Cannabis on the Brain: Is it Neurotoxic or Neuroprotective? *

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Recent studies have further confirmed that cannabis produces its psychological and behavioural effects by acting on specific cannabinoid receptors in the brain and that the brain also contains naturally occurring cannabinoids known as 'endocannabinoids'. Although some recent studies suggest that cannabis may be neurotoxic and that this may explain the effects of cannabis on short-term memory, this review argues that this evidence is not convincing and that there is increasing evidence that many cannabinoids have neuroprotective effects, and may be useful in the treatment of some neuropsychiatric disorders.

The aim of this review is to provide a summary of the most recent evidence relating to the neurotoxic and neuroprotective effects of cannabis and cannabinoids on the brain and to critically evaluate their significance. In doing so I will not attempt to provide an exhaustive summary of the increasingly vast field of cannabinoid neuropharmacology but will focus on key recent developments which have occurred in the last 2 years. The term 'cannabinoid' will be used throughout to refer to chemicals which activate the cannabinoid receptors. Some of these are chemically similar to the active ingredient in cannabis - delta^9-tetrahydrocannabinol (delta^9-THC) - others are synthetic and chemically dissimilar but in some cases still activate the cannabinoid receptors. Collectively, all of these substances are now referred to as 'cannabinoids' in a way that is analogous to the term 'opioids', which is used to refer to natural and synthetic morphine-like compounds that activate opiate receptors (Mechoulam, Fride & Di Marzo, 1998).

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A Brief History Of Cannabinoid Neuroscience

Until the 1980's, delta^9-tetrahydrocannabinol (delta^9-THC) was thought to affect neurons in the brain by dissolving into the lipid (i.e., fatty acid) component of their membranes and disrupting cellular function in a non-specific way. Since cannabis is highly soluble in lipids, it seemed very likely that this was the way that cannabis produced its various psychological and behavioural effects. However, cannabinoid pharmacology changed in the late 1980's when William Devane and colleagues at the University of St Louis Medical School in Missouri demonstrated a specific receptor protein for cannabinoids on the surface of neurons (i.e., an extracellular receptor) which appeared not to bind any other neurochemical (Devane, Dysarz, Johnson, Melvin & Howlett, 1988; see Feldman & Glass, 1998; Ameri, 1999 for recent reviews). This finding was quickly replicated and the study of cannabinoids became one of the most active fields of neuropharmacology throughout the 1990's.

It was also demonstrated in these early experiments that activation of the cannabinoid receptor resulted in biochemical effects within neurons, in particular a reduction in the intracellular second messenger, cyclic adenosine-3',5' monophosphate (cAMP). Activation of the cannabinoid receptor produced this effect by activating a so-called 'G-protein' (guanine nucleotide binding protein), which acted as a switching mechanism to produce biochemical effects inside neurons (Bidaut-Russell, Devane & Howlett, 1990). By the early 1990's, cannabinoid receptors had been extensively mapped in almost every area of the central nervous system (CNS), including the post-mortem human brain, and the cannabinoid receptor gene had also been identified and cloned (see Feldman & Glass, 1998; Ameri, 1999 for reviews).

Cannabinoid receptors were divided into 2 categories: the CB1 receptors which are located in the nervous system (both the central and peripheral nervous systems) and the testis; and the CB2 receptors which are located in many other areas of the body, including various parts of the immune system (see Feldman & Glass, 1998; Ameri, 1999

for recent reviews).

In addition to their numerous functions in the nervous system, it is now clear that cannabinoid receptors exist in sperm and may regulate their function; they may influence the response of the immune system to bacterial and viral infections, and they may also be important in cardiovascular function (see Feldman & Glass, 1998; Mechoulam, Fride & Di Marzo, 1998; Ameri, 1999, for reviews). Most recently, cannabinoid receptors have been implicated in the feeding responses of the very primitive organism, *Hydra*, suggesting that they may have developed at an early stage in evolutionary history (De Petrocellis, Melck, Bisogno, Milone & Di Marzo, 1999). Like many other G-protein-coupled receptors, there is evidence that when cannabinoid receptors are 'over-exposed' to cannabinoids, they not only become desensitized (i.e., less responsive) to these drugs but that they actually become internalized within the cell where they are modified in a process involving the addition of phosphate groups ('phosphorylation') before being recycled back to the cell surface as functional receptors (Hsieh, Brown, Derleth & Mackie, 1999; Jin, Brown, Roche, Hsieh, Celver, Kovoor, Chavkin & Mackie, 1999). The breeding of transgenic mice, in which certain genes have been deleted or knocked out ('knock-out mice'), has made it possible to discover what the nervous system is like without cannabinoid receptors. Studies using CB1 receptor knock-out mice have shown that without the CB1 receptor, animals are unresponsive to cannabinoids (Ledent, Valerde, Cossu, Petitet, Aubert, Beslot, Bohme, Imperato, Pedrazzini, Roques, Vassart, Fratta & Parmentier, 1999; Steiner, Bonner, Zimmer, Kitai & Zimmer, 1999; Zimmer, Zimmer, Hohmann, Herkenham & Bonner, 1999). There is increasing evidence that there are some glial cells in the CNS that have another subtype of cannabinoid receptor which is distinct from the CB1 receptor (Sagan, Venance, Torrens, Cordier, Glowinksi & Giaume, 1999). Further cannabinoid receptor subtypes are likely to exist (e.g., Jarai, Wagner, Varga, Lake, Compton, Martin, Zimmer, Bonner, Buckley, Mezey, Razdan, Zimmer & Kunos, 1999).

From the initial discovery of the cannabinoid receptor, one question in particular challenged researchers, and that was why should there be a specific cannabinoid receptor in the brain? Pharmacologists had puzzled over this kind of question before when the opiate receptors were discovered; in that case it was not long before a naturally occurring or 'endogenous' opioid was discovered. For the same reason, from the time of the cannabinoid receptor discovery, researchers vigorously pursued the identification of an endogenous cannabinoid. Once again, Devane and colleagues were the first to publish on a putative endogenous cannabinoid in 1992, which they named 'anandamide' after the chemical structure and the Sanskrit word for 'bliss' (Devane, Hanus, Breuer, Pertwee, Stevenson, Griffin, Gibson, Mandelbaum, Etinger and Mechoulam, 1992). This result was soon replicated (see Feldman & Glass, 1998; Ameri, 1999 for recent reviews).

However, it became apparent that there may be an entire family of endogenous cannabinoids, which have now been named 'endocannabinoids'. It is now well accepted that there are a number of different endocannabinoids in the body and over the last 5 years a great deal has been learned about their synthesis and metabolism (see Mechoulam, Fride & Di Marzo, 1998 for a review). Some recent studies have shown that anandamide and other endocannabinoids exist in small concentrations in chocolate and it has been suggested that they may be responsible for the rewarding properties of cocoa (see Bruinsma & Taren, 1999 for a review); however, it is likely that the endocannabinoid concentrations in chocolate are too small to exert any psychological or behavioural effect (Di Marzo, Sepe, De Petrocellis, Berger, Crozier, Fride & Mechoulam, 1998).

Are Cannabinoids Neurotoxic?

Many researchers believe that cannabinoids are neurotoxic, at least at high concentrations in the CNS (e.g., Hall & Solowij, 1998). That some cannabinoids are neurotoxic at some concentration is probably trivially true, since many drugs produce cell damage at very high concentrations; the difficulty is in determining at what concentration neurotoxicity may develop. Many experimental studies in the field of cannabinoid neuropharmacology have been flawed by the use of unrealistically high concentrations of delta^9-THC and other cannabinoids. For example, early studies demonstrated some morphological changes in the brains of rats treated with high doses of delta^9-THC; however, the doses used were very high by human standards and the changes induced were still very small (see Feldman & Glass, 1998; Ameri, 1999 for recent reviews). More recently, *in vitro* studies have provided incontrovertible evidence that delta^9-THC may be toxic to hippocampal neurons (Chan, Hinds, Impey & Storm, 1998); however, *in vitro* studies using neurons in cell culture are not necessarily reliable predictors of the effects of drugs *in vivo* in either animals or humans. One problem which arises even with *in vivo* studies is that the doses and routes of administration may not be similar to those used by humans. For example, cannabis itself is most often inhaled rather than injected or administered orally and therefore the pharmacokinetics (i.e., the processes of absorption, distribution, metabolism and excretion) which regulate the amount of delta^9-THC which penetrates the CNS and the timecourse of its actions, are quite different from many animal studies using other routes of administration. The effects of pharmacokinetics on the nature of the chemical stimulus to the CNS also impacts on the frequency of cannabis use. If the various pleasurable behavioural effects of cannabis are achieved more effectively by smoking cannabis, the moderate recreational user may not be inclined to use it as often. On the other hand, the kind of chronic injection schedules employed in many laboratory studies probably reflect more accurately the behaviour of 'heavy' cannabis users.

There is a body of evidence indicating that cannabis use in humans disrupts short-term memory (see Hampson & Deadwyler, 1999 for a recent review). As would be expected, these memory deficits, which in many studies are quite small and subtle, are more obvious in heavy users and with advancing age. It also needs to be pointed out that

disruption of short-term memory is not the same thing as neurotoxicity and that many other recreational drugs, including alcohol, have similar effects on short-term memory. It has been demonstrated, in perhaps what represents the most ecologically valid neurophysiological studies of the effects of cannabinoids, that delta^9-THC administered to alert non-human primates by inhalation results in both short-term memory deficits and a disruption of neuronal function in the dentate gyrus of the hippocampus (see Hampson & Deadwyler, 1999 for a recent review). In particular, delta^9-THC has been shown to alter the timing of neuronal firing in the dentate gyrus in relation to the performance of behavioural tasks (Hampson & Deadwyler, 1999). Likewise, cannabinoid administration results in many neurochemical changes, including changes in dopamine, noradrenaline and GABA release (e.g., Diana, Melis, Muntoni & Gessa, 1998; Kathmann, Bauer, Schickler & Gothert, 1999; Katona, Sperlagh, Sik, Kafalvi, Vizi, Mackie & Freund, 1999). However, this is not surprising since a disruption of neuronal electrophysiological and neurochemical function in an area of the brain associated with memory formation would be expected to underlie the behavioural changes that have been documented. It has been demonstrated that cannabinoids can disrupt the induction of long-term potentiation (LTP), the neural model of memory, and that drugs which selectively block cannabinoid receptors ('selective cannabinoid receptor antagonists') prevent this disruption (see Feldman & Glass, 1998; Ameri, 1999 for recent reviews). More recently, knock-out mice lacking the CB1 receptor gene have been shown to have enhanced memory in a two-trial object recognition test and to demonstrate enhanced LTP (Reibaud, Obinu, Ledent, Parmentier, Bohme & Imperato, 1999; Bohme, Laville, Ledent, Parmentier & Imperato, 2000). However, these results merely demonstrate at a neural level what psychologists and others have already observed at a behavioural level and, once again, do not constitute evidence of neurotoxicity.

Are Cannabinoids Neuroprotective?

There is increasing evidence that some cannabinoids are neuroprotective against neuronal damage induced by stroke and head trauma. Dexanabinol (HU-211), a cannabinoid analogue without psychotropic effects, is currently in Phase II clinical trials for the prevention of neuronal damage induced by head trauma (Fishman, 1998). The effects of dexanabinol appear not to be mediated by CB1 or CB2 receptors, since they are not blocked by selective cannabinoid receptor antagonists. However, dexanabinol appears to work by blocking the calcium ion channel associated with the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, whose overactivation under conditions of neural damage is well known as a major contributor to the biochemical cascade leading to neuronal degeneration (Leker, Shohami, Abramsky & Ovadia, 1999).

Until recently, it was thought that the only cannabinoids that might be neuroprotective were those that had no action on the CB1 receptors. However, Nagayama, Sinor, Simon, Chen, Graham, Jin and Greenberg (1999) have reported that the synthetic cannabinoid agonist, R(+)-WIN 55212-2, reduced hippocampal neuronal loss after temporary interruption of the blood supply to the brain from one carotid artery ('transient global cerebral ischemia') and also reduced the area of neuronal damage ('infarct volume') following permanent occlusion of the middle cerebral artery ('focal cerebral ischemia'). In both cases, the neuroprotective effect of the cannabinoid was blocked by a selective CB1 receptor antagonist. Because this was an *in vivo* study, it is possible that these results are directly relevant to the treatment of stroke in humans. Other *in vitro* studies showed that the same cannabinoid could protect against neuronal death in response to hypoxia and glucose deprivation in cell culture conditions, but this effect was not mediated by CB1 or CB2 receptors. This latter finding emphasises the difficulty in extrapolating some results obtained *in vitro* to complex cellular systems *in vivo*. Nagayama and colleagues suggested that previous results demonstrating the neurotoxicity of cannabinoids *in vitro* (e.g., Chan, Hinds, Impey & Storm, 1998) were due to the nature of the *in vitro* testing conditions. Further *in vitro* studies using cell culture systems have demonstrated that a variety of cannabinoids can exert neuroprotective effects. Hampson, Grimwald, Axelrod and Deadwyler (1998) have reported that, in rat cortical neuron cultures, the naturally occuring cannabinoid delta^9-THC and the synthetic non-psychotropic cannabinoid, cannabidiol, both protect against glutamate-induced neurotoxicity but not via the CB1 receptor. The authors present evidence instead that these cannabinoids exert antioxidant effects by preventing hydroperoxide-induced oxidative damage and suggest that this is their mechanism of neuroprotective action. Whether this result will be replicated *in vivo* remains to be seen.

Conclusions

Cannabinoids have a number of potential therapeutic uses, including antispastic, analgesic and anti-emetic effects, as well as the treament of glaucoma and wasting in diseases like AIDS (see Feldman & Glass, 1998; Ameri, 1999 for reviews). There is increasing evidence that at least some cannabinoids may have neuroprotective effects in conditions such as stroke and head trauma. Some of the most recent evidence (e.g., Nagayama, Sinor, Simon, Chen, Graham, Jin & Greenberg, 1999) has been obtained using *in vivo* animal models and therefore may be directly relevant to neuroprotective therapy in humans. On the other hand, the evidence that cannabinoids have neurotoxic effects is inconclusive and based largely on *in vitro* data. There is substantial evidence that some cannabinoids disrupt short-term memory; however, this does not constitute neurotoxicity nor should it prevent medical scientists from exploiting the potential therapeutic uses of cannabinoids. In terms of potential therapeutic uses, smoking cannabis would be the least desirable option since, like tobacco smoke, cannabis smoke promotes the development of lung tumours (see Feldman & Glass, 1998; Ameri, 1999 for recent reviews). Other routes of administration, such as oral or i.v. administration, would be preferable. Furthermore, there are more potent synthetic analogues of delta^9-THC that could

be used for medical treatment and many of these have no psychotropic effects. Therefore, in discussing the risks and benefits of the recreational and medical uses of cannabinoids, a clear distinction needs to be made between smoking cannabis and the administration of synthetic cannabinoids by other routes of administration.

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