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Epigenetic control of neurogenesis by the brain endocannabinoid system: Involvement of mitogen-activated protein kinases

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Endocannabinoids in the central nervous system (CNS) have recently emerged as instructive cues in the developing of the CNS and they are able to attenuate detrimental effects on neurogenesis and neuroinflammation that are associated with ageing. Emerging evidence suggests that cannabinoid signalling regulates gene expression by inducing epigenetic modification such as DNA methylation or histone modification in the regulation of a range of neurobiological processes in the brain, including CNS development, learning, memory and neurodegeneration associated with ageing. This could be highly significant since epigenetics is itself emerging as a possible key determinant of brain ageing and the study of these modifications will help to distinguish between a "young" and an "old" cell. The aim of this study is two-fold: firstly to determine if pharmacological targeting of the CB1 or CB2 subtype of endocannabinoid receptors can regulate epigenetic activity in neural stem cells (NSC), and secondly to determine the potential involvement of mitogen-activated protein kinase (MAPK) in the regulation of this epigenetic activity. We analysed if cannabinoid signals regulate DNA methylation in NSC by modifying the DNA methyltransferases (DNMT) activity using a quantification kit (AbCam, UK). This study was performed with a mini library of 5 highly selective cannabinoids (CB1 agonist: ACEA 0.5μM, t=48h, n=4; CB2 agonist: JWH133, 0.5μM, t=48h, n=4; CB1 antagonist: AM251, 1μM, t=48h, n=4; and CB2 antagonist: AM630, 1μM, t=48h, n=4), and an inhibitor of the diacylglycerol lipase (DAGL): RHC-80267, 5μM, t=48h, n=4), all commercially available (Tocris, UK). All experimental doses of cannabinoids and incubation times were based on previously published works from our laboratories. Experiments were performed in nuclear extracts prepared by using a nuclear extraction kit (AbCam, UK) from embryonic NSC from C57BL6/J mice (Harlan, UK) that have been characterized, expanded and banked in liquid nitrogen in our stem cell bank. The potential involvement of MAPK was evaluated by using a MAPK Multi-target sandwich ELISA kit (NewEngland Biolabs, UK). DNA methylation was significantly increased after exposure to ACEA (25% increases, $P<0.01$ vs. control) or the CB2 antagonist AM630 (one-fold increase, $P<0.001$ vs. control). In contrast, DNA methylation was blocked when NSC were exposed to the CB1 antagonist AM251 ($P<0.001$ vs. control) or the CB2 agonist JWH133 ($P<0.001$ vs. control). The inhibitor of the DAGL activity RHC-80267 was able to reduce DNA methylation by a 15% approximately ($P<0.01$ vs. control). In addition, our results suggest that ACEA is able to regulate DNA methylation in NSC, through a SAP/JNK, and to a lesser extent p38-MAPK-dependent pathway triggered by CB1 receptors. CB2 cannabinoid receptors activation abolished DNA methylation in NSC, through a p42/44-MAPK-dependent pathway. The modulation of DNA methylation by endocannabinoids may affect the expression of a number of genes that regulate many cell functions in response to these substances. These studies therefore indicate a novel mode of epigenetic modification for the endocannabinoid system in neurogenesis that may be of therapeutic interest in the emerging field of brain repair.

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