

CBD is a promiscuous modulator of voltage- and ligand-gated ion channels

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After academic research as a neuroscientist and 20 years of commercial ion channel drug discovery, Marc is now a freelance blogger, advisor and consultant for clients worldwide, where he shares his expertise and enthusiasm for all aspects of ion channel screening. He is particularly interested in automated patch clamp, and exploiting the potential of human iPS stem cell assays to facilitate the successful translation of new drugs into the clinic.

Cannabidiol (CBD) is a natural product found in the cannabis plant, alongside the psychoactive compound Δ^9 -THC, and it has been shown to be a promiscuous ligand with pleiotropic actions on a wide range of proteins and their signaling cascades, including many ion channels. Significantly, CBD does not activate CB G-protein coupled metabotropic receptors so these effects likely occur by direct binding to ion channel proteins, although there is also speculation about lipophilic CBD affecting membrane fluidity. Many health claims have been made for CBD, and the relaxation of laws around medicinal and recreational use of cannabis has opened up work on CBD as a neuromodulator and anti-inflammatory for applications in sleep, mood, glaucoma, GI disease, pain and epilepsy, and its commercial sale as a food additive and nutraceutical is now widespread across the globe.

Some may not view CBD as a drug due to its seemingly non-specific effects, as this contradicts the current reductionist model of drug discovery where a compound must be selective for a single protein or gene, pushing CBD into the realm of herbal medicines and nutraceuticals. This approach also makes sense to some as CBD is a natural product, and selling it as such is easier and faster than taking the prolonged and expensive route of traditional drug approval, especially as there is still major resistance to legalization of THC in many countries. Nevertheless, the powerful effects of CBD to reduce neuronal excitability and treat seizures was enough for the UK company GW Pharma (now part of Jazz Pharmaceuticals) to develop CBD as an anti-convulsant, especially for pediatric patients suffering from treatment-resistant and life-altering seizures, eventually winning FDA approval for Epidiolex in 2018. This

clinical success and the commercial buzz around CBD has supported ongoing preclinical work on modulation of ion channels and cellular excitability, which is outlined below.

While the anticonvulsant and analgesic mechanism of CBD is still uncertain, many studies have shown that CBD affects ligand-gated ionotropic nAChR, 5-HT₃, Glycine, GABA_A, Glutamate and TRP receptors (reviewed in refs. 1, 2) and voltage-gated Na_v1.x, Ca_v3.x, K_v7.x and HCN channels involved in peripheral and CNS neuron excitability and synaptic transmission (reviewed by refs. 3 - 5).

Neuronal Na_v1.x channels are inhibited by CBD

CBD at concentrations of 2 - 20 μ M inhibit neuronal Na_v1.1-1.8 channels in a state-dependent manner, decreasing peak currents with moderate potency and favoring stabilization of inactivated states with higher potency. This effect has been seen with TTX-sensitive Na_v1.1, Na_v1.2, Na_v1.3, Na_v1.6 and Na_v1.7 channels expressed in heterologous systems, and against TTX-resistant Na_v1.8 channels in peripheral dorsal root ganglion (DRG) sensory neurons and endogenous inward currents in CNS neurons ⁶⁻⁹ (reviewed in refs. 5, 10). CBD is moderately state-dependent but not particularly selective for any specific Na_v1.x isoform; Ghovanloo et al. (2018) found that CBD inhibited inactivated state current through hNa_v1.1 – Na_v1.7 channels expressed in HEK cells with IC₅₀ values of 1.9 – 3.8 μ M, and 10 fold lower potency for block of resting state channels ⁶, whilst Milligan and collaborators found that CBD inhibited resting state Na_v1.1, Na_v1.2, Na_v1.6 and Na_v1.7 channels with potencies of 11.9 to 18.5 μ M, with Na_v1.7 being the most sensitive isoform ⁸.

In most cases CBD stabilizes the inactivated state(s) of neuronal Na_v1.x channels through hyperpolarizing shifts in channel availability and slowing of the rate of recovery, but the degree of this effect varies across isoforms; shifts in activation are usually smaller and less common across isoforms ^{6,8}. CBD preferentially promotes entry into and stabilization of the slow inactivated state of native DRG TTX-R Na_v1.8 currents with a notably potent affinity of 150 nM, compared to KD values of 600 nM for the fast inactivated state and 5 μ M for resting channels ⁹. Other notable biophysical effects include preferential inhibition by 1 μ M CBD of resurgent and persistent currents carried by wildtype Na_v1.2 and Na_v1.6 channels and epilepsy-associated gain-of-function mutations in Na_v1.2 channels,

whilst persistent currents carried by $\text{Na}_v1.1$ channels associated with Dravet epilepsy were unaffected^{11,12}. The latter also found that CBD reduced peak $\text{Na}_v1.2$ current amplitude at sub-threshold potentials that was attributed to a positive shift in voltage-dependence of activation rather than inactivation. The selective inhibition of persistent and resurgent currents of some CNS Na_v channels associated with seizures by CBD may thus offer superior functional selectivity over current state-dependent anti-convulsants, and a wider therapeutic window with fewer side-effects in epileptic patients.

Inhibition of heterologously expressed TTX-S and TTX-R Na_v channels found in sensory neurons is assumed to contribute to the analgesic actions of CBD, but few studies have confirmed this by recording effects on neuronal firing in native tissue. An excellent recent example showed that 0.5 - 2 μM CBD inhibited mouse DRG neuronal excitability by reducing repetitive action potential firing, and also reduced action potential height and widened their duration⁹. Concentrations of 2 - 5 μM CBD inhibited both TTX-S (presumably $\text{Na}_v1.7 > \text{Na}_v1.6 > \text{Na}_v1.2 > \text{Na}_v1.3$) and TTX-R (mostly $\text{Na}_v1.8$) currents in a tonic and use-dependent manner with similar potency, which is significant as both types of channel contribute to DRG action potential firing in different ways and their inhibition could combine to profoundly reduce nociceptor excitability and create analgesia. It will be interesting to see if CBD has any effects against gain-of-function painful channelopathy mutations

(reviewed in ref. 13) in $\text{Na}_v1.7$ and $\text{Na}_v1.8$ channels, as most researchers have focused on pharmacogenetics of common local anesthetic and anti-convulsant analgesics.

Similarly, inhibition of CNS TTX-S Na_v channels is assumed to drive the anti-convulsant effects of CBD, although this may be a simplistic theory as many in the field have noted that therapeutic concentrations of CBD, especially in the brain, can fall below the IC_{50} for inhibition of central Na_v isoforms, and many other important ion channels in the brain are also affected by CBD. However, CBD can alter CNS neuronal excitability *in vitro* at similar concentrations that inhibit $\text{Na}_v1.x$ channels. For example, Hill et al. (2014) showed that a relatively high CBD concentration of 10 μM was required to inhibit native $\text{Na}_v1.x$ currents in mouse cortical neurons, and also reduced action potential duration and repetitive firing in rat CA1 hippocampal neurons⁷. Also, Patel et al. (2016) found that while 1 μM CBD did not inhibit peak Na_v currents in mouse striatal neurons, there was a reduction in resurgent and persistent currents, presumably carried by $\text{Na}_v1.6$ channels bound to endogenous $\beta 4$ subunits, and CBD reduced the amplitude of single action potentials and decreased neuronal excitability through elevated firing threshold and a reduction in repetitive firing¹¹. Finally, Ghovanloo et al. (2018) showed that 1 μM CBD reduced inward $\text{Na}_v1.x$ currents in GABA-ergic iPSC CNS neurons, and produced similar effects on action potential firing in an *in silico* cortical neuron model to those seen above⁶.

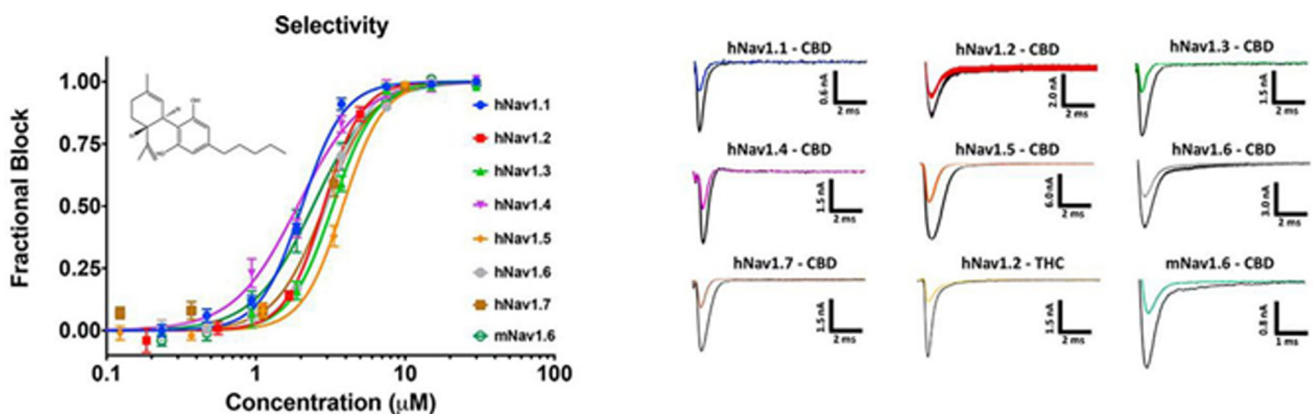


Figure 1 Non-selective inhibition of $\text{Na}_v1.x$ channels by CBD (Ghovanloo et al., 2018: CC BY license), Left: CBD dose-dependently inhibits human $\text{Na}_v1.1$ - $\text{Na}_v1.7$ and mouse $\text{Na}_v1.6$ channels with similar μM potency. Right: Current traces for each $\text{Na}_v1.x$ isoform exposed to a concentration of CBD closest to its IC_{50} value.

Other neuronal voltage-gated ion channels are modulated by CBD

Importantly, other neuronal voltage-gated ion channels are also inhibited in a state-dependent manner by CBD with varying potencies, and these may contribute to its analgesic and anti-seizure efficacy. A recent paper showed that HCN1 channels are modulated by cannabinoids, with CBD stimulating and Δ^9 -THC inhibiting hyperpolarization-activated current independently of CB receptors, albeit with relatively low IC_{50} values of 20 - 30 μM ¹⁴. Neither cannabinoid affected HCN1 channel voltage-dependence or gating kinetics, and initial experiments to assess the role of membrane fluidity were inconclusive. Australian researchers showed that both CBD and Δ^9 -THC inhibited $Ca_v3.x$ channels in HEK cells and mouse sensory neurons, favoring $Ca_v3.1$ and $Ca_v3.2$ channels over $Ca_v3.3$ ¹⁵. The effect occurred in a state-dependent manner by stabilizing the inactivated state, but unlike Δ^9 -THC CBD did not affect gating kinetics or activation voltage-dependence. Significantly, the potency of CBD inhibition of $Ca_v3.x$ channels increased 10 fold to 100 nM against channels evoked from an inactivated holding potential close to the resting potential of DRG neurons, making this another example of CBD's potent preference for inactivated channels. Similarly, a search for novel activators of the M-current carried by $K_v7.2/7.3$ channels discovered CBD to be a very potent ligand, increasing current size by shifting the voltage-dependence of activation to hyperpolarizing potentials with 30 - 300 nM potency¹⁶. The effect was seen in heterologous cells expressing human $K_v7.x$ channels as well as in rodent peripheral ganglion and CNS neurons, where CBD would presumably decrease action potential firing and neuronal excitability consistent with its action as an analgesic and anti-convulsant and analgesic in a similar fashion to the archetypal KCNQ modulator retigabine.

Skeletal and cardiac muscle ion channels are inhibited by CBD

Two groups reported that CBD inhibited $Na_v1.4$ channels in a state-dependent manner but with relatively low potency, exhibiting an IC_{50} for the resting state of heterologously expressed h $Na_v1.4$ channels of ~10 - 100 μM ^{17,18} which increased to 1 - 4 μM as the membrane was depolarized above the resting potential of skeletal muscles. CBD did not significantly affect the voltage-dependence of activation, and there was little evidence for any use-dependence.

Stabilization of the inactivated state of $Na_v1.4$ channels by CBD varied across these studies, with one group showing an effect of 1-2 μM CBD to shift fast inactivation, and the other failing to see this effect with 3 - 100 μM CBD but noting a strong preference for the slow inactivated state with IC_{50} values of 5 - 10 μM and a KD of 1.2 μM ¹⁸, and both publications showed that CBD slowed recovery from inactivation. Ghovanloo et al. (2021) extended this work to show that 1 μM CBD could also inhibit inactivated state and persistent currents through P1158S gain-of-function mutant $Na_v1.4$ channels associated with myotonia and hypokalaemic periodic paralysis, and reduced myotonic action potential amplitude, repetitive firing and after-depolarization and hypoPP depolarization block in an *in silico* skeletal muscle biophysical model, suggesting that CBD could be helpful in patients suffering from some $Na_v1.4$ channelopathies¹⁷.

CBD also modulates native cardiac ion channels and prolongs the action potential duration and ECG QT interval in animal preparations, suggesting there may be a cardiac safety risk with CBD at concentrations above 2 - 5 μM when repolarization reserve is weakened or drug metabolism impaired¹⁹. CBD inhibited hERG channels at 2 μM and the delayed rectifier I_{Kr} current at 6.5 μM , as well as late $Na_v1.5$ and L-type $Ca_v1.x$ inward currents at 10 μM in native cardiomyocytes, similar to CBD effects on neuronal isoforms of these ion channels. $Na_v1.5$ channels appear to be slightly less sensitive to inhibition by CBD than neuronal isoforms; Ghovanloo et al. (2018) reported an IC_{50} of 3.8 μM ⁶, whilst Fouda et al. (2020) found that 5 μM inhibits heterologous h $Na_v1.5$ currents by retarding activation and promoting steady-state fast inactivation²⁰. The latter authors also find that CBD appears to be cardio-protective as it reverses biophysical changes in $Na_v1.5$ activation and inactivation and persistent currents produced by high glucose and inflammatory mediators²¹.

Ligand-gated ionotropic channels are also modulated by CBD

CBD exhibits similar promiscuity to modulate ligand-gated receptor channels as seen against voltage-gated channels, with a net effect to inhibit excitatory and potentiate inhibitory ionotropic receptors which would be expected to decrease neuronal excitability in the CNS and periphery (reviewed by refs. 3, 4, 22). Notably, many of these effects are also elicited

"The promiscuous effects of CBD to decrease the activity of excitatory and increase the activity of inhibitory ion channels and ionotropic receptors alters neuronal and cardiac cell excitability and function, which has driven interest in CBD as a therapeutic ligand for a wide range of human diseases."

Marc Rogers, Founder and Director, Albion Drug Discovery Services Ltd

by endogenous cannabinoids and natural and synthetic phytocannabinoids independently of CB receptors ².

Inhibition of excitatory nAChR and GluRs by CBD

CBD (10 μ M) inhibits a range of nAChRs including α 1 heteromers at the frog neuromuscular junction, α 7 homomers expressed in HEK cells and native choline-evoked currents and norepinephrine release from hippocampal neurons ^{2,23}. CBD appears to be a non-competitive antagonist as nAChR currents are reduced without changes in agonist affinity. CBD is also a potent antagonist of 5HT₃ ionotropic receptors, with inhibition seen at IC₅₀ concentrations of 0.1 - 1 μ M in HEK cells and *Xenopus* oocytes with a similar non-competitive, voltage-independent manner to nAChRs and notable changes in desensitization kinetics ^{2,24}.

Similarly, CBD inhibits GluA heterologous currents (IC₅₀ of 22.5 μ M) and excitatory AMPA receptor-mediated synaptic transmission in hippocampal neurons ²⁵. The *in vitro* inhibitory mechanism include promotion of GluA1 and GluA2 deactivation kinetics and slowed recovery from desensitization, which were more prominent when GluA receptors were co-expressed with TARP γ 8 subunits and required an intact N-terminal domain of the GluRs. Inhibition of GluR synaptic transmission by CBD appeared to be mostly postsynaptic (decreased miniature and evoked current amplitude), although a possible presynaptic effect is indicated by a change in spontaneous current frequency.

CBD potentiates inhibitory GABA_A and GlyRs

Bakas et al. (2017) were the first to find that CBD is a PAM across most inhibitory GABA_A receptor isoforms, with some preference for heteromers containing α 2, β 2 and β 3 subunits (EC₅₀ of 1 - 4 μ M) ²⁶. CBD potentiates sub-threshold receptor activation by shifting the GABA affinity to the left, in a similar fashion to benzodiazepines and neurosteroids but independently of their known binding site. CBD also potentiates extra-synaptic GABA_A receptors containing the δ subunit, albeit with lower potency (EC₅₀ of 23 μ M) than at synaptic isoforms. An extension of these *Xenopus* oocyte studies showed that native GABA_A receptors extracted from the brains of Dravet epilepsy and tuberous sclerosis patients were also potentiated by 2 μ M CBD, independently of the benzodiazepine-binding γ subunit and likely including a component of α 4-containing extra-synaptic GABA_A receptors ²⁷.

CBD also potentiates inhibitory glycine receptors containing α 1 and α 3 subunits typically found in adult spinal cord, brainstem and hippocampus, with a PAM effect seen at EC₅₀ values of 1 - 18 μ M ²⁸ and gating effects including acceleration of activation and slowing of deactivation kinetics as well as a canonical left-shift in glycine affinity ²⁹. It would appear that the CBD binding site involves the S267 and S296 residues in the 2nd and 3rd transmembrane domains of α 1 and α 3 subunits, respectively, based on NMR spectra and molecular dynamic

modeling data that show this pocket is buried deep in the plasma membrane and closely apposed by phospholipids ^{29,30}. *In vivo* analgesia of CBD and structural analogues correlates with their potentiation of α 3 subunits, and is reduced in α 3-/- GlyR knockout mice ²⁹. More recently, the NMR structure of the α 1 hGlyR transmembrane domain was used to virtually screen a library of ~1500 FDA approved drugs, resulting in 7/13 hits that functionally potentiated GlyR currents at 1 μ M and whose efficacy and potency (EC₅₀ values from 0.3 - 3 μ M) were affected by membrane lipids ³¹.

Compared to numerous *in vitro* studies with ionotropic receptors expressed in heterologous systems, there is much less data showing that CBD can modulate synaptic transmission in *ex vivo* neuronal systems, although it is widely assumed that such actions contribute to changes in neuronal excitability and the anti-convulsant and analgesic actions of CBD *in vivo*. No doubt the high lipophilicity and low aqueous solubility of CBD makes such native tissue experiments very challenging, so some have used more stable analogues such as dehydroxycannabinoidiol (DH-CBD) to explore *ex vivo* mechanisms ²⁹.

CBD modulation of TRP channels

Many TRP channels are modulated by endogenous and phytocannabinoids, and this includes CBD which is an agonist at TRPVx and TRPA1 receptors and antagonizes TRPM8 activation at nM - μ M concentrations (reviewed in ref. 1). For example, TRPV2 receptors are sensitive to Δ 9-THC and CBD, with the latter inducing sustained Ca²⁺ influx in heterologous cells and CGRP release from sensory neurons at 3-30 μ M across species ³². CBD (1 - 10 μ M) can also sensitize TRPV1, V2 and V3 receptors to activation by the low potency and non-selective agonist 2-APB, but not to heat, via changes in channel open probability ^{33,34}. However, the potent agonism of CBD at TRPV and TRPA1 receptor channels also results in desensitization as measured by patch clamp electrophysiology ³⁵, so CBD may also act as a functional antagonist in a similar fashion to other desensitizing agonists such as capsaicin and resiniferatoxin which are under development as analgesics.

In summary, the analgesic effects of CBD may be linked to pleiotropic effects on state-dependent Na_v1.x, K_v7.x, Ca_v3.x and ligand-gated GlyR and GABA_A ion channels that influence nociceptive signaling in DRG sensory neurons and spinal pain pathways. Also, CBD inhibition of TRPM8 receptors may be analgesic, alongside agonism of CBD at TRPA1 and TRPVx which can lead to desensitization. Similarly, CBD's anti-epileptic efficacy may be linked to its ability to synergistically inhibit excitatory (Na_v1.x, HCN1, Ca_v3.2, GluR) and activate inhibitory ion channels (K_v7.x, GlyR, GABA_A) which control neuronal excitability and are associated with seizure phenotypes, although the rank order of sensitivity and prime anticonvulsant target of CBD remains unclear. Many of these ion channels are targets of ongoing preclinical and clinical drug discovery programs for epilepsy, depression and other CNS indications, suggesting that CBD may act as a

polypharmacological ligand in contrast to target-selective modulators.

CBD ion channel binding sites and modulatory mechanisms

There has been extensive speculation on the mechanism-of-action for CBD modulation of voltage- and ligand-gated ion channels, falling into two general possibilities which may both contribute to its promiscuity:

- CBD is very lipophilic (LogD of 6.6), so favors interaction with hydrophobic domains of transmembrane proteins where it can affect conformational changes important for their function.
- CBD inserts into the lipid bilayer where it can affect membrane fluidity and tension to regulate mechano-sensitive transmembrane ion channel proteins.

It is still not clear if CBD modulation of various ion channels occurs by the same mechanism, and if this is a specific effect or a non-selective phenomena. Similarly, it is not clear if the same region of the CBD molecule is exerting these effects on different ion channel types, making it very difficult for medicinal chemistry SAR optimization of CBD analogues and the development of small molecule mimetics. Just as we are seeing across all fields of ion channel research and

drug discovery, additional insights on CBD binding sites and mechanism-of-action are being revealed by recent high resolution cryo-EM structures and molecular modeling studies

CBD binding sites on ion channels

It is clear from biophysical and pharmacological data that multiple CBD molecules appear to bind to Nav1.x channels, as Hill slopes greater than 2 are seen for CBD inhibition of inactivated state $\text{Na}_v1.1$ - 1.7 channels⁶ and resting state $\text{Na}_v1.1$, $\text{Na}_v1.2$, $\text{Na}_v1.6$ and $\text{Na}_v1.7$ channels⁸. Similarly, CBD inhibition of $\text{Na}_v1.4$ channels is reduced but not abolished in local anesthetic (LA) binding site mutants^{6,17}, and CBD is an effective inhibitor but only exhibits weak state-dependence, which both suggest there may be multiple binding sites and mechanisms that affect closed, open and inactivated states of Nav1.x channels. Thus it shouldn't be surprising to find that a recent high resolution cryo-EM structure of the human $\text{Na}_v1.7$ channel showed not one but two CBD binding pockets³⁶. One CBD molecule was visualized within the canonical central pore cavity just beneath the selectivity filter, a site also occupied by classical $\text{Na}_v1.x$ state-dependent blockers such as carbamazepine, lacosamide and vixotrigine, and which lies adjacent to a domain I/IV subunit interface fenestration that is thought to allow compound access to the central pore and/or co-ordinate large head groups of small molecule inhibitors. For example, molecular dynamic modeling of $\text{Na}_v1.4$ channels

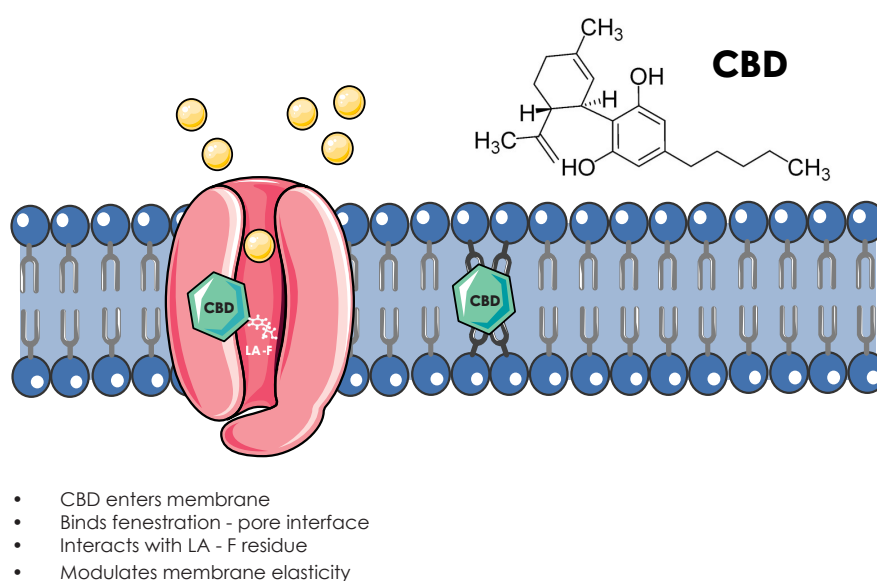


Figure 2 Potential binding sites and mechanism of CBD modulation of $\text{Na}_v1.x$ channels Schematic shows that after CBD enters the plasma membrane lipid bilayer, it can modulate $\text{Na}_v1.x$ channels by i) binding to specific sites on the channel protein, possibly gaining access via lateral fenestrations, and ii) by altering membrane lipid elasticity which can affect channel biophysics. (Figure 2 has been created with Servier Medical Art, licensed under CC BY 4.0.)

based on the cockroach cryo-EM structure showed that mutations in this fenestration prevented CBD access to the pore from the lipid membrane, and abolished CBD inhibition of peak, but not inactivated, $\text{Na}_v1.4$ currents^{6,17}. Interestingly, CBD adopts a similar binding pose near the fenestration of $\text{Na}_v1.7$ channels and rTRPV2 receptors (see below), with the cyclohexene ring pointing towards the central cavity and the n-pentyl tail exposed to the lipid membrane. The 2nd CBD binding site is novel, and sits adjacent to the classical LA binding site but outside the bottom of the pore on the S5-S6 linker between domains III and IV, where it mimics LAs by stabilizing fast inactivation but through different residues and conformational changes to the classical IFM motif. The authors confirmed that CBD is a potent and state-dependent blocker of $\text{Na}_v1.7$ channels, with an estimated K_D of 150 nM for the resting state and 50 nM for the inactivated state; mutations in one or the other binding site reduces, but does not abolish, the effect of CBD to reduce $\text{Na}_v1.7$ current amplitude and stabilize inactivation, strongly suggesting that both are functionally important and may be present in other human $\text{Na}_v1.x$ channels.

Affinity of lipophilic CBD for VSDs buried in the plasma membrane may also explain its state-dependent modulation of other voltage-gated ion channels such as HCN, $\text{Ca}_v3.x$ and $\text{K}_v7.2/7.3$, in a similar fashion to aryl and acyl sulphonamide and peptide toxin inhibition of $\text{Na}_v1.7$. Indeed, a recent paper showed 2 molecules of CBD bound to high resolution cryo-EM structures of closed and open $\text{K}_v7.2$ homomeric channels in a hydrophobic cleft between the S5-S6 pore helix regions of domain I and II³⁷. One CBD molecule occupies a conserved pocket near the fenestration formed between domain I S6

helix and the S5-S6 region of domain II (overlapping with the region that the clinical anti-epileptics retigabine and HN37 also occupy, especially Trp236), whilst the 2nd CBD density is seen above this site in a novel, shallow pocket formed between S5 helix and pore helix of domain II and the S1 region of domain III (especially F104 residue); Thr296 in S6 helix of domain I appears to interact with both CBD molecules. One caveat from this study is the use of a KCNQ2 homomeric structure, but the authors confirmed similar voltage-dependent CBD modulation in $\text{K}_v7.2/7.3$ heteromers³⁷. Both putative CBD binding sites were confirmed by mutagenesis studies that showed reduced voltage sensitivity of $\text{K}_v7.2$ current activation, revealing a potential mechanism by which CBD acts as a KCNQ agonist to modulate electro-mechanical coupling between the VSD and pore, as well as induce large conformational changes in the C-terminal domain, promoting PIP2 binding and destabilizing calmodulin binding. Significantly, CBD can act additively to agonist binding to the canonical retigabine fenestration site, demonstrating its importance as a clinical anti-epileptic and analgesic that may be able to augment the efficacy of current treatments, and reduce the high doses that cause side-effects.

CBD is also an agonist at various ligand-gated ionotropic receptors, but the binding sites and mechanism-of-action are still largely unknown and may differ across the different multimeric protein families. Work on this has begun, with high resolution 3.2 Å cryo-EM structures of CBD bound to the rat TRPV2 receptor identifying a novel binding site created by a hydrophobic pocket between the S5 and S6 helices of adjoining domain-swapped subunits, just below and adjacent to the pore helix and buried in the plasma

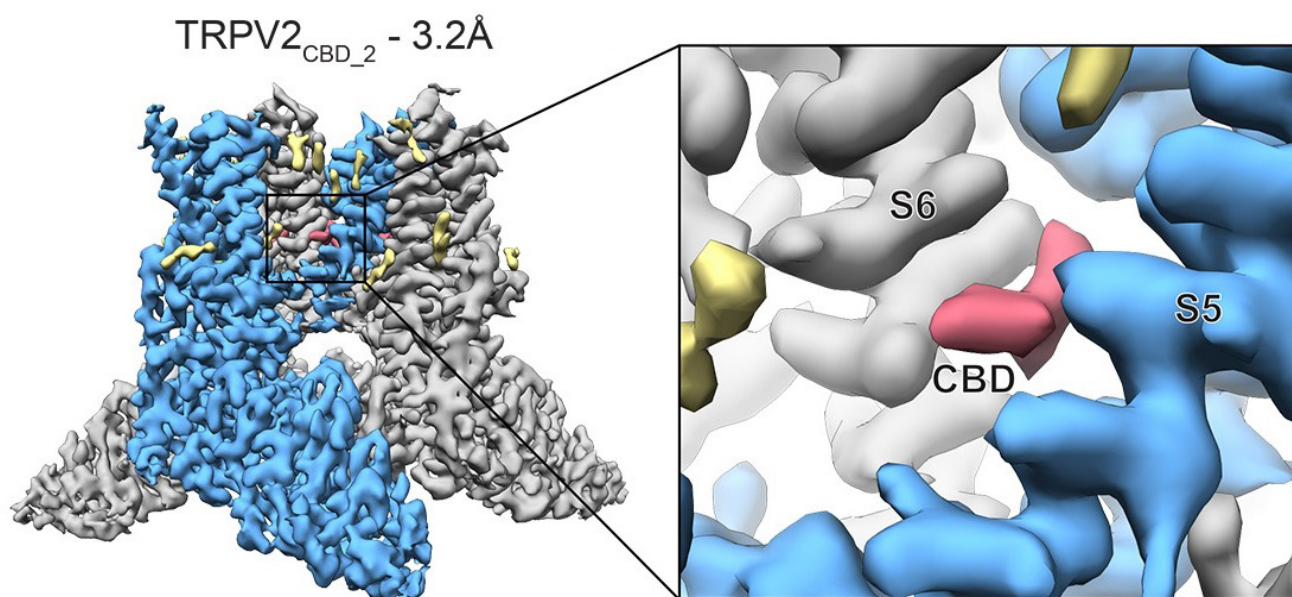


Figure 3 TRPV2 cryo-EM structure with CBD bound to 1st pore helix site (Pumroy et al., 2019; CC license 4.0) Cryo-EM structure of rat TRPV2 shows CBD tightly bound to a pocket between S5 and S6 helices of adjacent subunits lining the pore (shown in grey and blue), underpinning CBD effects on channel activation.

membrane³³. This site is analogous to the fenestrations in $\text{Na}_v1.x$ channels where CBD has also been found to bind, but in the TRPV2 homotetramer there are 4 separate 'window' binding sites. Comparison of apo and ligand-bound TRPV2 structures revealed that CBD may alter receptor gating via large conformational changes in the S4-S5 linker, which could underlie agonistic effects of CBD in other TRPV and TRPA1 receptors as the binding site is conserved across this diverse receptor family. There were also large changes in the cytoplasmic TRP helix and ankyrin repeat domains produced by CBD binding, but their significance for functional effects remains unclear. Significantly, both CBD-bound TRPV2 channel structures appeared to be non-conducting, suggesting they were either in a pre-open or closed-desensitized state (which is also seen in functional experiments after CBD application). A more recent paper confirmed this novel CBD binding site in rat TRPV2, and also revealed a 2nd CBD binding site in the lower pore near the S5 and S6 helices and the S4-S5 linker, close to the canonical vanilloid binding site³⁴. Both CBD agonist binding sites are some distance from the VSD S4 binding pocket for the agonist resiniferatoxin, but are adjacent to the S5 - S4-S5 linker pocket that binds 2-APB agonist in TRPV2 channels³³.

The recent cryo-EM structural data shows that CBD can bind within canonical sites in $\text{Na}_v1.x$, $\text{K}_v7.2$ and TRPV2 channels, and also occupies novel binding pockets on these proteins, suggesting that both mechanisms could contribute to its pleiotropic pharmacological effects. It will be interesting to see where CBD binds to modulate other voltage- and ligand-gated channels, and how this could underlie CBD's clinical efficacy in various disease processes.

CBD may also modulate ion channels through changes in membrane lipid fluidity

The high lipophilicity of CBD (LogD of 6.6, LogP of 6.3) and relatively high μM concentrations required to affect ion channel activity, and its striking polypharmacology, have lead many to suggest that CBD could be modulating transmembrane protein function via non-specific effects on the lipid plasma membrane (reviewed in ref. 3). Whilst recent publication of high resolution cryo-EM structures reveal specific binding sites for CBD on voltage- and ligand-gated channels, it remains possible that CBD could produce additional effects through perturbations in membrane fluidity, especially as many ion channels and receptors exhibit varying degrees of mechano-sensitivity. One collaborative group in the US have explored this aspect of CBD action in some detail, as part of their study of CBD modulation of $\text{Na}_v1.x$ channels (reviewed in ref. 5). Firstly, they noted that counter to expectations of a biomolecular binding mechanism, the effects of CBD on several $\text{Na}_v1.x$ channels was greater at lower temperatures⁶. Secondly, MD simulations clearly showed CBD's preference for inserting within the lipid bilayer, as expected for an amphiphilic compound¹⁷. Thirdly, experimental testing of this lipid hypothesis confirmed that CBD disturbs the lipid membrane (by NMR) and increases lipid order and stiffness

and decreases membrane elasticity (through effects on gramicidin channel flux), opposite to the effects of Triton X-100 detergent. However, the same authors subsequently showed that CBD had the opposite effect to weakly increase gramicidin channel activity (a correlate of membrane elasticity) measured by patch clamp electrophysiology in HEK cells, compared to the previous vesicle flux assay³⁹. Again, the effect of CBD was opposite to the detergent Triton X-100.

Thus, it is clear that CBD can affect lipid membrane properties, and that various voltage- and ligand-gated ion channels may be sensitive to these changes, but that the magnitude and direction of these effects may vary between different proteins and native membrane compositions. Moreover, CBD entry into the lipid bilayer is conducive to accessing ion channel fenestrations and binding to the central pore cavity of $\text{Na}_v1.x$ channels, and in addition changes in membrane fluidity can also produce some of the specific changes in $\text{Na}_v1.x$ channel biophysics (stabilizing inactivation, inhibiting resting current without changes in activation) that are also seen after application of other amphiphiles⁵.

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