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Original Article

Melanocortin-4 receptor in subthalamic nucleus is involved in the modulation of nociception

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Abstract: Deep brain stimulation of the subthalamic nucleus (STN-DBS) stimulation produces significant improvement of overall pain related to Parkinson disease; however, the mechanisms underlying analgesic effects of STN-DBS are still unknown. This report describes direct neuroanatomical evidence for the central melanocortinergic-opioidergic circuits in the STN. We investigated melanocortin-4 receptor (MC4R) and mu-opioid receptor (MOR)-positive expression of the STN in MC4R-GFP transgenic mice using fluorescence immunohistochemical detection. Immunohistochemistry showed a large number of MC4R-GFP- and MOR-positive neurons within the STN region, and approximately 50% of MC4R-GFP-positive neurons coexpressed MOR. The results of this study showed direct neuroanatomical evidence for the central melanocortinergic-opioidergic signaling in the STN region. These findings contribute to the view of melanocortinergic-opioidergic circuits in the subthalamic nucleus as a reliable source of modulating of nociception with therapeutic potential for alleviating pain.

Keywords: Subthalamic nucleus, melanocortin-4 receptor, mu-opioid receptor, nociception

Introduction

Several studies have shown that deep brain stimulation of the subthalamic nucleus (STN-DBS) stimulation produces significant improvement of overall pain related to Parkinson disease (PD) in patients with advanced PD [1]. However, the mechanisms underlying analgesic effects of STN-DBS are still unknown. In a previous study, we demonstrated MC4R positive expression in different subpopulations of STN neurons [2, 3]. There is growing evidence that MC4R, a well-established mediator in the regulation of energy homeostasis, may play an important role in pain sensation [4-6]. Mu-opioid receptor (MOR) is necessary for the analgesic effects of opioids, which are important mediators of the nociceptive response [7]. Pagano et al demonstrated that cortical stimulation increases the nociceptive threshold of naive conscious rats with opioid participation [8]. The study from Fonoff et al showed that epidural electrical motor cortex stimulation elicited a substantial and selective antinociceptive effect, which was mediated by opioids [9]. We explore the hypothesis that possible mechanism of subthalamic nucleus stimulation for alleviating pain may involve in the central opioidergic-melanocortinergic circuits. The main objective of this study is to provide direct neuroanatomical evidence for the central melanocortin-opioidergic circuits in the STN in melanocortin-4 receptor (MC4R)-green fluorescent protein (GFP) transgenic mice [2, 10-21], using fluorescence immunohistochemical detection.

Materials and methods

Animals

The procedures used in this study were approved by the Institutional Animal Care and Use Committee. All efforts were made to prevent animal suffering and to use the minimum number of animals. Male transgenic melanocortin-4 receptor (MC4R)-green fluorescence protein (GFP) knock-in mice (25-30 g), obtained from Dr. Joel Elmquist (UT Southwestern Medical Center, USA) and then bred to generate male and female mice, were used for this study [3, 5, 22-28]. Mice were genotyped as described by Rossi and colleagues [29]. Mice were group-housed in a stress-minimized facility where they had free access to food and water and

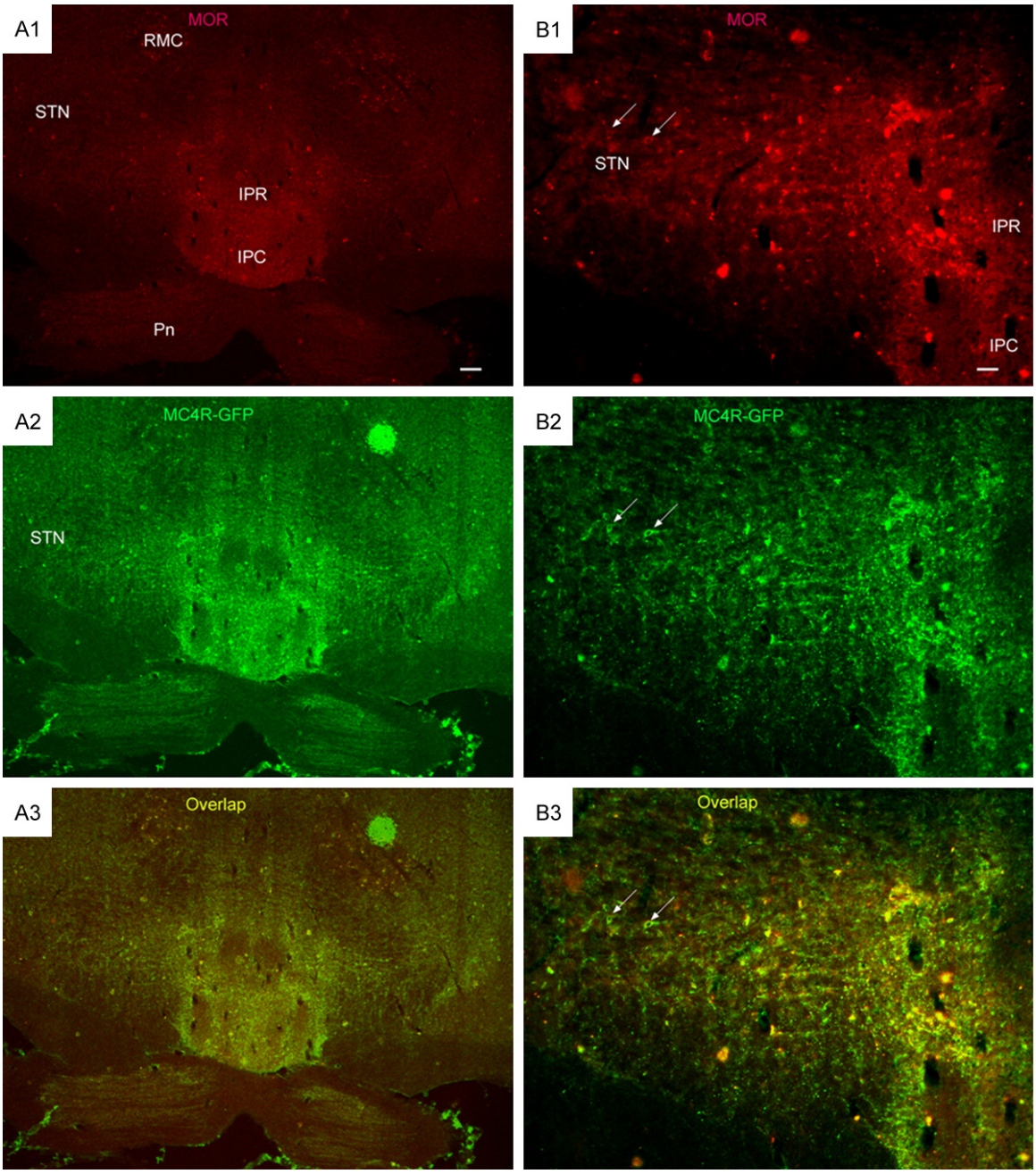

Figure 1. MC4R-GFP and MOR positive cells in the STN. (A1) MOR expressing neurons; (A2) MC4R-GFP expressing neurons in same section as (A1); (A3) overlap of (A1) and (A2), depicting distribution of MC4R-GFP-IR and MOR-bearing neurons. (B1-B3), amplified views of (A1-A3), respectively. RMC, Red nucleus, magnocell part; IPR, Interpedunc nucleus, rostral; IPC, Interpedunc nucleus, caudal. Pn, Pontine nucleus. Scale bar: 100 μm for (A1-A3); 50 μm for (B1-B3).

were maintained on a 12 h light/dark cycle (8:00 a.m.-8:00 p.m.).

Preparation of tissue sections

The mice were deeply anesthetized with the mixture of ketamine (10 mg/ml) and xylazine (0.5 mg/ml) by intraperitoneal injection and perfused transcardially with normal saline, followed by a fixative containing 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4). The brains were removed and placed in 4% formaldehyde for 24 h and then cryoprotected in 30% sucrose (in 0.1 M PB). The brains were

sectioned (25 μm) on a freezing microtome (Leica Microsystems Inc., Nussloch, Germany) and collected into 0.1 M PBS (phosphate buffer solution).

Fluorescence immunohistochemistry

The freezing sections were first incubated in 0.3% 10× Triton for 30 minutes at 37°C to get cell permeabilization and then washed by the 0.1 M PBS for 3 times. To block endogenous peroxidase activity, the sections were exposed to 10% normal Donkey Serum for 30 min at room temperature. Then, the sections were cut at 30 μm using a cryostat and used for double immunofluorescence detection according to published protocols. Briefly, they were incubated in the anti-GFP rabbit serum (A6455, life technologies, 1:1000) for 12 h at 4°C and then washed again as above, followed by Biotin-sp-conjugated AffiniPure Donkey anti-Rabbit IgG (Jackson ImmunoResearch, 1:2000) for 2 h at room temperature without any light. Then they were stained by Cy3-conjugated streptavidin (Jackson ImmunoResearch, 0.5 μg/ml) for 30 minutes at temperature in a dark circumstance. This was the first course of our double label immunohistochemistry. These sections were then incubated in Goat polyclonal MOR (c-20) Antibody (sc-7488, Santa Cruz, 1:200) overnight at 4°C after washing for 3 times. Then they were natured in the FITC-conjugated Mouse-anti-Goat IgG (H+L) (Jackson ImmunoResearch, 1: 1000). Finally, the sections were washed in 0.1 M PBS, mounted on gelatin-coated slides, dried, and observed via the fluorescence microscope (Leica DM2500). When taking pictures, we defined the FITC as the red while the Cy3 as the green.

Tissue analysis

The sections were visualized by using an Olympus IX81 photomicroscope equipped with epifluorescence with a filter set for visualization. MOR-positive cells were identified with red fluorescence; MC4R-GFP-expressing cells were recognized by green fluorescence. Images were overlaid using Adobe Photoshop, and double-labeled cells were presented as yellow (green/red).

Results

We checked the green fluorescent protein expression in the melanocortin-4 receptor-GFP reporter mouse by immunohistochemistry staining, and found a large number of GFP-positive neurons within the STN region.

Fluorescence immunohistochemistry showed a large number of MC4R-GFP-and MOR-positive neurons within the STN region (**Figure 1**), and approximately 50% of MC4R-GFP-positive neurons coexpressed MOR, indicating that they were opioidergic.

Discussion

Using a line of mice expressing GFP under the control of the MC4R promoter, we provided neuroanatomical evidence of MC4R expression in the STN for the modulation of nociception in transgenic mice. Fluorescence immunohistochemistry revealed that approximately 50% of MC4R-GFP-positive neurons coexpressed MOR, indicating that they were opioidergic. These findings extend our knowledge about the distribution of MC4R and MOR in rodent STN neurons.

The study from Pellaprat et al confirmed that STN-DBS induced a substantial beneficial effect on pain in PD, independently of its motor effects and mood status of patients [30]. Ciampi et al reported that STN-DBS contributed to relieve pain associated with PD and specifically modulated small fiber-mediated sensations [31]. Otherwise, Pagano pointed that deep brain stimulation increased the nociceptive threshold of naive conscious rats with opioid participation [8]. Therefore, opioidergic signaling in the subthalamic nucleus may involve in modulation of nociception.

The study from Kapoor showed MC4R antagonists and their emerging role in pain management [32]. Starowicz also reported peripheral antinociceptive effects of MC4R antagonists in a rat model of neuropathic pain [33]. Otherwise, Chu pointed that MC4R induced hyperalgesia and allodynia after chronic constriction injury by activation of p38 MAPK in dorsal root ganglion and spinal cord [34, 35]. Thus, we hypothesized that STN-DBS inhibited the up-regulation of MC4R in the spinal and peripheral nociceptive pathways in painful neuropathy, which were in agreement with a previous study in which clinical pain alleviation after STN-DBS may be considered as a consequence of a direct central modulation of pain perception, via increased mechanical pain and tolerance thresholds [36].

In conclusion, data presented here provide direct neuroanatomical evidence for the central melanocortinergic-opioidergic circuits in the STN region. Based on the above analyses, we propose a hypothesis that melanocortinergic-opioidergic signaling in the subthalamic nucleus involves in modulation of nociception, suggesting that deep brain stimulation of the STN may alleviate pain by the central melanocortinergic-opioidergic circuits.

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Disclosure of conflict of interest

None.

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