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Hypoxia/ischemia a key player in early post stroke seizures: Modulation by opioidergic and nitrergic systems

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ABSTRACT

Stroke is a leading cause of death, disability, and socioeconomic loss worldwide. All attempts at pharmacological reduction of the complications of stroke (e.g. post-stroke seizure, and brain's vulnerability to hypoxic/ischemic injury) have failed. Endogenous opioids and nitric oxide (NO) overproduction has been documented in brain hypoxia/ischemia (H/I), which can exert pro-convulsive effects. In this study, we aimed to examine the possible involvement of opioidergic and nitrergic pathways in the pathogenesis of post-stroke seizure. H/I was induced by right common carotid ligation and sham-operated mice served as controls. We demonstrated that right common carotid ligation decreases the threshold for clonic seizures induced by pentylenetetrazole (PTZ), a GABA antagonist. Furthermore, pro-convulsive effect of H/I following right common carotid ligation was blocked by naltrexone (NTX) (3 mg/kg), NG-Nitro-L-arginine methyl ester (L-NAME) (10 mg/kg), and aminoguanidine (AG) (100 mg/kg) administration ($P < 0.001$). Interestingly, co-administration of non-effective doses of NTX and L-NAME (1 and 0.5 mg/kg, respectively) reverses epileptogenesis of H/I ($P < 0.001$). In the same way, co-administration of non-effective doses of NTX and AG (1 and 5 mg/kg, respectively), reverses epileptogenesis of H/I ($P < 0.001$). Indeed, the histological studies performed on mice exposed to H/I confirmed our previous data. These findings suggest hyper-susceptibility to PTZ induced seizure following H/I is mediated by interaction of opioidergic, and iNOS/NO pathways. Therefore, our results identify new pharmacological targets and provide the rationale for a novel strategy to promote recovery after stroke and possibly other brain injuries.

1. Introduction

Stroke is a devastating disease and third leading cause of death following cardiovascular disease and cancer in major industrialized countries (Palmer et al., 2005). Stroke is considered by some authorities as a major cause of seizures in elderly population. Seizures after stroke can be early onset (occur within 2 weeks) or late onset (occur within months to years), variously ranging from 2% to 33% (Bladin et al., 2000; Jordan, 2004). Post stroke seizure also has a negative role in recovery after stroke and quality of life (Burneo et al., 2010).

Early post stroke seizures express a wide range of clinical presentations; they can be generalized and associated with tonic–

Abbreviations: (NO), Nitric oxide; (H/I), Hypoxia/ischemia; (PTZ), Pentylenetetrazole; (NTX), Naltrexone; (EEG), Electroencephalogram; (ATP), Adenosine triphosphate; (ROS), Reactive oxygen species; (NOS), Nitric oxide synthase; (L-NAME), NG-Nitro-L-arginine methyl ester; (AG), Aminoguanidine; (UNOP), Unoperated group; (SHOP), Sham operated; (RCC ligated), Right common carotid ligated; (CST), Clonic seizure threshold; (ANOVA), Analysis of variance; (MCAO), Middle cerebral artery occlusion; (CNS), Central nervous system

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clonic convulsions or partial and subclinical, being exclusively detectable by the electroencephalogram (EEG) (Jordan, 2004; Menon and Shorvon, 2009). Currently, the pathophysiology of early-onset post stroke seizures is poorly understood, and a standardized treatment regimen is yet to be established (Kwan and Wood, 2010).

Decreasing cerebral blood flow during stroke, leads to oxygen and glucose depletion, a condition generally referred to hypoxia/ischemia (H/I). This further causes a series of cellular processes, including adenosine triphosphate (ATP) deprivation, mitochondrial dysfunction, reactive oxygen (ROS) and nitrogen species production, and endogenous opioids levels alteration. These factors eventually can cause hypoxic/ischemic injury and seizure (Baskin et al., 1985; Broughton et al., 2009; Dirnagl et al., 1999; Doehner et al., 2012; Faden, 1983; Kao et al., 2008; Shi and Liu, 2007; Tuttolomondo et al., 2008).

Endogenous opioid peptide family, including dynorphins, enkephalins, and β-endorphins are widely distributed throughout the CNS, mediating several physiological and pathological processes (Hauser and Mangoura, 1998; Satoh and Minami, 1995). So far, opioid receptors at least have been divided into three groups including μ, δ, and κ (Satoh and Minami, 1995). Evolving data have shown neuroprotective and/or neurodestructive properties of endogenous opioids peptides during CNS related diseases such as hypoxy/ischemic injury and seizures based on their doses and models (Foote and Gale, 1984; Frenk, 1983; Lauretti et al., 1994).

Nitric oxide is synthesized by nitric oxide synthase (NOS) (Bolanos et al., 1997), either constitutive (eNOS, and nNOS) or inducible isoforms (iNOS) (Moncada and Higgs, 1993). NO has been regarded as a crucial factor in modulation of some types of seizures (Gholipour et al., 2008; Homayoun et al., 2002; Nidhi et al., 1999). In addition, increased CNS NO contents during H/I might exert neurodestructive effects (Brown and Bal-Price, 2003; Helps and Sims, 2007). Besides, it is thought to play a role in opioids anti- and pro-convulsant effects (Homayoun et al., 2002; Khavandgar et al., 2003).

Regardless of the difference between animal models of stroke, several similar molecules might be involved in their consequent H/I and early seizure (Mergenthaler and Meisel, 2012; Small et al., 2013). This raises the possibility that neuroprotective agents might be able to prevent the development of post stroke seizure. This study examined the postulation that ischemic stroke in mice may change the threshold of early clonic seizure induced by PTZ, acting as a GABA antagonist and a reliable discriminative stimulus for the induction of seizure (Payandemehr et al., 2014; Pollack and Shen, 1985). Likewise, involvement of endogenous opioids and NO in this process was also examined.

2. Materials and methods

2.1. Ethics

The procedures implemented throughout the study were approved by the Ethics Committee of Tehran University of Medical Sciences in accordance with the Standards for the Care and Use of Laboratory Animals.

2.2. Drugs and chemicals

The following compounds were used throughout the study: Pentylenetetrazole (PTZ) (Sigma, UK); NG-Nitro-L-arginine methyl ester (L-NAME); naltrexone (NTX); aminoguanidine (AG); cresyl violet (Sigma, St Louis, MO, USA). All drugs were freshly made in 0.9% saline. All injections were done intraperitoneally in volumes of not more than 10 ml/kg of the body weight of the mice.

2.3. Experimental animals

108 male NMRI mice (Razi Institute, Karadj, Iran) aging between 4 and 9 months were used to model ischemic brain injury and post-ischemic seizures, which may roughly correspond to a human age range of 20–40 years (Fox et al., 2006). The animals were housed in standard polycarbonate cages in groups of 4–5 and kept in a temperature-controlled room (22°C) with 12 h light/12 h dark cycle. Animals were acclimated at least 2 days before experiments with free access to food and water. The experiments were conducted between 09:00 and 13:00. All procedures were carried out in accordance with institutional guidelines for animal care and use. Groups consisted of at least six animals and each animal was used only once. In addition, efforts were made to reduce animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.4. Hypoxia/ischemia

H/I was induced by permanent unilateral double ligation of the carotid artery (Alberi et al., 2010). Briefly, animals were anesthetized with sodium pentobarbital (45 mg/kg) and supplemented during surgery as required. The anesthesia time for the animals lasted an average of 33.040 ± 10.089 (mean ± STD) min. The right common carotid artery was isolated and care was taken to avoid damage to the vagus nerve and other blood vessels. Then, it was ligated in two sites with 6-0 surgical silk. The first ligature was placed just proximal to the common carotid artery bifurcation into the internal and external carotid arteries. The second ligature was placed approximately 3 mm proximal. Then, to guarantee the absence of blood flow through the ipsilateral carotid artery, the common carotid was transected between the ligatures. The outer skin was closed with 6-0 monofilament nylon. SHOP animals were treated identically except for the carotid ligation. Prior evaluation of preoperative temperatures with this protocol found that rectal temperatures remained at 37 ± 0.5 °C and did not vary significantly between ligated and SHOP groups. During surgery, a body temperature of 37 °C was maintained, with the mice on a heating pad. Mice underwent RCC ligation and sham operation were observed for the following criteria: maintenance of dilated pupils, absence of a corneal reflex when exposed to strong light stimulation, and maintenance of rectal temperature at (37 ± 0.5 °C). The animals that did not match these criteria and showed seizures were excluded from the study (Kadam et al., 2009; Rahimian et al., 2011; Yager et al., 2002).

2.5. Experimental design

A total number of 108 animals were assigned randomly to 14 groups, each comprising 6–8 mice. Mice were treated according to following schemas:

In the first step of this study, mice were assigned randomly to 3 groups, including 1) unoperated group (UNOP), 2) sham operated group (SHOP), and 3) right common carotid ligated group (RCC ligated).

In order to study the effect of L-NAME, NTX, or AG on the epileptogenesis properties of right common carotid ligation mice were randomly assigned to the following groups: 1) NTX (3 mg/kg, ip), 2) L-NAME (10 mg/kg, ip), 3) AG (100 mg/kg, ip), 4) NTX Non-ligated, 5) L-NAME Non-ligated, 6) AG Non-ligated, 7) NTX (1 mg/kg, ip), 8) L-NAME (0.5 mg/kg, ip), 9) AG (5 mg/kg, ip), 10) additive (NTX (1 mg/kg)+L-NAME (0.5 mg/kg), ip), and 11) additive (NTX (1 mg/kg)+AG (5 mg/kg), ip).

The doses of NTX, L-NAME, and AG, as well as the time interval between drug injection and stroke induction (30 min for NTX, and L-NAME and 45 min for AG) were chosen according to a literature