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Perspective

Enantiomer-specific positive allosteric modulation of CB_1 signaling in autaptic hippocampal neurons

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ABSTRACT

The cannabinoid signaling system is found throughout the CNS and its involvement in several pathological processes makes it an attractive therapeutic target. Because orthosteric CB1 cannabinoid receptor ligands have undesirable adverse effects there has been great interest in the development of allosteric modulators – both negative (NAMs) and positive (PAMs) – of these receptors. NAMs of CB_1 appeared first on the scene, followed more recently by PAMs. Because allosteric modulation can vary depending on the orthosteric ligand it is important to study their function in a system that employs endogenous cannabinoids. We have recently surveyed first generation NAMs using cultured autaptic hippocampal neurons. These neurons express depolarization induced suppression of excitation (DSE), a form of synaptic plasticity that is mediated by CB_1 and 2-arachidonoyl glycerol (2-AG); they are therefore an excellent neuronal model of endogenous cannabinoid signaling in which to test CB_1 modulators.

In this study we find that while two related compounds, GAT211 and ZCZ011, each show PAM-like responses in autaptic hippocampal neurons, they also exhibit complex pharmacology. Notably we were able to separate the PAM- and agonist-like responses of GAT211 by examining the enantiomers of this racemic compound: GAT228 and GAT229. We find that GAT229 exhibits PAM-like behavior while GAT228 appears to directly activate the CB_1 receptor.

Both GAT229 and ZCZ011 represent the first PAMs that we have found to be effective in using this 2-AG utilizing neuronal model system. Because these compounds may exhibit both probe selectivity and biased signaling it will be important to test them with anandamide as well as other signaling pathways.

1. Introduction

Cannabinoid receptors mediate most of the salient effects of marijuana consumption [1]. First identified in the late-1980s [2,3] these receptors are now known to be key components of an endogenous signaling system that is found throughout the body. Orthosteric agonists (i.e. WIN55212-2 and CP55940) and antagonists (i.e. SR141716, AM251) were quickly identified and have been studied extensively. There is, however, a strong interest in developing allosteric modulators of CB_1 signaling, partly due to the sometimes unfavorable therapeutic profiles of orthosteric ligands. Negative allosteric modulators (NAMs) act by inhibiting binding and/or signaling, while positive allosteric modulators (PAMs) act by potentiating binding and/or signaling, by an orthosteric agonist [4]. NAMs/PAMs may act in various means but the essential concept is that some receptors have one or more secondary 'allosteric' sites. When engaged by allosteric ligands these sites modulate orthosteric signaling. This generally occurs via alteration of binding kinetics of the orthosteric ligand and/or potentiation/inhibition of receptor signaling. One attractive quality of allosteric modulators is the potential to modulate only those receptors that are being actively engaged by endogenous ligands. For the broadly distributed cannabinoid CB1 receptors the risk of off-target action is considerable, making such selectivity especially desirable.

We recently reported on first-generation CB_1 allosteric modulators on synaptic transmission in autaptic hippocampal neurons [5]. These neurons serve as a well-characterized model of endogenous

Abbreviations: DSE, depolarization-induced suppression of excitation; 2-AG, 2-arachidonoyl glycerol; NAM, negative allosteric modulator; PAM, positive allosteric modulator; EPSC, excitatory postsynaptic current.

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cannabinoid signaling that possesses the machinery to synthesize, release and metabolize endogenous cannabinoids and presynaptic CB_1 receptors, the activation of which inhibits neurotransmitter release [6–8]. Depolarization of these neurons yields depolarization induced suppression of excitation (DSE) a form of plasticity that involves retrograde signaling via 2-arachidonoyl glycerol (2-AG) that act on presynaptic CB_1 receptors [6,9]. Because this model is well-characterized and DSE is mediated by an endogenous cannabinoid, this makes autaptic neurons well-suited to an examination of allosterism with endocannabinoids. In our recent test of allosteric modulators (five negative and one positive allosteric modulator) we found that three acted in a manner consistent with allosteric modulation of CB_1 receptor function.

ZCZ011 was recently reported to serve as a PAM albeit with a mixed pharmacological profile that included agonist properties [10] (Fig. 1A). This compound is derived from 3-(2-nitro-1-phenylethyl)-2-phenyl-1H-indole [11]; CAS Registry Number: 102704-40-5; F-0870-0064) referred to here as GAT211 (Fig. 1B). GAT 211, a racemic compound, has been recently characterized as a CB_1-selective ago-PAM with its allosteric agonist activity residing in the *R*-(+)-enantiomer GAT228 and PAM activity in *S*-(−)-enantiomer GAT229 [12]. We now report our study of these candidate positive allosteric modulators in a neuronal model of endogenous CB_1 signaling.

2. Methods

2.1. Hippocampal culture preparation

All procedures used in this study were approved by the Animal Care Committee of Indiana University and conform to the Guidelines of the National Institutes of Health on the Care and Use of Animals. Mouse hippocampal neurons isolated from the CA1-CA3 region were cultured on microislands as described previously [13,14]. Neurons were obtained from mice (C57Bl/6, postnatal day 0–2, of indeterminate sex) and plated onto a feeder layer of hippocampal astrocytes that had been laid down previously [15]. Cultures were grown in high-glucose (20 mM) DMEM containing 10% horse serum, without mitotic inhibitors and used for recordings after 8 days in culture and for no more than three hours after removal from culture medium.

2.2. Electrophysiology

When a single neuron is grown on a small island of permissive substrate, it forms synapses—or "autapses"—onto itself. All experiments were performed on isolated autaptic neurons. Whole cell voltage-clamp recordings from autaptic neurons were carried out at room temperature using an Axopatch 200A amplifier (Axon Instruments, Burlingame, CA). The extracellular solution contained (in mM) 119 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 30 glucose, and 20 HEPES. Continuous flow of solution through the bath chamber (~2 ml/min) ensured rapid drug application and clearance. Drugs were typically prepared as stocks, and then diluted into extracellular solution at their final concentration and used on the same day.

Recording pipettes of 1.8–3 MΩ were filled with (in mM) 121.5 KGluconate, 17.5 KCl, 9 NaCl, 1 $MgCl_2$, 10 HEPES, 0.2 EGTA, 2 MgATP, and 0.5 LiGTP. Access resistance and holding current were monitored and only cells with both stable access resistance and holding current were included for data analysis. Conventional stimulus protocol: the membrane potential was held at −70 mV and excitatory postsynaptic currents (EPSCs) were evoked every 20 s by triggering an unclamped action current with a 1.0 ms depolarizing step. The resultant evoked waveform consisted of a brief stimulus artifact and a large downward spike representing inward sodium currents,

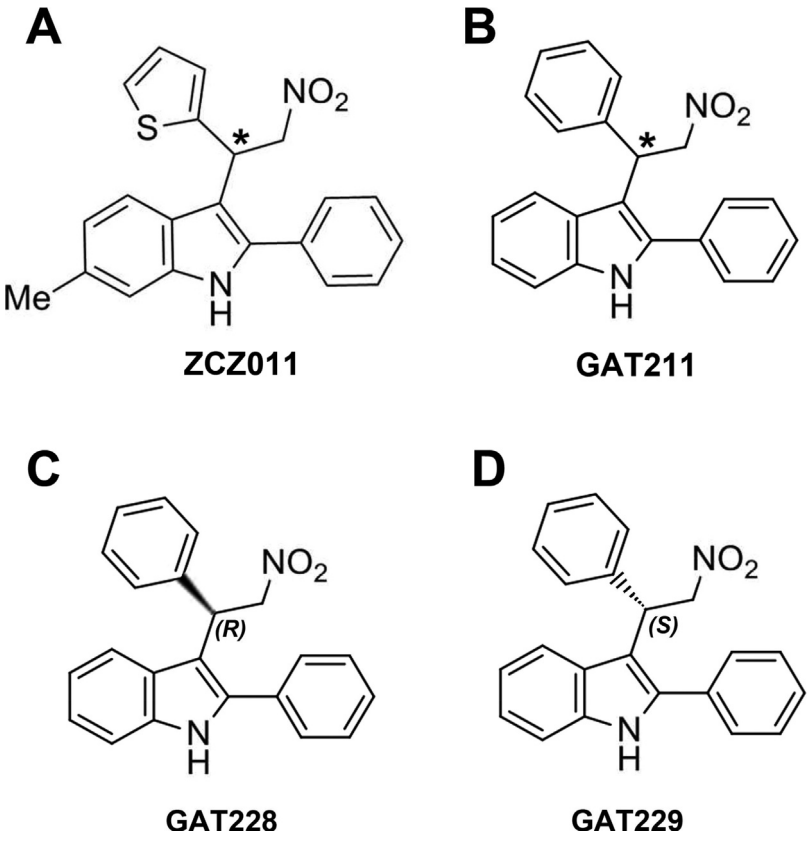

Fig. 1. Structures of ZCZ011 and GAT211 and its enantiomers GAT228 and GAT229.

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