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Phenotypic differences between fast and slow methionine sulfoximine-inbred mice: Seizures, anxiety, and glutamine synthetase

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Summary Seizures induced by the convulsant methionine sulfoximine (MSO) resemble human ''grand mal'' epilepsy, and brain glutamine synthetase is inhibited. We recently selected two inbred lines of mice: sensitive to MSO (MSO-Fast) and resistant (MSO-Slow). In the present study, the selection pressure was increased and consanguinity established. To gain insight into the mechanisms of epileptogenesis, we studied the behaviour of MSO-Fast and MSO-Slow mice based on their responses to various convulsants and anticonvulsants, and also the kinetics of glutamine synthetase. The results show that increasing the number of generations of sib-crossings resulted in an increase in the differences between MSO-Fast and MSO-Slow mice. The dose—response curve of MSO-dependent seizures demonstrated that the MSO-Slow mice were highly insensitive to MSO-dependent seizures compared with MSO-Fast inbred mice that were highly sensitivity. The MSO-Slow were resistant to convulsions induced by various convulsants having different mechanisms of action, whereas those in the MSO-Fast line were more sensitive to kainic acid-induced seizures. These data, in addition to the effects of anticonvulsant, strongly suggest that glutamatergic pathways are most likely involved in MSO-dependent seizures, rather than GABAergic ones. This hypothesis is corroborated by the glutamine synthetase activity, which is more elevated in the MSO-Slow line. Behaviour tests showed that MSO-Slow were less anxious than MSO-Fast. Collectively, these results showed that glutamatergic pathways could be involved in the epileptogenic action of MSO, which may be related to the glutamate/glutamine cycle in the brain.

Introduction

According to the World Health Organisation 1% of the worldwide population develop an epileptic syndrome. About 70% of the patients are treated using drugs, whereas the

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remainder are pharmacoresistant and undergo surgery whenever possible (Andrade and Minassian, 2007; Meldrum, 2007; Scharfman, 2007; Schmidt, 2009). The lack of an etiological treatment of epilepsy is due to poor knowledge on the basic mechanisms of seizure genesis, i.e., a sudden and temporary synchronization of neural activities (McNamara et al., 2006; Stafstrom, 2006; Scharfman, 2007; Reid et al., 2009). One difficulty in the study of epileptogenesis is how to find models which precisely mimic the human disease. Different animal models have been developed, corresponding essentially to spontaneous or genetic epilepsy, or to physical- or chemical-induced seizures. Among these latter models, one was described many years ago where its clinical pattern resembled the most striking form of human epilepsy (Wolfe and Elliot, 1962). This model depended upon methionine sulfoximine (MSO), which is known as a powerful inhibitor of glutamine synthetase (GS) (Griffith and Meister, 1978). Nevertheless many chemoconvulsants induce tonic and clonic seizures with similarity to human pathologies. MSO generates epileptiform seizures in a large variety of animals, and was shown in earlier studies to increase brain glycogen content (Folbergrova et al., 1969). Recently, an increase in brain glycogen was associated with human temporal lobe epilepsy (Dalsgaard et al., 2007). On account of the high resemblance of MSO models to human epilepsy, and because of the recent metabolic data on the condition, we became interested in looking for basic mechanisms that generate epileptiform activities in an MSO model, with the aim to better understand epileptogenesis. Our approach was based on selecting mice that respond differently to the administration of MSO, and on comparing the metabolism and the behaviour of selected inbred lines in order to find out abnormalities which can trigger seizures. In preliminary investigations, based on the first few stages of inbreeding, we observed that MSO separated types of mice that develop seizures minutes after MSO injection (MSO-Fast) from another type that developed seizures latter after administration of the same drug (MSO-Slow) (Cloix et al., 2010b). Moreover, metabolic differences were observed between these two types of mice, such as brain glycogen level and neurotransmitter contents (Cloix et al., 2010a). In the present study we aimed to increase the selection pressure to emphasise the difference between the two types of mice, after varying the doses and then making further crosses within lines in order to enhance the visible effects which could help to explain the seizures genesis.

Glutamate and GABA are two essential neurotransmitters acting as activator and inhibitor of brain activities, respectively. The neurotransmitters were described as being involved in the control of seizures in both patients and in animal models of epilepsies (Mohler, 2006; Kanner, 2008; Moult, 2009; Schmidt, 2009; Mares et al., 2010). Moreover, brain glutamate content and glutamate receptors, particularly metabotropic glutamate receptors (mGluR), are linked to the control of various physiological and physiopathological pathways, such as those involved in behaviour, mood and psychiatric disorders (Moult, 2009; Wieronska and Pilc, 2009; Krystal et al., 2010; Mares et al., 2010). As a relationship between epilepsy and psychiatric disorders has been hypothesized (Kanner, 2008; LaFrance et al., 2008; Hixson and Kirsch, 2009; Kanner, 2009), the present investigation is principally devoted to study the responses to various convulsants and anticonvulsants, and the behaviour of MSO-Fast and MSO-Slow mice in relation to GS activity.

Experimental procedures

Animals

MSO-Slow and MSO-Fast mice were selected according to a previously described procedure based upon the crossing of eight parental strains: ABP/Le, A/J, BALB/c, C3H/HeJ, C57BL/6J, CBA/H, DBA/2J, and SWXL-4 (Cloix et al., 2010b). Thereafter, 3 additional MSO-challenges were performed in order to increase the selection pressure: MSO-Slow mice were challenged with 100 mg/kg MSO, while MSO-Fast ones were challenged using 50 mg/kg MSO. As previously described (Cloix et al., 2010b) two groups of 16 breeding pairs were established after each MSO challenges in such a way that no brother sister mating was allowed. The MSO-Fast group comprised mating pairs of 16 females and 16 males having the shortest seizure latencies; while MSO-Slow group comprised mating pairs of 16 females and 16 males having the longest or no seizure latencies. This was followed by 7–9 inbreeding generations of brother–sister crosses to generate mice that were 76.9 ± 8.5 days old at the time of the experiments. All protocols were approved by the local ethical committee with the agreement number CREEA CL2007-023, and were in accordance with the European Community Council Directive of 24 November 1986 (86/609/ECC).

Chemicals and seizure latency

Methionine sulfoximine (MSO), pentylenetetrazole (PTZ, an GABA antagonist), kainic acid (KA, an glutamatergic agonist), pilocarpine (PC, an cholinergic agonist), valproic acid (VPA, considered as an GABA agonist as it increased GABA concentration through various mode of action) and MK-801 (an NMDA antagonist) were obtained from Sigma–Aldrich (Saint Quentin Fallavier, France). The chemicals were dissolved in 0.9% NaCl and the pH was adjusted to 7.4 for KA and VPA only. They were administered intraperitoneally, and the latencies to seizures were determined as the duration between injection and seizure onset, as previously described (Cloix et al., 2010b). A maximal latency score was given to mice that did not seize during the observation period: PTZ, 180 s, KA, 120 min, PC, 120 min, MSO, 600 min. Doses of various convulsants and anticonvulsants were as follows: MSO, various doses as indicated hereafter; PTZ, 75 mg/kg; KA, 25 mg/kg, PC, 300 mg/kg, VPA, 250 mg/kg; MK-801, 1 mg/kg. The ED_{50} for MSO were calculated using GraphPad Prism software with a non-linear fitting of data, normalised response and variable slope, and simply corresponded to the concentration required to provoke a response halfway between the baseline and maximum responses. In this analysis, only the generalised convulsions were considered. Twenty mice were used (10 females and 10 males) for the study of latency to generalised seizures, and 40 mice (20 females, 20 males) for the observation of seizure stages. The various stages (I–V) of MSO-dependent seizures were also determined accordingly to Racine's scale (Racine, 1972). In brief, the various stages and the main characteristics of each stage are as follows. Stage I: ataxia, akinesia, facial myoclony, sniffing. Stage II: head clony, head shaking. Stage III: myoclony of anterior members. Stage IV: myoclony of posterior members, tail whipping. Stage V: generalised seizures.

Glutamine synthetase activity

Mice were sacrificed by decapitation, and heads were immediately frozen in liquid nitrogen and conserved at −80 °C. Dorsal cortices were dissected out from the brain at −20 °C and pulverised with a liquid nitrogen frozen homogeniser, and then kept at −80 °C