Kynurenine pathway properties and applications

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INTRODUCTION

Kynurenine pathway

The kynurenine pathway (KP) is a major route for the metabolism of an essential amino acid, tryptophan which results in the production of biologically active molecules, kynurenines. Most of the intermediates of the kynurenine pathway are neuroactive and known to play roles in the regulation of N-methyl-D-aspartate (NMDA) receptor function and free radical production. A central compound of the pathway is Kynurenine (KYN) which can be metabolized in two separate ways: to Kynurenic acid (KYNA), or to 3-hydroxykynurenine (3-OH-KYN) and quinolinic acid (QUIN), the precursors of NAD. Most of the importance has been given to KYNA due to its broad spectrum antagonistic properties (Ganong and Cotman, 1986; Stone and Connick, 1985)

Epilepsy

Epilepsy is characterized by recurrent spontaneous seizures due to hyperexcitability and hypersynchrony of brain neurons. Although there are limited clinical evidence to support the deregulation of kynurenine pathway in epilepsy, but the understanding of the proinflammatory cytokine signaling regulated kynurenine pathway and neuroinflammation in the recurrence of epileptic seizure activity may strengthen this possibility. Further based on the pre-clinical data (Gleeson et al., 2010; Lehrmann et al., 2008), it may be predicted that in some forms of epilepsy, over activation of microglial branch with respect to the astrocytic branch of the KP leads to accumulation of QUIN and 3-HK in the CNS. It has been proposed that adjunctive treatment with

KMO inhibitor along with anti-convulsants can improve the treatment outcome, as inhibition of KMO increase the KYNA production and decrease the 3-HK and QUIN production in the CNS (Campbell et al., 2014).

KYNURENINES

Kynurenic acid

More than 20 years ago various neurophysiological experiments revealed the neuroinhibitory properties of KYNA. (Perkins and Stone, 1982) discovered that KYNA is a broad spectrum antagonists of glutamate receptor which can inhibit three types of ionotropic receptors- *N*-methyl-D-aspartate (NMDA), kainate and a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors. KYNA act as a competitive antagonist at the glycine site on NMDA receptors (Kessler et al., 1989). KYNA also block the α7- nicotinic acetylcholine receptor and this inhibition is noncompetitive in nature (Hilmas et al., 2001). In addition to this in past few years some other receptor targets have been identified. For example, KYNA is also reported to interact with GPR35 (Wang et al., 2006) and arylhydrocarbon receptors (DiNatale et al., 2010). However, further studies are required to elaborate their role in neuronal disorders.

Various studies have shown that KYNA alters the progression of kindled seizures. (Thompson et al., 1988) have shown that pretreatment with administration of intracerebroventricular KYNA prior to administration of electrical kindling stimulus in rats significantly reduced the rate of kindling. A study by Szyndler and co-workers demonstrated that during the electrically induced kindling of seizures, the seizures development is associated with

significant alterations in the levels of KYNA and its precursor, tryptophan (TRP). The levels of KYNA and ratio of KYNA/TRP (theoretical index of kynurenine pathway activity) was found to be increased in the amygdale and hippocampus of kindled animals whereas concentration of tryptophan in the prefrontal cortex and hippocampus was found to be decreased (Szyndler et al., 2012). Carpenedo et al. 1994 demonstrated that the administration of inhibitors of kynurenine hydroxylase and kynureninase, evoked a significant increase in brain levels of KYNA which protected the rats from electroshock induced seizures or DBA/2 mice from audiogenic seizures (Carpenedo et al., 1994).

An enhanced liberation of KYNA was reported in the hippocampus of electrically kindled animals and in the extracellular fluid (ECF) elevation of KYNA levels was not found to be associated with electrical stimulation per se but with the progression of kindling development to the stage of generalized tonic or clonic convulsions (Wu et al., 1995). Whereas in another study, it has been found that in nucleus accumbens kindling induced a lasting increase in kynurenate although no significant changes were seen in hippocampus, striatum, cerebral cortex, olfactory bulb, thalamus, tectum, pons/medulla, cerebellum, or plasma (Loscher et al., 1996). Nemeth et al (2004) found that PTZ induced seizures was inhibited by KYNC, and it also protected the animals from the death induced by repeated PTZ injections (Nemeth et al., 2004). Maciejak et al. (2009) also reported a significant decrease in tissue concentrations of KYNA in some brain structures (the caudate entorhinal cortex, piriform cortex, putamen, amygdala and hippocampus) after PTZ-induced kindling (Maciejak et al., 2009). Scharfman et al (1999) have

demonstrated that KYNA converted *in situ* from KYN was potent enough to block the epileptiform discharges induced in Mg2+-free medium and KYN applied at 200 uM prevents spontaneous activity in the hippocampal CA3 region (Scharfman et al., 1999). However, a study carried out by Rozsa et al., 2008 on hippocampal slices from young animals showed that KYN pretreatment was effective on neural hyperecxitability even at low concentration of 16uM. This study also found that the KYNA produced by conversion of KYN→KYNA was sufficient to prevent the neuroexcitatory effect of PTZ. This study thus supports the hypothesis that the various neuronal disorders which are affected by neuronal hyperexcitation, the kynurenine pathway might be a valuable drug target for these disorders (Rozsa et al., 2008).

Szyndler et al 2012 have shown an increase in the glutamate/GABA ratio and a decrease in GABA levels in the amygdale of kindled animals. Further in the amygdala of kindled rats, between the levels of KYNA and glutamate, negative correlation was found (Szyndler et al., 2012). This finding suggested that the disrupted balance between the excitatory and primary inhibitory brain systems may change the local KYNA levels as an adaptive reaction. Further onset of seizures can modify the function of the GABA system, and in some studies in response to kindling the stimulation as well as the inhibition of local GABAergic activity has been found (Kamphuis et al., 1990; Kaura et al., 1995). Reduction in the extracellular concentration of GABA has been found in PTZ-induced kindling in the rat hippocampus (Szyndler et al., 2008). These studies indicated that the imbalance between KYNA synaptic

transmission and glutamatergic, GABAergic characterizes hyperexcitable, epileptic and local neuronal circuits.

Mechanisms responsible for the increase level of KYNA were not clearly understood. During the kindling of seizers, few studies have indicated the association of hypertrophied astrocystes in brain regions with increase activity of KAT which is main enzyme for synthesis of KYNA synthesis (Wu et al., 1995). Thus, the astrocytic changes and process of kindling was found to be accompanied by KYNA elevation (Du et al., 1993). In contrary to this no significant changes were found in KAT activity in the amygdala of electrically kindled animals (Wu et al., 1995). The lasting elevation of KYNA levels appeared to be different in fully kindled animals from the increase which occurs immediately following generalized convulsions. Wu and Schwarcz 1996 have suggested that it may occur due to desensitization of presynaptic glial amino acid receptors which regulate local KYNA release, in response to repeated release of glutamate (Wu and Schwarcz, 1996).

Kynurenine, 3-hydroxykynurenine and other metabolites of kynurenine pathway

Some other metamobolities of kynurenine pathway metabolites, including 3-hydroxyanthranilic acid (3-HANA), kynurenine, 3-hydroxykynurenine (3-HK), and anthranilic acid , have failed to show direct effects on neuronal activity (Stone, 1993). Recently kynurenine has been described as an endogenous ligand of the human aryl hydrocarbon receptor (Opitz et al., 2011), and the activation of metabotropic glutamate receptors has been shown by xanthurenic (Copeland et al., 2013; Schwarcz et al., 2012). 3-

hydroxyanthranilic acid has been described as neurotoxin and known to play a role in immunoregulation (Chen and Guillemin, 2009; Lopez et al., 2008). In the brain various kynurenine pathway metabolites may participate in pro- and anti-oxidative processes(Giles et al., 2003). Goldstein et al , 2000 have shown that 3HK and 3HAA produce hydrogen peroxide and superoxide in a copper-dependent manner(Goldstein et al., 2000). This study has suggested that in the oxidative damage of proteins (such as alpha-crystallin), both these metabolites may act as cofactors by interacting with redox-active metals. Thus these findings may have important implications in the understanding of cataractogenesis and other degenerative conditions, where kynurenine pathway is activated (Goldstein et al., 2000).. However, these metabolites also have antioxidant properties, scavenging peroxyl radicals more effectively than equimolar concentrations of ascorbic acid or Trolox analogue of vitamin E) (Christen et al., 1990). Further Like quinolinic acid, intracerebral injection of 3-hydroxyanthranilic acid in rats also leads to a decrease in activity of choline acetyltransferase, but QUIN were found as more potent (Jhamandas et al., 1990).

Quinolinic acid

The first evidence that matabolites of kynurenine pathway can influence the brain function was provided by Lapin (Lapin, 1978), who after an intracerebroventricular QUIN injection observed convulsions in mice. Quinolinic acid is a selective agonist at the neuronal NMDA subtype of glutamate receptors (Stone and Perkins, 1981). Inspite of its low cerebral content and low receptor affinity, its potency as an excitotoxin is due to its selective interaction with NMDAR2 receptor subunit (de Carvalho et al.,

1996). For example QUIN has 10-fold higher affinity for NR2B subunit of the NMDA receptor, which predominates in the forebrain as compared to hindbrain specific NR2C subunit (Schwarcz et al., 2012). Several mechanisms have been found to be associated with neurotoxic properties of QUIN. In addition to agonist of NMDA receptor, it has been found to induce the lipid peroxidation (Rios and Santamaria, 1991), and produce reactive oxygen species (ROS) (Rodriguez-Martinez et al., 2000) (Rodriguez-Martinez et al, 2000; Santamaria et al., 2001) which may accounts for its neurotoxic properties. Its role in lipid peroxidation was found to be modulatd by its intetraction with Fe2+ ions to form Quin Fe2+ (Stipek et al., 1997). The production of ROS by QUIN was found as secondary to the generation and auto-oxidation of Fe2+- QUIN complexes and it can be inhibited by iron chelation (Platenik et al., 2001). Binding of QUIN to the NMDA receptor is also controlled by endogenous iron (St’astny et al., 1999).

All the studies discussed above suggest that alterations in levels of certain metabolites of KYN pathway (mostly KYNA, QUIN and 3-OH-KYN), either alone or in combination affect the pathology of some brain disorders. In most of the neurodegenerative disorders, these changes were appeared as secondary to the basic pathological process, and in some conditions might be the mediators of CNS pathology (Schwarcz and Pellicciari, 2002). Astrocytes now known to lack KYN-3- hydroxylase, thus favor the synthesis of KYNA, on the other hand microglial cells consist little KYN-aminotransferase (KAT) and thus likely generate the intermediate metabolites of the QUIN branch of the KA pathway (Guillemin et al., 2000). It has been found that astrocytes when present alone act as neuroprotectant

by elevating the synthesis of KYNA and diminishing the QUIN production, whereas it become neurotoxic in the presence of macrophages and/or microglia, by the production of large amount of Kynurenine which can be metabolized to form the neurotoxin QUIN by neighboring or infiltrating monocytic cells (Guillemin et al., 2001).

Combine role of cytokine and kynurenine in Epilepsy

Lehrmann et al have shown that inoculation of hamster neurotrophic measles virus in mice increases the microglial activation and brain levels of QUIN and 3-HK. These changes produced the subclinical seizure activity, behavioral seizures and neurodegeneration (Lehrmann et al., 2008). It has been found that administration of kainic acid in rat, induced an inflammatory response in the hippocampus which was characterized by activation of microglia (elevated expression of CD11b), increased expression of pro-inflammatory cytokines such as IFN- γ and IL-1 β and induction of cytokine-inducible enzymes IDO, iNOS and KMO (Gleeson et al., 2010). Further proinflammatory cytokines IL-1β, TNF-α, and IFN-γ have been found as inducers of IDO and IFN-γ also induced the expression of KMO (Mandi and Vecsei, 2012). Both the enzymes IDO and KMO, increased the production of 3-HK and QUIN. Role of QUIN as neurotoxin or excitotoxin has been described in various studies. Thus it appears that there is close interaction between the cytokine, kynurenine pathway and nervous system. Further studies are required to examine the interaction between the kynureniney and cytokine signaling pathway in case of epilepsy.

Therapeutic applications of kynurenines

Based on kynurenine pathway modulation, different approaches has been suggested for the development of therapeutic agents. One approach is to use kynurenic acid analogues as antagonists at glutamate receptors. Other is to inhibit the enzymes activities which are responsible for quinolinic acid synthesis. For example inhibition of enzyme kynurenine hydroxylase leads to decrease endogenous quinolinic acid levels and an increase kynurenic acid levels (Stone, 2001). It has been proposed that by maintaining the balance between these metabolites, i. e towards the neuroprotectant and away from the excitotoxin could have neuroprotective and anticonvulsant properties in stroke (Pellicciari et al., 1994; Varasi, 1996).

Antagonists of kunurenic acid

Several studies have used the kynurenate structure to target the glycine-2 receptor site as a prelude for the development of therapeutic agents (Manallack, 1990; Stone, 2001). Various authors have examined the diffrenet portions of kynurenat to explore the different approaches of their modifications(Bigge, 1993; Carling et al., 1993; Leeson et al., 1992; Leeson et al., 1993). These modifications have been reviewed by Stone 2001 (Stone, 2001). For example the halogen atoms substitution, yielded the potent analogue 5, 7-dichlorokynurenic (Baron et al., 1990). This formula has been retained in many of the analogues. Compound 4-carboxymethylamino-5, 7-dichloroquinoline-2-carboxylicacid(MDL 100, 748) and 3-(4, 6-dichloro-2-carboxyindol-3-yl)-propionic acid (MDL 29, 951) was found as potentanticonvulsantsafter their i. c. v. administration to audiogenic seizure-susceptible DBA/2J mice (Baron et al., 1992; Harrison et al., 1990). A kynurenate analogues with a 3-phenyl substituent resulted in lipid soluble

compounds which retained potent activity at the glycine-2 site(McQuaid et al., 1992). For example MDL 104, 653 (3-phenyl-4-hydroxy-7-chloro-quinolin-2(1 H)-one), has been found as protective against sound-inducedclonic seizuresin DBA/2 mice following intracerebroventricular, intraperitoneal or oral administration. In rats, fully amygdala-kindled motor seizures were found to be significantly reduced and the duration of the after-discharge was significantly shortened after the i. p. administration (Chapman et al., 1995). The enlargement of nitrogenous ring of KYNC into a 7-membered ring has produced benzazepinedione compounds which were found to reduce seizures in DBA/2 mice(Jackson, 1995).

Prodrugs of KYNC

Moore et al., 1993 have used agents which act as prodrugs to deliver kynurenic acid into the brain. For example, L-4-chlorokynurenine and 4, 6-dichlorokynurenine when transported into brain, converted into 7-chlorokynurenic acid and 5, 7-dichlorokynurenic acid respectively (Hokari et al., 1996; Moore, 1993). Different esters have been developed by linking 7-chlorokynurenic acid to D-galactose or D-glucose (Bonina et al., 2000). One such example is 7-chlorokynurenic acid-glucopyranos-3ylester which has been found to be rapidly metabolized in the brain into 7-chlorokunurenic acid and suppressed the NMDA induced seizures (Battaglia et al., 2000).

Modulators of kynurenic acid concentrations

The kynurenine 3-hydroxylase and kynureninase, the degradative enzymes of L-KYN are recognized as important targets for kynurenergic drug development. It has been proposed that hampering with these enzymes,

which act at a branching point of the KP can selectively alter the QUIN/KYNA ratio and thus repair the chemical impairments in the brain. Connick et al., 1992; Russi et al., 1992 have shown that administration of Nicotinylalanine with L-kynurenine and probenecid increase the level of kynurenic acid in brain and prevent the induction of seizures (Connick et al., 1992; Russi et al., 1992). Miranda et al. (1997, 1999) have shown the protective potential of nicotinylalanine for the nigrostriatal neurons against damage caused by the local injection of NMDA or quinolinic acid (Miranda et al., 1997; Miranda et al., 1999). Cerebral kynurenic acid levels were not found to be increased more than 3. 3-fold. Scharfman and Ofer, 1997 have found that epileptiform bursting activity generated by the prefusion of brain slices with the precursor L-kynurenine was inhibited by the small increase of kynurenic acid (Scharfman and Ofer, 1997) .

Meta-nitrobenzoylalanine and the related compound ortho-methoxybenzoylalanine were found as potent inhibitors of kynurenine-3-hydroxylase and kynureninase respectively (Natalini and Moroni, 1995; Pellicciari et al., 1994). Both these compounds were able to increase the levels of kynurenate in hippocampal extracellular spaces. This l effect was found to be associated with a protection from audiogenic convulsions in DBA/2 mice and decrease in locomotion in rats (Chiarugi et al., 1995). The 3, 4- dichlorobenzoylalanine, also a kynurenine-3-hydroxylase inhibitor was found as more effective than meta-nitrobenzoylalanine (Speciale et al., 1996). *S* -aryl-L-cysteine *S, S* -dioxides have been found as kynureninase inhibitors (Dua, 1993). *S* -(2- minophenyl)-L-cysteine- *S, S* -dioxide has been found as particularly strong inhibitor. The 5-methyl derivative was 3 times

more efficient against human kynureninase and found to reduce the stimulation of quinolinic acid synthesis induced by interferon-γ in human macrophages (Drysdale and Reinhard, 1998). The 4-aryl-2-hydroxy-4-oxobut-2- enoic acids and esters were found as the most potent kynurenine-3-hydroxylase inhibitors with nanmolar potencies (Drysdale et al., 2000).

Modulators of quinolinic acid concentrations

Another approach to prevent the synthesis of quinolinic acid is to inhibit 3-hydroxyanthranilic acid oxygenase. Compound 4-halo-3-hydroxyanthranilic acids have been found as inhibitor of this enzyme and thus lead to reduction in quinolinic acid formation (Walsh et al., 1991; Walsh et al., 1994). This compound, as well as norharmane and 6-chlorotryptophan were found to be effective in several cell lines including peripheral monocytes and attenuate quinolinic acid synthesis (Saito et al., 1994). Another compound 4, 6-dibromo-3-hydroxyanthranilic acid (NCR-631) has been found to inhibit 3-hydroxyanthranilic acid oxygenase and minimize the loss of hippocampal cells produced by anoxia, bacterial lipopolysaccharide or injurious cytokines such as interleukin- 1 β (Luthman et al., 1998).