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A brief history of cannabinoid and endocannabinoid pharmacology as inspired by the work of British scientists

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British scientists have played a leading role in the long history of cannabinoid and endocannabinoid research. Such research has progressed from the first crucial evaluation of the medicinal properties of *Cannabis sativa* in the Western world to pioneering studies of the chemical constituents of this plant, the development of *in vitro* biological assays to study cannabinoids, the identification of the mechanism of action of cannabinoids, the discovery of endocannabinoids and the assessment of their therapeutic implications. Stemming from the many innovative ideas and achievements of these researchers, I provide a personal view of where these studies have led us thus far and where they are likely to take us in the future.

The origins of cannabis research

Recently, the possible therapeutic applications of *Cannabis sativa* have been the subject of so many heated discussions that they have almost effaced the revival of the recreational use of this drug during the 1960s. Although used widely for millennia in Far Eastern folklore medicine, probably only few people know that it was mostly through the work of Sir William B. O'Shaughnessy, an Irish physician working in Calcutta, that the therapeutic value of cannabis was assessed scientifically for the first time and publicized in the Western world in the early 19th century [1]. In the words of Ethan Russo [2], 'His contribution was a model of modern investigation […] finally culminating with a series of human clinical trials with appropriate cautious dose titration. His treatise on the subject demonstrated the apparent clinical utility of cannabis in a range of disorders including cholera, rheumatic diseases, delirium tremens and infantile convulsions.' Indeed, O'Shaughnessy's seminal work probably marks the beginning of 'cannabis research', which became termed 'cannabinoid research' in the second half of the 20th century and evolved eventually into 'endocannabinoid research' (Figure 1). This work also exemplifies the important contributions of British scientists to this field. I use these contributions as an inspiration to discuss how our knowledge of the endocannabinoid signalling system (Figure 2) was generated in the late 1990s, and how it is taking us towards new frontiers in pharmacology and therapeutics.

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'Cannabinoid chemistry': the pioneers

The mechanism of action of cannabis would not have been discovered, and its possible therapeutic exploitation never realized, if its active principles had not been purified and characterized chemically [3]. In pioneering work, Thomas Wood and colleagues were the first to isolate cannabinol, and Robert Cahn elucidated most of its structure. Based on these studies, Lord Alan Todd, a giant in organic chemistry and a Nobel Laureate, completed the first synthesis and, hence, the full chemical elucidation of this compound in the UK [4] simultaneously with Roger Adams and colleagues in the USA [5]. Although we now know that cannabinol is not the most pharmacologically interesting constituent of cannabis, and is mostly an oxidation product of other cannabinoids, this discovery opened the way to the isolation, characterization and full chemical synthesis of the most important members of this family of natural products. These include, but are not limited to, (–)-Δ^9-tetrahydrocannabinol (THC), which is responsible for the psychotropic activity of cannabis [6] and thus has attracted most of the attention since its discovery, and cannabidiol (CBD), which, therapeutically, might be even more promising than THC [3,6,7]. However, the identification of THC was not followed immediately by the full understanding of its molecular mechanism of action: more than two decades of synthetic chemistry, pharmacological efforts, and the development of the modern techniques of molecular biology were needed before the first THC-binding site, the cannabinoid CB_1 receptor, was identified from several previously cloned 'orphan' G-protein-coupled receptors (GPCRs) (Figures 1,2) [8]. In 1993, 3 years later, a second cannabinoid receptor subtype was cloned by homology at the Medical Research Council in Cambridge, UK by Sean Munro and co-workers [9]. By contrast, the specific molecular target(s) of CBD still await(s) identification [7].

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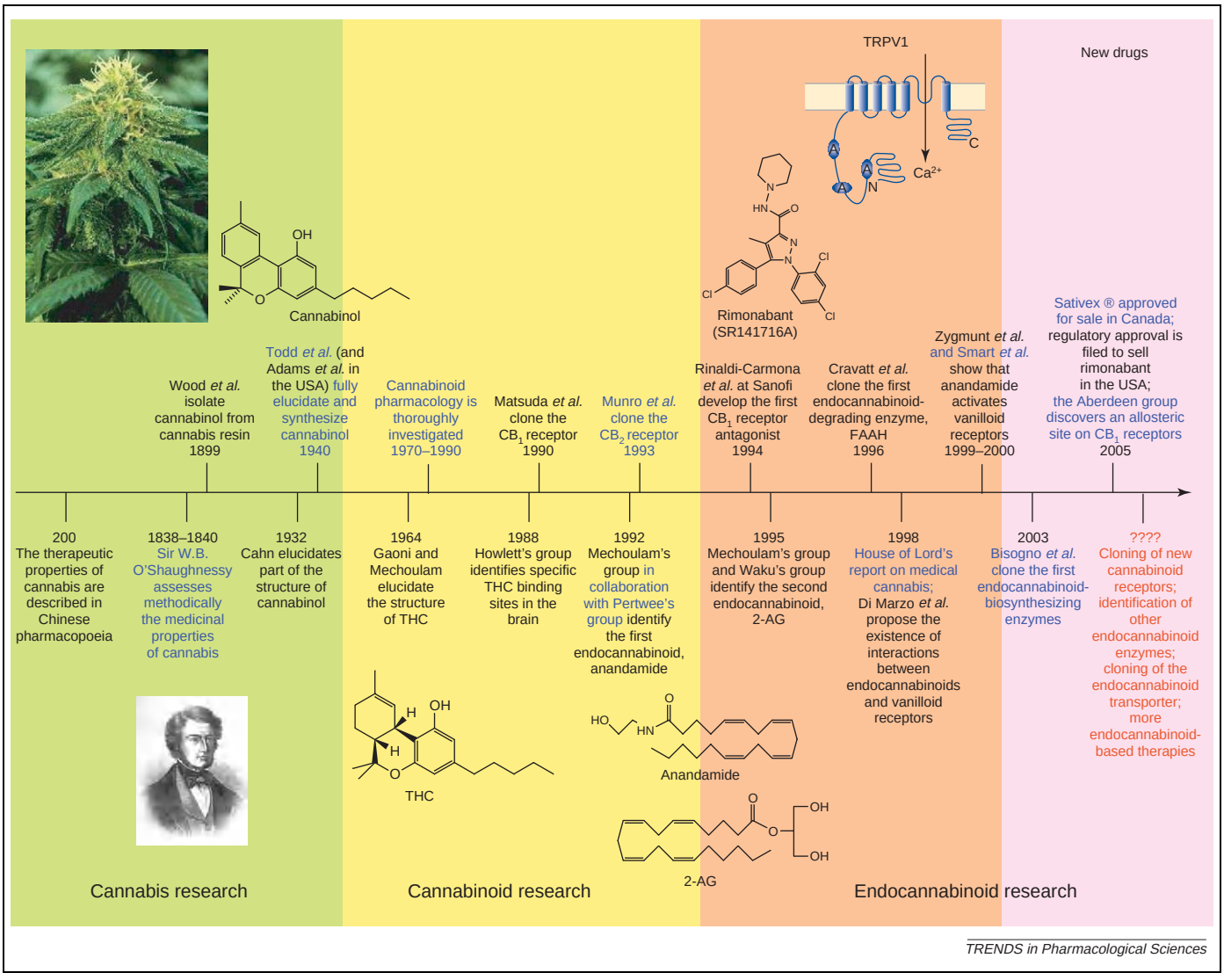

Figure 1. Major breakthroughs in the recent history of cannabis, cannabinoid and endocannabinoid research. Employed in the Far East for medicinal, recreational and ritual purposes since the third millennium Bc, *Cannabis sativa* and its components have been studied intensively in the Western world from the 19th century onwards. Research has moved progressively from the plant to its components, their receptors and their endogenous counterparts in mammals. The development of first cannabinoid-based medicines, Marinol® and Cesamet®, which were developed in the 1980s and are still used to treat nausea and weight-loss in patients with cancer and AIDS [6], has been followed by that of the first cannabis- and endocannabinoid-based medicines, Sativex® and rimonabant. The contributions of British scientists are highlighted in blue. The chemical structures of cannabinol (the first cannabinoid to have its structure elucidated) [4,5], (–)-Δ^9-tetrahydrocannabinol (THC) (the main psychotropic component of cannabis) [66], rimonabant (the first CB_1 receptor antagonist–inverse agonist to be developed) [43,44], the two endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) [11–13] and the two-dimensional structure of the TRPV1 channel [67] (which is part of the endocannabinoid system) [59,61] are shown. Possible further advances are predicted in red. Abbreviation: FAAH, fatty acid amide hydrolase.

'Cannabinoid biochemistry': a lesson from the endorphins

In the 1970s two pharmacologists at Marischal College in Aberdeen, Hans Kosterlitz and John Hughes, postulated that if the mammalian brain makes receptors for morphine, as suggested 2 years earlier by Pert and Snyder in the USA, it should also make their ligands. Such receptors would not have been selected by evolution merely to be activated by a plant product. Kosterlitz and Hughes were correct because they later discovered and, together with Howard Morris in London, characterized the enkephalins, the first two endogenous agonists of the then uncloned opiate receptors [10]. Later, several other endogenous 'morphine-like' compounds were identified, and the general name endorphin (from endogenous and morphine) was proposed for this family of neuropeptides by the French-born, American neuroendocrinologist and Nobel laureate, Roger Guillemin. This lesson was later well received by Raphael Mechoulam and his group in Jerusalem, who also believed that the CB_1 receptor, which is possibly the most abundant GPCR in the mammalian brain, is activated by endogenous agonists. However, the isolation and chemical identification of anandamide, the first endogenous ligand of CB_1 receptors to be identified (Figures 1,2), proved to be difficult because of the lipophilic nature of this compound [11]. Mechuolam asked Roger Pertwee (see later) at Marischal College to establish whether anandamide exhibited THC-like behaviour in selected bioassays [11]. Later, these two groups and, independently, a Japanese team identified a second endogenous ligand of CB_1 receptors and CB_2 receptors, 2-arachidonoylglycerol (2-AG) (Figures 1,2) [12,13].

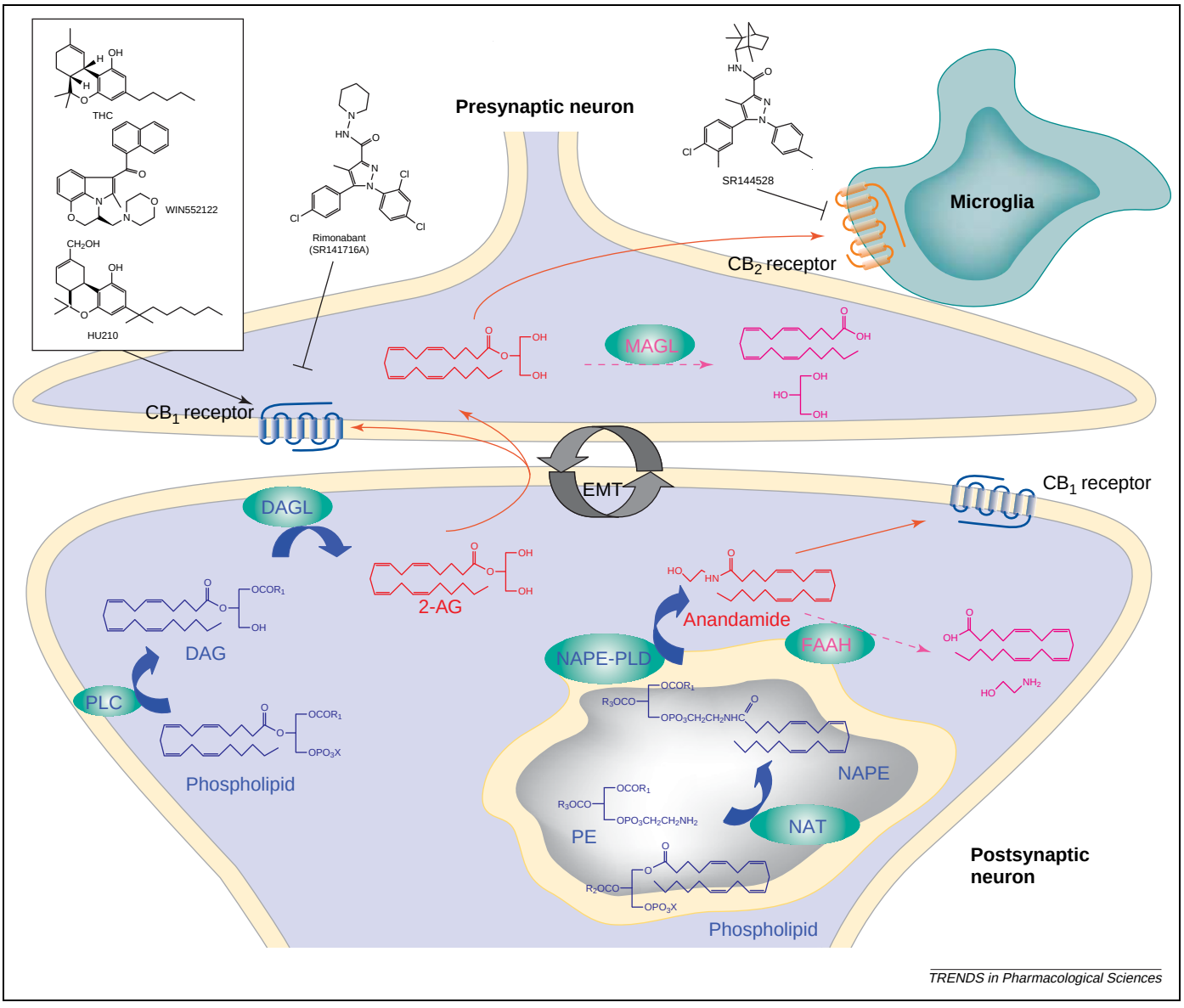

Figure 2. The endocannabinoid system in the brain. Of the ~66 cannabinoids isolated from *Cannabis sativa* [3], (–)-Δ^9-tetrahydrocannabinol (THC) [66] is responsible for most of the psychotropic activity of this plant, mainly by interacting with cannabinoid CB_1 receptors in the brain. The identification of CB_1 receptors, which have the highest density in the hippocampus, basal ganglia, cerebellum and cerebral cortex, paved the way to the discovery of the endocannabinoids. Synthetic agonists of cannabinoid receptors, such as WIN552122 and HU210, and antagonists–inverse agonists such as rimonabant and SR144528, which are selective for CB_1 and CB_2 receptors, respectively [38,43], have been developed. The metabolic pathways of the two major endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG) are shown, with their most likely localization in presynaptic and postsynaptic neurons [68,69]. CB_1 receptors are depicted as both presynaptic (the most frequent localization) and postsynaptic. CB_2 receptors do not appear to be present in most CNS neurons but they are expressed by microglial cells, particularly during neuroinflammation, and are activated by 2-AG and, less effectively, by anandamide [6]. However, recent studies carried out in Canada by the British-born pharmacologist Keith Sharkey and collaborators reveals the presence of functional CB_2 receptors in brainstem neurons [70]. Anandamide biosynthesis occurs from a phospholipid precursor, *N*-arachidonoyl-phosphatidylethanolamine (NAPE), which is synthesized from phosphatidylethanolamine (PE) and another phospholipid by an *N*-acyl-transferase (NAT). NAPE is then hydrolyzed to anandamide by a specific phospholipase D (NAPE-PLD) [18,22]. These enzymes are localized in intracellular membranes, although it is not known whether they are presynaptic or postsynaptic. The biosynthesis of 2-AG occurs through the formation from phospholipids of a diacylglycerol (DAG) precursor, which is catalyzed by a phospholipase C (PLC), followed by the hydrolysis of DAG by DAGLs [21]. Similar to PLC, DAGLs are in the plasma membrane (postsynaptic in the adult brain and presynaptic in the developing brain) [21]. Degradation of anandamide by fatty acid amide hydrolase (FAAH) occurs postsynaptically at intracellular membranes [20] [68,69], whereas degradation of 2-AG by monoacylglycerol lipases (MAGLs) [71] occurs presynaptically in the cytosol and at intracellular membranes. Endocannabinoids diffuse through the plasma membrane depending on their intracellular–extracellular concentration gradient by an endocannabinoid membrane transporter or binding protein (EMT) that is still to be characterized [72]. The endocannabinoid system is a regulatory apparatus that is present in the brain and most of the tissues and organs that have been studied. It is activated 'on demand' to re-establish transient perturbations of the homeostasis of other mediators (such as neurotransmitters, hormones and cytokines) whose levels and actions are modulated by endocannabinoids and which, in turn, control endocannabinoid levels [68,69]. The activation and malfunction of this system in numerous tissues and organs is involved in many physiological and pathological conditions, and provides targets for the development of new therapeutic drugs [69,72]. Solid arrows denote either activation or movement, blunted arrows denote antagonism, thick blue arrows denote enzymatic reactions and dashed arrows denote degradation pathways. Abbreviation: X, phospholipid base.

More recently, the same role has been proposed for other arachidonate-derived compounds. A later contribution from British pharmacologists to the biochemistry of cannabinoids came when David Kendall and co-workers at the University of Nottingham gained evidence that oleamide, a previously identified endogenous sleep-inducing lipid [14], might also act as an endocannabinoid [15]. Although controversial because of the many molecular

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targets that have been proposed for oleamide, this finding might increase in importance because recent experience with anandamide indicates that endocannabinoids, similar to other lipid mediators, are 'promiscuous' and interact with several types of receptors [16].

In 1995, in recognition of the inspirational role of endorphins on the discovery of endogenous agonists of cannabinoid receptors, I proposed the name endocannabinoids for these compounds [17]. From this came the term 'endocannabinoid system' to identify the ensemble of cannabinoid receptors, their ligands, and the enzymes and proteins that regulate ligand concentrations (Figure 2). The identification of endocannabinoids was not the last step in determining the function of cannabinoid receptors because understanding how the tissue concentrations of these compounds are regulated under physiological and pathological conditions was also needed. Several groups then started to develop quantitative analytical methods to measure endocannabinoids and to identify the mechanisms of their biosynthesis and degradation [18,19]. Finding the enzymes that catalyze these reactions [20–22] was crucial for the subsequent development of selective pharmacological inhibitors that regulate the endocannabinoid concentrations and hence, indirectly, the activity of cannabinoid receptors (Figure 2). Whereas a multidisciplinary effort led in 1996 to the purification and cloning of fatty acid amide hydrolase, the main enzyme responsible for the metabolism of anandamide and oleamide and, to some extent, 2-AG [20], the molecular characterization of the enzymes that synthesize endocannabinoids seemed to lag behind for some years. It was clear that the biosynthesis of 2-AG required a *sn*-1-selective diacylglycerol lipase (DAGL). This enzyme had been studied previously because it is involved in the breakdown of diacylglycerols and in regulating the activity of protein kinase C. However, it was only identified when Pat Doherty, a cell biologist at King's College in London, became interested in this field during studies of the molecular mechanisms by which fibroblast growth factor 2 (FGF-2) induces axon sprouting in the brain during development. Doherty and co-workers were convinced that 2-AG and CB_1 receptors were important intermediates in the effects of FGF-2 and they used a bioinformatics approach and knowledge of the genome sequence of many species, including some human chromosomes, to identify and express two putative DAGLs. They then asked my group to provide biochemical and pharmacological evidence that these were the long-sought enzymes that biosynthesize 2-AG. Indeed, we showed that they are [21]; they were also the first endocannabinoid-biosynthesizing enzymes to be identified, and were followed soon by the cloning of a possible anandamide-biosynthesizing enzyme (Figure 2) [22]. This opened the way to the future development of specific inhibitors of endocannabinoid biosynthesis.

'Cannabinoid pharmacology': British researchers at their best

Thanks to its long-standing, prestigious tradition, British pharmacology has contributed most to advancing cannabinoid and endocannabinoid research. In the 1970s Sir William Paton and his group at Oxford University pioneered the study of the pharmacological actions of cannabis and cannabinoids, and were among the first to investigate the analgesic and sedative properties of THC and its metabolism in mammals [23,24]. Paton was not convinced of the existence of specific cannabinoid receptors, favouring the hypothesis that THC acted in a similar, but not identical, way to general anaesthetics: 'Should one think of a cannabinoid receptor? I prefer the idea of hydrophobic spaces in membranes or macromolecules, the average shape and dimensions of which limit their capacity to accept larger lipophilic molecules…' [23]. Now, we know that interaction with CB_1- and CB_2-receptor-binding sites explains most, but not all, of the pharmacological actions of THCs [6], and that the plasma membrane has an important role in determining that cannabinoids assume the optimal conformation for interaction with their receptors [25]. With such a master in his background, Roger Pertwee also made fundamental contributions to the field. He used an assay of his own design, mouse immobility on a 'ring', to evaluate catalepsy and confirm that THC is the main psychoactive constituent of cannabis. For many years this assay has formed part of the 'tetrad' of behavioural assays in mice that was developed by Billy Martin in the USA to assess the cannabimimetic properties of compounds. After moving to Aberdeen Pertwee provided evidence that THC lowers body temperature by inducing animals to adjust heat-gain and heat-loss both autonomically and behaviourally. He also found that cannabinoids interact synergistically with benzodiazepines and identified the globus pallidus as a site where THC alters motor function [26]. This discovery was crucial when Jonathan Brotchie's group, at that time at the University of Manchester, and my group showed that elevated concentrations of endocannabinoids in this brain area might underlie some of the symptoms of Parkinson's disease [27]. Opioid research served again as an inspiration when the mouse isolated vas deferens assay was used to test cannabimimetic compounds. The availability of this preparation in Kosterlitz's laboratory in Aberdeen facilitated the discovery by Pertwee that it provides a sensitive and quantitative assay for agonists of CB_1 receptors. This assay, and others, were used later in Pertwee's laboratory to provide evidence that anandamide is an endogenous cannabinoid [11] and to test fundamental pharmacological tools with unique features (e.g. the first agonists selective for CB_1 receptors versus CB_2 receptors [28], the first water-soluble CB_1 receptor agonist [29], and one of the first neutral CB_1 receptor antagonists [30] to be developed). It was also used to investigate the mechanism of action of other plant cannabinoids, such as Δ^9-tetrahydrocannabivarin and cannabidiol, which act, in part, by antagonizing CB_1 receptors [7,31,32]. The 'Aberdeen school' as a whole has been very active in this field and has contributed to the understanding of the function of the CB_1 receptor in, for example, the functions of sensory and central neurons [33,34], the gastrointestinal tract [35], and the regulation of bone mass, osteoblasts and osteoclasts [36], and by identifying an allosteric site on this protein [37]. This latter discovery, again from

Pertwee's laboratory, plus the earlier cloning of enzymes that biosynthesize and metabolize endocannabinoids [20–22], provide a way to manipulate the concentration and action of endocannabinoids. Thus, they are a possible alternative to cannabinoid receptor agonists and antagonists for regulating the activity of CB_1 and CB_2 receptors [38].

The vasodilating effects of endocannabinoids were also investigated initially in the UK, thus giving rise to one of the most complex, unsolved conundrums of cannabinoid pharmacology. In 1998 Michael Randall and his collaborators at the University of Nottingham proposed that anandamide might be the long-sought endothelium-derived hyperpolarization factor [39]. This hypothesis was based on the observation that endothelium-dependent and nitric oxide-independent vasorelaxation of the rat mesenteric artery is blocked by a CB_1 receptor antagonist, and that endothelial cells produce an anandamide-like lipid. However, the hypothesis did not hold up when challenged by subsequent experiments by several groups, including that of Randall [40]. Nevertheless, it did generate a tremendous interest in the vasodilatory properties of anandamide. Also thanks to the work of other British teams, we now know that these properties result from several distinct mechanisms, which are both endothelium-dependent and endothelium-independent [41]. These mechanisms include the participation of putative new endothelial receptors, vanilloid receptors on perivascular neurons (see later), K^+ channels, Ca^{2+} channels, gap junctions and the formation of arachidonate metabolites. These studies provided the first hint of the 'promiscuity' of anandamide, which interacts with many molecular targets at submicromolar concentrations [16], and opened the way to the discovery of new cannabinoid receptors [42].

If asked to give an example of a new, synthetic drug that is likely to result from cannabinoid research, I would answer rimonabant, the first selective antagonist–inverse agonist at CB_1 receptors. This compound was developed in 1994 by Sanofi in France (now Sanofi–Aventis) [43] and might be soon on the market to treat obesity and related metabolic disorders [44]. Yet again, it was a British pharmacologist, Tim Kirkham at the University of Reading, who showed first that the well-known stimulatory effect of THC on food intake is reproduced by anandamide and antagonized by rimonabant and, hence, mediated by CB_1 receptors [45]. Two years later this finding led to studies showing that rimonabant might suppress food intake in obese mice by antagonizing the permanent upregulation of endocannabinoids in the hypothalamus, rather than by acting as an inverse agonist [46].

'Cannabinoid therapeutics': the future?

In 1998, following in O'Shaughnessy's path, and with the purpose of enquiring whether there was a sound basis for the renewed interest in possible therapeutic effects, the House of Lords ordered a scientific report on the potential advantages and disadvantages of the medical use of cannabis and cannabinoids. Together with some of the scientists mentioned above, the British pharmacologist Leslie Iversen was invited as a specialist advisor and reporter at scientific conferences on this subject. The report (available at http://www.parliament.the-stationery-office.co.uk/pa/ld199798/ldselect/ldsctech/151/15101.htm) was thorough and objective, and might be seen as an update of the 12-volume report on cannabis published by the British Government in India at the end of the 19th century [2]. Together with a real-time picture of the status of medical research into cannabinoids, it provides a series of useful recommendations, many of which have been translated into appropriate actions by institutions in the UK and overseas. For example, the recommendations that 'clinical trials of cannabis for the treatment of multiple sclerosis (MS) and chronic pain should be mounted as a matter of urgency' and 'research be promoted into alternative modes of administration (e.g. inhalation, sublingual, rectal) which would retain the benefit of rapid absorption offered by smoking, without the adverse effects' have produced promising results. The first rigorous experimental assessments of the beneficial actions of cannabinoids against the symptoms of MS were carried out in animal models and humans by British scientists. David Baker and his co-workers at University College London showed that stimulation of CB_1 receptors alleviates spasticity and tremors in mice with chronic relapsing experimental allergic encephalomyelitis [47]. Furthermore, the largest clinical study on a cannabinoid and a cannabis extract, which assessed their potential for the treatment of spasticity and other MS-related symptoms, originated from a collaboration between John Zajicek in Plymouth and Alan Thompson in London. The trial [48] and particularly its 12-month follow-up [49] indicated that a well-tolerated dose of THC produces subjective and, following a more prolonged treatment, objective alleviation of spasticity and other symptoms.

Analgesia, another potential therapeutic application of cannabinoid-based drugs that is supported by preclinical studies, has been investigated thoroughly in the UK by two groups in particular. Andrew Rice and co-workers at Imperial College London were among the first to investigate the actions of anandamide in visceral and somatic inflammatory pain [50]. Victoria Chapman and her collaborators at the University of Nottingham studied how cannabinoid receptor agonists and antagonists modify the behaviour of sensory neurons exposed to chronic and inflammatory pain stimuli [51]. The findings of these teams have been pivotal in establishing a role for the endocannabinoid system in the control of inflammatory and chronic pain, and in generating a rationale for the clinical study of cannabinoids in these conditions [52,53].

The recommendation of the House of Lord's report to develop alternative modes of administration was followed in the UK by Geoffrey Guy and his firm, GW Pharmaceuticals, whose innovative strategy was to select cannabis strains with well-determined contents of THC and CBD, prepare cannabinoid-enriched extracts from the flowers of the plant, and administer them in the form of oromucosal sprays. Three medical preparations, which differ in the ratio between THC and CBD, are being tested in clinical trials. Initially, these aimed for analgesia in several pathological conditions while allowing patients to self-titrate to the most efficacious, best-tolerated dose. In four

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Phase III clinical trials on MS patients in the UK, Sativex® (which contains a ~1:1 ratio of THC:CBD) is well tolerated and efficacious against spasticity, lower urinary tract symptoms and neurogenic symptoms that are unresponsive to standard treatments, and had no significant adverse effects on either cognition or mood and only mild intoxication [53–56]. The recent news that Sativex® has been granted approval in Canada for the symptomatic relief of neuropathic pain in MS raises expectations that it marks the start of what, in Raphael Mechoulam's words [3], has been 'the dream of several generations of scientists'.

Concluding remarks: a soft spot for the 'British approach'

From this brief historical overview one might get the impression that most of the major breakthroughs in cannabinoid and endocannabinoid research were achieved only thanks to British pharmacologists. However, this is obviously not true because progress in science is never the result of the efforts of one group, one discipline or one country. Many of the milestones depicted in Figure 1 have been built by the painstaking efforts of chemists, biochemists, pharmacologists and clinicians from all over the world. However, although my judgement might be biased by my past pleasant experience in a UK laboratory, it is undeniable that research on cannabinoids and the endocannabinoid system 'beyond the channel' has added a special flavour to this field. In the UK, the pragmatism and objectivity with which a socially and politically delicate matter such as the medical use of cannabis has been dealt with have been accompanied by a general open-mindedness, which is a prerequisite when working at the frontiers of any scientific discipline. A typical example of this absence of dogmatism is offered by the attitude with which British researchers approached the hypothesis that anandamide might be both an endocannabinoid and an agonist of the receptor for capsaicin, the pungent principle of chilli peppers [57,58]. Although initially declared *a priori* devoid of either physiological or pathological significance by some opinion leaders in the cannabinoid field, the stimulatory action of anandamide on transient receptor potential vanilloid type 1 (TRPV1) receptors has been investigated thoroughly by UK pharmacologists, who have contributed >20% of the >200 articles published on this subject during the past 7 years. For example, studies by researchers from GlaxoSmithKline in Harlow and academic institutions in Nottingham, Aberdeen and London have contributed significantly to our understanding that this intriguing phenomenon might contribute to the multifaceted functions of anandamide in nociception and local vasodilatation, particularly during inflammatory conditions [58–65].

The dedicated effort of British scientists in performing and fostering research in all aspects of the endocannabinoid system is also witnessed by their continued participation in the annual meetings of the International Cannabinoid Research Society (http://www.cannabinoidsociety.org), and by the successful series of reviews on this subject that have been published recently by the *British Journal of Pharmacology*, the official journal of the British Pharmacological Society, who selected the topic of cannabinoids for one of its Special Interest Groups. This effort will continue in the future and I and many others in this field are looking forward to its outcome.

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