Neurochemistry International 99 (2016) 85–93

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Activation of α_1 adrenoceptors in ventrolateral orbital cortex attenuates allodynia induced by spared nerve injury in rats

Lai Wei [b, f, 1], Yuan-Mei Zhu [b, 1], Yu-Xiang Zhang [b], Feng Liang [b], Hong Jia [a, d], Chao-Ling Qu [a, d], Jing Wang [e], Jing-Shi Tang [a, d], She-Min Lu [c, d], Fu-Quan Huo [a, d, *], Chun-Xia Yan [b, d, **]

[a] Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi 710061, China
[b] College of Forensic Medicine, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi 710061, China
[c] Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi 710061, China
[d] Key Laboratory of Environment and Genes Related to Diseases (Xi'an Jiaotong University), Ministry of Education, China
[e] Key Laboratory of Orthopedics of Gansu Province, The Second Hospital of Lanzhou University, Lanzhou, Gansu 730030, China
[f] Division of Forensic Medicine, School of Basic Medical Sciences, Hubei University of Medicine, Shiyan, Hubei 442000, China

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form
5 June 2016
Accepted 9 June 2016
Available online 11 June 2016

Keywords:
α_1 adrenoceptor
Neuropathic pain
Ventrolateral orbital cortex
Periaqueductal gray
PLC
PKC

ABSTRACT

Recent studies have demonstrated that noradrenaline acting in the ventrolateral orbital cortex (VLO) can potentially reduce allodynia induced by spared nerve injury (SNI), and this effect is mediated by α_2 adrenoceptor. The present study examined the effect of the α_1 adrenoceptors in the VLO on allodynia induced by SNI in the rats. The mechanical paw withdrawal threshold (PWT) was measured using von-Frey filaments. Microinjection of selective α_1 adrenoceptor agonist methoxamine (20, 50, 100 μg in 0.5 μl) into the VLO, contralateral to the site of nerve injury, increased PWT in a dose-dependent manner. This effect was antagonized by pre-microinjection of the selective α_1 adrenoceptor antagonist benoxathian into the same VLO site, and blocked by electrolytic lesion of the ventrolateral periaqueductal gray (PAG). Furthermore, pre-administration of non-selective glutamate receptor antagonist kynurenic acid, phospholipase C (PLC) inhibitor U73122, and protein kinase C (PKC) inhibitor chelerythrine to the VLO also blocked methoxamine-induced inhibition of allodynia. These results suggest that activation of α_1 adrenoceptors in the VLO can potentially reduce allodynia induced by SNI. This effect may be direct excitation of the VLO neurons, via PLC-PKC signaling pathway, projecting to the PAG or facilitating glutamate release and then indirectly exciting the VLO output neurons projecting to the PAG, leading to activation of the PAG-brainstem descending inhibitory system which depresses the nociceptive transmission at the spinal cord level.

1. Introduction

Anatomical studies have established that the ventrolateral orbital cortex (VLO) receives projections from spinal and medullary dorsal horn lamina I via the thalamic nucleus submedius (Sm) and contains neurons that project to the ventrolateral periaqueductal gray (vlPAG) (Coffield et al., 1992; Craig and Burton, 1981; Craig et al., 1982; Hardy and Leichnetz, 1981; Yoshida et al., 1991, 1992), a key region involved in descending modulation of nociception (Fields and Basbaum, 1999; Sandkuhler and Gebhart, 1984). Previous series of studies in our laboratory have demonstrated that electrolytic lesions of or microinjection of γ-aminobutyric acid into

Abbreviations: AUC, areas under the time-course curve; Beno, benoxathian; Cl, claustrum; DMSO, dimethyl sulfoxide; DR, dorsal raphe nucleus; fmi, forceps minor corpos callosum; i.p., intraperitoneally; Meth, methoxamine; KYNA, kynurenic acid; PAG, periaqueductal gray; PLC, phospholipase C; PKC, protein kinase C; PWT, paw withdrawal threshold; Sm, thalamic nucleus submedius; SNI, spared nerve injury; VLO, ventrolateral orbital cortex; vlPAG, ventrolateral periaqueductal gray.

* Corresponding author. Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi 710061, China.
** Corresponding author. College of Forensic Medicine, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi 710061, China.
E-mail addresses: huofq@mail.xjtu.edu.cn (F.-Q. Huo), yanchunxia@mail.xjtu.edu.cn (C.-X. Yan).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neuint.2016.06.006
0197-0186/

the VLO eliminates antinociceptive effects induced by peripheral electrical stimulation, or by activation of the Sm (Zhang et al., 1995a, b, 1998, 1999), while electrically or chemically induced activation of VLO depresses spinal and trigeminal nocifensive reflexes, such as the tail-flick reflex and jaw opening reflex, and these antinociceptive effects are eliminated by lesion or functional blocking of the vlPAG (Zhang et al., 1997a, 1997b, 1998). These data suggest that the VLO is involved in an endogenous analgesic system consisting of a spinal/medulla cord-Sm-VLO-PAG-spinal/medulla cord loop (Tang et al., 2009).

Noradrenaline, a principal monoaminergic neurotransmitter, distributes widely throughout the central nervous system and is involved in pain modulation (Pertovaara, 2006). Anatomic studies have indicated that the cerebral cortex including the VLO receives innervations from noradrenergic nuclei of the pons (Cooper et al., 2003; Jones and Yang, 1985). The recent studies from our laboratory have demonstrated that microinjection of the non-selective adrenoceptor agonist noradrenaline or selective α_2 adrenoceptor agonist clonidine into the VLO can potentially reduce allodynia induced by spared nerve injury (SNI) in a dose-dependent manner, and these inhibitory effects can be antagonized by pre-microinjection of the selective α_2 adrenoceptor antagonist yohimbine into the same VLO site (Zhu et al., 2013). These results suggest that the noradrenergic system plays a key role in anti-allodynia in the VLO, which is mediated by α_2 adrenoceptors. It is well known that the actions of noradrenaline are mediated by α_1, α_2 and β adrenoceptors (Bylund et al., 1994). Previous studies have demonstrated that β adrenoceptors may predominantly mediate epinephrine induced modulation of pain (Pertovaara, 2006). However, the α_1 adrenoceptors, differing from α_2 and β adrenoceptors, are coupled to phospholipase C (PLC) through Gq-protein or they are coupled directly to Ca^{2+} influx and produce excitatory effect on neurons (Summers and McMartin, 1993). Moreover, morphological studies have indicated that α_1 adrenoceptors are widely distributed throughout the brain including the VLO (Day et al., 1997; Domyancic and Morilak, 1997). Therefore, the aim of the present study was to investigate whether α_1 adrenoceptors were involved in mediating the VLO-evoked inhibition of allodynia induced by SNI in the rat, and the underlying mechanisms were further examined.

2. Materials and methods

2.1. Animals

Experiments were performed on 120 male Sprague-Dawley rats (250–300 g) which were provided by the Medical Experimental Animal Center of Xi'an Jiaotong University, Shaanxi Province, China. Animals were housed under a 12/12 h light-dark cycle (lights on at 07:00) with *ad libitum* access to food and water. The experimental protocols were approved by the Institutional Animal Care Committee of Xi'an Jiaotong University and in accordance with ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983). All efforts were made to minimize the number of animals used and their suffering.

2.2. Spared sciatic nerve injury

Spared nerve injury (SNI) was performed as previously described (Arsenault and Sawynok, 2009; Decosterd and Woolf, 2000). Briefly, the rats were intraperitoneally (*i.p.*) anesthetized with sodium pentobarbital (50 mg/kg, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China), and an incision was made along the back of the thigh. The biceps femoris muscle was separated to expose the three terminal branches of the sciatic nerve. The tibial and common peroneal nerves were tightly ligated using 4.0 silk and were transected distal to the ligation, following removal of 5 mm of nerve stump. The sural nerve was left intact, and the wound was closed. The sham-operated rats were treated in the same way but the nerves were neither ligated nor sectioned. Following surgery, the rats were allowed to recover for 1 week before implantation of intracerebral cannulas.

2.3. Intracerebral guide cannula placement

Rats were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*), and the head was immobilized in a stereotaxic frame. A small craniotomy was performed just above the VLO, contralateral to the site of nerve injury (since the SNI-induced nociceptive signals transmit to the contralateral VLO). A stainless steel guide cannula (0.8 mm in diameter) was stereotaxically inserted, with the tip 2.0 mm dorsal to the VLO at the following coordinates: 3.2 mm anterior to bregma, 2.0 mm lateral, and 2.6 mm below the cortical surface (Paxinos and Watson, 1986), followed by attachment to the skull with three microscrews and dental cement. Once the rats recovered from anesthesia, they were administered sodium penicillin (0.2 million U/day for 3 days, *i.p.*) to prevent wound and intracerebral infections. The rats were carefully nursed and still housed in groups in clean cages.

2.4. Electrolytic lesion of the ventrolateral PAG

Rats were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*), and the head was immobilized in the stereotaxic frame. The stainless steel concentric electrode, insulated with epoxylite to within 0.5 mm of its tip, was stereotaxically placed in the ventrolatetal parts of PAG (vlPAG) (7.8 mm caudal to Bregma, 0.7 mm lateral, 5.5 mm below cortical surface) on both sides (Paxinos and Watson, 1986; Zhang et al., 1995a), and 0.4 mA direct current was passed for 30 s on each side. Subsequently, the rats were implanted with guide cannulas aimed at the VLO as above-mentioned and allowed 7 days to recover from surgery.

2.5. Mechanical paw withdrawal threshold measurement

One week after intracerebral guide cannula placement (i.e. 2 weeks after SNI), paw withdrawal threshold (PWT) was measured in response to mechanical stimulation (von Frey filaments) using the up-down method (Chaplan et al., 1994; Dixon, 1980). The rats were placed in a transparent plastic box ($280 \times 250 \times 210$ mm^3) with a metal wire mesh floor, which allowed full access to the paws from below. Behavioral adaptation was allowed until cage exploration and major grooming activities had ceased for approximately 30 min. Ten von Frey filaments (Stoelting Company, Wood Dale, IL, USA), with approximately equal logarithmic incremental (0.17) bending force, were chosen (von Frey numbers: 3.61, 3.84, 4.08, 4.17, 4.31, 4.56, 4.74, 4.93, 5.07 and 5.18, equivalent to: 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10, and 15.0 g, respectively). Starting with filament 4.31 (2.0 g), which was the middle filament in the series, von Frey filaments with different intensities were repeatedly applied over a 2-s time interval from below and perpendicular to the fourth and fifth toes of the hind paw with sufficient force to cause slight bending against the paw for approximately 6–8 s. If response to filament stimulation was positive, the next lower force was delivered. If there was no withdrawal response (negative), the next higher force was delivered. Positive and negative responses were recorded and converted to a 50% threshold using a formula provided by Dixon (1980) and Chaplan et al. (1994) (Chaplan et al., 1994; Dixon, 1980). If PWT was reduced to <4.0 g, mechanical allodynia was considered to be successfully established. PWT