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Lateral hypothalamus orexinergic system modulates the stress effect on pentylenetetrazol induced seizures through corticotropin releasing hormone receptor type 1

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ABSTRACT

Stress is a trigger factor for seizure initiation which activates hypothalamic pituitary adrenal (HPA) axis as well other brain areas. In this respect, corticotropin releasing hormone (CRH) and lateral hypothalamus (LH) orexinergic system are involved in seizure occurrence. In this study, we investigated the role of LH area and orexin expression in (mediation of) stress effect on pentylenetetrazol (PTZ) -induced seizures with hippocampal involvement.

Two mild foot shock stresses were applied to intact and adrenalectomized animals; with or without CRHr1 blocking (NBI 27914) in the LH area. Then, changes in orexin production were evaluated by RT-PCR. Intravenous PTZ infusion (25 mg/ml) -induced convulsions were scored upon modified Racine scale. Finally, hippocampal glutamate and GABA were evaluated to study excitability changes.

We demonstrated that the duration and severity of convulsions in stress-induced as well as adrenalectomized group were increased. Plasma corticosterone (CRT) level and orexin mRNA expression were built up in the stress and/or seizure groups. Furthermore, glutamate and GABA content was increased and decreased respectively due to stress and seizures. In contrast, rats receiving CRHr1 inhibitor showed reduced severity and duration of seizures, increased GABA, decreased glutamate and corticosterone and also orexin mRNA compared to the inhibitor free rats.

Stress and adrenalectomy induced augmenting effect on seizure severity and duration and the subsequent reduction due to CRHr1 blocking with parallel orexin mRNA changes, indicated the likely involvement of CRH1r induced orexin expression of the LH in gating stress effect on convulsions.

1. Introduction

Stressful experiences have a profound effect in shaping an organism's physiological response (Blumcke et al., 1999; Pitkanen and Lukasiuk, 2009). In particular, exposure to stress induces neuroendocrine responses (Edwards et al., 2002; Goddard, 1967) as well as alteration of hypothalamic pituitary adrenal (HPA) function and other neuroendocrine axes. The activation of the HPA axis, in turn, releases the adrenal steroids and other mechanisms that have been shown to alter seizure susceptibility in humans as well as animal models (Weinstock, 2005). Swim stress has been shown to exacerbate convulsion intensity following acute and chronic stresses in animals (Rhodes et al., 2004), and also increases mortality and morbidity in patients with epilepsy due to emotional and psychological stresses (Yuen et al., 2007). Even, adult male offspring of prenatally stressed dams were kindled faster than non-stressed animals (Edwards et al., 2002). On the other hand, stress activates the paraventricular hypothalamic nucleus (PVN) which initiates HPA axis by corticotropin-releasing hormone (CRH) (Owens and Nemeroff, 1991), as the major player of many anxiety disorders (Joels, 2006; Johnson et al., 2012) with involvement of the amygdala. Such psychiatric disorders could be a potential precipitant for stress influenced seizures (Privitera et al., 2014). There are two G

Abbreviations: PTZ, Pentylenetetrazol; OrxR, Orexin Receptor; LHA, Lateral Hypothalamic Area; CRH, Corticotropin Releasing Hormone; HPA, Hypothalamic Pituitary Adrenal; CRT, Corticosterone; PVN, Paraventricular Nucleus; ACTH, Adrenocorticotropic Hormone.

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protein-coupled CRH receptor subtypes, namely, CRHr1 and CRHr2. CRHr1 receptors, which are the preferred receptors for CRH (Hauger et al., 2006) playing a major role in mediating the HPA axis response to stress (De Souza, 1995; Smith et al., 1998) are the main destination, in the lateral hypothalamic area (LHA), for excitation of orexinergic neurons (Winsky-Sommerer et al., 2004).

Orexins produced from LHA neurons, promote arousal and alertness by suppressing REM sleep and lowering the arousal threshold (Sutcliffe and de Lecea, 2002). In a study by Winsky-Sommerer et al., they demonstrated numerous LHA localized orexin-producing neurons express CRH receptors. They suggested that orexins activate brain circuits that modulate arousal associated with stress response paradigms (Winsky-Sommerer et al., 2004). Furthermore, reduced activation of orexin neurons has been demonstrated during stress in CRHr1 knock-out mice (Smith et al., 1998). The CRH receptor 1 antagonist, R121919, also has been attenuated stress-elicited sleep disturbances in rats, particularly in a high innate anxiety strain (Lancel et al., 2002).

The hippocampus, as the site of plastic changes in learning and memory processes, is susceptible to seizures and epilepsy. Stress activates the HPA axis which in turn alters hippocampal function, including glutamate and GABA changes (O'Toole et al., 2014). In addition, orexins released in the hippocampus originated from lateral hypothalamic neurons may influence hippocampal excitability as application of Orx-A to hippocampal slices modulated the balance between GABAergic and glutamatergic neurotransmissions (Selbach et al., 2004). In addition, we demonstrated, in our previous study, that blocking hippocampal orexin receptors suppresses behavioral convulsions, more prominently through Orx1Rs and changes the glutamate and GABA content toward seizure inhibition (Goudarzi et al., 2015). Therefore, the involvement of orexin receptors (OrxRs) in seizure severity and duration through hippocampal neurotransmission (Goudarzi et al., 2015) and LHA inactivation induced seizure cessation, in one hand, and CRHr1 involvement in stress neuropathology, on the other hand, raises the idea that stress may affect seizure occurrence as well as hippocampal excitability (Akbari et al., 2014) through CRHr1 of orexin neurons. Therefore, this study is exploring the effect of functional role of LHA-CRHr1 blocking following stress on the behavioral seizures and hippocampal glutamate and GABA content.

2. Material and methods

2.1. Animals

Adult, male Wistar rats (200 ± 20 g) were housed five per cage (50 cm × 26 cm × 25 cm) in a 12-h light/dark normal cycle at 22–24 °C, with food and water ad libitum. All experiments were done in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 23–80, revised 1996) which were approved by the research ethical standards for the care and use of animals in Damghan University. Additionally, care was taken to minimize the number of animals used in each experiment and their suffering.

2.2. Drugs

The CRH receptor 1 inhibitor (NBI 27914 hydrochloride; Tocris) was injected (intra-LHA.) in a dose of 10 μg/μl in a volume of 0.25 μl. The antagonist was dissolved in DMSO (Dimethyl sulfoxide) and diluted with a 0.9% physiological saline solution to reach the appropriate concentration in DMSO (2%). PTZ (Pentylenetetrazol; cat, P6500) from Sigma Aldrich was used in this research dissolved in saline.

2.3. Experimental groups

Animals used for this study were divided into seven experimental groups, including two sets of groups, with or without CRHr1 antagonism, to compare convulsive behavior.

1. Intravenous PTZ induced convulsion (control for behavioral convulsions; PTZ),
2. Acute foot shock stress plus intravenous PTZ (Stress + PTZ)
3. Bilateral adrenalectomy plus intravenous PTZ (ADX + PTZ)
4. Bilateral adrenalectomy plus acute foot shock stress, plus intravenous PTZ (ADX + Stress + PTZ)
5. Intra-LHA DMSO (Dimethyl Sulfoxide) treatment plus intravenous PTZ (Sham vehicle; DMSO + PTZ)
6. Intra-LHA CRHr1 antagonist (NBI 27914 hydrochloride; NBI for short) plus intravenous PTZ (NBI + PTZ)
7. Acute foot shock stress, plus intra-LHA NBI plus intravenous PTZ (Stress + NBI + PTZ)
8. Bilateral adrenalectomy, plus acute foot shock stress plus intra-LHA NBI plus intravenous PTZ (ADX + Stress + NBI + PTZ)

There are also four groups added to compare biochemical data;

1. Saline injected control (control for biochemical measurements; Saline)
2. Bilateral adrenalectomy (ADX)
3. Acute foot shock stress consisting of two mild foot shocks of 0.70 mA, 2 s in duration in a 5.5 min session (for CRT comparison; Stress)
4. Bilateral adrenalectomy plus acute foot shock stress (for CRT comparison; ADX + Stress).

Pentylenetetrazol was infused through the tail lateral vein 30 min following intra-LHA drug infusion.

2.4. Seizure induction procedure

The model was conducted in rats based on the previously established method in our laboratory (Goudarzi et al., 2015) as a modification from the older one (Mandhane et al., 2007). Pentylenetetrazol (25 mg/ml, i.v.), dissolved in saline, was infused with a constant rate (0.5 ml/min) using a syringe pump (WPI instruments, USA) connected with a polyethylene tube and heparinized needle inside the tail lateral vein. Every PTZ receiving rat was freely moving and behaviorally monitored for 20 min in a transparent Plexiglas box with ventilation holes in a blind manner. Pentylenetetrazol was infused through the tail lateral vein 30 min following intra-LHA infusion of CRHr1 inhibitor. The infusion was terminated when the first myoclonic twitches were appeared: to decrease high mortality of non-stop infusion and let the seizure wave to propagate through intact brain pathways without intensifying by PTZ infusion. Convulsions were scored as follows; 0, no response; 1, ear and facial twitching; 2, convulsive waves through the body; 3, myoclonic jerks; 4, tonic-clonic convulsions, rearing; 5, generalized tonic-clonic (TC) seizures, turnover into side position, loss of postural control (modified from Racine et al (Corda et al., 1990; Racine et al., 1972).). Following variables were measured during behavioral demonstration; 1. Seizure stage: appearance of each seizure stage upon Racine scale. 2. Stage duration: the duration of each seizure stage. Stage distribution was constructed to demonstrate the appearance of different stages following treatment. The animals were sacrificed quickly (to prevent brain tissue reaction to convulsions) after behavioral demonstration took over using Guillotine, hippocampus was extracted freshly and stored in −70 freezers until biochemical evaluations.