Neuroscience Letters 649 (2017) 78–84

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Intracranial self-stimulation-reward induces neurite extension in PC12m3 cells and activation of the p38 MAPK pathway

Yutaka Gomita[a,*], Satoru Esumi[a], Naoya Sugiyama[a], Yoshihisa Kitamura[a], Yoshihisa Koike[b], Hirotoshi Motoda[c], Toshiaki Sendo[a], Yoshio Kano[c]

[a] Department of Pharmacy, Okayama University Hospital Pharmacy, 2-5-1 Shikata-cho, Okayama 700-8558, Japan
[b] Department of Occupational Therapy, Faculty of Health and Welfare, Prefectural University of Hiroshima, 1-1 Gakuenmachi, Mihara City, Hiroshima 722-0053, Japan
[c] Department Occupational Therapy, School of Health of Science, Kibi, International University, 8 Iga-machi Takahashi City, Okayama 716-8508, Japan

HIGHLIGHTS

- There is a possibility which indicates promoting influence in neurite outgrowth of PC12-variant cell for the intracranial self-stimulation (ICSS) reward.
- Stimulated PC12-variant cell also exhibits heightened activity of the p38 MAPK pathway.
- Reward states lead to not only morphological changes but also increases in p38 MAPK activity at the neuronal level in the central nervous system.

ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form 9 March 2017
Accepted 4 April 2017
Available online 6 April 2017

Keywords:
Emotionality
Motivation
Intracranial self-stimulation reward
PC12-variant cells
p38 MAPK pathway

ABSTRACT

Factors that trigger emotional expression may be divided into two patterns according to the type of motivation, acquiring reward (pleasure) and avoiding aversion (punishment). Repeated exposure to certain external stimuli accompanied by aberrant motivation may produce psychiatric diseases such as bipolar disorder and addiction via dysregulation of the central nervous system. However, neurobiological underpinnings of such diseases have not been clarified, especially at the neuronal level. In the present study, plasma from rats undergoing intracranial self-stimulation (ICSS) produced neurite outgrowth in PC12-variant cells (PC12m3). Stimulated PC12m3 cells also exhibited heightened activity of the p38 MAPK pathway. These findings indicate that reward states lead to not only morphological changes but also increases in p38 MAPK activity at the neuronal level in the central nervous system.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Generally, when humans take action in a normal manner, a psychological motivation exists in manifesting the behavior. Concerning the motivational events that underlie behavior, it is said that an internal factor that becomes a driving force to cause the behavior (drive) and an external factor that represents a trigger (incentive) interact to cause motivated behavior [1,2]. Factors that trigger emotional expression may be divided into two patterns according to the type of motivation: acquiring reward (pleasure) and avoiding aversion (punishment). Two types of motivation underlie emotional behaviors, i.e., one type for acquiring reward that associates with pleasure and another type for avoiding aversion that is associated with the concept of punishment. It is also known that both pleasurable states and negative hedonic stimuli regulate endocrine, neural, and/or immune systems [3,5].

Importantly, it is widely believed that abnormalities induced by continuous emotional strain may cause various psychiatric diseases such as bipolar disorder, and drug addiction. These diseases may be attributed to specific abnormalities within the nervous system [4,6,7].

The intracranial self-stimulation (ICSS) procedure has used as one methodology for measuring reward, due to its ability to quantify the degree or intensity of motivational drive as it relates to reward [8–11]. ICSS behavior is experimentally useful to investigate reward system function as measured via lever pressing to acquire brain stimulation reward in a Skinner box. ICSS behavior is intricately linked with brain reward and is associated with changes in neurotransmitters (such as dopamine and endorphins) that regulate reward behavior [8,9,12,13]. However, few studies exist concerning the influence of emotional stimuli on neural system substrates at the cellular level.

* Corresponding author at: Department of Hospital Pharmacy, Okayama University Medical and Dental School, 2-5-1 Shikata-cho, Okayama 700-8558, Japan.
E-mail address: ygomita@gmail.com (Y. Gomita).

http://dx.doi.org/10.1016/j.neulet.2017.04.010
0304-3940/© 2017 Elsevier B.V. All rights reserved.

To investigate the regulation of dopamine system neurons in detail, PC12 cells derived from a pheochromocytoma of the rat adrenal medulla are often used as a model [14]. It is known that PC12 cells exhibit phenotypical changes such as cellular proliferation, differentiation, and apoptosis in response to nerve growth factor, cytokine exposure, and/or other specific external stimuli [14]. In addition, cellular activity in PC12 cells is primarily regulated by the MAPK signaling pathway [15–22]. During continuous culture, PC12 cells are subjected to spontaneous mutations that lead to the generation of PC12 variants. Recently, Kano's group has succeeded in making various novel variant PC12 cell lines in which NGF-induced outgrowth of neuronal processes is impaired [15–20].

In previous experiments, when PC12 cells were cultured for 2 weeks under acidic conditions, several surviving clones appeared [15,16]. Using the ring procedure, the colonies were selected and propagated on a mass culture and were termed PC12 m1, PC12 m2, PC12 m3, PC12 m5 to m10, and PC12 m12 [15,16]. Among the PC12 mutant clones obtained, PC12 m3 and m12 cells show poor neurite outgrowth similar to the others despite normal sustained activation of MAP kinase by NGF treatment [15–20]. Importantly, these PC12 mutant cells show not only impaired or poor NGF-induced neurite outgrowth but also almost the same morphology (small and round) as that of parental PC12 cells [15–20].

We observed as pre-trial basis that ICSS reward stimulated neurite extension in the PC12m12 cell. Therefore, in the present experiment, we first confirmed this preliminary finding in PC12m12 cells and in PC12m3 cells with almost the same characteristics as PC12m12 cells [23]. We further attempted to clarify the involvement of p38 MAPK activity, which is related to PC12 phenotypical appearance for cellular senescence, apoptosis, cell cycle regulation, differentiation, and proliferation.

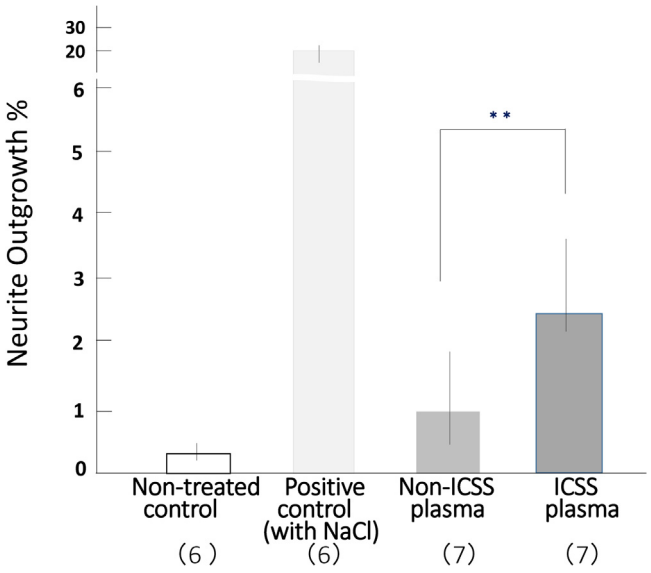

Fig. 1. Frequencies of neurite outgrowth in PC12m3 cells induced by ICSS reward (40× magnification).
PC12m3 cells were exposed for 7 days to plasma obtained from rats experiencing intracranial self-stimulation (ICSS plasma) for 15 min or without any stimulation (Non-ICSS plasma) as a control for ICSS treatment, as well as to control conditions without any blood (Non-treated control) or control with NaCl (Positive control with NaCl). Non-treated and positive controls were set as the standard control. The frequencies of outgrowths of PC12m3 cells in the culture were measured through a microscopic magnification of 40×. Each bar is shown as median percentage (with interquartile range) of the neurite-outgrowth in whole all cells which counted in a fixed same area through respective microscopic view, in the cultured cells obtained from 3 independent experiments. The number in parentheses is the number of microscopic site in cultured cells. Data were analyzed with the Mann-Whitney *U* test. ** $p < 0.01$, significant different from the non-ICSS plasma.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 250–50 g at the beginning of the experiment were obtained (Charles River Laboratories). They were housed in the Experimental Animals Center of Okayama University Medical and Dental School at a controlled ambient temperature of 22 ± 1 °C with approximately 60% relative humidity and with a 12 h light-dark cycle (light on 07:00 h). Animals were allowed free access to food and water until the experiment. The experiments were performed according to the Guideline for Animal Experiments in Okayama University Medical and Dental School.

2.2. ICSS reward

2.2.1. Implantation of electrode for brain stimulation

A chronic electrode was implanted at a specific brain site in the rat. Current would flow in that brain site from the electrode when the rat pressed a lever in a Skinner box to elicit the brain stimulation. The medial forebrain bundle (MFB) in the lateral hypothalamus and tegmentum mesencephali represent primary brain reward sites. The method for implanting the chronic electrode in the brain has been described before [24,25].

Rats were anesthetized with 40 mg/kg sodium pentobarbital, intraperitoneally (i.p.), and stereotaxically implanted with bipolar stainless steel electrodes at the MFB in the lateral hypothalamus for ICSS reward, according to the brain atlas of Paxinos & Watson [26].

2.2.2. Training for brain stimulation reward

At least 10 days were allowed for recovery before commencing the training for intracranial self-stimulation reward. The experiments were carried out using a Skinner box (25 cm wide, 30.8 cm depth, 27.7 cm height). Each animal was placed in the box, and a stimulating cable was connected to the electrode plug mounted on the animal's head. A lever press activates a counter and results in brain stimulation.

Each animal was trained to press a lever for brain stimulation reward, obtainable on a continuous reinforcement schedule [9,12]. Each brain stimulation reward consisted of a 60 Hz sinusoidal current lasting for 0.2 s, and was individually adjusted for each rat. The stimulation current was gradually increased until the animal began to respond at a heightened activity level (0–200 μA).

Five to 10 daily training sessions (15 min/day) were given to each animal. The stimulation current intensity was determined to be the approximate level that maintained the maximum response rate (high rate) of intracranial self-stimulation without producing gross motor disturbances or convulsion.

The training for self-stimulation was performed for 15 min, at least once a day for more than 3 days. Among rats displaying any ICSS behavior, we used animals that exhibited lever pressing of over 50 times per minute.

For observing these behaviors, operant conditioning control equipment and cumulative recording equipment aside from the Skinner box was required. The cumulative recording equipment could operate at the output from the operant conditioning control equipment.

2.3. Experimental procedure and blood sampling

In the experiments involving ICSS reward, immediately after the 15 min ICSS session, 200 μl of blood samples were collected using a 60 μl heparinized capillary tube from the tail vein by making a small incision with a razor under light local anesthesia with xylocaine jelly.

Download English Version:

https://daneshyari.com/en/article/5738410

Download Persian Version:

https://daneshyari.com/article/5738410

Daneshyari.com