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Invited review

Chemically-induced TLE models: Topical application

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HIGHLIGHTS

- Topical application of convulsant drugs recapitulates epileptogenesis.
- Most common substances are kainic acid and pilocarpine.
- Intracranial administration has many advantages over systemic drug application.

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ABSTRACT

Epilepsy is a condition of the brain that occurs in many different forms. For obvious reasons, understanding the complex mechanisms underlying the process of epileptogenesis cannot be fully acquired in clinical studies or analyses of surgically resected human epileptic specimens. Accordingly, a variety of animal models have been developed that recapitulate different aspects of the various forms of epilepsies. In our review we mainly focus on those chemically induced models that recapitulate characteristics typically seen in human temporal lobe epilepsies. By comparing models based on topical application of different agents, advantages and disadvantages are discussed with respect to parameters including reliability and mortality, as well as the similarity with the human condition of functional and morphological alterations occurring in different brain regions in the course of epileptogenesis and in the chronic state.

Contents

1. Topical application of convulsant drugs

Epilepsy is a condition of the brain characterized by recurrent seizures that affect 1–2% (Hesdorffer et al., 2011) of the population worldwide. Mesial temporal lobe epilepsy (MTLE) is the most frequent form of focal epilepsy in adults and at least 70% of patients presenting with MTLE are resistant to currently available medication (Schmidt and Löscher, 2005; Engel, 2001).

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Most patients with refractory TLE display severe unilateral hippocampal atrophy, so-called hippocampal sclerosis (HS), histopathologically characterized by segmental neuronal cell loss in the CA1 and CA4 subfields, astrogliosis, granule cell dispersion and axonal reorganization (Blümcke et al., 1999). Although in about 60% of cases the etiology of TLE is unknown (idiopathic) (Blumcke et al., 2007), the disorder is frequently associated with an initial precipitating injury including febrile seizures, trauma, stroke, brain infections or status epilepticus (SE). There is general agreement that such injuries can cause pathological changes in the brain that trigger the process of epileptogenesis and, after a latent period of months to years, lead to epilepsy. Attempts to recapitulate the process of epileptogenesis have been made using several animal models in which the initial brain insult is induced either physically or chemically. The most common substances for chemically-induced TLE are convulsant drugs, such as kainic acid (KA) or pilocarpine (Dudek et al., 2006; Cavalheiro et al., 2006). Following systemic or intracranial injection these drugs trigger SE, which initiates epileptogenesis and the development of spontaneous recurrent seizures (SRS) after a latent period. Systemic (intraperitoneal, intravenous or subcutaneous) administration of chemoconvulsants has been widely used as a model for TLE with HS in rats, since it reproduces many features of the disorder. A main advantage compared to intracranial administration is the simplicity of the procedure. However, the systemic approach has also several disadvantages: (i) neuronal damage is not limited to limbic structures but produces far more extrahippocampal damage than observed in human MTLE; (ii) chemoconvulsants evoke bilateral hippocampal damage, while HS in TLE patients usually occurs unilaterally; (iii) high mortality due to systemic secondary effects; and (iv) at least in mice the reliability to induce epileptogenesis as well as the survival rate is relatively low. Accordingly, our laboratory (at Bonn) failed to establish a systemic pilocarpine or KA model in mice, due to a very high mortality and the observation that surviving animals lacked the severe morphological alterations typically characterizing human MTLE.

In contrast, intracranial (intrahippocampal, intraamygdala or intracortical) administration of convulsants leads to spatially restricted brain injury and, consequently, the induced epilepsy is also more focal (Dudek et al., 2006). Moreover, this procedure usually evokes unilateral HS, which recapitulates the human pathology more closely. A further advantage of intracranial application is a much lower mortality rate which, of course, depends on the skills of the investigator. The main disadvantage is the need for anesthesia and surgery, which makes this approach technically more demanding and time-consuming than systemic drug administration.

2. Unilateral intraamygdala injection of KA

Microinjection of KA into the amygdala of rats was established in the late 1970s by Ben-Ari et al. (1978). In this and subsequent studies it was shown that focal administration of KA triggers convulsive seizures, which start at the site of injection and then spread via the cortex and ipsilateral hippocampus to the contralateral amygdala and contralateral hippocampus (Ben-Ari et al., 1978; Ben-Ari, 1985). Intriguingly, several studies demonstrated that early after KA injection, the most prominent neurodegeneration in rodents occurred at the injection site and in the CA3 area of the ipsilateral hippocampus where it extended from the septal to the temporal pole (Mouri et al., 2008; Li et al., 2008, 2012). In this model, hippocampal lesion cannot be attributed to a direct excitotoxic effect of KA, and thus provides strong evidence for the assumption that seizures per se can induce neuronal death. Indeed, blockade of KA-induced seizure activity through diazepam prevented neurodegeneration in the hippocampus, without affecting damage at the site of injection (Ben-Ari et al., 1978). At later time-points post SE, significant neuronal damage was also found in the ipsilateral hippocampal CA1 and CA4 areas, the contralateral hippocampus and in several extra-hippocampal structures in rats (Ben-Ari et al., 1980). In mice, however, seizure-induced neurodegeneration was reported to be unilateral and mainly confined to the hippocampus (Mouri et al., 2008). In addition to neuronal damage, astrogliosis and mossy fiber sprouting were found ipsilaterally (Mouri et al., 2008; Li et al., 2008, 2012; Gurbanova et al., 2008). Gurbanova et al. (2008) reported that after a latent period of 13 d, rats developed generalized SRS at a frequency of about 4 per day. A similar frequency of convulsive SRS was observed by Mouri et al. (2008) in mice, but after a much shorter latent period (3–4 d). In contrast, Li et al. (2008, 2012) detected only focal seizures (about 3 per hour) after a latent period of 12 d, which originated either in the amygdala or from the hippocampal CA3 area, but never spread beyond these regions. The reliability of inducing SE and chronic seizures was reported to be very high in this model and most studies found a very low mortality (less than 10%) (Araki et al., 2002; Mouri et al., 2008; Li et al., 2008, 2012). Hence, unilateral injection of KA into the rodent amygdala represents a suitable model for investigating putative mechanisms leading to human MTLE.

3. Unilateral intrahippocampal injection of KA

The first study showing that unilateral injection of 5–20 nmol KA into the hippocampus of rats induces seizures and unilateral hippocampal atrophy, gliosis and neurodegeneration was performed by Schwarcz et al. (1978). In later studies, KA injection into the hippocampal CA1 or CA3 region of anesthetized rats (0.4–2.0 μg) evoked convulsive SE and the development of SRS after a latent period of 5–21 d (Cavalheiro et al., 1982; Bragin et al., 1999). However, Bragin et al. (1999) reported that only 40% of the KA treated rats developed SRS while Cavalheiro et al. (1982) described that EEG and clinical seizures last only for a period of about 30 d. A remarkable improvement in the reliability of the model was achieved by injection of KA into awake rats (Bragin et al., 2004, 2005; Raedt et al., 2009; Rattka et al., 2013). In these studies, epilepsy was induced by injection of 0.4 μg/0.2 μl KA unilaterally into the CA3 region of the posterior hippocampus. This treatment very reliably evoked convulsive SE which lasted up to 20 h. SRS were recorded after a latent period of about 4 weeks in 73–86% of the animals and several studies reported survival rates of 100% (Bragin et al., 2004; Raedt et al., 2009; Rattka et al., 2013). Seizure frequency during the chronic phase varied considerably between animals but significantly increased over time, demonstrating the persistent and progressive nature of epilepsy in this model (Raedt et al., 2009; Rattka et al., 2013). Raedt et al. (2009) noted that 66% of seizures were partial with secondary generalization, and 34% without the latter. The most striking histopathological alteration induced by intrahippocampal injection of KA in rats is an almost complete loss of neurons in the CA3 region and dentate hilus of the injected hippocampus, while pyramidal neurons in the CA1 subfield are less affected (Cavalheiro et al., 1982; Rattka et al., 2013; Levesque and Avoli, 2013). This neuropathology, however, does not replicate classical human MTLE-HS, which usually exhibits more serious neuronal damage in the CA1 region than in CA3. Further neuropathological consequences of intrahippocampal KA administration include granule cell dispersion, gliosis and aberrant mossy fiber sprouting (Schwarcz et al., 1978; Cavalheiro et al., 1982; Babb et al., 1995; Longo and Mello, 1998; Bragin et al., 1999; Rattka et al., 2013).

In mice, unilateral injection of KA (20 mM/50 nl; 0.2 μg) into the CA1 area of the dorsal hippocampus is usually used to induce

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