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A novel kynurenic acid analog (SZR104) inhibits pentylenetetrazole-induced epileptiform seizures. An electrophysiological study

Special issue related to Kynurenine

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Abstract The concentration of kynurenic acid (KYNA) in the cerebrospinal fluid, which is in the nanomolar range, is known to decrease in epilepsy. The experimental data suggest that treatment with L-KYN dose dependently increases the concentration of the neuroprotective KYNA in the brain, which itself hardly crosses the blood–brain barrier. However, it is suggested that new synthetic KYNA analogs may readily cross the blood–brain barrier. In this study, we tested the hypothesis that a new KYNA analog administered systemically in a sufficient dose results in a decreased population spike activity recorded from the pyramidal layer of area CA1 of the hippocampus, and also provides protection against pentylenetetrazole-induced epileptiform seizures.

Keywords Neuroprotection · Epilepsy · Blood–brain barrier · Synthetic analog · Hippocampus · Pentylenetetrazole

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Introduction

The tryptophan metabolism, which occupies a central route in the brain, is known to be responsible for the formation of kynurenines (Beadle et al. 1947). Kynurenic acid (KYNA) is produced directly by irreversible transamination from L-kynurenine by the action of kynurenine aminotransferases (KATs) (Okuno et al. 1991a, b).

Endogenous kynurenines, KYNA and synthetic derivatives of kynurenines have been studied and found to be neuroprotective, and may be of therapeutic value in different neurological disorders (Gellert et al. 2011; Schwarcz and Pellicciari 2002; Vamos et al. 2009; Zadori et al. 2009).

Kynurenine administered intraperitoneally (i.p.) increases the concentration of KYNA in the brain, and exerts antiepileptic effects in pentylenetetrazole (PTZ)- and NMDA-induced seizures (Vecsei et al. 1992).

In some forms of human epilepsy, such as the West syndrome, the concentration of KYNA in the human cerebrospinal fluid is reduced relative to the normal level (Yamamoto et al. 1995; Stone 2001; Kaminski et al. 2003).

It is well known that in most forms of epilepsy the activation of excitatory amino acid receptors may play a decisive role. Excitatory amino acid agonists, and mainly those that act at NMDA receptors, can produce seizures (Stone 1993). The only known endogenous antagonist of excitatory amino acid receptors in the brain is KYNA (Swartz et al. 1990; Stone 2000), which has only a very limited ability to cross the blood–brain barrier.

Endogenous KYNA is neuroprotective (Hicks et al. 1994), and is also an anticonvulsant (Foster et al. 1984; Klivenyi et al. 2004).

Previous in vitro studies have confirmed that KYNA can suppress electrophysiological signs of epilepsy in brain slices (Stone 1988; Scharfman and Ofer 1997).

As a new KYNA analog, SZR104, earlier demonstrated an outstanding ability to cross an artificial blood–brain barrier in vitro, it was decided to examine whether peripherally administered SZR104 exerts any effect on the hippocampal electrical activity and whether it provides protection against PTZ-induced epileptiform seizures.

Methods

Animals

Experiments were performed on male Sprague–Dawley (SPRD) rats ($n = 18$) weighing 200–250 g. The animals were housed under controlled environmental conditions, under a 12-h light/dark cycle and had free access to food and water. All experiments had been approved by the Hungarian Health Committee and were performed in accordance with the 86/609/EEC directive.

Drugs

Pentylenetetrazole was purchased from Sigma (Steinheim, Germany) and was administered i.p. in a dosage of 60 mg/kg.

SZR104 was prepared in the Institute of Pharmaceutical Chemistry and Research Group for Stereochemistry, University of Szeged and was administered i.p. in a dosage of 358.2 mg/kg. This dosage is equimolar with that of kynurenine proved to be effective in protection against PTZ-induced epileptic seizures (Nemeth et al. 2004).

Electrophysiology

Male SPRD rats were anesthetized with urethane (1.25 g/kg, i.p.). The drugs were administered i.p., through a syringe implanted at the beginning of the experiments. The method was described in detail earlier (Nemeth et al. 2004). In brief: a 2- to 3-mm diameter hole was drilled over the dorsal hippocampus for the recording electrode in area CA1: 3.0- to-3.8 mm posterior and 1.8- to-2.3 mm lateral to the sagittal suture and lowered 2.2–2.8 mm from the cortical surface. Contralaterally, for the CA3 stimulating electrode, a 1- to 2-mm hole was drilled 3.7-mm posterior to the bregma, and 3.3-mm lateral to the sagittal suture, the final electrode depth being 3.8 mm below the dura. The recording electrode was lowered slowly and the final position was adjusted so that the maximum CA1 population spike was obtained in response to contralateral CA3 stimulation (Fig. 1, insert). Hippocampal areas CA1 and CA3 were confirmed histologically.

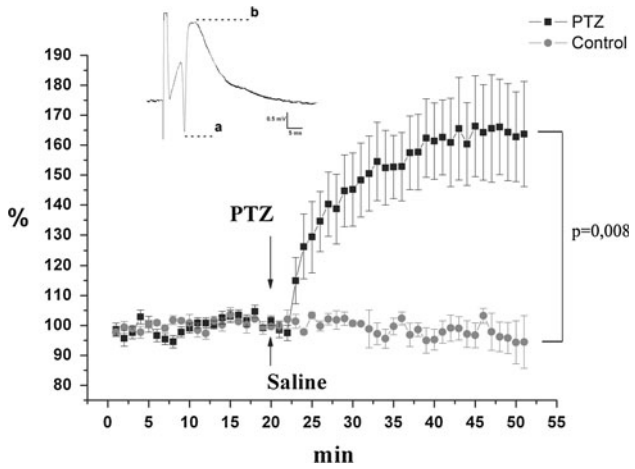

Fig. 1 Changes in population spike amplitudes after the administration of pentylenetetrazole (PTZ) (60 mg/kg i.p.). Saline administration resulted in hardly any changes in population spike amplitudes, whereas PTZ injection induced significant increases in amplitudes. Statistical analysis was achieved only on amplitudes registered after PTZ treatment ($F = 11.666$; $p = 0.008$). *Insert* a typical population spike, the amplitude of which was measured between levels **a** and **b**. Abscissa: time in min. Ordinate: spike amplitudes as percentages of the controls (between 0 and 20 min)

Statistical analysis

Population spike amplitudes evoked by CA3 stimulation in the pyramidal layer of area CA1 were measured from peak to peak (Fig. 1, insert). Differences between amplitudes were determined statistically. Statistical analysis was performed with the linear mixed-effects model in all cases (General linear model/PASW Statistics 18 data analysis package, SPSS Inc. Chicago, USA). The effects of the different rats were used as random effects and the different treatments were used as fixed effects in the mixed effect linear model (p value set at 0.05 for significance).

Results

The animals were divided into 4 groups: *group 1* (controls, injected with saline), *group 2* (injected with PTZ), *group 3* (injected with SZR104) and *group 4* (injected with SZR104 + PTZ).

Effects of PTZ

After a 20-min control period, PTZ was administered i.p. in a dose of 60 mg/kg. This resulted in a significant increase in the amplitude of the CA1 responses induced by contralateral CA3 stimulation, to over 70% of the control amplitude ($F = 11.666$; $p = 0.008$). The level remained elevated throughout the 30-min registration period (Fig. 1).

Effects of SZR104

After a 20-min control period, SZR104 was administered i.p. in a dose of 358.2 mg/kg, with registration for 180 min. After 30 min, the amplitude gradually decreased ($F = 9.509$; $p = 0.018$) (Fig. 2).

Effects of SZR104 + PTZ

The effects of PTZ were followed in SZR104-pretreated animals. After a 20-min control period, SZR104 was administered i.p. in a dose of 358.2 mg/kg. During the next 80 min, a gradual decrease in amplitude was observed.

Eighty minutes after SZR104 administration, PTZ was injected in a dose of 60 mg/kg, which resulted in an increase of the amplitude to the baseline. However, during the following 30 min, the amplitude remained on the baseline and did not exceed the control level (Fig. 3). Statistical analysis was achieved only on amplitudes registered after PTZ treatment ($F = 0.135$; $p = 0.726$).

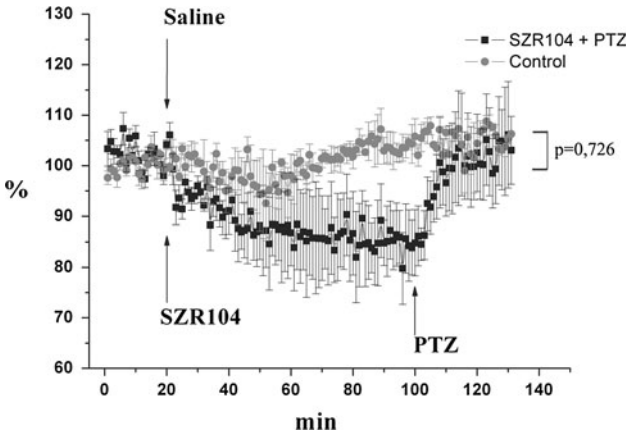

Fig. 3 Changes in amplitudes after SZR104 and PTZ administration. Saline administration did not induce any changes in the amplitudes (control). After SZR104 injection, the amplitudes decreased (as seen previously). PTZ administration (80 min after SZR104 injection), resulted in slightly increased amplitudes, but they did not exceed the control level. Statistical analysis was achieved only on amplitudes registered after PTZ treatment ($F = 0.135$; $p = 0.726$). Abscissa and ordinate are as in Fig. 1

Discussion

Pentylenetetrazole is a GABA A antagonist that has been used experimentally to investigate the seizure phenomenon. One of our previous electrophysiological and behavioral studies confirmed that the combination of kynurenine and probenecid inhibits PTZ-induced seizures (Nemeth et al. 2004). An in vitro electrophysiological study has demonstrated that exogenously administered L-kynurenine can be converted to KYNA, which not only decreases the amplitude of the hippocampal CA1 responses evoked by Schaffer collateral stimulation but is also sufficient to prevent the neuroexcitatory effect of PTZ (Rozsa et al. 2008).

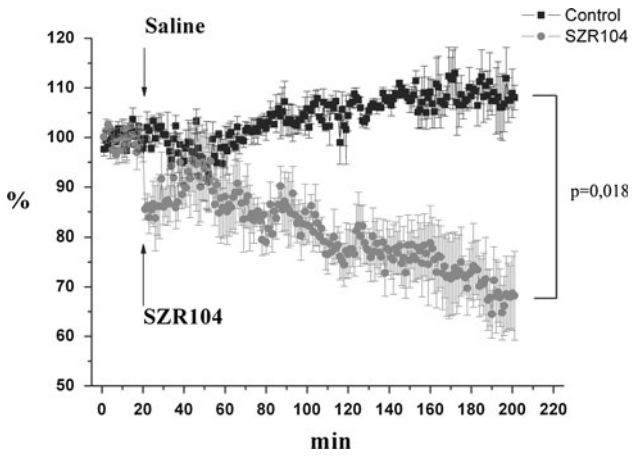

Fig. 2 Examples of the effects of the KYNA analog (SZR104) on the population spike amplitude recorded in area CA1. SZR104 (358.2 mg/kg, i.p.) resulted in a significant decrease in amplitude. Statistical analysis was achieved only on amplitudes registered after SZR104 treatment ($F = 9.509$; $p = 0.018$). Abscissa and ordinate are as in Fig. 1

During the past few years, several new KYNA analogs have been synthesized and tested by our research group (Fulop et al. 2009).

Firstly, KYNA analogs were tested in an in vitro BBB model. In parallel experiments, Na-fluorescein (SF) and Evans blue albumin (EBA) permeabilities were measured and compared to that of KYNA's permeability. Among SF, EBA and several KYNA analogs, permeability of SZR104 proved to be noteworthy higher. In accordance with these unpublished in vitro data, SZR104 administered i.p., significantly decreased the amplitude of hippocampal field EPSP's amplitudes.

All these results suggest that SZR104 crosses the blood–brain barrier and support our present ex vivo results.

Recent results indicated the enhancement of KYNA production by anticonvulsants (carbamazepine, phenytoin, phenobarbital, felbamate and lamotrigine), which directly elevate the activity of KAT I, as a novel mechanism in the control of epilepsy (Kocki et al. 2006). However, if the KYNA level in the central nervous system is increased by a peripherally administered KYNA analog which crosses the blood–brain barrier, this might also exert an effective anticonvulsant effect.

As expected, the systemic administration of SZR104 in a sufficient dose resulted in a decreased population spike activity recorded from the pyramidal layer of area CA1 of

the hippocampus, and additionally provided protection against PTZ-induced epileptiform seizures.

We have synthetized several new KYNA analogs during the past 8 years (Fulop et al. 2009), but SZR104 seems to be one of the most promising molecules as a neuroprotectant.

The question raises whether the pharmacological targets for KYNA and SZR104 are the same or not? Our experimental data suggest that the target for both molecules is probably the same: the strychnine-insensitive glycine-binding site of the NMDA receptor. However, the proof of this assumption needs further studies.

Presently the facts are as follows: (1) the new synthetic KYNA analog SZR104 administrated systemically in sufficient dose results in a decreased population spike activity recorded from the pyramidal layer of area CA1 of hippocampus, (2) SZR104 not only decreased the population spike activity but also provided protection against pentylenetetrazol-induced epileptiform seizures.

Conclusions

The findings of the present study have revealed that SZR104 is an effective neuroprotectant and provides protection against seizures. It appears to be a potentially promising candidate for future preclinical trials.

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References

Beadle GW, Mitchell HK et al (1947) Kynurenine as an intermediate in the formation of nicotinic acid from tryptophane by neurospora. Proc Natl Acad Sci USA 33(6):155–158

Foster AC, Vezzani A et al (1984) Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. Neurosci Lett 48(3):273–278

Fulop F, Szatmari I et al (2009) Syntheses, transformations and pharmaceutical applications of kynurenic acid derivatives. Curr Med Chem 16(36):4828–4842

Gellert L, Fuzik J et al (2011) Neuroprotection with a new kynurenic acid analog in the four-vessel occlusion model of ischemia. Eur J Pharmacol 667(1–3):182–187

Hicks RR, Smith DH et al (1994) Kynurenate is neuroprotective following experimental brain injury in the rat. Brain Res 655(1–2):91–96

Kaminski RM, Zielinska E et al (2003) Deficit of endogenous kynurenic acid in the frontal cortex of rats with a genetic form of absence epilepsy. Pol J Pharmacol 55(5):741–746

Klivenyi P, Toldi J et al (2004) Kynurenines in neurodegenerative disorders: therapeutic consideration. Adv Exp Med Biol 541: 169–183

Kocki T, Wielosz M et al (2006) Enhancement of brain kynurenic acid production by anticonvulsants–novel mechanism of antiepileptic activity? Eur J Pharmacol 541(3):147–151

Nemeth H, Robotka H et al (2004) Kynurenine administered together with probenecid markedly inhibits pentylenetetrazol-induced seizures. An electrophysiological and behavioural study. Neuropharmacology 47(6):916–925

Okuno E, Nakamura M et al (1991a) Two kynurenine aminotransferases in human brain. Brain Res 542(2):307–312

Okuno E, Schmidt W et al (1991b) Measurement of rat brain kynurenine aminotransferase at physiological kynurenine concentrations. J Neurochem 57(2):533–540

Rozsa E, Robotka H et al (2008) The pentylenetetrazole-induced activity in the hippocampus can be inhibited by the conversion of L-kynurenine to kynurenic acid: an in vitro study. Brain Res Bull 76(5):474–479

Scharfman HE, Ofer A (1997) Pretreatment with L-kynurenine, the precursor to the excitatory amino acid antagonist kynurenic acid, suppresses epileptiform activity in combined entorhinal/hippocampal slices. Neurosci Lett 224(2):115–118

Schwarcz R, Pellicciari R (2002) Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities. J Pharmacol Exp Ther 303(1):1–10

Stone TW (1988) Comparison of kynurenic acid and 2-APV suppression of epileptiform activity in rat hippocampal slices. Neurosci Lett 84(2):234–238

Stone TW (1993) Neuropharmacology of quinolinic and kynurenic acids. Pharmacol Rev 45(3):309–379

Stone TW (2000) Development and therapeutic potential of kynurenic acid and kynurenine derivatives for neuroprotection. Trends Pharmacol Sci 21(4):149–154

Stone TW (2001) Kynurenines in the CNS: from endogenous obscurity to therapeutic importance. Prog Neurobiol 64(2): 185–218

Swartz KJ, During MJ et al (1990) Cerebral synthesis and release of kynurenic acid: an endogenous antagonist of excitatory amino acid receptors. J Neurosci 10(9):2965–2973

Vamos E, Pardutz A et al (2009) The role of kynurenines in disorders of the central nervous system: possibilities for neuroprotection. J Neurol Sci 283(1–2):21–27

Vecsei L, Miller J et al (1992) Kynurenine and probenecid inhibit pentylenetetrazol- and NMDLA-induced seizures and increase kynurenic acid concentrations in the brain. Brain Res Bull 28(2):233–238

Yamamoto H, Murakami H et al (1995) Studies on cerebrospinal fluid kynurenic acid concentrations in epileptic children. Brain Dev 17(5):327–329

Zadori D, Klivenyi P et al (2009) Kynurenines in chronic neurodegenerative disorders: future therapeutic strategies. J Neural Transm 116(11):1403–1409