Experimental Neurology 263 (2015) 263–271

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Regular Article

Sucrose consumption test reveals pharmacoresistant depression-associated behavior in two mouse models of temporal lobe epilepsy

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ARTICLE INFO

Article history:
Received 28 March 2014
Revised 16 August 2014
Accepted 4 September 2014
Available online 16 September 2014

Keywords:
Anhedonia
Drug resistance
Animal model
Pilocarpine
Kainic acid

ABSTRACT

Among the comorbidities observed in epilepsy patients depression is the most frequent one. Likewise, depression by itself is accompanied by an increased risk to develop epilepsy. Both epilepsy and depression are characterized by a high incidence of pharmacoresistance, which might be based on overactivity of multidrug transporters like P-glycoprotein at the blood–brain barrier. Using genetically modified mice in preclinical epilepsy research is pivotal for investigating this bidirectional relationship. In the present study, we used the sucrose consumption test (SCT) in the pilocarpine and the intrahippocampal kainate mouse post-status epilepticus model to reveal anhedonic behavior, i.e. hyposensitivity to pleasure, as a key symptom of depression. Mice were repetitively investigated by SCT during early epilepsy and the chronic phase of the disease, during which response to antidepressant drug treatment was assessed. SCT revealed long-lasting anhedonia in both models. Anhedonia appeared to be pharmacoresistant, as neither chronic treatment with imipramine in the pilocarpine model nor chronic treatment with fluoxetine in the kainate model could annihilate the differences in sucrose consumption between control and epileptic mice. Moreover, knock-out of P-glycoprotein did not improve the treatment effect of fluoxetine. In conclusion, our findings show for the first time that the SCT is suited for detection of depression-like behavior in mouse models of temporal-lobe epilepsy. Both models might serve as tools to further investigate the neurobiology and pharmacology of epilepsy-associated pharmacoresistant depression.

Introduction

Depression is the most frequent psychiatric comorbidity in epilepsy (Valente and Busatto Filho, 2013, Kanner, 2003b). Up to 50% of pharmacoresistant epilepsy patients, especially those with temporal-lobe epilepsy (TLE), are affected by depression (Kanner, 2003a). Moreover, suicide risk in epilepsy patients is increased ten times compared to the general population (Kanner, 2003b). Otherwise, epidemiologic data underline that people with depression have an up to 7-fold increased risk of developing epilepsy (Garcia, 2012). The bidirectional relationship between epilepsy and depression is still poorly understood. A common feature of both diseases is the high percentage of pharmacoresistant patients. In general, seizures in one third of epilepsy patients do not respond to antiepileptic drug intake (Kwan and Brodie, 2000). Similar to epilepsy, pharmacoresistance to antidepressants is defined as non-response to adequate treatment with two or more antidepressants of different mechanism of action (El Hage et al., 2013). Prevalence of non-response to the first trial antidepressant is around 50% (Nemeroff, 2007, Wiborg, 2013). One widely studied mechanism of pharmacoresistance in epilepsy is increased antiepileptic drug efflux mediated by overexpression of multidrug transporters at the blood–brain barrier in the epileptic focus region (Löscher and Potschka, 2005). As several antidepressants are substrates of such multidrug transporters this mechanism could also be of relevance in depressive patients (Löscher and Potschka, 2005, O'Brien et al., 2012b).

Characterizing animal models of TLE with respect to occurrence of depression as comorbidity is pivotal for understanding pathomechanisms underlying the association of epilepsy and depression and for development of new treatments. Mouse models, in which epilepsy develops after an initial status epilepticus (SE), provide a high potential to identify mechanisms of epileptogenesis and pharmacoresistance, particularly because of the availability of genetically modified mice. We recently investigated depression-like symptoms in two mouse models of TLE (pilocarpine, intrahippocampal kainate) in which epileptogenesis is promoted by induction of a self-sustaining SE (Gröticke et al., 2007, Gröticke et al., 2008, Müller et al., 2009a, Müller et al., 2009b). For this

Abbreviations: Pgp, P-glycoprotein; SCT, sucrose/saccharin consumption test; SE, status epilepticus; SRS, spontaneous recurrent seizures; TLE, temporal-lobe epilepsy.

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http://dx.doi.org/10.1016/j.expneurol.2014.09.004
0014-4886/

purpose, we applied two widely used tests to identify depression-like behavior in rodents, the forced swimming test and the tail suspension test. Both tests assess the rodents' response to an inescapable situation (threat of drowning and being suspended by the tail), measured as time spent swimming or struggling and time spent immobile. Increased immobility is interpreted as the behavioral equivalent of "hopelessness" or "depressed mood" as a major symptom of depressive disorders (Crawley, 2007). Surprisingly, we found that in both tests, epileptic mice did not show the behavior which is usually characterized as depression-like, but presented opposite paradoxic behavior (Gröticke et al., 2007, Gröticke et al., 2008, Müller et al., 2009b, Müller et al., 2009a). We suggested that in both tests, the epileptic mice do not understand the context of the experimental situation, i.e. they do not realize that they are in a hopeless situation, this might be explained by simultaneous presence of impaired learning and memory and anxiety-associated behavior which both have also been found in these mice. Thus, there is still a need for suitable behavioral tests to elucidate depression-like behavior in epileptic mice. The sucrose or saccharin consumption test (SCT) is not based on despair induction but builds on the innate preference of rodents towards sweet food. The test evaluates anhedonia, i.e. hyposensitivity to pleasure, which is a cardinal symptom of depression in human patients (Kanner et al., 2012). In rodents, anhedonic behavior can be measured by simultaneously offering them access to both tap water and sweetened fluid. A healthy subject will prefer the latter, while an anhedonic animal will consume less sweet solution but equal amounts of water compared to controls. Advantageously, the SCT can be performed in the rodents' home cage, hereby minimizing a potential stress- or anxiety-induced bias of results. The SCT has already been studied following rapid kindling in immature rats (Mazarati et al., 2007), in rat models of genetic epilepsy (Jones et al., 2008, Russo et al., 2013b, Sarkisova et al., 2003), in PTZ-kindled mice (Russo et al., 2013a), and in the pilocarpine model of TLE in rats (Mazarati et al., 2008, Mazarati et al., 2010), but no studies are available in mouse models of chronic limbic epilepsy.

Here, we investigated whether SCT is an appropriate experimental set up for revealing depressive-like behavior during early and late epilepsy in the pilocarpine and intrahippocampal kainate mouse models. Furthermore, we tried to overcome anhedonic behavior in both TLE models by chronic antidepressive treatment, and finally evaluated whether P-glycoprotein (Pgp) deficiency improves effectiveness of antidepressive treatment with fluoxetine.

Materials and methods

Animals

Mice were randomly allocated to the eight experimental groups included in the present study (Table 1). For experiments in the pilocarpine model, female FVB/N wild-type mice were purchased from Charles River (Sulzfeld, Germany) at the age of 6 weeks. For experiments in the intrahippocampal kainate model, female FVB/N wild-type mice and Pgp knock-out (*mdr1a/b*$^{(-/-)}$) mice (generated on a FVB/N background) were obtained from Taconic (Ejby, Denmark) at the age of 5 to 6 weeks. After arriving at our department, mice were housed individually under controlled environmental conditions (temperature: 22–24 °C; 12-h light-dark cycle, lights on at 6.00 a.m.) with free access to standard laboratory chow (Altromin 1324 standard diet, Altromin Spezialfutter GmbH, Lage, Germany) and tap water. Mice were kept in separate rooms without male subjects or other species. Female mice were used to allow for data comparison with data generated in our previous studies (Gröticke et al., 2008, Müller et al., 2009b). Experiments started after an acclimatization period to laboratory conditions of at least 5 days. All experiments were performed in accordance with the European Communities Council Directive of November 24th, 1986 (86/609/EEC) and the German Law on Animal Protection ("Tierschutzgesetz"). Ethical approval for the study was granted by an ethical committee (according to §15 of the Tierschutzgesetz) and the government agency (Lower Saxony State Office for Consumer Protection and Food Safety; LAVES) responsible for approval of animal experiments in Lower Saxony. All efforts were made to minimize pain or discomfort as well as the number of animals.

Table 1
Experimental groups.

TLE model	Sucrose/saccharin	Mouse type	Group size at beginning of experiments	
			Control	SE group
Pilocarpine	Sucrose	FVB/N wild-type	5 (1†)	8
Pilocarpine	Saccharin	FVB/N wild-type	6 (1†)	6
Kainate	Sucrose	FVB/N wild-type	10 (3†)	20 (7†)
Kainate	Sucrose	Pgp knock-out	6 (1†)	15 (9†)

TLE, temporal-lobe epilepsy; SE, status epilepticus; Pgp, P-glycoprotein; †, number of mice that died during the experiment.

Chemicals

Methyl scopolamine, pilocarpine hydrochloride, kainate, imipramine hydrochloride, and saccharin were purchased at Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and dissolved in aqua ad injectabilia, except for kainate which was dissolved in saline, and saccharin which was dissolved in tap water. Sucrose was bought in a local grocery and dissolved in tap water as well. Chloral hydrate was purchased at AppliChem GmbH (Darmstadt, Germany), and fluoxetine hydrochloride was kindly provided by Sanofi-Aventis Deutschland GmbH (Berlin, Germany). Both were dissolved in saline. Marbofloxacin (Marbocyl® FD) and diazepam (Faustan®) were bought as commercial solutions from Vetoquinol GmbH (Ravensburg, Germany) or Temmler Pharma GmbH & Co. KG (Marburg, Germany), respectively. Administration volume was 10 ml/kg for all compounds injected i.p. Marbofloxacin was injected s.c. at a volume of 4 ml/kg.

Induction of status epilepticus by pilocarpine

In order to avoid peripheral cholinergic effects, methyl scopolamine (1 mg/kg i.p.) was administered 30 min before starting pilocarpine treatment. Based on preliminary experiments with pilocarpine in FVB/N wild-type mice, a ramping-up dosing protocol modified from a previous protocol (Gröticke et al., 2007) with repeated i.p. injections of pilocarpine was used, which allows a more individual dosing, resulting in a high percentage of mice developing and surviving SE. First, a bolus of 200 mg/kg pilocarpine was administered, followed by injections of 50 or 100 mg/kg every 20 min until onset of self-sustaining SE. If no SE had established after the 10th injection no further pilocarpine was administered. SE was interrupted 90 min after onset by diazepam (10 mg/kg i.p.). Control mice received the same drug treatment except for pilocarpine.

Induction of status epilepticus by focal administration of kainate

For SE induction by kainate, we used a protocol described previously (Gröticke et al., 2008, Riban et al., 2002). To avoid surgery-associated infections, marbofloxacin (2 mg/kg s.c., twice daily) was administered for 7 days starting 2 days before surgery. Mice were anesthetized with chloral hydrate (400 mg/kg i.p.), and kainate (0.21 μg in 50 nl saline) was stereotaxically injected into the right CA1 area of the dorsal hippocampus (AP − 1.8 mm, LL − 1.6 mm and DV − 2.0 mm relative to bregma) over a period of 60 s using a 0.5 μl microsyringe. After injection, the needle of the syringe was maintained in situ for additional 2 min to limit reflux along the injection track. For EEG recordings, animals were implanted with bipolar electrodes aimed at the right dorsal (injected) hippocampus using the same coordinates as for kainate injection. Electrodes were held in place by modeling a "headset" consisting of three scull screws (positioned over the left and right frontal cortex and