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RESEARCH HIGHLIGHT

Buzzkill: the consequences of depleting anandamide in the hippocampus

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The legalization and social acceptance of cannabis has been increasing dramatically over the past few years with recreational cannabis now being legal in Canada and in 10 states of the USA (with a total of 33 states also having legal cannabis in a medical context as of the beginning of 2019). This increase in the visibility of cannabis in North America (and other parts of the Western world) has also generated a new wave of interest in the effects of cannabis on the brain, and in turn, an increased focus on the important physiological roles that the endocannabinoid system governs. As what was found with opiates and the endogenous opioid system, many of the neurobehavioral processes that are impacted by cannabis use are naturally modulated by changes in endocannabinoid signaling. Given that the regulation of stress and anxiety is the primary reason people report as to why they use recreational cannabis, it is not surprising that it has become increasingly apparent that the endocannabinoid system is an important regulator of emotional behavior [1].

The role of endocannabinoids in the regulation of emotional behavior, such as fear and anxiety, has progressively been untangled as we have made progress in the development of tools allowing us to modify endocannabinoid signaling. The first reports of this nature were a series of studies that came out in sequence in the early 2000s after the development of both specific antagonists at the cannabinoid type 1 (CB1) receptor as well as mouse lines in which the CB1 receptor had been deleted. Both of these streams of research indicated that compromising CB1 receptor function resulted in an increased sensitivity to stress, elevated behavioral indices of anxiety and sustained fear behavior [1]. These findings proved to be relevant for humans as well, as following the release of a CB1 receptor antagonist, as a treatment for obesity, the drug was removed from the European market, and prevented from entering the US market, due to the fact that its consumption resulted in the development of psychiatric side effects, particularly anxiety and depression.

While the impact of disrupting CB1 receptor signaling was readily apparent, disentangling the role of individual endocannabinoid molecules proved to be more difficult. Within the brain, both anandamide (AEA) and 2-arachidonoylglycerol (2-AG) act as endogenous ligands at the CB1 receptor. The experimental approach to identify a function role of each of these molecules in a given physiological or pathological process has been to investigate the impact of either depleting or elevating these molecules (usually through an inhibition of their biosynthesis or hydrolysis, respectively) through pharmacological or genetic means. The metabolic pathways of 2-AG have been fairly well established [2], as the synthesis of 2-AG is dependent upon the enzyme diacylglycerol lipase (DAGL) and the hydrolysis of 2-AG is primarily driven by the enzyme monoacylglycerol lipase (MAGL; with additional support from the enzyme α/β-hydrolase domain containing 6, ABHD6). Within the past few years, specific pharmacological tools to block, as well as genetic lines to produce global or cell specific deletions of, DAGL or MAGL have been developed which have allowed the field to explicitly parse apart the role of 2-AG. Depleting 2-AG through the administration of a DAGL inhibitor, or the deletion of DAGL, consistently produces increases in fear and anxiety and increases susceptibility to the effects of stress [3, 4]. Elevating 2-AG signaling through disruption of MAGL, while generally showing stress-reducing and anti-anxiety effects [3, 5], has also been found to result in increased aspects of fear behavior [6]. This would suggest that the loss of 2-AG signaling indeed increases stress, anxiety, and fear, but also indicates that elevating 2-AG signaling does not always produce a consistent effect. The divergence of these data likely relate to the impacts of 2-AG signaling in different circuits or at CB1 receptors on different cell types. Interestingly, this complex effect on emotional behavior is generally consistent with what is seen with cannabis use in humans, where both anti-anxiety and anxiogenic effects can occur in response to cannabis consumption. Ongoing work in the field is continuing to investigate this question, and with the discovery of novel tools, we will hopefully have some clarity in the near future.

The case of AEA is somewhat more complicated. The hydrolysis of AEA is almost entirely driven by one enzyme, fatty acid amide hydrolase (FAAH), and genetic deletion or pharmacological inhibition of this enzyme results in profound elevations in AEA signaling [2]. The development of a specific FAAH inhibitor in 2003 allowed for the initial determination that elevating AEA signaling produces anxiolytic effects [7]. Additional work in the field expanded this finding to show that this was specific to the development of anxiety in response to aversive, stressful or challenging environmental conditions [8]. However, examining the impact of AEA depletion through inhibition of its synthesis, has proven to be very complicated given that there are multiple redundant pathways of AEA synthesis. In their recent article, Zimmermann and colleagues [9] have found a way to bypass this issue by the development of an AAV vector allowing for the overexpression of FAAH in a Cre-dependent manner. Specifically, these authors demonstrate that delivery of this FAAH-AAV to the hippocampus of NEX-Cre mice resulted in a specific overexpression of FAAH within glutamatergic pyramidal neurons in the CA3-CA1 region of the hippocampus, which in turn produced a local depletion of AEA signaling [9]. This approach allowed for the first time to determine the impact of depleting AEA signaling within a

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discrete brain region. Consistent with the effects of CB1 receptor blockade or depletion of 2-AG, these data demonstrate that depleting AEA within the hippocampus shifted excitability of this circuit, elevated indices of anxiety and fear, impaired hippocampal-dependent recognition memory and altered stress coping behavior [9]. As such, these data add an important contribution to the field and provide the first support for the hypothesis that depleting AEA signaling may increase indices of stress, anxiety, and fear behavior.

But of course, since we are dealing with endocannabinoids here, nothing is clear-cut and straightforward. In tandem with this manuscript, our group has also been investigating the impact of depleting AEA on emotional behavior, however, we have focused on the basolateral amygdala (BLA) as opposed to the hippocampus. While we predicted that our findings would parallel what was reported by Zimmermann and colleagues herein, we were quite surprised by the fact that we found the exact opposite. That is, using a herpes simplex virus vector to rapidly, yet transiently, overexpress FAAH in pyramidal neurons of the BLA and locally deplete AEA signaling, we found that this approach reduced stress responses, dampened anxiety-like behavior and inhibited the recall and expression of cued conditioned fear [10]. Therefore, as has been observed with 2-AG, the impacts of modulating AEA signaling are not always what we would expect and involve differential effects in discrete brain circuits, likely through the engagement of different neuronal populations. While these findings are a first step in moving forward, they demonstrate that the exact modes of action of endocannabinoids in the regulation of emotional states and fear memory are still far from being fully elucidated. The importance of this work is highlighted by the fact that there is significant clinical development in pharmacological agents that modify AEA and 2-AG signaling and, as these drugs move into Phase II trials for psychiatric conditions, the nuance of these details will become increasingly relevant.

DISCLOSURES

Both authors declare no conflict of interest.

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