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Research Report

Effects of herbimycin A in the pilocarpine model of temporal lobe epilepsy

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ABSTRACT

Pilocarpine-induced status epilepticus (SE) causes widespread tyrosine phosphorylation in the brain. It has been postulated that this intracellular signal may mediate potentially epileptogenic changes in the morphology and physiology of particular brain regions, including the hippocampus. The present study evaluated the effects of herbimycin A, a protein tyrosine kinase (PTK) inhibitor, over the acute (during which intense biochemical and electrophysiological activation occurs) and the chronic phase (characterized by spontaneous and recurrent epileptic seizures and the presence of synaptic reorganization, e.g., mossy fiber sprouting) of the pilocarpine model of epilepsy. The administration of a single dose of 1.74 nmol of herbimycin A (i.c.v., 5 μL) 5 min after the onset of SE did not change the acute behavioral manifestation of seizures despite significantly decreasing c-Fos immunoreactivity in different areas of the hippocampus and of the limbic cortex. Herbimycin-treated animals developed spontaneous recurrent seizures, as did control animals, with a similar latency for the appearance of the first seizure and similar seizure frequency. Neo-Timm staining revealed that all animals experiencing SE, regardless of whether or not injected with herbimycin, showed aberrant mossy fiber sprouting in the supragranular region of the dentate gyrus. Herbimycin did not obviously affect neuronal cell death as evaluated in Nissl-stained sections. These results indicate that the PTK blockade achieved with the current dose of herbimycin reduced the acute c-Fos expression but failed to alter the spontaneous seizure frequency or to attenuate the morphological modifications triggered by the SE.

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Abbreviations:
BDNF, brain-derived neurotrophic factor
DAB, 3,3′-diaminobenzidine
DG, dentate gyrus
dHilus, dorsal hilus
Ent, entorhinal cortex
GL, grey level
HERB, herbimycin A
NSE, non-status epilepticus
NT-3, neurotrophin-3
OD, optical density
Pir, piriform cortex
PTK, protein tyrosine kinase
SE, pilocarpine-induced status epilepticus
SGi, supragranular region infrapyramidal blade
SGs, supragranular region suprapyramidal blade
vHilus, ventral hilus

1. Introduction

Pilocarpine-induced status epilepticus (SE) produces permanent changes in the morphology and physiology of different brain areas, including the hippocampus thus generating recurrent spontaneous limbic seizures (Turski et al., 1989). Among such changes, it has been described that SE acutely triggers protein tyrosine phosphorylation (Funke et al., 1998). This phosphorylation could occur at least through three different intracellular pathways: (1) the Trk receptors for neurotrophins; (2) the Eph receptors for the ephrins cell-to-cell adhesion molecules and (3) the Src non-receptor tyrosine kinase (Purcell and Carew, 2003). In fact, all of the above-mentioned kinases have been implicated in the epileptogenesis process. McNamara and collaborators have demonstrated the importance of TrkB receptor in the epileptogenesis process (Binder et al., 1999; He et al., 2004). The literature regarding the other two pathways, however, is not so copious. It has been demonstrated, however, the involvement of EphA/ephrin-A family interactions in kindling epileptogenesis and mossy fiber sprouting (Xu et al., 2003). The Src family of protein tyrosine kinase enhances the epileptiform activity generation in the CA_3 region of the hippocampus (Sanna et al., 2000). In addition, it has been previously suggested that the Eph/ephrin effects could be mediated by the Src family of protein kinases (Murai and Pasquale, 2002). Indeed, the Src kinases have recently been described as "a hub for NMDA receptor regulation" (Salter and Kalia, 2004), as it controls synaptic strength and consequently activity-dependent plasticity. Thus, the characterization of protein tyrosine kinase (PTK) inhibitors that could interfere with the process of epileptogenesis might be of clinical interest for human application.

Herbimycin A, a cell permeable ansamycin antibiotic isolated from *Streptomyces sp.*, has specific inhibitory activity on tyrosine residues phosphorylation catalyzed by various protein kinases, in particular by Src kinase (Uehara and Fukazawa, 1991). Herbimycin A is able to attenuate the increase in excitability in different experimental paradigms (e.g., hippocampal LTP (Abe and Saito, 1993) and 5HT-induced potentiation in Aplysia (Purcell and Carew, 2003)).

Here we investigated the effect of herbimycin A on epileptogenesis using the pilocarpine model of epilepsy. We hypothesized that blocking the protein tyrosine phosphorylation with herbimycin A during sustained epileptic activity (i.e., the SE) might prevent epileptogenesis. Animals were evaluated for the development of spontaneous recurrent seizures and associated morphological alterations in the hippocampus (e.g., cell death and mossy fiber sprouting).

2. Results

2.1. *Pilocarpine induced status epilepticus*

The first signs of pilocarpine systemic administration could be observed approximately 10 min after the intraperitoneal injection, as described previously (Longo et al., 2003; Mello et al., 1993). First, the animal freezes and presents some chewing behavior. Piloerection, increased salivation and diarrhea followed by body tremors and stereotyped oral movements are observed after 20 min of the injection. This pattern could progress to partial seizures, characterized by eye blinking, ears and jaw movements and myoclonic twitching of the head muscles. After that, the animals evolved to one of three different states: (1) occasional presence of this behavior for the next hour evolving to rest (normal) behavior (the non-SE group); (2) evolve to a tonic seizure, normally followed by death or (3) evolve to episodic motor generalized seizures (Racine's stage V) with increasing frequency until reaching *status epilepticus* (SE). The SE time course was characterized by robust muscle contractions for 20 min and thereafter with a progressive decline of the intensity of limbs and body jerks. The administration of herbimycin A did not change the behavioral evolution of the SE animals or the frequency of