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Annual Review of Pharmacology and Toxicology

Synthetic Cannabinoids: A Pharmacological and Toxicological Overview

Rita Roque-Bravo,[1,*] Rafaela Sofia Silva,[1,*] Rui F. Malheiro,[1] Helena Carmo,[1] Félix Carvalho,[1] Diana Dias da Silva,[1,2] and João Pedro Silva[1]

[1]Associate Laboratory i4HB - Institute for Health and Bioeconomy, and UCIBIO, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; email: jpmsilva@ff.up.pt, dsilva@ff.up.pt

[2]Toxicology Research Unit (TOXRUN), University Institute of Health Sciences, IUCS-CESPU, Gandra, Portugal

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*These authors contributed equally to this article

Keywords

designer drugs, drugs of abuse, endocannabinoid system, new psychoactive substances, Spice, substance use disorders

Abstract

Synthetic cannabinoids (SCs) are a chemically diverse group of new psychoactive substances (NPSs) that target the endocannabinoid system, triggering a plethora of actions (e.g., elevated mood sensation, relaxation, appetite stimulation) that resemble, but are more intense than, those induced by cannabis. Although some of these effects have been explored for therapeutic applications, anticipated stronger psychoactive effects than cannabis and reduced risk perception have increased the recreational use of SCs, which have dominated the NPS market in the United States and Europe over the past decade. However, rising SC-related intoxications and deaths represent a major public health concern and embody a major challenge for policy makers.

Here, we review the pharmacology and toxicology of SCs. A thorough characterization of SCs' pharmacodynamics and toxicodynamics is important to better understand the main mechanisms underlying acute and chronic effects of SCs, interpret the clinical/pathological findings related to SC use, and improve SC risk awareness.

1. SYNTHETIC CANNABINOIDS: HIGH POTENCY, MAJOR PUBLIC HEALTH THREAT

Synthetic cannabinoids (SCs): a diverse group of man-made new psychoactive substances designed to mimic, with higher potency, the psychoactive action of Δ^9-THC

New psychoactive substances: novel narcotic/psychotropic drugs, not controlled by the United Nations Drug Control Conventions, that may pose a public health threat comparable to substances listed in those conventions

Endocannabinoid system: a complex signaling network comprising endogenous cannabinoids, enzymes responsible for the synthesis and degradation of endocannabinoids, and cannabinoid receptors

Cannabinoid receptors: cell membrane G protein–coupled receptors belonging to the endocannabinoid system that act as main targets for endogenous and exogenous cannabinoids

Synthetic cannabinoids (SCs) are a structurally diverse group of new psychoactive substances (NPSs) designed to target the endocannabinoid system. These substances have a higher affinity for cannabinoid receptors (CBRs) than Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive substance in cannabis (1, 2), inducing several effects that resemble, but are more intense and short-lived than, those induced by Δ^9-THC (3).

The first SC dates back to 1964 and was a synthetic version of Δ^9-THC designed by Gaoni & Mechoulam (4). The synthesis of other SCs ensued to help understand how the endocannabinoid system regulates critical biological processes, leading to the discovery of CBRs in the 1980s (5). More recently, the search for more potent and legal alternatives to cannabis led to the emergence of several SCs, whose recreational use began in the mid-1990s (6). SCs dominated the NPS market between 2009 and 2019, but the number of new SCs reaching the market per year decreased during 2014–2018 (7). Nevertheless, a total of 209 SCs have been detected in the European Union's member states since 2008, accounting, alongside synthetic cathinones, for about 60% of the total NPS seizures in 2019 (8). In Europe, SCs are most popular among the population aged 15–34 years (comprising adolescents and young adults), with prevalence rates varying between 0.1% (Netherlands) and 1.5% (Latvia) (3). Moreover, the emergence of new structurally different SCs, along with their short half-life in plasma circulation, hinders their monitoring and detection, probably leading to an underestimation of their prevalence (9), and represents a major challenge to policy makers (10).

SCs are usually dissolved in an organic solvent (e.g., acetone, methanol) and sprinkled over plant-based materials (e.g., lemon balm, mint, thyme) (3). They are then sold without any quality or quantity control and in attractive packages with appealing names (e.g., Spice, K2), mostly over the internet (e.g., dark web). These herbal mixtures may contain unknown molecules or other illicit/noxious substances (e.g., bath salts, ecstasy, rodenticides), which may further contribute to their adverse effects (3). SCs are usually smoked (e.g., using a pipe/water pipe or paper or e-cigarettes) but may also be orally ingested as tablets, powders (11), and herbal infusions (12, 13).

The mechanisms underlying SCs' pharmacological action and toxicological effects remain mostly underexplored. This is especially concerning, as SC use has been increasingly associated with severe intoxications and deaths, thus representing a global public health concern (8, 9).

Here, we comprehensively review the most recent updates on the pharmacology and toxicology of SCs, which is important to better interpret the SC use–related clinical/pathological findings as well as to improve SC risk awareness.

2. PHYSICOCHEMICAL PROPERTIES AND ANALYTICAL METHODOLOGIES

In their pure form, SCs are usually described as white or yellowish crystalline fine powders with no odor, low water solubility, and high solubility in nonpolar organic solvents and aliphatic alcohols (e.g., ethanol, methanol, acetone, isooctane, ethyl acetate, acetonitrile) (14, 15).

Their structure may be generally divided into four main elements: the core, tail, linker, and ring/linked group, as depicted in **Figure 1** (14, 16). SCs can be categorized into distinct groups and subgroups depending on their structure (15, 16).

The timely detection of SCs in biological samples can be challenging, thus compromising proper diagnosis of SC-related intoxications. So far, different analytical methods can be used to detect and quantify SCs in different matrices. The gold standard for qualitative analysis of SCs is gas chromatography coupled with mass spectrometry (GC-MS) due to its notorious

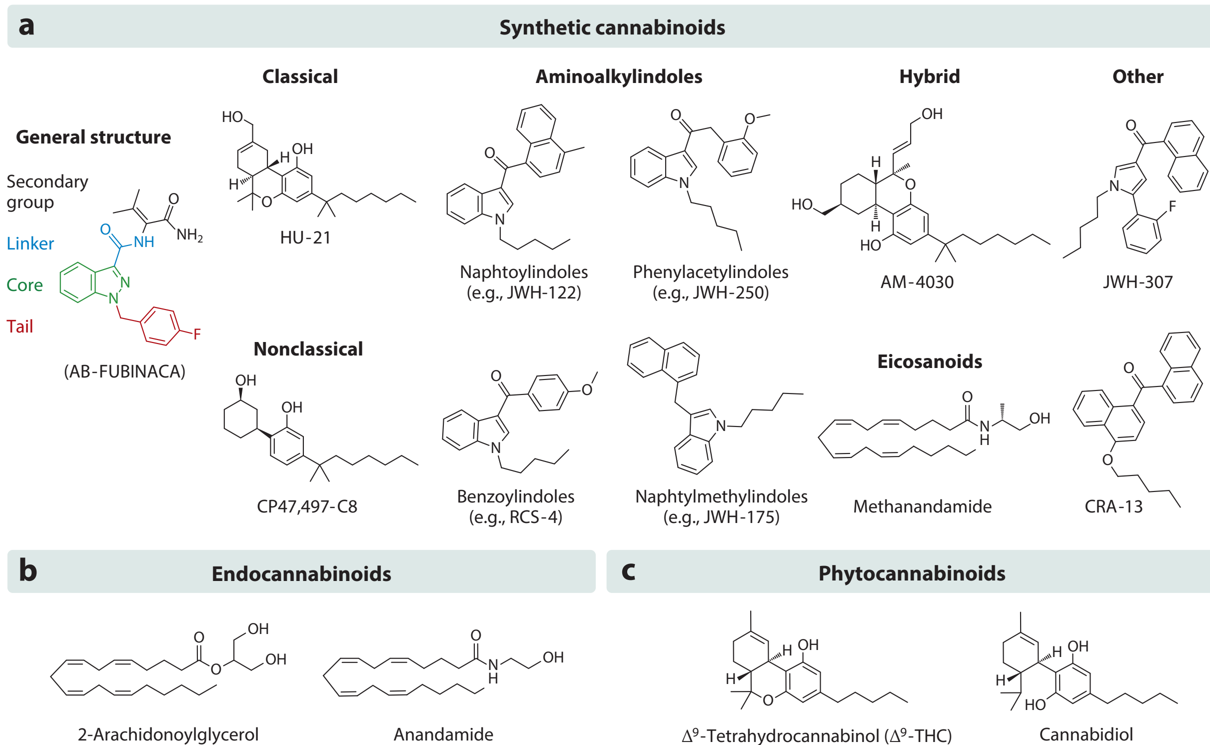

Figure 1

General classification of synthetic cannabinoids (SCs). (*a*) Although different groups of SCs display structural variations, these substances exhibit a general structure (the structure of AB-FUBINACA is shown as an example), comprising a core and a secondary structure, joined by a linker with a tail group attached, as presented on the left side of the panel. The structures of the two main endocannabinoids (*b*) and the two main phytocannabinoids (*c*) are presented for comparison. Notably, some SC classes present structural similarities to these molecules. For example, classical SCs are similar to phytocannabinoids, whereas SCs from the eicosanoid class are structurally similar to endocannabinoids.

chromatographic resolution. Nevertheless, GC-MS may present limitations regarding the analysis of closely related isomers. Techniques such as nuclear magnetic resonance (NMR) or infrared spectroscopy, gas chromatography coupled with flame ionization detector, and liquid chromatography coupled with mass spectrometry (LC-MS) may also be used (15, 17). Liu et al. (17) used GC-MS and NMR to detect 10 distinct indole/indazole SCs in 36 herbal blends, noting that both techniques had similar sensitivity and detection range (1.9–50.6 mg/g for CG-MS, 1.5–49.0 mg/g for NMR). Recently, Mercieca et al. (18) developed a time- and cost-effective extraction technique, based on the pairing of dispersive liquid-liquid microextraction with ultrasound, to improve SC detection via GC-MS.

The most common quantitative method is LC-MS, which only requires a single mass spectrometer (either with or without high resolution; LC-HRMS), or tandem mass spectrometry (LC-MS/MS). The analysis is preceded by an extraction step (either solid phase extraction or liquid-liquid extraction) and is mostly suited for instances where elevated amounts of fatty acids are present (14, 15). A set of parent indole and indazole (e.g., 5F-PB-22, 5F-ADB) and other SCs (e.g., EG-018, MDMB-CHMCZCA, CUMYL-PeGACLONE) were successfully detected (in addition to their respective metabolites) in wastewater up to 29 days using LC-HRMS and

LC-MS/MS, validating these parent compounds (not just their metabolites) as biomarkers for SC detection in wastewater (19).

Using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QToF-MS), Giorgetti et al. (20) recently developed an LC-MS/MS-based method that allowed the quantification of the 7-azaindole 5F-AB-P7AICA in serum samples—with good linearity, accuracy, and precision—and in urine. Similarly, Haschimi et al. (21) detected the new derivatives CUMYL-CBMICA and CUMYL-CBMINACA and the products of their phase I metabolism in human urine samples using LC-QToF-MS.

The use of immunoassays to screen blood and/or urine for the presence of SCs is not recommended, due to the restricted concentration range of these assays, the structural variability of SCs, and the fast metabolism of these substances in the body, which often produce metabolites that may not be detected by this method (14, 18). For example, Mogler et al. (22) unsuccessfully attempted to detect the novel analog CUMYL-PeGACLONE in 30 biological samples from SC users using SC-specific immunoassays, even after lowering cut-off values.

3. PHARMACODYNAMICS

The pharmacology of SCs is generally similar to that of Δ^9-THC, as these substances act on two of the main receptors in the endocannabinoid system, the cannabinoid receptors 1 and 2 (CB1R and CB2R, respectively), which are G protein–coupled receptors (GPCRs). In contrast to Δ^9-THC (partial agonist), most SCs are full agonists of these receptors, thus displaying higher efficacy compared to Δ^9-THC, which accounts for both the more intense psychoactive effects and the greater severity of undesired effects. Moreover, SC mixtures lack cannabidiol, a CBR antagonist present in cannabis that helps counter the psychoactive effects of Δ^9-THC (14, 16). A structure-activity analysis concerning CB1R activation, performed by Banister et al. (23) in transfected murine AtT-20 neuroblastoma cells, showed that SCs with an L-*tert*-leucinamide linker generally displayed greater potency at CBRs than did their L-valinamide counterparts, regardless of the core being an indole or an indazole. The same group also demonstrated that *tert*-leucinate SCs were more potent than their respective valinate counterparts (similarly, no correlation was found between the presence of an indole or indazole core and CBR activation), having observed different potencies for distinct *N*-alkyl substitutions (cyclomethylhexyl < 4-fluorobenzyl < pentyl < 5-fluoropentyl) (24). Moreover, indazole analogs showed higher potency (reflecting improved binding and affinity) for the CBRs, followed by the indole and the 7-azaindole moieties (25).

Activation of the CB1R leads to a decrease in cyclic adenosine monophosphate (cAMP) by inhibiting adenylyl cyclase activity (14, 16). Additionally, CB1R activation causes the βγ subunits to activate the mitogen-activated protein kinase (MAPK) family, including the signal-regulated extracellular kinases 1 and 2 (ERK1/2). CB1R phosphorylation (following its activation) by G protein receptor kinases (GRKs) may induce β-arrestin 1 and 2 translocation to the cell membrane, causing CB1R desensitization and internalization, a mechanism reportedly associated with tolerance development (14, 26).

Similarly to other GPCR-activating molecules, SCs were found to act via biased agonism/functional selectivity, a concept in which the activation of CB1R favors a specific receptor conformation, promoting one signaling pathway over another (26), which may possibly explain the distinct pharmacological actions produced by SCs binding to the same receptor (27). Indeed, activation of the $G_{\alpha i/o}$ heterotrimer over β-arrestin 1 triggers the $G_{\beta\gamma}$ subunit release, inhibiting voltage-dependent calcium channels and activating G protein–gated inwardly rectifying potassium channels. The $G_{\alpha i/o}$ subunit then inhibits adenylyl cyclase, stimulating the phosphorylation

and early activation of ERK1/2. In contrast, β-arrestin 1 recruitment triggers the late activation of ERK1/2 (28). For example, although JWH-018 and JWH-081 bind to CB1R, JWH-018 decreases phospho-ERK1/2 expression (29), whereas JWH-081 impairs phospho-CaMKIV and phospho-CREB levels (e.g., associated with neuronal plasticity regulation) (30). Wouters et al. (31) assessed the preference of a set of SCs for the β-arrestin 2 or $G_{\alpha i}$ pathways in HEK293T cells stably transfected with CB1R and found balanced agonists (favoring neither pathway) such as MMB-CHMICA, SCs showing bias toward β-arrestin 2 recruitment (e.g., 5F-APINACA, CUMYL-PeGACLONE, JWH-018), and a single SC (e.g., EG-018) showing bias toward $G_{\alpha i}$ recruitment. Since EG-2201 and MDMB-CHMCZCA, which contain a carbazole group like EG-018, also displayed a tendency for $G_{\alpha i}$ signaling, the authors suggested that this carbazole may influence the bias (31). Interestingly, the consequences of favoring CB1 recruitment of β-arrestin 1 or 2 remain unclear. Agonists often activate both β-arrestins, with specificity likely stemming from a cell-specific context (32). Patel et al. (26) observed that, compared to the reference ligand WIN55,212-2, the indazole derivatives 4CN-CUMYL-BUTINACA, 5F-AMB-PINACA, and 5F-MDMB-PICA exhibited a strong bias toward cAMP inhibition (and away from cAMP stimulation and β-arrestin recruitment and/or internalization) in CB1R-transfected HEK293 cells, supporting a strong CB1R twin toggle switch (ranging residues F200 and W356 in the TM2/TM6 binding pocket) interaction. Notably, the aromatic interaction of indole or indazole-based SCs (e.g., MDMB-FUBINACA, 5F-MDMB PICA) with the toggle switch has been shown to stabilize the active conformation of the receptor, further increasing these substances' binding ability (33). 5F-CUMYL-P7AICA and XLR-11 only displayed partial efficacy in cAMP stimulation and β-arrestin translocation. Most importantly, SCs showed higher potency and efficacy than Δ^9-THC in the recruitment and internalization of β-arrestins and $G_{\alpha s}$ signaling. Specifically, the relatively low efficacy of Δ^9-THC in β-arrestin pathways compared to SCs suggests that these pathways may be key contributors to SCs' effects (26). Zagzoog et al. (34) further noted that the indazole core and halogen-substituted pentyl or butyl tails [e.g., JWH-018 2′-napthyl-*N*-(3-methylbutyl) isomer, 4-fluoro MDMB-BINACA] biased SCs toward cAMP inhibition and β-arrestin 2 recruitment.

Biased agonism: a ligand-based induction of a particular receptor conformation that favors a specific signaling transduction pathway, relative to a reference agonist

Endocannabinoidome: expanded endocannabinoid system, which further includes several mediators biochemically related to endocannabinoids, their receptors, and metabolic enzymes

SCs can also modulate signaling pathways independently of CBRs, in line with the discovery of the endocannabinoidome, an expanded endocannabinoid system. For example, aminoalkylindole derivatives, arylpyrazole derivatives, and synthetic analogs of phytocannabinoids have been reported to target the transient receptor potential cation channel subfamily V member 1 (TRPV1) (35). The desensitization of these channels by WIN55,212-2 has been found to promote analgesic effects (36). Nevertheless, despite sharing a strong ability to stimulate CBRs, SCs display limited efficacy in opening TRPV1 channels, with only XLR-11 and its analogs inducing a significant activation of these channels (37).

SCs may also activate peroxisome proliferator-activated receptors (PPARs), a family of nuclear hormone receptors that form heterodimers with the retinoid X receptor and alter gene transcription by binding to DNA sequences called PPAR response elements (38). CB1R- and CB2R-mediated activation of PPARγ by WIN55,212-2 has been suggested to underlie its neuroprotective and anti-inflammatory effects against amyloid-β (Aβ)-induced damage (39). Similarly, modulation of PPARα via WIN55,212-2-induced activation of CBRs has been reported as the main mechanism responsible for WIN55,212-2 anticonvulsant properties in a pentylenetetrazol-induced clonic seizure mice model (40). Also, Vara et al. (41) demonstrated that the antiproliferative action of JWH-015 on hepatocellular carcinoma was shown to be modulated, in vitro and in vivo, by the upregulation of PPARγ signaling.

SCs may further modulate the orphan GPCRs (e.g., GPR55, GPR18) (42, 43). For example, JWH-015 has been shown to activate GPR55 in distinct cell types, increasing intracellular calcium

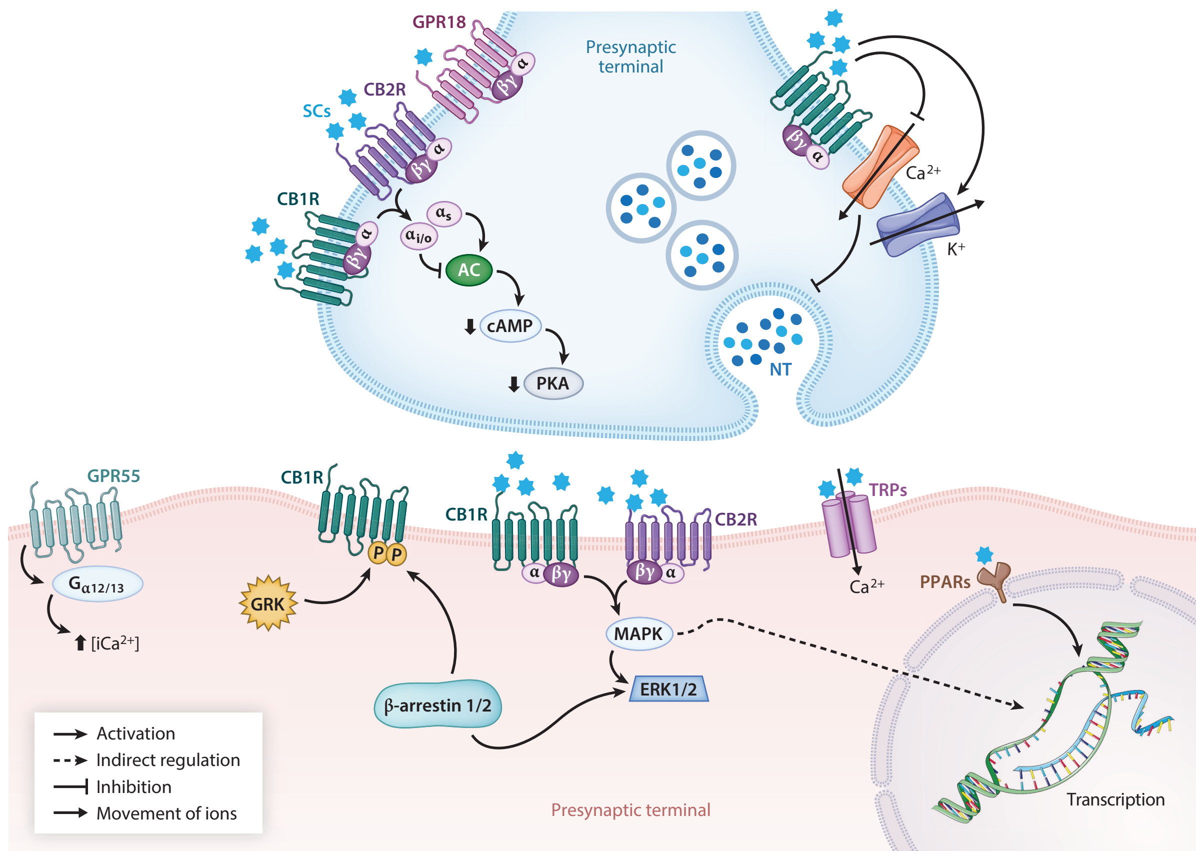

Figure 2

Intracellular signaling pathways triggered by synthetic cannabinoids. Activation of cannabinoid receptors 1 and 2 (CB1R and CB2R, respectively) in the presynaptic terminal leads the $\alpha_{i/o}$ subunit of the G protein–coupled receptor to inhibit adenylyl cyclase (AC), causing a decrease in cyclic adenosine monophosphate (cAMP) and, subsequently, in protein kinase A (PKA) activity. CB1R activation also triggers the opening of inward-rectifying K^+ channels with concomitant inactivation of Ca^{2+} channels (through the βγ subunits of the receptor), which block the release of neurotransmitters (NTs) into the synaptic cleft. At the postsynaptic level, the βγ subunits are also responsible for the activation of mitogen-activated protein kinase (MAPK), which may further activate signal-regulated extracellular kinases 1 and 2 (ERK1/2) at an early stage. Moreover, MAPKs may regulate nuclear transcription factors and thus influence gene expression. Phosphorylation of CB1R by G protein receptor kinases (GRKs) marks the receptor and signals for β-arrestin 1/2 translocation while promoting CB1R desensitization and internalization. β-arrestin may also activate ERK1/2 at a later timepoint than MAPK. Other elements of the endocannabinoid system may also be stimulated by synthetic cannabinoids (SCs): G protein–coupled receptor 55 (GPR55) activation leads to an increase in intracellular Ca^{2+} concentration; GPR18 also couples with the $\alpha_{i/o}$ receptor subunit, being also able to inhibit AC; transient receptor potential channels (TRPs) stimulate the neuronal uptake of Ca^{2+}; and activation of peroxisome proliferator-activated receptor γ (PPARγ) regulates gene transcription. Some figure elements adapted from Servier Medical Art, provided by Servier (CC BY 3.0).

levels (44). Activation of GPR55 has also been suggested to underlie the antinociceptive effects of the atypical SC O-1602 in a rat model of acute joint inflammation (45). O-1602 may also act as an agonist to GPR18, promoting calcium influx and triggering ERK1/2 phosphorylation in HEK293 cells overexpressing GPR18 (46).

The main signaling pathways activated by SCs are summarized in **Figure 2**.

3.1. Modulation of Neurotransmitter Signaling

SCs mainly target the brain, modulating neurotransmitter signaling, among other processes. Ossato et al. (47) showed that JWH-018 and AKB48 induced a CB1R activation-dependent psychostimulant effect in mice by facilitating striatal and nucleus accumbens' dopamine release. Since the ventral tegmental area and the nucleus accumbens, and the portion of the medial forebrain bundle that links both regions, are key structures of the brain's reward circuitry, SC-induced dopamine neural firing in these regions increases the reward response, accounting for these substances' addictive potential (48). However, Ma et al. (49) observed that CB2R activation in mouse ventral tegmental area slices by different CB2R-selective agonists (e.g., JWH-133) instead inhibited dopamine neuron firing in a concentration-dependent manner. SCs have also been reported to be more effective than Δ^9-THC in inhibiting glutamatergic synaptic transmission (50). MAM-2201 was shown to suppress glutamate and γ-aminobutyric acid (GABA) release in mice by activating presynaptic CB1Rs in Purkinje cells (51). WIN55,212-2 was shown to increase glutamate uptake via the overexpression of glutamate transporter 1 (GLT1) and excitatory amino acid carrier 1 (EAAC1) in the rat frontal cerebral cortex (52). Additionally, Sánchez-Zavaleta et al. (53) observed that the synthetic CB2R agonists GW833972A, GW405833, and JHW-133 inhibited glutamate release by modulating P/Q channels in rat subthalamic-nigral terminals in a CB2R activation–dependent manner.

SC exposure may also modulate serotonergic neurotransmission. For example, Yano et al. (54) observed the positive allosteric modulation of the 5-HT_{1A} receptor by SCs comprising an indole moiety (e.g., JWH-018, AM-2201), indicating that such SCs may target serotonin receptors independently of CBR activation. Bambico et al. (55) reported a decline in the firing rates of serotoninergic neurons in the dorsal raphe nucleus of rats exposed to high WIN55,212-2 doses, also through a CBR-independent mechanism. In contrast, at low doses, WIN55,212-2 enhanced serotonergic neuronal activity and promoted dose-dependent antidepressant-like effects in the rat forced-swim test, through a CB1R activation-dependent mechanism. Moreover, subchronic administration of CP55,940 to male Sprague-Dawley rats has been shown to increase the membrane-associated expression of dopaminergic D2 and serotonergic 5-HT_{2A} receptors, resulting in the formation of 5-HT_{2A}-D2 receptor heterodimers, whose dysregulation has been suggested to contribute to the pathophysiology of neuropsychiatric disorders (56).

4. PHARMACO- AND TOXICOKINETICS

Analysis of SCs' pharmaco- and toxicokinetics is essential to (*a*) understand the extent (i.e., duration and intensity) of the putative (noxious) effects users may experience and (*b*) determine the time window for their detection in biological samples.

SCs are mainly consumed by inhalation (i.e., smoking), which leads to a rapid absorption by the alveoli, resulting in fast peak blood concentrations, redistribution, and onset of effects (14, 16). For example, Adamowicz et al. (57) observed a peak in JWH-018 and JWH-073 concentration 5 min after smoking herbal incenses, which rapidly declined to less than a tenth of peak concentration within the following 3 h. Although SCs may also be consumed as infusions, their oral administration is not very common, which is possibly explained by the inconsistent absorption inherent to this administration route, which depends on multiple factors (e.g., presence/absence of food in the stomach, particularly fat; gastric pH; first-pass effect). Evidence for the use of the intravenous route of administration remains scarce, although it is possible that high-risk marginalized individuals may, at times, inject SCs (14, 16).

The high lipophilicity of most SCs anticipates their extensive binding to plasma proteins, which may in turn result in increased distribution volumes (16, 58). Brandon et al. (59) determined that

the log $D_{7.4}$ of indole- and indazole-3-carboxamide SCs ranged from 2.81 for AB-FUBINACA (least lipophilic) to 4.95 for MDMB-4en-PINACA (most lipophilic) among the SCs tested. Interestingly, these in-silico predictions contrasted the experimental data, in which SCs with an indole core scored higher log P values than SCs containing an indazole core.

Schaefer et al. (60) observed that the biodistribution of 200 μg/kg JWH-210 and RCS-4 administered intravenously to pigs followed a three-compartment model, with central volumes of distribution of 0.2 and 0.67 L/kg and clearances of 0.048 and 0.093 (L/min)/kg for JWH-210 and RCS-4, respectively. These substances demonstrated similar patterns of distribution, with the exception of the lungs and the kidneys. The authors detected high levels of RCS-4 and low levels of JWH-210 in the lungs and the opposite in the kidneys, which could possibly be due to the more lipophilic nature of JWH-210 (compared to RCS-4), which favors distribution into tissues with higher fat content (e.g., kidneys) and facilitates its elimination from the lungs (60, 61). Indeed, high concentrations of both SCs (40–60 ng/g) were found in abdominal and perirenal adipose tissue. Recently, a pulmonary dose in pigs of 200 μg/kg body weight (using a nebulizer) of the same SCs showed that this administration route led to lower SC concentrations in certain tissues (e.g., cerebellum, muscle), compared to intravenous administration. The authors attributed this effect to the distinct SC bioavailabilities, putative pulmonary uptake, and first-pass metabolism, which is more common following pulmonary administration (61).

The structure of SCs is a determining factor for metabolism. The high lipophilicity of most SCs makes them prone to phase I (e.g., alkyl and aromatic oxidation) and phase II (e.g., glucuronidation, sulfation) reactions before excretion (14, 16, 62). Oxidative metabolism generally leads to the formation of mono-, di- and trihydroxylated metabolites. SCs possessing aromatic cores (e.g., 7-azaindole, indole, indazole, and γ-carbolinone) have been reported to result in mono- and dehydroxylated, as well as dihydrodiol, metabolites (14, 16). Walle et al. (63) recently noted, in in vitro (pooled human liver S9 fraction, pooled human liver microsomes, pig liver microsomes) and in vivo (rat and pig) systems, that the 7-azaindole derivative CUMYL-5F-P7AICA underwent oxidative defluorination, monohydroxylation, ketone formation, and carboxylation as the most prevalent phase I reactions and detected the formation of sulfated and glucuronidated phase II metabolites. Interestingly, the parent compound was still detected in all models. Franz et al. (62) observed that the main metabolites of *tert*-leucine- and valine-derived SCs (e.g., 5F-MDMB-PINACA, AB-CHMINACA) derived from four types of reactions: *N*-dealkylation, hydrolysis of the methyl ester group or terminal amide, hydroxylation (of the core ring, amino acid moiety, or *N*-alkyl side chain), and dehalogenation of 5-fluoropentyl side chain. The authors thus postulated that (*a*) indazole-containing compounds were more prone to undergo hydrolysis of the terminal functionality and the secondary amide, as well as dihydrodiol formation, than their indole counterparts (more likely to undergo *N*-dealkylation), and (*b*) a valine moiety provides greater susceptibility for hydrolysis of the terminal functionality, whereas a *tert*-leucine group favors dehalogenation and hydroxylation (of the amino acid moiety) (62). **Supplemental Figure 1** summarizes the structural alterations induced by phase I and phase II reactions, using CUMYL-5F-P7AICA and 5F-MDMB-PINACA as examples.

Supplemental Material >

Takayama et al. (64) described oxidation at the *N*-(1-amino-alkyl-1-oxobutan) moiety as the main metabolic reaction of AB-FUBINACA in human liver microsomes. *N*-hydroxylation (mainly at N-4 and N-5) and carboxylation were identified as the main reactions of MAM-2201 and JWH-122 metabolism, both in vitro (human liver microsomes) and in vivo (human urine) (65). Some SCs such as THJ-2201 have also been reported to undergo oxidative defluorination (66). A recent study analyzed the metabolism of third generation SCs in zebrafish larvae, observing that ABD-CHMINACA and MDMB-CHMCZCA shared monohydroxylation as the main metabolic pathway, whereas MMB-CHMINA mainly underwent *O*-demethylation (67).

SCs are further transformed into more hydrophilic compounds to facilitate their renal excretion through conjugation with sulfate and/or glucuronic acid. Notably, this near ubiquity of SC metabolites in urine makes it the matrix of choice for SC detection. However, prior to analysis, urine must be subjected to β-glucuronidase treatment to cleave conjugated metabolites (14, 16). The metabolites derived from the acidic hydrolysis of AB-FUBINACA and AMB-FUBINACA, for example, were shown to have a very long detection window (putative detection phase >1 year), so their detection in urine does not necessarily reflect a recent consumption (68).

Interestingly, some SC metabolites have been shown to retain pharmacological activity. For example, the hydroxypentyl metabolites of SCs like AB-PINACA, 5F-ADB, CUMYL-5F-PINACA, and APINACA were reported to retain full efficacy at human CB1R and CB2R, despite their reduced potency and affinity compared to the respective parent compounds (69). On the other hand, SC metabolism may lead to the formation of toxic metabolites. For example, carboxylesterase-mediated hydrolysis of the ester bond present in some SCs (e.g., XLR-11) may cause the blood accumulation of a metabolite with toxicological mechanisms similar to those of *N*-acetyl-*p*-benzoquinone imine (acetaminophen metabolite) (70).

5. BIOLOGICAL AND TOXICOLOGICAL EFFECTS OF SCs

The diversity and extent of effects induced by SCs are influenced by factors such as the route of administration; the user's vulnerability; and the dose, lipophilicity, and pharmacological properties of the drug (71, 72).

SC consumers often search for some of the drug's known psychotropic effects, including increased relaxation, elevated well-being, and social disinhibition, which arise quickly after consumption (73). However, SC-related adverse effects often develop, mostly due to (*a*) biotransformation into toxic metabolites, (*b*) by-products of pyrolysis of smoked herbal blends, and/or (*c*) drug-drug interactions (e.g., shared metabolic pathways with frequently used medications). Notably, the contribution of SCs per se to the onset of adverse effects remains undetermined (74). Similarly to their psychoactive effects, adverse symptoms may also appear minutes or hours after consumption, and last from minutes to several hours (11, 75). These symptoms, which are summarized in **Figure 3**, can be grouped according to the target systems, that is, cardiovascular, digestive, dermatological, ophthalmological, neurological, pulmonary, and hepatic (72, 75, 76).

Acute intoxications have been particularly associated with neurological perturbations, including cognitive impairment, such as short-term memory loss, flashbacks, and suicidal ideation (11). Other neurological symptoms include delirium, confusion, hallucinations, agitation, panic attacks, and convulsions that have been associated with the use of SCs of the JWH series or AM-694 (77). Chronic SC consumption has also been correlated with an increased risk of developing neuropsychiatric disorders (78). Psychotic symptoms are frequent following SC use. Although these are usually temporary (only lasting a few hours), SCs may lead to prolonged psychotic episodes both in vulnerable subjects and individuals with no previous history of psychosis (6, 77). Novel third-generation fluorinated SCs, 5F-ADBINACA, AB-FUBINACA, and STS-135, were shown to cause hypothermia and increased pain threshold to noxious mechanical and thermal stimuli, besides reducing motor activity and impairing sensorimotor responses in mice (79). SCs also target the human cardiovascular system, reportedly causing increased heart rate, tachycardia, and in the most severe cases, myocardial infarction or stroke (77). A series of case reports associated with 5-fluoro-ADB use have also provided evidence for cardiomegaly as an SC-related effect in humans (80).

Severe intoxications have also been associated with rhabdomyolysis and liver and kidney toxicity and failure (81, 82). Notably, acute tubular necrosis has been reported in the biopsies of individuals that had consumed XLR-11 (83), leading the Centers for Disease Prevention and

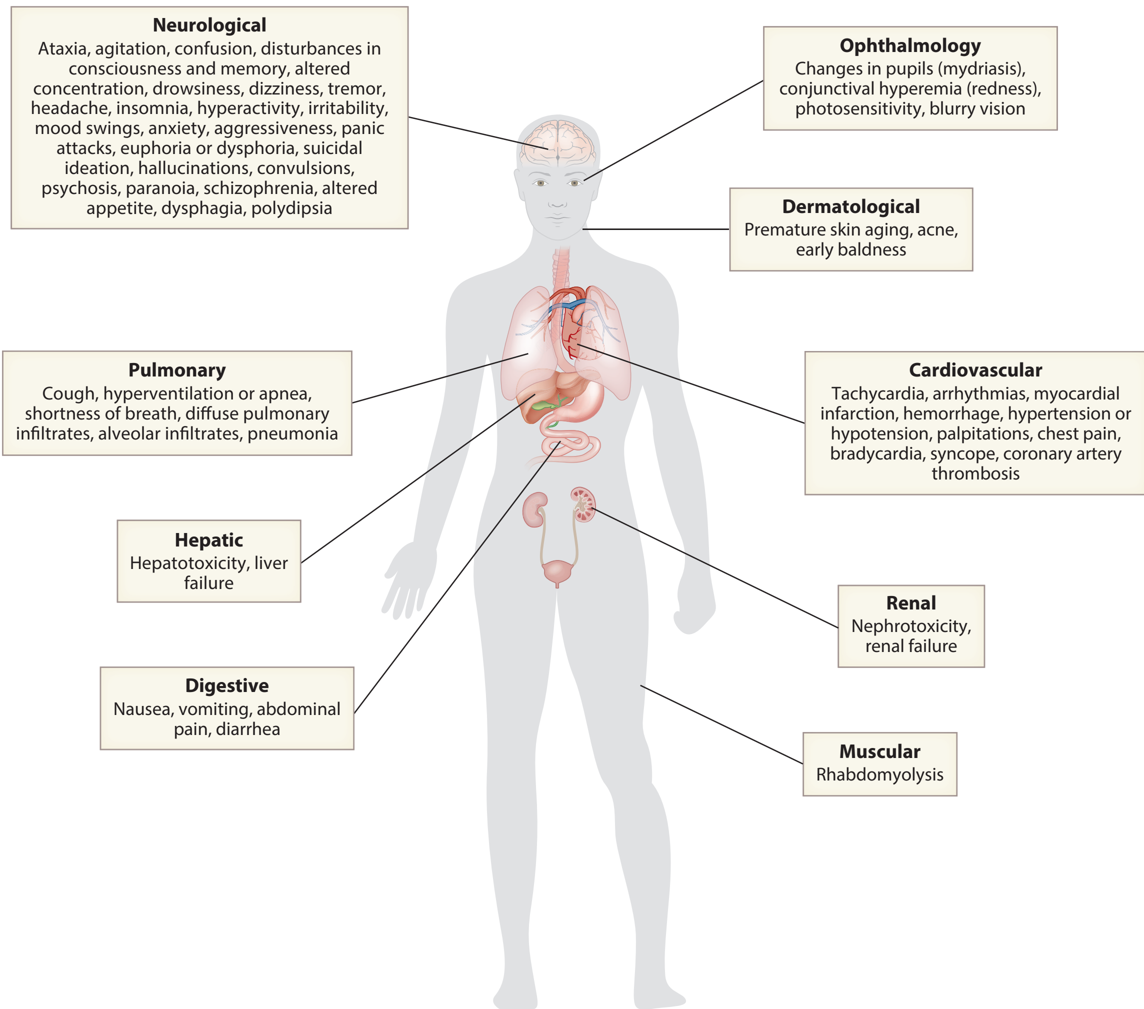

Figure 3

Summary of the most common adverse effects associated with the use of synthetic cannabinoids (SCs). In view of the wide distribution of cannabinoid receptors throughout the body, SCs may target different organs and trigger adverse effects in cardiovascular, digestive, dermatological, ophthalmological, neurological, pulmonary, and hepatic systems.

Control (CDC, USA) to establish a direct link between XLR-11 and acute kidney injury (84). Lung injuries (e.g., pneumothorax, pneumomediastinum) are also frequent and may be attributed to local injuries caused either directly by the SCs or by impurities in the SC mixtures (10), with patients often requiring oxygen supplementation (85).

Supplemental Material >

Cannabinoid hyperemesis syndrome: a rare condition causing recurrent, severe vomiting in daily long-term cannabis users

Hopkins & Gilchrist (86) described the first case of cannabinoid hyperemesis syndrome caused by SCs in a patient whose urine revealed the presence of JWH-018, JWH-073, and AM-2201.

Examples of clinical cases reporting SC-related poisonings are summarized in **Supplemental Table 1**.

SC withdrawal may also cause adverse symptoms, including restlessness, headache, irritability, drug craving, high blood pressure, nausea, tremors, diaphoresis, and nightmares (10, 11, 87). Seizures and cardiovascular arrest may also occur in more severe cases (73, 88).

Consumption of SCs, either alone or in combination with other recreational/prescription drugs, has also been associated with fatal intoxications, mainly attributed to poisoning, cardiac arrest, asphyxiation, multiple organ failure, suicide, or traumatic accident (73, 89, 90). Acyl indoles, indole carboxylates, and indazole carboxamides are the SC classes most implicated in death reports (13, 90). For example, MAB-CHMINACA, AMB-FUBINACA, MDMB-CHMICA, and 4F-MDMB-BICA have been associated with major death outbreaks in the United States and Europe (12, 13, 89, 91). Nevertheless, it is often difficult to establish a direct correlation between an SC and the cause of death, since (*a*) the lack of proper reference standards usually prevents the correct identification and quantification of SCs present in biological samples, and (*b*) postmortem blood concentrations may vary depending on the type of sample, individual idiosyncrasies, and time elapsed since death (92).

Most mild SC intoxications only require ambulatory symptomatic treatment, whereas severe intoxications (e.g., those involving seizures, severe agitation, neuropsychiatric perturbations, arrhythmias, stroke, thoracic pain) often lead to hospitalization (93). Acute SC intoxication treatment usually includes intensive monitoring and supportive therapy (10, 82, 94, 95). Intravenous fluids are often administered to expand the volume of the circulatory system, control vomiting, and prevent dehydration and renal failure.

Sedation with benzodiazepines (e.g., lorazepam) is the first-line treatment to reduce anxiety and agitation, though psychiatric evaluation and antipsychotics administration are often necessary (95, 96). Intubation and mechanical ventilation may be required in severe cases (10). In case of per os administration, gastric lavage and ingestion of activated carbon may be required depending on the amount of SC ingested and the time elapsed after intake (88). Recently, Aksel et al. (97) described a new treatment for SC intoxications, referred to as intravenous lipid emulsion (ILE), which showed promising results as an effective antidote in poisonings by lipophilic drugs like SCs, allowing recovery from cardiovascular collapse and reversing the neurological symptoms caused by these drugs. ILE retains drugs found in the intravascular space and distributes the fat-soluble drugs to a circulating phase, reducing their concentration and toxicity. Treatment for SC withdrawal symptoms is based on benzodiazepines, antiemetics, and other symptomatic care (94).

6. MOLECULAR MECHANISMS OF TOXICITY

Some of the mechanisms underlying SC toxicity have already been evidenced. For example, Tomiyama et al. (98) observed that eight SCs (e.g., CP series, JWH series) induced apoptosis (indicated by caspase-3 activation and a high count of annexin-V-positive cells) of cultured mouse forebrain neurons after 2 h. These effects were CB1R (but not CB2R) activation and concentration dependent (up to 30 μM). Similarly, MAM-2201-induced toxicity to human primary neuron-like cells and astrocytes (D384 cell line) was time (3–48 h) and concentration (1–30 μM) dependent and had a significant impact on cell viability and morphology, metabolic function, expression of neuronal markers, and apoptosis, which was only noted above 5 μM MAM-2201 (99). AB-FUBINACA, 5F-ADBINACA, and STS-135 have been reported to decrease the mitochondrial membrane potential in the murine neuroblastoma Neuro-2a cells at 60 μM, although STS-135 neurotoxic effects manifested starting at 3 μM after 1 h (79). Exposure of neuroblastoma SH-SY5Y cells to 100 and 200 μM APINACA for 24 h resulted in excessive reactive oxygen species (ROS) formation. Furthermore, 25 μM APINACA increased messenger RNA levels of CB1R and MAPK8, whereas at 100 and 200 μM, APINACA increased interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) levels (100). Recently, Sezer et al. (101) also observed an increase in oxidative stress (e.g., reduction of glutathione reductase and catalase activities, increased lipid peroxidation and protein carbonylation) in SH-SY5Y cells exposed for 24 h to JWH-018 at concentrations between 5 and 150 μM.

Neurogenesis: the set of processes (e.g., differentiation, migration, maturation) leading to the formation of new neurons

Notably, most of these studies assessed the cytotoxicity of SCs at high micromolar concentrations. However, cannabinoids generally exhibit nanomolar affinities at CB1R and CB2R, and it is broadly accepted that testing them at concentrations higher than 1 μM (three orders of magnitude above their affinity) is likely to induce off-target effects (101). Alexandre et al. (102), for example, have shown that THJ-2201 and 5F-PB22 at biologically relevant concentrations (≤1 μM) interfered with neuronal differentiation but did not compromise metabolic activity, lysosomal or plasma membrane integrity, or intracellular ATP levels of undifferentiated NG108-15 neuroblastoma X glioma cells after 24 h. However, the authors observed an increase in mitochondrial membrane potential for 1 pM THJ-2201 and 1 μM 5F-PB22, suggesting an impairment of mitochondrial activity. Hebert-Chatelain et al. (103) have demonstrated that 1 μM HU-210 reduced mitochondrial mobility in mice hippocampal neurons via activation of mitochondrial CB1Rs, which was correlated with the animal's memory impairment.

SCs may also elicit toxic effects on other organs, especially those involved in their metabolism and/or excretion. For example, Silva et al. (104, 105) demonstrated that, after 3 h, XLR-11, JWH-122, and THJ-2201 primarily targeted mitochondria in human proximal tubular (HK-2) cells, at biologically relevant concentrations (≤1 μM). These SCs induced a transient mitochondrial membrane hyperpolarization and increased intracellular ATP levels, as well as chromatin condensation and caspase-3 activation, suggesting the activation of apoptotic pathways. Moreover, these events depended on CB1R and/or CB2R activation (104). Koller et al. (106) reported that CP-47,497-C8 at concentrations higher than 7.5 μM for 24 h impaired mitochondrial function, protein synthesis, and lysosomal activity and caused cell membrane damage in hepatic (HepG2)- and buccal (TR146)-derived cell lines. Moreover, CP-47,497-C8 induced DNA damage, as evidenced by the high number of single- and double-strand breaks (at 10 μM for TR146 cells and ≥15 μM for HepG2 cells). 5F-EMB-PINACA, JWH-200, and A-796260, for 48–72 h, were also shown to be hepatotoxic (HepG2 cells) at 7.8 and 125 μM, impacting cell number, plasma membrane integrity, nuclear size, cytosolic calcium levels, and mitochondrial membrane potential (107). Furthermore, CP-55,940 induced a concentration-dependent (5–50 μM) decrease in cell viability of human embryonic rhabdomyosarcoma cells, an effect accompanied by loss of mitochondrial membrane potential, caspase-3 activation, and annexin V accumulation (for 0.5–2 h), thus evidencing apoptosis induction (108).

After 48 h, UR-144, JWH-122, and JWH-018 were shown to disrupt the plasma membrane and cause cell cycle arrest in the G2/M phase of human placental cytotrophoblast cells (109). Additionally, JWH-122 and JWH-018 increased ROS formation, and UR-144 and JWH-122 reduced mitochondrial membrane potential. Interestingly, while all these SCs were shown to increase caspase-9, -3, and -7 activities, the cytotoxic effects of UR-144 appeared to solely depend on CB1R activation, both CB1R and CB2R seemed to mediate the effects of JWH-018, and JWH-122's toxicity was shown to be CBR independent.

6.1. Impact of SCs on Neuronal Development

The impact of SC use on neurodevelopment represents a core issue, as adolescents and young adults (which include women of child-bearing age/pregnant women) are the main SC users (110). SCs modulate the endocannabinoid system, which is in turn involved in several biological processes, including cell fate and neurogenesis-related mechanisms (e.g., neuronal differentiation, migration, maturation, synaptic pruning) in a well-defined spatiotemporal manner, from the earliest stages of ontogenetic development to late adolescence (110, 111). The developing brain is thus especially vulnerable to SC-elicited effects, which may alter the neural circuitry and trigger the onset of neurodevelopmental disorders (e.g., psychoses, autism spectrum) (110, 111). Moreover, SCs can easily cross the placental barrier into embryonic tissues due to their high lipophilicity (112).

The link between pre- and postnatal exposure to SCs and neurogenesis dysfunction has been strongly supported by preclinical studies. For example, Mereu et al. (113) showed that the daily administration of the CB1R agonist WIN55,212-2 (0.5 mg/kg) to pregnant rats caused the impairment of memory retention capacities in their offspring at 40 and 80 days old. These effects were accompanied by a reduction in hippocampal presynaptic glutamate release and alterations of hippocampal long-term potentiation, which is associated with learning and memory consolidation. Recently, Pinky et al. (114) reported that administration of the same SC (WIN55,212-2) at 2 mg/kg body weight per day to pregnant rats significantly altered the levels of several biochemical markers of the adolescent offspring, including a reduction of oxidative stress and apoptotic marker levels and an increase of mitochondrial function in the cerebellum (a brain region playing an important role in learning and motor function). Interestingly, while Glu_{A1} levels (a major glutamate receptor subtype) and tyrosine hydroxylase activity remained unaffected, total monoamine oxidase (MAO) activity significantly declined in the cerebellum, supporting the notion that SCs affect monoamine neurotransmitter levels in this brain region.

Multiple in vitro studies have also uncovered the key role played by CBR stimulation in the SC-mediated modulation of neurogenic processes (115, 116). For example, Kim et al. (117) observed that 300 nM WIN55,212-2 markedly prevented new synapse formation in rat hippocampal neurons obtained from 17-day-old embryos by inhibiting the forskolin-induced cAMP increase. Remarkably, WIN55,212-2 did not block the effects evoked by a membrane-permeant cAMP analog, suggesting that it inhibited new synapse formation by preventing cAMP synthesis rather than cAMP actions (e.g., neurotransmitter release). Jordan et al. (118) reported a significant concentration-dependent increase of neurite outgrowth in Neuro-2A cells exposed to up to 10 μM HU-210, which was mediated by CB1R and Rap1 signaling. Jiang et al. (119) reported that chronic (but not acute) treatment of hippocampal neural stem cells isolated from embryos of E17 Long Evans rats with 100 μg/kg HU-210 promoted neuronal proliferation, but not differentiation, through ERK pathway activation, associating this effect with anxiolytic- and antidepressant-like effects of HU-210. Recently, Alexandre et al. (102) described that THJ-2201 and 5F-PB22 increased differentiation ratios and total neurite length of NG108-15 cells in a CB1R activation-dependent manner, at 1 pM and 1 nM.

Notably, the CB2R also seems to play a key role in neurodevelopment-related processes. For example, Palazuelos et al. (120) reported that HU-308 (a selective CB2R agonist) induced the proliferation of rat HiB5 hippocampal progenitor cells via stimulation of CB2Rs and subsequent activation of the PI3K/Akt/mTOR1 pathway. Oudin et al. (116) observed that 1 μM JWH-133 promoted neuronal cell migration of COR-1 neural stem cells and rostral migratory stream neuroblasts via CB2R activation. Miranda et al. (121) showed that chronic exposure to THJ-018 and EG-018 during neurogenesis promoted a premature neuronal and glial differentiation of human-induced pluripotent stem cells that led to abnormal functioning of voltage-gated calcium channels of newborn neurons when stimulated by extracellular potassium.

Some studies have highlighted the importance of SCs' interaction with the brain-derived neurotrophic factor (BDNF), a neuroplasticity modulator involved in the regulation of neuronal differentiation, maturation, and survival (122). Recently, Ferreira et al. (123) showed that WIN55,212-2 regulated adult neurogenesis, as exposure of subventricular zone (SVZ) and dentate gyrus (DG) neurosphere cultures from young (postnatal days 1–3) rats to this SC increased cell proliferation in the DG but not in the SVZ. This process seemed to be modulated independently of CBR2 activation, as the selective CB2R agonist HU-308 did not interfere with neuronal cell proliferation in either neurogenic niche. Nevertheless, WIN55,212-2 and HU-308 promoted cell differentiation in both DG and SVZ.

Epigenetic modifications: modifications to gene function, without any alteration in DNA sequence, that are mitotically or meiotically heritable

Li et al. (124) detected a CB1R-mediated reduction of BDNF levels in the hippocampus, which was associated with memory impairment, in adult mice administered JWH-018 (1 mg/kg, intraperitoneal). Notably, these changes correlated with an increased content of the endocannabinoids anandamide and 2-arachydonyl glycerol, suggesting that SCs may influence BDNF suppression and neurogenesis-related processes by disrupting endocannabinoid-mediated homeostasis.

Assessment of the outcome of pre- and postnatal SC exposure to human neurodevelopment is substantially challenging since (*a*) the cognitive, motor, and behavioral parameters can only be evaluated in retrospect (121); and (*b*) several confounding factors can introduce major variations to the outcome, hampering the isolation of the direct consequences of SC use without interpretation bias (125, 126). Data on perinatal SC-related toxicity are thus restricted to just a few case studies reporting the absence of mortality or morbidity features in newborns (127).

6.2. Epigenetic Modulation

Epigenetic-elicited disruptions have been reported in the brain and peripheral organs after Δ^9-THC exposure (128), but thus far only a few studies have reported the epigenetic mechanistic outcomes of SC exposure (129). For example, Ibn Lahmar Andaloussi et al. (130) observed an increase in global DNA methylation and transcription of DNA methyltransferases 1 (DNMT1) and 3 (DNMT3) in the prefrontal cortex of adolescent male rats exposed for one week to WIN55,212-2, suggesting that these epigenetic modifications contributed to the anxiogenic-like effects observed in the exposed mice, as well as in their offspring. Scherma et al. (131) observed histone hyperacetylation and reduced histone deacetylase 6 (HDAC6) levels in the prefrontal cortex of adolescent rats exposed to WIN55,212-2 for 11 days, which was found to enhance the brain's initial molecular and epigenetic response to cocaine in adult rats. Aguado et al. (132) demonstrated that HU-210 and JWH-133 (selective CB1R and CB2R agonists, respectively) modulated glioma cell differentiation by increasing histone methylation (increased H3K9me3-positive cells) in a CBR activation–dependent manner. Tomas-Roig et al. (133) observed that long-term administration of WIN55,212-2 during rats' adolescence increased anandamide levels and promoted DNA hypermethylation at the intragenic region of the intracellular signaling modulator Rgs7 (an intracellular antagonist of GPCR signaling), which was found to alter Rgs7 expression in adulthood. Administration of HU-210 to female rats during pregnancy and to their offspring for 14 days after postnatal day 35 was shown to modify the expression of microRNA in the left hemisphere of the entorhinal cortex, a brain area associated with schizophrenia (134).

7. THERAPEUTIC POTENTIAL OF SCs

Accumulating findings have suggested the potential of the endocannabinoid system as a therapeutic target, leading to the exploitation of SCs as candidate agents to treat several disorders (135). In fact, the synthetic analogs of Δ^9-THC dronabinol and nabilone (Marinol and Cesamet, respectively) have been approved by the US Food and Drug Administration to treat chemotherapy-associated nausea and vomiting in cancer patients, after first-line antiemetics have failed, or as adjunct analgesics to alleviate chronic pain (136). Also, there are already marketed nabiximols (standardized combination of equal amounts of synthetic Δ^9-THC and cannabidiol), namely Sativex, which have shown moderate evidence to treat spasticity associated with multiple sclerosis (135). Nevertheless, most efforts to develop SC-based therapeutic agents have been discontinued, mainly due to the CB1R activation–related adverse events triggered at the central nervous system (111).

SCs' ability to bind CB2Rs has suggested the safe targeting of the endocannabinoid system due to their potential to modulate inflammatory processes. For example, WIN55,212-2 was shown

to suppress nitric oxide production; TNF-α release; and the formation of CXCL10, CCL2, and CCL5 chemokines in IL-1-stimulated astrocytes (137). Similarly, Aguirre-Rueda et al. (138) observed that WIN55,212-2 reverted the increased oxidative stress, inflammatory response, and cell viability loss of primary cultured astrocytes elicited by $A\beta_{1-42}$. However, the discovery of the endocannabinoidome has further increased the complexity of signaling events triggered by SCs, thus limiting their potential therapeutic use (111).

Most importantly, there is still scarce evidence supporting the use of cannabinoid-based products for most suggested therapeutic applications. As such, the potential benefits arising from SC use are outweighed by their associated risks, thus deterring their medical use.

8. CONCLUSIONS

Research on the biological relevance of the endocannabinoid system has greatly expanded over the past years, with SCs playing a major role as research tools to help understand how this system regulates key biological processes. Nevertheless, the widespread recreational use of SCs has become a major public health and social concern.

The ability of SCs to interact with CBRs (i.e., CB1R, CB2R) as well as with non-CBRs (e.g., TRPV, GPR55, PPARs, 5-HT receptors), along with the biased agonism of SCs upon binding to CBRs, increases the complexity of the signaling pathway network modulated by these substances and hampers the understanding of such signaling modulation.

Moreover, as SCs' targets are widely distributed throughout the body, their action and adverse outcomes extend to all major organs and tissues. Most importantly, the scarce information available on SCs' toxicological signatures is often equivocal, as (*a*) toxic effects may be associated with the presence of other toxic substances in the SC herbal blends, besides the SC itself; (*b*) several confounding factors (e.g., genetic, environmental, frequency/type of SC used) may influence their action; (*c*) in vitro effects vary according to the cell model (e.g., primary cultures versus cell lines, species of origin) and experimental design (e.g., concentration, timepoint, exposure protocol); and (*d*) only a few studies have addressed the toxicological effects of SCs at biologically relevant concentrations.

SC-mediated modulation of mitochondrial function and activity and apoptotic signaling induction, for example, have been proposed as important mechanisms underlying the toxicity of these substances. Additionally, SC-associated perturbations in neurogenesis are likely to contribute to the onset of neurodevelopmental/neuropsychiatric disorders, which is especially alarming considering that adolescents and young adults are the main SC users. SCs may also interfere with the epigenetic machinery and promote epigenetic changes that may predispose individuals to distinct pathologies that can then be inherited by their progeny.

Interestingly, although the therapeutic value of SCs has been evidenced by the clinical use of synthetic Δ^9-THC analogs to treat nausea and vomiting in patients undergoing chemotherapy, their potential use for other therapeutic applications still lacks scientific evidence. Further research is crucial to understand the pharmacological and toxicological mechanisms underlying the short- and long-term consequences of SC use and how these may condition consumers' health and quality of life, as well as to improve the interpretation of SC-related clinical/pathological findings.

SUMMARY POINTS

1. Synthetic cannabinoids (SCs) are designed to mimic the action of Δ^9-tetrahydrocannabinol (Δ^9-THC) but with stronger potency and efficacy at cannabinoid receptors.

2. Recreational SC use is globally widespread and is often associated with reports of acute intoxications and deaths.
3. SCs target both cannabinoid and non-cannabinoid receptors, triggering an intricate network of signaling pathways that contribute to the modulation of key biological processes.
4. Timely detection and quantification of SCs in biological samples remain a challenge for forensic toxicologists/pathologists, considering their high metabolic rate and lack of proper reference standards for parent compounds and respective metabolites.
5. SCs induce a plethora of adverse outcomes at different organ systems that are more severe and longer-lasting than those induced by Δ^9-THC.
6. Chronic SC use and/or use by particularly vulnerable groups (e.g., adolescents and young adults) may promote the onset of neurodevelopmental/neuropsychiatric disorders (e.g., psychosis, autism spectrum) in the long term by, for example, disrupting proper neurogenesis or inducing epigenetic changes.
7. SCs have been proposed as candidate agents for a few different therapeutic applications, but there is scarce evidence of their therapeutic potential, which is currently limited to chemotherapy-associated nausea and vomiting treatment.
8. Further research is required to clarify the main mechanisms underlying SC-mediated short- and long-term effects, which will hopefully reduce SC misuse by high-risk groups.

FUTURE ISSUES

1. Identification of major molecular initiating events and other key events triggered by SCs is crucial to establish the pathways leading to the main SC-related adverse outcomes.
2. Research into the mechanisms of SC-related toxicity has mostly focused on naphthoylindoles (e.g., JWH series, AM-2201), and further research is required to understand the toxicological profiles of other SC classes (e.g., indole- and indazole-based SCs, cumyl derivatives).
3. The long-term impact of SC use remains to be addressed, since thus far no clinical studies have followed the long-term behavioral, cognitive, or mood changes following acute and chronic SC use.
4. The ability of SCs to interfere with the epigenetic machinery, thus promoting epigenetic modifications (e.g., DNA methylation, histone methylation/acetylation/phosphorylation) needs to be further addressed, as it could explain some of the long-term effects of SC use.
5. The specific contribution of SCs to the onset of neuropsychiatric disorders remains unclear and is often equivocal, requiring a more in-depth understanding of how SCs may dysregulate the mechanisms underlying key biological processes such as neurogenesis.
6. Development of new SCs deprived of the noxious cannabinoid receptor 1–associated psychoactive effects and long-term toxicity, but maintaining some of their potential beneficial actions (e.g., anti-inflammatory), could represent a major advance for the establishment of SCs as interesting candidate therapeutic agents.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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