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Roles of Transient Receptor Potential Vanilloid Subtype 1 and Cannabinoid Type 1 Receptors in the Brain: Neuroprotection versus Neurotoxicity

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Abstract Transient receptor potential vanilloid subtype 1 (TRPV1), also known as vanilloid receptor 1 (VR1), is a nonselective cation channel that is activated by a variety of ligands, such as exogenous capsaicin (CAP) or endogenous anandamide (AEA), as well as products of lipoxygenases. Cannabinoid type 1 (CB1) receptor belongs to the G protein-coupled receptor superfamily and is activated by cannabinoids such as AEA and exogenous Δ-9-tetrahydrocannabinol (THC). TRPV1 and CB1 receptors are widely expressed in the brain and play many significant roles in various brain regions; however, the issue of whether TRPV1 or CB1 receptors mediate neuroprotection or neurotoxicity remains controversial. Furthermore, functional crosstalk between these two receptors has been recently reported. It is therefore timely to review current knowledge regarding the functions of these two receptors and to consider new directions of investigation on their roles in the brain.

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Abbreviations

TRPV1	Transient receptor potential vanilloid 1
VR1	vanilloid receptor 1
CAP	capsaicin
AEA	anandamide
CB1	cannabinoid type 1
THC	Δ-9-tetrahydrocannabinol
CNS	central nervous system
DA	dopaminergic
SN	substantia nigra
12-HPETE	12-hydroperoxyeicosatetraenoic acid
$[Ca^{2+}]_i$	intracellular Ca^{2+}
CZP	capsazepine
GABA	γ-aminobutyric acid

Introduction

Characteristics of Transient Receptor Potential Vanilloid Subtype 1 (TRPV1)

Capsaicin (CAP), the hot component in Capsicum peppers, is a powerful stimulant for a specific subset of primary sensory neurons. There is a large body of evidence that the excitatory effect of CAP on sensory neurons is caused by its ability to increase the open state of a channel previously defined as the CAP receptor. Cloning of this molecule has revealed that it is a 426 amino-acid protein that was originally termed vanilloid receptor 1 (VR1) [1]. VR1 was found to belong to the transient receptor potential family of ion channels, which comprises three main subclasses: canonical, melastatin, and vanilloid. The CAP-activated TRPV1 belongs to the latter group [2]. TRPV1 has an

oligomeric structure formed by subunits having six transmembrane segments, with a pore domain formed by the fifth and sixth transmembrane regions, and intracellular N and C termini [3, 4]. This receptor is a nonselective cation channel activated by vanilloids [3, 5–9] such as CAP and by endogenous ligands including anandamide (AEA) [10–12] and *N*-arachidonoyl-dopamine [13, 14] as well as lipoxygenase products such as 12-hydroperoxyeicosatetraenoic acid (12-HPETE) [15].

TRPV1 is also opened by noxious heat or extracellular acidic pH [16, 17], suggesting that it mediates both thermal and chemical pain. Paradoxically, CAP not only causes pain but also produces analgesia [18]. This analgesic effect may be caused by the desensitization of nociceptive terminals after prolonged exposure to CAP. Notably, stimulation of TRPV1 receptors in the periaqueductal grey (PAG) produces an analgesic effect by acting on the same rostral ventromedial medulla-projecting neurons that mediate glutamate-induced analgesia in the PAG or by desensitizing the activity of other neurons involved in nociception in this region [19, 20]. Recently, TRPV1-mediated antinociception has been reported in the PAG of the healthy animals, for example, following inhibition of fatty acid amide hydrolase, which enhances the levels of AEA, an endogenous ligand of TRPV1 [21].

Several lines of evidence indicate that TRPV1 is expressed in at least three cellular compartments, namely, the cytoplasmic membrane, endoplasmic reticulum, and cytoplasmic vesicles [22, 23]. TRPV1s in the cytoplasmic membrane are responsible for the TRPV1-mediated effects, such as inward currents or transmitter release, whereas those in the cytoplasmic vesicles seem to serve as a reserve that can be quickly translocated to the cytoplasmic membrane, for example following the activation of protein kinase C [23]. The role of TRPV1 in the endoplasmic reticulum, however, is not clear. The finding that activation of these receptors by CAP or its ultrapotent analogue, resiniferatoxin [24], evokes Ca^{2+} mobilization from intracellular stores shows that these receptors are also functional, and they may be involved in the regulation of Ca^{2+} homeostasis [25, 26].

TRPV1 mRNA is highly expressed in a subset of primary sensory neurons that respond to chemical, mechanical, and thermal stimuli and are classified as polymodal nociceptors. TRPV1 mRNA is also expressed in diverse areas of the central nervous system (CNS), including the limbic system (e.g., hippocampus, central amygdala, and both medial and lateral habenula), striatum, hypothalamus, centromedian and paraventricular thalamic nuclei, substantia nigra, reticular formation, locus coeruleus, cerebellum, and inferior olive [27, 28]. There is also an evidence that TRPV1 mRNA and protein are produced and expressed in nonneuronal cells, including the epithelial cells of the urothelium [29, 30], keratinocytes [31], microglia in the substantia nigra (SN) [32], astrocytes around blood vessels in the brain [33], and some spinal astrocytes [34].

Opening of the TRPV1 channel produces an excitatory effect on terminals of primary sensory neurons, with subsequent depolarization of the nerve fiber, and initiation of an action potential. Ca^{2+} influx into the nerve endings, driven either indirectly by antidromic conduction of the action potential or directly by TRPV1 gating, causes the local release of neuropeptides, including calcitonin gene-related peptide, substance P, and neurokinin A [13, 35, 36]. In addition, activation of TRPV1 increases the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) [10, 37]. Moreover, TRPV1 agonists cause excessive mitochondrial Ca^{2+} loading in neurons, resulting in cell death [9, 37, 38]. On the other hand, recent in vivo studies show that TRPV1 activation protects neurons against excitotoxicity [39] and ischemia [40].

Characteristics of Cannabinoid Type 1 (CB1) Receptor

The CB1 receptor was first cloned as an orphan receptor from a rat cDNA library based on its homology to the bovine substance K receptor [41]. CB1 belongs to the superfamily of G protein-coupled receptors and couples to inhibitory G proteins including Go and Gi [41, 42]. CB1 receptors inhibit adenylyl cyclase and activate mitogen-activated protein kinases [43], presynaptic N- and P/Q-type calcium channels, and inwardly rectifying potassium channels [44].

CB1 receptors were first described on neuronal elements in the rat brain, including postsynaptic sites in specific cell populations, such as pyramidal neurons in the hippocampus [45], and at presynaptic sites in γ-aminobutyric acid (GABA)-ergic interneurons [46, 47] and GABAergic projection neurons [48]. CB1 receptors have also been found in dopaminergic (DA) neurons in the SN of the monkey brain [49]. The widespread distribution of CB1 receptors in the brain has suggested that they play an important role in the CNS. Since these discoveries, a large body of in vivo and in vitro evidence has accumulated supporting the idea that the main function of the endocannabinoid system is in the control of neuronal activity. Furthermore, many of the effects of exogenous and endogenous cannabinoids have been explained by the presence of these receptors in discrete neuronal circuits. Recent studies in rats also show that CB1 receptors are expressed in perivascular astrocytes [50] and microglia [51, 52].

The distribution of the CB1 receptor in the brain is best characterized in the rat. Autoradiographic [53], immunohistochemical [54], and in situ hybridization studies [55] have allowed generation of a detailed map of CB1 localization in the rat CNS. A very recent immunohisto-

chemical study examined the localization of the CB1 receptor in the mouse brain [56]. Some striking features of these receptors include their atypical location during developmental stages (i.e., they are mostly found in fiber-enriched areas) [57] and their abundant and selective presence in discrete anatomical regions and neuronal circuits within the CNS, such as the cortex, hippocampal formation, basal ganglia, and cerebellum [53].

In general, CB1 receptors are known to mediate glutamatergic and GABAergic transmission in the CNS [14, 58–60], but activation of these receptors may produce different effects in different types of neurons. This has been specifically demonstrated in basal ganglia circuits, where the neuronal populations have a variety of phenotypic and electrophysiologic characteristics [48]. For instance, the inhibition of both GABA uptake and release has been reported in striato-efferent neurons [61]. Furthermore, electrophysiological differences among striato-efferent GABAergic projecting neurons and subthalamonigral glutamatergic neurons are thought to support differences in the motor effects of cannabinoids at the basal ganglia level [48, 61]. As in the case of TRPV1, whether agonist stimulation of CB1 receptors mediates neurotoxicity [9, 62–65] or survival [66–72] remains controversial.

Roles of TRPV1 and CB1 Receptor in the Brain

The widespread distribution of TRPV1 in the brain suggests that it plays an important role in the CNS [1]. This is supported by recent evidence of TRPV1-mediated activities in several regions of the rat brain, including the hypothalamus [56, 73], locus coeruleus [74], hippocampus [13, 56], and SN [14, 75]. The TRPV1 agonist CAP has also been reported to cause glutamate release in nigral slices [75] and in the dorsal vagal complex, promoting GABA release [76]. This also enhances motor behavior [77], causes hypokinesia and reduces the activity of nigrostriatal DA neurons [78, 79], and modulates the effects of high levels of L-DOPA on motor activity [80]. Collectively, these findings suggest that TRPV1 plays a functional role in the SN.

CB1 receptors are also expressed in various brain regions [53, 54, 56, 81], and they play important roles in the CNS, such as in the regulation of glutamatergic and GABAergic transmission [14, 58–60]. Endocannabinoids mediate retrograde synaptic signaling by suppressing the release of neurotransmitters, such as glutamate and GABA, suggesting that the CB1 receptor is an important modulator of short- and long-term synaptic plasticity [58–60]. In addition, endocannabinoids can modify the inducibility of nonendocannabinoid-mediated forms of plasticity [60], influence action potential back-propagation and timing and influence oscillations that are involved in higher cognitive functions such as feature binding during learning and memory processes [58]. The CB1 receptor also regulates motor behavior in the basal ganglia by mediating both excitatory and inhibitory inputs to the substantia nigra reticulate and globus pallidus [48].

AEA [10–12, 82] and *N*-arachidonoyl-dopamine [14] activate TRPV1 and CB1 receptors, both of which are expressed in areas of the brain involved in the control of motor behavior, including the SN [27, 83] and striatum [54, 84]. This suggests that the endovanilloid and/or endocannabinoid systems participate in neurodegenerative disorders, including Parkinson's disease [85, 86, 88] and Huntington's disease [87, 88]. Furthermore, there has been growing interest in the ability of TRPV1 [9, 37–40] and CB1 receptors [6, 62–69] to control cell survival and death, particularly in neurons.

Neuroprotection via TRPV1 and the CB1 Receptor

Neuroprotection via TRPV1

TRPV1 has been shown to mediate protection of neurons against excitotoxicity in vivo [39]. In particular, it was shown that arvanil, a synthetic AEA analog, attenuates excitotoxic brain injury and that capsazepine (CZP) reduces the neuroprotective effect of arvanil, indicating that the effect is mediated by TRPV1. The authors proposed that these effects are caused by stimulation of CB1 receptors and desensitization of TRPV1 by its agonist [89]. Furthermore, Pegorini et al. [90] recently reported that CZP can block the ability of the CB1 receptor antagonist rimonabant to protect against global ischemia-induced memory impairment and loss of CA1 hippocampal neurons. This suggests that TRPV1 participates in rimonabant's neuroprotective effects and the effect of CZP is not mediated by CB1 receptors. They also reported that desensitization following activation of TRPV1 can attenuate ischemia-induced hyperlocomotion and memory impairment [40], suggesting that desensitization of TRPV1 protects against excitotoxicity. Moreover, Veldhuis et al. [39] recently reported that TRPV1-mediated neuroprotection against excitotoxicity in vivo is caused by desensitization of TRPV1. Notably, they also showed that CZP alone reduces brain injury caused by excitotoxicity. These neuroprotective actions of CZP imply that TRPV1 mediates neurotoxicity. Although this argues against the possibility that TRPV1 participates in neuroprotection, the neuroprotective effects of CZP may not always be mediated by TRPV1 [91].

Neuroprotection via the CB1 Receptor

The majority of experimental evidence indicates that cannabinoids protect neurons from toxic insults. For

example, cannabinoid receptor agonists protect neurons against excitotoxicity in vivo [69] and in vitro [68, 71], and this neuroprotective effect is blocked by CB1 antagonists. The CB1 receptor has also been found to mediate the neuroprotective effect of the CB1 receptor agonist Δ-9-tetrahydrocannabinol (THC), the principal psychoactive component of marijuana, against *N*-methyl-D-aspartate-induced retinal neurotoxicity [70]. In addition, a recent study in CB1 gene-deleted mice suggested that the endocannabinoid system mediates neuroprotection against kainic acid-induced seizure [45, 92].

Endocannabinoids are neuroprotective molecules that are rapidly released in response to pathological stimuli. For example, under ischemic conditions, the endocannabinoid 2-arachidonylglycerol is produced in the ventral tegmental area, and activation of CB1 receptors by the CB1 receptor agonist WIN55,212-2 protects ventral tegmental area DA neurons in vivo and in vitro [93], suggesting that the CB1 receptor is a good target for the protection of DA neurons against ischemic injury. Notably, these studies also revealed that CB1 agonists mediate neuroprotection against ischemia only at very low concentrations and that higher concentrations can produce neurotoxicity, suggesting that CB1 receptors have biphasic effects on ischemic injury. Additionally, Kim et al. [94] reported that CB1 receptors mediate neuroprotection against oxidative neuronal injury and that this effect depends on the suppression of cyclic AMP signaling through cAMP-dependent protein kinase.

Although this evidence suggests that CB1 receptors mediate neuroprotection, some evidence suggests that cannabinoids have receptor-independent neuroprotective effects. Nagayama et al. [67] showed that the synthetic cannabinoid receptor agonist WIN 55212-2 decreases hippocampal loss following transient global cerebral ischemia via the CB1 receptor, but this compound also protects cultured cerebral cortical neurons from the effects of hypoxia and glucose deprivation by a mechanism independent of the CB1 receptor. Furthermore, THC and nonpsychotropic cannabinoids decrease glutamate toxicity in rat cortical neuronal cultures by a receptor-independent mechanism that is not blocked by CB1 receptor antagonists [66].

G_q/G_{11}-coupled receptors, which mediate synaptic transmission by signaling through phospholipase C, protein kinase C, and intracellular Ca^{2+} mobilization, are involved in the formation of endocannabinoids and neuroprotection against kainic acid-induced seizure. Recently, Wettschureck and colleagues demonstrated that forebrain-specific $G\alpha_q/G\alpha_{11}$ double-knockout mice exhibit increased susceptibility to kainic-acid-induced seizure and impaired activation of neuroprotective mechanisms [95]. This study also showed that endocannabinoid levels are reduced under both basal and excitotoxic conditions and that increased susceptibility to kainic acid can be normalized by increasing the endocannabinoid levels with an endocannabinoid reuptake inhibitor. In contrast, the competitive CB1 receptor antagonist SR141716A did not cause further aggravation of excitotoxicity.

Interestingly, CB1 receptor agonists block the activation of microglia by beta-amyloid peptide in vivo and in vitro and abrogate microglia-mediated neurotoxicity [72]. Furthermore, cannabinoids inhibit lipopolysaccharide-induced cytokine expression in cultured rat microglia [51, 52, 96]. It is therefore likely that both synthetic and endogenous cannabinoids block the activation of microglia, but this effect appears to be independent of the CB1 receptor because the CB1 receptor-selective antagonists SR141716A and AM251 are unable to reverse the inhibition of microglia activation by CB1 agonists [52, 96].

Two types of cannabinoid receptor have been cloned and characterized. Whereas CB1 receptors are ubiquitously expressed in neurons of the CNS, G protein-coupled CB2 receptor have been thought to be absent from the CNS and expressed exclusively on cells and organs of the immune system, and is unrelated to cannabinoid psychoactivity [97]. However, recent data support the expression of CB2 receptors in microglial cells [98], astrocytes and even some neuron subpopulations [97]. It has been shown that CB2 receptors affect the migration [98] and production of cytokines [96] and nitric oxide (NO) [99] by microglial cell cultures in vitro. Another recent study showed that activation of CB2 receptors prevents neurotoxicity by inhibiting beta-amyloid peptide activation of microglia [72], although the CB2 receptor-selective antagonist SR144528 was unable to reverse the ability of cannabinoids to inhibit lipopolysaccharide-induced cytokine mRNA expression in cultured microglia [52, 96].

Neurotoxicity via TRPV1 and the CB1 Receptor

Neurotoxicity via TRPV1

Although TRPV1-mediated cell death has been explored in a variety of cell types, including human neuroblastoma and lymphoma cell lines [100], Jurkat cells [101], VR1-transfected human kidney cells [37], and sensory neurons [37, 38, 102], little is known about TRPV1-mediated neurotoxicity in the CNS. Recently, we showed that CAP and AEA can induce the degeneration of mesencephalic DA neurons in vivo and in vitro by increasing the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and by producing mitochondrial damage via TRPV1 [9]. These results support the hypothesis that CAP and AEA exert neurotoxic effects by activating TRPV1 [27, 83, 103, 104]. Alternatively, TRPV1 agonists may induce TRPV1-independent cell death because CZP, a TRPV1 antagonist, was unable to prevent

AEA-induced death in PC12 cells although they constitutively express endogenous TRPV1 [105].

Neurotoxicity via the CB1 Receptor

THC, a CB1 receptor agonist, induces the degeneration of cultured hippocampal [62) and cortical neurons (64, 65, 106]. This neurotoxicity is accompanied by cytochrome c release and/or caspase-3 activation and is blocked by the receptor-selective antagonist AM251 [106]. In agreement with these findings, our recent report showed that AEA induces cytochrome c release and casapse-3 activation, leading to the degeneration of DA neurons, and that these effects are blocked by AM251 [9]. We also found that the CB1 receptor agonist HU210 induces the death of DA neurons, an effect reversed by AM251.

Interaction Between TRPV1 and the CB1 Receptor

In neuron-enriched mesencephalic cultures, the effects of CAP, AEA, HU210, and the CB1 receptor agonist WIN 55,212-2 on $[Ca^{2+}]_i$ were not observed in Ca^{2+}-free medium containing CZP or AM215 [9]. In addition, in cultured hippocampal neurons [62] and resting T cells [107], THC-induced increases in $[Ca^{2+}]_i$ were not observed in the

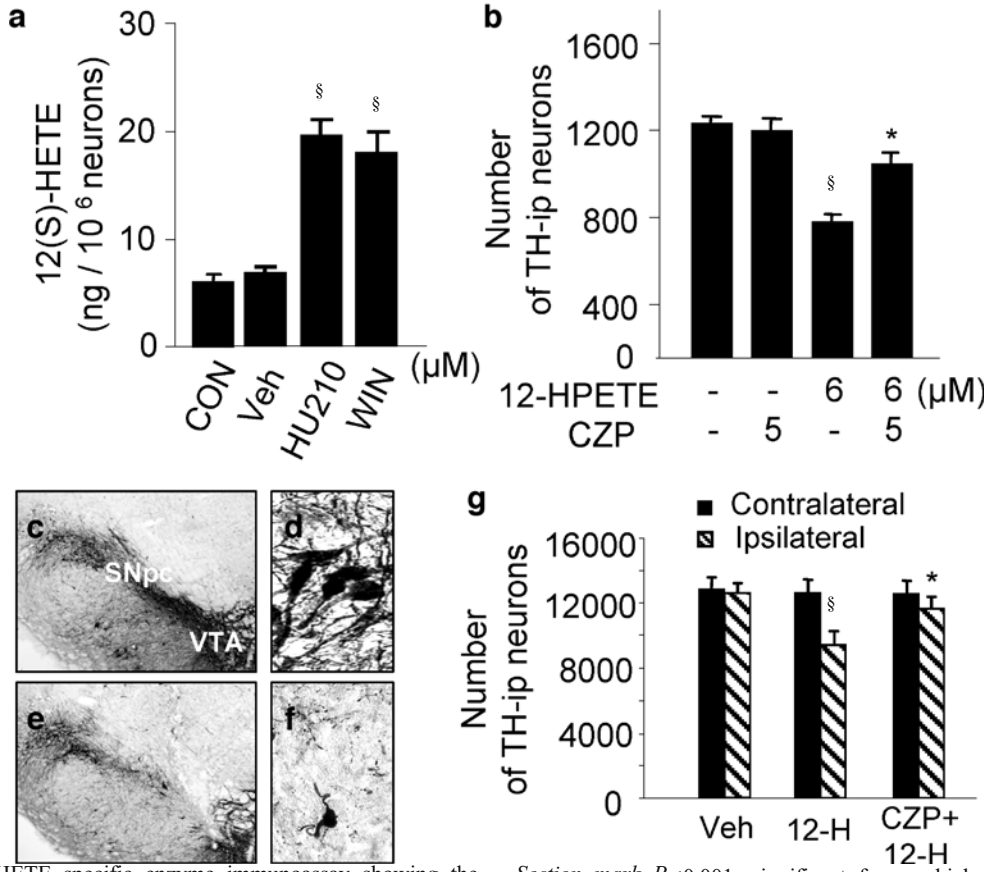

Fig. 1 **a** 12(S)-HETE specific enzyme immunoassay showing the increase of 12-HETE, a downstream metabolite of 12-HPETE, at 30 min after treatment with 3 μM HU210 or 6 μM WIN 55,212-2 (*WIN*) in neuron-enriched rat mesencephalic cultures. CON, non-treated control. Veh, 0.5% DMSO. The statistical significance of differences was assessed using one-way ANOVA, followed by Student-Newman-Keuls analyses. All values represent the mean±SEM of triplicate cultures in four separate plating. *Section mark* $P<0.001$, significant from control (*CON* or *Veh*). **b** 12-HPETE-induced neurotoxicity in neuron-enriched rat mesencephalic cultures treated with 6 μM 12-HPETE for 24 h. Where indicated, cells were pretreated with 5 μM CZP for 5 min before treatment with 12-HPETE and immunostained with antibodies to tyrosine hydroxylase (*TH*) for dopamine neurons. Death of dopaminergic neurons was assessed by counting the number of TH-immunopositive (i.p.) cells. All values represent the mean±SEM of triplicate cultures in four separate plating. *Section mark* $P<0.001$, significant from vehicle; *asterisk* $P<0.01$, significant from 12-HPETE. **c**–**f** For in vivo experiments, animals were administered a unilateral injection of (**e**, **f**) 200 pmol 12-HPETE in 3 μl of PBS containing 14% ethanol or (**c**, **d**) vehicle into the SN and sacrificed 7 days later. Brain tissues were immunostained with antibodies to TH. *SNpc*, substantia nigra pars compacta; *VTA*; ventral tegmental area. **g** Number of TH-i.p. neurons in the SN. Animals receiving intranigral 12-HPETE (200 pmol) with or without administration of CZP (500 pmol) were sacrificed 7 days after injection. Brain tissues were cut and immunostained with antibody to TH. TH-ip neurons were counted using a stereological technique in the whole SN. All values represent the mean±SEM of six to eight animals for each experimental group. *Section mark* $P<0.001$, significant from vehicle or control; *asterisk* $P<0.05$, significant from 12-HPETE (*12-H*; B.K. Jin unpublished data)

absence of extracellular Ca^{2+} or in the presence of SR141716A. Collectively, these results suggest that the TRPV1- and/or CB1 receptor-mediated increases in $[Ca^{2+}]_i$ depend on the influx of extracellular Ca^{2+}. This is also supported by the recent finding that thapsigargin, an inhibitor of the endoplasmic reticulum Ca^{2+} pump, has no effect on the increase in $[Ca^{2+}]_i$ induced by CAP, AEA, or HU210 in neuron-enriched mesencephalic cultures [9].

Although the increase in $[Ca^{2+}]_i$ induced by CAP, AEA, HU210, and WIN 55,212-2 appears to be caused by Ca^{2+} influx, the patterns of Ca^{2+} influx induced by these various agents differ. Similar to previous reports [37, 62, 103], we found that application of CAP to neuron-enriched mesencephalic cultures evokes an initial rapid rise in $[Ca^{2+}]_i$, peaking at 25–30 s, followed by a slow recovery over the next 2 min [9]. In contrast, application of AEA causes a delayed increase in $[Ca^{2+}]_i$ that peaks after 160–170 s and remains elevated for more than 6 min [9]. This may explain the finding that the time required for AEA-induced mitochondrial damage is at least twice that required for CAP-induced damage. Furthermore, addition of HU210 or WIN 55,212-2 causes a delayed increase in $[Ca^{2+}]_i$ that reaches a maximum after 220–240 s and returns to the basal level after 320–330 s. Although the reason for this apparent discrepancy remains unclear, it is likely that AEA activates both TRPV1 and CB1 receptors, and that their interactions modulate Ca^{2+} influx. This is supported by the findings that AEA-induced Ca^{2+} influx is significantly higher in cells coexpressing TRPV1 and CB1 receptors than in cells expressing TRPV1 alone and that this effect is abolished by pretreatment with the CB1 receptor antagonist SR141716A [82].

Unexpectedly, the neurotoxicity of TRPV1 and CB1 receptor agonists is inhibited by pretreatment with AM251 and CZP, respectively. These results agree with the fact that Ca^{2+} influx caused by CAP stimulation of TRPV1 leads to the biosynthesis of AEA, which can activate both TRPV1 and CB1 receptors [78, 108]. In addition, intracellular Ca^{2+} mobilization caused by the purinergic receptor agonist ATP, the muscarinic receptor agonist carbachol, or the sarcoplasmic reticulum ATPase inhibitor thapsigargin promotes the formation of AEA and subsequent TRPV1-dependent Ca^{2+} influx in transfected cells and rat dorsal root ganglia neurons [109]. This production of AEA following Ca^{2+} influx can explain why CAP-induced neuronal death is inhibited by AM251. It is therefore likely that CB1 receptor-mediated Ca^{2+} influx enhances the formation of AEA. Furthermore, a recent report showed that WIN 55,212-2 increases AEA biosynthesis in human embryonic kidney 293 cells by promoting Ca^{2+} release because of CB_1 receptor coupling to $G_{q/11}$ and downstream activation of phospholipase C [94, 109, 110]. These events could also underlie CZP inhibition of HU210-induced neuronal death, although the effect of HU210 on AEA formation is unknown.

We hypothesized that in mesencephalic cultures expressing both TRPV1 and CB1 receptors, there is a functional interaction between these two receptors in response to Ca^{2+} influx that contributes to neuronal cell death [9]. Indeed, we recently found that there is neurotoxic crosstalk between these two receptors mediated by the endogenous TRPV1 agonist 12-HPETE [15], which is produced by 12-lipoxygenase (Figs. 1 and 2; B.K. Jin, unpublished observations). In addition, activation of the CB1 receptor with HU210 and WIN 55,212-2 increased the level of 12 (*S*)-hydroxyeicosatetraenoic acid, a downstream metabolite of 12-HPETE (Fig. 1a), and 12-HPETE induced the death of mesencephalic neurons via activation of TRPV1 in vitro (Fig. 1b) and in vivo (Fig. 1c–g). Furthermore, we found that the ability of HU210 and WIN 55,212-2 to induce neuronal death via TRPV1-mediated Ca^{2+} influx is attenuated by the 12-lipoxygenase inhibitor baicalein. Collectively, these results suggest that, in addition to the direct neurotoxicity mediated by each receptor, the interaction of the two receptors in response to Ca^{2+} influx and the biosynthesis of 12-HPETE contributes to the death of cultured mesencephalic neurons including DA neurons.

These results may be unrelated to the protective effect of CB1 receptor activation against TRPV1-mediated toxicity.

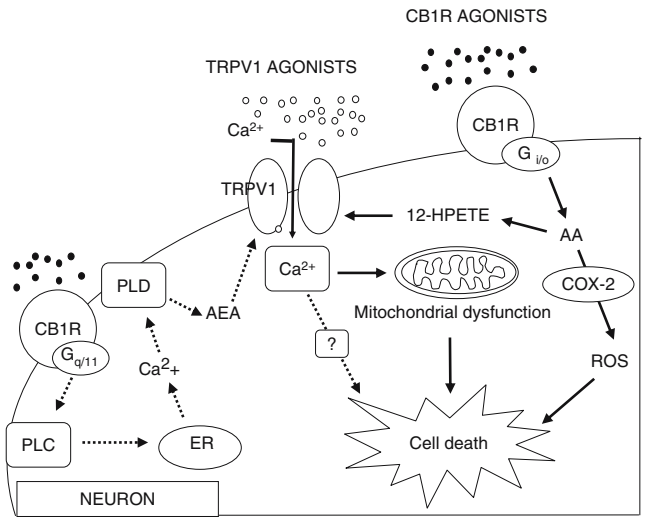

Fig. 2 Proposed schematic diagram showing neurotoxic interaction between TRPV1 and CB1 receptors. The *soild line arrows* indicate that stimulation of G protein-coupled CB1 receptors with its agonists leads to biosynthesis of TRPV1 agonist, 12-HPETE from arachidonic acid (*AA*). And then 12-HPETE can activate TRPV1, resulting in elevation of Ca^{2+} influx via TRPV1, mitochondrial disruption and eventual neuronal cell death. Additionally, activation of CB1 receptors enhances cyclooxygenase-2 (*COX-2*) expression, leading to neuronal cell death in a TRPV1-independent manner. The *dotted line arrows* indicate that stimulation of CB1 receptors coupled to $G_{q/11}$ activates the PLC/IP_3 pathway and results in Ca^{2+} mobilization from the endoplasmic reticulum (ER). The increases in $[Ca^{2+}]_i$ activates the biosynthetic pathway of AEA, activating TRPV1 to gate Ca^{2+} entry into the cytoplasm. *ROS*, reactive oxygen species; *PLC/IP*$_3$, phospholipase C/inositol 1,4,5-triphosphate; *PLD*, phospholipase D

Maccarrone et al. [100] showed that the CB1 receptor antagonist SR141716 enhances AEA-induced death of rat C6 glioma cells, which express both TRPV1 and CB1 receptors. They concluded that this was probably because of the inhibition of TRPV1 by AEA-activated CB1 receptors, resulting in increased Ca^{2+} influx, mitochondrial damage, and cytochrome c release from mitochondria. In contrast, AM251, also a CB1 receptor antagonist, almost completely abolished AEA-activated increases in $[Ca^{2+}]_i$ and prevented mitochondrial damage and cytochrome c release from mitochondria in cultured rat mesencephalic neurons [9]. Thus, the effect of AEA on Ca^{2+} influx appears to be entirely mediated by TRPV1, and its costimulation of CB1 receptors appears to modulate the kinetics of this effect. This is also supported by recent findings that AM251 inhibits neurotoxicity and increases in $[Ca^{2+}]_i$ caused by CB1 agonists such as HU210 [9] and WIN 55,212-2 (B.K. Jin, unpublished data). Alternatively, the apparent discrepancy between the two studies may be as a result of the different cell types (neurons vs glioma cells) employed.

Summary and Conclusions

Accumulating evidence indicates that both TRPV1 and CB1 receptors have many functions in the brain, some of which are neuroprotective and others neurotoxic. This apparent discrepancy is probably caused by the use of different cell types and experimental conditions. Direct ligand binding of these receptors appears to be neurotoxic, whereas the neuroprotective effects are mainly observed under conditions of toxic insult, which could affect the physiological functions of these receptors. In addition to direct neurotoxicity caused by activation of these two receptors, interaction between them may contribute to neurodegeneration. Collectively, these results indicate that the function of TRPV1 and/or CB1 receptors in the CNS may depend on the concentration of locally released endogenous ligands and the specific physiological conditions.

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