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ORIGINAL ARTICLE

Seizures Induced by in Vivo Latrunculin A and Jasplakinolide Microperfusion in the Rat Hippocampus

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Abstract

The molecular basis for developing epilepsy remains under debate. It is hypothesized that increased excitatory synaptic activity might activate the *N*-methyl-D-aspartate receptor/Ca^{2+} transduction pathway, which induces long-lasting plasticity changes leading to recurrent epileptiform discharges. To determine if these effects are caused by disruption of F-actin in the dendritic spines, we have perfused the hippocampus of conscious rats with the F-actin-depolymerizing agent latrunculin A and the actin filament stabilizer jasplakinolide. Single perfusions of latrunculin A and jasplakinolide decrease and increase picrotoxin seizure threshold, respectively. Repeated perfusions of both latrunculin A and jasplakinolide induce epileptic seizures and a long-term increase in neuronal excitability. These results suggest that actin disruption might not be just a consequence but also a possible cause of epileptic seizures. We propose a new experimental model in rats to study the biochemical changes that might lead to chronic seizures and a method for testing new antiepileptic drugs.
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Index Entries: Seizures; latrunculin A; jasplakinolide; microdialysis; actin; hippocampus.

Introduction

The neurochemical events underlying the hyperexcitability of neurons and neuronal networks that might lead to epileptic seizures are still under debate. There is a considerable volume of scientific data indicating that the pathogenesis of epileptic seizures might result from alterations of the synaptic function and several intrinsic properties of neurons. Although development of circuitry with recurrent excitatory synapses is emerging as a common theme in many experimental models of epilepsy, it seems probable that the intrinsic properties of the neurons within a network will have a powerful influence on its excitability (McNamara, 1999; Scharfman, 2002).

Much research has focused on the role of intracellular Ca^{2+} concentration mediated by *N*-methyl-D-aspartate receptor (NMDAR) activation. In several animal models, prolonged activation of the NMDAR-Ca^{2+} transduction pathway induces long-lasting plasticity changes in hippocampal neurons causing increased excitability leading to the occurrence of recurrent epileptiform discharges (Stasheff et al., 1989; Dingledine et al., 1990; DeLorenzo et al., 1998).

Ca^{2+} influx through NMDARs causes a depolymerization of F-actin (Bonfoco et al., 1996; Shorte, 1997; Fischer et al., 2000; Ackerman and Matus, 2003) and inhibits its interaction with NMDARs through

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the competitive inhibition of α-actinin binding by Ca^{2+}-calmodulin (Wyszynski et al., 1997; Zhang et al., 1998). Although this induces a Ca^{2+}-dependent inactivation of NMDA currents (Rosenmund and Westbrook, 1993; Krupp et al., 1999), which might be responsible for seizure arrest (Sierra-Paredes et al., 1999), it is commonly accepted that NMDAR-mediated Ca^{2+} influx is the trigger for long-term potentiation (LTP) and other neurochemical events increasing neuronal excitability (DeLorenzo et al., 1998).

The integrity of dendritic spines also seems to be important for neuronal excitability. NMDARs and other glutamate receptor subtypes are clustered in dendritic spines (Craig et al., 1994; Kornau et al., 1995; Rao and Craig, 1997; O'Brien et al., 1998), which serve as integrative units in synaptic circuitry and participate in synaptic plasticity (Harris and Kater, 1994; Yuste and Denk, 1995; Brünig et al., 2004; Matus, 2005). The accumulation of glutamate receptor clusters in spines is governed by excitatory synaptic activity (Rao and Craig, 1997; O'Brien et al., 1998); and F-actin, a cytoskeletal protein that is concentrated in dendritic spines (Kaech et al., 1997; Matus, 2005), provides the main structural basis for their cytoskeletal organization (Halpain, 2000; Matus, 2000). The localization of NMDARs at synaptic sites is achieved through interactions between their intracellular domains, cytoskeletal elements (Allison et al., 1998; Ehlers et al., 1998; Zhang et al., 1998; Roelandse et al., 2003), and other cytoplasmically located submembrane proteins in the postsynaptic density (Ponting et al., 1997). F-actin might be responsible for targeting NMDARs to synaptic sites, because treatment with actin-depolymerizing agents selectively reduces the numbers of synaptic NMDAR clusters without affecting nonsynaptic clusters (Allison et al., 1998, 2000; Sattler et al., 2000). Furthermore, cultured hippocampal neurons exposed to NMDA, L-glutamate, AMPA, and ionomycin exhibit a rapid and extensive loss of dendritic spines (Halpain et al., 1998).

When neurons from both hippocampus and neocortex are examined from patients with chronic focal epilepsy, they often show dramatic dendritic abnormalities (Sheibel and Sheibel, 1973; Isokawa and Levesque, 1991; Sheibel et al., 1974; Müller et al., 1993; Multani et al., 1994; Belichenko and Dahlstrom, 1995). Dendritic spine loss has been reported repeatedly and is suggested to be more severe with increasing duration of a seizure disorder (Multani et al., 1994). A decrease in dendritic branching is commonly observed. Also, redistribution of glutamate receptors and glutamate receptor subunits has been reported (de Lanerolle et al., 1998). However, it is difficult to know if these alterations are a cause or a consequence of epileptic seizures. Mizrahi et al. (2004) have reported that spines remain structurally stable after 30 min of experimental induction of epileptic seizures; however, they begin to disappear several hours after induction of epileptic activity.

The sequence of biochemical events leading to increased neuronal excitability is still unknown. There are multiple sites by which Ca^{2+} might enter the neuron, and Ca^{2+} can activate numerous specific enzyme systems or trigger specific Ca^{2+} transduction pathways (Bading et al., 1993; Ghosh and Greenberg, 1995; Brünig et al., 2004). Also, other stimuli apart from Ca^{2+} influx through NMDARs might induce alterations in dendritic spines and cytoskeletal proteins (Halpain, 2000).

In this study the effects of the actin-depolymerizing agent latrunculin A and actin filament stabilizer jasplakinolide have been tested for the first time in the hippocampus of freely moving rats. Latrunculin A (Spector et al., 1983) affects actin polymerization by the formation of a 1:1 molar complex with G-actin, causing net actin depolymerization (Spector et al., 1989). Latrunculin A has been widely used (Allison et al., 1998; Halpain et al., 1998; Sattler et al., 2000) to study the role of F-actin in anchoring NMDARs to synaptic sites. Jasplakinolide stabilizes actin filaments in vitro and induces in vivo polymerization of actin (Bubb et al., 2000).

For this purpose we have used a new experimental design on a whole-animal model in which partial seizures can be elicited repeatedly on different days without changes in threshold or seizure patterns. Picrotoxin seizure thresholds remain constant in the same animal in repeated experiments for time periods as long as 6 mo, thus providing a good model to study possible modifications in neuronal excitability (Sierra-Paredes et al., 1996). Hippocampal microperfusion in freely moving rats has been demonstrated to be a good method for investigating the in vivo effect of substances with intracellular or extracellular action on neurons (Sierra-Paredes et al., 1999, 2001b).

Latrunculin A, picrotoxin, and jasplakinolide were dialyzed through the probe to avoid possible dynamic effects imposed by the blood–brain barrier on certain systemic administered drugs (Aguilar-Veiga et al., 1991).

A preliminary report of some of the findings has been published in abstract form (Sierra-Paredes et al., 2001a).

Materials and Methods

Drugs and Solutions

All compounds were purchased from Sigma Chemical Co., excluding latrunculin A and jasplakinolide, which were purchased from Molecular Probes (Eugene, OR). Stock solutions of latrunculin A and jasplakinolide were prepared in ethanol (100 μg/μL) and kept at –20°C until used. Solutions in Ringer's fluid appropriate for microperfusion, containing a maximum of 1:18750 ethanol, were prepared weekly at concentrations of 2, 4, and 6 μg/mL. Control Ringer's fluid and picrotoxin solutions containing 1:18750 ethanol were prepared weekly.

Animals and Surgical Procedure

Twenty adult male Sprague-Dawley rats, initially weighing 250–300 g, were used. They were housed in groups of 3, under controlled environmental conditions (ambient temperature 21 ± 1.8°C, humidity 50%–60%, 12:12-h light/dark cycle), with free access to food and water except during testing. Rats were obtained from the University of Santiago. All experiments were performed in a laboratory under controlled environmental conditions and at the same time in the morning to avoid circadian variations. All efforts were made to minimize animal suffering, and our chronic animal protocols were designed to reduce the number of animals used (Sierra-Paredes et al., 1996). Animal care followed Spanish legislation on Protection of Animals Used in Experimental and Other Scientific Purposes, in agreement with the European Union regulations (O.J. of E.C. L358/1 18/12/1986).

The rats were anesthetized with pentobarbital (40 mg/kg) and placed in a stereotaxic instrument (D. Kopf, Tujunga, CA). Under aseptic conditions, two stainless-steel microscrews to be used as electrodes for EEG recording were positioned in the skull above the frontal and occipital areas of each hemisphere; one screw, used as a reference electrode, was anchored in the mid-line, 7–9 mm rostrally to the coronal suture. The intracerebral guide for the microdialysis probe (CMA/12, CMA/Microdialysis AB, Stockholm, Sweden) was implanted vertically into the ventral hippocampus. Stereotaxis coordinates, derived from the atlas of Paxinos and Watson (1997), were 5 mm posterior, 4.8 mm lateral, and 4 mm ventral for the tip of the cannula relative to bregma and dural surface.

Wires from the microscrews were soldered to a miniature plug (Cannon MD1-9SL1, ITT Cannon, Santa Ana, CA) and fixed firmly to the skull with dental cement. After surgery, the rats were housed in individual cages and received antibiotic therapy for 4–5 d.

The experiments were carried out on conscious, freely moving rats 10 d after surgery. From the fourth day the animals were placed daily for 3 h in the experimental unit for habituation. Bipolar cortical EEGs were recorded on magnetic tapes, using a holter-EEG system (Oxford-Medilog 9200, Oxford, U.K.), and also with a Minihuit electroencephalograph (Alvar Electronic, Paris, France).

Seizure Thresholds

In our experiments, seizure threshold was defined as the lowest picrotoxin concentration that produced a specific pattern of EEG and/or behavioral seizures after a 5-min perfusion through the rat hippocampus. Only one picrotoxin dose was perfused in each experimental session. The lowest picrotoxin concentration used was 100 μ*M*, and the dose was increased slowly (+25 μ*M* each step) in each animal in successive experimental sessions at 7-d intervals until an EEG-behavioral seizure was induced. This seizure was defined as the threshold seizure. Each animal was randomly perfused four times with the same picrotoxin concentrations that had induced seizures to ensure that these doses would produce the same type of seizure on different days. Seizure types and rest periods between experimental sessions have been described previously in detail (Sierra-Paredes et al., 1996).

Microdialysis

We used the CMA/120 system for freely moving animals (CMA/Microdialysis AB, Stockholm, Sweden) and CMA/12 microdialysis probes with 4 mm of membrane length. The probe was connected via polyethylene tubing to a syringe selector (CMA/111) and to 1-mL syringes mounted on a microinjection pump (CMA/100). Before starting each experiment, the probe was perfused with ethanol and distilled water. After checking the integrity of the probe under a light microscope, it was perfused with sterile Ringer's solution (147 m*M* NaCl, 4.0 m*M* KCl, 2.4 m*M* $CaCl_2$) for 10 min, tested routinely for in vitro recovery before every experiment, and then introduced into the rat hippocampus through the chronically implanted intracerebral guide.

For control experiments, Ringer's solution was perfused at a constant flow rate of 2 μL/min throughout the experiment.

We used latrunculin A for selective actin depolymerization at the dendrite level (Allison et al., 1998) and jasplakinolide for its two possible actions: stabilization of actin filaments (Halpain et al., 1998), and disruption of actin filaments inducing polymerization of monomeric actin in amorphous masses (Bubb et al., 2000).

For latrunculin-A experiments, rats were divided into four groups: In the first group, after the picrotoxin seizure threshold was established, latrunculin A was perfused for 2 h and a subthreshold dose of picrotoxin was administrated. In the second group, to test the acute effect of latrunculin A alone, it was dissolved in Ringer and perfused daily for 8 h/d with continuous EEG monitoring for 5 consecutive days. In the third group, to test the chronic effect of a single latrunculin-A perfusion, the picrotoxin threshold was established, and after a week of rest latrunculin A was perfused for 8 h with continuous EEG monitoring. In the fourth group, latrunculin A was perfused weekly for 8 h for a maximum period of 8 wk. The picrotoxin threshold was checked every month and latrunculin-A concentrations of 2, 4, and 6 μg/mL were used. Frequent control EEGs were recorded in all of the animals during the experimental periods (at least twice a week), and they were directly observed and videotaped in search of spontaneous seizures.

The same protocol was repeated to test the effect of jasplakinolide.

EEG records were analyzed using Medilog 9200 software (v. 7.2). Spike and wave discharge duration, seizure duration, and seizure latencies were evaluated after picrotoxin administration. Statistical significance of the difference in number of seizures, seizure duration, and seizure onset times was determined by Student's paired t-test.

At the end of the experiments rats were anesthetized with Nembutal and killed by decapitation. A probe was introduced and perfused with Sudan black to help locate the position of the probe. Then the brain was removed and placed in 4% phosphate-buffered formaldehyde solution. A week later, 50-μm coronal sections were cut and stained with cresyl violet, and the position of the probe was checked under light microscopy.

Results

In Vivo Actin Depolymerization

Latrunculin A induces a short-term increase in neuronal excitability in the rat hippocampus, which leads to epileptic seizures and a permanent effect as measured by picrotoxin seizure threshold (PCTS threshold). The degree of hyperexcitability is not dose-dependent at the concentrations studied (2, 4, and 6 μg/mL) but related to the perfusion duration. In animals not treated with picrotoxin, no modifications were detected in basal EEG during wakefulness and sleep periods in a single 2-h perfusion of latrunculin A. However, latrunculin-induced seizures were observed after 6 h of continuous perfusion in 20% of the animals studied (Fig. 1A). When latrunculin A was perfused for several consecutive days (8 h a day [*see* Materials and Methods]), seizures were observed in 80% of the animals studied (Fig. 1A,C). The severity, duration, and onset time of the seizures showed considerable variation among animals, but 90% ± 6% of the seizures were observed during the second and third days of consecutive perfusion (Fig. 1A). Weekly perfusions of latrunculin A induced seizures during perfusion after the fourth week. As in the consecutive perfusion experiments, the onset of the first seizures showed considerable variation, but all of the animals studied presented several seizures between weeks 4 and 6 (Fig. 2A,C). Those animals also show an abnormal EEG response to handling and tapping with slow waves and a significant increase in sleep periods as compared with control EEGs in the same animals (Fig. 2E). Other sensory stimuli, such as auditory random stimuli, did not elicit any EEG response. A few sporadic spontaneous seizures (0.4 ± 0.3 seizures/mo) were observed during controls in the 3 mo following the last latrunculin-A perfusion (Fig. 3A). The effect observed here seems to be receptor activation-independent and related solely to actin depolymerization. In all of the animals in which PCTS threshold was tested, a significant decrease up to 56% ± 7% of the basal PCTS threshold was observed after a 2-h perfusion (Fig. 4). The chronic experiments showed that the decrease in PCTS threshold is permanent and progressive for a period of 3 mo in all of the animals studied (Fig. 3B).

Induced Actin Polymerization

Repeated and consecutive jasplakinolide perfusions (*see* Materials and Methods) into the hippocampus of living rats induces an increase on neuronal excitability in the rat hippocampus, which leads to epileptic seizures in 30% of the animals studied, a permanent effect as measured by PCTS threshold in 80% of the rats and sporadic spontaneous seizures in 60%. The degree of hyperexcitability is also related to the perfusion duration and is not dose-dependent at the concentrations studied (2, 4, and 6 μg/mL). In animals not treated with picrotoxin, no modifications were detected in basal EEG during wakefulness and sleep

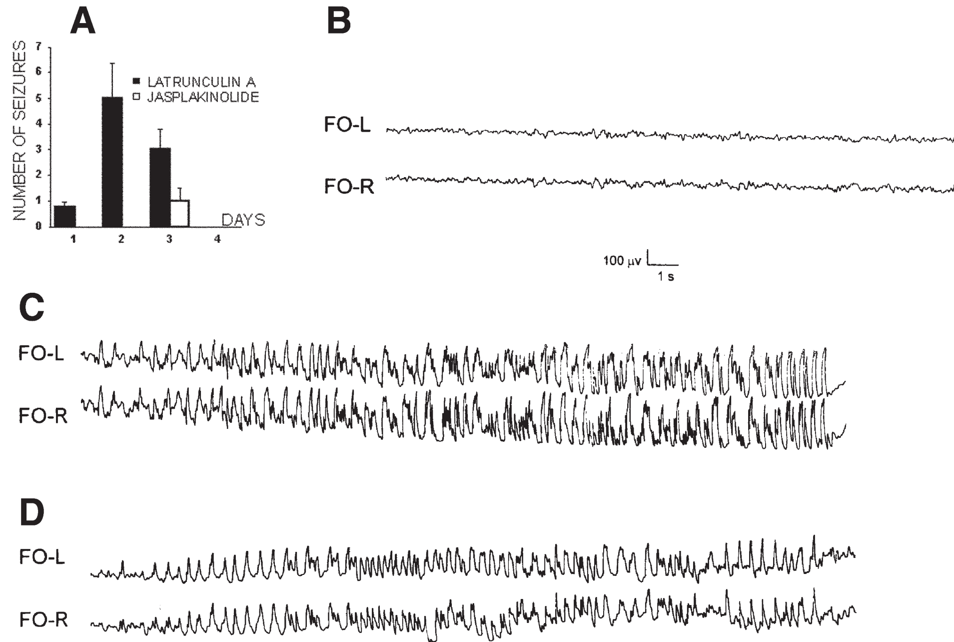

Fig. 1. **(A)** Consecutive, daily 8-h hippocampal microperfusion of latrunculin A and jasplakinolide in the hippocampus of conscious rats induces epileptiform seizures. The average number of seizures induced by latrunculin A and jasplakinolide is 10 each from days 1–4 of consecutive perfusion. **(B)** Control EEG recording. FO-R, fronto-occipital right; F-OL, fronto-occipital left. **(C)** EEG seizure induced by latrunculin A (4 μg/mL), correlated with rearing, alimentary automatism, and forelimb clonus. **(D)** EEG seizure induced by jasplakinolide (4 μg/mL), with a similar behavioral outcome.

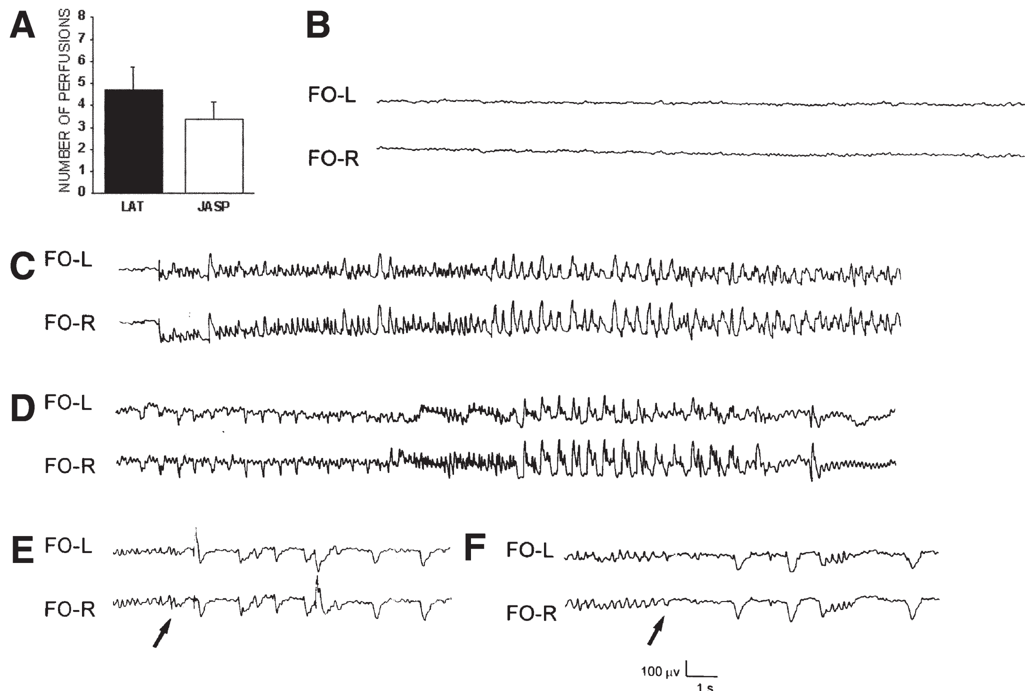

Fig. 2. Consecutive, weekly 8-h hippocampal microperfusion of latrunculin A and jasplakinolide in the hippocampus of freely moving rats induces epileptiform seizures with a variable frequency. **(A)** Summary of the average number of weekly perfusions of latrunculin A ($n = 10$) and jasplakinolide ($n = 10$) to induce seizures in rats. **(B)** Control EEG recording. F-OR, fronto-occipital right; F-OL, fronto-occipital left. **(C)** EEG seizure induced by latrunculin A (4 μg/mL). **(D)** EEG seizure induced by jasplakinolide (4 μg/mL). **(E)** EEG response to handling (this pattern appears 10–15 s after stimulation) in a rat treated with latrunculin A. **(F)** EEG response 5 s after whisk stimulation in a rat treated with jasplakinolide.

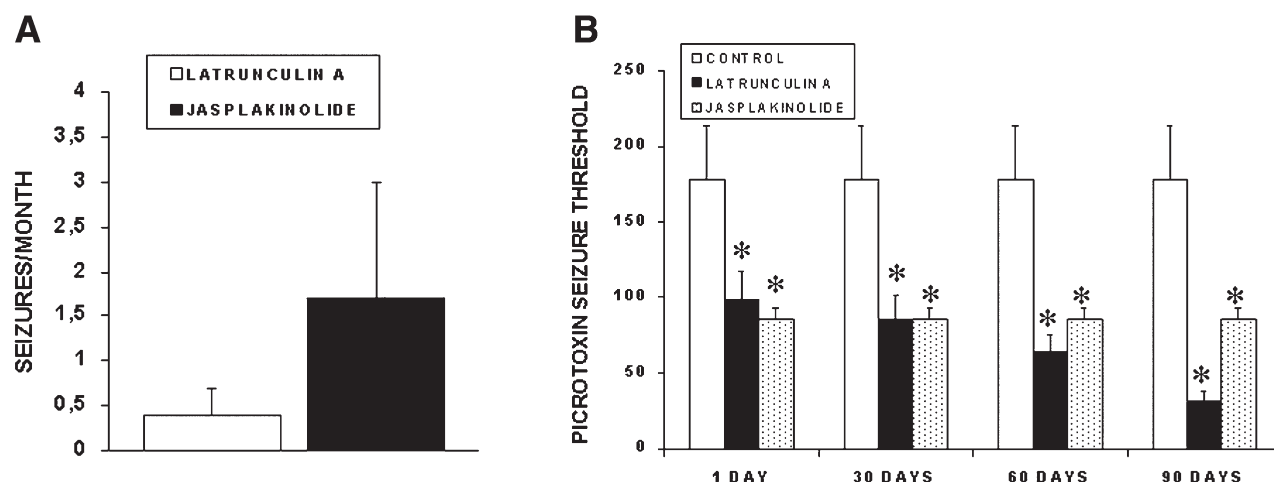

Fig. 3. Repeated latrunculin-A and jasplakinolide administrations (a minimum of 3 perfusions, 4 μg/mL, daily or weekly) produce a long-lasting effect in neuronal excitability. **(A)** Average number of seizures per month observed 3 mo following weekly latrunculin A ($n = 10$) and jasplakinolide ($n = 10$) administration. **(B)** Repeated latrunculin-A perfusions induce a permanent and progressive decrease in picrotoxin seizure threshold (*$p < 0.01$), which lasts for 3 mo after the last latrunculin-A administration ($n = 10$). Repeated jasplakinolide perfusions produce a significant (*$p < 0.01$) decrease that lasts unmodified for 3 mo ($n = 10$).

periods during a single jasplakinolide perfusion. Jasplakinolide-induced seizures were observed after 3 consecutive days in 30% of the animals studied (Fig. 1A,D). In all of the animals in which PCTS threshold was tested, a significant increase up to 33% of the basal PCTS threshold was observed after a 2-h perfusion (Fig. 4). However, chronic experiments showed that a permanent decrease in PCTS threshold was detected 1 wk after jasplakinolide administrations, lasting for a period of 3 mo in 80% of the animals studied (Fig. 3b). Weekly perfusions of jasplakinolide-induced seizures during perfusion after the third week (Fig. 2A,D) and 80% of the animals studied presented several sporadic seizures between weeks 3 and 12 (1.7 ± 1.3 seizures/mo [Fig. 3A]). The severity, duration, and frequency of the seizures showed considerable variation among animals, ranging from rapidly recurrent seizures during 24 h to short (48 ± 21 s) partial seizures. During the 3 mo following the last jasplakinolide administration, spontaneous focal discharges and abnormal EEG responses to handling, tapping, and whisk stimulation were observed in 80% of the animals (Fig. 2F), as well as a significant increase in sleep periods as compared with control EEGs in the same animals (data not shown).

Discussion

Previous studies in vitro and cell cultures showed that AMPA and NMDA receptors are anchored in the dendritic spine by actin cytoskeleton (Allison et al., 1998; Halpain, 2000). Several findings support the use of latrunculin A to perturb NMDA and AMPA receptor clusters (Allison et al., 1998; Sattler et al., 2000; Fukuzawa et al., 2003) but do not establish an in vivo functional significance for this effect. Because the actin-depolymerizing agents failed to show any effect on macroscopic whole-cell NMDA currents (Sattler et al., 2000), it was presumed that although NMDAR localization might be rearranged, function might be grossly unaffected (Allison et al., 2000). More recently, it was shown that inhibition of actin polymerization with latrunculin A impairs late-phase LTP in hippocampal slices (Fukazawa et al., 2003). Here, for the first time, we show that actin-disrupting agents can be perfused in conscious, freely moving animals to investigate the in vivo effect of modifying actin dynamics. We show also for the first time that latrunculin A and jasplakinolide, probably through F-actin depolymerization or abnormal polymerization, modify neuronal excitability leading to both acute and chronic epileptic seizures. However, the molecular mechanisms leading to latrunculin A- and jasplakinolide-induced epileptogenesis are still unclear.

Much experimental evidence shows that an increase in glutamate excitatory action modifies the excitability of postsynaptic neurons via the actin cytoskeleton (Fischer et al., 2000; Ackerman and Matus, 2003; Brünig et al., 2004). Alterations in other

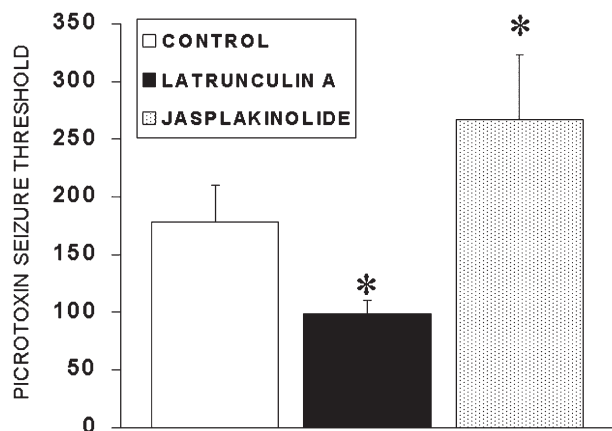

Fig. 4. A single 2-h perfusion of a 4 μg/mL latrunculin-A solution decreases significantly (*$p < 0.01$) the picrotoxin seizure threshold in freely moving rats ($n = 8$). A 2-h jasplakinolide perfusion (4 μg/mL), however, increases significantly (*$p < 0.01$) the picrotoxin seizure threshold ($n = 8$).

actin filament-related proteins, such as acidic calponin (Ferhat et al., 2003), have been linked to experimental epileptic seizures. It is well known that excitatory synaptic activity modulates the distribution of AMPA and NMDA receptors in the postsynaptic sites of hippocampal neurons. The mechanism for receptor redistribution involves calcium-mediated actin depolymerization (Bonfoco et al., 1996; Halpain et al., 1998; Lin et al., 2000; Ackerman and Matus, 2003) and is related to rapid dendritic spine plasticity (Fischer et al., 1998; Shi et al., 1999; Halpain, 2000). This receptor redistribution has been shown to participate in mechanisms such as LTP and long-term depression (Carroll et al., 1999; Lin et al., 2000; Fukazawa et al., 2003), as well as pathological processes leading to epileptogenesis (de Lanerolle et al., 1998; Fellin et al., 2004). Most of these studies have centered on the increased or decreased number of postsynaptic receptors; however, receptor location also might be an important factor in neuronal excitability (Sierra-Paredes et al., 2000; Fellin et al., 2004) and might be changed by alterations in the actin cytoskeleton (Sattler et al., 2000). Alterations in receptor density or distribution on dendritic segments would be expected to significantly modify the effectiveness of synaptic transmission. Furthermore, alterations in the distribution of recurrent excitatory synapses on dendrites could lead to an enhanced ability of these synapses to produce action potentials and, in turn, promote the reverberation of recurrent excitation in networks of mutually excitatory pyramidal cells.

A differential action of actin-disrupting agents among excitatory pyramidal cells and GABAergic interneurons cannot be ruled out. F-actin is necessary for the proper synaptic localization of AMPA receptors in GABA cells (Allison et al., 1998), and receptor relocation and complete recovery might be more difficult in GABA cells than in the dendritic spines of pyramidal cells, maintaining a permanent lack of recurrent inhibition.

We observed a differential effect between latrunculin A and jasplakinolide in acute administration. Depolymerization of actin by latrunculin A has been reported to transiently promote neurotransmitter release by a mechanism independent of extracellular Ca^{2+}, and this effect is antagonized by jasplakinolide (Morales et al., 2000). However, this presynaptic effect does not explain the chronic effect of both latrunculin A and jasplakinolide. In cell cultures, a 24-h treatment with latrunculin A is required for maximum effect (Allison et al., 1998), and we obtain the maximum effect after three perfusions of 8 h each. It is probable that in vivo spine disruption needs at least several hours to be effective (Mizrahi et al., 2004). It has been shown that jasplakinolide can disrupt actin filaments and induce polymerization of monomeric actin in amorphous masses (Bubb et al., 2000), which might explain the similar effect when both compounds are perfused for several consecutive days. The permanent decrease in seizure threshold is more likely to be related to morphological changes in the number or shape of dendritic spines, or a permanent reorganization in the location of glutamate receptors or other proteins within the postsynaptic density that are highly dependent on F-actin for their localization, such as calmodulin Kinase II, spectrin, myosin V, α-adducin, neurabin, neurabin II/spinophilin, cortactin, and many others (Allison et al., 2000). The major function of those actin-associated component proteins of the postsynaptic membrane appears to be in signal transduction and modification of the microfilament arrays in response to synaptic activation—events thought to mediate long-term synaptic plasticity.

The F-actin cytoskeleton is vulnerable to disruption by elevated intracellular calcium, a condition observed in several neuropathologies such as epilepsy, neurotrauma, and other degenerative neurological diseases. The results reported here support the idea that NMDARs and voltage-dependent Ca^{2+} channels remain suitable targets for future antiepileptic drugs, but they also support the search for other intracellular pharmacological targets such as calcineurin (Halpain et al., 1998), calpain (Sierra-Paredes et al., 1999),

calmodulin Kinase II (MacDonald et al., 1996), and several other proteins related to actin dynamics (Allison et al., 2000), including synaptic adhesion molecules (Yamagata et al., 2003). Furthermore, the seizures induced by latrunculin A or jasplakinolide present distinctive characteristics, such as low frequency and a permanent threshold decrease, which are completely different from the chemical convulsants widely used and could provide a new alternative experimental model of epilepsy for testing antiepileptic drugs.

Spontaneous seizure activity was never present in the first month after latrunculin A or jasplakinolide administration. This latency period is consistent with the time needed for plasticity changes in the hippocampus, such as in the kindling model of epilepsy (Brandt et al., 2004). It is not completely clear when sprouting occurs after seizures (Brandt et al., 2004), although in models involving a period of status epilepticus (i.e., kainic acid or pilocarpine) it appears that sprouting begins some time in the first 1–2 wk and is most robust after many weeks (Scharfman, 2002).

In summary, our results, suggesting that epileptic seizures might be caused by the direct in vivo disruption of the actin cytoskeleton, indicate that the biochemical study of actin-dependent receptor-anchoring and transduction pathways seems to be an important approach to the neuropathology and neuropharmacology of epilepsy. However, other molecular mechanisms for latrunculin A- and jasplakinolide-induced seizures, different from actin disruption, cannot be ruled out at this stage. Further research is required to define the mechanisms of the permanent neuronal alterations induced by latrunculin A and jasplakinolide microperfusion.

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