Endocannabinoid 2-arachidonoylglycerol levels in the anterior cingulate cortex, caudate putamen, nucleus accumbens, and piriform cortex were upregulated by chronic restraint stress

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論文題目

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（前帯状皮質、尾状核、側坐核、梨状皮質におけるエンドカンナビノイド 2-アラキドノイルグリセロールレベルは慢性拘束ストレスにより上昇した）

論文の内容の要旨

[Introduction]

Endocannabinoid 2-arachidonoylglycerol (2-AG) is one of the main endogenous cannabinoid ligands in the central nervous system. It could be biosynthesized from 1-oleoyl-2-arachidonoylsn-glycerol (OAG) and 1-stearoyl-2-arachidonoylglycerol (SAG) in the brain tissues by diacylglycerol lipases (DAGLs). Monoacylglycerol lipase (MGL) is a key enzyme for 2-AG hydrolysis. 2-AG is an essential lipid messenger in the brain, functioning as a retrograde synaptic messenger. Revealing the spatial distribution of 2-AG in brain tissue will be beneficial for the study of related diseases, such as stress-related disorders. Desorption electrospray ionization-mass spectrometry imaging (DESI-MSI) is a technique that allows the spatial intensity distribution to be recorded directly from histological sections. In this study, we used DESI-MSI to investigate and reveal the 2-AG distribution in mice brain tissues and the effect of chronic restraint stress (CRS) on 2-AG levels in the anterior cingulate cortex (ACC), caudate putamen (CP), nucleus accumbens (NAc), and piriform cortex (PIR). Doing so will allow for a clearer understanding of 2-AG expression and changes in the brain and extend the horizon for future etiological and therapeutic studies of related diseases.

[Materials and Methods]

The animal study protocol was approved by the Institutional Animal Care and Use Committees of Hamamatsu University School of Medicine (protocol code: 2022021). Mice were randomly grouped into stress and control groups. Mice in the stress group were separately restrained for 30 min in a tube with several small holes (for ventilation) for 8 consecutive days. Mice in the control group were left undisturbed in their home cages. Mice brain samples were coronally sectioned for DESI-MSI, hematoxylin and eosin staining, and immunohistochemistry analysis. Pearson’s Chi-square test and Two-tail t-test were performed to evaluate the differences between the two groups.

[Results]

Firstly, the endogenous 2-AG in the mice brain sections at m/z 417.2409 was detected the same as standard 2-AG. We confirmed that the signal intensity of 2-AG varied

depending on its concentration. Secondly, 2-AG in the coronal brain sections of mice were visualized via DESI-MSI. The 2-AG levels were highest in the hypothalamus (HY) and lowest in the hippocampal (HIP). Thirdly, 2-AG levels were upregulated and 2-AG precursor levels were downregulated in the ACC, CP, NAc, and PIR by CRS. The quantification of the immunohistochemical expression of MGL in the ACC, CP, NAc, and PIR was not affected by CRS. CRS upregulated 2-AG levels in the non-layer I area but not in layer I of ACC.

[Discussion]

Others have reported that 2-AG augmentation reduces stress-induced anxiety-like behavior and participates in habituation to repeated stress. Our DESI-MSI results provide direct evidence that 2-AG in the ACC, CP, NAc, and PIR is upregulated under CRS conditions and may be involved in habituation to stress. The CRS not only upregulated 2-AG but also downregulated OAG and SAG. DAGLα immunoreactivity has been reported in the ACC, CP, NAc, and PIR. We speculate that the demand for 2-AG in the ACC, CP, NAc, and PIR increases after CRS exposure; thus, OAG and SAG are hydrolyzed more by DAGLα, leading to a decrease in them and an increase in their degradation product 2-AG. Therefore, the downregulation of OAG and SAG may be a reason for the upregulation of 2-AG after CRS exposure. Moreover, our results show MGL expression levels in the ACC, CP, NAc, and PIR were not different between the control and stressed groups. Therefore, we suggest that the CRS-induced 2-AG increase was not caused by the change in MGL expression levels.

Previous studies have reported that CB1 receptors were detected in the ACC, CP, NAc, and PIR. Therefore, a functional 2-AG signaling system composed of cannabinoid receptors and the complete machinery for the synthesis and degradation of 2-AG existed in the ACC, CP, NAc, and PIR. It has been reported that 2-AG contributes to stress response termination by inhibiting glutamate release and restraint following anxiety arousal by stimulating presynaptic CB1 receptors. 2-AG can also act on astrocytic CB1 to indirectly regulate glutamate release via the release of gliotransmitters. These reports and our results suggest that CRS-induced upregulated 2-AG in the ACC, CP, NAc, and PIR plays a role in contributing to stress response termination by regulating glutamate release through targeting presynaptic or astrocytic CB1 receptors.

[Conclusion]

This study shows that 2-AG levels are highest in the HY and lowest in the HIP. Moreover, this study reveals that CRS upregulates 2-AG levels and downregulates its precursors' levels in the ACC, CP, NAc, and PIR. The expression levels of MGL were not affected by CRS. Our findings extend the horizon for future etiological and therapeutic studies of 2-AG-related diseases, such as stress-related neuropsychiatric

diseases.