, gerbils and mice while CSF and brain tissue KYNA levels are increased along with increases in QUIN levels, the magnitude of the increases in QUIN is always larger than the increases in KYNA [27, 29, 31, 33–38]. These results show that the balance between QUIN and KYNA favours excitotoxicity.

It is important to note that the increases in CSF QUIN and KYN levels in patients with inflammatory neurologic disease cannot be attributed simply to brain atrophy, dementia, motor disturb-
Figure 3
Model of kynurenine pathway metabolism in inflammatory neurologic disease

Note that QUIN and KYNA may originate within the CNS or from systemic tissues, that macrophages may be an important intra-cerebral source of kynurenine pathway metabolites, and that QUIN and KYNA may produce both cytolytic and non-cytolytic neurologic deficits. Abbreviations used: AA, anthranilic acid; 3-HAA, 3-hydroxyanthranilic acid; IFN, interferon; L-KYN, L-kynurenine.

ances, disturbances in food intake, non-specific stress, glossis or seizures, as CSF QUIN and KYNA are not increased in patients with either Huntington's disease, Alzheimer's disease, seizures, depression, anorexia nervosa or bulimia nervosa [27, 39–44]. CSF and brain tissue KYNA levels tend to be lower in patients with Huntington's disease [27, 45] or Alzheimer's disease [27], although the significance of this reduction with respect to excitotoxicity remains to be established.

Animal models of inflammatory neurologic disease

Generalized immune activation

Macques infected with either the simian immunodeficiency virus or the type-D retrovirus have proved to be excellent models of HIV-1 infection of systemic and CNS tissue. Our results to date show that increases in CSF and brain tissue QUIN concentrations also occur in retrovirus-infected macaques, and that such increases are largest in macaques with neurologic disease [27, 36, 37, 46]. The activity of IDO is also increased within the CNS in proportion to the concentrations of QUIN in the CSF and brain tissue [27, 47]. Induction of IDO was also found in the lung, whereas the activity of hepatic tryptophan-2,3-dioxygenase was not affected.

In many of the inflammatory diseases studied, immune stimulation was present not only within the CNS, but also in systemic tissues. Consequently, it is difficult to differentiate CNS-restricted changes in kynurenine pathway metabolism from the contribution of systemic responses. We have proposed that it is highly likely that changes in systemic kynurenine pathway metabolism have a strong influence in the changes in QUIN and KYNA levels within the CNS, particularly in circumstances where immune...
activation is predominantly or exclusively systemic [27, 34, 38]. We have described a rodent model which can replicate the kynurenine pathway responses in both CNS and systemic tissues following systemic immune activation that supports a contributory role for systemic kynurenine pathway responses in the CNS changes [20-22, 33, 34]. However, the following two studies in experimental models have clearly established that the CNS is indeed capable of synthesizing QUIN and KYNA, independently of systemic contributions.

**CNS-restricted immune activation**

**Poliovirus infection of rhesus macaques.** Macaques received an intraspinal injection of poliovirus as a model of localized inflammatory neurologic disease [35]. Seventeen days later, spinal cord IDO activity and QUIN concentrations in both spinal cord and CSF were increased in proportion to the degree of inflammatory responses and neurologic damage in the spinal cord, as well as the severity of motor paralysis. The absolute concentrations of QUIN in CSF and tissue (μM), exceeded levels reported to kill spinal cord neurons in vitro (nM) [48]. Smaller increases in IDO activity and QUIN concentrations also occurred in parietal cortex, a poliovirus target area. In contrast, IDO activity was not affected in the frontal cortex, a region that is not a target for poliovirus. Immunocytochemical studies showed that IDO was localized predominantly in inflammatory lesions in the grey matter. Spinal cord slices from poliovirus-infected macaques converted [13C6]-tryptophan to [13C6]QUIN in vitro. Because spleen macrophages stimulated with interferon-γ were also able to convert [13C6]-tryptophan to [13C6]QUIN, we postulated that QUIN may be produced in localized macrophage infiltrates into the CNS, and that such cells contain kynurenine-3-hydroxylase and kynureninase but not 3-hydroxyanthranilate-3,4-dioxygenase. Subsequent studies established that the activities of kynurenine-3-hydroxylase, kynureninase and 3-hydroxyanthranilate-3,4-dioxygenase were increased in the spinal cord of poliovirus-infected macaques (K. Saito and M. Heyes, in preparation).

No changes in serum l-kyurenine or QUIN concentrations occurred in the poliovirus-infected macaques. Notably, in control macaques, the ratio of serum:CSF QUIN levels was > 40:1. However, in poliovirus-infected macaques, QUIN concentrations in spinal cord and CSF exceeded serum QUIN levels in 29% and 19% of macaques, respectively. Clearly, the increases in CSF QUIN cannot be attributed simply to a breakdown of the blood-brain barrier.

**Ischaemic brain injury.** Inflammatory lesions, including macrophage infiltrates and reactive gliosis, occur in damaged brain regions following transient cerebral ischaemia. We have found proportional increases in IDO activity and QUIN concentrations in brain 4 days after 10 min of cerebral ischaemia, with both responses decreasing in different regions as follows: hippocampus > striatum > cerebral cortex > thalamus. These increases paralleled the severity of local brain injury and inflammation. IDO activity and QUIN concentrations were unchanged in the cerebellum of post-ischaemic gerbils, which is consistent with the preservation of blood flow and resultant absence of pathology in this region. Blood QUIN and l-kyurenine concentrations were unaffected by ischaemia. Brain tissue QUIN levels at four days post-ischaemia exceeded blood concentrations, minimizing a role for breakdown of the blood-brain barrier. Marked increases in the activity of kynureninase, kynurenine 3-hydroxylase and 3-hydroxyanthranilate-3,4-dioxygenase were also detected in hippocampus, but not in cerebellum. Synthesis of [13C6]QUIN from [13C6]-tryptophan in vivo was demonstrated in hippocampus of four-day post-ischaemic animals. No changes in kynurenic acid concentrations in either hippocampus, cerebellum or cerebrospinal fluid were observed in the post-ischaemic gerbils compared with controls, in accordance with the unaffected activity of kynurenine aminotransferase activity.

**Macrophages**

A number of cellular changes occur in the CNS following infection or injury, including infiltration by macrophages and glial proliferation. While the cellular localization of QUIN synthesis within the CNS in situations of CNS immune activation is not known, studies in vitro of human peripheral blood macrophages [49] and macaque spleen macrophages [35] have shown that these cells can convert [13C6]-tryptophan to [13C6]QUIN. Interferon-γ substantially increased the accumulation of [13C6]QUIN in a dose- and time-dependent manner. Notably, the micromolar QUIN concentrations achieved exceeded levels reported in both CSF and blood of patients and non-human primates with inflammatory diseases. Activated macrophages may be an important source of accelerated l-tryptophan conversion to QUIN in inflammatory diseases. Other cells may also be involved in converting l-tryptophan and other substrates to QUIN [37, 46, 47].
Therapeutic implications

Collectively, these observations support a role for QUIN in the pathogenesis of neurodegenerative changes in patients with a broad spectrum of inflammatory neurologic disease. The increases in QUIN and KYNA are attributable to increased activities of IDO, kynureninase, kynurenic acid, kynurenine 3-hydroxylase and 3-hydroxanthranilic acid, 3,4-dioxygenase, either within the CNS or systemic tissues. Both QUIN and KYNA may contribute to neurologic dysfunction by interfering with excitatory amino acid neurotransmission or NMDA receptors’ function. Strategies to attenuate the receptor-mediated effects of QUIN, KYNA and other neuroactive kynurenines are potential avenues to therapy, although drugs which block NMDA receptors could conceivably accentuate neurologic deficits under some circumstances. In view of the role for interferon-γ and other cytokines in IDO induction, drugs such as interferon-γ antibodies or anti-inflammatory agents may also be useful therapies [34]. Additional approaches include inhibitors of kynurenine pathway enzymes, such as 4-chloro-3-hydroxyanthranilic acid [50].

Characterization of the allosteric modulatory protein associated with non-NMDA receptors

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Excitatory amino acid receptors (glutamate receptors; GluRs) mediate most fast excitatory transmission in the vertebrate central nervous system (CNS); they are involved in formation and stabilization of synapses, their prolonged activation is potently neurotoxic and their dysfunction has been implicated in a variety of neurodegenerative diseases [1]. Historically, three distinct classes of ionotropic GluRs have been categorized, the N-methyl-D-aspartate (NMDA), kainate and a-amino-3-hydroxy-5-methylisoxazolepropionate (AMPA) subtypes [2]. However, recent electrophysiological, biochemical and molecular biological data have suggested that, at least in a number of cases, AMPA and kainate act at the same class of receptors; thus, the last two categories are often referred to simply as non-NMDA receptors [1, 3].

There has been rapid progress in the past few years in the biophysical and molecular biological characterization of GluRs [1, 4]. Nonetheless, despite the cloning of an increasing number of cDNAs from rat brain that encode functional non-NMDA and NMDA receptor subunits [1, 4, 5], there is still relatively little known about the stoichiometric arrangement of GluRs in vivo or about their physiological regulation at the molecular and cellular levels. These are particularly important questions since changes in the regulation of GluRs are believed to be the primary event underlying changes in synaptic plasticity such as long-term potentiation (LTP).

Several putative control mechanisms that are capable of regulating non-NMDA receptors have been proposed. These include alternative RNA splicing and RNA editing at the nucleic acid level [4] and receptor phosphorylation and dephosphorylation at the protein level [6]. As discussed below, yet another layer of control to which non-NMDA receptors are subjected is mediated by an associated modulatory protein.

The presence of a non-NMDA-receptor-associated modulatory protein (GluR-MP) was first demonstrated in rat cortex [7] by radiation inactivation (Table 1). This technique correlates the inactivation of proteins by high-energy electron bombardment with their molecular weight. Target theory suggests that the natural log of the binding activity remaining when plotted against the dose of...