Chapter 5

Therapeutic Potential of Nonpsychoactive Cannabinoids by Targeting at Glycine Receptors

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Additional information is available at the end of the chapter

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Abstract

The glycine receptors (GlyRs) have been identified as major inhibitory neurotransmission receptors in the brain since the mid of last century. Unfortunately, no therapeutic agent has been developed from targeting these receptors. Accumulating evidence has suggested that GlyRs are one primary target for exogenous and endogenous cannabinoids in the central nervous system. Cannabinoids enhance the function of GlyRs in various neurons in the brain. However, this line of research has been largely ignored since little is known about the molecular mechanism and behavioral implication of cannabinoid modulation of GlyRs. Recent studies using various experimental approaches have explored molecular insights into cannabinoid-GlyR interaction and shed light on the molecular basis of nonpsychoactive cannabinoid modulation of GlyRs. Emerging evidence has suggested that cannabinoid modulation of GlyRs can contribute to some of the cannabis-induced therapeutic effects. In this chapter, I discuss recent development in studies of mechanism and therapeutic potential of cannabinoid modulation of GlyR subunits. This research direction shows considerable promise toward the development of novel therapeutic agents acting at defined modulatory sites of GlyRs in the treatment of various chronic pain, neuromotor disorders, and other GlyR deficiency diseases.

Keywords: glycine, receptor, cannabinoid, pain, nonpsychoactive, therapeutics, action of mechanism

Abbreviations

AEA, anandamide; THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; GABA, γ-aminobutyric acid; $I_{Gly,}$ glycine-activated current; TM, transmembrane domain; VTA, ventral tegmental area

1. Molecular composition and tissue distribution

Glycine receptors (GlyRs) belong to the Cys-loop ligand-gated ion channel (LGIC) family, a group of membrane ion channel receptors including γ-aminobutyric acid type A ($GABA_A$), neuronal nicotinic acetylcholine (nACh), 5-$HT_{3,}$ and GlyRs. These receptors are critical for fast synaptic neurotransmission in the central nervous system. GlyRs are known to predominantly mediate fast synaptic inhibitory neurotransmission in the spinal cord and brain stem [1]. To date, four GlyRs subunits have been identified in humans including three α subunits (α1–3) and one β subunit [1]. The α subunits share a high degree of homology in the amino acid sequence (>90%), especially in the large extracellular domain that bears agonist- and antagonist-binding sites. This has posted a challenge to the development of selective ligands for specific GlyR subunits. Two very recent studies have resolved crystal structures of GlyRα1 and α3 subunits with high level of resolution (3.0 A) [2, 3]. These studies have detailed the molecular insights of GlyR-agonist/antagonist interaction and channel-gating dynamics.

It is well established that GlyR β subunits are expressed at postsynaptic sites where they can assemble with the α subunit to form heteromeric functional channels [4]. A cytoskeleton protein, gephyrin, plays a critical role in targeting heteromeric GlyRs at postsynaptic sites. While the GlyRs represent the primary inhibitory neurotransmission in spinal cord, the role of GlyRs in most supraspinal areas has been less clear [5, 6]. Although the β subunit mRNA is relatively abundant in all brain areas at the adult stage, the β subunit protein expression in many brain regions appears very low for an unknown mechanism [5]. Coincidently, glycinergic synaptic transmission in all brain areas except the spinal cord and brain stem are nearly absent at the adult stage [1]. While the α2 subunit represents the dominant form of GlyRs at early development stage, it gives way to the α1 subunit after postnatal stage [7, 8]. The α1β subunits are found to serve as the dominant functional form of GlyRs in the spinal cord and brain stem at the adult stage [9]. The biological switch between the α1 and α2 subunits occurs at a time point of ~postnatal 16–20 days [6, 10]. This timing is consistent with a shift from GABAergic to glycinergic transmission representing the maturity of brain stem and spinal inhibitory systems [6, 10]. In some brain areas such as forebrain and hippocampus, however, the mRNA levels of the α2 subunit remain to be at the steady state from developmental to adult stage [11–15]. Distinct expression of GlyR subunits is consistent with their physiological and pathological roles. For instance, the α3 subunits are restrictively expressed in the superficial layers of the spinal cord dorsal horn, consistent with the involvement of their role in the regulation of nociceptive process [16]. On the other hand, the dominant expression of GlyRα1 subunits in spinal cord and brain stem motor neurons explains well how the functional deficiency in the α1 subunits can cause human hyperekplexia disease, a neuromotor disorder [17, 18].

2. Presynaptic and extrasynaptic GlyRs

While postsynaptic GlyRs have been the major interest of many previous and current studies [1], evidence has emerged to suggest that functional GlyRs are also located at presynaptic terminals and extrasynaptic sites in many brain areas [19–25].

2.1. Presynaptic receptors

Presynaptic GlyRs are first described in calyceal synapses in the medial nucleus of the trapezoid body (MNTB) in rat brainstem [19]. These receptors are thought to play an important role in the modulation of glutamate release [6, 10, 23, 26]. Presynaptic GlyRs have also been reported from studies of other brain areas such as spinal cord, ventral tegmental area (VTA), hippocampus and periaqueductal gray area (PAG), and brain stem hypoglossal nucleus [22, 24, 25, 27, 28].

Presynaptic GlyRs are believed to regulate releases of major neurotransmitters including GABA, DA, and glutamate. All three α [1–3] subunits have been identified to contribute to presynaptic glycinergic activity in different brain regions. While the α2 subunits mediate the facilitation of presynaptic GABAergic transmission in VTA at early development stage [20], the α1 subunits emerge and facilitate glutamate release at presynaptic sites of brain stem calyx in the postnatal stage [6, 26]. A very recent study has shown that the α3 subunits are involved in presynaptic glycine release in brain stem hypoglossal motor neurons [25].

Different from postsynaptic heteromeric GlyRs, presynaptic GlyRs are the likely homomeric α subunits [23, 27, 28]. There are a number of evidence to support this idea. First, the β subunit is always bound with postsynaptic cytoskeleton protein, gephyrin [4, 29]. Second, low concentrations of picrotoxin (PTX) that are found to preferentially inhibit homomeric α GlyRs in vitro selectively alter presynaptic GlyR functionality in the spinal cord and brainstem [23, 27, 30–33]. Finally, this idea is consistent with microscopic observation that the GlyRs at presynaptic terminals of calyceal synapses are composed of homomeric α1 subunits [23]. The presynaptic GlyRs have been the interest of recent research because they disinhibit GABA-mediated synaptic inhibition of VTA dopaminergic neurons [20, 34]. There is evidence suggesting that these receptors are involved in the reward mechanism of drugs of abuse [34].

Presynaptic GlyRs are a potential therapeutic target for the treatment of hyperekplexia disease [26]. A very recent study has shown that streptozotocin-induced diabetic nerve injury caused a decrease in the paw withdrawal latency to mechanical stimuli and reduced the mean frequency of glycinergic miniature inhibitory post-synaptic current (mIPSC) in spinal dorsal horn neurons [35]. This effect is selectively mediated through a presynaptic mechanism because there is no change in miniature inhibitory post-synaptic current rise, decay kinetics, and mean mIPSC amplitude following streptozotocin injection.

2.2. Extrasynaptic GlyRs

Extrasynaptically located GlyRs have been identified in many brain regions, including hippocampus, supraoptic nucleus, and prefrontal cortex (PFC) [13, 36–39]. Functional extrasynaptic GlyRs are likely α homomers because clustering and synaptic targeting of GlyR β subunit requires postsynaptic protein gephyrin [4]. The endogenous agonists of nonsynaptic GlyRs have been postulated to be glycine and taurine [37, 39–41]. While glycine is originated from either synaptic spillover or via release from glia [39, 42], taurine is released from glial cells where the synthesizing enzyme and the transporter for taurine are present [40, 43–45]. Taurine can be released in high levels in response to physiological and pathological

conditions. For instance, taurine is released in response to hypotonic stimulus [46]. There is strong evidence to suggest that ethanol can promote the release of taurine in mesolimbic structure [47–49]. The biological role of tonic activation of extrasynaptic GlyRs remains elusive. Accumulating evidence has suggested that these extrasynaptic GlyRs are likely the target for ethanol modulation in vitro and in vivo [48, 50, 51].

Although our knowledge about presynaptic and extrasynaptic GlyRs is still limited, these receptors could represent emerging targets attractive for future mechanistic and therapeutic studies.

3. GlyR-related disease

3.1. GlyRs in chronic pain

The GlyRs mediate fast synaptic inhibitory neurotransmission and regulate pain formation at spinal level. The α3GlyRs are thought to be the key player involving in spinal antinociceptive process [16, 52].

3.1.1. α3GlyRs in inflammatory pain

α3GlyR knockout mice demonstrate a reduction in pain hypersensitivity in several lines of chronic pain models. Prostaglandin E_2 (PGE_2), which promotes central and peripheral pain sensitization, selectively inhibits α3GlyRs channel activity through the activation of receptor phosphorylation in vitro [16]. Consistent with this, PGE_2 inhibits the glycinergic inhibitory postsynaptic currents in spinal cord slices of wild type (WT), but not in α3GlyRs knockout mice [16]. These α3 knockout mice reduce thermal hyperalgesia induced by the intrathecal injection of PGE_2 [16, 52]. PGE_2 inhibition of the α3GlyRs is attributed to the mechanism of chronic inflammatory pain induced by the intra-plantar injection of complete Freund's adjuvant (CFA) [16, 52]. The α3GlyRs are not involved in all inflammatory pain animal models. While the α3GlyR knockout mice show reduced pain hypersensitivity to spinal PGE_2 injection and CFA- or zymosan-induced peripheral inflammation, these mice do not display altered pain hypersensitivity after the injection of capsaicin, carrageenan, kaolin/carrageenan, or monosodium iodoacetate, which produces rheumatoid and osteoarthritis [53]. A very recent study suggested that glucose at 5 mM can allosterically increase α3GlyR receptor activity, and this interaction between the α3 subunit and sugar may underlie some of the analgesic effects of glucose [54].

3.1.2. α3GlyRs in neuropathic pain

Similarly, the α3GlyRs are also found to play a selective role in some forms of neuropathic and visceral pain models. For instance, there is no significant difference in pain behaviors between α3GlyR knockout mice and wild-type littermates following partial sciatic nerve ligation and colorectal distension [53]. On the other hand, evidence is also available suggesting that these receptors are involved in some forms of neuropathic pain models. For instance,

there is a substantial reduction in the frequency of GlyR-mediated mIPSC of lamina I neurons in rat diabetic neuropathic pain after treatment with streptozotocin in rats [35]. Intrathecal injection of glycine reverses streptozotocin-induced tactile pain hypersensitivity. Moreover, the intrathecal injection of α3GlyR siRNA can reduce the anti-allodynia effect of platelet-activating factor antagonists in three different nerve injury animal models including partial sciatic nerve ligation injury, streptozotocin-induced diabetic nerve injury, and infraorbital nerve injury [55]. Overall, these data indicate that the α3GlyRs are involved in the mechanism of neuropathic pain pathway.

The role of the α2GlyR subunit in antinociception is unclear. A previous study has reported that the mice lacking the α2 subunits showed prolonged mechanical hyperalgesia induced by the peripheral injection of zymosan [56]. The α2 subunits are unlikely to play a role in persistent neuropathic pain (partial sciatic nerve ligation) as the mice lacking either α2 subunit demonstrated a normal nociceptive behavior after spinal nerve injury [56]. So far, the α1GlyRs have not been reported to play any role in pain modulation [57].

Taken together, the α3GlyRs have been the interest of many research interest because of their unique role in nociceptive process and their therapeutic potential in the development of new anti-pain drugs [52, 58–60].

3.2. Alcohol use disorder

Several lines of studies have provided consistent evidence to suggest that GlyRs are one primary target that mediates alcohol-induced behaviors in the brain [61–65]. Activation of VTA GlyRs reduces GABAergic transmission and increases the activity of dopaminergic neurons originated from VTA [20, 34]. GlyRs in the nAc are involved in modulating both basal- and ethanol-induced dopamine output in the same brain region as local injection of strychnine can inhibit ethanol-induced DA release in nAc [48, 66]. There is strong evidence that extrasynaptic GlyRs are the candidate that, at least in part, mediates ethanol-induced dopamine elevation and reward system in nAc [49, 51, 67, 68]. These receptors are likely activated by taurine, which is released from glial cells upon exposure to ethanol [49]. Microinjection of glycine into the VTA reduced the intake of ethanol in rats chronically exposed to ethanol under the intermittent-access and continuous-access procedures and decreased lever-press responding for ethanol under an operant self-administration procedure [69]. VTA microinjection of strychnine completely reversed glycine inhibition of alcohol consumption behaviors, suggesting that GlyRs in the VTA may play a critical role in ethanol self-administration in animals [69]. Consistent with this idea, a recent study in α2- and α3GlyR knockout mice has shown that the depletion of the α2GlyRs decreased ethanol intake and preference in the 24-h two-bottle choice test, whereas the depletion of the α3GlyRs increased ethanol intake and preference in the 24-h intermittent access test [70]. It appears that these GlyR subunits are selectively involved in ethanol consumption behavior but not acute ethanol intoxication-induced behaviors such as motor incoordination, loss of righting reflex, and acoustic startle response [70]. By contrast, mice carrying knock-in mutations in the GlyR α1 subunit alter the behaviors induced by acute ethanol intoxication [71, 72]. Thus, the α2- and α3GlyR subunits are involved in the reward

mechanism of chronic ethanol consumption, while α1GlyR subunits are attributed to acute alcohol intoxicating-induced behaviors.

3.3. Rare genetic disease: hyperekplexia

Human exaggerated startle disease, also known as hyperekplexia, is a rare genetic neurological disorder caused by deficiency in glycinergic neurotransmission [73]. Missense point mutations in the human GlyRs α1 subunit gene disrupt GlyRs function resulting in familial startle disease, an autosomal-dominant disorder [74, 75]. Although rare, this disease is often characterized by an exaggerated startle reaction to sudden, unexpected auditory and tactile stimuli. The most frequently occurring mutation causing human hyperekplexia is the R271Q/L mutation in the α1 subunit [75]. Mice carrying the R271Q mutation exhibit severe neuromotor defects that resemble human hyperekplexia disease [57]. Except for the mutations occurring in the GlyR α1 subunit, point mutations in the GlyR β subunit are also linked to recessive human hyperekplexia disease [76].

4. Cannabinoid interaction with GlyRs

4.1. Cannabinoid potentiation of GlyRs

4.1.1. Allosteric modulation

A previous study from our laboratory has shown first evidence that both exogenous and endogenous cannabinoids such as Δ^9-tetrahydrocannabinol (THC), the principle psychoactive component of marijuana, and endocannabinoid anandamide (AEA) potentiate the amplitude of glycine-activated current (I_{Gly}) in cells expressing homomeric α1 and heteromeric α1β GlyRs and in acutely isolated VTA neurons [77]. The modulation by cannabinoids is not dependent on CB1 receptors. This initial finding has been tested and supported by a number of studies [58, 78–82]. The EC_{50} values for the THC-induced potentiation are 73 nM for human α1GlyRs, 109 nM for human α1β GlyRs expressed in *Xenopus* oocytes, and 320 nM for native GlyRs in rat VTA neurons [83]. THC at low concentrations of 100 and 300 nM can significantly enhance I_{Gly} in HEK-293 cells expressing the α1 and α3 subunits [58]. This concentration range of THC has been found to induce psychotropic and antinociceptive effects in humans [84]. The concentrations of THC in human blood can peak as high as 800 nM for 15 min after a casual marijuana inhalation and stay at 100 nM for 60 min after the smoke. The potentiation of I_{Gly} by either exogenous or endogenous cannabinoids depends on the concentration of glycine [58, 78, 81–83]. Maximal potentiation of GlyRs induced by cannabinoids occurs at the lowest concentration of glycine. With increasing glycine concentrations, the cannabinoid potentiation decreases [83].

4.1.2. Subunit-specific modulation

Both endogenous and exogenous cannabinoids modulate GlyRs in a subunit-specific manner [58, 78, 81, 82]. AEA has been found to produce various effects on I_{Gly} in different

neurons [82, 83, 85]. Among all three GlyRs α subunits (α1, α2, and α3) expressed in HEK-293 cells, the α1 subunit is most sensitive to AEA-induced potentiation [78, 81, 82]. In addition to AEA, other cannabinoids and cannabinoid-mimic lipids such as *N*-arachidonyl-glycine (NA-glycine) exhibit complex action (both potentiation and inhibition) of I_{Gly} in a subunit-specific manner [81]. NA-glycine potentiated the amplitude of I_{Gly} in HEK-293 cells expressing the α1 subunits and inhibits the amplitude of I_{Gly} in HEK-293 cells expressing the α2 and α3 subunits [81]. Similarly, THC has been shown to potentiate GlyRs in a subunit-specific manner expressed in HEK-293 cells [58]. The most significant difference among the three subunits appears to be the efficacy of the THC potentiation [58]. For instance, the magnitudes of the THC (1-μM)-induced potentiation of I_{Gly} are 1156, 1127, and 232% in HEK-293 cells expressing the α1, α3, and α2 subunits, respectively. It should be mentioned that heteromeric α1β1 subunits are less sensitive than their counterpart homomeric α1 receptors to THC-induced potentiation [58, 83]. This is also the case that DH-cannabidiol (CBD), a modified cannabidiol, selectively rescues the function of mutant homomeric α1GlyR subunits [26].

4.2. Molecular mechanisms

4.2.1. Direct interaction and the site

The α1, α2, and α3GlyR subunits are differentially sensitive to THC- and AEA-induced potentiation of I_{Gly} [58]. Molecular analysis has identified single amino acid residue, serine (S), in the TM3, the α1 and α3 subunits critically involved in cannabinoid-GlyR interaction [58, 82]. Substituting the serine (S) at 296 of the α1 subunit and at 307 of the α3 subunit with an alanine (A) converts the α1/α3 subunits from cannabinoid high-sensitive receptors to cannabinoid low-sensitivity receptors. This suggests that S296 is a molecular determinant of cannabinoid potentiation of GlyRs. This idea has gained support from an experiment involving nuclear magnetic resonance (NMR) chemical shift measurement [58]. THC selectively shifts the S296 residue in a concentration-dependent manner in the purified proteins of the full-length four TMs of the human α1 subunit. This hypothesis is further tested by NMR titration and nuclear Overhauser effect spectroscopy (NOESY) analysis of the interaction between cannabidiol and purified α3GlyR protein. The data from these experiments favor a direct interaction of cannabidiol with residue S296 of the GlyR α3 subunit. The analysis of the α3GlyR transmembrane (TM) domains indicates that S296 is located near the intracellular end of the TM3 helix. Direct interaction of CBD with α3GlyR-TM protein is confirmed by the intermolecular NOESY cross-peaks between CBD and the protein. This finding also favors a protein conformational change at S296 in the presence of CBD.

Electrophysiological experiments using mutagenesis analysis indicate a hydrogen-bonding interaction between cannabinoid and S296 residue [58, 86]. Consistent with this idea, chemically the removal of both hydroxyl and oxygen groups from THC abolishes the efficacy of THC in potentiating GlyRs [58]. However, the compound with retaining oxygen group is still potent in potentiating GlyR function but demonstrates significantly reduced binding affinity to CB1 receptors.

4.2.2. A common molecular basis for endogenous and exogenous cannabinoids

It has been proposed that exogenous and endogenous cannabinoids potentiate GlyRs via a common molecular basis. This idea is based on the following evidence. First, the point mutation at the S296 residue in the TM3 is critical for both THC and AEA potentiation of the α1 and α3 subunits [58, 83, 86]. Second, the hydroxyl/oxygen groups are essential for AEA and THC potentiation of GlyRs. Third, the deletion of these groups results in reduction in the efficacy of AEA and THC potentiation. Finally, desoxy-AEA and didesoxy-THC are found to inhibit AEA- and THC-induced potentiation of GlyRs in a similar manner.

5. Therapeutic potential of glycinergic cannabinoids

5.1. Suppression of acute and chronic pain by targeting α3GlyRs

One popular medical benefit from the use of cannabis is its therapeutic relief of chronic pain. There is evidence showing that some of the THC-induced cellular and behavioral effects are independent of CB1 receptors.

5.1.1. α3GlyR dependent

A previous study has shown that the THC-induced analgesic effect in tail-flick reflex (TFR) test remained unchanged in CB1 and CB1-CB2 double-knockout mice, suggesting a different target that may mediate THC analgesia [87]. In view of this observation, we tested whether or not GlyRs are involved in the THC-induced analgesia in the TFR. Both THC and 5-desoxy-THC, a nonpsychoactive cannabinoid, produced a strong analgesic effect in TFR test, and this effect was completely abolished by the administration of strychnine. Cannabinoid-induced analgesic effect was completely absent in the α3GlyR knockout mice. By contrast, the analgesic effect induced by THC remains unchanged in both CB1 and α2GlyR subunit knockout mice [58]. The THC-induced hypothermia did not significantly differ between the α3GlyR knockout and wild-type mice. While 5-desoxy-THC is analgesic, it does not significantly affect locomotor activity and body temperature of mice. Collectively, these data have provided first evidence that α3GlyRs are the target that selectively mediates some of cannabinoid analgesic effects.

The α3GlyRs contribute to the mechanism of chronic inflammatory pain induced by the intraplantar injection of complete Freund's adjuvant [16, 53]. Intrathecal injection of cannabidiol, the major nonpsychoactive component of cannabis, and DH-CBD, a chemically modified CBD, suppress pain hypersensitivity following CFA intra-plantar injection [52]. In addition, DH-CBD significantly attenuates both mechanical and heat-induced pain hypersensitivity following spinal sciatic nerve ligation [52]. Both DH-CBD- and CBD-induced analgesic effects in CFA-induced pain hypersensitivity were significantly reduced in mice lacking the α3 subunits. On the other hand, CBD- and DH-CBD-induced analgesic effects remained unchanged in either CB1 or CB2 knockout mice as compared to their WT littermates.

5.1.2. A correlation between cannabinoid potentiation of I_{Gly} and cannabinoid analgesia

To explore the interrelationship between cannabinoid in vitro and in vivo effects, 11 synthetic cannabinoids structurally similar to CBD were collected and their structural and functional activity was evaluated. Overall, there is a strong correlation between the cannabinoid-induced potentiation of GlyRs and cannabinoid-induced analgesic effect in chronic inflammatory pain in mice. By contrast, there is no such interrelationship between cannabinoid-induced analgesia and cannabinoid-binding affinity for either CB1 or CB2 receptors. Neither cannabinoid-induced potentiation of GlyRs nor cannabinoid-induced analgesia is significantly correlated with cannabinoid-induced psychoactive effects such as hypothermia, hypolocomotion, and incoordination. Collectively, these data suggest that cannabinoids selectively target at α3GlyRs to produce some of the analgesic effects.

5.2. Rescue of hyperekplexia by targeting presynaptic α1GlyRs

Despite overwhelming evidence for functional deficiency of GlyRs in hyperekplexia disease, current therapeutic agents do not target GlyRs [88]. While postsynaptic GlyRs as α/β heteromers attract the most research attention, little is known about the role of presynaptic GlyRs, likely α homomers, in diseases. Therefore, two testable questions emerge. Can DH-CBD treat exaggerated startle response by restoring deficiency in GlyR function? What is the role of presynaptic α1GlyRs in hyperekplexia disease?

5.2.1. Cannabinoid restoration of exaggerated startle response

DH-CBD, in a concentration-dependent manner, rescued the functional deficiency caused by α1R271Q-mutant GlyRs expressed in HEK-293 cells in spinal neurons isolated from α1R271Q-mutant mice [26]. Intraperitoneal injection of DH-CBD at 10–50 mg/kg suppressed both acoustic noise and tactile-induced exaggerated reflex displayed in α1R271Q-mutant mice. Similarly, DH-CBD restored a hind feet-clenching behavior and exaggerated tremor when picked up by the tail demonstrated in these hyperekplexia mice. 9 hyperekplexic-mutant α1GlyRs are classified as cannabinoid-sensitive and -insensitive receptors based on their response to cannabinoid potentiation of I_{Gly} and rescue of startle behavior. A correlational analysis was conducted between DH-CBD potentiation of mutant GlyR function and DH-CBD therapeutic efficacy of 4 hyperekplexia-mutant α1GlyR knock-in mice. The efficacy of DH-CBD rescue of GlyR function is correlated with its restoration of exaggerated startle behaviors. This suggests that DH-CBD restoration of hyperekplexic-mutant receptors and mice appears to be a site/genotype-specific effect.

5.2.2. Therapeutic potential of presynaptic GlyRs

There is strong evidence to suggest that presynaptic GlyRs are a potential therapeutic target of dominant hyperekplexia disease [26]. First, hyperekplexic point mutations in the α1 subunits disrupted the function of homomers more significantly than that of heteromers when expressed in HEK-293 cells. Consistent with this, the hyperekplexic mutation was found to

preferentially impair I_{Gly} recorded in presynaptic terminals but not that from postsynaptic sites of calyceal/MNTB synapses. Second, hyperekplexic-mutant homomers were more sensitive than heteromers to DH-CBD-induced rescue. Third, DH-CBD potentiated presynaptic homomeric α1GlyRs without significantly altering postsynaptic GlyR activity recorded in calyx slices isolated from hyperekplexic-mutant mice. In line with this observation, DH-CBD preferentially restored the diminished frequencies of Gly sIPSCs and mIPSCs, whereas DH-CBD did not significantly alter the amplitudes of Gly sIPSCs and mIPSCs in spinal cord slices from hyperekplexic-mutant mice. PTX at a concentration preferentially blocked DH-CBD rescue of functional deficiency of homomeric-mutant GlyRs but not their heteromeric counterparts. Finally, the observation that DH-CBD increased pre-pulse ratio (PPR) suggests an enhanced probability of glycine release in the spinal cord slice of adult hyperekplexic-mutant mice.

6. Summary

Recent progress as summarized in this chapter has indicated that GlyRs are the target that mediates some of the therapeutic effects of nonpsychoactive cannabinoids in the brain. The widespread medical use of cannabis has been so controversial because the plant can produce both therapeutic and unwanted effects. The cannabinoid-GlyRs interaction opens up a new avenue to separate cannabis-induced analgesic effects from cannabis-induced psychoactive effects [89]. For instance, a very recent study has successfully developed a strategy to discover and develop analgesic drugs based on NMR structure of the GlyR and the critical role of residue S296 in THC potentiation of GlyRs [60]. The therapeutic potential for nonpsychoactive cannabinoids by targeting GlyRs has been implied to hyperekplexia disease. Unlike $GABA_A$-acting agents that are plagued by various side effects [90], DH-CBD does not produce significant psychoactive or sedative effects even at high concentrations [58]. Finally, presynaptic GlyRs are proposed to be an emerging target for the pathological mechanism of hyperekplexia disease. This idea is consistent with recent research trend toward the roles of presynaptic and extrasynaptic GlyRs in various neurological disorders [25, 63, 66, 69, 91, 92]. Thus, like postsynaptic GlyRs, presynaptic and extrasynaptic GlyRs should emerge as therapeutic targets for nonpsychoactive cannabinoids in the treatment of various neurological diseases with GlyR deficiency.

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