Review Article:

Possible role interaction between cannabinoid and TRPC3, TRPC6 and TRPV2 in behavioral process

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ABSTRACT

Most studies have found that changes in concentration of intracellular ions can change cell activation. Calcium ion is one of the most vital one of these ions which is the most vital second messenger within cells. Ca2+ is necessary for a wide range of cellular processes such as cell proliferation, muscle contraction and exocytosis and apoptosis. Different kinds of TRPCs (transient receptor potential cation channels) have a wide range of activity in cell but are known as Ca2+ blocker channel. On the other hand, cannabinoids are a class of different chemical compounds that act on cannabinoid receptors in cells that suppress neurotransmitter release in the brain. Ligands for these receptor proteins consist of endocannabinoids (produced naturally in the body of humans and animals), the phytocannabinoids (exist in cannabis and some other plants), and synthetic cannabinoids (artificially made). The most important cannabinoid is the phytocannabinoid tetrahydrocannabinol (THC), the primary psychoactive compound of cannabis. Cannabidiol (CBD) is another major part of the plant. It is showed that endocannabinoid biosynthesis is a calcium-dependent process, though the physiological source of calcium is not identified. Many of biochemical experiments have been investigating the contributions of extracellular and intracellular calcium in the biosynthetic process. Some studies found that extracellular calcium is a vital factor for the endogenous cannabinoids biosynthesis, while a simply releasing intracellular calcium store such as endoplasmic reticulum does not have an effect on endocannabinoid biosynthesis. In addition to introduction of cannabinoid, blocker of Ca2+ channel (TRPC) and investigating interaction among them in this review, the possible role of cannabinoid and TRPC3, TRPC6 and TRPV2 in behavioral process are induced in conclusion.

Keywords: Cannabinoid; TRPC; Behavioral process

INTRODUCTION

Cannabinoid is among the class of different oxygen-containing C21 aromatic hydrocarbon compounds which is naturally found in the plant Cannabis sativa [1] which act on cannabinoid receptors in cells that suppress neuromodulators release in brain [2]. Three types of cannabinoid receptor have been identified which are referred to as CB1, CB2 and non CB1 and CB2 [3]. CB1 receptors are identified in the brain, in the basal ganglia and in the limbic system, in the hippocampus [2] in the cerebellum, in both male and female reproductive systems and in the human anterior eye and retina. CB1 receptors are not presented in the medulla oblongata, part of the brain stem on the top of spinal cord [4].

CB2 receptors are predominantly found in the immune system or immune-derived cells [5] with the highest density in the spleen, in the peripheral nervous system which are expressed by a subpopulation of microglia in the human cerebellum [6]. They are responsible for the anti-inflammatory and perhaps other therapeutic effects in animal models [5].

These receptors are involved in a huge range of physic-pathological processes including

neurotransmitter release, suppress formation and storage of long-term and short-term memory [7] [8], energy balance and metabolism [9], stress response [10], exploration, social behavior, anxiety [11], regulation of pain perception, cardiovascular and gastrointestinal functions [12]. Moreover, colonic inflammation enhances the antinociceptive action of CB1 and CB2 receptor agonists, and activates an endogenous CB1 receptor mediated antinociceptive pathway [13].Distinction between these receptors depends upon the differences in their predicted amino acid sequences, signaling mechanisms, tissue distribution and sensitivity to certain potent agonists and antagonists that show important selectivity for one or the other receptor type [14].

Cannabinoid receptors CB1 and CB2 show 48% amino acid sequence identity [15]. Both receptor types are coupled through G proteins which activate both adenylyl cyclase and mitogen-activated protein kinase. CB1 receptors are also coupled through G proteins, activating several types of calcium and potassium channels [16]. These receptors are firstly present on central and peripheral neurons. One of their functions is to prevent neurotransmitter release. In fact, it is probable that endogenous CB1 agonists act as retrograde synaptic messengers [17]. It has been revealed that cannabinoids receptors are found in presynaptic terminals of both inhibitory and excitatory neurons [18]. Although the release of most of neurotransmitters happen between presynaptic and post synaptic terminal, the signal transduction of CB1 follows retrograde pathway, as shown in fig1.

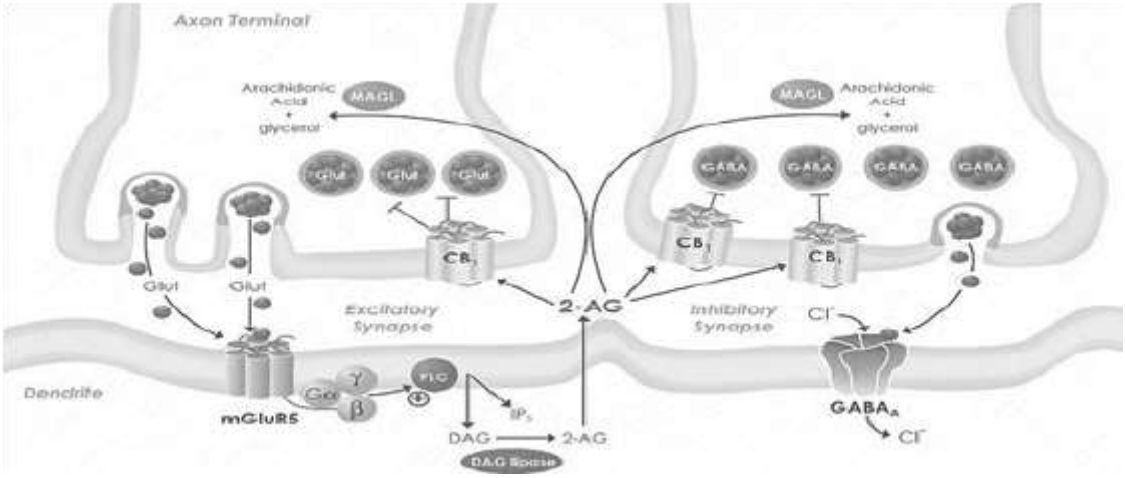

Figure 1. Retrograde signaling by the endocannabinoid 2-AG at the neuronal synapse

2-Arachidonoyl glycerol (2-AG) is an endogenous cannabinoid, affecting both excitatory synapse and inhibitory synapse by releasing GABA and glutamate respectively from excitatory synapse and inhibitory synapse. The entrance of CL- happens on the right side while exocytosis of Ca2+ occurs from endoplasmic reticulum. As you can see in the picture, CB1 receptors are G-protein coupled receptors which are involved in retrograde signaling and are extensively distributed on presynaptic terminals in brain regions, involved in cognition, particularly learning and memory [19] [20]. Glutamate, dopamine and acetylcholine are three neurotransmitter systems that are thought to play a role in mediating the memory effects of cannabis [21]. The previous data strongly indicate that excitatory synaptic transmission in forebrain areas is directly modulated by CB1 expressed on presynaptic axon terminals originating from glutamatergic neurons [22]. CB1 and CB2 receptors in their inactive state are collaborated with inactive heterodimer G-proteins that include GDP-bound α subunit with β and γ subunits. Stimulation by an agonist induces conformation changes which start a series of intracellular signal cascades by catalyzing an exchange of GTP for GDP on α

subunit and trigger the disassociation of the α subunit from the βγ subunits. Signal transduction is finished by the hydrolysis of GTP to GDP [23]. The classical sample of agonist-induced GPCR mediated signal transduction involves agonist-induced dissociation of the G-proteins from the GPCR and subsequent G-protein regulation of secondary messengers [24], [25]. Both CB1 and CB2 receptors are coupled with the Gαi/o G-protein. The human brain has more cannabinoid receptors than any other G protein-coupled receptor (GPCR) type [18]. Activation of these cannabinoid receptors causes inhibition of adenylate cyclase (AC) and activation of mitogen-activated protein kinases (MAPK) such as ERK1/2, c-Jun N-terminal kinase (JNK), and p38 MAPK [26]. Recent finding has showed that neuronal CB1 receptors can regulate ERK1/2 signaling through Gi/o and multiple tyrosine kinase receptors [27] and neuronal CB2 receptors can modulate ERK1/2 signaling through β-arrestin2 [28]. In fact, recent studies have found that β-Arrestins can recruit proteins such as ERK1/2 to GPCR to form scaffolding complexes which can regulate the activation of signaling cascades [24], [25]. CB2 receptor transactivation of tyrosine kinase receptors has not been investigated. CB1 receptors can inhibit N- and P/R- type calcium channels, stimulate G-protein coupled inside revise potassium (GIRK) channels and increase activation of A-type potassium channels (Figure 2).

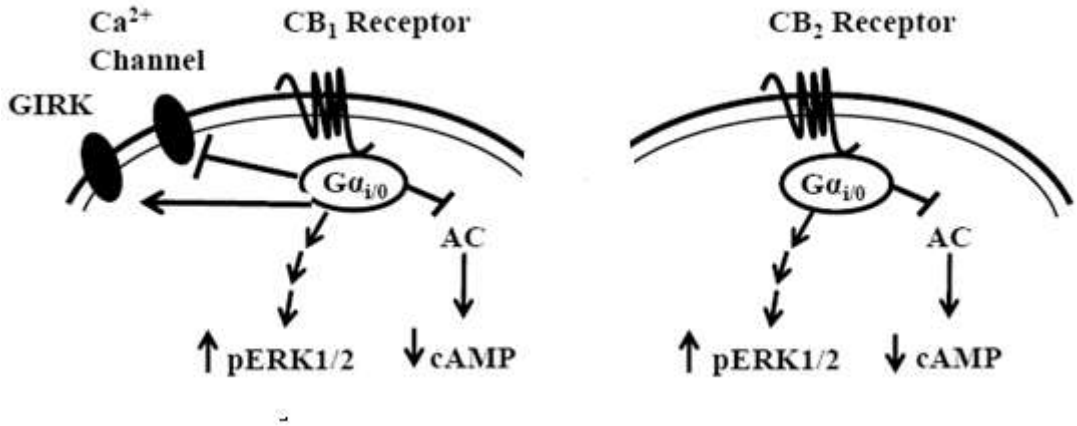

Figure 2. Signaling mechanisms associated with CB1 and CB2 receptors

Some endogenous ligands for CB1 and CB2 receptors have also been presented to bind and activate transient receptor-potential vanilloid receptor 1 (TRPV1) and peroxisome proliferator-activated receptors γ (PPARγ) [29], [30]. Transient receptor -potential channels (TRP channels) are a set of ion channels that is mostly found on the plasma membrane of animal cell. These ion channels are basically non-selectively permeable to cations, including sodium, calcium and magnesium. About 28 TRP channels have been identified that have collaboration with each other [31]. They are divided into two groups: Group 1 consists of TRPC (“C" for canonical), TRPV ("V" for vanilloid), TRPM ("M" for melastatin), TRPN and TRPA. In group 2, we have TRPP ("P" for polycystic) and TRPML ("ML" for mucolipin). Most of them mediate several sensations such as the sensations of pain, heat, warmth or coldness, pressure and vision. In the body, some TRP channels act like microscopic thermometers and are used in animals hot or cold feeling sensations [32]. Some food such as garlic (allicin), chili pepper (capsaicin), and wasabi (allyl isothiocyanate) can stimulate their activation; yet, others are activated by molecules found in cannabis (i.e. THC, CBD and CBN). A common point about TRP channels is their activation or modulation by phosphatidylinositol signal transduction pathways. As it can be seen in fig. 3, the channel subunits have six tranmembrane domains which provide tetramers to form non-selective cationic channels, which in turn allow for the influx of calcium ions into cells. Three subgroups comprise the TRP channel family which answers to painful stimuli. Other

suggested functions consist of repletion of intracellular calcium stores, receptor-mediated excitation and modulation of the cell cycle [33]. The mammalian proteins which show the greatest sequence to Drosophila TRP belong to the TRPC subfamily. These proteins share 30%–47% amino acid homology over the N-terminal ~800 amino acids, that encompass 3–4 ankyrin repeats, the six transmembrane segments, and a widely conserved 25 amino acid segment which are referred to as the TRP domain (Figure 3).

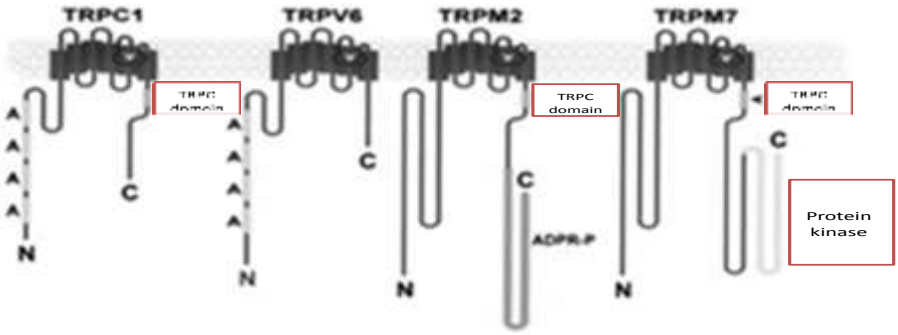

Figure 3. Members of the TRPC, TRPV and TRPM subfamilies

The ankyrin repeats (A) TRP domain, protein kinase domains and ADP-ribose pyro phosphatase (ADPR-P) domains are indicated. The TRPV proteins also consist of 3–4 ankyrin repeats but lack the TRP domain, whereas the TRPM proteins contain a TRP domain, but without any ankyrin repeats [34]. All TRP channels, except for TRPM4 and TRPM5 are cation channels which let Ca2+ influx. However, there is a daunting variety in the way of activation and regulation in each case. Special TRP channels may be activated by different stimuli such as vasoactive agents, oxidative stress, mechanical stimuli and heat. TRP channels may change these stimuli into changes in the cytosolic Ca2+, finally coupled with different vascular responses. Studies have been performed to purpose the involvement of at least the following TRP channels in vascular function: TRPC1, TRPC4, TRPC6 and TRPV1 to control vascular permeability; TRPC4, TRPV1 and TRPV4 in the regulation of vascular tone; TRPC4 in hypoxia-induced vascular remodeling; and TRPC3, TRPC4 and TRPM2 in oxidative stress–induced responses. In spite of the huge amount of available data, the functional role of many endothelial TRP channels still needs more researches (35). TRPC channels are a subset of the transient receptor potential (TRP) proteins that have been widely found in mammalian cells. They are considered to be firstly involved in detecting calcium or sodium entry and have broad-ranging functions which take part in modulation of cell proliferation, motility and contraction. The channels do not respond to one stimulator but more are activated or regulated by a multiple of factors which exist as effector in the plasma membrane. Though data is restricted, the lipid profiles are stable with TRPCs that have close relation with phospholipase C and A2 enzymes [36]. Three mammalian TRP channels (TRPC5, TRPM2, TRPA1) are chosen based on our studies interest, new knowledge of the channels, and a clear common role of the channels in cells and tissues to respond to questionable subjects [37]. In the peripheral nervous system, stimuli of temperature, pressure, inflammatory agents and receptor activation affect TRP-mediated responses. In the central nervous system, TRPs participate in neuritis outgrowth, receptor signaling and excitotoxic cell death resulting from anoxia. TRP channels are the main part of cell that allow animals to respond to their environments [38]. TRP channels have finally been considered as one of the most important GPCR effectors. Most of the time the signals from GPCRs to TRPs are regulated via lipid signals [39]. Study about exocytosis of Ca2+ stores has attracted growing attention, triggered

by new discoveries which fill the gap in the chain of reactions which cause activation of store-operated channels and Ca2+ entry. Ca2+ independent phospholipase A2 appears as a target for CIF (Calcium influx factor), and a major factor for the SOCE(store-operated calcium entry) mechanism [40].

As mentioned before, Cannabinoids are a structurally different group of lipophilic molecules which bind to cannabinoid receptors. Properly, endogenous cannabinoids (endocannabinoids) are a set of signaling lipids including amides and esters of long-chain polyunsaturated fatty acids. They are made from lipid precursors in plasma membranes via Ca2+ or G-protein-dependent processes and show cannabinoid-like actions through binding to cannabinoid receptors [41].

CONCLUSION

Before 1980s, it was considered that cannabinoids create their physiological and behavioral effects through nonspecific interaction with cell membranes instead of interacting with specific membrane-bound receptors. The first study on cannabinoid receptors in 1980s helped solve this discussion [42]. The mechanisms of cannabinoid effects present a high variability and may also involve transient receptor potential channels (TRP) and N-type voltage-gated Ca2+ channels. One study showed that the CB 1 receptor antagonist AM251 blocked neuroprotection mediated by WIN while the CB 2 receptor antagonist AM630 didn't have any effects. Injection of the TRPA1 blocker HC-030031 raised the neuroprotective efficiency of high WIN concentrations and the number of degenerating neurons became equal after the application of the most effective WIN dose. On the other hand, the application of TRPA1 agonist icilin or allylisothiocyanate (AITC) caused a stronger neurodegeneration. The injection of TRPV1 blocker 6-iodo-nordihydrocapsaicin did not affect WIN-mediated neuroprotection [43].

These investigations suggested intracellular Ca2+ [Ca2+ (i)] changes induced by Delta(9)-THC, CP55,940 and by low concentrations of virodhamine involve mobilization and subsequent CCE (capacitive Ca2+ entry) mechanisms, while such responses by high virodhamine concentrations involve NCCE (non-CCE) pathways [44]. All in all, these studies suggest that [Ca2+]i alterations induced by Δ9-THC, CP55,940 and by low concentrations of endocannabinoid virodhamine involve mobilization and subsequent CCE mechanisms, while such responses by high virodhamine concentrations involve NCCE pathways [44]. Many of the effects such as modulating numerous sensory sections, homeostatic, and inflammatory reactions are mediated by metabotropic cannabinoid receptors. A new finding has manifested that multiple members of the transient receptor -potential (TRP) ion channel family can act as "ionotropic cannabinoid receptors". On the other hand, many of these same TRP channels are involved in processes that are related to skin such as the initiation of pain, temperature, and itching perception, the maintenance of epidermal homeostasis, the regulation of hair follicles and sebaceous glands, and the modulation of dermatitis. Ionotropic cannabinoid receptors show potentially interesting intentions for the therapeutic use of cannabinoids to treat sensory and dermatological diseases. Furthermore, they show the interactions between neurons and other kinds of cells which are mediated by cutaneous ionotropic cannabinoid receptors [45] .The pattern for endocannabinoid biosynthesis was investigated into MES 23.5 cells which is a neuronal cell line. Researchers realized that AEA biosynthesis in MES 23.5 cells may happen as a result of the activation of TRPC 3/6/7-type channels [46] [47]. They also determined the role of calcium as a 'switch' to activate the synthesis of anandamide and at the same time diminish uptake. Of course, [3H] anandamide uptake was decreased in the presence of Ca2+. Scientists studies propose a mechanism indicative of calcium-modulated activation of anandamide synthesis and simultaneous finishing of the uptake [47]. One study presented that the thermoregulatory effects of cannabinoids is specifically related to endogenous systems and transient receptor potential (TRP) channels [48]. Endocannabinoids are released by large increase of calcium concentration in the postsynaptic neuron that is usually induced by strong depolarization (depolarization-induced suppression of inhibition/excitation, DSI/DSE), strong activation of G protein-coupled receptors (receptor-driven endocannabinoid release,

RER), or at the same time small raise of calcium concentration and weak receptor activation (Ca2+assisted RER, Ca2+-RER) and acts as presynaptic ally to delete neurotransmitter release. Unlike DSE/DSI, Ca-RER can be induced by slight raise of Ca2+ that can be mediated by calcium sources other than the voltage-operated calcium channel (VOCC) like the Endoplasmic reticulum (ER) calcium store or TRPC (Fig4). As it was showed that ER calcium release do not have a role in the induction of Ca2+-RER [49], it is worth investigating the role of TRPC in endocannabinoid release. They studied whether endocannabinoid-induced retrograde signaling is modulated by the TRPC-induced slack current. They infused transient depression of synaptic transmission by PF burst stimulation in current clamp pattern and associative stimulation of parallel fiber (PF) burst and Purkinje cell (PC) depolarization in voltage clamp mode, though neither was influenced by TRPC blockers. This suggests that TRPC mediated slack currents and calcium transients do not have an important role in the endocannabinoid signaling and that calcium sources other than TRPC is needed for Ca2+-RER(receptor-driven endocannabinoid release) [50]. Two major mGluR1(Metabotropic glutamate receptors)-evoked calcium signaling pathways are identified: (1) slow-kinetic inward current carried by TRPC channel that is permeable to Ca2+; (2) IP3-induced calcium release from intracellular calcium store [43]. SKF96365 has been reported to increase intracellular calcium ion levels [51], and inhibit VOCCs[52] [53] especially T-type calcium channels in cerebellar Purkinje cells [54] , potassium channels [55] [56] and calcium ATPase [57] [58].

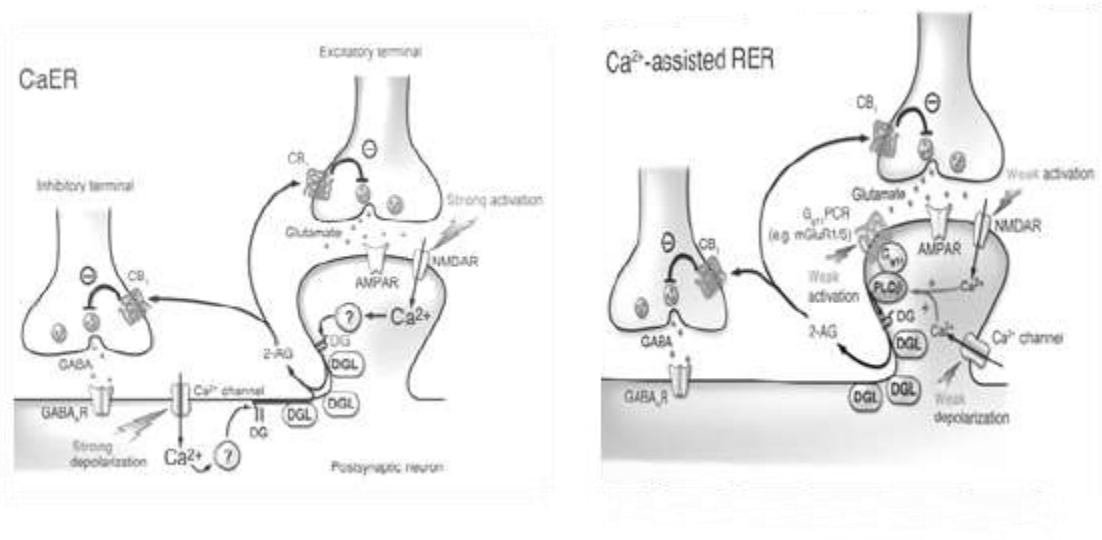

Figure 4. The two terminals has been comparing with each other

Picture on the left shows the terminal has been affected by strong activation whereas on the right side the stimulus is weak activation. Moreover, whole-cell voltage-clamp recordings shows that prenatal WIN (a synthetic cannabinoid agonist) exposure significantly increased Ca2+ channel current talent in origin Purkinje neurons compared to control cells. Furthermore, the data showed here strongly propose that maternal exposure to cannabinoids can cause long-term changes in complex spike finish activity, which in turn may cause changes in neuronal output [58]. The mGluR1-evoked slow EPSC (excitatory postsynaptic conductance) is mediated by the TRPC1 cation channel. TRPC1 is expressed in perisynaptic sites of the cerebellar parallel fibre-Purkinje cell synapse and is physically coworked with mGluR1. Manipulations which interfere with TRPC1 block the mGluR1-evoked slack EPSC in Purkinje cells, though rapid transmission mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors stays uncomplicated. Besides, co-expression of mGluR1 and TRPC1 in a heterologoussystem reconstituted a mGluR1-evoked conduction that closely favors the slow EPSC inPurkinje cells [59].

SKF96365 blocked the endocannabinoid-induced depression effectively. The SKF96365

inhibited endocannabinoid release by decreasing postsynaptic calcium raise through TRPC is insured, though BTP2 (3, 5-bistrifluoromethyl pyrazole) as another blocker of TRP channel did not block RER (rough endoplasmic reticulum), and yet same concentration of BTP2, Ca 2+ release-activated (CRAC) channels could block the most of the slow current. This means the action of SKF96365 doesn't have specific role. BTP2 has been identified to function as a store-operated Ca2+ channel/Ca2+ release-activated Ca2+ channel blocker [60] [61]. However, based on the observation that BTP2 did not influence endocannabinoid release, we propose that TRPC does not associate with endocannabinoid release although 50 μM of BTP2 may be not effective [50]. Moreover, Chang and his colleagues reported that for biosynthesis of endocannabinoid, VOCC(voltage-operated Ca2+ channels) is more considerable and effective than TRPC [50]. For clarifying the data, other findings showed that the intra-medial septum infusion of inhibitors of transient receptor potential TRPC3, TRPC6 and TRPV2, SKF-96365(Ca2+ channel blocker) at the applied dose did not alter memory by itself, while restore amnesia induced by co-administration of ACPA(Arachidonylcyclopropylamide) plus CP94253(5HT1 receptor agonist), GR127935(5HT1 receptor antagonist) , αm5htm(5HT2 receptor agonist) and Cinancerine (5HT2 receptor antagonist) in the medial septum region indicate the involvement of the TRPC3, TRPC6 and TRPV2 channels in this phenomenon [62].

Endogenous cannabinoids are released from postsynaptic neurons and induce transient and long-term decrease of neurotransmitter release through activation of presynaptic cannabinoid receptors. Freedom of endocannabinoid is induced either by raised postsynaptic Ca2+ levels or by activation of Gq/11-coupled receptors. When these two stimuli concur, endocannabinoid release is highly increased which is carried to the Ca2+ dependency of phospholipase Cβ (PLCβ). This Ca2+-helped receptor-driven endocannabinoid release is proposed to associate in different forms of synaptic plasticity, consisting of short-term associative plasticity in the cerebellum and spike-timing-dependent long-term depression in the somatosensory cortex. In these shapes of plasticity, PLCβ seems to act as a concurrence detector of presynaptic and postsynaptic functions [63]. Both pathways, including receptor-operated or store-operated which are active TRPC3 and 6 channels [64] [65] play an important role in cell functions [66] [67]. In the receptor-operated pathway, diacylglycerol which is created by phospholipase C in the G-protein-coupled receptors or receptor tyrosine kinases can directly activate TRPCs to modulate Ca2+ signaling [65]. IP3 receptor–dependent free of intracellular Ca2+ is other direction, that terminates influx of extracellular Ca2+ into the cell by TRPCs [63]. Both the receptor-operated direction and the store-operated pathway play a vital role in cell proliferation [68] . For example, CB1 receptors pair to Gq/11 proteins [69] [70] and its stimulation induce activation of the phospholipase C direction, which results in the activation of several TRP channels [71] . In order to understand how memories can be restored by injection of SKF-96365, some findings indicated that 5-HT motivated a fast raise in intracellular Ca2+, followed by a keep plateau phase that was dependent on extracellular Ca2+. One finding showed that SKF-96365 is a blocker of T-type Ca2+ channels under physiological circumstances. For example, SKF-96365 has been injected to find out the correlation of non-selective cation channels in keeping intracellular Ca2+ levels and rapidly firing within the midbrain dopamine neurons [72], although a study presented that SKF-96365 may not be the optimal select to investigate TRPC in several tissues [54]. SKF 96365 repressed glioblastoma cell growth by enhance [Ca2+] i without considering whether TRPC channels were blocked or not. The effect of SKF 96365 initially resulted from raised reverse operation of the Na+/Ca2+ exchanger (NCX) with an EC50 of 9.79 μM. SKF 96365 got the glioblastoma cells in the S and G2 stages and activated p38-MAPK and JNK, which were all avoided by the Ca2+ chelated BAPTA-AM or EGTA. The expression of NCX in glioblastoma cells was significantly more than the amount in normal human astrocytes. Separation of the NCX1 isoforms reduced the effect of SKF 96365 on glioblastoma cells. It also presents that modulation of the Na+/Ca2+ exchanger is a considerable method to separate Ca2+ homeostasis [73].

Scientists also studied the effect on TRPM8 of: (1) a series of compounds previously presented

to stimulate or antagonize TRPV1 (transient receptor potential channel of vanilloid type 1), and (2) co-stimulation of transiently co-expressed cannabinoid CB1 receptors. Both brominated derivative of cyclic adenosine monophosphate (cAMP) (8-Br-cAMP) and forskolin right-shifted the dose-response curves for the TRPM8-mediated effect of icilin and menthol on intracellular Ca2+. The restrain effects of 8-Br-cAMP and forskolin were reduced by the selective PKA inhibitor (Rp-cAMP-S). Stimulation of human CB1 receptors spontaneously co-expressed in TRPM8-HEK-293 cells also contained TRPM8 response to icilin. Eventually, some TRPV1 agonists and antagonists, (not iodinated antagonists) influenced TRPM8 activation. The endovanilloids/endocannabinoids, anandamide and NADA antagonized TRPM8 activation at submicromolar concentrations [69]. Even though endocannabinoid release is induced by either depolarization or activation of Gq/11-coupled receptors, it is basically increased by the conformity of depolarization and receptor activation. It has been reported that this coincidence is identified by phospholipase Cβ1 (PLCβ1) in hippocampal neurons. By measuring cannabinoid-sensitive synaptic commons, scientists showed that the receptor-driven endocannabinoid release was dependent on physiological levels of [Ca2+] i, and basically raised by depolarization-induced [Ca2+] i increase. By and large, they measured PLC activity in intact neurons through employing exogenous TRPC6 channel as a biosensor for the PLC product diacylglycerol and gain that the receptor-driven PLC activation showed similar [Ca2+]i dependence to that of endocannabinoid release. Neither endocannabinoid release nor PLC activation was influenced by receptor activation in PLCβ1 knockout mice. It can be derived that PLCβ1 serves as a conformity detector through its Ca2+ dependency for endocannabinoid release in hippocampal neurons [74]. Several reports also realize that endocannabinoids can extract effects that are independent of cannabinoid receptors. Therefore, in pharmacologically suitable concentrations, endocannabinoids have been proved to adjust the functional characteristics of voltage-gated ion channels consisting of Ca2+ channels, Na+ channels and various types of K+ channels, and ligand-gated ion channels such as 5-HT3 (5-hydroxytryptamine receptor3), and nicotinic Ach (acetylcholine) receptors. Furthermore, the functional regulation by endocannabinoids of other ion-transporting membrane proteins such as transient potential receptor-class channels, gap junctions, and neurotransmitter transporters have also been reported [75].

In response to the endocannabinoid virodhamine and the non-selective synthetic agonist CP 55,940, the CB2R signaling direction is predominant, terminating to inhibition of adenylyl cyclase and of interleukin-8 (IL-8) release from these cells [76]. Several findings suggest that [Ca2+]i enhances in 16HBE14o- cells by THC, CP 55,940 and low concentrations of virodhamine are implanted via mobilization and subsequent CCE while at the high dose of virodhamine, NCCE (non-capacitive Ca (2+) entry) pathways get involved [44]. Basically, stimulation of CB1R was reported to restrain voltage-operated Ca2+ channels and to activate internally-rectifying K+ channels in neurons and vascular smooth muscle [77] [78] [79]; more recently cannabinoid excited enhances in [Ca2+]i have been realized as well in a various type of cell [41]. Intracellular Ca2+ mobilization and Ca2+ influx happen upon stimulation of DDT1 MF-2 smooth muscle cells with the phytocannabinoid Δ9-tetrahydrocannabinol (THC), which were rather CB1R mediated [80], while excitation with CP 55,940 a predominantly CB1R-mediated Ca2+ influx was perceived [81] [82] . While this compound was independent of intracellular Ca2+ store vacation and subsequent capacitive Ca2+ entry (CCE), arachidonic acid (AA)-operated Ca2+ channels were complicated in this response [81] [82]. Remarkably, THC (not CP 55,940) excited a large Ca2+ influx and a low dose of Ca2+ component in a non-CB1R-, non-CB2R-dependent way in T cells[81] ,engaging Ca2+ influx through the transient receptor potential (TRP) channel TRPC1 [83]. Another member of the TRP superfamily (TRPV1) can be worked out as well by the endocannabinoid anandamide [84]. Generally speaking, these studies want to show that cannabinoid-dependent Ca2+ mobilization from intracellular stores and Ca2+ influx through various entry mechanisms may underlie these intracellular Ca2+ responses. Recently, a powerful THC excited Ca2+ influx was reported in T cells that was also independent of CB1R and CB2R (83), and was

finally sort of mediated by TRPC1 channels [83]. The potent synthetic non-selective CBR agonist CP 55,940 [85] indicated to be less effective than virodhamine in limiting cAMP association in 16HBE14o- cells (human bronchial epithelial cell line) [76], and induced a normal influx of Ca2+, squired by a stable division mobilized from intracellular stores. This influx was restrained by Ni2+ indicating the influence of a CCE mechanism, perhaps mediated by TRPC1 channels. Yet, researchers have found that the CP 55,940-induced increase of intracellular Ca2+ in DDT1 MF-2 cells (cell line in smooth muscle) could be carried to a CB1R-mediated production of AA and subsequent Ca2+ influx predominantly via a non-capacitive Ca2+ entry(NCCE)mechanism [82], while in renal tubular epithelial cells, CP 55,940 is enhanced [Ca2+]i via Ca2+ entry following Ca2+ release from thapsigargin non-competitive inhibitor of the sarco/endoplasmic reticulum Ca ATPase (SERCA) -sensitive pools in a CBR-independent way [86].

Considerably, with virodhamine, the concentration range implanting Ca2+entry in 16HBE14o- cells was several times higher than CB1R-mediated inhibition of c AMP accumulation in these cells [76]. This observation and the steep concentration-dependence of the Ca2+ response of this endocannabinoid strongly propose Ca2+ entry via CBR-independent channel activation. According to this data, CCE as well as the NCCE responses elicited by virodhamine were enhanced rather than reduced in the presence of CB1R or CB2R antagonists. A special actions on cation channels by CBR antagonists have previously been explained [81]. Release of AA and subsequent Ca2+ influx via NCCE could be involved in DDT1 MF-2 cells [80] and not through CB1R or CB2R, since both antagonists were ineffective in working out the virodhamine-induced AA release. These comparisons were done for virodhamine-induced AA release and Ca2+ entry, proposing that they are part of the equal mechanism.

Receptor-independent effects by endocannabinoids may be translated when created by their lipophilic nature [41]. This does not prevent possible physiologically vital effects, such as activation of TRP channels, since the concentrations as used in our current study are physiologically not unusual [87].

Regulation of ion channels is an important component of neurotransmission modulation by endogenous cannabinoid compounds released in response to depolarization and Ca2+mobilizing hormones. However, evidence exists that CB1Rs can also stimulate adenylyl cyclase via Gs, induce receptor-mediated Ca2+ fluxes and stimulate phospholipases in some experimental models [88]. Ca2+ signaling after CB1R stimulation has also been reported while the mechanism of this response is not clear. CB1R stimulation leads to an increase in Ca2+ levels in NG108-15, a hybrid cell line of the mouse neuroblastoma N18TG-2 cells, but not in C9 (Caspase 9) cells [89].

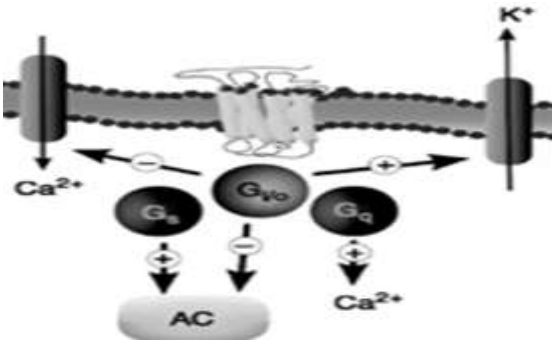

Figure 5 . Main signal transduction pathways of CB1R activation.

This figure shows main signal transduction of CB1R activation and the role of G-protein activation and modulation of ion channels. The main mediators of CB1R are the G proteins of the Gi/o family, which stop adenylyl cyclases in most cells, and moderate ion channels, consisting calcium and potassium ion channels. Moderation of ion channels is a vital component of neurotransmission fluctuation by endogenous cannabinoid compounds rescue in response to

depolarization and Ca2+mobilizing hormones [88]. As mentioned in the fig. 5, the activation of Gs has positive effect on AC while Gi/o inhibits the activation of AC.

CB1Rs can also influence the function of voltage-gated calcium channels. Inhibition of L-type calcium channels by cannabinoid stimulation was detected in cerebral vessels [90], inside retinal bipolar cells [91]. Inhibition of N-type calcium channels was detected in a number of experiments [92]; this effect may have a role in the presynaptic inhibition and retrograde signaling induced by cannabinoids [91]. P/Q-type calcium channels are also negatively modulated by CB1R [94]. Although the expression of DSI (depolarization-induced suppression of inhibition) is presynaptic, DSI does not influence the sensitivity of the postsynaptic membrane to GABA or analysis size of miniature GABAergic events [95]. So DSI is mediated by retrograde signals, initiated by Ca2+ influx into the postsynaptic cell. Nowadays findings suggest that cannabinoids can be that kind of a signal (Fig4) [96]. In hippocampal slices, antagonist of cannabinoid receptor-1 (CB1) which is localized on GABAergic axon terminals, blocks DSI. A synthetic CB1 agonist or natural CB1 ligand can unloose greatly GABAergic transmission. Furthermore, postsynaptic Ca2+ releasing emulates DSI which can be blocked by the CB1 antagonist. So endogenous cannabinoids delivered by the depolarized pyramidal neurons can intercede a transient down-regulation of GABAergic transmission. Physiological functions introduced so far for TRPV1 and TRPV4, including invention of heat and H+ ions and osmolarity sensing [84] may have a vital role in bronchial epithelial signal transduction. Moreover, modulation of TRPV1 channels by the endocannabinoid anandamide is well documented [84]. Ca2+ entry inducted by high dose of virodhamine could be prevented in 16HBE14o- cells by CPZ and RR, blockers of TRPV1 and TRPV1/TRPV4, respectively. MRNA encoding these channels is expressed suggesting their possible influence in these answers. Various mediators involved in airway disease, e.g. growth factors or stimulation of protease-activated receptor 2 have been presented to distaste these channels [97]. Overall, TRPC6 might be involved in this Ca2+ entry process, since this channel mediates NCCE [98] and is highly sensitive to low doses of La3+ and Gd3+ [84]. Accordingly, TRPC6 mRNA was expressed in 16HBE14o- cells. The results show that each TRPC subtype is forcefully prevented by DAG-induced PKC activation, eventuating a likely global feedback control on TRPCs, and that DAG-mediated PKC-independent activation of TRPC channels is widely subtype-specific. The deep yet distinguished control by PKC and DAG of the activation of TRPC channel subtypes is possibly the foundation of a spectrum of regulatory phenotypes of expressed TRPC channels [99]. Studies suggest that WIN triggers a cascade of events: it activates the CB1 receptor and the IP3 signaling pathway stimulates the release of Ca2+ from intracellular stores, raises the cytosolic Ca2+ levels, and inhibits the NMDA-mediated Ca2+ influx and cell death through a process that remains to be determined [100].

Abbreviation:

8-Br-cAMP (8-Bromoadenosine 3', 5'-cyclic monophosphate) TRPM8 (transient receptor potential channel of melastatin type 8)

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