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The Endocannabinoid System in Ageing: A New Target for Drug Development

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Abstract: Endocannabinoids are a new class of lipids, which include amides, esters and ethers of long chain polyunsaturated fatty acids. Anandamide (N-arachidonoylthetanolamine; AEA) and 2-arachidonoylglycerol are the main endogenous agonists of cannabinoid receptors able to mimic several pharmacological effects of Δ9-tetrahydrocannabinol, the active principle of Cannabis sativa preparations like hashish and marijuana. AEA is released “on demand” from membrane lipids, and its activity at the receptors is limited by cellular uptake followed by intracellular hydrolysis. Together with AEA and congeners, the proteins which bind, synthesize, transport and hydrolyze AEA form the “endocannabinoid system”. Endogenous cannabinoids are present in the central nervous system and in peripheral tissues, suggesting a physiological role as broad spectrum modulators. This review summarizes the main features of the endocannabinoid system, and the latest advances on its involvement in ageing of central and peripheral cells. In addition, the therapeutic potential of recently developed drugs able to modulate the endocannabinoid tone for the treatment of ageing and age-related human pathologies will be reviewed.

Key Words: ageing, cannabinoids, nervous system, immune system, inflammation, excitotoxicity, osteoarthritis, neurodegenerative disorders.

THE ENDOCANNABINOID SYSTEM

Cannabis sativa preparations (marijuana, hashish) are among the most widely consumed drugs of abuse around the world. The main active component of Cannabis was identified in 1964 as Δ9-tetrahydrocannabinol (THC; Fig. (1A)) [1] and the use of powerful THC analogues, such as HU-210, in 1988 led to the identification of plasma membrane cannabinoid receptors (CBRs) [2]. Type-1 (CB1R) [3] and type-2 (CB2R) [4] cannabinoid receptors have been cloned. In a few years, different endogenous ligands were found to bind and activate CBRs, mimicking the psychotropic effects of THC. These compounds, collectively termed in 1995 “endocannabinoids” (eCBs), are derivatives (amides, esters and ethers) of long-chain polyunsaturated fatty acid, and exhibit different selectivity for CB1R and CB2R [5-8]. Although structurally different from plant cannabinoids (Fig. (2)), eCBs share critical pharmacophores with THC [9,10]. The two best-studied eCBs are anandamide (N-arachidonoylthetanolamine, AEA; Fig. (2B)) and 2-arachidonoylglycerol (2-AG; Fig. (2B)) [7,11]. Also 2-arachidonoyl-glycerol-ether (noladin ether, an ether-type eCB), N-arachidonoyldopamine (NADA) and virodhamine have been added to the cohort of these lipid mediators (Fig. (1B)) [12-14]. eCBs, their molecular target and the enzyme involved in their metabolism are collectively called endocannabinoid system (ES). The molecular relationship between the different components of ES and their cellular localizations are illustrated in Fig. (2).

Molecular Targets of Endocannabinoids

The molecular targets of eCBs are CBRs [15]. CB1Rs are among the most abundant receptors in the central nervous system (CNS), but are also expressed in peripheral cells, like those of the immune and reproductive systems [16-18]. CB2Rs, which are virtually absent on neurons, have been found primarily on immune cells, although they have been recently described also in non-immune cell types such as the cerebrovascular endothelial cells [19]. Other molecular targets of eCBs are non-CB1/non-CB2 cannabinoid receptors (CBxRs), the non-CBRs and the vanilloid receptors [20-22]. Both CB1R and CB2R have been classified into the class A rhodopsin-like family of the seven transmembrane G-protein coupled receptors (GPCR) [3,4,23-27]. Signal transduction pathways regulated by CBR-coupled G proteins include the activation of focal adhesion kinase (FAK), of mitogen-activated protein kinase (MAPK), of cytosolic phospholipase A2 and of nitric oxide synthase (NOS) [21,22,28]. A new molecular target of AEA which has attracted great interest is the type-1 vanilloid receptor (now called transient receptor potential vanilloid 1, TRPV1) [29]. TRPV1 is a ligand-gated and non-selective cationic channel, activated by molecules derived from plants, such as capsaicin, the pungent component of “hot” red peppers [30], and resiniferatoxin, and also by stimuli like heat and protons [31]. In the last few years, a number of studies suggested a physiological role for AEA as TRPV1 agonist [32], leading to the concept that AEA, besides being an eCB, is also a true “endovanilloid” [33]. The interaction of AEA with TRPV1 occurs at a cytosolic binding side (Fig. (2)) [30,32], raising the possibility that AEA, when biosynthesized by cells that express TRPV1, may activate this protein before being released from the cells. On the other hand, when extracellular AEA activates sequentially CB1R and TRPV1 in the same cell, this lead to an enhancement of its TRPV1-mediated responses [34]. Binding of AEA to TRPV1 triggers activation of non-selective ion channels and of protein kinases, and increases intracellular Ca2+ concentration, mitochondrial uncoupling and cytochrome c release [35,36].

Synthesis of Endocannabinoids

It was postulated that AEA and 2-AG are not stored in resting cells, but are synthesized and released “on demand” following physiological and pathological stimuli, such as neuronal depolarization and bacterial lipopolysaccharides. Their production is due to receptor-stimulated cleavage of phospholipid precursors via a Ca2+-dependent mechanism [37]. The AEA precursor is an N-arachidonylphosphatidylethanolamine (NArPE), which is believed to originate from the transfer of arachidonic acid from the sn-1 position of 1,2-sn-arachidonoylphosphatidylcholine to membrane-bound phosphatidylethanolamine, catalyzed by a calcium-dependent N-acetyltransferase (trans-acylase) [38,39]. Subsequent hydrolysis of NArPE releases phosphatidic acid and AEA. This reaction is catalyzed by a specific phosphodiesterase of the PLD type (NAPE-PLD) that was recently cloned and expressed [40].
The biosynthesis of 2-AG is more complex, due to the fact that this compound is at the crossroads of several metabolic pathways. In most cases, 2-AG is produced from the hydrolysis of 2-arachidonate-containing diacylglycerols (DAGs), catalyzed by an \textit{sn}-1 selective DAG lipase (\textit{sn}-1 DAGL). DAGs can be produced in turn from the hydrolysis of 2-arachidonate-containing phosphatidic acid (PA), catalyzed by a PA phosphohydrolase, or phosphoinositides (PI), catalyzed by a PI-selective phospholipase C (PI-PLC). Two \textit{sn}-1 DAGL isoenzymes have been cloned and enzymatically characterized [41]. They are mostly found in the plasma membrane, and are stimulated by Ca$^{2+}$. 

Fig. (1). Chemical Structures of Plant and Endogenous Cannabinoids. (A) Cannabinoids are found in \textit{Cannabis sativa} preparations. \Delta^8\text{-tetrahydrocannabinol (THC)} is the main psychoactive component of \textit{Cannabis}, while THC acid, cannabidiol (CBD) and cannabidiolic acid are non-psychoactive cannabinoic acids, which could become promising therapeutical agents. (B) Endogenous cannabinoids are derivatives of long-chain polyunsaturated fatty acid. Although structurally different from plant cannabinoids, they mime the psychotropic effects of THC. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the best-studied endogenous cannabinoids, while virodhamine, noladin ether and N-arachidonoyldopamine (NADA) have been identified more recently.
Inactivation of Endocannabinoids

The first step in the inactivation of eCBs consists of their rapid removal from the extracellular space. In fact, the biological activity of AEA at CBRs is terminated by a two-step process: (i) cellular uptake by a high affinity transporter, followed by (ii) intracellular degradation by a membrane bound amide hydrolase. AEA is taken up by cells via a selective, saturable, temperature-dependent and Na⁺-independent “facilitated transporter” mechanism, catalyzed by the AEA membrane transporter (AMT) [42-45]. Though several properties of this selective carrier have been characterized, its molecular structure remains unknown [6,46,47].

Once taken up by cells, AEA acts as substrate for the enzyme fatty acid amide hydrolase (N-arachidonoylphosphatidylethanolamine-phospholipase D (NAPE-PLD) from membrane-bound phospholipids, and it binds the cytosolic site of vanilloid receptors (TRPV1). Alternatively, AEA can be transported by AEA membrane transporter (AMT) outside the cell, where it binds cannabinoid type-1 (CB1R) and/or type-2 (CB2R) receptors at an extracellular side. CBRs can be located in the same cell or in the neighbouring cells. The action of AEA is terminated by its removal from extracellular space, throughout AMT, and its subsequent hydrolysis to arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH). The latter is bound to intracellular membranes.

Full and functional ES has been found virtually in all body’s districts. Even if brain was the first tissue in which ES was characterized, outside the CNS eCBs seem to play crucial role on the modulation of the autonomic nervous, reproductive, endocrine and immune systems (for reviews, see [58,59]). Within the immune system, THC and other cannabimimetic agents have long been shown to modulate a broad variety of cell functions, generally with suppressive effects. Interestingly, CB1R expression has been documented in immune cells [16-18,60,61], that contain predominantly CB2Rs. These receptors are virtually absent in neurons, and have been recently described also in non-immune cell types such as the cerebromicrovascular endothelial cells [19]. More recently, in several tissues and cells, including the murine spleen, mast cells, dendritic cells, the brain-resident macrophage-like microglia and astrocytes, coexpression of mRNAs for both CB1R and CB2R has also been documented [62-66].

eCBs and their receptors are also present from the early developmental stages, and are therefore likely to be important in the maturation of the nervous system [67]. Fetal brain CB1Rs are functionally active not only in regions which contain CB1Rs throughout life, but also in white matter and in proliferative zones [69]. These findings suggest a role for eCBs in neuronal development [70,71].

As dysregulation of CNS and immune system has a pivotal role in ageing and age-related diseases, in the following sections we will briefly review the modulatory effects of ES in these systems.
NEUROMODULATORY EFFECTS OF ENDOCANNABINOID SYSTEM

eCBs and the proteins that bind, transport and metabolize these compounds form a neurotransmitter system that controls neuronal excitability at the junctions between nerve cells (synapses). In particular, AEA and 2-AG are the only neurotransmitters as yet known to act as retrograde synaptic messengers [72-74]. The postsynaptic neuron releases AEA and 2-AG that diffuse to the presynaptic neuron and that either inhibit or stimulate it [75]. eCBs in the brain seem to be important not only as a neurotransmitter/neuromodulatory system, but also as neuroprotective compounds against the damage of excessive neuronal activity (excitotoxicity) [76]. In fact, protective signaling systems exist that are able to provide on-demand defense in case of abnormally high spiking activity. Exogenous cannabinoids, both natural and synthetic, have been shown to exert neuroprotective functions in several models of neurotoxicity [77-80], and in fact neuronal depolarization increases the production of eCBs [37,62,77,81]. Endogenous activation of CB1R in principal forebrain neurons promotes neuronal survival during excitotoxicity and consistently AEA levels rapidly increase in the kainic acid model of excitotoxicity [82]. CB1R activation is known to induce hyperpolarization of neuronal membranes, mainly by increasing K+ and decreasing Ca2+ conductance [62]. This hyperpolarization, caused by an autocrine or paracrine activation of CB1Rs by eCBs (presumably AEA), would also decrease the L-glutamate release evoked during excitotoxicity [82].

Since the presence of ES in developing brain, activation of CB1R in postnatal rats (7 days old) prevented neuronal loss in a model of acute asphyxia [83], and a remarkable increase of AEA precursors was observed in the infant rat brain after head trauma [84]. These data are consistent with a role for eCBs as endogenous neuroprotectants in the adult brain, and also demonstrate that ES appears to be critical during early CNS development and maturation. In this line, NAPE-PLD activity and expression have been shown to increase with advancing age of the brain [85], though the impact of this increase on eCBs and CNS physiology remains to be ascertained.

In view of the fact that eCBs are present not only within the adult and developing CNS but also in peripheral tissues, the action of these cannabimimetic compounds should not be restricted to synapses. As a matter of fact, eCBs play a role in the control of cell cycle, apoptosis and differentiation of peripheral tissues.

IMMUNOMODULATORY EFFECTS OF ENDOCANNABINOID SYSTEM

From the last two decades, the potential roles of the eCBs in regulating the immune system [58,59,86], inflammation [47,87,88] and neuroinflammation [89] have extensively been investigated and discussed in comprehensive reviews [87,88,90-92].

Although the precise mechanisms of cannabinoid-induced immune modulation are not yet clear, it is largely accepted that most of the cannabinoid effects are mediated through the binding to CB1R and/or CB2R on the immune cells, even if emerging evidence also points to a more indirect action on the hypothalamopituitary-adrenal axis, a known modulator of the immune functions [93,94]. Like in the nervous system, it is has been largely shown that engagement of CBRRs in immune cells initiates complex signaling events, whose immunosuppressive effects are determined by a number of molecular and cellular processes, including (i) inhibition of cell proliferation and activation, (ii) induction of apoptosis, (iii) modulation of cytokine gene expression, and (iv) modulation of hormonal networks. An important immunomodulatory function of the cannabinoids is their well-documented modulation of T helper (Th) cells development. In particular, several in vivo and in vitro studies demonstrated that, in a CBR-dependent manner, THC decreases Th1 responses (cell-mediated immunity) and increases Th2 responses (humoral immunity), likely by differential regulation of the development of Th cells and/or of the expression of cytokine genes [59].

Like cannabinoids, eCBs, and primarily AEA and 2-AG, can be also considered immunomodulators, even if their role in normal homeostasis and development of immune system disorders has been recently questioned. In fact, these compounds have been found to exert various effects on immune cell functions, some of which (e.g. modulation of cytokine release from macrophages and inhibition of lymphocyte proliferation) resemble those of THC, while others (e.g. stimulation of hematopoietic cell proliferation and cell migration) seem to have rather immunostimulatory effects [58,86]. Moreover, based on a set of notable issues, such as relative abundance in tissues, relative binding affinities and agonist activities (reviewed in [95]), it has been recently proposed that 2-AG rather than AEA is the true natural ligand for the CBRs, at least in the immune system. This new view is sustained by recent experimental results showing that CB2R and 2-AG play essential stimulatory roles in inflammation, rather than being suppressive. To further support this hypothesis, there are also a number of findings, describing 2-AG as a chemoattractant. For example, in a macrophage-like cell line, HL-60, it has been found that 2-AG induces major metabolic and physiological changes, such as (i) activation of p42/44 and p38 mitogen-activated protein kinases and c-Jun N-terminal kinase pathways, (ii) actin rearrangement and morphological changes, (iii) release of various pro-inflammatory chemokines and, finally, (iv) migration of HL-60 cells differentiated into macrophage-like cells [95]. The latter effect was observed also in human monocytes, natural killer cells, and eosinophils, while actin rearrangements have been recently reported also in human platelets [96]. The active involvement of 2-AG into cell migration has also been found in myeloid and normal splenocytes [97], and in other different lymphoid lineages, such as macrophage-like cells, HL-60, U937, THP-1 and human peripheral blood monocytes [98], through a CB2R-mediated mechanism. Moreover, another study described that in eosinophils the activation of CB2R by 2-AG, but not by AEA, induces cell to migrate, suggesting that 2-AG may be closely involved in allergic responses by promoting eosinophils infiltration [99]. These data strengthened the notion that 2-AG acts as a chemoattractant and as a chemokine agent via stimulation of CB2R. On the contrary, AEA seems to be ineffective on the stimulation of immune cell migration, or at least in the case of T lymphocytes, it has been described to have inhibitory effects [100].

AGE-RELATED CHANGES OF ENDOCANNABINOID SYSTEM IN THE CENTRAL NERVOUS SYSTEM

Senescence is a physiological process characterized by a slow and progressive impairment of motor capabilities, without symptoms of disease [101]. Ageing is a process that occurs in all members of species, after the time of reproduction, and is distinct from age-related disease. Behavioral research on ageing has demonstrated robust declines in abilities such as encoding new memories of episodes or facts, working memory and processing speed [102-104]. Similar features are also characteristic of normal senescence [105].

A decline in learning and memory ability is due to a dysfunction of the medial temporal lobe structures, while psychomotor performance and motor coordination are neurobiological process associated with different brain areas, such as basal ganglia, hippocampus, cerebellum and limbic nuclei. Based on the distribution of CBR binding and mRNA levels in the brain [106-108], and on the well-known pharmacological effects of plant and synthetic cannabinoids [109], it has been proposed that the ES would be involved in the regulation of motor behaviour, cognition, learning and memory, as well as in brain development [71]. It is interesting to note that the impairment of functions associated with marijuana consumption, in terms of psychomotor performance, immediate memory, and motor coordination, decline with age [110]. As CBRs
are located in brain areas related to those neurobiological processes [106,107], it is likely that the ES could be modulated in CNS during ageing, by analogy with the binding and metabolism of neurotransmitters other than eCBs [111-113]. A possible loss of CB1Rs in aged rats has been proposed in motor areas, such as striatal efferent neurons, which may occur in parallel to the possible progressive degeneration of these neurons during normal senescence. Therefore, a potential use of this system as a molecular marker for normal and pathological ageing could be suggested [114]. Both levels of CB1R binding and agonist-stimulated [35S]GTPγS binding decreased significantly in striatal efferent neurons of aged rats, and this decrease was accompanied by a reduction in CB1R mRNA levels [114]. A marked decrease in CB1R binding was found in the cerebellum of aged rats, but no changes of gene transcripts were detected in the granular layer, where most of the cell bodies of CBR–containing cerebellar neurons are clustered [108]. In other structures, like the Ammon’s horn of the hippocampus, the changes observed during senescence mainly affected gene expression, but not the binding levels [115]. It can be proposed that the ageing-induced decrease in gene expression, although not affecting the basal levels of receptor binding, might originate a plausible deficit of these neurons to respond to a variety of circumstances that demand the de novo synthesis of receptors. By contrast, increased levels of mRNA for CB1Rs have been found in the brainstem of aged rats, an area that contains a relatively low population of CB1Rs [106-108]. However, this up-regulation seems more likely due to the expression of CB1Rs in non-neuronal elements, such as glial cells [115]. This would be similar to what occurs in the developing brain, where transient expression of these receptors in glial cells has been also proposed [26,71,116,117]. It has been reported that the neuronal death that appears during normal and pathological ageing is also associated with the appearance of glial elements that replace dead neurons [118].

Besides psychomotor performance and motor coordination, ES seems to be involved in learning and memory, as demonstrated by long-lasting memory impairments in various learning and memory tasks, and by morphological changes in the hippocampus due to chronic cannabinoid administration to rodents [119-123]. Basal levels of CB1R protein expression in the entorhinal and temporal cortices were increased in aged rats as compared with young adults [124], while there was a significant decrease in CB1R expression in the aged postrhinal cortex. As the entorhinal and postrhinal cortices are important components of the medial lobe memory system, whereas the temporal cortex is a critical auditory structure, it is possible that altered CB1R protein levels may be associated with age-related memory impairments and auditory dysfunction [124]. Further support to a functional role of an altered expression of CB1R in aged brains come from recent findings showing that the age-dependent decline in ethanol preference of rodents may be linked to a parallel decline in eCBs and CB1R signaling [125]. The same mechanism may be involved in the age-dependent decline in food-intake. There is no change in the cellular density of CB1R in different regions of aged brain, while CB1R coupling to G proteins, as measured by agonist-stimulated [35S]GTPγS binding, is reduced in the limbic forebrain of old mice [125]. Therefore, the localized decline of CB1R function may account for the reduced alcohol and food-intake preference.

Although CB1R expression seems to be regulated in several brain areas during ageing, there is no significant difference between young adult and aged mice with respect to the cannabinomeditated inhibition of acetylcholine release in the hippocampus, a function of the CB1Rs which is preserved in aged mice [126].

Taking into account that ES appears to be critical during early CNS ontogenesis, it could be speculated that any deficiency in ES during early CNS development may contribute to premature ageing. In fact, improper functioning of ES during prenatal and postnatal developmental stages has been demonstrated to produce long-term effects in the offsprings [127-129]. Many of these effects are typical of senescence, like disruption of memory, motor behaviors and higher cognitive functions.

CB1Rs are not the only elements of ES that change in aged brain. In fact, age-related changes of AEA metabolism were found in mice carrying a constitutive knockout of the CB1R gene [130]. Old mice, when compared with young mice, showed a significant decrease in hippocampal content of AEA, but not of 2-AG, and this was associated with significant increases in hippocampal and cortical AMT and FAAH activity [131]. The increase of AMT and FAAH activity in ageing animals suggests that AEA degradation might become more critical. In fact, AEA can be converted into noxious hydroperoxy-derivates by lipoxygenase [10,132] and cyclooxygenase [133], which in fact are more active in the brain of ageing animals [134]. A summary of age-related modification of ES can be found in Table 1.

Table 1. Age-Related Changes of Endocannabinoid System

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CB1R Binding</th>
<th>CB1R mRNA</th>
<th>Agonist-stimulated [35S]GTPγS Binding</th>
<th>AEA Content</th>
<th>FAAH</th>
<th>AMT</th>
<th>Refs.</th>
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<tr>
<td>Striatal Efferent Neurons</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>[114]</td>
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<tr>
<td>Cerebellum</td>
<td>↑</td>
<td>=</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>[114]</td>
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<tr>
<td>Ammon’s horn of the hippocampus</td>
<td>=</td>
<td>↓</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>[115]</td>
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<tr>
<td>Brainstem</td>
<td>↑</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>[115]</td>
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<tr>
<td>Postrhinal Cortex</td>
<td>nd</td>
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<td>nd</td>
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<td>[124]</td>
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<tr>
<td>Limbic Forebrain</td>
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<td>nd</td>
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<td>[125]</td>
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<tr>
<td>Hippocampus</td>
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<td>nd</td>
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<td>↓</td>
<td>↑</td>
<td>↑</td>
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<td>Sertoli Cells</td>
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<td>↓</td>
<td>↓</td>
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<td>[135]</td>
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</table>

* down-regulated; ↑ up-regulated; ↓ unchanged; nd not determined.
AGE-RELATED CHANGES OF ENDOCANNABINOID SYSTEM IN PERIPHERAL TISSUES

Very little is known about the modifications of the peripheral ES during ageing. In the context of the reproductive system, the only study regarding the age-dependent modulation of ES has been done in Sertoli cells from 4- and 16-days old mice. In this investigation, an age-dependent down-regulation of the expression and activity of both FAAH and AMT has been described (see Table 1), together with the up-regulation of FAAH by follicle-stimulating hormone [135].

Regarding the age-related changes of ES in the immune system, the studies carried out on this topic described the immunomodulatory effects of THC in cultured lymphoid cells transplanted from spleen and lymph nodes of young, adult and old mice. From these studies, it emerged that cells of different ages respond differentially, in terms of proliferation and cytokine release, when mitogenic stimuli, like concanavalin A, phytoemagglutinin or anti-CD3 antibody, are co-administered together with THC. In particular, when co-stimulated with concanavalin A (or phytoemagglutinin) and THC, cells do not proliferate, independently of their age [136]. In contrast, THC has been found to up-regulate the proliferative response of splenocytes from adult mice, but not from young or old mice, both in vitro and in vivo [136-138]. The stimulation was found to reflect an increase in the number of both suppressor/cytotoxic T cells as well as mitogen-stimulated T cells. Interleukin-2, a Th1 cytokine involved in cell-mediated immunity, was found to be enhanced under these conditions. This up-regulation was not detected in either lymph node cells of adult or young mice, or in spleen cells of young mice. The THC modulating activity was directly related to its effect on both interleukin-2 activity and generation of mitogen-stimulated cells in spleen and lymph node cells of both adult and young mice.

Obviously, in light of these data, it is difficult to identify any clear age-related malfunctioning of the ES in immune cells. It seems more likely that, with time passing, the immune system declines and responds in a different way to cannabinoids. In any case, the knowledge of these changes will be essential for the development of ES-based therapeutics directed to the treatment of the diseases in the elderly people.

PERSPECTIVES FOR AGE-RELATED DISEASES

Ageing is a complex of changes in an organism that progressively and deleteriously leads to a general decline of its biological functions, rendering it more prone to illness and, ultimately, to death. Although it is very difficult to unravel the complexity of this process, that includes genetic and environmental causes, growing evidence points to a pivotal role of the immune system. With the passing of time, our immune system goes towards a slow and inexorable functional decline, generally coupled to a low-grade inflammatory state and, in some pathologies, to an excessive inflammatory condition. This state of local or generalized chronic inflammation (which is accompanied by typical cellular ageing phenomena such as telomeric loss, oxidative stress, DNA defects) damages all the organs, leading in the course of time to the development of age-related diseases, such as osteoporosis, osteoarthritis, atherosclerosis, neurodegenerative disorders and cancer. In this direction, recent data provide important evidence that the increase of pro-inflammatory cytokines such as interleukin-6, and the alteration of innate and adaptive immunity, are a likely key-mechanism through which biological, chemical and psychological chronic stressors may have potent health consequences for older adults, even accelerating the risk of age-related diseases [139]. Moreover, growing evidence suggests that pro-inflammatory genotypes are related to unsuccessful ageing, and, inversely, controlling inflammatory state may allow a better chance of “healthy” ageing. The impressive expansion of cannabinoid research in the past decade provided a large body of data regarding the potential involvement of ES in an ever-increasing number of pathological conditions, including cancer and brain injury (reviewed in [140,141]). For this reason, in the past few years the pharmacological research focused on the development of synthetic compounds that, by modulating the ES, could become potential drugs (Fig. (3)). The role of ES in neuro- and immune-modulation described above implies that, apart from CBR agonists, like THC and WIN55,212-2, other components of the ES could represent suitable targets for designing new therapeutics. In fact, besides selective reverse agonist/antagonists (SR141716A for CB1R or SR144528 for CB2R) a number of inhibitors of AMT (OMDM-1 and UCMI707) or of FAAH (URB597 and methylarachidonyl fluorophosphonate (MAFP)) have been developed (Fig. (3)), and have been useful in ameliorating a variety of pathological conditions (Table 2). All of these compounds, by virtue of their high potency and selectivity, could represent suitable drugs that, depending on the type of disorder, may either prolong the half-life of eCBs, or prevent their formation or action.

In the following section we will review data pointing to a potential therapeutic value of ES in the treatment of some age-related pathologies. In particular we show that, through its well-documented immunomodulatory activity, and together with its neuroprotective and antioxidative effects, drugs that are able to modulate the eCB metabolism might be also effective anti-ageing therapeutics.

Endocannabinoids and Arthritis

Osteoarthritis (OA), the most common of all joint disorders, is a disabling and painful disease whose incidence increases with age. The aetiology of OA is unknown but several mechanisms could be involved including genetic/age-related alterations in extracellular matrix components, biomechanical stresses or an imbalance in synovial homeostasis. The disease is characterized by hypertrophy of the bone and breakdown of the cartilage matrix, followed by development of fibrillations and fissures, which ultimately lead to the arthritic pain. The production of both anabolic and catabolic cytokines and growth factors by the articular chondrocytes and synovial lining cells could contribute to the coexistence of repair and destructive processes in OA joints [142]. It has been observed that cytokine imbalances, particularly Th1-biased immune states, predispose patients to additional risk of developing osteoarthritis as well as osteoporosis, another skeletal condition related to ageing. In fact, increased release of Th1 cytokines such as interleukin-1 and tumor necrosis factor (TNF)-z has been reported to induce cartilage breakdown in patients with arthritis [143-145], whilst Th2 cytokines, such as interleukin-4, interleukin-13, transforming growth factor-ß1 and insulin-like growth factor-1 had protective effects against cartilage degradation [146-148]. Overall, several of these diseases are attributable to a systemic inflammation associated with dysregulation of cytokines network and NO production [149-152]. To date, nonsteroidal anti-inflammatory drugs (NSAIDs) have been the mainstay of the treatment of all forms of arthritis. However, it is generally accepted that NSAIDs relieve the symptoms of arthritis such as pain and swelling without changing the course of underlying disease. Moreover, their use is associated with adverse drug reactions, such as increased risk of ulcer perforation, upper gastrointestinal bleeding, and renal dysfunctions. In the last years, there have been considerable efforts to develop new drugs to prevent and/or contrast molecular events that lead to arthritis state. In the light of the analgesic properties of ES and, particularly, of its well-described influence on Th development, several studies have explored the possibility that this system may be a candidate target for future new arthritis therapeutics. As a matter of fact, some natural and synthetic cannabinoids, devoid of psychoactive and toxic actions, displayed promising anti-arthritic effects [153]. In particular, enhanced signaling of CBRs by WIN55,212-2 (Fig. (3A)) has been recently shown to block NO production in interleukin-1ß-stimulated chondrocytes, as well as interleukin-1ß-induced proteoglycan breakdown in bovine nasal cartilage explants [154]. It has also been found that AEA...
inhibited, even though to a minor extent compared to WIN55,212-2, cartilage degradation, while it had no effects on NO synthesis [154]. These results suggest that the anti-arthritis properties of eCBs might be due in part to the preservation of cartilage matrix integrity through their ability to inhibit NO production and metalloprotease activation, possibly via CBR-dependent mechanisms.
In this line a beneficial therapeutic action of cannabidiol (CBD), see Fig. (1A), the major nonpsychoactive component of cannabis, has been reported on established collagen-induced arthritis, depicting its anti-arthritis potency as a combination of immunosuppression, especially of the Th1 response, and an anti-inflammatory action by way of reducing TNF in the synovium [155]. Apart from these major effects, other in vitro anti-inflammatory effects of CBD, which may contribute to its anti-arthritic action, were demonstrated such as the inhibition of the release of reactive oxygen species (ROS) and NO by stimulated lymphoid cells.

The therapeutic potency of a novel CBD derivative, HU-320 (Fig. (3A)), has also been assessed in a mouse model of arthritis [156]. Clinical improvement was associated with protection of the joints against severe damage. The mode of action of this novel compound has been partially evaluated in vitro, showing that it displays profound suppressive effects on cellular immune responses by inhibiting lymphocyte proliferation, TNF and ROS production from mouse macrophages and RAW 264.7 cells, respectively. Moreover, HU-320 administration has been found to be well-tolerated, yielding no adverse psychotropic effects in mice.

Another synthetic cannabinoid, the ajulemic acid (also known as AjA, CT-3 and IP-751; see Fig. (3A)), has been developed from plant cannabidiol acid as a new nonpsychoactive anti-inflammatory agent [157]. AjA displays, both in vitro and in vivo, analgesic effects [158,159], revealing remarkable anti-arthritic properties. Oral administration of AjA has been found to reduce joint tissue damage in rats with adjuvant arthritis, a well-established model for chronic inflammation and joint tissue injury. The most notable effect of AjA was seen in the histomorphologic evaluation of the tibiotarsal joints at the end of the treatment. While the control group showed moderate to severe changes, the treated rats displayed only mild synovitis with a marked decrease in joint tissue injury [160]. The data thus far available on its mechanism of action suggest a receptor-dependent activation of the transcription factor peroxisome proliferator-activated receptor-γ, followed by down-regulation of pro-inflammatory cytokine production, like interleukin-1β [161] and interleukin-8 [162]. It has also been proposed that its immuno-suppressive effects might be related to induction of apoptosis in T lymphocytes [163]. AjA has a pharmacological profile similar to that of a typical NSAID (reviewed in [164]); however, unlike these compounds, it is not ulcerogenic at therapeutic doses, making it a promising analgesic and anti-inflammatory drug. It is important to note that, since both CBD and AjA are known to be only weak CB1/2Rs agonists, the anti-inflammatory effects displayed by these cannabinoids are likely due to the activation of other receptors, which at least in the case of the CBD-induced anti-hyperalgesy, could belong to the TRPV family [165].

Overall, these data suggest that modulation of ES signaling, for example by ligating classical or still unknown CBRs, may have therapeutic implications for the management of several arthritic disorders that afflict most of the elderly people.

**Cannabinoids and Neurodegenerative Disorders**

Neurological pathologies are another promising field of therapeutic application of ES-related compounds. The aetiology of neurodegenerative diseases is multifactorial and consists of a complex interplay among ageing, environment, and genetic factors. Neuronal cell loss in specific areas of the CNS and the resulting clinical symptoms are used to characterize different neurological syndromes, among which the more common in older people are Alzheimer’s disease (AD) and Parkinson’s disease (PD). These pathologies are caused by the gradual death of specific neurons leading to a progressive impairment in movement control, memory, and thinking abilities. While the selectivity of neuronal cell death is not clearly understood, should be partly due to the physiological role and microenvironment of the impacted cells. In particular, loss of neurons, neuritis and synaptic connections is thought to be mediated primarily by the cytotoxic action of the brain’s immune system, formed by astrocytes and microglia. Under neurodegenerative conditions, these macrophage-like cells, particularly microglia, are overstimulated to a prolonged release of several pro-inflammatory cytokines.
factors, including cytokines, proteases, ROS and excitotoxins, that, at elevated concentrations, are directly responsible for the destruction of the neuronal elements [166,167]. Other proposed mechanisms contributing to neuropathology include excitotoxicity, oxidative stress, impaired energy metabolism, abnormal protein interactions and apoptosis, in which again glial cells seem to play an important role. Of special interest is the contribution of excitotoxicity into neurodegenerative disorders [168]. It has been found that, in the course of exacerbated neuroinflammation, microglia cells release quinolinic acid and glutamate in concentrations that are known to act as excitotoxins [169]. Because glutamate can also activate microglia to enhance its cytokine release, a vicious cycle is created in which immune cytokines can stimulate the release of glutamate, and glutamate in turn enhances cytokine production and release [170]. Moreover, cytokines inhibit glutamate retrotransport, which plays a critical role in removal of excess extracellular glutamate. Intimately linked to excitotoxicity is the generation of destructive free radicals, especially the reactive nitrogen species, like peroxynitrite and nitrosoperoxycarbonate [171].

Since AD and PD, as well as other neurodegenerative disorders, are invariably accompanied with exacerbated neuroinflammation, the molecular mechanisms underlying the production of immune-related mediators released by microglial cells constitute a promising target for therapies aimed at reducing these pathological states. In this context, the role of ES has been explored due to its well-documented immunomodulatory activities.

The first evidence for a physiological role of ES in neurodegenerative disorders came from the finding that, in traumatic brain injury and experimental autoimmune encephalomyelitis (both of which are associated with exacerbated neuroinflammation), there is a substantial increase in production of eCBs in brain parenchyma [172,173]. The increased eCB concentrations, and subsequent signal transduction, is thought to prevent cell injury in multiple ways: (i) on neurons, by inhibiting excitotoxicity [82,173-175]; (ii) on endothelial cells, by inducing hypotension and decreasing oedema [176]; and (iii) on microglial cells and other invading immune cells, by controlling their immune response [177,178]. In the light of these actions, a potential role of the ES in AD (reviewed in [179]) and in PD (reviewed in [180]) has recently attracted great interest, and is currently object of intense investigation. In the last few years, eCB signaling has been found to increase in several animal models of AD and PD: in the hippocampi of beta-amyloid treated rats (a model of AD) and in the basal ganglia of reserpine- or 6-hydroxydopamine-treated rats (two models of PD) [181]. In addition, enhanced eCB signaling has been documented also in the brain and spinal cord of mice with chronic relapsing experimental allergic encephalomyelitis, a model of multiple sclerosis [173]. Although at present it is not possible to establish a cause-effect relationship between these changes and the clinical symptoms of the neurodegenerative disorders, an intriguing issue concerns the effectiveness of an ES re-modulation as potential therapeutic strategy for these pathologies. However, the importance of CB1R signaling for neuron survival appears more complex than imagined so far [182]. It is interesting that, at least in some experimental model of PD, suppression, rather than enhancement, of CB1R signaling by specific antagonist, i.e. SR141716A, has been ascertained to improve clinical symptoms. For example, in 1-methyl-4-phenyl-1,2,3,6-tetrahydroxypridine-treated marmosets, a primate PD model, the blockade of CB1Rs with SR141716A (Fig. 3B) reduced L-DOPA-induced dyskinesia without affecting the anti-parkinsonism efficacy of L-DOPA [183]. Moreover, another study reported that combined administration of quinpirole, a D2 receptor agonist, and SR141716A produced a full restoration of locomotion in reserpine-treated rats [181].

Very recently, some of the components of the ES, i.e. FAAH, CB1R and CB2R, have been measured in postmortem brains from patients with AD. It has been found that both FAAH and CB2Rs are abundantly and selectively expressed in neuritic plaque-associated astrocytes and microglia, respectively, whereas the expression of CB1Rs remains unchanged [184]. These data have provided new evidence for a potential role also of CB2R signaling in AD, speaking in favour of an involvement of the non-neuronal ES in the regulation of AD-associated neuroinflammation. In fact, as proposed by Pazos and colleagues [179], in response to Aβ deposition it is possible that AEA and 2-AG are released from neurons and glia, thus activating CB1Rs and CB2Rs placed on neurons and glial cells, respectively. CB1R ligation should provide protection against excitotoxicity by blocking release of glutamate and activating appropriate intracellular signaling cascades [82,185]. In addition to this, CB1R-dependent anti-excitotoxic action, ligation of CB2Rs located in glia should depress the release of pro-inflammatory cytokines from these cells, thereby reducing the intensity of neuroinflammation and its devastating effects on neurons [186]. Finally, it has been also speculated that the increased FAAH activity in astrocytes serves to release arachidonic acid from AEA, thus leading to an increase in pro-inflammatory arachidonate derivatives, such as prostaglandins and leukotrienes, in the proximity of the neuritic plaques. This model foresees that either CB1R or CB2R agonists, as well as AMT and FAAH inhibitors, could have beneficial effects on the prevention of the degenerative processes linked to Aβ deposition, and also on the release of other pro-inflammatory compounds related to ageing, such as those involved in AD and PD. In particular, inhibitors of FAAH activity have become subject of intense investigation, and a number of interesting reviews appeared in the last year, pointing out that drugs able to enhance the biological activity of eCBs by reducing their degradation may become novel medicines, devoid of the psychotropic effects typically associated with CB agonists [79,187-190]. For example, inhibition of FAAH by MAFP has been found to reduce glutamatergic spontaneous activity in 6-hydroxy-dopamine-lesioned (6-OHDA) animals, suggesting that targeting this specific component of ES signaling may provide a novel approach to treat the abnormal striatal glutamatergic activity observed in PD [190]. Of interest is the fact that the recently resolved X-ray crystal structures of FAAH have revealed that this enzyme possesses a remarkable collection of channels, which seem to grant the simultaneous access of substrates to both the membrane and cytoplasmic compartments of the cell [49,50]. These FAAH channels may represent novel targets for inhibition, possibly inspiring new strategies for the design of inhibitors with higher selectivity towards FAAH, compared to the many other serine hydrolases present in the human proteome [191].

In view of the non-secondary role of reactive oxygen and nitrogen species damages in neurodegeneration, it has been long tested the neuroprotective potency of some natural and synthetic cannabinoids, such as the nonpsychoactive cannabinoic acids, cannabiol and CBD (see Fig. 1A), that, together with an anti-inflammatory action, display also remarkable anti-oxidant properties. In particular, it has been recently reported that CBD displays cyto-protective actions on Aβ-induced toxicity in cultured rat pheochromocytoma PC12 cells [192]. Treatment of these cells with CBD prior to Aβ peptide exposure significantly elevated cell survival, while it decreased ROS production, lipid peroxidation, activated-caspase 3 levels, DNA fragmentation and intracellular Ca²⁺. These results demonstrated that CBD exerts a very promising combination of neuroprotective, anti-oxidative and anti-apoptotic effects against Aβ peptide toxicity.

CONCLUSIONS

An altered balance within the functions of the nervous, endocrine and immune systems characterizes ageing and age-related diseases as schematically depicted in Fig. (4). In particular, the onset of a chronic inflammation state, together with genetic and environmental factors, seems to modify complex interplays among these systems, leading to dysfunctions and even to pathological consequences, which greatly reduce life quality of the ageing peo-
For example, it has been proposed that at an old age the increased risk to develop neurodegenerative pathologies is attributable to the general inflammatory tone associated with ageing, and is characterized by an increase of “early” cytokines, such as interleukin-1, interleukin-6 and TNF [193].

Although it is not yet known how this functional alteration of the immune system takes place, its origin has probably to be searched in the entropic decline associated with ageing, which inevitably and irreversibly damages the homeostatic networks of the whole organism. In the context of these complex regulatory mechanisms, eCBs may play an important role by virtue of their well-known neuro- and immuno-modulatory effects, inside and outside the CNS. From the complex picture on the properties and functions of the ES, that we have summarized in the present review, the possibility emerges that this system is involved in the control of some relevant physiological phenomena including neuronal excitability and immune response. To this end, ES should be placed at the interface among neuronal, immune and endocrine systems where it may dynamically regulate the intensity and/or the duration of specific activities (Fig. (4)). In our simplified model, the ligation of CBRs present on these cells leads to a generally negative regulation of inflammation and excitotoxicity, two of the potentially most dangerous activities associated with the normal functioning of the immune and nervous systems, respectively. This picture underlines the protective role that eCBs exert against ageing-promoting processes, although 2-AG has recently been shown to act as a pro-inflammatory mediator, and although the real nature of the ES/endocrine system interplay needs to be still clarified.

To date, only few clinical data exist regarding the potential therapeutic applications of ES modulation on ageing and age-related diseases, yet it can be anticipated that a better understanding of how this complex regulation takes place, both at molecular and cellular level, will be of extreme importance also for the design of new therapeutics for the management of ageing and age-related diseases.

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ABBREVIATIONS

Aβ = β-amyloid peptide
AD = Alzheimer’s disease
AEA = Anandamide (arachidonoylethanolamide)
2-AG = 2-arachidonoylglycerol
AjA = Ajulemic acid
AMT = AEA membrane transporter
CB1R = Type-1 cannabinoid receptor
CB2R = Type-2 cannabinoid receptor
CBD = Cannabidiol
CBR = Cannabinoid receptor
CBxR = Non-CB1/CB2 receptor
CNS = Central nervous system
DAG = 2-Arachidonate-containing diacylglycerol
DAGL = DAG lipase
eCB = Endocannabinoid
ES = Endocannabinoid system
FAAH = Fatty acid amide hydrolase
FAK = Focal adhesion kinase
GPCR = G-protein coupled receptor
GTPyS = Guanosine-5’-O-(3-thiotriphosphate)
MAFP = Methyl-arachidonoyl fluorophosphonate
MAGL = Monoacylglycerol lipase
MAPK = Mitogen-activated protein kinase
NADA = N-arachidonoyldopamine
NAPE = N-acyl-phosphatidylethanolamine
NarPE = N-arachidonoyl-phosphatidyl ethanolamine
NO = Nitric oxide
NOS = Nitric oxide synthase
NSAID = Non-steroidal anti-inflammatory drugs
OA = Osteoarthritis
OEAs = N-oleoylethanolamine
OHDA = 6-hydroxy-dopamine
PD = Parkinson’s disease
PEA = N-palmitoylethanolamine
PLD = Phospholipase D
ROS = Reactive oxygen species
SEA = N-stearoylethanolamine
Th = T helper cells
THC = Δ⁶-tetrahydrocannabinol
TNF = Tumor necrosis factor
TRPV1 = Transient receptor potential channel vanilloid receptor subunit 1

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