Neuroscience 150 (2007) 984–992

GALANIN GENE TRANSFER CURTAILS GENERALIZED SEIZURES IN KINDLED RATS WITHOUT ALTERING HIPPOCAMPAL SYNAPTIC PLASTICITY.

I. KANTER-SCHLIFKE,[1a] A. TOFT SØRENSEN,[1a] M. LEDRI,[a] E. KUTEEVA,[b] T. HÖKFELT[b] AND M. KOKAIA[a]*

[a]*Experimental Epilepsy Group, Wallenberg Neuroscience Center, BMC A-11, Lund University Hospital, 221 84 Lund, Sweden*

[b]*Department of Neuroscience, Retzius väg 8, Karolinska Institutet, 171 77 Stockholm, Sweden*

Abstract—Gene therapy–based overexpression of endogenous seizure-suppressing molecules represents a promising treatment strategy for epilepsy. Viral vector–based overexpression of the neuropeptide galanin has been shown to effectively suppress generalized seizures in various animal models of epilepsy. However, it has not been explored whether such treatment can also prevent the epileptogenesis. Using a recombinant adeno-associated viral (rAAV) vector, we induced hippocampal galanin overexpression under the neuron specific enolase promoter in rats. Here we report that in animals with galanin overexpression, the duration of electrographic afterdischarges was shortened and initiation of convulsions was delayed at generalized seizure stages. However, the hippocampal kindling development was unchanged. Short-term plasticity of mossy fiber–cornu ammonis (CA) 3 synapses was unaltered, as assessed by paired-pulse and frequency facilitation of field excitatory postsynaptic potentials (fEPSPs) in hippocampal slices, suggesting that despite high transgene galanin expression, overall release probability of glutamate in these synapses was unaffected. These data indicate that hippocampal rAAV-based galanin overexpression is capable of mediating anticonvulsant effects by lowering the seizure susceptibility once generalized seizures are induced, but does not seem to affect kindling development or presynaptic short-term plasticity in mossy fibers.

Key words: galanin, epilepsy, gene therapy, rAAV, kindling, synaptic plasticity.

The neuropeptide galanin, first isolated from porcine intestine (Tatemoto et al., 1983), is widely expressed in the peripheral nervous system and CNS, and is involved in a variety of brain functions such as food intake (Kalra and Horvath, 1998; Leibowitz, 2005), cognition (Crawley, 1996), mood (Weiss et al., 1998; Barrera et al., 2005), and pain sensation (Xu et al., 2000; Liu and Hokfelt, 2002). In the rat hippocampus, a common area of seizure generation in temporal lobe epilepsy, galanin is present in afferent cholinergic and noradrenergic fibers originating from the septum–basal forebrain complex and the locus coeruleus, respectively (Melander et al., 1986; Gabriel et al., 1995; Xu et al., 1998). Among the three G-protein-coupled galanin receptors (GalRs) cloned (Branchek et al., 2000), subtypes 1 and 2 are expressed in the hippocampus (Lu et al., 2005), with GalR1 being primarily found in the ventral cornu ammonis (CA) areas, and GalR2 in the ventral and dorsal dentate gyrus (DG) (O'Donnell et al., 1999; Burazin et al., 2000).

Following experimental seizures, galanin-immunoreactivity (IR) disappears in hippocampal fibers normally containing galanin (Mazarati et al., 1998), indicating depletion of stored galanin. At the same time, seizure activity induces galanin mRNA expression in the entire hippocampus (Wilson et al., 2005), as well as de novo galanin synthesis in interneurons of the polymorphic cell layer of the DG, as revealed by galanin-IR (Mazarati et al., 1998). These changes have led to the suggestion that galanin might play a role in epilepsy. Indeed, infusion of galanin into the DG of rats before or during self-sustained status epilepticus (SE) was able to shorten or completely abolish seizures (Mazarati et al., 1998; Mazarati and Wasterlain, 2002). In addition, galanin knockout mice were shown to be more susceptible to SE, whereas mice overexpressing galanin under the dopamine beta-hydroxylase (DBH) promoter were more resistant to the induction of SE (Mazarati et al., 2000). In line with these findings, we have previously shown that transgenic mice ectopically overexpressing galanin under the platelet-derived growth factor B (PDGF-B) promoter exhibited delayed epileptogenesis induced by kindling stimulations (Kokaia et al., 2001). In addition, electrophysiological recordings in hippocampal slices from these mice revealed that ectopically overexpressed galanin was most likely released during repetitive high-frequency afferent stimulation causing reduced glutamate release via GalR-mediated action (Kokaia et al., 2001). Other studies have also shown that binding of galanin to GalRs can modulate neuronal excitability. Activation of GalR subtypes 1 and 3 causes the opening of ATP-dependent K^+ channels (Zini et al., 1993; Kask et al., 1997) and/or blockade of voltage-gated Ca^{2+} channels (Palazzi et al., 1991). These are well-known mechanisms of inhibiting presynaptic gluta-

*Corresponding author. Tel: +46-0-46-2220547; fax: +46-0-46-2220560.
E-mail address: merab.kokaia@med.lu.se (M. Kokaia).

Abbreviations: aCSF, artificial cerebrospinal fluid; AD, afterdischarge; AP, anterior–posterior; CA, cornu ammonis; CMV, cytomegalovirus; DCG-IV, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl) glycine; DG, dentate gyrus; EEG, electroencephalogram/electroencephalographic; fEPSP, field excitatory postsynaptic potential; FF, frequency facilitation; FIB, fibronectin; GalOE, galanin overexpressing; GalR, galanin receptor; IR, immunoreactivity; ISI, interstimulus interval; KA, kainic acid; ML, medio-lateral; NSE, neuron specific enolase; PC, piriform cortex; PDGF-B, platelet-derived growth factor B; PPF, paired-pulse facilitation; P_r, release probability; rAAV, recombinant adeno-associated virus; SE, status epilepticus; SSC, standard saline citrate; V, ventral.

[1] These authors contributed equally to this work.

0306-4522/07$32.00+0.00
doi:10.1016/j.neuroscience.2007.09.056

mate release, which could provide an explanation for the seizure suppressing effects of galanin. While GalR1 and GalR3 probably suppress seizures through presynaptic inhibition of excitatory transmission, the GalR2 possibly also inhibits seizures via a postsynaptic component (Mazarati, 2004). However, the mechanisms of action of the GalR2 remain unclear (Mazarati, 2004; Lu et al., 2005).

Targeting galanin as an anticonvulsant molecule will require a feasible approach to deliver the peptide into the brain. Gene therapy represents such a novel approach, allowing local delivery and expression of genes for peptides in the epileptic focus, with possibly less side effects and more efficacy as compared with conventional antiepileptic drugs. Recently, it has been shown that transduction of hippocampal neurons with a recombinant adeno-associated viral (rAAV) vector encoding for galanin under the neuron specific enolase (NSE) promoter could decrease the time spent in seizures induced by hippocampal kainic acid (KA) injection (Lin et al., 2003). In another study, using a similar vector, but containing a cytomegalovirus (CMV) promoter and the fibronectin (FIB) release sequence, overexpressed and constitutively released galanin increased the threshold of wild running seizures induced electrically in the inferior collicular cortex (Haberman et al., 2003). In addition, transduction of the piriform cortex (PC) with rAAV–CMV–FIB–galanin in previously kindled animals increased the threshold current for seizure induction (McCown, 2006). Moreover, rAAV–CMV–FIB–galanin injection in the PC suppressed the generalization of seizures induced by systemic KA-administration (McCown, 2006). These data demonstrate that local galanin overexpression mediated by a rAAV vector is able to suppress already generalized seizures. However, it remains unknown whether such viral vector–induced galanin overexpression could also delay kindling development, as was shown in transgenic galanin overexpressing (GalOE) mice (Kokaia et al., 2001).

In the present study, we used an rAAV–NSE vector to induce galanin overexpression in the hippocampus of rats subsequently undergoing hippocampal kindling, a model of temporal lobe epileptogenesis. We also explored possible alterations in synaptic transmission and plasticity caused by transgene galanin in hippocampal mossy fiber–CA3 synapses. We chose these synapses since (i) we have previously observed a reduction of frequency facilitation (FF) of field excitatory postsynaptic potentials (fEPSPs) in mossy fibers of mice overexpressing galanin under the PDGF-B promoter (Kokaia et al., 2001), and (ii) rAAV–NSE–galanin vector injections in the present study caused a prominent galanin overexpression in the mossy fibers.

EXPERIMENTAL PROCEDURES

Animals

Totally 15 adult male Sprague–Dawley rats (B&K, Sollentuna, Sweden), weighing 250 g at the beginning of the experiment, and 22 pups (Sprague–Dawley; B&K) from two litters were used for kindling and electrophysiological recordings, respectively. Animals were housed at a 12-h light/dark cycle with *ad libitum* access to food and water. All experiments were performed according to international guidelines on the use of animals as well as to Swedish Animal Welfare Agency guidelines and approved by the local Ethical Committee for Experimental Animals. All experiments were designed to minimize the number and suffering of animals.

Viral vector injection

Viral vectors were produced as described elsewhere (Lin et al., 2003). Adult animals, subsequently used for the kindling experiment, were anesthetized by i.p. injection of ketamine (80 mg/kg) and xylazine (15 mg/kg), and placed into a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Drill holes were made in the skull at the designated coordinates (see below), and viral vector suspensions were injected through a glass pipette attached to a 5 μl Hamilton syringe (Hamilton, Reno, NV, USA). To achieve maximal spread of the virus throughout the adult hippocampus, viruses (with genomic titers of 1×10^{12} for rAAV-galanin and 1×10^{13} for rAAV-empty, respectively) were diluted 3:1 with heparin (2000 IU/ml) and injected bilaterally in two sites of the dorsal (anterior–posterior (AP) -3.6, medio-lateral (ML) ±2.0, ventral (V) -3.0 and -2.1) and ventral (AP -4.8, ML ±5.2, V -6.5 and -3.8) hippocampus (in mm). Reference points for all coordinates were bregma, midline and dura, tooth bar at -3.3 mm (Paxinos and Watson, 1996). A schematic presentation of the injection sites is shown in Fig. 1A. On each side of the hippocampus, 6 μl of viral vector dilution was infused at a rate of 0.2 μl per min. After injection, the pipette was left in place for additional 3 min to prevent backflow through the injection track when retracting the pipette. Newborn animals (postnatal days 1–3), later used for electrophysiological recordings, were anesthetized by cooling and mounted on a pup tray attached to the stereotaxic frame. Pure viral vector was injected into the DG, at (in mm): AP -1.5 (from bregma), ML $+1.9$ (from midline) and V -1.8 (from dura). Totally 0.5 μl viral vector was infused at a rate of 0.2 μl per min.

Electrode implantation

Two weeks following viral vector injection, adult animals were anesthetized as described above. A bipolar stainless-steel stimulating/recording electrode (PlasticsOne, Roanoke, VA, USA) was implanted stereotaxically into the ventral hippocampus at the following coordinates (in mm): AP -4.8 (from bregma), ML $+5.2$ (from midline), V -6.3 (from dura), tooth bar at -3.3. A reference electrode was inserted into the temporal muscle, and electrodes were fixed to the skull with dental cement. Animals were allowed to recover for 1 week before undergoing electrical stimulation.

Electrical kindling stimulation

Kindling stimulations (1 ms square wave pulses of 100 Hz for 1s) were given once daily at an initially sub-convulsive threshold current. The threshold was determined on the first days of kindling by delivering stimulations at increasing current intensity in 10 μA steps, starting with 10 μA, until focal epileptiform activity (afterdischarge, AD) of at least 5 s duration was detected by electroencephalographic (EEG) recording. Behavioral seizures were scored according to the scale of Racine (1972): stage 0, no behavioral changes; stage 1, facial twitches; stage 2, chewing and head nodding; stage 3, unilateral forelimb clonus; stage 4, rearing, body jerks, bilateral forelimb clonus; stage 5, imbalance. EEG activity was recorded on a MacLab system (ADInstruments, Bella Vista, Australia) before, during, and at least 1 min after the end of each AD. Animals were considered fully kindled after having exhibited five stage 5 seizures.

Electrophysiology

Viral vector–injected rats were randomly selected 35–57 days after surgery, and following decapitation their brains were rapidly