Endocannabinoids and multiple sclerosis: a blessing from the 'inner bliss'?

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The Chinese emperor Huang Ti certainly did not suspect that the *Cannabis sativa* preparations he advised for the treatment of, among other things, rheumatic pains, migraine and constipation[1] had, in his own body, endogenous counterparts that are potentially capable of exerting similar beneficial effects. Indeed, more than four millennia passed before two receptor subtypes[2,3] for the psychoactive principle of cannabis, Δ^9-tetrahydrocannabinol (THC)[4], were discovered. Soon afterwards, endogenous THC-like molecules, the 'endocannabinoids'[5], were isolated, of which anandamide (from the Sanskrit word 'ananda' for 'inner bliss'[5]) certainly bears the most picturesque name. In a recent report, David Baker and colleagues[6] presented evidence that both exogenous and endogenous cannabinoids alleviate spasticity and tremors in an animal model of multiple sclerosis (MS), which finally supports previous anecdotal reports[7] that the use of cannabis could ameliorate the symptoms of this disorder.

Baker *et al.* used mice with chronic relapsing experimental allergic encephalomyelitis (CREAE), which can be induced by the injection of homogenates of mouse spinal cord in Freund's complete adjuvant. This treatment causes demyelinization and axonal loss of nerve fibres, which results in a disorder that bears several similarities to MS, including relapsing–remitting paralytic episodes, and tremor and spasticity of limb muscles during post-relapse remission. In particular, in this model, tremors occur during voluntary movement after sudden disturbance of the animal and with higher frequency than in MS. Apart from limited clinical studies that claim that THC analogues could ameliorate the symptoms of MS (Ref. 8), several other clues, including the neuromodulatory actions of cannabinoids[9] and the beneficial effects of these compounds on dyskinesia in animal models of Parkinson's disease[10], suggested that activation of cannabinoid receptors could improve tremor and spasticity in CREAE. In their study, Baker and co-workers[6] showed that intravenous administration of THC and, at lower doses, *R*(+)-WIN552122, a potent synthetic agonist of CB_1 and CB_2 receptors (the two cannabinoid receptor subtypes identified so far[2,3]), rapidly decreased both the frequency and amplitude of tremors in limbs of mice with this disease. Moreover, these compounds also reduced hind-limb spasticity in animals with CREAE, as assessed by measurement of hind-limb resistance to flexion. Two lines of evidence suggest that these two beneficial effects are mediated by cannabinoid receptors. First, the *S*(−)-enantiomer of WIN552122, and cannabidiol, which are both very weak agonists at CB_1 and CB_2 receptors, did not reduce spasticity. Second, SR141716A, which is a selective CB_1 receptor antagonist, and SR144528, which is a selective CB_2 receptor antagonist, prevented *R*(+)-WIN552122 from inhibiting tremor.

These findings are very important because they: (1) represent the first report that cannabinoids alleviate the symptoms of CREAE, as assessed by measurement of specific and well-controlled parameters of this disease; and (2) might lead to novel strategies for the treatment of MS-induced tremor and spasticity, for which no efficacious remedy has yet been developed. The report by Baker *et al.*, however, also raises several questions, only a few of which could be addressed in their study. First, the respective roles of CB_1 and CB_2 receptors in these beneficial effects of cannabinoids are not completely understood. Although there is no sound evidence that CB_2 receptors have a functional role in the CNS (Ref. 11), these receptors do appear to be involved, at least in part, in the control of spasticity exerted by *R*(+)-WIN552122. This hypothesis was also supported by the finding that the CB_2-receptor-selective agonist JWH133, at doses that are unlikely to activate CB_1 receptors, also reduces spasticity in mice with CREAE (Ref. 6). Because CB_2 receptors are abundant only in blood leukocytes[3] and microglia[12], the role of these cells, as well as of brain-resident mast cells, in the generation of MS symptoms needs to be clarified. Moreover, the development of CB_2-receptor knockout mice will offer the possibility of examining further the participation of these receptors in the effects described by Baker and colleagues. Another issue that needs to be addressed is whether and how the many actions mediated by CB_1 receptors at the cellular level, such as the modulation of the release and action of neurotransmitters[9], explain their involvement in the improvement of CREAE-induced tremor and spasticity. In particular, mechanisms analogous to those that underlie exogenous and endogenous cannabinoid-induced generation of symptoms of Parkinson's disease in animal models (e.g. enhancement of GABA-mediated neurotransmission in the basal ganglia[7,10,13]) might be worth exploring.

A crucial point that deserves further investigation, at least if therapeutic applications are to be developed, is the full assessment of the possible psychotropic side-effects of intravenous cannabinoids. These compounds were used by Baker and co-workers at concentrations (5–10 mg kg^{-1})[6] that were previously shown to induce strong neurobehavioural activity in mice[14]. Apart from the above-mentioned action of the CB_2 receptor agonist JWH133, two other findings of the authors provide encouraging information regarding the possible development of drugs that are useful for the alleviation of the symptoms of MS without undesired 'central' effects. First, the observation that methanandamide, a CB_1-receptor-selective and metabolically stable analogue of the endocannabinoid anandamide[5], was almost as potent as *R*(+)-WIN552122 against hind-limb spasticity in mice with CREAE. This finding implies that drugs based on endocannabinoids, which have been reported to have very

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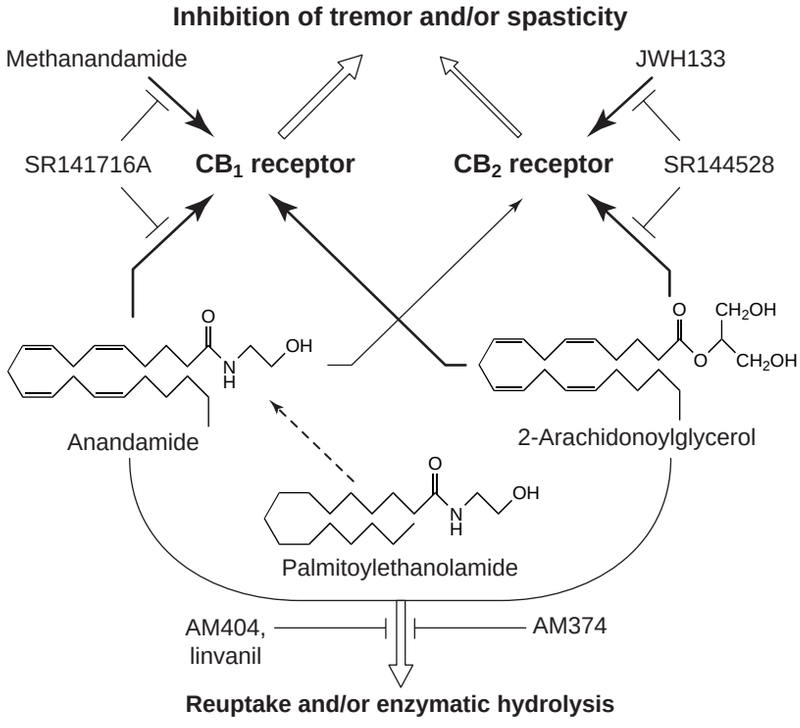

Fig 1. The possible involvement of endocannabinoids in the tonic inhibition of tremor and spasticity in chronic relapsing experimental allergic encephalomyelitis (CREAE) mice is shown. Counteraction of an endocannabinoid-mediated tone by the cannabinoid CB_1 receptor antagonist SR141716A and the CB_2 receptor antagonist SR144528 might explain why these antagonists exacerbate spasticity and tremor in CREAE mice. CB_2 receptors might contribute less than CB_1 receptors to the inhibition of tremor and spasticity. Whether inhibitors of anandamide reuptake (e.g. AM404, linvanil[17,18]), or of the enzymatic hydrolysis of both anandamide and 2-arachidonoylglycerol [e.g. AM374 (Ref. 18)] ameliorate these CREAE signs remains to be determined. 'Enhancers' of endocannabinoid actions, such as palmitoylethanolamide and 2-linoleoylglycerol[5,19–21], which are inactive at CB_1 and CB_2 receptors, might also exert a beneficial effect if endocannabinoids are released during CREAE. Thin arrows denote activation, thick arrows denote strong activation, blunt arrows denote inhibition, broken arrow denotes enhancement and open arrows denote 'leads to'.

low potential for physical dependence[15], could also be used in the treatment of MS-induced spasticity. Second, another non-psychoactive endogenous compound, the anti-inflammatory mediator palmitoylethanolamide[16], also induced a significant, albeit transient, inhibition of spasticity[6]. However, the mechanism of action of this compound, which does not bind appreciably to CB_1 or CB_2 receptors, is still a matter for speculation[16] (see below) and was not investigated by Baker *et al*.

Indeed, possibly the most intriguing suggestion of the authors was that endogenous cannabinoids might be involved in the control of CREAE-induced tremor and spasticity (Fig. 1). In fact, the CB_1 receptor antagonist SR141716A, when administered alone, produced a significant worsening of both tremor and spasticity of hind limbs and tail in post-remission CREAE mice but not in healthy or pre-acute CREAE mice. The CB_2 receptor antagonist SR144528 was less potent but its adverse effects on spasticity increased after treatment of mice with SR141716A. Interestingly, these effects were short-lasting, which could suggest the onset of compensatory mechanisms such as the formation of endogenous agonists that counteract the action of the antagonists. These observations raise the possibility that endocannabinoids such as anandamide and 2-arachidonoylglycerol[5] might be produced during CREAE. This hypothesis, an alternative to the possibility that the antagonists act as reverse agonists on constitutively active cannabinoid receptors, can be validated only by measuring the levels of endocannabinoids in nervous tissues of CREAE mice. The possible finding of increased amounts of endocannabinoids in these damaged tissues could open even wider horizons for therapeutic intervention in MS with few psychotropic side-effects. In fact, if endocannabinoids are produced during MS to compensate for some of the noxious effects of this disease, it will be possible to counteract the symptoms of MS not only with cannabinoid receptor agonists but also with inhibitors of endocannabinoid degradation. Several of these compounds, such as selective inhibitors of anandamide uptake and enzymatic hydrolysis[17,18], are now available and could serve as a basis for the development of other drugs that might be helpful for the treatment of MS. Moreover, compounds with no psychotropic activity, which are capable of enhancing endocannabinoid actions through not fully understood 'entourage' effects[16,19], are also known. Interestingly, palmitoylethanolamide is one of these endogenous 'enhancers' of anandamide-mediated pharmacological effects[20,21] and this property, rather than its activity on yet to be discovered 'CB_2-like' receptors[20], might explain its beneficial effects on spasticity observed by Baker and colleagues[6].

In conclusion, further studies will be required to appreciate fully the involvement of the endocannabinoid system in MS. Given the modulatory effects of both exogenous and endogenous cannabinoids in the immune system of other animal models of MS (see Ref. 22 for an example), it is possible that future investigations will reveal a multi-sited action of these compounds not only on the symptoms but also on the onset and development of MS, which might eventually result in drugs for this life-impairing disease that are not just palliative.

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Chemical names

AM374: palmityl-sulfonyl-fluoride

AM404: *N*-(4-hydroxy-phenyl)-arachidonylamine

JWH133: 3-(1′,1′-dimethylbutyl)-1-deoxy-Δ^8-tetrahydrocannabinol

SR141716A: *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazone-3-carboxamide

SR144528: *N*-([1s]-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazolone-3-carboxamide

WIN552122: (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphathalenylmethanone

Reply: a sanguine approach to cannabis

The comments of Di Marzo *et al.* provide a balanced synopsis of our findings[1] and raise some important questions for which answers are emerging. One comment that pertains to our initial study was the dose level used and the possible psychotropic side-effects of intravenous cannabinoids. We did not carry out a rigorous dose–response study of the compounds used and, although some doses could be considered to be in the 'high' range, these compounds could produce anti-spastic effects with no evidence of a loss of body temperature. This latter aspect forms part of the tetrad test (body temperature, ring catalepsy, activity in the open field and analgesia) to elucidate psychoactive neurobehavioral activity of cannabinoids in rodents. Owing to the presence of CB_1 receptors in motor and cognitive centres, it is possible that CB_1 receptor agonism might lead to psychotropic effects, which is one of the challenges that must be overcome in the clinical use of cannabis. Although apparently evident in mice, it remains to be seen to what extent an acceptable 'threshold' for symptomatic benefit over adverse psychoactivity can be achieved in patients, if direct CB_1 receptor agonism is the target.

Our data suggest that the beneficial effects of cannabinoids on spasticity and tremor were transient (lasting for hours) but could be observed following repeated administration (D. Baker *et al.*, unpublished). This indicates the likelihood that regular administration of cannabinoids is required to sustain effects in humans, which probably makes the direct intravenous route impractical therapeutically. However, this route was examined for experimental purposes in the first instance because routes of administration made an impact on the therapeutic outcome. This was particularly evident with Δ^9-tetrahydrocannabinol (THC), which had minimal effect when injected intraperitoneally[1]. This raises the question of appropriate routes for clinical application, a problem again highlighted recently in a clinical anecdote[2]. Although smoking is not an acceptable option, cannabinoids are highly lipophilic and are slowly released following ingestion. They are susceptible to 'first pass' effects when administered via the venous portal system as could be encountered via the oral and intraperitoneal routes. There might therefore be therapeutic advantage from a more direct intravenous route, such as spraying or inhaling directly into the vascular beds in the mouth or lungs to provide a 'hit' to the receptor system. We now have an experimental system to address these issues. Is this the beginning, or the beginning of the end, of cannabis as a medication? Can we move from the plant into the arena of pharmaceutical-based medicines for the treatment of human diseases? The future holds the answers.

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