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NeuroToxicology 26 (2005) 969–979

NeuroToxicology

Comparison of Neuroprotective Effects Induced by α-Phenyl-*N*-*tert*-butyl nitrone (PBN) and *N*-*tert*-Butyl-α-(2 sulfophenyl) nitrone (S-PBN) in Lithium-Pilocarpine Status Epilepticus

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Received 7 January 2005; accepted 4 April 2005
Available online 10 May 2005

Abstract

The status epilepticus (SE) induced in rats by lithium-pilocarpine (Li-pilo) shares many common features with soman-induced SE including extensive limbic neuropathology. Reactive oxygen species are hypothesized to play a role in the SE induced neuropathology and we propose that the free radical scavengers α-phenyl-N-tert-butyl nitrone (PBN) and N-tert-butyl-α-(2 sulfophenyl) nitrone (S-PBN) may be neuroprotective. PBN or S-PBN were administered either immediately following pilocarpine (exposure treatment) or 5 min after the onset of SE as determined by ECoG activity. SE was allowed to continue for 3 h before termination with propofol. The rats were sacrified 24 h following pilocarpine administration. S-PBN induced minor effects to reduce SE duration and improve neurological deficit 24 h following pilocarpine administration. One hundred and fifty milligrams per kilograms PBN administered 5 min after SE onset produced significant neuroprotection in the parietal, occipital, perirhinal and piriform cortices as well as the lateral amygdala. One hundred and fifty milligrams per kilograms S-PBN was neuroprotective only in the occipital and perirhinal cortex while 300 mg/kg S-PBN exacerbated cortical neuropathology. S-PBN administered 5 min after SE onset exacerbated neuropathology in thalamic regions. In contrast, PBN and S-PBN administered as exposure treatment exacerbated neuropathology in thalamic and CA3 regions. The differential neuroprotective effects of PBN and S-PBN may be the result of the poor brain penetration by S-PBN. The results suggest that free radical scavenger activity is neuroprotective in cortical regions during cholinergic convulsions. Regional variations in drug-induced neuroprotectant activity in Li-pilo SE are common and suggest multiple mechanisms of neuropathology.

Keywords: α-Phenyl-*N*-*tert*-butyl nitrone; *N*-*tert*-Butyl-α-(2 sulfophenyl) nitrone; Neuroprotection; Lithium; Pilocarpine; Status epilepticus

INTRODUCTION

Lithium administration in rats followed 20–24 h later by pilocarpine induces a continuous status epilepticus (SE) of several hours duration. Lithium-pilocarpine (Li-pilo) SE induces a characteristic pattern of limbic neuropathology that is particularly severe in midline thalamus regions, hippocampus, piriform cortex, perirhinal cortex and amygdala (Clifford et al., 1987). Following a latent period of several weeks the animals develop spontaneous recurrent seizures (Andre et al., 2001; Leite et al., 2002; Loscher, 2002). Because

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doi:10.1016/j.neuro.2005.04.002

of the pharmacological, behavioral and pathological similarities, Li-pilo SE is considered a model of human temporal lobe epilepsy (Leite et al., 2002; Loscher, 2002) and has been tested as such (Andre et al., 2001, 2003; Rigoulot et al., 2003).

Li-pilo convulsions may serve as a model of the effects induced by the warfare nerve agent soman. Both Li-pilo (Jope et al., 1986) and soman-induced SE (Shih et al., 1997; McDonough and Shih, 1997) have an initial muscarinic phase that is followed by a secondary glutamatergic phase (Ormandy et al., 1989; McDonough and Shih, 1997). Both produce a similar pattern of neuropathology that is particularly severe in the limbic system (Clifford et al., 1987; McDonough et al., 1998). Li-pilo (Jope et al., 1986) and soman (Shih et al., 1997; McDonough and Shih, 1997) produce convulsions that are relatively refractory to drug therapy. Because such convulsions are resistant to anticonvulsant activity, we propose that neuroprotectants that reduce neuronal damage even during ongoing seizure activity would be of value.

α-Phenyl-*N*-*tert*-butylnitrone (PBN) is neuroprotectant in models of neuronal injury. PBN is neuroprotective in brain ischemia models in gerbils (Yue et al., 1992; Oliver et al., 1990) and in rats (Cao and Phillis, 1994; Li et al., 2001), even when administered as late as 12 h after the ischemic episode (Cao and Phillis, 1994). PBN has also been shown to be neuroprotective in experimental models of epilepsy. PBN (200 mg/kg) administered 30 min before kainic acid had no effect on the SE but induced a neuroprotective effect as measured by cytochrome C oxidase activity and energy metabolism (Milatovic et al., 2001). PBN (100 mg/kg) was neuroprotective in flurothyl-induced SE (He et al., 1997) and PBN (30 mg/kg) reduced hydroxyl radical formation in seizures induced by a pentylenetetrazol kindling paradigm (Rauca et al., 2004). PBN (two doses of 100 mg/kg) induced neuroprotection in the hippocampus of postictal day 25 (P25) rats subjected to Li-pilo SE (Rejchrtova et al., 2005).

N-*tert*-Butyl-α-(2 sulfophenyl) nitrone (S-PBN) is a sulfonated derivative of PBN that is significantly more water-soluble. The major difference is pharmacokinetic in that PBN has a plasma half-life of 3 h and readily penetrates the blood–brain barrier (BBB) (Chen et al., 1990) while S-PBN has a plasma half-life of 9 min and poor BBB penetration (Marklund et al., 2001a; Yang et al., 2000). Despite these reports systemically administered S-PBN induces significant CNS neuroprotection when tested using in vivo models of neural injury. For example, systemically administered S-PBN decreased central lesions induced by numerous excitotoxins (Schulz et al., 1995a). Systemic S-PBN reduced hypoxic lesion volume when tested as late as 6 h following the ischemic episode (Schulz et al., 1995b). Intraperitoneal PBN or S-PBN were equally effective in reducing infarct volume following embolic stroke in rats (Yang et al., 2000). Intravenous PBN or S-PBN were equally effective neuroprotectants in traumatic brain injury (TBI) models in rats (Marklund et al., 2001a,b, 2002). In two of the TBI studies S-PBN induced significantly greater neuroprotective effects than PBN (Marklund et al., 2001a, 2002). In spite of reportedly unfavorable pharmacokinetic properties, S-PBN induces significant neuroprotection in a wide variety of experimental models of excitotoxicity.

The purpose of this study was to test the relative activities of S-PBN and PBN as neuroprotectants in Li-pilo-induced SE as an experimental model of nerve agent exposure. As spin-trapping agents it was hypothesized that S-PBN and PBN will reduce SE-induced neuropathology mediated by reactive oxygen species.

METHODS

Animals

Male, Sprague–Dawley rats obtained from Harlan (Indianapolis, IN) and weighing 290–325 g at the time of seizure test were used for these experiments. The animals were maintained in a climate-controlled vivarium at 21 °C on a 12 h light/12 h dark cycle with food and water available ad libitum. All animal care and use conformed to the policies of the University of New Mexico Health Sciences Center.

Intracranial Implants

Rats were anesthetized with equithesin (a mixture of chloral hydrate, pentobarbital, magnesium sulfate, ethanol, propylene glycol and water) for the surgical placement of the electrocorticogram (ECoG) recording electrodes. Stainless steel ECoG recording screws were placed bilaterally in the skull 3 mm lateral to midline and equidistant between bregma and lambda. The screws were attached to connector pins by insulated wire. A third screw assembly was placed over the frontal sinus as a reference electrode and additional screws were set in the skull to serve as anchors. All