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European Journal of Pharmacology 281 (1995) 55–61

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L-α-Aminoadipic acid as a regulator of kynurenic acid production in the hippocampus: a microdialysis study in freely moving rats

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Received 19 January 1995; revised 5 April 1995; accepted 6 April 1995

Abstract

L-α-Aminoadipic acid is a lysine metabolite with neuroexcitatory properties, and has previously been shown to inhibit the production of the broad spectrum excitatory amino acid receptor antagonist kynurenic acid in brain tissue slices. The effects of L-α-aminoadipic acid on the levels of extracellular kynurenic acid were now studied by microdialysis in the dorsal hippocampus of freely moving rats. Application of L-α-aminoadipic acid through the microdialysis probe dose dependently decreased both the concentration of endogenous kynurenic acid and of kynurenic acid which was produced de novo from its bioprecursor L-kynurenine (500 μM applied through the probe). 500 μM L-α-aminoadipic acid lowered the kynurenic acid concentration in the dialysate by 47% and 28% with and without precursor loading, respectively, whereas D-α-aminoadipic acid was without effect. Co-administration of 500 μM L-α-aminoadipic acid with 50 μM veratridine, which by itself produces a substantial decrease in the levels of extracellular kynurenic acid, did not result in a further reduction in kynurenic acid concentrations. Extensive neuronal degeneration caused by an intrahippocampal injection of quinolinic acid (120 nmol) did not interfere with the effect of L-α-aminoadipic acid. Taken together, these data suggest that the effect of L-α-aminoadipic acid on extracellular kynurenic acid levels is likely due to its direct action on astrocytes, which are known to harbor kynurenic acid's biosynthetic enzyme, kynurenine aminotransferase. L-α-Aminoadipic acid may modulate kynurenic acid function in the brain and thus play a role in the pathogenesis of neurodegenerative and seizure disorders.

Keywords: L-α-Aminoadipic acid; Excitotoxicity; Kynurenic acid; Kynurenine; Microdialysis; Neurodegeneration

1. Introduction

Kynurenic acid is a broad spectrum excitatory amino acid receptor antagonist which possesses relatively high affinity for the glycine co-agonist site of the *N*-methyl-D-aspartate (NMDA) receptor (Stone, 1993). In addition to its common use as a convenient pharmacological tool, kynurenic acid has received attention as a possible endogenous modulator of excitatory amino acid receptor function in the brain. Recent evidence has suggested that kynurenic acid, which is present in the mammalian brain in nanomolar to micromolar concentration, may serve as the brain's own defense against overactivation of excitatory amino acid receptors, and against pathological consequences such as excitotoxicity and seizures (Schwarcz et al., 1992). Thus, kynurenic acid levels increase during periods of seizure activity (Wu and Schwarcz, 1994), and neuroprotection and seizure reduction have been reported in situations where brain kynurenic acid concentrations were elevated experimentally (Russi et al., 1992; Nozaki and Beal, 1992; Vécsei et al., 1992; Carpenedo et al., 1994). It was also shown that non-specific kynurenic acid synthesis inhibitors such as aminooxyacetic acid (Urbańska et al., 1991; Beal et al., 1991; McMaster et al., 1991) and γ-acetylenic GABA (McMaster et al., 1993) are capable of causing excitotoxic lesions upon intracerebral application to experimental animals. The

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SSDI 0014-2999(95)00224-3

idea that kynurenic acid plays a role in excitatory amino acid receptor function is further supported by neuroanatomical studies which have demonstrated the presence of kynurenic acid's biosynthetic enzyme, kynurenine aminotransferase, in astrocytic processes closely apposed to glutamatergic synapses (Schwarcz et al., 1992).

In the rat brain, extracellular kynurenic acid concentrations are low (approximately 20 nM) but readily increase after the administration of L-kynurenine, the natural substrate of kynurenine aminotransferase (Speciale et al., 1990; Swartz et al., 1990; Wu et al., 1992a,b). Several reports, studying either endogenous kynurenic acid or the de novo production of kynurenic acid from kynurenine, have demonstrated that kynurenic acid levels in the extracellular compartment can be influenced, for example, by depolarizing agents, experimental neuronal ablation or pharmacological manipulation of the kynurenine pathway (Turski et al., 1989; Wu et al., 1992a,b; Carpenedo et al., 1994). In most cases, these effects were first noted in studies with brain tissue slices and were subsequently confirmed in vivo using brain microdialysis.

Extracellular kynurenic acid concentrations *decrease* under depolarizing conditions, i.e. after tissue exposure to high K^+ concentrations, veratridine or certain excitatory amino acids. The mechanisms by which these agents reduce kynurenic acid levels are not fully understood but are likely to involve both indirect and direct actions on kynurenic acid-producing astrocytes (Wu et al., 1992a,b). Since this phenomenon could constitute a means to regulate brain kynurenic acid function, we now selected the most potent endogenous agent found in vitro, L-α-aminoadipic acid (Gramsbergen et al., 1989), for a detailed in vivo microdialysis study in rats. L-α-Aminoadipic acid is a lysine metabolite which exists in mammalian brain in micromolar concentration (Bernasconi et al., 1988; Chang et al., 1990; Rao and Chang, 1990; Tsai, 1992). Its properties as a glutamate uptake inhibitor (Fletcher and Johnston, 1991; Robinson et al., 1991), postsynaptic excitatory amino acid receptor agonist (Hall et al., 1977), excitotoxin (Garthwaite and Regan, 1980; Olney et al., 1980) and gliotoxin (Olney et al., 1980; Huck et al., 1984a; Takada and Hattori, 1986; Kato et al., 1990) are well documented, but only sporadic efforts have been made to examine its putative neuromodulatory role. In the present set of experiments, L-α-aminoadipic acid was applied through the microdialysis probe to investigate its pharmacological effect on kynurenic acid metabolism and, possibly, to mimic its function as an endogenous regulator of kynurenic acid levels. All studies were performed in the rat hippocampus, a brain area that is exquisitely sensitive to excitotoxic insults (Köhler, 1984). Some of the data described here have been published in abstract form (Schwarcz and Wu, 1991).

2. Materials and methods

2.1. Chemicals

L-α-Aminoadipic acid, D-α-aminoadipic acid, kynurenic acid, L-kynurenine, quinolinic acid, veratridine and tetrodotoxin were purchased from Sigma (St. Louis, MO). All other chemicals were of the highest commercially available purity.

2.2. Animals

Male Sprague-Dawley rats (180–220 g), housed under standard laboratory conditions at a 12 h/12 h light/dark cycle with free access to food and water, were used in all experiments.

2.3. Quinolinic acid lesion of the hippocampus

Under chloral hydrate (360 mg/kg, i.p.) anesthesia, animals were placed in a David Kopf stereotaxic frame (Tujunga, CA), with the upper incisor bar set at 2.4 mm below the interaural line. 7 days prior to the microdialysis experiments, a unilateral injection of quinolinic acid (120 nmol/1 μl, pH 7.4) was made over 10 min into the dorsal hippocampus (A: 3.4 mm behind bregma, L: 2.3 mm from the midline, V: 3.0 mm below dura) as described previously (Wu et al., 1992b).

2.4. Microdialysis

On the day before a microdialysis experiment, the animals were anesthetized with chloral hydrate (360 mg/kg, i.p.) and mounted in the stereotaxic frame. A guide cannula (outer diameter: 0.65 mm) for the microdialysis probe was positioned over the dorsal hippocampus (1 mm below the dura), using identical stereotaxic coordinates as for intrahippocampal injections (cf. above), and secured to the skull with acrylic dental cement. In experiments with quinolinic acid-lesioned rats, the guide cannula was implanted 6 days after the intrahippocampal injections.

1 day after the surgery, a microdialysis probe (CMA/10, membrane length: 2 mm, Carnegie Medicin, Stockholm, Sweden) was inserted through the guide cannula, extending vertically throughout the dorsal hippocampus. Ringer solution (144 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.7 mM $CaCl_2$, pH 6.7) was delivered at a flow rate of 1 μl/min with a microinjection pump (CMA/100, Carnegie Medicin) for 1 h to establish stable conditions for dialysis. Freshly prepared kynurenine (500 μM in Ringer solution; Wu et al., 1992b) was then perfused for 2–3 h. Subsequently, the test compounds (L-α-aminoadipic acid, D-α-aminoadipic acid, veratridine, tetrodotoxin) were added to the solution containing kynurenine, and perfusion

continued for an additional 4 h. Control animals were perfused with 500 μM kynurenine for a total of at least 7 h. Dialysates were collected every 30 min. In the experiments designed to study the fate of *endogenous* kynurenic acid, the animals were first perfused with Ringer solution for 3 h. L-α-Aminoadipic acid or D-α-aminoadipic acid, dissolved in Ringer solution, was then delivered through the dialysis probe for 2.5 h. After discontinuation of the perfusion with L- or D-α-aminoadipic acid, recovery from the effect induced by the test compounds was studied for another 2.5 h (perfusion with Ringer solution only).

2.5. Determination of kynurenic acid in the dialysate

For the determination of kynurenic acid in the dialysate in the presence of kynurenine, each 30 μl fraction was mixed with 30 μl of 1 M HCl and applied to a Dowex 50 W ion exchange column (H^+-form). The column was then washed with 1 ml 0.1 M HCl and 1 ml distilled water. Kynurenic acid was eluted with 2 ml of distilled water (Turski et al., 1989), and the eluate was lyophilized. The samples were resuspended in 300 μl water, and 200 μl were applied to a 3 μm C_{18} high performance liquid chromatography (HPLC) column (100 × 3.2 mm i.d., Bioanalytical Systems, West Lafayette, IN). Kynurenic acid was eluted isocratically at a flow rate of 0.5 ml/min utilizing a mobile phase of 50 mM ammonium acetate containing 5% methanol. Kynurenic acid was detected spectrophotometrically at 340 nm.

Endogenous kynurenic acid in the dialysate was determined according to the methodology developed by Shibata (1988) as modified by Wu et al. (1992a). Briefly, each 30 μl dialysate was directly applied to an HPLC system coupled with a fluorescence detector (Perkin Elmer, LC 240) set at an excitation wavelength of 344 nm and an emission wavelength of 398 nm. The system utilized a mobile phase of 50 mM sodium acetate, 0.25 M zinc acetate and 4% acetonitrile (pH 6.3), pumped through an 8 cm HR-80, 3 μm reverse-phase column (ESA, Bedford, MA) at a flow rate of 1.0 ml/min.

2.6. Histological analysis

The correct position of the microdialysis probe was examined in every animal by routine histological procedures. Briefly, animals were deeply anesthetized with chloral hydrate (400 mg/kg, i.p.), perfused transcardially with 4% paraformaldehyde, and their brain was removed and postfixed for 48 h. Coronally cut 30 μm cryostat sections were stained with thionin and observed in a light microscope. Only data from animals with proper placement of the probe were used for experimental analysis.

The success of intrahippocampal quinolinic acid injections was also inspected by light microscopy. Only animals which showed the characteristic massive neuronal degeneration in the dorsal hippocampus (Schwarcz et al., 1983) were used for data analysis.

2.7. Data analysis

Kynurenic acid concentrations were not corrected for recovery through the dialysis probe (cf. Wu et al., 1992b). Kynurenic acid levels after treatment with test compounds were expressed as a percentage of basal levels, i.e. the mean of the last three samples collected prior to drug administration (= time 0). Kynurenic acid levels in control animals were also calculated as a percentage of the last three samples prior to time 0. The effects of the treatment with test compounds, compared to respective controls, were evaluated by two-way analysis of variance (ANOVA) with Newman-Keuls post-hoc analysis. Changes in endogenous kynurenic acid following L-α-aminoadipic acid application and withdrawal were statistically evaluated in comparison to control data obtained at the same time-points using one-way ANOVA followed by Dunnett's test.

3. Results

3.1. De novo kynurenic acid production from kynurenine: controls

In agreement with a previous report (Wu et al., 1992b), steady-state concentrations of extracellular kynurenic acid were obtained within 2 h of perfusion with 500 μM kynurenine alone. During continuous perfusion with 500 μM kynurenine for an additional 4 h, kynurenic acid concentrations did not deviate from basal levels by more than 15% at any timepoint (Fig. 1).

3.2. L-α-Aminoadipic acid-induced inhibition of de novo kynurenic acid production

The effects of different concentrations of L-α-aminoadipic acid on the extracellular levels of kynurenic acid were examined in the presence of 500 μM kynurenine. In a dose-dependent fashion, L-α-aminoadipic acid caused a decrease in the kynurenic acid content of the dialysate (Fig. 1). 300 μM and 500 μM of L-α-aminoadipic acid resulted in a 25 ± 4% and 47 ± 5% decrement, respectively, by 1.5 h of perfusion. No further decrease in extracellular kynurenic acid occurred when the L-α-aminoadipic acid concentration was raised to 1 mM. D-α-Aminoadipic acid (1 mM) did not affect the production of kynurenic acid from kynurenine (Fig. 1).

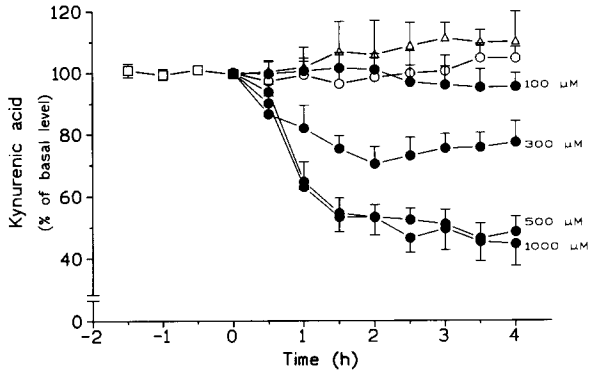

Fig. 1. Effect of various concentrations of L-α-aminoadipic acid on the levels of de novo produced extracellular kynurenic acid in hippocampal dialysate. Rats were perfused with a combination of kynurenine (500 µM) and various concentrations of L-α-aminoadipic acid (●) (indicated in the Fig.) or 1 mM D-α-aminoadipic acid (△). Baseline data (□) represent cumulative values compiled from all six experimental groups. Data (means ± S.E.M.) were obtained from six animals per group and are expressed as a percentage of basal levels computed from the last three samples prior to time 0 (15.2 ± 1.2 pmol kynurenic acid/30 µl; $n = 36$). Control animals were treated with 500 µM kynurenine alone (○). D-α-Aminoadipic acid [$F(7,70) = 0.76$; $P > 0.05$] and 100 µM L-α-aminoadipic acid [$F(7,70) = 1.76$; $P > 0.05$] showed no effect on kynurenic acid production. 300 µM [$F(7,70) = 2.42$; $P < 0.05$], 500 µM [$F(7,70) = 13.20$; $P < 0.001$] and 1 mM [$F(7,70) = 26.86$; $P < 0.001$) L-α-aminoadipic acid caused statistically significant decreases in kynurenic acid production.

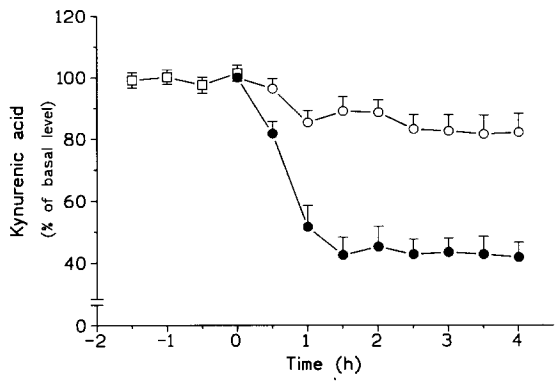

Fig. 2. Effect of L-α-aminoadipic acid on the levels of extracellular kynurenic acid in the quinolinic acid-lesioned hippocampus. Hippocampi lesioned with quinolinic acid were perfused with 500 µM kynurenine in the absence (○) or presence (●) of 500 µM L-α-aminoadipic acid. Lesions were made by unilateral intrahippocampal injection of quinolinic acid (120 nmol/1 µl) 7 days prior to the experiment. Baseline data (□) represent cumulative values compiled from both experimental groups. Data (means ± S.E.M.) were obtained from six animals per group and are expressed as a percentage of basal levels computed from the last three samples prior to time 0 (32.1 ± 4.2 pmol kynurenic acid/30 µl; $n = 12$). L-α-aminoadipic acid-treated and control animals differed significantly for each other [$F(7,70) = 4.93$; $P < 0.01$].

3.3. Effect of L-α-aminoadipic acid on de novo kynurenic acid production in quinolinic acid-lesioned hippocampus

Intrahippocampal infusion of quinolinic acid (120 nmol/1 µl) 7 days prior to microdialysis caused extensive neuronal degeneration and pronounced astrogliosis throughout the dorsal hippocampus. Basal levels in the neuron-depleted hippocampus were approximately twice as high as in the unlesioned hippocampus (cf. legends to Figs. 1 and 2, and also Wu et al., 1992b). 500 µM L-α-aminoadipic acid, applied through the dialysis probe, caused a 54 ± 6% decrease in de novo produced kynurenic acid in the lesioned hippocampus by 1.5 h, as compared to lesioned controls (perfused with kynurenine only) (Fig. 2).

3.4. Comparison of L-α-aminoadipic acid- and veratridine-induced inhibition of de novo kynurenic acid production

L-α-Aminoadipic acid (500 µM) and veratridine (50 µM) had very similar effects on de novo kynurenic acid production, resulting in a nadir of approximately 50% of control levels by 2 h of perfusion (Fig. 3). Co-administration of 500 µM L-α-aminoadipic acid and 50 µM veratridine did not result in additive effects. Thus, joint perfusion of the two compounds did not differ from the effect of either L-α-aminoadipic acid alone or veratridine alone, respectively (Fig. 3). However, in contrast to the veratridine effect (Wu et al., 1992b), the L-α-aminoadipic acid-induced decrease in kynurenic acid levels was not prevented by the inclusion of 5 µM tetrodotoxin in the perfusion solution ($n = 6$; data not shown) [$F(7,70) = 2.08$; $P > 0.05$].

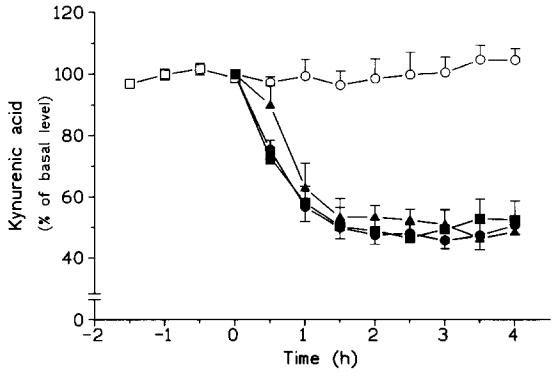

Fig. 3. Effects of L-α-aminoadipic acid (500 µM; ▲) and veratridine (50 µM; ■) on the levels of extracellular kynurenic acid in the presence of 500 µM kynurenine. Control animals were perfused with 500 µM kynurenine for a total of 7 h (○). Solid circles illustrate the effect of co-perfusion with 500 µM L-α-aminoadipic acid and 50 µM veratridine. Baseline data (□) represent cumulative values from all four experimental groups. Data (means ± S.E.M.) were obtained from 6 animals per group and are expressed as a percentage of basal levels computed from the last three samples prior to time 0 (15.5 ± 1.3 pmol kynurenic acid/30 µl; $n = 24$). Joint perfusion of the two compounds did not differ from the effect of either L-α-aminoadipic acid [$F(7,70) = 1.26$; $P > 0.05$] or veratridine [$F(7,70) = 0.34$; $P > 0.05$] alone.

3.5. Effect of L-α-aminoadipic acid on endogenous kynurenic acid levels

The concentration of extracellular endogenous kynurenic acid did not deviate by more than 15% from basal levels (defined as the average of the three 30 min sampling periods prior to time 0) at any timepoint during continuous perfusion with Ringer solution (Fig. 4). In a dose-dependent fashion, L-α-aminoadipic acid lowered the levels of endogenous extracellular kynurenic acid in the hippocampus (Fig. 4), though the effect of L-α-aminoadipic acid appeared to be less pronounced than its reduction of de novo produced kynurenic acid (cf. Fig. 1). Thus, 500 μM and 2 mM L-α-aminoadipic acid decreased the endogenous kynurenic acid concentration in the dialysate by 28 ± 3% ($P < 0.05$) and 37 ± 3% ($P < 0.05$), respectively, 1.5 h after initiation of the perfusion. Discontinuation of perfusion with 500 μM L-α-aminoadipic acid resulted in a return to basal kynurenic acid levels within the next 1.5 h. In contrast, discontinuation of perfusion with 2 mM L-α-aminoadipic acid caused a slower return of extracellular kynurenic acid towards control levels. Perfusion with this high concentration of L-α-aminoadipic acid kept kynurenic acid levels depressed for at least 2.5 h after drug withdrawal. No changes in kynurenic acid levels were observed in animals treated with 2 mM D-α-aminoadipic acid (Fig. 4).

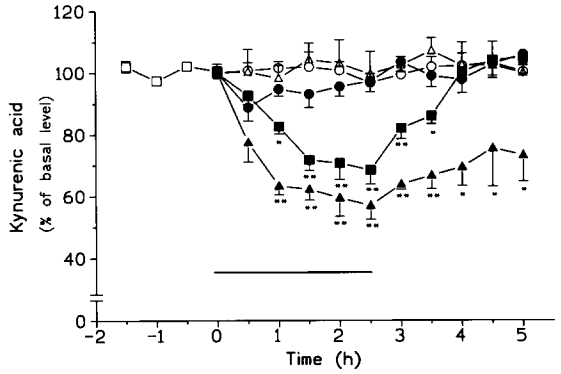

Fig. 4. Effect of 200 μM (●), 500 μM (■) or 2 mM (▲) L-α-aminoadipic acid or 2 mM D-α-aminoadipic acid (△) on the levels of endogenous kynurenic acid in hippocampal dialysate. α-Aminoadipic acid was delivered through the dialysis probe for 150 min (bar). After discontinuation of the perfusion with α-aminoadipic acid, microdialysis continued for an additional 150 min with Ringer solution alone. Controls (○) were continuously perfused with Ringer solution alone. Baseline data (□) represent cumulative values compiled from all five experimental groups. Data (means ± S.E.M.) were obtained from six animals per group and are expressed as a percentage of basal levels computed from the last three samples prior to time 0 (94.5 ± 5.1 fmol kynurenic acid/30 μl; $n = 30$). $^{*}P < 0.05$, $^{**}P < 0.01$ as compared to controls at the same timepoint (one-way ANOVA and Dunnett's test).

4. Discussion

The primary finding of the present study is that L-α-aminoadipic acid is capable of stereospecifically reducing the extracellular concentration of kynurenic acid in the rat hippocampus. Since kynurenic acid is an endogenous antagonist of excitatory amino acid receptors, and may serve a regulatory role in excitatory amino acid neurotransmission, the data therefore suggest that L-α-aminoadipic acid may be an indirect modulator of excitatory amino acid function in the hippocampus, augmenting glutamatergic tone by decreasing the levels of an endogenous glutamatergic antagonist.

Although the presence of L-α-aminoadipic acid in the mammalian brain (approx. 30 μM) has been reported (Bernasconi et al., 1988), very little is currently known about its metabolism and possible function in the brain. In the periphery, L-α-aminoadipic acid is a product of lysine catabolism, with pipecolic acid and L-α-aminoadipic acid δ-semialdehyde as important metabolic intermediates (Rao and Chang, 1990; Chang et al., 1990). The presence of key enzymes of L-α-aminoadipic acid's biosynthetic cascade in the brain has as yet not been ascertained (Chang, 1978; Tsai, 1992), though it is noteworthy that patients suffering from Zellweger syndrome, a peroxisomal disease characterized by the absence of pipecolate oxidase, show distinct neurological and psychiatric symptoms, which are conceivably linked to the presumed deficit in brain L-α-aminoadipic acid (Wanders et al., 1988; Mihalik et al., 1989).

In mammalian cells, L-α-aminoadipic acid is degraded by transamination to α-oxoadipic acid. Interestingly, the enzyme responsible for L-α-aminoadipic acid catabolism, L-α-aminoadipic acid aminotransferase, shares several physico-chemical and biochemical characteristics with kynurenine aminotransferase and was until recently thought to be identical with kynurenic acid's biosynthetic enzyme (Tobes and Mason, 1977). However, the two enzyme activities are now known to be associated with two different proteins (Mawal and Deshmukh, 1991), and the presence and cellular localization of L-α-aminoadipic acid aminotransferase in the brain remains to be examined.

Although no attempts have been made to examine the neurobiology of L-α-aminoadipic acid systematically, the neuropharmacological properties of exogenously supplied L-α-aminoadipic acid have been the subject of several studies. When applied to central neurons, L-α-aminoadipic acid is a rather weak direct excitatory amino acid receptor agonist (Hall et al., 1977). In synaptosomes, L-α-aminoadipic acid is an effective inhibitor of L-glutamate uptake, and has been successfully used as a pharmacological tool to distinguish region-specific glutamate transport sites in the

rat brain (Fletcher and Johnston, 1991; Robinson et al., 1991). L-α-Aminoadipic acid has also been shown to compete with the vesicular uptake of L-glutamate, a potentially critical step in the recycling of neurotransmitter glutamate (Fykse et al., 1992).

It is not immediately apparent how the reduction of extracellular kynurenic acid levels by L-α-aminoadipic acid might be related to its documented interference with excitatory amino acid neurotransmission. Conceivably, L-α-aminoadipic acid may inhibit kynurenic acid production by preventing the cellular uptake of kynurenine (Speciale and Schwarcz, 1990), though L-α-aminoadipic acid does not seem to fulfil the requirements for transport by the large neutral amino acid carrier (Christensen, 1984). It is more likely, however, that the effect of L-α-aminoadipic acid on kynurenic acid levels can be traced to intracellular events which take place after L-α-aminoadipic acid is transported into astrocytes through efficient Na^+-dependent (Huck et al., 1984b) or Na^+-independent (Tsai, 1992) processes. Thus, astrocytes contain the vast majority of kynurenine aminotransferase, which is singularly responsible for the production of kynurenic acid in the rat brain (Okuno et al., 1991; Du et al., 1992). Although L-α-aminoadipic acid does not compete directly with kynurenine for transamination (Okuno et al., 1991), it may interfere with the ability of kynurenine to enter mitochondria, which harbor a substantial proportion of kynurenine aminotransferase (Okuno et al., 1991). Alternatively, L-α-aminoadipic acid may reduce kynurenine aminotransferase activity indirectly, for example by allosteric inhibition, or impede the efflux of kynurenic acid from astrocytes (Turski et al., 1989).

The present study provides direct evidence in support of the idea that astrocytes are at the focus of the interactions between L-α-aminoadipic acid and kynurenic acid. Thus, the effect of L-α-aminoadipic acid was seen in both normal and neuron-depleted tissue, i.e. kynurenic acid production in controls and in the excitotoxin-lesioned hippocampus was reduced to the same degree (cf. Figs. 1 and 2). In absolute terms, L-α-aminoadipic acid in fact caused a *greater* decrease in extracellular kynurenic acid in the degenerated tissue where astrogliosis resulted in a doubling of both endogenous and de novo produced kynurenic acid levels by 7 days after the lesion (Wu et al., 1992a,b). This is in marked contrast to studies with the depolarizing agent veratridine. As described previously (Wu et al., 1992a,b), the reduction of extracellular kynurenic acid levels by veratridine is dependent on the presence of neurons, indicating that this compound, unlike L-α-aminoadipic acid, affects astrocytes indirectly. It has been postulated that veratridine first interacts with tetrodotoxin-sensitive neuronal Na^+ channels and subsequently releases an endogenous agent(s) (such as glutamate or L-α-aminoadipic acid) from neurons, which in turn reduces kynurenic acid production in astrocytes (Wu et al., 1992b). This interpretation is in line both with the lack of additivity of the effects of L-α-aminoadipic acid and veratridine (Fig. 3) and the ineffectiveness of tetrodotoxin in preventing the actions of L-α-aminoadipic acid.

L-α-Aminoadipic acid was more potent in reducing the extracellular concentrations of newly synthesized kynurenic acid than in decreasing endogenous kynurenic acid levels. This discrepancy and the relative resistance of endogenous kynurenic acid to pharmacological depletion in general has been observed previously in studies with veratridine and the non-specific transaminase inhibitor aminooxyacetic acid, and may be due to the existence of two intracellular kynurenic acid pools (Wu et al., 1992a,b). It is also noteworthy that L-α-aminoadipic acid failed to cause a greater than 50% decrement in de novo synthesized kynurenic acid in the present study. This is in contrast to veratridine and aminooxyacetic acid, which at high concentrations reduce the levels of newly produced kynurenic acid by 80% or more (Wu et al., 1992b). The lesser potency of L-α-aminoadipic acid, as well as the rapid reversibility of its action upon withdrawal, might be related to efficient degradation of the amino acid in situ, which could limit L-α-aminoadipic acid's ability to reduce extracellular kynurenic acid levels.

Regardless of its precise mechanism of action, which clearly needs to be elucidated in greater detail, the present data indicate that L-α-aminoadipic acid should be considered as an endogenous modulator of kynurenic acid function in the brain. Decreases in the extracellular levels of kynurenic acid may contribute to excitotoxic neuropathology in a wide variety of neurodegenerative and seizure disorders (Schwarcz et al., 1992), and several lines of recent evidence indicate that pharmacological manipulation of brain kynurenic acid can decisively influence neuronal viability. The further study of L-α-aminoadipic acid neurobiology may therefore reveal interesting clues regarding both physiological excitatory amino acid receptor function and the pathogenesis of excitotoxic brain diseases.

Acknowledgements

We thank Mrs. Joyce Burgess for excellent secretarial assistance. This work was supported by USPHS grants NS 16102 and NS 28236.

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