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Are endocannabinoids involved in muscarinic acetylcholine receptor-mediated plasticity of the skeletal neuromuscular junction?

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ABSTRACT

The *Cannabis sativa*, or marijuana, plant has long been used by humans as a means of altering mental state. Recently, there has been a growing interest in therapeutic applications of cannabinoids, the chemical compounds that are derived from marijuana. The discovery of endocannabinoids, which are synthesized naturally in the body, and receptors for these chemicals has generated a great deal of interest in their roles in neurotransmission. Much has been learned about cannabinoid signaling in the central nervous system (CNS); however, within the context of the peripheral nervous system (PNS), relatively little is known about endocannabinoid activity. Muscarinic acetylcholine receptors (mAChRs), which, in addition to being widely found in the CNS, are present in the PNS, may have important interactions with endocannabinoids. In this review, we are interested in the possibility of interactions between mAChRs and endocannabinoids at the vertebrate neuromuscular junction. We will begin by providing an overview of cannabinoid research and drawing connections between cannabinoids and mAChRs. We will then discuss the current understanding regarding mAChR presence at the vertebrate muscular junction. We will also examine what is known about cannabinoid signaling and mAChRs in the CNS and correlate this information with the physiological effects of cannabinoids. We propose that knowledge about endocannabinoid function in the CNS can perhaps reveal information about possible roles for cannabinoids in the PNS, particularly at the vertebrate neuromuscular junction.

INTRODUCTION

Overview of Cannabinoid Research

Cannabinoids are chemical compounds derived from the plant *Cannabis sativa*, more commonly known as marijuana. Δ^9-tetrahydrocannabinol, or Δ^9THC, is the chief psychoactive chemical component of marijuana. It has been found that the presence Δ^9THC in vertebrates is correlated with a "tetrad" of physiological effects: reduced motility, catalepsy, lowering of body temperature, and reduced sensitivity to pain. Studies of Δ^9THC activity, along with the activity of other structurally homologous cannabinoids, on a molecular biological scale concluded that cannabinoids activate G-protein-coupled receptors in both the central nervous system (CNS), particularly in the brain, and in immune cells. Receptors expressed in the CNS are known as CB_1 receptors, while those expressed in cells of the immune system are known as CB_2 receptors (Elphick and Egertová 2001).

The neurobiology of cannabinoid activity can best be understood by focusing on the CB_1 receptors. To elucidate the mechanisms of CB_1 receptor mediation of cannabinoid effects, both CB_1 receptor agonists and antagonists have been synthesized. Of particular importance is the identification of two endogenous cannabinoids, or endocannabinoids: arachidonylethanolamide (anandamide) and 2-arachidonylglycerol (2-AG). These endocannabinoids are similar to Δ^9THC in terms of biological activity. (Elphick and Egertová 2001). The presence of endocannabinoids and corresponding receptors in the CNS suggests that cannabinoids have inherent, perhaps significant, biological roles in the body.

Cannabinoid Signaling

CB_1 receptors in the CNS have been shown to be expressed most heavily in the substatia nigra pars reticulata, globus pallidus, hippocampus, and cerebellum (Herkenham et al. 1990). Additionally, there is evidence for the presence of CB_1 receptors on the presynaptic terminals of nerves innervating peripheral, visceral organs of the reproductive, urinary, and digestive systems, such as the small intestine, bladder, and heart (Elphick and Egertová 2001; Pertwee 2001). The fact that CB_1 receptors are located on presynaptic terminals has important functional implications. Following postsynaptic depolarization, endocannabinoids have been shown to act presynaptically to suppress the release of both excitatory and inhibitory neurotransmitters in the hippocampus (Wilson and Nicoll 2001; Wilson et al. 2001) and in the cerebellum (Kreitzer and Regehr 2001; Yoshida et al. 2002) via retrograde signaling mechanisms. These types of suppression are known as depolarization-induced suppression of excitation (DSE) or inhibition

(DSI) (Alger and Pitler 1995; Freund et al. 2003).

Endocannabinoids and Muscarinic Receptors

Interestingly, it was demonstrated that activation of muscarinic acetylcholine receptors (mAChRs) enhances endocannabinoid release, and therefore enhances DSI, in the hippocampus (Kim et al. 2002). In their research, McQuiston and Madison (1999) found that mAChR activation in the hippocampus results most frequently in interneuronal depolarization. This suggests an important relationship between mAChRs and endocannabinoids: mAChR activation mediates endocannabinoid activity, which in turn modulates presynaptic neurotransmitter release.

Van der Kloot (1994) found evidence for a cannabinoid receptor at the motor nerve terminal of the frog neuromuscular junction; however, Elphick and Egertová (2001) consider Van der Kloot's conclusion to be an isolated case and maintain that there is no evidence suggesting cannabinoid receptor expression by motor neurons or muscle cells that mediate voluntary skeletal muscle control. Nevertheless, as little research has been done on cannabinoid expression at the vertebrate neuromuscular junction, the assumption that there is no such expression is premature.

Muscarinic acetylcholine receptors are expressed at the vertebrate neuromuscular junction (Slutsky et al. 1999; Graves et al. 2004). Therefore, based on the relationship between endocannabinoids and mAChRs in the CNS, it is reasonable to suggest that cannabinoid receptors do, in fact, occur at the vertebrate neuromuscular junction. Discovery of cannabinoid receptor expression at the neuromuscular junction could offer further insight into the modulation of excitatory and inhibitory neurotransmitter release in the peripheral nervous system.

MUSCARINIC ACETYLCHOLINE RECEPTORS AT THE VERTEBRATE NEUROMUSCULAR JUNCTION

Over the years, various studies have attempted to resolve whether muscarinic receptors increase or depress cholinergic release at motor neuron terminals. It is clear that these effects are presynaptic due to the fact that resulting changes affect acetylcholine release and quantal content (Graves et al. 2004). Slutsky et al. (1999) have provided evidence for a dual inhibitory/enhancive effect on presynaptic receptors observed only at low depolarization potentials and diminished as the potential is increased. Thus, a physiological study of the cholinergic modulation of these presynaptic effects would enhance our knowledge of synaptic transmission under extreme conditions that compromise the probability of acetylcholine release (Nikolsky et al. 2004). With regards to the concomitant inhibitory and enhancing effects of muscarinic receptors on presynaptic neurons, M_2 receptors have been associated with a depression of acetylcholine release while M_1 receptors appear to modulate increased release of acetylcholine (Slutsky et al. 1999). Research conducted by Graves et al. (2004) also supports the idea of biphasic muscarinic modulation of acetylcholine release involving M_1 (enhancive) and M_3 (inhibitory) receptors.

While it is known that activation of muscarinic receptors alters channel activity through direct binding or indirect second messenger pathways (Nicholls et al. 2001), the possible underlying mechanisms of muscarinic modulation have not yet been fully defined. The roles of protein kinase A and intracellular calcium as potential 'second messengers' have been closely investigated since it is known that the G-protein coupled muscarinic receptor can be physically remote from the channel that it affects (Hoshi et al. 2003). With regards to cAMP-dependent protein kinase A (PKA), Graves et al. (2004) demonstrated that this molecule plays a role only in the M_1 receptor-associated delayed enhancement of acetylcholine release. In addition, both phases of the muscarinic modulation model proposed by Graves et al. (2004) were found to be dependent on nitric oxide. Inhibition of acetylcholine release seems to proceed via a Ca^{2+}- independent feedback mechanism (Slutsky et al. 2002); however, increased Ca^{2+} has been linked to enhanced acetylcholine release (Slutsky et al. 1999). In order to further understand the role of muscarinic receptors in endocannabinoid signaling in the PNS, we will be focusing on Ca^{2+}-dependent activation pathways.

ENDOCANNABINOID SIGNALLING IN THE CENTRAL NERVOUS SYSTEM

Within the CNS, CB_1 is the most abundant member of the heptahelical G-protein coupled receptor family and is highly localized in the substatia nigra, basal ganglial, cerebellar, and hippocampal regions of the brain (Herkenham et al. 1990). Recent studies have shown that endocannabinoids mediate retrograde signaling at inhibitory synapses in the hippocampus and cerebellum (Ohno-Shosaku et al. 2002; Wilson & Nicoll 2001).

Endogenous cannabinoids mediate retrograde signaling to regulate transmission at both excitatory and inhibitory synapses by inhibiting the release of antereograde neurotransmitters. When action potentials arrive at the presynaptic terminal, neurotransmitters are released from internal stores and cross the synaptic terminal to bind to corresponding receptors on the

postsynaptic cell. Upon neurotransmitter binding and receptor signaling, postsynaptic cells are depolarized and Ca^{2+} enters the terminal. In a Ca^{2+}-dependent pathway, endocannabinoids are released and diffuse back across the synapse to bind to CB_1 receptors on the presynaptic cell. This retrograde signaling mediates depolarization-induced suppression of inhibition (DSI) or excitation (DSE) by inhibiting either inhibitory or excitatory neurotransmitter release from the presynaptic cell (Alger 1995; Elphick and Egertová 2001; Freund et al. 2003).

The Ca^{2+}-dependent release of endocannabinoids from postsynaptic cells during retrograde signaling has been suggested to occur through several mechanisms (Figure 1). Activation of voltage-dependent Ca^{2+} channels in the depolarized postsynaptic cells will increase transient Ca^{2+}. Transient Ca^{2+} may also be released from intracellular stores after activation of IP_3 through the G-protein-mediated PIP_2 pathway after activation of metabotropic receptors. Additionally, activation of metabotropic glutamate receptors or muscarinic cholinergic receptors will activate phospholipase C (PLC) to produce DAG, which remains at the cell membrane after dissociating from IP_3. DAG may then convert to the endocannabinoid 2-AG by the enzyme 1,2-diacylglycerol (DGL) in the plasma membrane where it would quickly be released and migrate back to presynaptic CB_1. After cannabinoid binding to CB_1 receptors, neurotransmitter release by the presynaptic cell may be inhibited by one or more of the following: blockage of action potentials invading the nerve terminal; or a reduction of Ca^{2+} influx and an increase in K^+ efflux in the nerve terminal through G-protein-mediated signaling through the CB_1 receptor (Elphick and Egertová 2001; Freund et al. 2003; Kim et al. 2002).

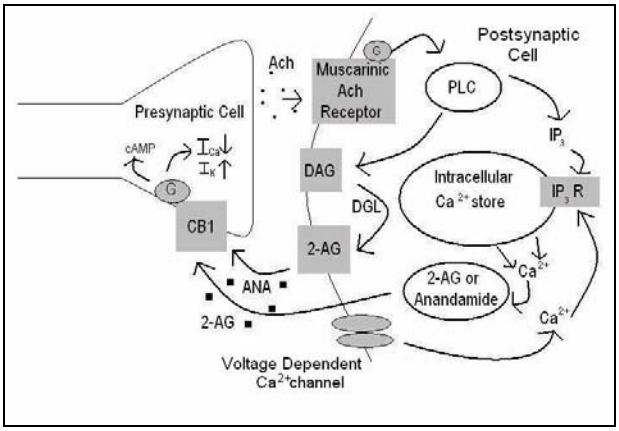

Figure 1. Possible mechanisms of endocannabinoid retrograde signaling and release from postsynaptic cells. Diagram adapted from a model of GABAergic signaling by Freund et al. (2003).

The involvement of CB_1 signaling in suppression of excitatory and inhibitory neurotransmitters through activation of group 1 metabotropic glutamate receptors (mGluRs) and GABAergic receptors in the CNS has been well documented (Alger 1995; Wilson and Nicoll 2001; Wilson et al. 2001). Recent studies have focused on the role of muscarinic ACh receptor-mediated endocannabinoid signaling within the hippocampus (Kim et al. 2002; McQuinston and Madison 1999). Kim et al. (2002) found that the activation of mAChRs enhances the release of endocannabinoids in the hippocampus. Endocannabinoids release though mAChR activation appears to utilize the same intracellular signaling pathways as mGluR, while activation of release via these receptors is accomplished through independently modulated pathways (Figure 1).

Muscarinic ACh receptor-mediated endocannabinoid retrograde signaling within the brain indicates that cannabinoid mediated DSE and DSI may occur in synapses within other regions of the central nervous system or within the peripheral nervous system. While the role of cannabinoid signaling has not been well investigated at the vertebrate neuromuscular junction, the presence of mAChRs at these junctions suggests that endocannabinoids may be involved in mAChR-mediated plasticity at the vertebrate neuromuscular junction.

PHYSIOLOGICAL EFFECTS OF CANNABINOIDS

In vertebrates, the presence of Δ-^{9}THC has been associated with a range of physiological effects including reduced motility, catalepsy, lowering of body temperature and reduced sensitivity to pain (Elphick and Egertová 2001; Freund et al. 2003). When exogenous cannabinoids bind to CB1 receptors in the brain, this may result in signaling in the brain's thermoregulatory, perceptive, cognitive, and motor movement centers which in turn will alter function. However, connections between the physiological effects cannabinoids on synaptic plasticity and transmission and role of the endogenous cannabinoids in these systems are not well established. The endocannabinoid system may provide a means for fine tuning within the CNS and may also function within the PNS by inhibiting neurotransmitter release at peripheral sites (Di Marzo and Le Petrocellis 1997; Pertwee 1997; Atha 2002).

Cannabinoids are well known anecdotally for their therapeutic value in the treatment of a variety of conditions including headaches, epilepsy, hypertension, discomfort during childbirth, and multiple sclerosis (Felder and Glass 1998). In the United States, human clinical studies are problematic due to the illegal status of marijuana and the lack of an effective placebo to mimic the psychoactive effects of cannabinoids (Di Marzo and

De Petrocellis 1997; Pertwee 1997 ; Baker 2003). However, anecdotal evidence from testimonies, newspaper articles, and surveys of self-medicating patients has provided some clues as to the possible roles of cannabinoids in motor function. Self-medicating patients, as well as patients treated in limited clinical trials, report dramatic improvement in symptoms associated with multiple sclerosis. In these patients cannabinoid treatment appears to reduce muscle pain, spasticity and tremor (Pertwee 1997; Atha 2002; Baker 2003).

Animal model studies of CB_1 mediation of motor function have shown cannabinoids to have an effect on muscle movement, coordination, posture and skeletal muscle tone (Van der Kloot 1994; Pertwee 1997). Cannabinoids and their receptors in the brain appear to mediate these effects on motor function. However, it is not known if the cannabinoid system plays a role in synaptic transmission and plasticity in the vertebrate skeletal neuromuscular junction.

The therapeutic effects of cannabinoid treatment on muscle spasticity, tremor, and catalepsy indicate that future studies must be undertaken in order to determine if the endocannabinoid system plays a role in mACh signaling pathways in the neuromuscular junction of vertebrates.

CONCLUSION

Anecdotal evidence and clinical trials have demonstrated a link between cannabinoids and relief of muscle spasticity in patients suffering from multiple sclerosis. Due to the abundance of CB_1 receptors in regions of the brain associated with locomotor function, many studies have examined endocannabinoid signaling in the CNS. Preliminary studies on the effects of cannabinoids on neurotransmission in the frog suggest the need for further investigation of endocannabinoid signaling and modulation of cholinergic release at motor endplates of the PNS (Van der Kloot 1994).

The frog sciatic nerve-sartorius muscle preparation has proven to be well-suited to the study of the effects of cannabinoids on cholinergic transmission at the motor endplates using various pharmacological tools. We propose that future studies investigating the role of CB_1 receptors in acetylcholine release should involve electrophysiological measurements of depolarizing potentials at these junctions in the presence of various CB_1 agonists and antagonists.

Arachidonylcyclopropylamide (ACPA) is the most widely used CB_1 agonist, while AM281 is a common CB_1 antagonist (Pertwee 2001). With these tools, it may be possible to determine the effects of the cannabinoid receptors on skeletal muscle resting membrane potentials following electrical stimulation of the *in vitro* nerve-muscle preparation. In addition, CB_1-deficient mice have been created (Zimmer et al. 1998) and may also prove useful in investigating the role of CB_1 receptors in acetylcholine release at motor endplates. The results of these studies could potentially have a significant impact on the debate concerning the medical use of marijuana to treat defects in motor functions. It is important to note that these studies would have to be safely replicated in humans before any definitive conclusions can be drawn.

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