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Systemic administration of kainate induces marked increases of endogenous kynurenic acid in various brain regions and plasma of rats

Halina Baran, Martina Gramer, Dagmar Hönack, Wolfgang Löscher *

Department of Pharmacology, Toxicology, and Pharmacy, School of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany

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Abstract

The endogenous neuroinhibitory and neuroprotective excitatory amino acid receptor antagonist kynurenic acid has been hypothetically linked to the pathogenesis of epilepsy and several other brain disorders. In the present study, alterations in kynurenic acid levels were examined in the kainate model of temporal lobe epilepsy. Kainate was systemically injected in rats at a dose (10 mg/kg s.c.) which induces a characteristic behavioural syndrome with stereotypies and focal (limbic) and generalized seizures, eventually progressing into severe status epilepticus. Kynurenic acid was determined 3 h after kainate injection in various brain regions (olfactory bulb, frontal cortex, piriform cortex, amygdala, hippocampus, nucleus accumbens, caudate/putamen, thalamus, superior and inferior colliculus, pons and medulla, and cerebellar cortex) and in plasma, using a sensitive high-performance liquid chromatographic method. When data were analysed irrespective of individual seizure severity, significant increases in kynurenic acid were determined in all brain regions examined except the hippocampus, nucleus accumbens and pons/medulla. The most marked (200–500%) increases above controls were seen in the piriform cortex, amygdala, and cerebellar cortex. Furthermore, a significant kynurenic acid increase of about 200% above control was determined in plasma. When kynurenic acid levels were determined in subgroups of rats with different behavioural alterations in response to kainate, the most marked kynurenic acid increases were seen in subgroups with status epilepticus. Rats which only developed mild (focal) seizures or stereotyped behaviours (wet dog shakes) also exhibited significantly increased kynurenic acid levels, thus indicating that the increase in kynurenic acid in response to kainate was not solely due to sustained convulsive seizure activity. Whereas it was previously proposed that kynurenic acid is involved only in later stages of seizure disorders, the present data demonstrate that marked increases in central and peripheral kynurenic acid levels occur early after the onset of neuroexcitation, at least in the kainate model.

Keywords: Epilepsy; Kynurenate; Kynurenine; Excitotoxicity; Anticonvulsant action; Dizocilpine; Piriform cortex

1. Introduction

Kynurenic acid, a product of the kynurenine pathway of tryptophan metabolism, was detected in the mammalian brain in 1988 (Moroni et al., 1988a; Turski et al., 1988). In view of the neuropharmacological effects of kynurenic acid (Stone, 1993), the presence of this compound in the brain has attracted an enormous interest in its potential role in brain functions and dysfunctions. Kynurenic acid is a broad-spectrum antagonist of ionotropic excitatory amino acid receptors which can antagonize competitively *N*-methyl-D-aspartate (NMDA), kainate, and quisqualate-induced neuronal excitation and, at lower concentrations, can also block the strychnine-insensitive glycine site associated with the NMDA receptor (Carter, 1992; Stone, 1993). Because these effects of endogenous kynurenic acid could be critically involved in physiological and pathophysiological processes in the brain, its hyper- and hypofunction has been speculatively linked to several brain disorders, including epilepsy (Schwarcz et al., 1992; Stone, 1993). However, until recently direct evidence for a role of endogenous kynurenic acid in seizure genesis and/or propensity was lacking. In animal experiments, administration of kynurenic acid was shown to exert anticonvulsant effects in different

* Corresponding author. Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany. Tel. (49) (511) 953 8721, fax (49) (511) 953 8581.

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seizure models, e.g. against sound-induced seizures in DBA/2 mice (Stone, 1993). However, the endogenous activity of the kynurenic acid synthesizing enzyme kynurenine aminotransferase is not altered in seizure susceptible mouse strains, including DBA/2 mice (Eastman et al., 1993). In contrast to the latter finding in models of generalized seizures, kynurenine aminotransferase activation in the hippocampus, piriform cortex and thalamus was recently determined in a rat model of chronic focal (limbic) epilepsy (Du et al., 1993), i.e. the most common type of epilepsy in humans. Similarly, systemic administration of kainate, a potent glutamate analogue which induces a characteristic convulsive syndrome resembling, at least in part, human limbic (temporal lobe) epilepsy (Ben-Ari, 1985), substantially increased kynurenic acid synthesis ex vivo in the piriform cortex, hippocampus, and striatum of rats (Baran et al., 1991; Wu et al., 1991). More recently, Wu and Schwarcz (1994) reported in vivo increases of extracellular kynurenic acid levels as measured by microdialysis in rat hippocampus in response to kainate and other convulsants. The latter authors proposed that increases in endogenous kynurenic acid may be a common occurrence in response to seizures. In the present study, we investigated the effects of systemic administration of kainate on endogenous kynurenic acid levels in various brain regions of rats, using a modification of recently described sensitive techniques to measure fmol quantities of kynurenic acid (Shibata, 1988; Swartz et al., 1990). Furthermore, kynurenic acid was analysed in plasma in order to examine if alterations in levels of this compound also occur in the periphery.

2. Materials and methods

2.1. Materials

Unless otherwise specified, all compounds were purchased from Sigma (Munich, Germany). All HPLC solvents were HPLC grade.

2.2. Animals

Sixty female Wistar rats (Harlan-Winkelmann, Borchen, Germany) weighing 210–230 g were used. The animals were purchased from the breeder at a body weight of about 200 g. Following arrival in the animal colony, the rats were kept under controlled environmental conditions (ambient temperature 24–25°C, humidity 50–60%, 12/12 h light/dark cycle, light on at 6:00 a.m.) for at least 2 weeks before being used in the experiments. Standard laboratory chow (Altromin 1324 standard diet) and tap water were allowed ad libitum. All experiments were done in the morning (to avoid circadian variations) in a laboratory with an ambient temperature of 24–25°C. Prior to experiments the rats were habituated to handling for at least 1 week. For this purpose, the animals were transferred in their home cages from the vivarium to the laboratory every day, picked up, weighed, injected subcutaneously (s.c.) with saline (2 ml/kg) and placed in observation cages (without sawdust flooring and grid covers) for 2–3 h.

2.3. Drug injection and behavioural observations

Kainate was dissolved in saline and injected s.c. at a dose of 10 mg/kg in the neck area of 30 rats. 30 control rats received s.c. injection of saline. The injection volume for drug or saline injections was 2 ml/kg. After drug or vehicle injection, rats were placed in plastic cages without sawdust flooring and grid covers. The behaviour of each animal was continuously evaluated during 3 h after kainate injection according to a rating scale previously described by Sperk et al. (1985) and Baran et al. (1985) with slight modifications, developed by direct comparison with seizure scores used in the amygdala kindling model (Racine, 1972). The following scores were used for rating the severity of kainate-induced seizures: 0, no seizures; 1, eye closure, twitching of vibrissae, sniffing, facial clonus, staring; 2, head nodding associated with more severe facial clonus; 3, unilateral or bilateral forelimb clonus; 4, rearing, often accompanied by bilateral forelimb clonus; 5, rearing with loss of balance and falling accompanied by generalised clonic seizures; 6, sustained generalised clonic convulsions (convulsive status epilepticus). Additionally, other behavioural alterations were observed following kainate injection: reduced movement activity, which occurred at about 5 min after kainate application, lasted up to about 30 min and was often followed by hyperlocomotion; generalized myoclonic twitches; Straub tail; salivation; wet dog shakes. The number of wet dog shakes was counted in each animal.

2.4. Kynurenic acid determination

3 h after injection of kainate or saline, all rats were decapitated, blood was collected in tubes containing EDTA, and the brain was rapidly removed, shortly immersed in liquid nitrogen, and then dissected within 4 min on a cold plate at −10°C into 11 brain regions as described elsewhere (Löscher et al., 1984, 1989). The following regions were dissected bilaterally: olfactory bulb, frontal cortex, piriform cortex, amygdala, hippocampus, nucleus accumbens, striatum (i.e., caudate/putamen), thalamus, tectum (i.e., superior and inferior colliculus), pons/medulla, and cerebellar cortex. After dissection, the individual (bilaterally pooled) regions were rapidly weighed and homogenized with an

Ultra-Turrax in 1.2 ml of 0.1 M HCl. After mixing for 10 min, the samples were centrifuged at 4°C for 15 min at 18000 r.p.m. 1 ml of the supernatants was applied to a Dowex-50W cation-exchange resin in a Pasteur pipette after prewashing with 1 ml 0.1 M HCl. After application of the supernatant onto the column, the column was washed with 1 ml of 0.1 M HCl and 1 ml of water, and the fraction containing kynurenic acid was eluted with 2 ml of water (Turski et al., 1989). The eluates were deep-frozen at −80°C, lyophilized under vacuum and then stored at −80°C until analysis. Immediately prior to analysis, the lyophilized samples were reconstituted in 300 μl of water. Kynurenic acid was measured by isocratic high-performance liquid chromatography (HPLC) using fluorometric detection. In short, a 100-μl aliquot of the resuspended residue was applied to a 3 μm Spherisorb ODS HPLC column (125 × 4 mm internal diameter; Bischoff Analysentechnik, Leonberg). Kynurenic acid was isocratically eluted with a mixture of two mobile phases (A and B) which were simultaneously pumped through the column by two pumps at a flow rate of 0.4 ml/min and a relation of 60% mobile phase A and 40% mobile phase B. Mobile phase A consisted of 0.5 M zinc acetate (to enhance the fluorescent yield of kynurenic acid; Shibata, 1988) and mobile phase B of 0.1 M sodium acetate/40% acetonitrile (adjusted to pH 6.3 with glacial acetic acid). The temperature of the column oven was set to 55°C. Under these conditions, kynurenic acid had a retention time of 4.5 min. Kynurenic acid was detected by a fluorescence detector at an excitation wavelength of 344 nm and an emission wavelength of 389 nm. Details of the HPLC apparatus and the fluorescence detector used have been given elsewhere (Löscher et al., 1993).

For determination of kynurenic acid in plasma, the blood samples were immediately centrifuged at 14000 r.p.m. for 5 min. To 0.6 ml plasma aliquots, 0.6 ml of 0.2 M HCl was added, the sample was mixed and then centrifuged for 5 min at 14000 r.p.m. 1 ml of the supernatants then underwent the same clean-up and concentration procedure as described above for brain tissue extracts, and 100 μl of the resuspended eluate was applied to the HPLC column.

The sensitivity limit of the HPLC method was 170 fmol of kynurenic acid per injection (signal/noise ratio = 3). Based on this sensitivity limit, several preliminary experiments were done to determine how much tissue per brain region had to be used to allow reliable measurement of endogenous kynurenic acid. These preliminary experiments showed that, dependent on tissue weight and endogenous kynurenic acid levels of the respective brain regions, kynurenic acid could be reliably determined in pons/medulla and cerebellar cortex of individual rats, whereas all other regions had to be pooled from several animals (two animals for olfactory bulb and striatum, three animals for frontal cortex, nucleus accumbens, thalamus, hippocampus and tectum, and five animals for piriform cortex and amygdala, respectively) to allow reliable determination well above the detection limit. Because of the relatively high kynurenic acid levels in plasma (see Results), kynurenic acid could be reliably measured in 0.6 ml plasma samples from individual rats. Standard curves for kynurenic acid added to plasma or brain tissue samples before the extraction and purification procedures gave linear responses over a wide range of concentrations. The reproducibility of kynurenic acid determinations in brain tissue was determined by adding 1 pmol kynurenic acid to a brain tissue homogenate and then repeating kynurenic acid analysis in 10 aliquots from this homogenate, which yielded a coefficient of variation of 8.8%. The overall loss of kynurenic acid during the extraction, purification and lyophilization procedures of brain samples amounted to 24% (mean of five determinations).

2.5. Statistics

The significance of differences in kynurenic acid levels between kainate-treated animals and controls was calculated by the Mann-Whitney U-test (two-sided). Linear regression analysis was used to calculate relationships between alterations in individual kynurenic acid levels and seizure severity in response to kainate.

3. Results

3.1. Kainate-induced seizures

Systemic administration of 10 mg/kg kainate (s.c.) caused the complex behavioural syndrome described in detail previously (Baran et al., 1985; Ben-Ari, 1985; Sperk et al., 1985; Sperk, 1994). The first behavioural alterations observed after administration of kainate were hypolocomotion, followed by hyperlocomotion, wet dog shakes, mouth clonus, salivation, sniffing and head nodding. The severity of seizures slowly increased and maximum seizure severity with sustained generalized clonic convulsions was observed during the last 40–60 min of the 3-h observation period. In contrast to the time course of seizure severity, maximum numbers of wet dog shakes were determined between 60 and 80 min after kainate injection. The severity of kainate-induced behavioural alterations differed between individual animals. About 60% of the 30 rats treated with kainate developed generalized (score 3–5) seizures. In most of these animals, there was little or no respite between seizure episodes (status epilepticus). 20% of the rats developed only mild (focal) seizures (mostly

score 2), i.e. head nodding and facial clonus. A further 20% of the rats exhibited sustained wet dog shakes but no clear signs of convulsive activity. In the 30 saline-treated controls, no behavioural alterations were noted.

3.2. Kainate-induced alterations in kynurenic acid levels

In controls, kynurenic acid levels showed an uneven regional distribution in the brain (Fig. 1). As reported previously (Swartz et al., 1990), the highest levels were determined in pons/medulla and olfactory bulb. In plasma, kynurenic acid levels were more than 10 times higher than in brain regions (Fig. 2).

Kainate induced marked kynurenic acid increases in both plasma and brain (Figs. 1 and 2). Except for pons/medulla, nucleus accumbens and hippocampus, the increases in kynurenic acid levels were statistically significant. The lack of statistical significance of increases in some regions was due to the large variation in kynurenic acid levels, which was the reason for using a non-parametric test for statistical evaluation.

In terms of percent increase, the most marked kynurenic acid increases were found in the cerebellar cortex (480% above controls), piriform cortex (310%), amygdala (250%), tectum (190%) and striatum (180%), whereas increases were less marked in frontal cortex (100%), thalamus (96%), and olfactory bulb (51%). In plasma, kynurenic acid levels increased by 210% above controls. Despite this significant average increase in kynurenic acid levels in the plasma of kainate-treated rats, it should be noted that four of the 30 rats (one with wet dog shakes, two with stage 2, one with stage 4 seizures, respectively) exhibited no plasma kynurenic acid increase but marked kynurenic acid increases in those brain regions in which kynurenic acid levels could be determined in individual rats, thus strongly indicating that the brain kynurenic acid increases were not secondary to peripheral alterations in kynurenine metabolism.

As described above, the individual seizure severity differed among the 30 animals treated with kainate. Thus, it was possible to compare seizure severity and kynurenic acid increases in those regions (pons/medulla, cerebellar cortex) in which kynurenic acid could be determined in individual rats. For respective

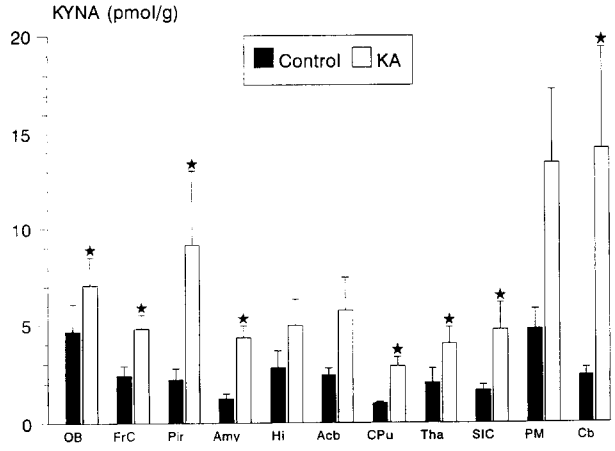

Fig. 1. Kynurenic acid levels in brain regions of rats 3 h after s.c. injection of saline (controls) or kainate (10 mg/kg s.c.). Data (pmol kynurenic acid per g wet weight) are shown as means+S.E. for 30 rats per group. Whereas data from pons/medulla and cerebellar cortex are means+S.E. from determinations in individual rats, all other regions had to be pooled from several animals (two animals for olfactory bulb and striatum, three animals for frontal cortex, nucleus accumbens, thalamus, hippocampus and tectum, and five animals for piriform cortex and amygdala) so that the number of determinations per brain region differ ($n = 30$ for pons/medulla and cerebellar cortex, $n = 15$ for olfactory bulb and striatum, $n = 10$ for frontal cortex, nucleus accumbens, thalamus, hippocampus and tectum, and $n = 6$ for piriform cortex and amygdala). Significant differences from controls (P at least < 0.05) are indicated by asterisks. Abbreviations: KYNA, kynurenic acid; KA, kainate; OB, olfactory bulb; FrC, frontal cortex; Pir, piriform cortex; Amy, amygdala; Hi, hippocampus; Acb, nucleus accumbens; Cpu, caudate/putamen; Tha, thalamus; SIC, superior and inferior colliculus; PM, pons/medulla; Cb, cerebellar cortex.

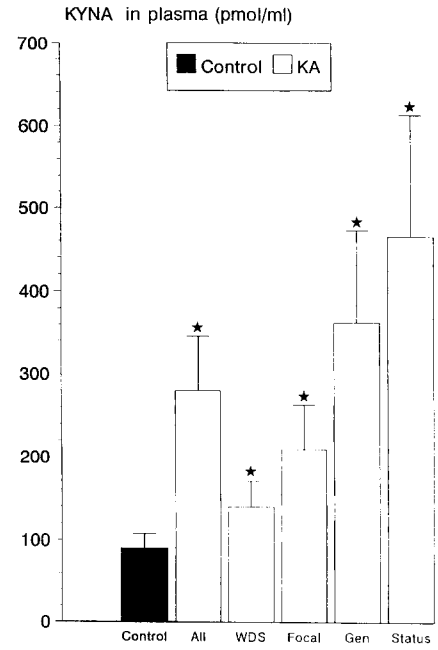

Fig. 2. Kynurenic acid levels in plasma of rats 3 h after s.c. injection of saline or kainate (10 mg/kg s.c.). Data (pmol kynurenic acid per ml plasma) are shown as means+S.E. Control data are from 30 rats. The data shown for 'all' are from 30 rats treated with kainate. The other bars are for subgroups of those 30 kainate-treated rats that were subdivided according to their behavioural syndrome. Wet dog shakes are data from seven rats which exhibited only stereotypies (wet dog shakes) in response to kainate. 'Focal' are data from six rats with score 1–2 seizures, whereas 'gen' are data from 17 rats with generalized (score 3–6) seizures in response to kainate. 12 of the 17 rats with generalized seizures had developed status epilepticus at time of kynurenic acid determinations; this subgroup with status is shown as a separate column ('Status'). Significant differences from controls are indicated by asterisks (P at least < 0.05). Abbreviations: KYNA, kynurenic acid; KA, kainate; WDS, wet dog shakes.

calculations, rats only exhibiting wet dog shakes were scored '0'. By use of linear regression analysis, no significant relationships between individual seizure severity and kynurenic acid levels were found for pons/medulla ($r = 0.015$) or cerebellar cortex ($r = 0.034$). Similarly, no significant relationship was calculated for seizure severity and kynurenic acid levels in plasma ($r = 0.39$).

For further exploration of the differences in kynurenic acid increases between rats differing in their convulsive response to kainate, kynurenic acid levels were separately calculated for subgroups with generalized seizures (score 3–6; further subdivided into animals with and without status), focal seizures (score 1–2), and wet dog shakes only. As shown in Fig. 2, significant kynurenic acid increases in plasma were seen in all subgroups of kainate-treated rats, but the extent of the kynurenic acid increase seemed to be related to the seizure severity. The highest kynurenic acid levels (415% above control) in plasma were determined in rats with status epilepticus, whereas increases in animals with wet dog shakes or focal seizures were 55% and 131%, respectively. In pons/medulla, where no significant kynurenic acid increase had been found when kynurenic acid levels from all kainate-treated rats were used for calculation (Fig. 1), a significant (285%) increase was calculated for rats exhibiting status epilepticus (Fig. 3). In cerebellar cortex, differences between the subgroups were less marked (Fig. 3). Again, the highest kynurenic acid increase (630% above controls) was seen in rats with status epilepticus, but significant increases of 385% above controls were also seen in rats without status epilepticus. As in plasma, cerebellar kynurenic acid levels were also significantly increased in the subgroups with focal (score 1–2) seizures ($P < 0.05$) and wet dog shakes ($P < 0.01$) only (not illustrated).

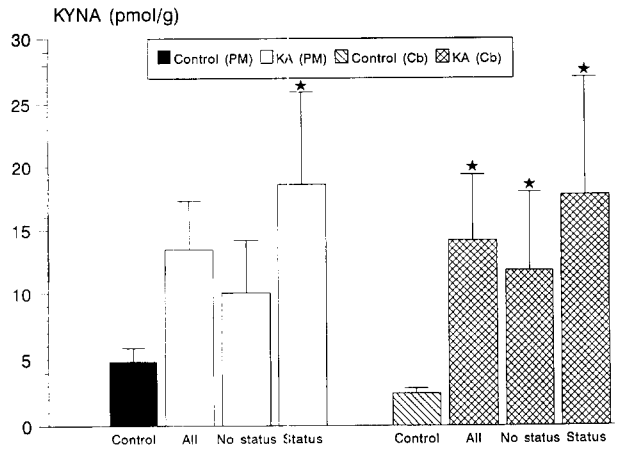

Fig. 3. Kynurenic acid levels in pons/medulla and cerebellar cortex of rats 3 h after s.c. injection of saline or kainate (10 mg/kg s.c.). Data (pmol kynurenic acid per g wet weight) are shown as means + S.E. Control data are from 30 rats. The data shown for 'all' are from 30 rats treated with kainate. The other bars are from subgroups of these 30 kainate-treated rats which were subdivided according to their behavioural syndrome. The data shown for 'no status' are from 18 rats which exhibited wet dog shakes, focal, and/or generalized seizures but no status epilepticus, whereas 'status' shows the data from 12 rats that developed status epilepticus in response to kainate. Significant differences to controls are indicated by asterisks (P at least < 0.05). There was no significant difference between kynurenic acid levels shown for 'status' and 'no status' in the two brain regions. Abbreviations: Cb, cerebellum; KYNA, kynurenic acid; KA, kainate; PM, pons/medulla.

4. Discussion

The present results demonstrate that systemic administration of a convulsant dose of kainate markedly enhanced endogenous levels of kynurenic acid in several brain regions and in the plasma of rats. Kainate is generally thought to be a useful tool in epilepsy research, but its ability to induce brain damage both directly by an excitotoxic mechanism and indirectly by seizure-related mechanisms poses problems in interpreting pathophysiological findings in the kainate model (Olney et al., 1986; Sperk, 1994). In contrast to a recent proposal (Wu and Schwarcz, 1994), the present data indicate that the increase in kynurenic acid levels in brain regions and in plasma was not only a response to sustained convulsive seizures induced by kainate, since kynurenic acid increases also occurred in rats that only exhibited stereotyped behaviour (i.e., wet dog shakes).

Kainate represents the prototype pharmacological agonist for pre-and postsynaptic kainate subtypes of glutamate receptors (Watkins et al., 1990). The long-lasting convulsive state induced by kainate is accompanied by widespread neuropathological damage in several brain areas, particularly in limbic structures (Sperk et al., 1983; Ben-Ari, 1985; Olney et al., 1986; Sperk, 1994). It has been proposed that kainate causes most of its neurotoxic effects through activation or presynaptic stimulation of glutamate or aspartate release (Ferkany et al., 1982; Olney et al., 1986), and that excessive activation of glutamate receptors, particularly the NMDA subtype, may be responsible for the seizures and neuronal damage in response to kainate (Olney et al., 1986). Indeed, NMDA receptor antagonists, such as MK-801 (dizocilpine), were shown to protect rats against kainate-induced seizures and neuronal damage (e.g., Fariello et al., 1989; Clifford et al., 1982; Virgili et al., 1992; Baran et al., 1994). Furthermore, the glycine site ligand D-cycloserine was recently reported to potently block kainate-induced seizures (Baran et al., 1994). Since kynurenic acid is a broad-spectrum antagonist of NMDA and non-NMDA receptors and has a particularly high affinity for the glycine site (Carter, 1992; Stone, 1993), increases in endogenous

kynurenic acid in response to kainate could be interpreted as an attempt of the brain to protect itself against the excitotoxic effects of this powerful convulsant. Indeed, kynurenic acid has been shown to possess neuroinhibitory, anticonvulsant and neuroprotective properties (Stone, 1993) which could serve to contain the spread of excitotoxic activity induced by kainate. In this respect it is important to note that intracerebroventricular application of kynurenic acid was shown to exhibit potent anticonvulsant activity on seizures induced by kainate (Turski et al., 1985).

In a previous study on kainate-induced alterations of kynurenic acid production in rats, kynurenic acid synthesis from its bioprecursor kynurenine was studied using brain slices and in vivo microdialysis (Wu et al., 1991). In vitro, the production of extracellular kynurenic acid in tissue slices obtained from rats 3 h after the injection of kainate (10 mg/kg s.c.) did not differ from control values in any of the three brain areas (i.e., piriform cortex, hippocampus, striatum) examined. In contrast, a significant increase in in vitro kynurenic acid production was observed in the piriform cortex and hippocampus 1 month after kainate injection. Similarly, in microdialysis experiments performed in the piriform cortex after exposure to kynurenine, no change in extracellular kynurenic acid concentration was detected within 4 h after kainate administration, whereas a significant increase was observed 1 month later (Wu et al., 1991). However, due to the relatively insensitive HPLC method used in this study, endogenous levels of kynurenic acid could not be determined in these experiments. Subsequent experiments of Wu et al. (1992) on quinolinate-induced excitotoxicity with a more sensitive HPLC method demonstrated that alterations in endogenous kynurenic acid may markedly differ from those in situations where exogenous L-kynurenine is used to study kynurenic acid production, possibly suggesting the existence of different intracellular kynurenic acid pools. The present study now shows that endogenous kynurenic acid levels in various brain regions markedly increase as early as 3 h after kainate treatment. This corresponds to a more recent report of Wu and Schwarcz (1994), only available in abstract form, in which extracellular kynurenic acid levels were found to rise within 1 h and gradually reached a plateau about 4 h after administration of kainate, 10 mg/kg, in rats.

Kynurenic acid biosynthesis in the rat brain takes place predominantly in astroglia (Stone, 1993). Although an increase in tissue content of kynurenic acid in brain regions as found in the present study does not necessarily mean that the functionally relevant extracellular kynurenic acid levels are increased too, recent studies by Wu et al. (1992) with the neurotoxic quinolinate showed that the induced increases in kynurenic acid tissue content were parallelled by comparable increases in extracellular kynurenic acid levels. Furthermore, as described above, Wu and Schwarcz (1994) recently reported that kainate increases extracellular kynurenic acid levels within 1 h after administration. The increase in kynurenic acid production by quinolinate and kainate is due either to a direct stimulatory action on glial kynurenic acid production or to (astro)glia proliferation in response to the produced neurodegeneration, or both (Wu et al., 1991, 1992; Stone, 1993).

Although kainate increased kynurenic acid levels in almost all brain regions examined in this respect in the present study, there were marked differences in the extent of the increases across brain regions. In addition to regional differences in synthesis rates of kynurenic acid and susceptibility to kainate, this might be due to the fact that a time course study was not performed. It appears likely that the maximal response of kynurenic acid levels occurs at different times in different brain regions. Furthermore, the maximal responses certainly do not occur just 3 h after kainate injection, possibly explaining the lack of significant alterations in the hippocampus, which has the highest density of kainate receptors in the brain (Ben-Ari, 1985). The high kynurenic acid increase in the piriform cortex was not unexpected since this region is known as the area which is most severely affected by systemic kainate treatment (Schwob et al., 1980; Sperk et al., 1983; Ben-Ari, 1985). Unexpected findings include the large increase in the cerebellum, since the latter region is not known to be particularly sensitive to systemic administration of kainate. Furthermore, the cerebellum displays lower activities of the kynurenic acid synthesizing enzyme kynurenine aminotransferase than, for instance, the hippocampus does (Okuno et al., 1991). The cerebellum is among those brain regions with high concentrations of high-affinity kainate binding sites (Sperk, 1994). Furthermore, time course studies of kainate-induced alterations in calmodulin kinase II activity, i.e. an enzyme that may contribute to kainate neurotoxicity, have shown a trend toward an increase in the cerebellum 2–4 h following administration of 10 mg/kg in rats (Wasterlain et al., 1992), which indicates that the cerebellum is involved in an early phase of the excitotoxic effects of kainate. With respect to the large cerebellar kynurenic acid increase in response to kainate found in the present experiments, it is interesting to note that kynurenic acid has been reported to antagonize responses to NMDA and non-NMDA receptor stimulation in this region, with a particularly high potency against responses to kainate (Kano et al., 1988).

In addition to the brain, kainate induced marked kynurenic acid increases in plasma which, to our knowledge, has not been reported previously. In the periphery, the kynurenic acid precursor kynurenine is

the primary product of tryptophan degradation (Bender, 1989). Kynurenic acid is present in several peripheral tissues, such as liver and kidney, in much higher levels than in the brain (Moroni et al., 1988a). The absolute magnitude of the kainate-induced kynurenic acid increases in plasma seen in the present study cannot be explained by an efflux of kynurenic acid from brain tissue but indicates either a direct effect of kainate on kynurenic acid production in peripheral tissues or interference with the degradation or elimination of kynurenic acid. Alternatively, the observed correlation between kynurenic acid levels in plasma with the intensity of seizure activity could suggest that plasma kynurenic acid levels increase in response to behavioural manifestations of seizure activity, rather than to kainate. However, as discussed above, the finding that plasma (and brain) kynurenic acid levels were also significantly increased in rats without signs of convulsive seizures argues against the latter possibility but suggests a direct (yet unknown) effect of kainate on kynurenic acid production or elimination in peripheral tissues. Theoretically, the elevation of kynurenic acid levels in the brain of kainate-treated rats may only be secondary to an activation of the kynurenine pathway in the liver. Such an activation would elevate plasma kynurenine, which could enter the brain and raise kynurenic acid levels. However, the finding that some rats with marked brain kynurenic acid increases did not exhibit any elevation of plasma kynurenic acid seems to rule out this possibility.

Since findings in the kainate model are thought to bear some relevance to processes which take place in chronic epilepsy (Ben-Ari, 1985; Olney et al., 1986; Sperk, 1994), the present data on kynurenic acid levels in the brain indicate that this endogenous anticonvulsant and neuroprotective compound may be critically involved to limit the spread of seizure activity. Indeed, an increased neosynthesis of kynurenic acid by pharmacological modification of kynurenine catabolism, resulting in similar regional increases of kynurenic acid levels in the brain as observed in the present study, was recently shown to block electroshock seizures in rats (Carpenedo et al., 1994). In contrast to previous suggestions that kynurenic acid is only involved in later stages of chronic epilepsy, i.e. once gliosis has developed (Wu et al., 1991; Du et al., 1993), the present data demonstrate that kynurenic acid levels increase early during the onset of epileptic activity. In a recent clinical study, a tendency to increased kynurenic acid levels was found interictally and postictally in the cerebrospinal fluid of patients with intractable complex partial seizures (Heyes et al., 1994). However, in contrast to the present findings in the kainate model, kynurenic acid levels were significantly decreased in the plasma of epileptic patients (Heyes et al., 1994). Since almost all patients were receiving polytherapy with antiepileptic drugs, this might have affected kynurenic acid levels, which was indeed suggested by entering drug concentrations as a covariate (Heyes et al., 1994).

In conclusion, the present data add to the accumulating evidence that endogenous kynurenic acid plays a pathogenic role in seizure genesis and/or propagation. The finding that kynurenic acid levels were not only increased in rats with severe kainate-induced seizures but also in animals without any overt convulsive activity might indicate that kynurenic acid production is immediately enhanced in response to neuroexcitation and serves as an endogenous anticonvulsant and neuroprotective agent to limit or, in some animals, even prevent seizure induction and spread. In line with this assumption, kynurenic acid has previously been shown to block kainate-and quinolinate-induced seizures and to retard kindling-induced epileptogenesis (Foster et al., 1984; Turski et al., 1985; Thompson et al., 1988). Although it has been argued that endogenous kynurenic acid levels are too low to be of physiological relevance, pharmacological increases in endogenous kynurenic acid levels in the brain have been shown to protect against chemically or electrically induced seizures (Stone, 1993; Carpenedo et al., 1994). Drugs capable of specifically affecting cerebral kynurenic acid metabolism may thus be useful not only for the study of the endogenous functions of kynurenic acid but also as a new strategy for treatment of epilepsy and other brain diseases where alterations in kynurenic acid metabolism have been suggested. However, in view of the importance of glutamate receptors, particularly those of the NMDA type, in the processes of learning and memory, it should be noted that elevated concentrations of kynurenic acid have been related to conditions associated with cognitive deficits (Moroni et al., 1988b; Heyes et al., 1990; Gramsbergen et al., 1992). Thus, such negative aspects of elevations in kynurenic acid should be considered in therapeutic strategies involving pharmacological enhancement of kynurenic acid formation in the brain. A possibility to protect NMDA receptors from inactivation while leaving intact the neuroprotective potential of kynurenic acid acting at other glutamate receptor types could be achieved by combining drugs which increase the cerebral formation of kynurenate with putative cognition enhancers such as D-cycloserine, which reverse kynurenic acid antagonism at NMDA receptors (Pittaluga et al., 1995) and exert anticonvulsant activity against different seizure types, including seizures induced by kainate (Baran et al., 1994).

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