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Role of Endocannabinoids in 5-HT₂ Receptor-Mediated Effects

William M. Connelly¹ and Matthew J. Baggott²

¹Department of Pharmacology and Toxicology, Otago School of Medical Sciences, University of Otago, Dunedin, New Zealand; and ²Helen Wills Neuroscience Institute, University of California Berkeley, Berkeley, California

Endocannabinoids are lipid retrograde messengers that can be released by postsynaptic depolarization and/or activation of certain metabotropic receptors. We review a recent report that activation of metabotropic 5-HT₂ receptors by endogenous serotonin induces the release of endocannabinoids in the olivary nucleus and suppresses glutamatergic input through a presynaptic action. This serotonin–endocannabinoid interaction has implications in the pathophysiology of pain and mental illness and raises the possibility that drugs targeting the 5-HT₂ receptor may act by modulating endocannabinoid release.

Receptor-driven endocannabinoid (eCB) release was first demonstrated in the cerebellum, where glutamate released from climbing fiber terminals acted on metabotropic glutamate subtype 1 receptors (mGluR1) expressed on Purkinje cells. The activation of mGluR1s subsequently caused the release of eCBs, thus depressing climbing fiber input. Later research showed receptor-driven eCB release is not confined to mGluRs. Orexin-B and muscarinic acetylcholine M1 and M3 receptors are all capable of inducing eCB release (reviewed by Hashimoto et al. 2007). eCB release can also be triggered by the Ca transient evoked by strong postsynaptic depolarization.

Because eCBs are lipid molecules and are released as soon as they are synthesized, synthesis is a key event in initiating eCB signaling. The pathways of eCB synthesis are not fully characterized. However, there appear to be independent mechanisms involving phospholipase C (PLC) in some cases (receptor-driven eCB release) and increased intracellular Ca²⁺ in others (i.e., depolarization-induced eCB synthesis). These two pathways can interact synergistically so, when combined, they allow eCBs to be released by levels of depolarization and metabotropic receptor activation that would not produce eCB release on their own (reviewed by Chevalyere et al. 2006).

eCBs can inhibit neurotransmitter release over both short- and long-term timescales. Short-term depression manifests via CB1R-mediated inhibition of presynaptic Ca²⁺ channels, enhanced presynaptic K⁺ conductance, and by affecting release machinery downstream of Ca²⁺ influx. Long-term depression is less well defined and is thought to involve coactivation of presynaptic *N*-methyl-D-aspartate receptors and CB1Rs. eCB-mediated synaptic plasticity has been implicated in learning and memory and the distribution of CB1Rs in the brain suggests specific roles in the control of pain, motivation, emotion, learning, and cognition (reviewed by Chevalyere et al. 2006).

In recent work, Best and Regehr (2008) add serotonin (5-HT) to the list of neurotransmitter systems with receptors able to trigger eCB release. In this study, both 5-hydroxytrypt-

amine 2 receptor (5-HT_{2R}) and 5-hydroxytryptamine 1B receptor (5-HT_{1BR}) activation decreased the probability of glutamate release in the inferior olive and the effect of 5-HT_{2Rs} was prevented by a CB1R antagonist. The authors took advantage of a novel brain stem slice that preserves serotonergic neurons and their synapses onto the inferior olive. Because these serotonergic inputs are physically separated from glutamatergic input from mesodiencephalic regions, they were able to study the interactions of these two inputs on olivary neurons (Fig. 1A). Whole cell voltage clamp of neurons in the dorsal principal olive revealed that exogenous 5-HT (10 μM) depressed the amplitude of evoked excitatory postsynaptic currents (EPSCs) by >80%. This effect was mimicked by the high-affinity 5-HT_{2R} agonist TCB-2, but was fully suppressed only by a combination of 5-HT_{2R} and 5-HT_{1BR} antagonists. This modulation was presynaptic because it increased the paired-pulse ratio of the evoked EPSCs and had no effect on the amplitude of currents evoked by exogenous glutamate application.

Given that other Gq-coupled receptors can evoke eCB release, Best and Regehr investigated whether the serotonergic suppression of evoked EPSC amplitude was cannabinoid dependent. CB1R agonists depressed the amplitude of evoked EPSCs to a similar extent as serotonergic agonists and, importantly, the reduction of evoked EPSC amplitude produced by selective activation of 5-HT_{2R} was blocked by selective CB1R antagonists.

Activating receptors with exogenous agonists does not replicate physiological recruitment. First, there is always the risk of nonselective effects caused by ligands straying onto other receptors. Second, 5-HT_{2A} receptors undergo agonist-directed trafficking, meaning that different agonists can preferentially recruit different second-messenger cascades (Parrish and Nichols 2006). Finally, the temporospatial characteristics of agonist stimulation will vastly differ between bath application and neuronal release of a neurotransmitter.

To stimulate 5-HT₂ receptors with greater physiological validity than afforded by exogenous agonists, the researchers electrically stimulated the slice dorsal to the inferior olive with a 1-s 50-Hz train. This initiated a slow 5-HT_{2R}-mediated postsynaptic inward current (presumably by activating serotonergic fibers from the nucleus reticularis paragigantocellularis; Fig. 1B). A single train induced a serotonergic current that lasted for about 10 s but depressed evoked EPSCs for about 25 s in a manner that was sensitive to 5-HT₂–5-HT_{1B} antagonist coapplication. The inhibition of glutamate release induced by endogenous serotonin was mediated by eCB release because a CB1R antagonist blocked it. Thus one effect of serotonin in the inferior olive is 5-HT_{2R}-mediated eCB re-

Address for reprint requests and other correspondence: W. M. Connelly, Pharmacology Department, University of Otago, P.O. Box 913, Dunedin, New Zealand (E-mail: bill.connelly@otago.ac.nz).

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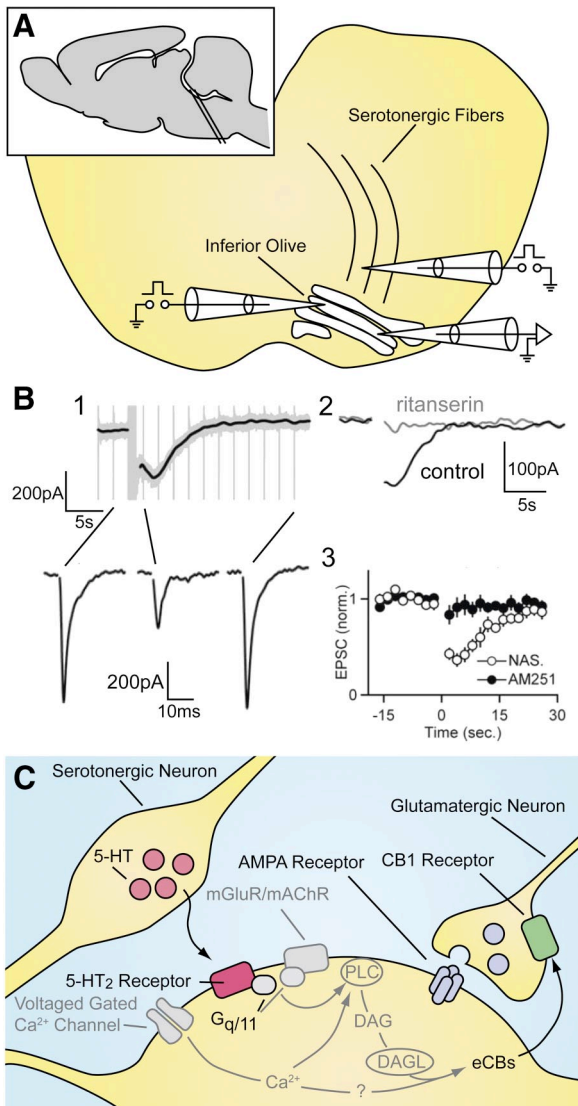


FIG. 1. Schematic of the experimental protocol and the pathway by which endocannabinoid (eCB) release is caused by 5-hydroxytryptamine 2 receptors (5-HT₂R). A: diagram showing the position of the stimulating electrode for recruiting glutamatergic input into the inferior olive (left electrode), location of the whole cell recording (bottom right electrode), and area where stimulation activated serotonergic fibers (top right electrode). Inset: plane and angle of the inferior olivary slice. B: by activating serotonergic fibers a 5-HT₂-mediated current could be induced that produced a depression in glutamate release: 1 shows the slow serotonergic current and the fast glutamatergic currents before, during, and after the evoked serotonin release; 2 shows the sensitivity of the serotonergic current to a 5-HT₂R antagonist, ritanserin; 3 shows the depression of excitatory postsynaptic current amplitude by the serotonergic current (time 0) and its sensitivity to the CB1 receptor antagonist AM251. C: 5-HT is released by serotonergic neurons and activates 5-HT₂Rs that produce diacylglycerol (DAG), via the action of phospholipase C (PLC). DAG is metabolized by DAG lipase (DAGL) to eCBs, which in turn cause eCB release, acting in a retrograde fashion to depress glutamate release. Depolarization, presumably by activating voltage-sensitive Ca²⁺ channels and increasing intracellular Ca²⁺ concentration, can also generate eCBs. Pathways outlined in gray were not directly demonstrated by Best and Regehr (2008) but exist in other brain regions and potentially act here too. A and B were modified from Best and Regehr (2008) with permission.

lease, which acts retrogradely via CB1R to suppress glutamate release.

Unfortunately, the role the 5-HT₁BR plays in the 5-HT-evoked depression of glutamate release remains unclear. The

authors repeatedly demonstrate that the blockade of both 5-HT₁B and 5-HT₂ receptors is required to block the effect of serotonergic agonists (either 5-HT or TCB-2). Although the suppression of glutamate release by 5-HT₂R activation is clearly shown to be CB1 receptor dependent, the study does not address whether the suppression of glutamate release by 5-HT₁B receptor activation also relies on CB1 receptor function.

It is also worth considering the 50-Hz stimulation frequency used to recruit a bundle of serotonergic fibers. How well this models the physiological firing rates of the individual neurons in these fibers remains unclear. Limited recordings from putative serotonergic neurons in the nucleus reticularis paraventricularis have shown rates between 30 and 70 Hz during or after painful stimuli (Leung and Mason 1995). Nonetheless, it would have been informative to see a “frequency–response” relationship between serotonergic fiber stimulation frequency and evoked EPSC depression, to document how hard one needs to stimulate 5-HT release to produce eCB release.

The finding that 5-HT₂R activation triggers eCB release is consistent with past evidence of possible functional recruitment of eCBs by 5-HT₂R. For example, 5-HT₂AR agonists cause a PLC-dependent increase in the release of the eCB 2-arachidonylglycerol in cultured fibroblasts (Parrish and Nichols 2006). 5-HT₂AR agonists induce a stereotypic “wet dog shake” in rats and these behaviors are abolished in the presence of CB1 receptor antagonists (Gorzalka et al. 2005).

Previous studies have also suggested a possible functional recruitment of eCBs by 5-HT₂R in pain states. eCB and 5-HT concentrations are elevated in many brain regions in models of neuropathic pain (Hohmann et al. 2005; Palzo et al. 2006). Both cannabinoids and selective serotonin reuptake inhibitors have analgesic effects, the latter putatively through 5-HT₂R mechanism (Honda et al. 2006). Indeed, stress-induced analgesia is both dependent on eCBs and blocked by 5-HT₂R antagonism (Hohmann et al. 2005; Tokuyama et al. 1993). Accordingly, it would be interesting to see whether the increase in eCB levels during the stress-induced analgesia paradigm were blocked by 5-HT₂R antagonism.

In their discussion, Best and Regehr suggest that many of the clinically significant effects of 5-HT₂R may be mediated by eCBs. This is an intriguing idea. 5-HT₂AR agonists, such as LSD, and high doses of CB1R agonists, such as Δ⁹-tetrahydrocannabinol, have similar hallucinogenic effects that may model some aspects of schizophrenia and recreational use of cannabinoids may increase susceptibility to schizophrenia/psychosis (reviewed in Roser et al. 2008). This raises the natural question of whether cannabinoid manipulations might have a role in treatment of psychosis. A 4-wk, controlled, double-blind clinical trial of cannabidiol, a weak partial CB1R antagonist, in 42 schizophrenic patients, showed that cannabidiol reduced acute psychotic signs and symptoms to a degree that did not differ from the antipsychotic D2/D3 receptor antagonist amisulpride. However, the selective CB1 antagonist rimonabant (SR141716) was no more effective than a placebo in a trial of 72 patients with schizophrenia or schizoaffective disorder (reviewed in Roser et al. 2008). Even if CB1R blockade does not prove useful on its own, it is possible that this approach may find use as an adjunct to dopamine D2 receptor antagonism, much in the same way as atypical antipsychotics derive additional efficacy from 5-HT₂R antagonism.

The interaction between serotonin and eCB systems reported by Best and Regehr (2008) suggests that therapeutic drugs acting via 5-HT₂R_s may produce their action, at least in part, by modulating eCB release. Because there are many (patho)physiological states that involve heightened serotonergic activity, it may prove fruitful to investigate the role of eCB release in these states. One may also ask how many more G_q-linked receptors may directly activate eCB synthesis. This study opens new vistas for understanding the serotonin system and reveals a potential rationale for therapeutic approaches to treating psychosis and neuropathic pain.

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