British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01280.x
www.brjpharmacol.org

Themed Issue: Cannabinoids in Biology and Medicine, Part I

REVIEW

The dual neuroprotective–neurotoxic profile of cannabinoid drugs

Yosef Sarne, Fadi Asaf, Miriam Fishbein, Mikhal Gafni and Ora Keren

The Adelson Center for the Biology of Addictive Diseases and The Mauerberger Chair in Neuropharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

Correspondence
Yosef Sarne, Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv 69978, Israel. E-mail: sarney@post.tau.ac.il

Keywords
neuroprotection; preconditioning; postconditioning; THC (Δ^9-tetrahydrocannabinol); PTZ (pentylenetetrazole); seizures; carbon monoxide intoxication

Received
1 December 2010
Revised
24 January 2011
Accepted
25 January 2011

Extensive *in vitro* and *in vivo* studies have shown that cannabinoid drugs have neuroprotective properties and suggested that the endocannabinoid system may be involved in endogenous neuroprotective mechanisms. On the other hand, neurotoxic effects of cannabinoids *in vitro* and *in vivo* were also described. Several possible explanations for these dual, opposite effects of cannabinoids on cellular fate were suggested, and it is conceivable that various factors may determine the final outcome of the cannabinoid effect *in vivo*. In the current review, we focus on one of the possible reasons for the dual neuroprotective/neurotoxic effects of cannabinoids *in vivo*, namely, the opposite effects of low versus high doses of cannabinoids. While many studies reported neuroprotective effects of the conventional doses of cannabinoids in various experimental models for acute brain injuries, we have shown that a single administration of an extremely low dose of Δ^9-tetrahydrocannabinol (THC) (3–4 orders of magnitude lower than the conventional doses) to mice induced long-lasting mild cognitive deficits that affected various aspects of memory and learning. These findings led to the idea that this low dose of THC, which induces minor damage to the brain, may activate preconditioning and/or postconditioning mechanisms and thus will protect the brain from more severe insults. Indeed, our recent findings support this assumption and show that a pre- or a postconditioning treatment with extremely low doses of THC, several days before or after brain injury, provides effective long-term cognitive neuroprotection. The future therapeutical potential of these findings is discussed.

LINKED ARTICLES

This article is part of a themed issue on Cannabinoids in Biology and Medicine. To view the other articles in this issue visit http://dx.doi.org/10.1111/bph.2011.163.issue-7

Abbreviations

2AG, 2-arachidonylglycerol; BAY 38-7271, [(–)-(R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate]; CBs, cannabinoids, the psychoactive ingredients of the cannabis plant, their synthetic analogues and the endogenous ligands that act through CB_1 and/or CB_2 receptors. This definition excludes, within the framework of the present review, the non-psychoactive ingredients of cannabis such as cannabidiol; CO, carbon monoxide; CP 55 940 [(–)-*cis*-3-(2-hydroxy-4-(1,1-dimethylheptyl)phenyl)-*trans*-4-(3-hydroxypropyl)cyclohexanol)]; ERK, extracellular signal-regulated kinase; HU-210 ((-)-11-hydroxy-Δ^8-tetrahydrocannabinol-dimethylheptyl); i.p., intraperitoneal; i.v., intravenous; JNK, c-Jun N-terminal kinase; NO, nitric oxide; PTZ, pentylenetetrazole; Raf1, murine leukaemia viral oncogene homolog 1; THC, Δ^9-tetrahydrocannabinol; TNFα, tumour necrosis factor α; WIN 55,212-2 [(R)-(+)-(2,3-fihydro-5-methyl-3-[(morphonolinyl)methyl]pyrrolol[1,2,3-del]-1,4-benzoxazin-yl)(1-naphtaleneyl)methanone mesylate]; WIN 55,212-3, [(3*S*)-2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenyl-methanone monomethanesulfonate

Cannabinoids (CBs), the psychoactive components of *Cannabis sativa* L. (marijuana) and their analogues, exert their effects by activating at least two specific receptors (CB_1 and CB_2) that belong to the seven transmembrane G-protein-coupled receptor family. CBs are known as neurosuppressive drugs. At the cellular level, CBs, through interaction with Gi/o proteins, attenuate cAMP production, reduce neuronal activity by modulating potassium channels and inhibit

voltage-gated calcium channels (Howlett, 1995; Pertwee, 1997; Howlett *et al.*, 2010). Gi/o proteins also mediate the effect of CBs on the mitogen-activated protein kinases extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 (Bouaboula *et al.*, 1999; Rueda *et al.*, 2000; Derkinderen *et al.*, 2001; Rubovitch *et al.*, 2004). *In vivo*, CBs inhibit nociception, suppress motor activity, impair cognitive processes and short-term memory and reduce body temperature (Ameri, 1999; Chaperon and Thiebot, 1999). Nevertheless, there are anecdotal reports on opposite, stimulatory effects of CBs: CBs were shown to induce aggressive behaviour, hyperalgesia, increased motor activity and elevated body temperature (Davis *et al.*, 1972; Taylor and Fennessy, 1977; Sulcova *et al.*, 1998). At the cellular level, CBs have also been shown to couple to Gs proteins (Glass and Felder, 1997; Bash *et al.*, 2003), increase cAMP production (Glass and Felder, 1997; Maneuf and Brotchie, 1997; Bash *et al.*, 2003) and elevate intracellular Ca^{++} levels (Sugiura *et al.*, 1997; Rubovitch *et al.*, 2002; Bash *et al.*, 2003). The dual (stimulatory and inhibitory) effects of CBs depended, in some cases, on the concentration of the drugs: while regular (high) concentrations induced the conventional inhibitory effects, low concentrations of CBs induced stimulatory effects (Sulcova *et al.*, 1998; Rubovitch *et al.*, 2002).

Many studies have demonstrated either neuroprotective or neurotoxic effects of CBs *in vitro* (Guzman *et al.*, 2002; Sarne and Mechoulam, 2005; van der Stelt and Di Marzo, 2005; Galve-Roperh *et al.*, 2008). *In vivo*, acute administration of CBs was found to be protective in various models of acute brain injuries (see below), while chronic exposure to CBs was found in some cases to result in neurotoxic consequences both in heavy cannabis users (Block, 1996; Pope and Yurgelun-Todd, 1996; Ehrenreich *et al.*, 1999; Solowij *et al.*, 2002; Matochik *et al.*, 2005; Arnone *et al.*, 2008; McHale and Hunt, 2008) and in animals exposed to repeated administrations of CB drugs (Fehr *et al.*, 1976; Stiglick *et al.*, 1984; Landfield *et al.*, 1988; Scallet, 1991; Lawston *et al.*, 2000). These apparently contradictory effects of CBs are not yet understood, although several possible explanations for these opposite findings were suggested (e.g., see Guzman, 2003; Sarne and Mechoulam, 2005; Di Marzo, 2008; Fowler *et al.*, 2010). The present review concentrates on one of the possible reasons for the dual neuroprotective/neurotoxic effects of CBs *in vivo*, namely, the opposite effects of low versus high doses of CBs.

Neuroprotective effects of CBs in acute brain injuries

Neuroprotective properties of CBs were demonstrated in several models of acute brain injuries. Acute *in vivo* administration of the synthetic CB agonist WIN-55,212-2 (1–10 $mg \cdot kg^{-1}$, i.p.), but not of its inactive enantiomer WIN-55,212-3, was found to protect against global and focal ischaemic damage in the hippocampus and cortex (Nagayama *et al.*, 1999). Application of the phytocannabinoid Δ^9-tetrahydrocannabinol (THC) (1 $mg \cdot kg^{-1}$, i.p.) (van der Stelt *et al.*, 2001a), or of the endocannabinoid anandamide (1–10 $mg \cdot kg^{-1}$, i.p.) (van der Stelt *et al.*, 2001b), was found to reduce the infarct volume through a CB_1-dependent mechanism in an *in vivo* model of ouabain-induced excitotoxicity. The endocannabinoid 2-arachidonylglycerol (2-AG, 5 $mg \cdot kg^{-1}$, i.p.) was found to reduce brain oedema and infarct volume following severe closed head injury (Panikashvili *et al.*, 2001; 2006) and the intravenous infusion of the CB_1/CB_2 agonist BAY 38-7271 protected against traumatic brain injury and focal ischaemia in rats (Mauler *et al.*, 2003). Since these early reports, numerous studies examined the involvement of CB drugs and the endocannabinoid system in neuroprotection (for recent reviews, see Di Marzo, 2008; Galve-Roperh *et al.*, 2008; Fowler *et al.*, 2010; Viscomi *et al.*, 2010).

Various mechanisms can account for the receptor-mediated neuroprotection that is induced by CBs. The main factor that induces neuronal cell death is the elevation in intracellular calcium ions during brain insult (ischaemia, epileptic seizure or mechanical trauma). This elevation of intracellular calcium concentration initiates a complex cascade of intracellular events such as the stimulation of numerous enzymes (including proteases like calpains and caspases that participate in apoptotic cell death) or other calcium-dependent protein–protein interactions, which affect cell homeostasis and lead to neuronal death. Another consequence of the rise in intracellular free calcium concentration is the production of free radicals that attack DNA, mitochondria and the cell membrane and are considered as major contributors for cell death. In addition, the elevation in intracellular calcium concentration induces an increased release of glutamate and the activation of postsynaptic NMDA receptors that stimulates calcium entry into adjacent cells. Thus, calcium ions have a major role in spreading the damage to additional brain regions (for review, see Doble, 1999). Hence, the inhibitory effect of CBs on voltage-gated calcium channels (Caulfield and Brown, 1992; Mackie and Hille, 1992; Twitchell *et al.*, 1997), which attenuates the elevation in intracellular Ca^{++} and consequently also the release of glutamate (Shen *et al.*, 1996; Shen and Thayer, 1998), was suggested as a possible mechanism for the neuroprotective effects of CBs against excitotoxicity. The modulation by CBs of other calcium-dependent mechanisms, such as the inhibition of NO synthesis (Hillard *et al.*, 1999) and the inhibition of the release of the pro-inflammatory cytokine tumour necrosis factor α (TNFα) (Facchinetti *et al.*, 2003), were also suggested. Other CB actions that may contribute to their neuroprotective effects include the induction of hypothermia (Leker *et al.*, 2003), vasodilatation (Wagner *et al.*, 2001), anti-inflammatory effects (e.g. Maresz *et al.*, 2007; Zhang *et al.*, 2007; Fernandez-Ruiz *et al.*, 2008) and neurogenesis (Galve-Roperh *et al.*, 2007). It was also suggested that CB_1 receptors activate intracellular mechanisms such as the phosphatidylinositol 3-kinase (PI3K/Akt) (Gomez Del Pulgar *et al.*, 2002; Molina-Holgado *et al.*, 2005; Ozaita *et al.*, 2007) and ERK (Valjent *et al.*, 2001; Derkinderen *et al.*, 2003; Tonini *et al.*, 2006; Moranta *et al.*, 2007) pathways that are considered as survival signals and may contribute to the protective effects of CBs. Other neuroprotective mechanisms of CBs in either healthy or pathologic conditions were also suggested (for recent reviews, see Pacher *et al.*, 2006; Di Marzo, 2008; Fowler *et al.*, 2010; Viscomi *et al.*, 2010).

The various studies that showed protective effects of CBs in acute brain injuries presented two common experimental features: (a) the doses that were administered ranged between 1 and 10 $mg \cdot kg^{-1}$ (the doses that also induce the conventional

acute effects of CBs), and (b) the drugs were administered immediately before or after the insult. For example, the CB agonist WIN-55,212-2 (1–10 mg·kg^{-1}, i.p.) was administered between 40 min before to 120 min after the induction of global or focal ischaemia in rats (Nagayama *et al.*, 1999); the CB agonist CP 55 940 (4 mg·kg^{-1}, i.p.) was administered 5 min after the induction of transient global ischaemia in gerbils (Braida *et al.*, 2000); THC (1 mg·kg^{-1}, i.p.) (van der Stelt *et al.*, 2001a) or anandamide (1–10 mg·kg^{-1}, i.p.) (van der Stelt *et al.*, 2001b) were applied 30 min before the induction of ouabain-induced excitotoxicity in rats; THC (10 mg·kg^{-1}, i.p.) was administered immediately before or 3.5 h after the induction of ischaemia in mice (Hayakawa *et al.*, 2007); the endocannabinoid 2-AG (5 mg·kg^{-1}, i.p.) was found to reduce brain oedema and infarct volume when applied 15 min after but not when applied 60 min after severe closed head injury in mice (Panikashvili *et al.*, 2001) and anandamide (10 mg·kg^{-1}, i.p.) protected the new born mouse brain against AMPA and kainate receptor–mediated excitotoxicity when injected within the first 4 h following the insult but not when injected 8–24 h after the insult (Shouman *et al.*, 2006).

As described above, the neuroprotective properties of CBs are attributed, among other factors, to their ability to suppress voltage-gated calcium channels (Mackie and Hille, 1992) and consequently to attenuate the release of glutamate (Shen *et al.*, 1996). *In vitro* findings, however, have shown that very low concentrations of CBs can potentiate, rather than suppress, calcium entry into cells (Okada *et al.*, 1992; Rubovitch *et al.*, 2002). These findings suggest that very low doses of CB drugs may elevate, rather than decrease, intracellular calcium levels in the brain, and consequently the release of glutamate, and thus may be neurotoxic *in vivo*. We therefore conducted studies in order to explore the possibility that very low doses of THC may have *in vivo* neurotoxic effects.

The neurotoxic properties of extremely low doses of THC

We first conducted some acute experiments in order to determine the doses of THC that will induce stimulatory effects in mice. Based on our *in vitro* experiments on the dual effect of CBs on calcium (Rubovitch *et al.*, 2002), we predicted a 3–4 orders of magnitude difference between the *in vivo* inhibitory and stimulatory doses of THC. Our experiments showed, as expected, that an i.p. injection of 10 mg·kg^{-1} THC to mice induced acute hypothermia, analgesia and decreased locomotion. On the other hand, an i.p. injection of 0.001–0.002 mg·kg^{-1} THC produced the opposite effects, that is elevation in body temperature, potentiation of the response to noxious stimuli and increased locomotor activity (Tselnicker, 2005). The doses of 0.001–0.002 mg·kg^{-1} THC were hence chosen for studies on the long-term effects of a low concentration of THC on cognitive functions in mice.

In order to test our assumption that ultra-low doses of THC may induce neuronal damage and impair cognitive functions, we first tested the long-term effect of THC (0.001 mg·kg^{-1}, i.p.) in two behavioural assays that assess spatial learning: the Morris water maze (in ICR mice) and the water T-maze (in C57B1 mice). THC significantly deteriorated the performance of the mice in both assays 3–7 weeks following the injection (Tselnicker *et al.*, 2007; Senn *et al.*, 2008). The effect of the ultra-low dose of THC was mild but reproducible and statistically significant and could be overcome by additional training (see, e.g., figure 1b in Tselnicker *et al.*, 2007 and figure 2 in Senn *et al.*, 2008). The ability of a single low dose of THC to induce cognitive deficits was blocked by the CB_1 receptor antagonist SR141716A, indicating the involvement of CB_1 cannabinoid receptors in this effect (Senn *et al.*, 2008). Since swimming can be stressful to mice, and since mice were shown to perform spatial tasks poorly in a swimming pool compared with dry land (Whishaw and Tomie, 1996), we next tested the effect of the low dose of THC in the oasis maze, a land-based spatial learning assay that was designed to approximate the spatial learning demands required by the Morris water maze (Clark *et al.*, 2005). Similar to our findings in the water maze assays, the mice that were injected 3 weeks earlier with a single low dose of THC exhibited a deficit in the acquisition of spatial learning in the oasis dry maze (Amal *et al.*, 2010). Moreover, in a modification of the test, that could assess the ability of the mice to acquire the strategy of actively looking for a well that contained water, the THC-injected mice performed poorly compared to vehicle-injected control mice (see figure 4 in Amal *et al.*, 2010). Thus, the administration of a single low dose of THC to mice had long-term effects on their learning of strategy as well. We then tested the long-term effect of the low dose of THC on the performance of the mice in two recognition tests that are believed to be less stressful and arousing to mice as they do not depend on reward or negative reinforcement but rather on their natural tendency to explore novel objects (Dere *et al.*, 2007). These tests examine spatial ('place recognition') or non-spatial ('object recognition') visual memory without a requirement for the learning of strategy. Long-term, statistically significant deteriorating influence of a single low dose of THC was observed in both tests and persisted for at least 5 months (Amal *et al.*, 2010). Our findings thus demonstrated that the administration of a single ultra-low dose of THC to mice resulted in a poor performance in a variety of behavioural tests that examined various aspects of cognitive functioning. These various long-term cognitive deficits may have resulted either from several distinct impairments that were induced by THC and affected different aspects of learning and memory, or from a more general deficit that affected the performance of the mice in all the assays that were employed. Our experimental observations supported the latter possibility since we have noticed a common behavioural characteristic in the mice that were injected few weeks earlier with THC, who seemed less curious and less prone to investigate their surroundings, compared with their controls. In the open-field test, no difference in motor activity was found between the THC- and the vehicle-injected mice when the test was performed in familiar surroundings (following habituation of the mice to the arena, as was required for measuring true 'motor activity'). However, during the first session of the test ('habituation'), when the mice were not familiar yet with their surroundings, the control group appeared more active than the THC-injected group. Similar behaviour of the THC-injected mice was noticed in the two recognition tests. In the first session, when the mice were introduced to two unfamiliar objects in the arena, control mice spent more time than THC-treated mice in investigating the two objects. On the second day, however,

their activity was reduced and was similar to that of THC-treated mice (Amal *et al.*, 2010). This lack of curiosity of THC-treated mice that can also be described as lack of motivation or reduced awareness or attention may, in our opinion, be the cause for the poor performance of the mice in all the behavioural tests that were employed. It is interesting to note that deficits of a similar nature were described following chronic use of cannabis: repeated administration of high doses of THC to rats resulted in reduced social interactions 2 weeks following the cessation of the treatment (Quinn *et al.*, 2008); repeated administration of THC to rats caused an attentional deficit that was detected 7 days later in a test of visuospatial divided attention (Verrico *et al.*, 2004) and monkey infants that were born to mothers treated chronically with THC showed an alteration in visual attention (Golub *et al.*, 1982). Furthermore, attentional dysfunction has been reported repeatedly in human heavy cannabis users (Solowij *et al.*, 1991; Fletcher *et al.*, 1996; Pope and Yurgelun-Todd, 1996; Ehrenreich *et al.*, 1999; McHale and Hunt, 2008).

The finding that a single extremely low dose of THC causes cognitive deficits similar to those caused by repeated treatments with high doses of the drug are in agreement with our previously published hypothesis on the deteriorating effects of chronic exposure to cannabis (Sarne and Keren, 2004). According to this hypothesis, the detrimental outcomes of intermittent applications of CBs result from the exposure of the organism to the very low concentration of the drug that is present in the body for a prolonged time following each application, due to the slow washout of the lipophilic drug.

The surprising finding that a single administration of such an ultra-low dose of THC causes cognitive deficits led us to test whether similar doses of THC would induce biochemical effects in the brain. Indeed, we found that the injection of 0.001–0.002 $mg \cdot kg^{-1}$ of THC triggered a biochemical pathway that led to a delayed activation of ERK1/2 in the cerebellum that peaked at 24 h and then declined (Senn *et al.*, 2008; Amal *et al.*, 2010). This finding was in contrast to the reported rapid activation of ERK following the injection of high doses of THC (1–10 $mg \cdot kg^{-1}$, doses that induce the conventional acute effects of the drug) that peaked at 10–30 min post-injection and then declined (Derkinderen *et al.*, 2003; Rubino *et al.*, 2004). We have also searched for long-term (weeks) neurochemical changes that develop in parallel to the long-term behavioural effects that were induced by the ultra-low dose of THC. We have therefore recently tested the amounts of total and phosphorylated (active) ERK in the cerebellum 7 weeks following the injection of 0.002 $mg \cdot kg^{-1}$ THC to mice. A consistent significant decrease in phosphorylated ERK was found in the cerebella of THC-injected mice compared to vehicle-injected mice, while there was no difference in the amount of total ERK between the two groups of mice. Interestingly, in other brain regions (hippocampus and frontal cortex), a sustained activation of ERK (namely, elevation in phosphorylated ERK with no change in total ERK) was observed 7 weeks after the injection of THC. These findings suggested that a single injection of an ultra-low dose of THC to mice can induce both short-term (days) and long-term (weeks) modifications in the brain. Further studies are needed in order to understand the relevance of the long-term decline in ERK activity in the cerebellum to our findings in the behavioural assays, since ERK has been shown to have an important role in regulating many processes of cellular homeostasis, including both cell survival and cell death (reviewed in Agell *et al.*, 2002).

To conclude, we have shown that a single administration of an extremely low dose of THC to mice induced long-term cognitive deficits that were detected by several tests that evaluated different aspects of memory and learning. The deficits were usually mild and were not accompanied by any apparent neurological (motor or sensory) damage. We have also shown that a single injection of the ultra-low dose of THC evoked long-term neurochemical processes that may affect brain plasticity. These findings led us to examine whether this low dose of THC, which induces minor damage to the brain, may activate preconditioning and/or postconditioning mechanisms and thus will protect the brain from more severe insults.

Pre- and postconditioning

The discovery of the phenomena of preconditioning, where a minor noxious stimulus protects various organs, including the brain, from a subsequent more severe insult (Murry *et al.*, 1986; Kitagawa *et al.*, 1991) and of postconditioning, where the protective intervention is applied following the insult (Zhao and Vinten-Johansen, 2006; Pignataro *et al.*, 2008), prompted studies that aimed to find ways to utilize therapeutically pre- and postconditioning mechanisms (Hausenloy and Yellon, 2009).

Preconditioning research was initially conducted in cardiology, following the finding of Murry and coworkers, who have shown that there was a considerable reduction in myocardial infarct size resulting from prolonged ischaemia, in dogs that had been submitted earlier to four cycles of brief coronary occlusion followed by reperfusion (Murry *et al.*, 1986). Since then, the molecular mechanisms of both early and delayed cardiac preconditioning have been extensively studied (Das and Das, 2008), and large clinical trials have confirmed the existence of myocardial preconditioning in humans (Dirnagl *et al.*, 2003). The phenomenon of preconditioning was later described in other organs, including the brain. The interest in ischaemic preconditioning ('tolerance') in the brain started with the finding that a brief bilateral carotid occlusion 2 days before global ischaemia protected neurons from death in several brain areas of the gerbil (Kitagawa *et al.*, 1991). Similar to the findings in the heart, preconditioning can protect the brain almost immediately ('early preconditioning') or after a delay of 1–7 days ('delayed preconditioning'). The molecular signalling cascades that are involved in both types of preconditioning were extensively studied (reviewed in Gidday, 2006; Dirnagl *et al.*, 2009). These cascades include, for example, the activation of ERK, Akt, nitric oxide synthase and various neurotrophins (Gidday, 2006; Dirnagl *et al.*, 2009). Preconditioning can be induced by different harmful stimuli like ischaemia, hypoxia, trauma, hyperthermia or by chemical substances and is not specific to the type of injury. The preconditioning stimulus can be different from the insult ('cross preconditioning') or even remote, since preconditioning of one organ can protect a different organ (Hausenloy and Yellon, 2008).

A novel protective approach that was recently described was ischaemic postconditioning, where the protective inter-

vention is applied following the insult. Postconditioning was found effective both in cardiac ischaemia (Zhao and Vinten-Johansen, 2006) and in the brain (Pignataro *et al.*, 2008), and recently the existence of remote postconditioning was also suggested (Hausenloy and Yellon, 2009). Similar to preconditioning, postconditioning can be produced by various chemical agents (reviewed in Gross and Gross, 2006). Moreover, it has been suggested that pre- and postconditioning share common signalling pathways (Hausenloy and Yellon, 2009).

Pre- and postconditioning treatments with an ultra-low dose of THC provide long-term neuroprotection

We have recently conducted experiments in order to examine whether a single ultra-low dose of THC, which induces minor cognitive deficits in mice, may activate preconditioning and/or postconditioning mechanisms and thus will protect the brain from more severe insults (a detailed account of our findings was recently published: Assaf *et al.*, 2011). Two different brain insults that cause cognitive damage and mimic different clinical situations were used as experimental models: (i) the injection of pentylenetetrazole (PTZ) that induces seizures that correlate to the petit mal type of epilepsy and (ii) repeated short sessions of exposure to carbon monoxide (CO) that induce partial anoxia and correlate to various hypoxic pathological conditions.

The epileptogenic drug PTZ was previously shown to impair cognitive functions (Lamberty and Klitgaard, 2000; Wang *et al.*, 2008). Epileptic seizures can cause neuronal damage via the release of glutamate that triggers the excitotoxic cascade (Charriaut-Marlangue *et al.*, 1996) and were shown to induce neuronal death (Sankar *et al.*, 1998; Huang *et al.*, 2002; Troy *et al.*, 2002) and memory impairment (Huang *et al.*, 2002; Rutten *et al.*, 2002). Moreover, various insults to the brain such as stroke or trauma are known to induce seizures that augment the risk of damage (Bladin *et al.*, 2000; De Reuck *et al.*, 2006; Christensen *et al.*, 2009; Pitkanen *et al.*, 2009). The CO intoxication model was shown to induce excitotoxicity accompanied by synaptic and cellular loss in the hippocampus and the development of learning deficits in mice (Ishimaru *et al.*, 1991; Maurice *et al.*, 1999; Meunier *et al.*, 2006). CO exposure induces anoxia, which is the main damage-inducing factor in brain insults such as stroke, cardiac arrest or suffocation. Thus, these two experimental models (PTZ and CO) represent a wide spectrum of pathological conditions.

In our experiments, a single injection of PTZ (60 mg·kg^{-1}) to mice caused acute clonic–tonic seizures (stage 5 according to Clement *et al.*, 2003) that lasted for 2–10 min. This treatment induced cognitive deficits that were detected 3 weeks later by the oasis maze. The potential of a single ultra-low dose of THC to protect the mice and prevent the development of PTZ-induced cognitive deficits was then studied. THC (0.002 mg·kg^{-1}) was initially injected to mice 3 days before the administration of PTZ (60 mg·kg^{-1}), and its effect on the performance of the mice three weeks later in the oasis maze was examined. The time point of 3 days before the insult was chosen since at 3 days the protection induced by ischaemic preconditioning had been reported to be maximal (Obrenovitch, 2008). We found that the mice that were injected 3 weeks earlier with PTZ needed a significantly longer time than the control mice to find the well that was filled with water in the oasis maze. The mice that had been pretreated with THC 3 days before the administration of PTZ needed a significantly shorter time than the PTZ-injected mice to find the water-filled well, indicating that pretreatment with THC protected the mice from the cognitive deficits that were induced when PTZ alone was injected. Moreover, no significant difference between the performance of the mice that were pretreated with THC before the injection of PTZ and the control group was found, suggesting the absence of cognitive deficits in the PTZ-injected THC-pretreated mice. Similar results were obtained when THC was injected either 1 day or 7 days before the administration of PTZ. Our findings thus suggested that a preconditioning treatment of mice with an ultra-low dose of THC, 1–7 days before the administration of PTZ, can protect the mice from PTZ-induced cognitive deficits.

We next tested whether preconditioning with a single low dose of THC will similarly protect the mice from cognitive deficits that were induced by another insult to the brain, namely CO intoxication. Mice were exposed to CO (for 12 s) thrice, with 45 min intervals between exposures (according to Ishimaru *et al.*, 1991; Meunier *et al.*, 2006). This treatment induced a cognitive deficit that was detected 3–7 weeks following the exposure to CO by the oasis maze. The injection of a single dose of THC (0.002 mg·kg^{-1}) 1 or 3 days before the exposure to CO significantly protected the mice and prevented the appearance of this cognitive deficit. These findings corroborate the results that were obtained with PTZ as the insult and suggest that THC may have a potential as a preconditioning treatment in a broader spectrum of brain injuries.

While the current review has been prepared, another group reported that a high (conventional) dose of WIN 55,212-2 (1 mg·kg^{-1}) protected rats from focal cerebral ischaemia when injected 24 h before the insult (Hu *et al.*, 2010). This report further supports the idea that CBs may produce conditioning effects, although it is not clear whether the protective effect in that study resulted from the high concentration of the injected drug or alternatively, from the low concentration that was present in the body several hours after the injection, as had been suggested by us before to explain the deteriorating effects of chronic exposure to cannabis (see above and Sarne and Keren, 2004).

Our next goal was to test whether the ultra-low dose of THC will also protect the mice from PTZ-induced cognitive deficits when injected after the administration of PTZ. In this set of experiments, THC (0.002 mg·kg^{-1}) was injected 1 or 3 days following the administration of PTZ (60 mg·kg^{-1}). Three weeks later, the mice were tested for cognitive deficits in the oasis maze. The results indicated that a postconditioning treatment with this single low dose of THC 1 or 3 days following the insult prevented the appearance of PTZ-induced cognitive deficits, as detected by the oasis maze assay.

To further establish the pre- and postconditioning potential of THC, the mice were also tested in the place- and object recognition assays, which assess spatial and non-spatial visual memory respectively. Similar to the results that were obtained using the oasis maze, the treatment with a single dose of THC 1–7 days before the injection of PTZ, or 1–3 days after the

injection of PTZ, prevented the development of PTZ-induced cognitive deficits that were detected 3–7 weeks later. The performance of the pre- and postconditioned mice in the tests was significantly better than the performance of the PTZ-injected mice and not different from the control mice, suggesting the absence of cognitive deficits that could be detected by the recognition assays.

The molecular mechanism(s) of THC-induced pre- and postconditioning is not defined yet. If, indeed, the protective effect of THC is secondary to its deteriorating effect and the mobilization of an endogenous compensatory mechanism(s) ('conditioning'), it is expected to involve CB_1 receptors, since these receptors were previously shown by us to mediate the cognitive deficits that were induced by THC (Senn *et al.*, 2008). Nevertheless, the introduction of selective CB_1 and CB_2 antagonists, or the use of CB receptor knockout mice, will reveal the involvement of CB receptors in the THC-induced pre- and postconditioning.

To conclude, our results suggest that a pre- or a postconditioning treatment with extremely low doses of THC, several days before or after brain injury, may provide effective long-term neuroprotection and be used as a therapeutical treatment in a wide spectrum of brain insults.

Therapeutical potential

Brain damage is a leading cause of long-term disability and mortality worldwide. Brain damage can be induced by stroke (ischaemic or haemorrhagic), by traumatic brain injury (TBI), by hypoxic or anoxic conditions (e.g. due to suffocation, cardiac arrest, complications of general anaesthesia or carbon monoxide poisoning), by epileptic seizures or by various toxins. The consequences of brain injury depend on the amount of brain tissue that was damaged and the part of the brain where the injury occurred and can range from transient or long-term cognitive, emotional or motor deficits in the case of mild or moderate injury, to coma or even brain death in the case of severe injury. The initial insult induces multiple processes that lead to a rapid apoptotic and necrotic cell death in the core area of the injury, including excitotoxicity (excessive release of glutamate that generates the accumulation of toxic concentrations of intracellular free calcium and of nitrogen and oxygen free radicals), acidotoxicity and ionic imbalance (for a detailed review, see Doyle *et al.*, 2008). The cells in the region surrounding the core area of the injury (penumbra) degenerate more slowly, over a period of hours or days following the initial insult, by mechanisms such as apoptosis and inflammation. These cells may be salvaged by therapeutical intervention, but, as the processes that lead to cellular death are already in progress, the time window for treatment is limited. Currently, two major therapeutical approaches for the rescue of penumbral cells are considered: (a) the use of neuroprotective drugs that suppress biochemical pathways that mediate cellular death (e.g. NMDA receptor antagonists, calcium channel blockers, antioxidants or anti-inflammatory drugs) and (b) neurotrophic factors that induce synaptogenesis, proliferation of dendritic spines and regeneration of neuronal cells. However, despite two decades of research, clinical trials did not yield any effective neuroprotectant drugs, and the only treatment available today is thrombolysis, which restores the interrupted blood flow in the case of stroke, using, for example, the recombinant tissue plasminogen activator that has a therapeutical time window of 3 h (Zaleska *et al.*, 2009).

The discovery of the phenomena of pre- and postconditioning presented a different therapeutical approach, namely, the possible activation of endogenous mechanisms by which the brain protects itself and recovers from damage. One of the main advantages of pre- and postconditioning stimuli is that the time window for their application is long (days) (Zhao, 2009), in contrast to the therapeutical time window for pharmacological intervention with neuroprotective drugs that is very short (hours). Thus, conditioning procedures may be used to either protect patients that are at risk of injury or to treat the insulted brain following injury.

Our findings demonstrate the potential of a single treatment with a very low dose of THC to induce pharmacological pre- and postconditioning and protect the brain from the development of cognitive deficits due to epileptic seizures and CO intoxication and probably from other insults that involve excitotoxicity. As described above, the therapeutical time window for protective intervention with the conventional doses of CBs is short ($\pm$4 h in rodents), while the preconditioning treatment with the ultra-low dose of THC can be employed 1–7 days before the insult and the postconditioning treatment can be applied for at least 3 days following the insult. In both cases, the ameliorating consequences of these treatments last for at least several weeks. Our findings with THC preconditioning thus render this drug a potential candidate for inducing neuroprotection in advance in patients that are at risk of cognitive damage due, for example, to heart or brain surgery or to complications of anaesthesia or percutaneous coronary intervention. Similarly, the ability of the low dose of THC to induce postconditioning may prove to be effective in treating the insulted brain following traumatic injury, stroke, suffocation or cardiac arrest, insults that are known to induce long-term cognitive decline.

Traditional preconditioning approaches use sublethal doses of otherwise damaging insults such as brief episodes of ischaemia, hyperthermia or hypoxia, or low doses of toxins (reviewed in Gidday, 2006), and therefore, their future therapeutical benefit is questioned, due to safety, efficacy and ethical considerations (Dirnagl *et al.*, 2009). THC is already safely used in the clinic for various pathological conditions (Pertwee, 2009). According to our findings in mice, the dose of THC that induced pre- and post-insult protection is 3–4 orders of magnitude lower then the dose that induced the acute conventional effects of THC. A treatment with such extremely low doses of THC may therefore have a potential to provide safe, long-term neuroprotection before or after brain injury, without the undesired psychotropic effects of the conventional dose of the drug. Moreover, since similar molecular mechanisms are involved in brain injuries and neurodegenerative diseases (Zemke *et al.*, 2004), it is possible that a chronic treatment with very low doses of THC may prove beneficial in neurodegenerative diseases as well.

What can be learned from in vitro *studies*

The current review focused on the *in vivo* neuroprotective/neurotoxic profile of CB drugs. Yet *in vitro* studies may shed more light on the cellular mechanisms that underlie this dual activity of CBs. As was mentioned above, many studies have demonstrated either beneficial or deleterious effects of CBs on

the survival of various cells in culture (reviewed in Guzman *et al.*, 2002; Sarne and Mechoulam, 2005; van der Stelt and Di Marzo, 2005; Galve-Roperh *et al.*, 2008). For example, CB agonists acting through CB_1 receptors protected hippocampal neurons in culture from synaptically mediated excitotoxicity (Shen and Thayer, 1998). In cultures of mouse spinal cord neurons, application of THC attenuated, via CB_1 receptors, kainate-induced toxicity (Abood *et al.*, 2001). Similarly, the CB agonist CP-55 940 protected cultured cortical neurons from glutamatergic excitotoxicity by CB_1 receptor–mediated inhibition of voltage-dependent calcium channels (Hampson and Grimaldi, 2001). CBs have also shown *in vitro* neuroprotective potential in models of neurodegenerative diseases. For example, CBs abrogated microglia-mediated neurotoxicity after amyloid addition to rat cortical cocultures through the activation of CB_1 and CB_2 receptors (Ramirez *et al.*, 2005), and CB_1 agonists were shown to be neuroprotective in an *in vitro* model of Huntington's disease (Scotter *et al.*, 2010). On the other hand, *in vitro* treatment of neuronal cell lines or cultured hippocampal neurons and cortical neurons or hippocampal slices with THC has been shown to induce neuronal death (Chan *et al.*, 1998; Guzman *et al.*, 2002; Downer *et al.*, 2003; 2007b). It should be noted that some of the *in vitro* protective effects of CBs were not mediated by CB receptors (e.g., see Hampson *et al.*, 1998; Nagayama *et al.*, 1999; Sinor *et al.*, 2000; Marsicano *et al.*, 2002), a fact that may correlate to their non-CB receptor–mediated protective effects in some of the *in vivo* studies (Shohami and Mechoulam, 2000; Lastres-Becker *et al.*, 2005).

While the *in vivo* protective and toxic effects of CBs may be the result of complex processes that involve various systems (e.g. effects on body temperature, inflammation, blood flow, etc.), the dual effects of CBs on survival *in vitro* occur at the cellular level, by the activation of intracellular pro-survival or pro-death signalling mechanisms. CBs, via CB receptors, were shown to activate different signal transduction mechanisms in different cell types. This differential activation may lead to diverse effects on cell survival. For example, two sub-clones of C6 glioma cells exhibited different sensitivity to the toxic, anti-tumoral action of THC: in C6.9 cells, THC induced cellular death that correlated to sustained ceramide accumulation and Raf1/ERK activation, while in C6.4 cells, THC did not induce cellular death, and no sustained accumulation of ceramide and activation of Raf1/ERK was found (Galve-Roperh *et al.*, 2000). Moreover, while THC increased the synthesis of ceramide and induced apoptosis in transformed glioma cells, it did not affect ceramide synthesis and failed to produce apoptosis in native astrocytes and even protected them from oxidative stress (Carracedo *et al.*, 2004). It is conceivable that CBs may activate distinct cellular pathways in different cell types *in vivo* as well. Hence, different neurons in different brain regions, which regulate different physiological functions, may be affected differently by CB drugs, leading to different functional consequences *in vivo*.

Recent reports have shown that CBs can mediate distinct signalling mechanisms in a single neuron, depending on the state of the neuron (Kellogg *et al.*, 2009; Roloff and Thayer, 2009). The physiological state of the neuron at the time of the application of the CB may therefore affect the outcome of the CB treatment. Our recent study (Bologov *et al.*, 2011) showed that various CB agonists (CP 55,940, THC, HU-210 and WIN 55,212-2) significantly reduced the viability of N18TG2 neuroblastoma cells that were grown under stressful conditions (glucose- and serum-free medium) but not when the same cells were grown under optimal conditions (normal medium). These *in vitro* experiments suggest that the consequences of the administration of CBs *in vivo* may be affected by the stressful or pathologic conditions of the organism (see, e.g., Di Marzo, 2008). Furthermore, THC was shown to activate pro-apoptotic mechanisms in cerebral cortical slices obtained from the neonatal rat brain but not from adult brain (Downer *et al.*, 2007a), indicating different sensitivity to the pro-apoptotic effect of THC of immature cortical cells, compared with mature, differentiated cortical cells. Similarly, we have recently shown that exposure of differentiating N18TG2 cells, but not of dividing cells, to CB agonists significantly increased their viability (Bologov *et al.*, 2011). These studies imply that the neuroprotective/neurotoxic effect of CBs *in vivo* may be affected by the age and stage of development of the organism (Downer and Campbell, 2010).

In vitro studies offer an experimental setup that enables a better analysis of the signalling pathways that lead to either the survival or the death of the cells, yet the relevance of the *in vitro* findings to the *in vivo* effects of CBs is not always clear. Nevertheless, a thorough research of the *in vitro* effects of CBs on the survival of isolated neurons may direct our future experiments with living organisms, in order to further elucidate the dual, neuroprotective/neurotoxic profile of CB drugs.

Acknowledgements

THC that was used in the experiments reported herein was donated by Prof R. Mechoulam of the Hebrew University of Jerusalem, Israel and by the National Institute on Drug Abuse (NIDA), USA.
The cannabinoid research in our laboratory has been supported by grants from the Israel Science Foundation and The Israel Antidrug Authority.

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