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# The Relationship between Muscarinic and Cannabinoid Receptors in Neuronal Excitability and Epilepsy: A Review

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**Keywords**
Cannabinoids · Epilepsy · Crosstalk · Endocannabinoids · Muscarinic receptors · Neural excitability

**Abstract**
***Background:*** Of the seventy million people who suffer from epilepsy, 40 percent of them become resistant to more than one antiepileptic medication and have a higher chance of death. While the classical definition of epilepsy was due to the imbalance between excitatory glutamatergic and inhibitory γ-aminobutyric acid (GABA)-ergic signalling, substantial evidence implicates muscarinic receptors in the regulation of neural excitability.
***Summary:*** Cannabinoids have shown to reduce seizure activity and neuronal excitability in several epileptic models through the activation of muscarinic receptors with drugs which modulate their activity. Cannabinoids also have been effective in reducing antiepileptic activity in pharmaco-resistant individuals; however, the mechanism of its effects in temporal lobe epilepsy is not clear.
***Key Messages:*** This review seeks to elucidate the relationship between muscarinic and cannabinoid receptors in epilepsy and neural excitability.



## Introduction

Epilepsy is defined as a disorder characterised mainly by the spontaneous recurrence of unprovoked seizures [1, 2]. Seizures are paroxysmal alterations of neurologic function caused by an excessive, hypersynchronous electrical discharge of neurons in the brain [3]. The condition affects 70 billion of the world's population and 40 percent of the affected persons suffer from pharmaco-resistant epilepsy otherwise known as intractable epilepsy, where the use of two adequately chosen drugs fails to produce a reduction in seizure activity [4, 5]. Individuals with pharmaco-resistant epilepsy have a higher incidence of sudden unexpected epileptic death; this occurs with a higher frequency in paediatric epilepsy syndromes with a 20% incidence rate [6].

A guiding principle on the aetiology of seizures highlights a disruption of mechanisms that normally create a balance between neuronal excitation and inhibition [7]. Glutamatergic and γ-aminobutyric acid (GABA)-ergic transmissions are the major excitatory and inhibitory physiologic mechanisms of the nervous system. However, these neurotransmission pathways may not have a simple and direct relationship to seizures due to the effects of desensitisation of receptors. Alternations in the gradients responsible for ion flow through ionotropic receptors may also influence seizure outcome, for example, GABA-ergic transmission can lead to depolarisation rather than hyperpolarization if the gradients responsible for chloride

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(Cl-) ion flow through GABA-A receptors are altered. Glutamatergic synapses innervate both glutamatergic neurons and GABA-ergic neurons in many neuronal systems and could also potentially impact the relationship between receptor activation and excitation [8]. Therefore, it is difficult to predict how glutamatergic or GABA-ergic modulation can influence seizure generation in vivo and the need for further interrogation of other neuroexcitatory or modulatory mechanisms to neuroexcitation is critical.

## The Role of Muscarinic Receptors in Central Neuronal Excitability and Epilepsy

Muscarinic receptors (mAChR) are G-protein coupled receptors present in neurons in the central and peripheral nervous system, cardiac and smooth muscles, and a variety of exocrine glands [8]. There are five different subtypes of mAChR, termed $M_1$–$M_5$, which are divided into two broad functional categories [9, 10]. $M_1$, $M_3$, and $M_5$ are coupled to the Gq family of G-proteins and upon activation lead to excitatory effects. $M_2$ and $M_4$ are coupled to the Gi family of G-proteins and upon activation lead to inhibitory effects. Acetylcholine (ACh) binds to these receptors centrally and peripherally. In the central nervous system, the neurotransmitter acts as a neuromodulator of neuronal excitability, presynaptic release probability, postsynaptic responsiveness and synaptic plasticity [11]. The $M_1$ subtype is the predominant mAChR in the cortex, hippocampus, striatum, and thalamus [12]. Immunoprecipitation followed by a radioligand binding assay in post-mortem tissue of the human hippocampus has quantified the proportion of mAChR as 60% $M_1$, 20% $M_2$, 15% $M_4$, and roughly 5% $M_3$ receptor expression [13].

The signal transduction pathways associated with the mAChR create a potential for overlapping pathways and modulatory functions, for example, the activation of mitogen-activated protein kinases (MAPK) by M1, M3, and M5 subtypes. Two (2) isoforms of MAPK have been discovered; extracellular regulated kinase 1 (ERK1) and extracellular regulated kinase 2 (ERK2). The signalling pathways produced by ERK in neurons are key steps in regulatory pathways of cell survival and neuronal plasticity [14]. Additionally, the production of inositol triphosphate ($IP_3$) by mAChR activation triggers the release of calcium ($Ca^{2+}$) from the endoplasmic reticulum (ER) stores in CA1 pyramidal neurons of the hippocampus in vitro and in vivo models [15]. Cholinergic innervation is enriched in the entorhinal cortex-hippocampus complex and plays an important role in the normal control of neuronal excitability. The entorhinal cortex-hippocampus complex is also the site of origin of seizure activity in the majority of patients with temporal lobe epilepsy (TLE) [16]. While current epileptic treatment strategies do not include drugs which act exclusively on mACh receptors, organophosphate-induced seizures occur primarily via perturbation of cholinergic signalling. Additionally, selectively blocking $M_1$ receptor with the $M_1$ antagonist VU0255035 delays status epilepticus and reduces hyperexcitability after exposure to an organophosphate [17]. Clinically, the use of $M_1$ antagonists has not been used as therapeutic treatments for epilepsy due to the widespread localisation of the receptor, both peripherally and centrally. Thus, to address neuronal excitability other therapeutic targets to modulate $M_1$ activation should be explored.

## The Endocannabinoid System

Cannabis has been used as a traditional treatment of epilepsy since the 1800s [18]. However, it was not until the 1930s that the first phytocannabinoid, cannabinol (CBN), was isolated from cannabis [19]. Cannabidiol (CBD) and delta-9 tetrahydrocannabinol (THC) were isolated subsequently [19, 20]. The biological effects of cannabis were postulated initially to disrupt the cell membrane non-specifically based on the lipophilic nature of cannabinoids [21]. However, the mechanism of action of the cannabinoids has been further explored upon the discovery of the endocannabinoid system (ECS) in the 1990s in vertebras [21, 22].

The ECS has important neuromodulatory functions in CNS development, synaptic plasticity, and the response to endogenous and environmental insults [23]. The intent of this section is not to provide reverberation of the endocannabinoid system but to provide a synopsis of its possible interactions with receptors involved in central neurotransmission which could impact neuronal excitability.

The system consists of endocannabinoids, anandamide and 2-arachindonyl glycerol (2-AG), cannabinoid receptors, CBR1 and CBR2, and the enzymes, which were responsible for the synthesis and degradation of the endocannabinoids. Anandamide and 2-arachidonoyl glycerol (2-AG) are the first discovered and best-characterized endocannabinoids. Additional endogenous substances (e.g., virodhamine and 2-arachidonoyl glycerol ether expand the list of endocannabinoids, but our review will be centred on mechanisms related anandamide and 2-AG interactions on the cannabinoid

receptors as the more characterised ligands [23]. Other cannabinoid related receptors such as the G protein-coupled receptor 55 (GPR55), transient receptor potential cation channels (TRVP) and peroxisome proliferator-activated receptor (PPAR) have also been shown to be activated by cannabinoid ligands [24–26]. Anandamide is postulated to be mainly produced from N-arachidonoyl phosphatidyl ethanol (NAPE), while 2-AG is produced from 2-arachidonoyl-containing phospholipids, primarily arachidonoyl-containing phosphatidyl inositol bisphosphate (PIP2) [24]. Anandamide is a partial agonist with low efficacy at CBR1 receptors and a very low efficacy at CB2 receptors. 2-AG is a high efficacy agonist for both CB1 and CB2 receptors [22, 24] Anandamide is mainly hydrolysed by fatty acid amide hydrolase (FAAH) into arachidonic acid and ethanolamine [27]. 2-AG degradation is primarily due to three hydrolytic enzymes, monoacylglycerol lipase (MGL) and alpha/beta domain hydrolases 6 and 12 [28]. However, they can also be broken down by cyclooxygenase-2, lipoxygenases, and cytochrome P450 enzymes [29].

### ECS' Role in Modulating Neurotransmission of GABA- and Glutamatergic Acid-Mediated Currents

According to brain topography, the ECS displays actions at various brain neuronal pathways and modifies their specific functions. It modulates neurotransmission indirectly, with endocannabinoids acting as retrograde neurotransmitters instead of direct neurotransmitters [30, 31]. As noted, prior, glutamatergic and GABA-ergic neurotransmission pathways are the major excitatory and inhibitory mechanism of the CNS. The modulation of GABA- and glutamatergic acid-mediated currents has been demonstrated by the application of endocannabinoids and phytocannabinoids and has been found to be clinically relevant [32–34].

Cannabinoid receptor agonists have been shown to attenuate glutamatergic and GABA mediated transmission in several regions of the brain for example, the hippocampus, prefrontal cortex, and nucleus accumbens by pre-synaptic effects [35–37]. The hippocampal neurons in the depolarised state have been shown to rapidly release both anandamide and 2-AG in a $Ca^{2+}$ dependent manner. Moreso, functional CBR1 cannabinoid receptors are present on glutamatergic terminals of the hippocampus and are co-localised with vesicular glutamate transporter 1 (VGluT1) [38]. Conditional deletion of the CBR1 gene in the cortical glutamatergic neurons and in the forebrain's GABAergic neurons, as well as virally induced deletion of the CBR1 gene in the hippocampus, demonstrate the neuroprotective role of CBR1 receptors in glutamatergic hippocampal neurons against kainic acid (KA)-induced seizures [38].

The CBR1 is also expressed by the GABA-mediated inhibitory neurons in the hippocampus, basal ganglia and thalamus where the receptor clusters on the axon terminal [35, 36]. Application of a synthetic CBR1 agonist ligand has shown a reduction in GABA release from hippocampal slices [36]. This finding supports the hypothesis that transient suppression of GABA-mediated transmission in depolarised hippocampal pyramidal neurons is mediated by retrograde signalling through the release of endogenous cannabinoids. Clinically, this mechanism of action on the GABA-mediated system has led to the study of cannabinoid receptor agonists such cannabidiol (CBD) in neurological conditions such as epilepsy among others [39].

### Muscarinic and Cannabinoid Co-Signalling Mechanisms in the CNS

As noted above, the cholinergic system in the CNS has important roles in various aspects of brain function, primarily through the muscarinic receptors. The diverse effects upon muscarinic receptor activation are generally considered to be related to modulation of ionic currents; however, the existence of endocannabinoids and their signalling have led to other possibilities [35, 40]. Studies have shown that sequential activation of muscarinic receptors has been able to demonstrate some of these possibilities. For example, muscarinic and cannabinoid CB1 receptors have been shown to produce contractile effects of the bovine ciliary muscle by involving the activation of Rho-kinase and Protein Kinase C mechanisms [41]. Further, muscarinic receptor activation also enhances transient endocannabinoid release (DSI) and induces persistent release in rat hippocampal slices in a dose dependent way [42]. The CB1 receptor has shown an inhibitory interaction with the muscarinic receptors on cholinergic neurons in the hippocampus of rodents. A study by Schulte et al. [43] demonstrated that the inhibitory effect of the muscarinic receptor agonist oxotremorine on acetylcholine release in murine hippocampal slices was augmented by genetic CB1 receptor ablation and with the use of CB1 antagonist rimonabant in the hippocampus of rats. However, this inhibitory effect was decreased by the application of a cannabinoid receptor agonist in a murine model. Further, it is known that seizures

induced by the muscarinic agonist pilocarpine are due to $M_1$ receptor activation and can be modulated by the cannabinoid CB1 receptor [44].

### Modulation of M Currents by M1 Receptor and Neuronal Excitability

Another possible role of the muscarinic receptor in regulating neuronal excitability and seizure-like activity is the inhibition of M-type potassium channels [45, 46]. M currents are non-inactivating potassium current found in many neuronal cell types. These channels belong to the Kv7 (KCNQ) $K^+$ channel family and comprise of 5 members with heteromers, KCNQ2/3 being localised in the brain. Mutations of KCNQ2 and KCNQ3 have led to the epileptic condition known as "benign familial neonatal convulsions" (BFNC) [47].

M currents can be modulated by a large array of receptor types, and the modulation can occur either by suppression or enhancement of these targets and has dramatic effects on neuronal excitability [47]. The main forms of the regulation of the M current are receptor-mediated modulation and the control of macroscopic current amplitude by intracellular calcium which are intrinsically linked to muscarinic receptor signal transduction mechanisms [46, 48]. Inhibition of M currents by pathways that stimulate phospholipase C activity controls excitability throughout the nervous system [47]. Muscarinic receptor activation inhibits M-channels in CA3 pyramidal hippocampal neurons and its efferents which causes depolarisation, activates voltage-gated calcium channels, and elevates the intracellular calcium concentration which leads to an increase in the release of glutamate on CA1 pyramidal neurons [12].

The inhibition of these currents through muscarinic activation has been due to the production of PLC which leads to the subsequent production of $PIP_2$ [46–48] While earlier work on the role of DAG and $IP_3$ as second messengers is developing, it is clear that M channels require $PIP_2$ to move into an open state and it switches to a closed state when membrane levels of $PIP_2$ are reduced, or its polar head becomes neutralised and not from the accumulation of products of its hydrolysis. This was further reaffirmed when the muscarinic inhibition of M channels is reduced when membrane $PIP_2$ levels were increased by over-expressing the PI5-kinase. If the inhibition was due to the products of hydrolysis of $PIP_2$ then the overexpression of PI5-kinase would have led to an increase of those products and thus an increase in the inhibition of the M currents [48, 49]. Interestingly, one of the novel cannabinoid receptors, GPR55, also inhibits M current. The proposed mechanism is shown in Figure 1.

### Muscarinic Receptors, NMDA Activation, ECS, and Neuronal Excitability

So far, it has been demonstrated that muscarinic receptor activation has been effective in inducing seizure activity [44, 51, 52]. Other studies have shown that the receptor's activation appears to be important for the induction phase of seizures only. Moreso, the blockade of muscarinic receptor's activation with atropine given before pilocarpine administration prevents the seizure onset but did not interrupt established seizures in animal studies [50, 53]. This suggests the participation of other neurotransmitter systems in maintenance of seizure activity. A study by Priel et al. [54] illustrated that pilocarpine, acting through different cholinergic muscarinic receptors, promotes distinct effects on GABA and glutamate release and shifts the balance of excitatory and inhibitory transmission resulting in the generation of status epilepticus (SE) in cultured rat hippocampal neurons.

Like muscarinic receptors, NMDA receptors are widely distributed in the hippocampal region [55]. These receptors have a critical role in sustaining SE by mediating the plasticity of GABA-A receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, neuronal loss, and epileptogenesis [56]. In electrical stimulation models of SE, NMDA receptor antagonists can effectively terminate SE and are considered more effective in the prolonged, self-sustaining phase of SE [56]. In a study by Mazarati et al. [57] blocking the NDMA receptor rapidly and irreversibly aborted both behavioural and electrographic manifestation of self-sustaining status epilepticus in rats. However, in cholinergic stimulation models of SE, the NMDA receptor antagonists alone are ineffective in terminating benzodiazepine-refractory SE. When these drugs are combined with benzodiazepines, they work synergistically to end seizures [56, 58]. The inability of NDMA antagonist monotherapy to terminate benzodiazepine-refractory SE in cholinergic stimulated models may be related to the multiple neurological pathways which are linked to cholinergic induced seizure activity [54]. Moreover, the extracellular signal-regulated kinase (ERK1,2 or ERK) pathway is an essential component of NMDA and M1, M3, and M5

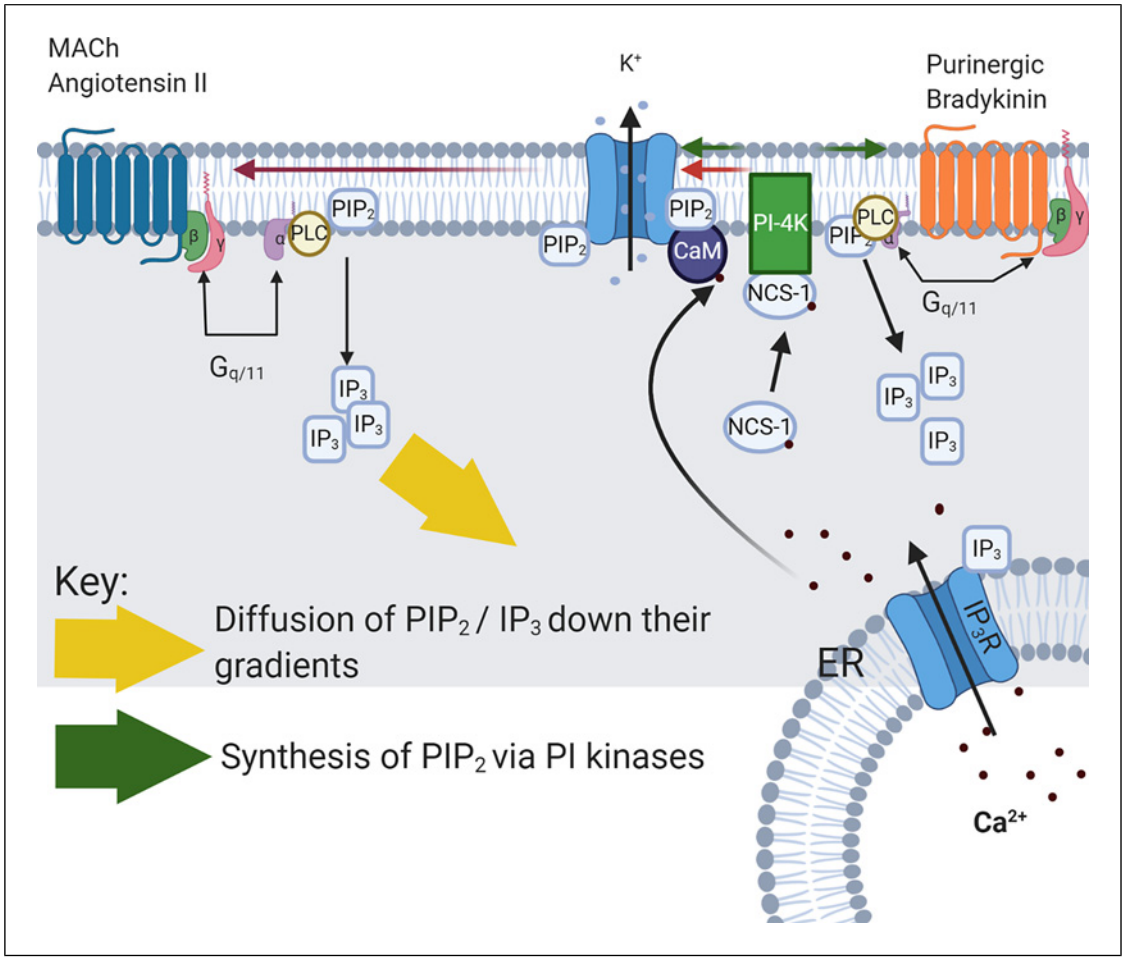


**Fig. 1.** Modulation of M channels by both phosphatidylinositol 4,5-bisphosphate (PIP2) and calcium-calmodulin ($Ca^{2+}$-CaM) complex in the superior cervical ganglion (SCG). Fig. 1 shows two mechanisms associated with the inhibition of M channels used by Gq/11 coupled receptors in the superior cervical ganglion (SCG). The M channels are modulated by both phosphatidylinositol 4,5-bisphosphate (PIP2) and calcium-calmodulin ($Ca^{2+}$-CaM) complex, with calcium ions released from the endoplasmic reticulum (ER) through the activation of inositol 1,4,5 trisphosphate receptor (IP3R). Activation of the M1 muscarinic acetylcholine receptors (M1AChR) and AT1 angiotensin II receptors (ATR) are believed to be ineffective releasing $Ca^{2+}$ from the ER due to the lack of spatial co-localisation between the IP3R and IP3 produced by the M1AChR and ATR [50]. Thus, the IP3 dissipates away (yellow arrow) after PIP2 is consumed by PLC. The second pathway is used by purinergic P2Y and bradykinin B receptors which due to their spatial localisation to ER IPR are capable of releasing Ca signals. The released $Ca^{2+}$ binds to neuronal $Ca^{2+}$ sensor-1 (NCS-1) and calmodulin (CaM). NCS-1 promotes PIP2 synthesis via acceleration of PI-4-kinase (green arrow), increasing concentration of PIP2 in the membrane and stabilising PIP2 levels which increases PLC activity. CaM binds to the carboxyl domains of the channel and is speculated to reduce the affinity of PIP2 which then unbinds from the channel since tonic PIP2 concentration is insufficient to maintain association with the channel [50].

receptor signal transduction mechanisms and a possible crosstalk mechanism may be related to this observation [14, 59].

As noted, prior, the activation of cholinergic muscarinic receptors promotes distinct effects on GABA and glutamate release and may shift the balance of excitatory and inhibitory neurotransmission. Depolarised hippocampal neurons have been shown to rapidly release both anandamide and 2-AG in a calcium ($Ca^{2+}$) dependent manner. Moreover, functional CBR1 cannabinoid receptors are present on glutamatergic and GABA-mediated inhibitory neurons of the hippocampus. The CBR1 is also expressed by the GABA-mediated inhibitory neurons in the basal ganglia and thalamus [35, 37, 38]. The absence or presence of retrograde signalling of the CB1 receptor demonstrates its possible neuroprotective role against seizure activity by either of the excitatory or inhibitory neurotransmission pathways in the CNS [38, 39].

**Table 1.** Summary of the interactions between muscarinic and endocannabinoid system

| | Reference |
|---|---|
| Cannabinoid antagonist CBD is produces an antiseizure effect in seizure models of muscarinic receptors | Jones 2010 [60] |
| Cannabinoid agonists have biphasic effects on ACH release in the hippocampus | Tzavara 2003 [61] |
| Muscarinic receptor in regulating neuronal excitability and seizure-like activity is the inhibition of M-type potassium channels | Sun 2012 [12] |
| Genetic disruption of M1 ablates seizure activity, while CB1 knockout models increase seizure activity | Kow 2014 [62] |
| Crosstalk between CB1 and muscarinic receptors has been investigated in Alzheimer's Disease mouse models where dysregulation of muscarinic neurotransmission was shown to increase CB1 expression | Liorente-Ovejero 2018 [63] |
| Synthetic cannabinoids WIN55212-2 and methanandamide when added methanandamide applied to CA1 neurons caused a decrease in non-inactivating voltage dependent $K^+$ M current ($I_M$) | Schweitzer 1999 [64] |

## Way Forward for Epileptic Research and ECS

Cannabinoid and muscarinic and receptors have pathways of modulating neural excitability, such as through the modulation of M currents as well as the modulation of NMDA and GABA receptors. Table 1 outlines studies which identify direct and indirect interactions between signal transduction mechanisms associated with cholinergic neurotransmission (mainly signal transduction associated with muscarinic receptors) and the endocannabinoid system.

The modulation of muscarinic M1-induced seizures by cannabinoids has long been a point of interest in the use of cannabinoids in the treatment of epilepsy and implies some interaction between the muscarinic and cannabinoid receptors [60]. Mechanisms of crosstalk between these receptors have also been shown in models of Alzheimer's disease where dysregulation of muscarinic receptor transmission results in an increase in cannabinoid receptor expression in early stages of Alzheimer's disease [61]. This review seeks to further highlight the need for further research into exploring the mechanisms of crosstalk of these receptors in epilepsy and neural excitability.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.


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