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Hypoxia/ischemia a key player in early post stroke seizures: Modulation by opioidergic and nitrenergic 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 nitrenergic 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 pentylentetrazole (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.

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Abbreviations: (NO), Nitric oxide; (H/I), Hypoxia/ischemia; (PTZ), Pentylentetrazole; (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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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-

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 hypoxia/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: Pentylentetrazole (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 \pm 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

review (Adabi Mohazab et al., 2012; Cordery et al., 2012; Shafaroodi et al., 2008).

2.6. Seizure paradigm

4 h after right common carotid ligation clonic seizure threshold (CST) was determined by inserting a 30-gauge dental needle into the lateral tail vein of the mouse (Payandemehr et al., 2012, 2014; Yager et al., 2002). The needle was then secured to the tail by a narrow piece of adhesive tape. With mouse moving freely, the PTZ solution (0.5%) was slowly infused into the tail vein at a constant rate of 1 ml/min using an infusion pump (NE 1000, New Era Pump System, Inc.), which was connected to the dental needle by polyethylene tubing. Infusion was halted when general clonus (forelimb clonus followed by full clonus of the body) was observed. The minimal dose of PTZ (mg/kg of mice weight) needed to induce general clonus was recorded as an index of clonic seizure threshold. As such, the seizure threshold is dependent on PTZ dose administered and time-related.

2.7. Histology

4 h after right common carotid ligation (see above), all animals from different groups were killed by an overdose of ether inhalation. The brains were kept at 4% PFA for at least 1 week and then were processed for histological studies as follows. Three series of 10- μ m thick coronal sections were cut every 100- μ m from 2.3 to 4.3 mm posterior to the bregma. Sections were stained with 1.0% cresyl violet. Slides were examined with a light microscope and digital photographs were taken from the hippocampal CA1 area of both hemispheres. Quantitative analysis of dark neurons at $\times 100$ and $\times 400$ magnification by the light microscope, was done by a blinded investigator (Karimzadeh et al., 2012; Ooigawa et al., 2006). On the other hand, The thickness of the pyramidal cell layer of the hippocampus was measured. Three measurements per section, for 3 sections, were averaged for CA1 region per mouse to calculate group means. All sections represented the same level along the longitudinal axis, and measurements were performed by two blinded investigators (Alvarez et al., 2014).

2.8. Statistical analysis

Data are expressed as the mean \pm S.E.M of CST for each experimental group. One-way analysis of variance (ANOVA) followed by a Tukey–Kramer multiple comparison test was used to analyze the data. $P < 0.05$ was considered the significance level between groups.

3. Results

4 h after RCC ligation, we assessed the animals matching study criteria (see above). The mortality rate was less than 10%.

3.1. Histological assessment of H/I in brain injury after RCC ligation

There are several criteria for recognizing dark neurons, including neuronal shrinkage, cytoplasmic eosinophilia, nuclear pyknosis, and surrounding spongiosis.

Dark neurons were revealed by cresyl violet staining in the CA1 region in different groups (Table 1 and Fig. 1). The mean number of dark neurons in the hippocampal CA1 area was 25.25 in RCC ligated, 4.25 in UNOP, 3.00 in SHOP, 20.50 in l-NAME (0.5 mg/kg), 11.50 in l-NAME (10 mg/kg), 20.25 in NTX (1 mg/kg), 10.75 in NTX (3 mg/kg), 21.00 in AG (5 mg/kg), 10.50 in AG (100 mg/kg), 3.00 in NTX Non-ligated, 3.00 in l-NAME Non-ligated, 5.50 in AG Non-ligated, 9.14 in

Table 1

Effect of right common carotid ligation in dark neuron number. The seizure threshold was measured 4 h after right common carotid ligation. Data represent mean \pm S.E.M from 6 to 8 animals and were analyzed using a multivariate analysis of variance (ANOVA) for exploration time and the percentage of arm entries, and one-way ANOVA for locomotor activity, followed by Tukey's post-hoc test.

Groups	Mean \pm S.E.M
UNOP	(4.25 \pm 0.95)
Sham operated (SHOP)	
Saline	(3.00 \pm 1.41)
NTX (3 mg/kg)	(3.00 \pm 0.40)
l-NAME (10 mg/kg)	(3.00 \pm 0.40)
AG (100 mg/kg)	(5.50 \pm 0.64)
Right common carotid ligated (RCC ligated)	
Saline	(25.25 \pm 1.03) ^{bbb, cc}
NTX (3 mg/kg)	(10.75 \pm 0.85) ^{aaa}
l-NAME (10 mg/kg)	(11.50 \pm 0.64) ^{aaa}
AG (100 mg/kg)	(10.50 \pm 1.25) ^{aaa}
NTX (1 mg/kg)	(20.25 \pm 2.09)
l-NAME (0.5 mg/kg)	(20.50 \pm 1.04)
AG (5 mg/kg)	(21.00 \pm 0.97)
Additive (NTX (1 mg/kg)+ l-NAME (0.5 mg/kg))	(11.75 \pm 1.75) ^{aaa}
Additive (NTX (1 mg/kg)+AG (5 mg/kg))	(9.14 \pm 1.76) ^{aaa}

^a $P < 0.05$, ^{aa} $P < 0.01$, and ^{aaa} $P < 0.001$ compared to RCC ligated group; ^b $P < 0.05$, ^{bb} $P < 0.01$, and ^{bbb} $P < 0.001$ versus the corresponding SHOP group; ^c $P < 0.05$, ^{cc} $P < 0.01$, and ^{ccc} $P < 0.001$ versus the corresponding SHOP group.

additive (NTX (1 mg/kg)+AG (5 mg/kg)), and 11.75 in additive (NTX (1 mg/kg)+ l-NAME (0.5 mg/kg)) groups. As shown in our table there was no significant alteration in the dark neuron density between SHOP saline group and UNOP group ($P > 0.05$), whilst a significant increase was observed in right common carotid ligated (RCC ligated) group in comparison to SHOP and UNOP ($P < 0.001$). Pretreatment with NTX (3 mg/kg), l-NAME (10 mg/kg), and AG (100 mg/kg) significantly decreases dark neuron density in comparison to RCC ligated group ($P < 0.001$). On the other hand, administration of NTX (3 mg/kg) in NTX Non-ligated group, l-NAME (10 mg/kg) in l-NAME Non-ligated, and AG (100 mg/kg) in AG Non-ligated, had no significant effect on dark neuron density in comparison to SHOP saline group ($P > 0.05$). Pretreatment with a low dose of l-NAME (0.5 mg/kg) and NTX (1 mg/kg), and AG(5 mg/kg) had no significant effect on dark neuron density in comparison to RCC ligated group ($P > 0.05$). However, a combination of low dose of NTX and l-NAME as an additive group had a significant effect on dark neuron density in comparison to RCC ligated ($P < 0.001$). Likewise, a combination of low dose of NTX and AG as an additive group had a significant effect on dark neuron density in comparison to RCC ligated ($P < 0.001$).

The mean thickness of the pyramidal cell layer of CA1 in different groups was calculated as below: 363.788 μ m in RCC ligated, 361.438 μ m in UNOP, 409.51 μ m in SHOP, 474.386 μ m in l-NAME (0.5 mg/kg), 273.837 μ m in l-NAME (10 mg/kg), 369.536 μ m in NTX (1 mg/kg), 262.12 μ m in NTX (3 mg/kg), 327.982 μ m in AG (5 mg/kg), 281.132 μ m in AG (100 mg/kg), 389.927 μ m in NTX Non-ligated, 419.942 μ m in l-NAME Non-ligated and 263.688 μ m in AG Non-ligated groups, 253.947 μ m in Additive (NTX (1 mg/kg)+AG (5 mg/kg)), and 327.492 μ m in additive (NTX (1 mg/kg)+ l-NAME (0.5 mg/kg)) groups.

3.2. The effect of RCC ligation on CST

As shown in Fig. 2 there was no significant alteration in the CST between SHOP group and UNOP group ($P > 0.05$), whilst a significant reduction was observed in right common carotid ligated (RCC ligated) group in comparison to SHOP and UNOP ($P < 0.01$).

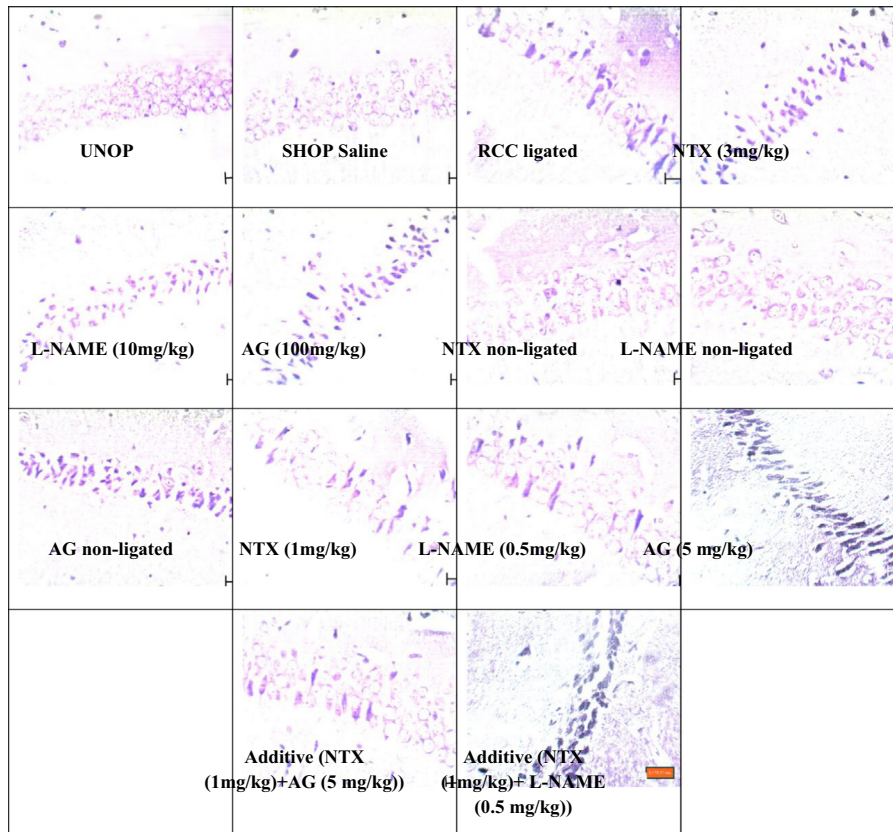


Fig. 1. Micrographs of cresyl-violet stained cells. Representative micrographs of cresyl-violet stained cells in the hippocampal CA1 region from different groups at 4 h after right common carotid ligation. Scale bars in different groups include: UNOP: 73.52 μm , SHOP: 108.17 μm , RCC ligated: 110.65 μm , NTX (3 mg/kg): 75.84 μm , L-NAME (10 mg/kg): 83.30 μm , AG (100 mg/kg): 83.30 μm , NTX Non-ligated: 78.33 μm , L-NAME Non-ligated: 80.81 μm , AG Non-ligated: 80.81 μm , NTX (1 mg/kg): 108.17 μm , L-NAME (0.5 mg/kg): 72.11 μm , additive (NTX (1 mg/kg)+AG (5 mg/kg)): 110.77, and additive (NTX (1 mg/kg)+ L-NAME (0.5 mg/kg)): 78.37.

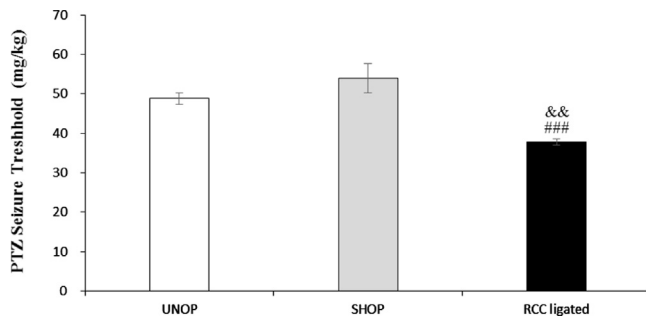


Fig. 2. Comparison of CST between right common carotid ligated group (RCC ligated) with that of SHOP and UNOP controls. The seizure threshold was measured 4 h after right common carotid ligation. Data represent means \pm S.E.M from 6 to 8 animals and were analyzed using a multivariate analysis of variance (ANOVA) for exploration time and the percentage of arm entries, and one-way ANOVA for locomotor activity, followed by Tukey's post-hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to RCC ligated group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ versus the corresponding SHOP group; & $P < 0.05$, && $P < 0.01$, &&& $P < 0.001$ versus the corresponding SHOP group.

3.3. The effect of NTX pretreatment in H/I after RCC ligation on CST

As shown in Fig. 3, a complete reversal was observed using high dose of NTX (3 mg/kg) ($P < 0.001$). Low dose administration of NTX (1 mg/kg) had no significant effect on CST in comparison to RCC ligated ($P > 0.05$). However, administration of NTX (3 mg/kg) in NTX Non-ligated group had no significant effect on CST in comparison to SHOP group ($P > 0.05$).

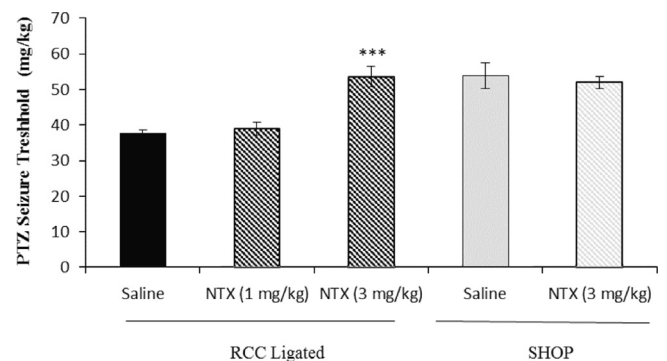


Fig. 3. The effect of administration of different doses of NTX (1 and 3 mg/kg, i.p. 30 min before testing) on right common carotid ligation induced decrement of CST. The seizure threshold was measured 4 h after right common carotid ligation. Data represent means \pm S.E.M from 6 to 8 animals and were analyzed using a multivariate analysis of variance (ANOVA) for exploration time and the percentage of arm entries, and one-way ANOVA for locomotor activity, followed by Tukey's post-hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to RCC ligated group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ versus the corresponding SHOP group.

3.4. The effect of L-NAME pretreatment in H/I after RCC ligation on CST

As shown in Fig. 4, in high dose pretreatment with L-NAME (10 mg/kg), a complete reversal was observed in comparison to RCC ligated ($P < 0.001$). Administration of low doses of L-NAME (0.5 mg/kg) had no significant effect on CST in comparison to RCC ligated ($P > 0.05$). Moreover, administration of L-NAME (10 mg/kg)

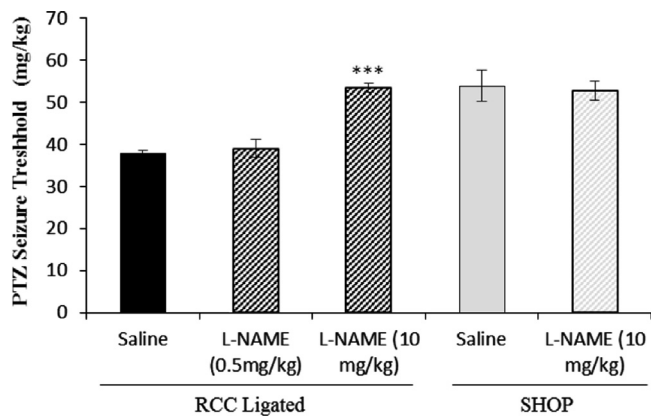


Fig. 4. The effect of administration of different doses of L-NAME (0.5, and 10 mg/kg, i.p. 30 min before testing) on right common carotid ligation induced decrement of CST. The seizure threshold was measured 4 h after right common carotid ligation. Data represent means \pm S.E.M from 6 to 8 animals and were analyzed using a multivariate analysis of variance (ANOVA) for exploration time and the percentage of arm entries, and one-way ANOVA for locomotor activity, followed by Tukey's post-hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to RCC ligated group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ versus the corresponding SHOP group.

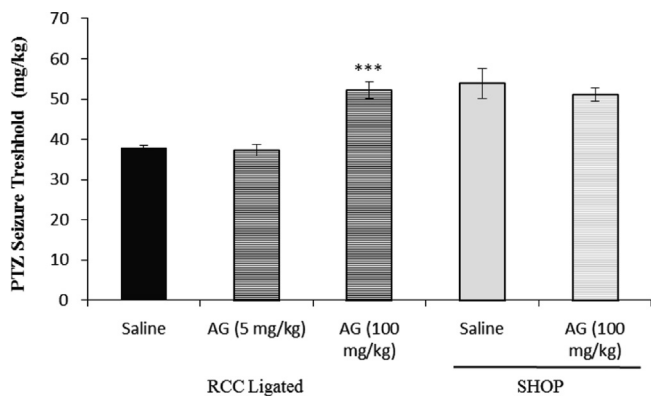


Fig. 5. The effect of administration of AG (5 and 100 mg/kg, i.p. 45 min before testing) on right common carotid ligation induced decrement of CST. The seizure threshold was measured 4 h after right common carotid ligation. Data represent means \pm S.E.M from 6 to 8 animals and were analyzed using a multivariate analysis of variance (ANOVA) for exploration time and the percentage of arm entries, and one-way ANOVA for locomotor activity, followed by Tukey's post-hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to RCC ligated group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ versus the corresponding SHOP group.

in L-NAME Non-ligated had no significant effect on CST in comparison to SHOP group ($P > 0.05$).

3.5. The effect of AG pretreatment in H/I after RCC ligation on CST

As shown in Fig. 5, in high dose pretreatment with AG (100 mg/kg), a complete reversal was observed in comparison to RCC ligated ($P < 0.001$). Administration of low doses of AG (5 mg/kg) had no significant effect on CST in comparison to RCC ligated ($P > 0.05$). Moreover, administration of AG (10 mg/kg) in AG Non-ligated had no significant effect on CST in comparison to SHOP group ($P > 0.05$).

3.6. The effect of additive L-NAME and NTX pretreatment in H/I after RCC ligation on CST

As shown in Fig. 6, pretreatment with low doses of L-NAME (0.5 mg/kg) and NTX (1 mg/kg) had no significant effect on CST in comparison to RCC ligated group ($P > 0.05$). However, the combination of low doses of NTX and L-NAME as additive group had a significant effect on CST in comparison to RCC ligated ($P < 0.001$).

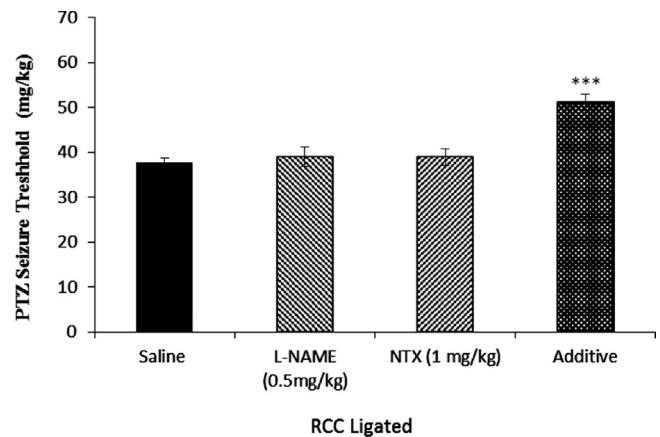


Fig. 6. The effect of concurrent administration of L-NAME (0.5 mg/kg i.p. 30 min before testing) and NTX (1 mg/kg, i.p. 30 min before testing) on right common carotid ligation induced decrement of CST. The seizure threshold was measured 4 h after right common carotid ligation. Data represent means \pm S.E.M from 6 to 8 animals and were analyzed using a multivariate analysis of variance (ANOVA) for exploration time and the percentage of arm entries, and one-way ANOVA for locomotor activity, followed by Tukey's post-hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to RCC ligated group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ versus the corresponding SHOP group.

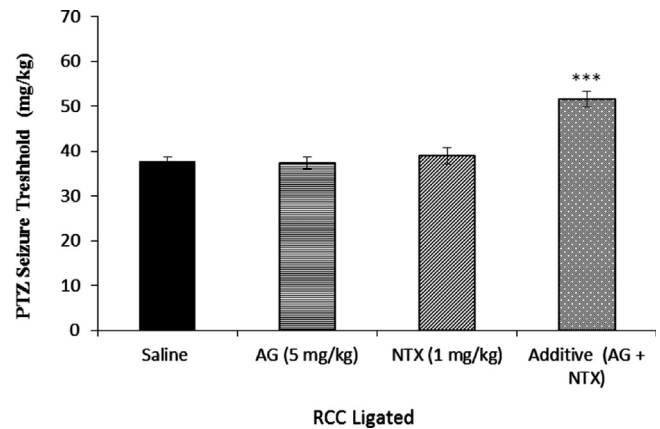


Fig. 7. The effect of concurrent administration of AG (5 mg/kg i.p. 45 min before testing) and NTX (1 mg/kg, i.p. 30 min before testing) on right common carotid ligation induced decrement of CST. The seizure threshold was measured 4 h after right common carotid ligation. Data represent means \pm S.E.M from 6 to 8 animals and were analyzed using a multivariate analysis of variance (ANOVA) for exploration time and the percentage of arm entries, and one-way ANOVA for locomotor activity, followed by Tukey's post-hoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to RCC ligated group; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ versus the corresponding SHOP group.

3.7. The effect of additive AG and NTX pretreatment in H/I after RCC ligation on CST

As shown in Fig. 7, pretreatment with low doses of AG (10 mg/kg) and NTX (1 mg/kg) had no significant effect on CST in comparison to RCC ligated group ($P > 0.05$). However, the combination of low doses of NTX and AG as additive group had a significant effect on CST in comparison to RCC ligated ($P < 0.001$).

4. Discussion

In this study, we demonstrated that opioidergic, nitroergic systems and their additive effects mediate the pro-convulsant effect of H/I following right common carotid ligation. Our results showed that in H/I induced mice, the threshold of PTZ-induced clonic seizure decreased dramatically (Fig. 2). Furthermore, the

histological studies performed on mice exposed to H/I, revealed that tissue damage is associated with pro-convulsive effect of RCC ligation (Table 1, and Fig. 1).

Our data demonstrated that both opioidergic and nitric systems were involved in pro-convulsive effect of ischemic stroke. Since, opioid receptors antagonist (NTX) and/or NOS inhibitors (L-NAME & AG) were able to restore the seizure threshold to the normal level (Figs. 3–5).

Several animal models are used to study the central nervous system (CNS) responses to stroke. Although, the most common method for inducing stroke in mice is the middle cerebral artery occlusion (MCAO) model, but our model investigates more specifically H/I rather than the other aspects of stroke. On the other hand, it provides several advantages, including inducing global ischemia instead of focal ischemia, modeling early seizure after stroke, excluding the effects of long term pathological changes and reperfusion, as well as clinical relevance to situations in which the brain is deprived of both oxygen tension and blood flow, such as coronary bypass surgery, cardiac arrest, and neurovascular surgeries (El-Hayek et al., 2011).

To examine the seizure susceptibility, PTZ a prototypical convulsant drug and GABAA receptor antagonist, has been used. It has been extensively utilized in animal models of seizures and produces a reliable discriminative stimulus. Also, it is a rapid and efficient measure of both seizure susceptibility and screening of new drugs (Loscher, 2009; Payandemehr et al., 2014; Pollack and Shen, 1985).

4.1. The role of H/I in the pathogenesis of early post stroke seizures

To investigate the role of H/I in the pathogenesis of post stroke seizure we recruited right common carotid ligation as a model of H/I. Generally, our observations demonstrated tissue damage as a consequence of H/I insults after right common carotid ligation. Interestingly, decreasing PTZ induced seizure threshold following right common carotid ligation coincided with this tissue damage. Thus, the decrease in PTZ induced seizure threshold might be a consequence of H/I induced neuronal death and associated morphological and physiological changes that are only partly elucidated.

Stroke like other neurological conditions leads to several morphological and physiological changes, causing subsequent complications such as paralysis, memory impairment, and seizures (Lim and Alexander, 2009; Ring and Weingarden, 2007). Indeed, there are evolving evidences suggesting that neural damages regardless of their etiologies can cause seizures (Haas et al., 2001).

H/I, as an integral part of stroke, results in disruption of neuronal metabolism and leads to depolarization of membrane potential that can promote excitotoxicity (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). On the other hand, H/I is further associated with other cellular changes including nuclear fragmentation, chromatin condensation, cell body shrinkage, and consequently resultant cellular death (Kovesdi et al., 2007). These concepts surmise that H/I during stroke might have a part to play in hyperexcitability and seizure genesis.

4.2. The role of opioidergic pathway in the pathogenesis of early post stroke seizures

On the one hand, endogenous opioid peptide family mediate several physiological processes, including nociception/analgesia, respiration, ion channel activity and immune responses, exerted by interaction with G-protein linked membrane receptors (Hauser and Mangoura, 1998; Roy and Loh, 1996; Satoh and Minami, 1995).

On the other hand, they are involved in several CNS related pathological conditions such as Hypoxic/ischemic insults and seizures (Doehner et al., 2012; Faden, 1983; Hong et al., 2002; Panuccio et al., 2009; Rocha et al., 2009).

Opioidergic system plays controversial role in pathogenesis of stroke, since both ameliorative and detrimental effects have been reported. Opioid receptor stimulation ameliorates the disease course (He et al., 2013; Kao et al., 2008; Maslov et al., 2013), whereas worsening of symptoms, and increasing ischemic injury is observed when plasma levels of endogenous opioids are elevated (Doehner et al., 2012; Faden, 1983; Hong et al., 2002). Indeed, it has been demonstrated that misadministration of exogenous opioids and drug abuse can cause brain ischemia (Xu et al., 2006). Therefore, administration of opioid receptor antagonists might have a protective effect in brain H/I (Baskin and Hosobuchi, 1981; Huang et al., 2008; Liao et al., 2003; Sareen, 2002).

Furthermore, the role of opioid system in epileptic seizures is still controversial. For example, it has been demonstrated that opioidergic system mediates anti-convulsive effect of hypoxic preconditioning (Feng et al., 2012; Rubaj et al., 2000). Controversially, increased endogenous opioids (e.g. enkephalin and dynorphin) production, and also opioid receptors up-regulation has been reported in the brain during epileptic seizures (Panuccio et al., 2009; Rocha et al., 2009).

Concluding remarks, raise the possibility of involvement of endogenous opioids over-production during H/I. Therefore, to examine the probable contribution of the opioidergic system in the pathogenesis of post stroke seizure, we used NTX, a non-selective opioid receptors antagonist (Fig. 3).

Our findings indicated that NTX reverses pro-convulsant effect of H/I following RCC ligation (3 mg/kg, ip). These data imply that increased opioidergic tone in stroke has serious effects on the CNS. Also, NTX, at the doses used, did not alter the seizure threshold in UNOP and SHOP control animals.

4.3. The role of iNOS/NO pathway in the pathogenesis of early post stroke seizures

Nitric oxide is an important brain messenger, which has been accredited with both pro-oxidant and anti-oxidant actions (Lipton, 1993; Wink et al., 1999). NO has also been implicated in several neurological and cellular cascades and compromises cellular energy metabolism by inhibiting components of the mitochondrial respiratory chain (Brown and Bal-Price, 2003).

Although, NO generation may lead to vasodilation, enhancement of blood flow, and hypoxic injury alleviation in endothelial cells, it causes neuronal free radical-induced injury by peroxynitrite formation, and glutamate excitotoxicity (Brown and Bal-Price, 2003; Helps and Sims, 2007). Multiple and distinct changes in cerebral NO content and signaling are an acute characteristics of ischemic stroke (Kader et al., 1993; Kumral et al., 2004; Malinski et al., 1993). It is most likely caused through increased calcium availability and activation of nNOS (Huang et al., 2008). Although, within the first few minutes after H/I insult, eNOS and nNOS activity increases simultaneously with NO level changes, it decreases significantly thereafter (Kader et al., 1993).

There are ample evidences that NO pathway could contribute to the pathogenesis of seizure. Excessive NO production has been found in different animal models of convulsion such as PTZ induced seizure (Itoh et al., 2004), and kainic acid induced seizure (Bolanos et al., 1997; Lipton, 1993). Furthermore, excessive NO can cause neuronal necrosis or apoptosis by conversion into peroxynitrite, a potent free radical (Bolanos et al., 1997; Leist and Nicotera, 1998; Lipton, 1993). NO pathway plays controversial role in seizure; while higher doses of L-arginine as a NO precursor decreased the PTZ-induced seizure threshold (Nidhi et al., 1999),

NOS inhibitors attenuated PTZ-induced convulsion (Gholipour et al., 2008; Homayoun et al., 2002; Riazi et al., 2006). Therefore, NO activation may act as “double edged sword” playing both anti-convulsive and pro-convulsive role.

Taking all these data together, post stroke NO overproduction might increase PTZ induced seizure susceptibility. In our study, nonselective inhibition of NOS by L-NAME increased seizure threshold approximately up to control level (10 mg/kg, ip) (Fig. 4). Therefore, it appears that NO overproduction is, in part, involved in epileptogenic properties of stroke in mice. Whilst, L-NAME, at the doses used, had no significant effect on seizure susceptibility in UNOP and SHOP control animals.

Administration of AG, a specific inhibitor of iNOS, might have a protective effect on seizures induced by kainic acid, but, interestingly, nonspecific inhibitors of NO have pro-convulsive effect. On the other hand, pathological production of NO from iNOS plays a detrimental role, while production of NO from eNOS and/or nNOS might have ameliorative effects depending on the magnitude of inhibition (Byun et al., 2009). Moreover, evidences suggest that expression of iNOS during H/I increases significantly (Hewett et al., 1996; Wang et al., 2011).

Thus, to examine the probable contribution of iNOS in the pathogenesis of post stroke seizure, we used AG, which is an irreversible specific inhibitor of iNOS (Nilsson, 1999) (Fig. 5).

Our data indicated, iNOS might be involved in epileptogenic properties of H/I in mice. In our study, selective inhibition of iNOS by AG (100 mg/kg, ip), exerts anti-convulsant effects after RCC ligation. On the other hand, it appears iNOS/NO pathway mediate pro-convulsant effects of stroke. While, effective dose of AG (100 mg/kg), had not significantly alter CST in UNOP and SHOP control animals.

4.4. Interaction of opioidergic and iNOS/NO pathways in the pathogenesis of early post stroke seizures

There are ample evidences supporting the interaction of nitricergic and opioidergic systems (Toda et al., 2009). Indeed, increasing NO production as a result of increased expression of iNOS is implicated in the enhanced effects of morphine (Merighi et al., 2013; Pol et al., 2005). Interestingly, sub-effective doses of NTX, L-NAME, and AG (1, 0.5, and 5 mg/kg, respectively), each alone had no significant alleviating effect on H/I injury and its pro-convulsive effects, while sub-effective co-administration of NTX and L-NAME (Fig. 6) or NTX and AG (Fig. 7) completely reversed it, down to the control level. A similar additive effect between two agents has been recently reported in our previous publications (Gholipour et al., 2008; Homayoun et al., 2002; Shafaroodi et al., 2011), and may imply a functional interaction between opioidergic, and iNOS/NO systems.

Association between the severity of H/I injury and PTZ induced seizure threshold alterations, highlights the notion that H/I might be an etiological factor in the pathogenesis of early post stroke seizure. Altogether, our results have demonstrated that acute H/I during stroke and its consequent hyper-susceptibility to PTZ induced seizure is mediated by the interaction of opioidergic, and iNOS/NO pathways.

References

Adabi Mohazab, R., Javadi-Paydar, M., Delfan, B., Dehpour, A.R., 2012. Possible involvement of PPAR-gamma receptor and nitric oxide pathway in the anticonvulsant effect of acute pioglitazone on pentylenetetrazole-induced seizures in mice. *Epilepsy Res.* 101, 28–35.

Alberi, L., Chi, Z., Kadam, S.D., Mulholland, J.D., Dawson, V.L., Gaiano, N., Comi, A.M., 2010. Neonatal stroke in mice causes long-term changes in neuronal Notch-2 expression that may contribute to prolonged injury. *Stroke* 41, S64–S71.

Alvarez, P.S., Simao, F., Hemb, M., Xavier, L.L., Nunes, M.L., 2014. Effects of under-nourishment, recurrent seizures and enriched environment during early life in hippocampal morphology. *Int. J. Dev. Neurosci.* 33, 81–87.

Baskin, D.S., Hosobuchi, Y., 1981. Naloxone reversal of ischaemic neurological deficits in man. *Lancet* 2, 272–275.

Baskin, D.S., Kuroda, H., Hosobuchi, Y., Lee, N.M., 1985. Treatment of stroke with opiate antagonists—effects of exogenous antagonists and dynorphin 1–13. *Neuropeptides* 5, 307–310.

Bladin, C.F., Alexandrov, A.V., Bellavance, A., Bornstein, N., Chambers, B., Cote, R., Lebrun, L., Pirisi, A., Norris, J.W., 2000. Seizures after stroke: a prospective multicenter study. *Arch. Neurol.* 57, 1617–1622.

Bolanos, J.P., Almeida, A., Stewart, V., Peuchen, S., Land, J.M., Clark, J.B., Heales, S.J., 1997. Nitric oxide-mediated mitochondrial damage in the brain: mechanisms and implications for neurodegenerative diseases. *J. Neurochem.* 68, 2227–2240.

Broughton, B.R., Reutens, D.C., Sobey, C.G., 2009. Apoptotic mechanisms after cerebral ischemia. *Stroke* 40, e331–e339.

Brown, G.C., Bal-Price, A., 2003. Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria. *Mol. Neurobiol.* 27, 325–355.

Burneo, J.G., Fang, J., Saposnik, G., 2010. Impact of seizures on morbidity and mortality after stroke: a Canadian multi-centre cohort study. *Eur. J. Neurol.* 17, 52–58.

Byun, J.S., Lee, S.H., Jeon, S.H., Kwon, Y.S., Lee, H.J., Kim, S.S., Kim, Y.M., Kim, M.J., Chun, W., 2009. Kainic acid-induced neuronal death is attenuated by aminoguanidine but aggravated by L-NAME in mouse hippocampus. *Korean J. Physiol. Pharmacol.* 13, 265–271.

Cordery, S.F., Taverner, A., Ridzwan, I.E., Guy, R.H., Delgado-Charro, M.B., Husbands, S.M., Bailey, C.P., 2012. A non-rewarding, non-aversive buprenorphine/naltrexone combination attenuates drug-primed reinstatement to cocaine and morphine in rats in a conditioned place preference paradigm. *Addict. Biol.* 24, 575–586.

Dirnagl, U., Iadecola, C., Moskowitz, M.A., 1999. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci.* 22, 391–397.

Doehner, W., von Haehling, S., Suhr, J., Ebner, N., Schuster, A., Nagel, E., Melms, A., Wurster, T., Stellos, K., Gawaz, M., Bigalke, B., 2012. Elevated plasma levels of neuropeptide proenkephalin A predict mortality and functional outcome in ischemic stroke. *J. Am. Coll. Cardiol.* 60, 346–354.

El-Hayek, Y.H., Wu, C., Chen, R., Al-Sharif, A.R., Huang, S., Patel, N., Du, C., Ruff, C.A., Fehlings, M.G., Carlen, P.L., Zhang, L., 2011. Acute postischemic seizures are associated with increased mortality and brain damage in adult mice. *Cereb. Cortex* 21, 2863–2875.

Faden, A.L., 1983. Neuropeptides and stroke: current status and potential application. *Stroke* 14, 169–172.

Feng, Y., He, X., Yang, Y., Chao, D., Lazarus, L.H., Xia, Y., 2012. Current research on opioid receptor function. *Curr. Drug Targets* 13, 230–246.

Foote, F., Gale, K., 1984. Proconvulsant effect of morphine on seizures induced by pentylenetetrazol in the rat. *Eur. J. Pharmacol.* 105, 179–184.

Fox, J.G., Barthold, S., Davisson, M., Newcomer, C.E., Quimby, F.W., Smith, A., 2006. *The Mouse in Biomedical Research: Diseases*, (2nd edn.). Academic Press, San Diego, Calif, USA.

Frenk, H., 1983. Pro- and anticonvulsant actions of morphine and the endogenous opioids: involvement and interactions of multiple opiate and non-opiate systems. *Brain Res.* 287, 197–210.

Gholipour, T., Riazi, K., Noorian, A.R., Jannati, A., Honar, H., Doratotaj, B., Nik, H.H., Sharifabrizi, A., Dehpour, A.R., 2008. Seizure susceptibility alteration following reversible cholestasis in mice: modulation by opioids and nitric oxide. *Eur. J. Pharmacol.* 580, 322–328.

Haas, K.Z., Sperber, E.F., Opanashuk, L.A., Stanton, P.K., Moshe, S.L., 2001. Resistance of immature hippocampus to morphologic and physiologic alterations following status epilepticus or kindling. *Hippocampus* 11, 615–625.

Hauser, K.F., Mangoura, D., 1998. Diversity of the endogenous opioid system in development. Novel signal transduction translates multiple extracellular signals into neural cell growth and differentiation. *Perspect. Dev. Neurobiol.* 5, 437–449.

He, X., Sandhu, H.K., Yang, Y., Hua, F., Belsner, N., Kim, D.H., Xia, Y., 2013. Neuroprotection against hypoxia/ischemia: delta-opioid receptor-mediated cellular/molecular events. *Cell. Mol. Life Sci.* 70, 2291–2303.

Helps, S.C., Sims, N.R., 2007. Inhibition of nitric oxide synthase with 7-nitroindazole does not modify early metabolic recovery following focal cerebral ischemia in rats. *Neurochem. Res.* 32, 663–670.

Hewett, S.J., Muir, J.K., Lobner, D., Symons, A., Choi, D.W., 1996. Potentiation of oxygen-glucose deprivation-induced neuronal death after induction of iNOS. *Stroke* 27, 1586–1591.

Homayoun, H., Khavandgar, S., Namiranian, K., Gaskari, S.A., Dehpour, A.R., 2002. The role of nitric oxide in anticonvulsant and proconvulsant effects of morphine in mice. *Epilepsy Res.* 48, 33–41.

Hong, X., Zheng, L., Chen, X., 2002. Changes of dynorphin A1–13 on the treatment of hypoxic-ischemic brain injury by the brain-derived neurotrophic factor in neonatal rats. *Zhonghua Yi Xue Za Zhi* 82, 1637–1640.

Huang, H.M., Yu, J.Y., Ou, H.C., Jeng, K.C., 2008. Effect of naloxone on the induction of immediately early genes following oxygen- and glucose-deprivation in PC12 cells. *Neurosci. Lett.* 438, 252–256.

Itoh, K., Watanabe, M., Yoshikawa, K., Kanaho, Y., Berliner, L.J., Fujii, H., 2004. Magnetic resonance and biochemical studies during pentylenetetrazole-kindling development: the relationship between nitric oxide, neuronal nitric oxide synthase and seizures. *Neuroscience* 129, 757–766.

Jordan, K.G., 2004. Emergency EEG and continuous EEG monitoring in acute ischemic stroke. *J. Clin. Neurophysiol.* 21, 341–352.

- Kadam, S.D., Mulholland, J.D., Smith, D.R., Johnston, M.V., Comi, A.M., 2009. Chronic brain injury and behavioral impairments in a mouse model of term neonatal strokes. *Behav. Brain Res.* 197, 77–83.
- Kader, A., Frazzini, V.I., Solomon, R.A., Trifiletti, R.R., 1993. Nitric oxide production during focal cerebral ischemia in rats. *Stroke* 24, 1709–1716.
- Kao, T.K., Ou, Y.C., Liao, S.L., Chen, W.Y., Wang, C.C., Chen, S.Y., Chiang, A.N., Chen, C.J., 2008. Opioids modulate post-ischemic progression in a rat model of stroke. *Neurochem. Int.* 52, 1256–1265.
- Karimzadeh, F., Hosseini, M., Mangeng, D., Alavi, H., Hassanzadeh, G.R., Bayat, M., Jafarian, M., Kazemi, H., Gorji, A., 2012. Anticonvulsant and neuroprotective effects of *Pimpinella anisum* in rat brain. *BMC Complement. Altern. Med.* 12, 76.
- Khavandgar, S., Homayoun, H., Dehpour, A.R., 2003. Mediation of nitric oxide in inhibitory effect of morphine against electroshock-induced convulsions in mice. *Pharmacol. Biochem. Behav.* 74, 795–801.
- Kovesdi, E., Pal, J., Gallyas, F., 2007. The fate of “dark” neurons produced by transient focal cerebral ischemia in a non-necrotic and non-excitotoxic environment: neurobiological aspects. *Brain Res.* 1147, 272–283.
- Kumral, A., Baskin, H., Gokmen, N., Yilmaz, O., Genc, K., Genc, S., Tatli, M.M., Duman, N., Ozer, E., Ozkan, H., 2004. Selective inhibition of nitric oxide in hypoxic-ischemic brain model in newborn rats: is it an explanation for the protective role of erythropoietin? *Biol. Neonate* 85, 51–54.
- Kwan, J., Wood, E., 2010. Antiepileptic drugs for the primary and secondary prevention of seizures after stroke. *Cochrane Database Syst. Rev.* 1, CD005398.
- Lauretti, G.R., Ahmad, I., Pleuvry, B.J., 1994. The activity of opioid analgesics in seizure models utilizing N-methyl-DL-aspartic acid, kainic acid, bicuculline and pentylenetetrazole. *Neuropharmacology* 33, 155–160.
- Leist, M., Nicotera, P., 1998. Apoptosis versus necrosis: the shape of neuronal cell death. *Results Probl. Cell Differ.* 24, 105–135.
- Liao, S.L., Chen, W.Y., Raung, S.L., Chen, C.J., 2003. Neuroprotection of naloxone against ischemic injury in rats: role of mu receptor antagonism. *Neurosci. Lett.* 345, 169–172.
- Lim, C., Alexander, M.P., 2009. Stroke and episodic memory disorders. *Neuropsychologia* 47, 3045–3058.
- Lipton, S.A., 1993. Prospects for clinically tolerated NMDA antagonists: open-channel blockers and alternative redox states of nitric oxide. *Trends Neurosci.* 16, 527–532.
- Loscher, W., 2009. Preclinical assessment of proconvulsant drug activity and its relevance for predicting adverse events in humans. *Eur. J. Pharmacol.* 610, 1–11.
- Malinski, T., Bailey, F., Zhang, Z.G., Chopp, M., 1993. Nitric oxide measured by a porphyrinic microsensor in rat brain after transient middle cerebral artery occlusion. *J. Cereb. Blood Flow Metab.* 13, 355–358.
- Maslov, L.N., Naryzhnaia, N.V., Tsubulnikov, S.Y., Kolar, F., Zhang, Y., Wang, H., Gusakova, A.M., Lishmanov, Y.B., 2013. Role of endogenous opioid peptides in the infarct size-limiting effect of adaptation to chronic continuous hypoxia. *Life Sci.* 93, 373–379.
- Menon, B., Shorvon, S.D., 2009. Ischaemic stroke in adults and epilepsy. *Epilepsy Res.* 87, 1–11.
- Mergenthaler, P., Meisel, A., 2012. Do stroke models model stroke? *Dis. Model Mech.* 5, 718–725.
- Merighi, S., Gessi, S., Varani, K., Fazzi, D., Stefanelli, A., Borea, P.A., 2013. Morphine mediates a proinflammatory phenotype via mu-opioid receptor-PKCvarepsilon-Akt-ERK1/2 signaling pathway in activated microglial cells. *Biochem. Pharmacol.* 86, 487–496.
- Moncada, S., Higgs, A., 1993. The L-arginine-nitric oxide pathway. *N. Engl. J. Med.* 329, 2002–2012.
- Nidhi, G., Balakrishnan, S., Pandhi, P., 1999. Role of nitric oxide in electroshock and pentylenetetrazole seizure threshold in rats. *Methods Find. Exp. Clin. Pharmacol.* 21, 609–612.
- Nilsson, B.O., 1999. Biological effects of aminoguanidine: an update. *Inflamm. Res.* 48, 509–515.
- Ooigawa, H., Nawashiro, H., Fukui, S., Otani, N., Osumi, A., Toyooka, T., Shima, K., 2006. The fate of Nissl-stained dark neurons following traumatic brain injury in rats: difference between neocortex and hippocampus regarding survival rate. *Acta Neuropathol.* 112, 471–481.
- Palmer, A.J., Valentine, W.J., Roze, S., Lammert, M., Spiesser, J., Gabriel, S., 2005. Overview of costs of stroke from published, incidence-based studies spanning 16 industrialized countries. *Curr. Med. Res. Opin.* 21, 19–26.
- Panuccio, G., Curia, G., Colosimo, A., Cruccu, G., Avoli, M., 2009. Epileptiform synchronization in the cingulate cortex. *Epilepsia* 50, 521–536.
- Payandemehr, B., Bahremand, A., Rahimian, R., Ziai, P., Amouzegar, A., Sharifzadeh, M., Dehpour, A.R., 2012. 5-HT(3) receptor mediates the dose-dependent effects of citalopram on pentylenetetrazole-induced clonic seizure in mice: involvement of nitric oxide. *Epilepsy Res.* 101, 217–227.
- Payandemehr, B., Rahimian, R., Gooshe, M., Bahremand, A., Gholizadeh, R., Berijani, S., Ahmadi-Dastgerdi, M., Aminzade, M., Sarresht-Dari, A., Dianati, V., Amanlou, M., Dehpour, A.R., 2014. Nitric oxide mediates the anticonvulsant effects of thalidomide on pentylenetetrazole-induced clonic seizures in mice. *Epilepsy Behav.* 34, 99–104.
- Pol, O., Sasaki, M., Jimenez, N., Dawson, V.L., Dawson, T.M., Puig, M.M., 2005. The involvement of nitric oxide in the enhanced expression of mu-opioid receptors during intestinal inflammation in mice. *Br. J. Pharmacol.* 145, 758–766.
- Pollack, G.M., Shen, D.D., 1985. A timed intravenous pentylenetetrazol infusion seizure model for quantitating the anticonvulsant effect of valproic acid in the rat. *J. Pharmacol. Methods* 13, 135–146.
- Rahimian, R., Daneshmand, A., Mehr, S.E., Barzegar-Fallah, A., Mohammadi-Rick, S., Fakhfour, G., Shabanzadeh, A.P., Dehpour, A.R., 2011. Tropisetron ameliorates ischemic brain injury in an embolic model of stroke. *Brain Res.* 1392, 101–109.
- Riazi, K., Roshanpour, M., Rafiei-Tabatabaei, N., Homayoun, H., Ebrahimi, F., Dehpour, A.R., 2006. The proconvulsant effect of sildenafil in mice: role of nitric oxide-cGMP pathway. *Br. J. Pharmacol.* 147, 935–943.
- Ring, H., Weingarden, H., 2007. Neuromodulation by functional electrical stimulation (FES) of limb paralysis after stroke. *Acta Neurochir. Suppl.* 97, S375–S380.
- Rocha, L., Orozco-Suarez, S., Alonso-Vanegas, M., Villeda-Hernandez, J., Gaona, A., Paldy, E., Benyhe, S., Borsodi, A., 2009. Temporal lobe epilepsy causes selective changes in mu opioid and nociceptin receptor binding and functional coupling to G-proteins in human temporal neocortex. *Neurobiol. Dis.* 35, 466–473.
- Roy, S., Loh, H.H., 1996. Effects of opioids on the immune system. *Neurochem. Res.* 21, 1375–1386.
- Rubaj, A., Gustaw, K., Zgodzinski, W., Kleinrok, Z., Sieklucka-Dziuba, M., 2000. The role of opioid receptors in hypoxic preconditioning against seizures in brain. *Pharmacol. Biochem. Behav.* 67, 65–70.
- Sareen, D., 2002. Current concepts in the management of acute ischemic stroke. *J. Assoc. Physicians India* 50, 407–414.
- Sato, M., Minami, M., 1995. Molecular pharmacology of the opioid receptors. *Pharmacol. Ther.* 68, 343–364.
- Shafaroodi, H., Baradaran, N., Moezi, L., Dehpour, S., Kabiri, T., Dehpour, A.R., 2011. Morphine sensitization in the pentylenetetrazole-induced clonic seizure threshold in mice: role of nitric oxide and mu receptors. *Epilepsy Behav.* 20, 602–606.
- Shafaroodi, H., Ghasemi, M., Dehpour, A.R., 2008. Elevation of pentylenetetrazole-induced seizure threshold in cholestatic mice: interaction between opioid and cannabinoid systems. *J. Gastroenterol. Hepatol.* 23, e251–e257.
- Shi, H., Liu, K.J., 2007. Cerebral tissue oxygenation and oxidative brain injury during ischemia and reperfusion. *Front. Biosci.* 12, 1318–1328.
- Small, S.L., Buccino, G., Solodkin, A., 2013. Brain repair after stroke—a novel neurological model. *Nat. Rev. Neurol.* 9, 698–707.
- Toda, N., Kishioka, S., Hatano, Y., Toda, H., 2009. Modulation of opioid actions by nitric oxide signaling. *Anesthesiology* 110, 166–181.
- Tuttolomondo, A., Di Raimondo, D., di Sciacca, R., Pinto, A., Licata, G., 2008. Inflammatory cytokines in acute ischemic stroke. *Curr. Pharm. Des.* 14, 3574–3589.
- Wang, H.Q., Xiong, Y., Guo, W.J., 2011. Expression of iNOS protein and gliocyte apoptosis in neonatal rats with white matter damage. *Zhongguo dang dai er ke za zhi=Chin. J. Contem. Pediatr.* 13, 309–312.
- Wink, D.A., Vodovotz, Y., Grisham, M.B., DeGraff, W., Cook, J.C., Pacelli, R., Krishna, M., Mitchell, J.B., 1999. Antioxidant effects of nitric oxide. *Methods Enzymol.* 301, 413–424.
- Xu, B., Wang, Z., Li, G., Li, B., Lin, H., Zheng, R., Zheng, Q., 2006. Heroin-administered mice involved in oxidative stress and exogenous antioxidant-alleviated withdrawal syndrome. *Basic Clin. Pharmacol. Toxicol.* 99, 153–161.
- Yager, J.Y., Armstrong, E.A., Miyashita, H., Wirrell, E.C., 2002. Prolonged neonatal seizures exacerbate hypoxic-ischemic brain damage: correlation with cerebral energy metabolism and excitatory amino acid release. *Dev. Neurosci.* 24, 367–381.