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Regulation of kynurenic acid levels in the developing rat brain

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Summary. Several brain-specific mechanisms control the formation of the endogenous excitatory amino acid receptor antagonist kynurenic acid (KYNA) in the adult rat brain. Two of these, dopaminergic neurotransmission and cellular energy metabolism, were examined in the brain of immature (postnatal day 7) rats. The results indicate that during the early postnatal period cerebral KYNA synthesis is exceptionally amenable to modulation by dopaminergic mechanisms but rather insensitive to fluctuations in cellular energy status. These findings may be of relevance for the role of KYNA in the function and dysfunction of the developing brain.

Keywords: Excitotoxicity – Glia – Kynurenines – Neuroprotection – Ontogeny

Introduction

The prominent role of excitatory amino acids (EAAs) in brain development was recognized in the late 1980s. Thus, it became clear that one of the three ionotropic EAA receptors, the N-methyl-D-aspartate (NMDA) receptor, needs to be activated during development in order for synaptic plasticity to occur (Artola and Singer, 1987; Balazs et al., 1988). Disruption of NMDA receptor function during the critical perinatal period has catastrophic consequences including aberrant neuronal migration (Komuro and Rakic, 1993) and cognitive abnormalities (Wangen et al., 1997). Moreover, chronic blockade of NMDA receptors during the neonatal period results in functionally significant behavioral changes (Gorter and de Bruin, 1992; Dall'olio et al., 1994) and increased seizure sensitivity (Gorter et al., 1991) in mature animals.

Kynurenic acid (KYNA), a metabolite of tryptophan, is present in the brain under physiological conditions. KYNA functions as an antagonist of EAA receptors, having a particularly high affinity to the glycine co-agonist site of the NMDA receptor complex (Perkins and Stone, 1982; Kessler et al., 1989), and has been suggested to serve as an endogenous regulator of EAA receptor function (Schwarcz et al., 1996). By implication, abnormal cerebral KYNA levels may therefore adversely affect the developing brain.

In the brain as in the periphery, KYNA is formed by enzymatic transamination of L-kynurenine (L-KYN), but no degradative enzymes or re-uptake processes for KYNA have been detected so far (Turski and Schwarcz, 1988). Therefore, after efflux, KYNA continues to be present in the extracellular milieu until it is removed from the brain through a probenecid-sensitive transport process (Moroni et al., 1988). These characteristics, as well as the demonstration that KYNA is neuroprotective in several *in vivo* models of neurodegeneration both in the adult (Foster et al., 1984; Germano et al., 1987; Gill and Woodruff, 1990) and in the immature (Nozaki and Beal, 1992) brain, prompted us to study KYNA metabolism in greater detail.

Studies with brain tissue slices (Gramsbergen et al., 1997) and *in vivo* (Wu et al., 1992) demonstrated that in adult rats extracellular levels of KYNA can be modified by a variety of experimental interventions. For example, KYNA production in the brain (but not in peripheral tissues) is strongly impaired in situations of compromised cellular energy metabolism. Similarly, brain KYNA levels are moderately reduced following the administration of the psychostimulant D-amphetamine (Rassoulpour et al., 1996). We have now extended the study of cerebral KYNA regulation to immature animals and detected quantitatively substantial differences between perinatal and adult rats.

Materials and methods

Animals and chemicals

Male Sprague-Dawley rats were used for all experiments. All biochemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Experiments in vivo

One hour after systemic administration of test compounds or vehicle (phosphate buffered saline), postnatal day (PND) 7 and 3 months-old ("adult") rats were killed by decapitation. Therefore brains and livers were rapidly dissected out and homogenized (1:10, w/v) in distilled water. After centrifugation (5 min, microfuge), KYNA was measured in the supernatant as described below.

Experiments in vitro

PND 7 and adult rats were killed by decapitation, and their brain was rapidly removed and chilled at 4°C. Cortical tissue slices (base: 1 mm × 1 mm) were prepared using a McIlwain chopper, and the tissue was kept in a minimal volume of ice cold Krebs-Ringer buffer (KRB) (NaCl: 118.5 mM, KCl: 4.75 mM, $CaCl_2$: 1.77 mM, $MgSO_4$: 1.18 mM, NaH_2PO_4: 16.2 mM, glucose: 5 mM) until the initiation of the experiment (< 1 h). KRB was oxygenated on ice for 30 min, and the pH adjusted to 7.4 prior to use.

Tissue slices were placed in incubation wells (routinely 7 slices per well) containing ice-cold KRB (1 ml final volume). Assays were performed in triplicate, using three separate wells for each experimental condition (Gramsbergen et al., 1997). Test compounds were dissolved in KRB and were added to the wells in a volume of 100 μl during a 10 min pre-incubation period. The reaction was started by the addition of 2 μM L-KYN. After incubation for 2 h at 37°C in an oxygenated chamber, the wells were

placed on ice, and the medium was rapidly separated from the tissue. 100 μl of 1 M HCl was then added to the medium, and the samples were processed for KYNA measurement as described below. Parallel incubations were performed at 4°C (blanks). The tissue was resuspended in 500 μl distilled water and frozen at –20°C for protein determination (Lowry et al., 1951).

KYNA measurement

For the determination of KYNA, brain tissue supernatant or samples of incubation medium were diluted (1:1, v/v) with HPLC mobile phase (see below), centrifuged for 5 min in a microfuge, and 200 μl aliquots of the supernatant were injected directly onto a 3 μm C_{18} reverse phase HPLC column (80 × 4.6 mm). KYNA was isocratically eluted using a mobile phase containing 0.25 M zinc acetate, 50 mM sodium acetate and 5% acetonitrile (pH 6.2) and detected fluorimetrically (Swartz et al., 1990).

Results

In the first set of experiments, the effect of D-amphetamine administration (5 mg/kg, administered i.p. 1 hour earlier) on tissue KYNA levels was examined in PND 7 and adult rats. As illustrated in Fig. 1, the drug caused a > 70% reduction in brain KYNA levels in the immature animals but only a 23% decrease in young adult rats. The D-amphetamine effect was not observed in the liver and, in PND 7 rats, was prevented by co-administration of the D1 receptor antagonist SCH 23390 (0.5 mg/kg, i.p.) or the D2 receptor antagonist raclopride (2 mg/kg, i.p.) (data not shown).

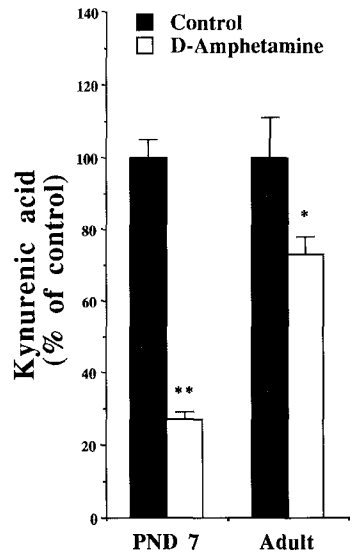

Fig. 1. Effects of D-amphetamine (5 mg/kg, given i.p. 1 hour before sacrifice) on forebrain KYNA levels in PND 7 and adult rats. Data (mean ± SEM, N = 6 per group) are expressed as a percentage of the respective control values (PND 7: 84 ± 6; adult: 195 ± 9 fmol KYNA/mg protein). * $p < 0.05$, ** $p < 0.01$ vs. control (repeated measures ANOVA with Bonferroni multiple comparisons test)

Follow-up studies were designed to explore if the effect of D-amphetamine on brain KYNA concentrations could be duplicated by selective agonists of D1 and D2 receptors. Both the D1 receptor agonist SKF 38393 (5 mg/kg, i.p.) and the D2 receptor agonist quinpirole (2 mg/kg, i.p.), again given 1 hour before sacrifice, caused substantial decreases in brain KYNA levels – albeit only in PND 7 rats. Notably, efforts to reduce KYNA levels further by administering a combination of SKF 38393 and quinpirole failed both in immature and adult rats (Fig. 2).

In a separate study, the effects of glucose deprivation and the possible compensatory role of alternative energy substrates on KYNA formation were explored *in vitro*. Comparison of cortical tissue slices from PND 7 and adult rats revealed significant differences. Thus, omission of glucose from the incubation medium was far less consequential in the immature tissue, merely causing a trend towards a reduction in *de novo* KYNA formation during the 2 hour incubation period. In contrast, the same treatment resulted in a 67% decrease in KYNA synthesis in slices from adult rats (Table 1). In such slices, pyruvate and, to a far lesser extent, lactate, acetoacetate and D(-)-3-hydroxybutyrate reversed the effect of glucose deprivation. Pyruvate also substantially increased KYNA formation in aglycemic tissue slices from PND 7 rats. Under identical conditions, lactate had only a moderate stimulatory effect, and addition of acetoacetate or D(-)-3-hydroxybutyrate did not influence KYNA synthesis (Table 1).

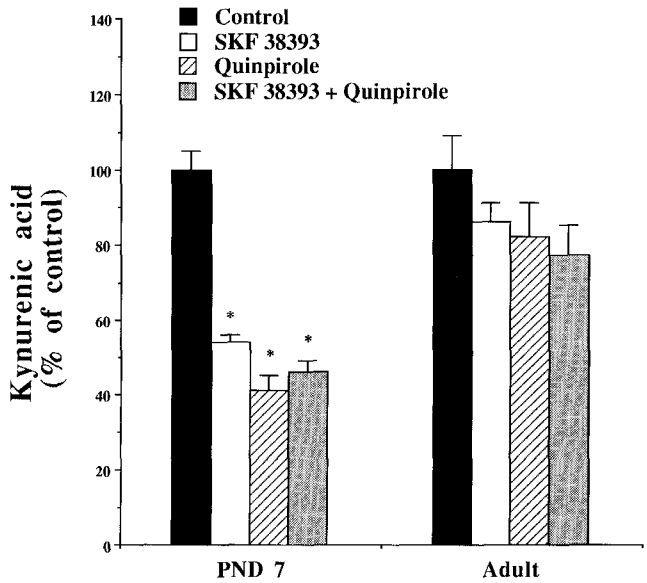

Fig. 2. Effects of the dopamine agonists SKF 38393 (5 mg/kg), quinpirole (2 mg/kg), and a combination of the two drugs, on forebrain KYNA levels in PND 7 and adult rats. Drugs were administered i.p. 1 hour before sacrifice. Data (mean ± SEM; N = 6 per group) are expressed as a percentage of control values (PND 7: 92 ± 5; adult: 202 ± 11 fmol KYNA/mg protein). * $p < 0.05$ vs. control (repeated measures ANOVA with Bonferroni multiple comparisons test)

Table 1. Effect of glucose deprivation and alternative energy substrates on the *de novo* formation of KYNA in cortical tissue slices

	PND 7	Adult
Control medium (KRB)	100.0 ± 7.6	100.0 ± 8.8
Glucose-free	83.2 ± 4.1	33.2 ± 2.0[a]
Glucose-free + pyruvate (5 mM)	191.7 ± 10.3[a,b]	128.0 ± 11.2[b]
Glucose-free + lactate (10 mM)	109.9 ± 6.6[b]	58.0 ± 6.4[a,b]
Glocose-free + acetoacetate (5 mM)	91.4 ± 3.3	61.0 ± 7.5[a,b]
Glucose + D(-)-3-hydroxybutyrate (5 mM)	86.6 ± 8.8	51.2 ± 0.4[a,b]

Data are expressed as a percentage of KRB controls and are the mean ± SEM of 3 separate experiments per age group. Under control conditions, 1.3 ± 0.1 and 3.3 ± 0.4 pmol KYNA/h/mg protein were produced in tissue slices from PND 7 and adult rats, respectively. [a]$p < 0.05$ vs. KRB controls; [b]$p < 0.05$ vs. glucose-free medium (repeated measures ANOVA with Student-Newman-Keuls multiple comparisons test).

Discussion

These experiments demonstrate a pronounced age-dependence of two of the mechanisms which control KYNA formation in the rat brain. Both cerebral energy metabolism and D1 and D2 receptors have distinct ontogenetic characteristics (Nehlig, 1997; Zouakia et al., 1997), which probably account for the age-related differences reported here. For example, functional interactions between dopaminergic activation and KYNA-producing astrocytes may preferentially facilitate the efflux of KYNA in young animals. The ability of D-amphetamine to reduce KYNA formation substantially in PND 7 animals also appears to be mediated by the same mechanism since both D1 and D2 receptor antagonists prevented the drug effect.

The dependence of the brain on glucose increases gradually during ontogeny (Nehlig, 1997). In a recent study (Hodgkins and Schwarcz, unpublished data), we obtained evidence that the brain-specific effect of glucose deprivation on KYNA formation in adult rats can be attenuated both by providing alternate cellular fuels (such as lactate or pyruvate) and by co-substrates of L-KYN transamination (such as pyruvate) (ef. Gramsbergen et al., 1997). The present data indicate that in the immature brain cellular energy metabolism is a far less important determinant of KYNA production, whereas co-substrate regulation is fully functional. Thus, acetoacetate, D(-)-3-hydroxybutyrate and lactate, all efficient energy substrates in the developing brain (Booth et al., 1980; Edmond et al., 1987; Medina et al., 1997; Nehlig, 1997), were marginally effective, while pyruvate more than doubled KYNA formation in the absence of glucose.

The functional significance of the age-dependent mechanisms described here needs to be elaborated in future studies. NMDA receptors show distinct developmental patterns (Monyer et al., 1994) and have been suggested to play a central role in developmental brain disorders and in perinatal hypoxic-ischemic complications (McDonald et al., 1987; Ikonomidou et al., 1989).

Moreover, dopaminergic mechanisms have been demonstrated to influence excitotoxic vulnerability by interaction with EAA neurotransmission (Garside et al., 1996). It remains to be seen if fluctuations in the brain levels of the EAA receptor antagonist KYNA, effected by the mechanisms described here and elsewhere (Gramsbergen et al., 1997), participate in the function and/or dysfunction of the immature brain.

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References

Artola A, Singer W (1987) Long-term potentiation and NMDA receptors in rat visual cortex. Nature 330: 649–652

Balazs R, Jorgensen OS, Hack N (1988) N-methyl-D-aspartate promotes the survival of cerebellar granule cells in culture. Neuroscience 27: 437–451

Booth RFG, Patel TB, Clark JB (1980) The development of enzymes of energy metabolism in the brain of a precocial (guinea pig) and non-precocial (rat) species. J Neurochem 34: 17–25

Dall'olio R, Facchinetti F, Contestabile A, Gandolfi O (1994) Chronic neonatal blockade of N-methyl-D-aspartate receptor by CGP 39551 increases dopaminergic function in adult rat. Neuroscience 63: 451–455

Edmond J, Robbins RA, Bergstrom JD, Cole RA, de Vellis J (1987) Capacity for substrate utilization in oxidative metabolism by neurons, astrocytes, and oligodendrocytes from developing brain in primary culture. J Neurosci Res 18: 551–561

Foster AC, Vezzani A, French ED, Schwarcz R (1984) Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. Neurosci Lett 48: 273–278

Garside S, Furtado J-CS, Mazurek MF (1996) Dopamine-glutamate interactions in the striatum: behaviourally relevant modification of excitotoxicity by dopamine receptor-mediated mechanisms. Neuroscience 75: 1065–1074

Germano IM, Pitts LH, Meldrum BS, Bartkowski HM, Simon RP (1987) Kynurenate inhibition of cell excitation decreases stroke size and deficits. Ann Neurol 22: 730–734

Gill R, Woodruff GN (1990) The neuroprotective actions of kynurenic acid and MK-801 in gerbils are synergistic and not related to hypothermia. Eur J Pharmacol 176: 143–149

Gorter JA, de Bruin JPC (1992) Chronic neonatal MK-801 treatment results in an impairment of spatial learning in the adult rat. Brain Res 580: 12–17

Gorter JA, Titulaer M, Bos NPA, Huisman E (1991) Chronic neonatal MK-801 administration leads to a long-lasting increase in seizure sensitivity during the early stages of hippocampal kindling. Neurosci Lett 134: 29–32

Gramsbergen JBP, Hodgkins PS, Rassoulpour A, Turski WA, Guidetti P, Schwarcz R (1997) Brain-specific modulation of kynurenic acid synthesis in the rat. J Neurochem 69: 290–298

Ikonomidou C, Price MT, Mosinger JL, Frierdich G, Labruyere J, Salles KS, Olney JW (1989) Hypobaric-ischemic conditions produce glutamate-like cytopathology in infant rat brain. J Neurosci 9: 1693–1700

Kessler M, Terramani T, Lynch G, Baudry M (1989) A glycine site associated with N-methyl-D-aspartic acid receptors: characteristics and identification of a new class of antagonists. J Neurochem 52: 1319–1328

Komuro H, Rakic P (1993) Modulation of neuronal migration by NMDA receptors. Science 260: 95–97

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–273

McDonald JW, Silverstein FS, Johnston MV (1987) MK-801 protects the neonatal brain from hypoxic-ischemic damage. Eur J Pharmacol 140: 359–361

Medina JM, Tabernero A, Tovar JA, Martin-Barrientos J (1996) Metabolic fuel utilization and pyruvate oxidation during the postnatal period. J Inher Metab Dis 19: 432–442

Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12: 529–540

Moroni F, Russi P, Lombardi G, Beni M, Carlà V (1988) Presence of kynurenic acid in the mammalian brain. J Neurochem 51: 177–180

Nehlig A (1997) Cerebral energy metabolism, glucose transport and blood flow: changes with maturation and adaptation to hypoglycemia. Diabetes Metab 23: 18–29

Nozaki K, Beal MF (1992) Neuroprotective effects of L-kynurenine on hypoxia-ischemia and NMDA lesions in neonatal rats. J Cereb Blood Flow Metab 12: 400–407

Perkins MN, Stone TW (1982) An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. Brain Res 247: 105–111

Rassoulpour A, Wu H-Q, Poeggeler B, Schwarcz R (1996) D-Amphetamine and reserpine modulate kynurenic acid levels in the rat brain in vivo. Soc Neurosci Abstr 22: 39.15

Schwarcz R, Guidetti P, Roberts RC (1996) Quinolinic acid and kynurenic acid: glia-derived modulators of excitotoxic brain injury. In: Aschner M, Kimelberg HK (eds) The role of glia in neurotoxicity. CRC Press, Boca Raton, pp 245–262

Swartz KJ, During MF, Freese A, Beal MF (1990) Cerebral synthesis and release of kynurenic acid: an endogenous antagonist of excitatory amino acid receptors. J Neurosci 10: 2965–2973

Turski WA, Schwarcz R (1988) On the disposition of intrahippocampally injected kynurenic acid in the rat. Exp Brain Res 71: 563–567

Wangen K, Myhrer T, Moldstad JN, Iversen EG, Fonnum F (1997) Modulatory treatment of NMDA receptors in neonatal rats affects cognitive behavior in adult age. Dev Brain Res 99: 126–130

Wu H-Q, Baran H, Ungerstedt U, Schwarcz R (1992) Kynurenic acid in the quinolinate-lesioned rat hippocampus: studies *in vitro* and *in vivo*. Eur J Neurosci 4: 1264–1270

Zouakia A, Guilloteau D, Zimmer L, Besnard J-C, Chalon S (1997) Evolution of dopamine receptors in the rat after neonatal hypoxia-ischemia: autoradiographic studies. Life Sci 60: 151–162

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