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論　文　内　容　の　要　旨

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論文題名	Hormonal mechanisms in the paraventricular nuclei associated with hyperalgesia in Parkinson's disease model rats (パーキンソン病モデルラットにおける室傍核のホルモン機構と痛覚過敏の関連性)

論文内容の要旨

Introduction

Pain, particularly hyperalgesia, is a significant non-motor symptom of Parkinson's disease (PD) that substantially affects a patient's quality of life. Although PD is primarily characterized by motor dysfunction, chronic pain is increasingly recognized as a debilitating non-motor feature of the disease, which is often under-treated in clinical settings. Hyperalgesia in PD is associated with pathological changes in the central nervous system, including the loss of dopaminergic neurons and disruption of pain modulation pathways, but its exact mechanism remains unclear. Recent research utilizing a PD rat model induced by injecting 6-hydroxydopamine (6-OHDA) has provided insights into these mechanisms, highlighting the changes in the brain's neurochemical environment, particularly in the paraventricular nucleus (PVN) of the hypothalamus. This region produces neuropeptides, including oxytocin (OXT), arginine vasopressin (AVP), and corticotrophin-releasing hormone (CRH), which all have analgesic properties. Previous studies have demonstrated that the analgesic effect of OXT is suppressed in 6-OHDA rats, contributing to increased hyperalgesia, but the roles of AVP and CRH remain poorly understood. The present study aimed to investigate the involvement of OXT, AVP, and CRH in PD-related hyperalgesia, utilizing a 6-OHDA-induced PD rat model.

Materials and Methods

Twenty-four male Wistar rats weighing 150–200 g were used. To induce Parkinsonian symptoms, 6-OHDA was injected into the medial forebrain bundle (MFB) of the rats, selectively damaging the dopaminergic neurons. Sham rats received saline injections, instead of 6-OHDA. At 2 weeks after the 6-OHDA injections, the rats underwent a methamphetamine-induced rotational behavior test, where the animals displaying ≥5 turns per minute were considered to be successfully induced with 6-OHDA. At 3 weeks after the surgery, the rats were randomly divided into the following two groups according to whether they received stimulation via subcutaneous (SC) injection of formalin or a saline solution into the vibrissa pad: formalin and saline injection groups. The formalin injection group received SC injection of 4% formalin. Blood samples were collected 5 min after the formalin injection, and the rats were perfused for histological analysis. Brain sections were prepared for immunohistochemistry, specifically to assess the number of p-ERK-immunoreactive (-IR) cells in the trigeminal spinal subnucleus caudalis (Vc), which served as a marker of neuronal activation in response to pain. Furthermore, we performed double immunofluorescence labeling for p-ERK/OXT, p-ERK/AVP, and p-ERK/CRH in the sections containing PVN to quantify the proportion of OXT, AVP, and CRH neurons activated by the SC injection of formalin into the vibrissa pad. The blood levels of OXT, AVP, and CRH were measured using enzyme immunoassays to determine the systemic response to formalin injection.

Results and Discussion

p-ERK in Vc

The number of p-ERK-IR cells in the formalin injection group was significantly higher than that of the saline injection group, indicating that the rats were stimulated by formalin. The number of p-ERK-IR cells was significantly higher in the 6-OHDA rats than in the sham rats in those that received formalin injections, indicating that the 6-OHDA rats are hyperalgesic as compared to the sham rats.

OXT and CRH

The percentage of OXT-IR and CRH-IR neurons that co-localized with the p-ERK protein in the PVN was significantly higher in the formalin injection group than in the saline injection group in the sham rats, but no significant difference was observed in the 6-OHDA rats. Moreover, the percentage of OXT- and CRH-IR neurons co-localized with p-ERK protein in the PVN of the formalin injection group was significantly lower in the 6-OHDA rats than in the sham rats. The serum OXT and CRH levels were significantly higher in the formalin injection group than in the saline injection group in the sham rats, but not significant difference was observed in the in 6-OHDA rats. OXT and CRH are known to be promoted to synthesize and secrete by nociceptive stimuli, resulting in pain modulation. These findings indicated that the activity of OXT and CRH neurons in the PVN induced by formalin injection was enhanced in sham rats, but this was suppressed in the 6-OHDA rats, suggesting that the dysfunction of the OXT- and CRH-mediated analgesic pathways in PD contributes to hyperalgesia.

AVP

AVP exhibited a different pattern of involvement in pain modulation. In both the sham and 6-OHDA rats, formalin injection did not result in a significant increase in AVP neuron activation, as indicated by the absence of a substantial change in the AVP-IR neurons co-localized with p-ERK protein in the PVN. This suggests that AVP was not activated by the nociceptive stimuli. Moreover, the serum AVP levels were consistently lower in the 6-OHDA rats than in the sham rats, regardless of the presence of nociceptive stimuli. This finding indicates that AVP may not play a direct role in pain modulation in PD, at least within the parameters of the present study. AVP is implicated in various physiological processes, including stress and fluid balance, and PD patients are known to exhibit reduced AVP secretion and symptoms, including urinary incontinence. However, the involvement of AVP in nociceptive modulation in PD appears to be limited. Thus, AVP does not seem to contribute directly to the hyperalgesia observed in the present PD rat model. The role of AVP in pain modulation may be more complex or indirect, as AVP receptors have been reported to be involved in analgesia.

Conclusion

In PD rats, the enhancement of neuronal activity and increase in the serum OXT and CRH levels in response to nociceptive stimuli were suppressed. These findings suggest that the reduced analgesic effects of OXT and CRH in response to nociceptive stimuli may contribute to hyperalgesia in PD rats. This finding could shed light on the mechanisms underlying hyperalgesia in PD and potentially contribute to the establishment of future treatments to alleviate hyperalgesia.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究は、パーキンソン病（Parkinson's disease: PD）モデルラットを用いて、痛覚過敏における３種類の神経ペプチド[oxytocin （OXT)、arginine vasopressin （AVP)、corticotropin-releasing hormone （CRH)]の役割を明らかにしようとしたものである。

その結果、鎮痛作用を有する OXT と CRH の室傍核における変化とそれに伴う血中濃度変化が PD モデルラットの痛覚過敏に関与している可能性を見出した。

本研究の結果は、PD モデルラットにおける痛覚過敏の背景を理解する上で重要な知見を与えるものであり、博士（歯学）の学位論文として価値のあるものと認める。