Neuroscience 284 (2015) 11–17

PREGABALIN ALLEVIATES THE NITROGLYCERIN-INDUCED HYPERALGESIA IN RATS

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Abstract—The association between the clinical use of nitroglycerin (NTG) and migraine suggests NTG as an animal model trigger for migraine. NTG-induced hyperalgesia in rats has been extensively used as a migraine model for pre-clinical research. Pregabalin is an anti-epileptic drug and may play a role in the preventive treatment of migraine; however, the mechanism of this action remains to be clarified. Herein, we performed the present study to investigate the effect of pregabalin on the NTG-induced hyperalgesia in rats. Sixty male Sprague–Dawley rats were divided equally into six groups. Thirty minutes before NTG injection, the rats were pretreated with pregabalin. von Frey hair testing was employed to evaluate tactile sensitivity. Enzyme-linked immunosorbent assay was used to analyze plasma calcitonin gene-related peptide (CGRP) levels in the jugular vein. Immunohistochemistry was applied to detect c-Fos-immunoreactive neurons and western blot was performed to detect c-Fos protein expression in trigeminal nucleus caudalis (TNC). We found that pregabalin pretreatment alleviated the NTG-induced hyperalgesia. Moreover, pregabalin suppressed peripheral CGRP release, c-Fos-immunoreactive neurons and the protein expression of c-Fos in TNC as well. These data suggest that pregabalin could alleviate the NTG-induced hyperalgesia. Further studies are required to determine the mechanisms of action for this effect.

Key words: pregabalin, migraine, hyperalgesia, calcitonin gene-related peptide, c-Fos, trigeminovascular system.

INTRODUCTION

Migraine is a common episodic neurological disorder characterized by typical throbbing and unilateral headache accompanied by nausea, vomiting, photophobia and phonophobia, affecting approximately 10–20% of the general population (Pietrobon and Moskowitz, 2013; Reddy, 2013). Epidemiological studies have documented the disorder can cause great economic burden and significant lowering of an individual's quality of life (Vos et al., 2012). Although migraine is a remarkably prevalent and disabling condition, it is still underdiagnosed and undertreated (Rizzoli, 2012). Despite great advances in the management of this disorder, the well-recognized optimal therapy remains unknown. Increasing evidence suggests that anti-epileptic drugs (AEDs) such as divalproex sodium, sodium valproate and topiramate are both effective and well tolerated in the prophylactic treatment of migraine (Tfelt-Hansen, 2013). These AEDs are considered as Level A recommendations and are offered for first-line migraine prevention.

Pregabalin is an AED widely used for the treatment of various neuropathic and other pain conditions including fibromyalgia, orofacial pain, diabetic neuropathy and spinal cord damage (Kumar et al., 2013). Given its mechanism of action, i.e., inhibition the release of excitatory neurotransmitters such as glutamate, noradrenaline and substance P via calcium channels, which is consistent with the available data concerning migraine physiopathology, pregabalin is postulated to be effective in migraine prevention (Pizzolato et al., 2011). Recent clinical studies indicated that pregabalin was effective and well tolerated as preventive treatment of migraine (Calandre et al., 2010; Pizzolato et al., 2011; Rizzato et al., 2011). Our previous study also showed that pregabalin exhibited good migraine prophylactic efficacy and tolerability similar to topiramate (data not shown). Pregabalin may represent a useful alternative prophylaxis for migraine.

The clinical use of nitroglycerin (NTG) reliably induces attacks of migraine in migraineurs (Christiansen et al., 1999). Systemic administration of NTG has been extensively used to make an animal model for pre-clinical migraine research because NTG induces hyperalgesia through activation of spinal and brain structures involved in nociception (Buzzi et al., 2003; Greco et al., 2010).

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Abbreviations: 10P + NTG, 10 mg/kg pregabalin plus nitroglycerin; 30P, 30 mg/kg pregabalin; 30P + NTG, 30 mg/kg pregabalin plus nitroglycerin; AEDs, anti-epileptic drugs; ANOVA, analysis of variance; c-Fos-ir, c-Fos immunoreactive; CGRP, calcitonin gene-related peptide; NS, normal saline; NS + NTG, normal saline plus nitroglycerin; NTG, nitroglycerin; TGVS, trigeminovascular system; TNC, trigeminal nucleus caudalis.

http://dx.doi.org/10.1016/j.neuroscience.2014.08.056
0306-4522/

Current studies indicate that trigeminovascular system (TGVS), comprising trigeminal nucleus caudalis (TNC), trigeminal nerve and intracranial arteries (May and Goadsby, 1999), may play a pivotal role in migraine pathophysiology, and TGVS activation via both peripheral and central sensitizations is considered as an essential neuropathogenic mechanism of migraine (Pietrobon and Moskowitz, 2013). Central afferent projections of TGVS and second-order neurons are contained within TNC, and TNC with its rostral connections plays a key role in nociceptive transmission and modulation (Dalkara et al., 2006). The intranuclear proto-oncogene protein c-Fos is a sensitive marker of neuronal activation following noxious stimulation, and expression of c-Fos has been widely used to identify areas of neuronal activation and to study neural correlates of nociception as well (Harris, 1998). Increase of c-Fos expression within TNC, particularly in layers I and II, may serve as an activation marker of TGVS (Bates et al., 2010; Ramachandran et al., 2012).

It has been proposed that calcitonin gene-related peptide (CGRP) may play an important role in migraine disorder and headache generation (Messlinger et al., 2012). CGRP is well poised to enhance neurotransmission in migraine by both peripheral inflammation and central modulation (Raddant and Russo, 2011). The good efficacy of CGRP receptor antagonists in migraine treatment further documents its role in the disorder (Olesen et al., 2004; Ho et al., 2008; Diener et al., 2011). Moreover, plasma CGRP has been found to be increased among migraineurs during ictal and interictal periods (Gallai et al., 1995; Gupta et al., 2009). Rather, plasma CGRP level during headache attack may be a potential tool for diagnosing and predicting migraine (Fan et al., 2009).

So far, to the best of our knowledge, evidence from animal study addressing the efficacy of pregabalin on migraine and the concerning mechanism is still lacking. Therefore, the present study was designed to evaluate the role of pregabalin in migraine treatment through investigating the effect of pregabalin on the NTG-induced hyperalgesia, peripheral CGRP release and TGVS activation in rats.

EXPERIMENTAL PROCEDURES

Animals

Sixty male Sprague–Dawley rats weighing 180–220 g obtained from the Laboratory Animal Center of Sun Yat-sen University (Guangzhou, China) were used in the study. The rats were housed in groups of 3–4 with a 12-h light–dark cycle and free access to water and food under specific pathogen-free and constant temperature (25 ± 1 °C) conditions. All experimental procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 80-23, revised in 1996) and approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University.

Drugs

Pregabalin (Lyrica; Pfizer Inc., New York, USA) was dissolved in sterilized normal saline (NS). Pregabalin administration was based on previous studies about the drug used in rat models of neuropathic pain (Tanabe et al., 2009; Baastrup et al., 2011; Bannister et al., 2011). NTG was purchased from Sigma–Aldrich (St. Louis., Missouri, USA).

Experimental protocol

Sixty rats were randomly divided equally into six groups, i.e., Control group, rats received an i.p. injection of NS; NTG group, rats received an i.p. injection of 10 mg/kg NTG; NS plus NTG group, rats received a s.c. injection of NS 30 min before NTG (10 mg/kg, i.p.); pregabalin (10 mg/kg) plus NTG group, rats received a s.c. injection of 10 mg/kg pregabalin 30 min before NTG (10 mg/kg, i.p.); pregabalin (30 mg/kg) plus NTG group, rats received a s.c. injection of 30 mg/kg pregabalin 30 min before NTG (10 mg/kg, i.p.); pregabalin (30 mg/kg) group, rats received a s.c. injection of 30 mg/kg pregabalin.

von Frey hair testing

After drug administration, tactile sensitivity was evaluated with calibrated von Frey hairs (Stoelting Co., Wood Dale, Illinois, USA) by the up-down method as described previously (Chaplan et al., 1994; De Felice et al., 2010). Briefly, the rats were placed in testing cages for 30 min of adaptation. A series of von Frey hairs with logarithmically incremental stiffness was applied to the periorbital region of the face and the mid-plantar surface of the hind paw for 6–8 s at intervals of 30 s between consecutive stimuli. Quick withdrawal of the face or hind paw in response to the stimulus was considered as a positive response. Maximum stimulus strengths for the face and hind paw were 8 g and 15 g, respectively. The withdrawal thresholds to tactile stimuli of von Frey hairs were analyzed at 0, 30, 60, 120, 180 and 240 min after NTG injection by the experimenters who were blinded to the grouping of each rat.

Enzyme-linked immunosorbent assay

After von Frey hair testing, rats were anesthetized with 10% chloral hydrate (3.5 ml/kg, i.p.) and then fixed in a supine position on a pad to expose its cervical region. The fur around the area was removed by shaving and wiped down with an alcohol swab. The blue jugular vein was revealed by pressure applied on its proximal to the collection site for the vein distended. By using a 1-mL syringe, a 23-gauge needle was inserted into the jugular vein through the skin right around the middle point between the sternum and shoulder area (Shirasaki et al., 2012). Approximately 1 mL of blood sample was collected in prechilled tubes containing 10% ethylene diamine tetraacetic acid (15 μl) and aprotinin (500 KIU/ml blood). Plasma was subsequently separated by cold centrifuge (4000*g* at 4 °C) and stored at −80 °C for further analysis. Plasma CGRP level was analyzed using a commercial enzyme-linked immunosorbent assay kit (SPI-BIO, Paris, France) according to the manufacturer's instructions.