Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 364 (2007) 131–137
BBRC
www.elsevier.com/locate/ybbrc

Cannabinoid receptor agonists are mitochondrial inhibitors: A unified hypothesis of how cannabinoids modulate mitochondrial function and induce cell death

Andriani Athanasiou [a], Anna B. Clarke [a], Amy E. Turner [a,b], Nethia M. Kumaran [a], Sara Vakilpour [a], Paul A. Smith [a], Dimitra Bagiokou [a], Tracey D. Bradshaw [c], Andrew D. Westwell [d], Lin Fang [e], Dileep N. Lobo [f], Cris S. Constantinescu [f], Vittorio Calabrese [g], Andrzej Loesch [h], Stephen P.H. Alexander [a], Richard H. Clothier [a], David A. Kendall [a], Timothy E. Bates [b,e,*]

[a] *School of Biomedical Sciences, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, UK*
[b] *New Use Therapeutics Limited, BioCity Nottingham, Pennyfoot Street, Nottingham NG1 1GF, UK*
[c] *School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, UK*
[d] *Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3XF, UK*
[e] *Nottingham UK–China Collaboration on Complementary and Alternative Medicine (NUCCAM), School of Community Health Sciences, Division of Epidemiology and Public Health, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, UK*
[f] *School of Medical and Surgical Sciences, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, UK*
[g] *Department of Chemistry, Biochemistry and Molecular Biology Section, Faculty of Medicine, University of Catania, Catania, Italy*
[h] *Department of Anatomy and Developmental Biology, Royal Free & University College Medical School, UCL, Rowland Hill Street, London NW3 2PF, UK*

Received 24 September 2007
Available online 2 October 2007

Abstract

Time-lapse microscopy of human lung cancer (H460) cells showed that the endogenous cannabinoid anandamide (AEA), the phyto-cannabinoid Δ-9-tetrahydrocannabinol (THC) and a synthetic cannabinoid HU 210 all caused morphological changes characteristic of apoptosis. Janus green assays of H460 cell viability showed that AEA and THC caused significant increases in OD 595 nm at lower concentrations (10–50 μM) and significant decreases at 100 μM, whilst HU 210 caused significant decreases at all concentrations. In rat heart mitochondria, all three ligands caused significant decreases in oxygen consumption and mitochondrial membrane potential. THC and HU 210 caused significant increases in mitochondrial hydrogen peroxide production, whereas AEA was without significant effect. All three ligands induced biphasic changes in either mitochondrial complex I activity and/or mitochondrial complex II–III activity. These data demonstrate that AEA, THC, and HU 210 are all able to cause changes in integrated mitochondrial function, directly, in the absence of cannabinoid receptors.

Keywords: Cannabinoid; Apoptosis; Mitochondria; Anandamide; Δ-9-Tetrahydrocannabinol; HU 210

Cannabinoids have been shown to have effects mediated by CB1 and CB2 as well as other receptor types, at the plasma membrane, e.g. TRPV1 receptors [1] and in the nucleus on PPAR receptors [2]. Many studies assume that the cell types/organs that respond to cannabinoid receptor ligands all contain CB1 and/or CB2 receptors, when this may not be the case, and the effects observed may be due to cannabinoid receptor ligands interacting with a variety of non-CB1, non-CB2-receptor targets [3]. Cannabinoid receptor ligands can affect cell

* Corresponding author. Fax: +44 115 912 4281.
E-mail address: timothy.bates@newusetherapeutics.co.uk (T.E. Bates).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.09.107

proliferation, apoptosis and necrosis in a concentration-dependent positive and/or negative fashion, both *in vitro* and *in vivo*, and because of the large amount of conflicting data a unified hypothesis of cannabinoid action on these processes has not been formulated. There has been particularly intense debate concerning the potential anti-carcinogenic or pro-carcinogenic effects of cannabinoids, which is complicated by the fact that the non-medical/"recreational" use of cannabinoids is often associated with inhalation of smoke containing complex mixtures of compounds from *Cannabis sativa*, rather than purified cannabinoids, as are used for therapeutic medicinal purposes. Studies have suggested that mitochondria are involved in cannabinoid-induced cell death [4], and also demonstrate the ability of TRPV1 (vanilloid) receptor agonists and antagonists (some of which have activity at CB1 and/or CB2 receptors) to modulate mitochondrial function, resulting in apoptotic cell death [5]. The aim of the present study was to investigate whether endocannabinoid, phyto-cannabinoid and synthetic cannabinoid receptor agonists such as AEA, THC, and HU 210 can induce apoptosis in H460 lung cancer cells, and to investigate whether these effects were directly as a result of modulation of mitochondrial function.

Materials and methods

Cells and chemicals. H460 cells (a human non-small cell lung cancer line) were obtained from the American Type Culture Collection, Manassas, VA, USA. All chemicals used were of the highest grade available and were from Merck Biosciences, Nottingham, UK, Sigma Chemical Company, Poole, UK, InVitrogen, Paisley, UK or Tocris Bioscience, Bristol, UK. AEA, THC, and HU 210 were dissolved in 100% ethanol at a stock concentration of 10 mM. For all cannabinoids used, the diluent (ethanol) was never present at >1.0%. Control culture flasks with H460 cells or mitochondrial incubations having the same concentration of diluent acted as negative controls and showed no statistically significant effects.

Time-lapse photomicroscopy of H460 cells. H460 cells were grown on Iwaki 35 mm glass based dish to 60–70% confluence in a water jacketed incubator at 37 °C in 95% air/5% CO_2 in RPMI 1640 with 10% foetal calf serum (FCS) and then treated with the cannabinoids. Digital images were captured every minute for 2 h using a Leica DMIRE2 microscope and a Hamamatsu ORCA II BT 1024 CCD camera [5].

Janus green viability/cell mass assays. H460 cells were seeded into 24-well plates and incubated with the cannabinoids at a range of concentrations for 2 h. The medium containing the cannabinoids was removed, the cells washed with phosphate-buffered saline (PBS), fixed with 50% ethanol for 10 min and washed again with PBS. The cells were then stained for 15 min with 0.2% (w/v) Janus green [6], washed three times with distilled water which was then removed and the plates left to air-dry in a fume cupboard. Janus green was eluted using 500 μL of 0.5 M HCl, added to each well for 20 min to ensure complete elution. One hundred and fifty microliters of each sample was pippeted in triplicate into a 96-well plate, which was read spectrophotometrically at 595 nm in a SPECTRAmax 384 spectrophotometer (Molecular Devices, Berkshire, UK).

Isolation of rat heart mitochondria. Rat heart mitochondria were prepared from male 250 g Lister rats as previously described [5].

Measurement of mitochondrial oxygen consumption. Rat heart mitochondrial oxygen consumption was measured polorographically in respiration state 3 (+ADP) at 37 °C with 10 mM malate + 10 mM glutamate as substrates as previously described [5]. AEA, THC or HU 210 were added to the mitochondrial suspension in the oxygen electrode chamber and incubated for 5 min before addition of ADP.

Measurement of mitochondrial membrane potential. Mitochondrial membrane potential was measured fluorimetrically (using Rhodamine 123) at 37 °C in a Hitachi F2500 fluorimeter as previously described [5].

Measurement of mitochondrial hydrogen peroxide production. Mitochondrial hydrogen peroxide production was measured fluorimetrically (using amplex red) at 37 °C in a Hitachi F2500 fluorimeter as previously described [5].

Mitochondria–cannabinoid binding experiments. Freeze thawed mitochondria from rat heart (0.25 mg/mL) were incubated in 250 μL of respiration buffer (100 mM KCl, 75 mM mannitol, 25 mM sucrose, 10 mM Tris, 0.1 mM EDTA, and 10 mM phosphate-Tris pH 7.4) in a 96-well plastic plate at 37 °C for 15 min to thermostabilise. Cannabinoids (or the diluent, ethanol) were then added in a volume of 5 μL to give a range of concentrations from 0 to 200 μM (i.e., 0.00, 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, 100, and 200 μM) of AEA, Δ-9-THC, HU 210, which were then incubated with the mitochondria for 5 min at 37 °C to allow for drug binding. Samples were then pipetted off from the cannabinoid/mitochondria suspensions and added to separate assay plates containing all the assay components needed for the assay of mitochondrial complex I activity or mitochondrial complex II–III activity, as detailed below.

Measurement of mitochondrial complex I activity and mitochondrial complex II–III activity. Mitochondrial complex I [EC 1.6.99.3] activity was measured spectrophotometrically as the rotenone sensitive rate of NADH oxidation (at 340 nm and 37 °C) [7]. Mitochondrial complex II–III [EC 1.8.3.1] activity was measured spectrophotometrically as the antimycin a sensitive rate of cytochrome *c* reduction (at 550 nm and 37 °C) using succinate as the substrate [7].

Protein assay. Protein concentration was determined using a modified microplate Lowry assay with bovine serum albumin (0–200 μg/mL) as a concentration standard [7].

Statistical analysis. All experiments were repeated $n = 3$–8 times. Statistical analysis was performed using Students' paired *t*-tests in the case of the mitochondrial oxygen consumption experiments and the mitochondrial hydrogen peroxide production experiments and ANOVA followed by Dunnett's multiple comparison tests for the complex I activity and mitochondrial complex II–III activity measurements. Significance was attributed when $P < 0.05$.

Results

Cannabinoid receptor agonists induce morphological features of apoptosis in H460 cells

Fig. 1A–F shows single images taken at time = 0 and time = 2 h in the time-lapse microscope in the presence of 100 μM AEA, 100 μM THC, or 100 μM HU 210, respectively. In the case of AEA, after 2 h (Fig. 1B) there was a general rounding of the H460 cells, with no apparent cell loss, but with many cells showing a marked increase in cytoplasmic granularity. In the case of THC, after 2 h (Fig. 1D) there was a general rounding of the H460 cells, with some marked "ballooning out" of the cytoplasm in some cells (indicated by a **C** on the images) and some cells detaching (indicated by **Det** on the image) from the culture plate surface, as shown by becoming rounded and slightly unfocussed with a dark cell membrane delineation in the image. In the presence of HU 210, after 2 h (Fig. 1F) many H460 cells

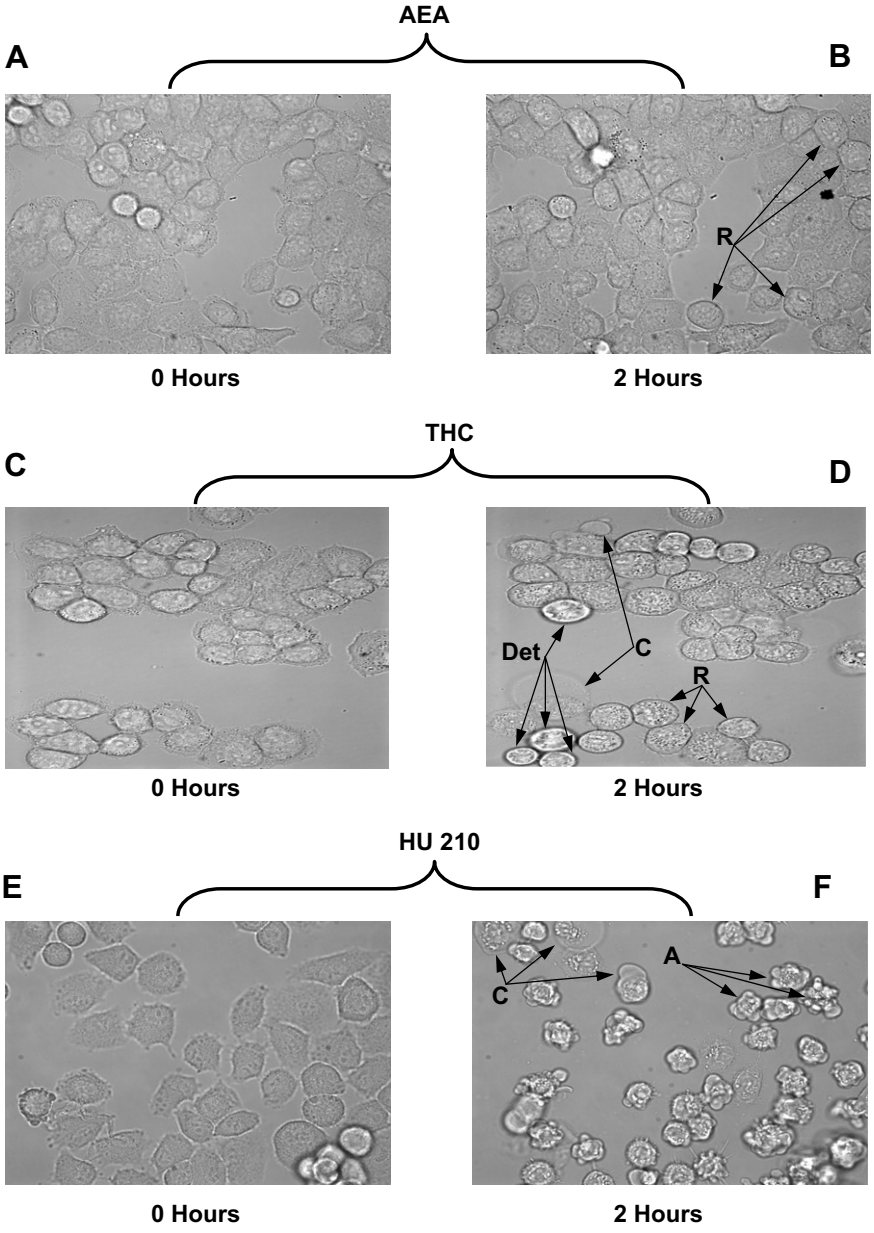

Fig. 1. Photomicroscopy of H460 cells (A–F) showing single images taken at time = 0 and time = 2 h in the presence of 100 μM AEA, 100 μM THC, or 100 μM HU 210. Rounding of the cells is indicated by **R** on the images, ''ballooning out'' of the cytoplasm indicated by **C** on the images, cell detachment is indicated by **Det** on the images, membrane blebbing with frank apoptosis is indicated by **A** on the images.

became detached from the tissue culture plate surface and showed frank morphological signs of apoptosis, including membrane blebbing.

Cannabinoid receptor agonists decrease mitochondrial membrane potential

Fig. 2 shows annotated fluorimeter traces of Rhodamine 123 fluorescence before and after subsequent additions of the various cannabinoid receptor agonists to isolated rat heart mitochondria. Fig. 2A shows that 2 equal additions of AEA (final concentration of 20 μM) caused a small cumulative decrease in membrane potential. The addition of the uncoupler Carbonyl Cyanide *m*-chlorophenylhydrazone (CCCP) caused a large increase in fluorescence, demonstrating that the mitochondria retained a significant membrane potential after the AEA additions. Fig. 2B shows that 2 additions of THC (final concentration of 20 μM) caused a cumulative decrease in membrane potential (which was intermediate to that due to AEA and HU 210). The addition of the uncoupler CCCP caused a further increase in fluorescence, demonstrating that the mitochondria had some membrane potential left after the cannabinoid additions. Fig. 2C demonstrates that two additions of HU 210 (final concentration of 20 μM) caused a rapid and greater decrease in mitochondrial membrane potential

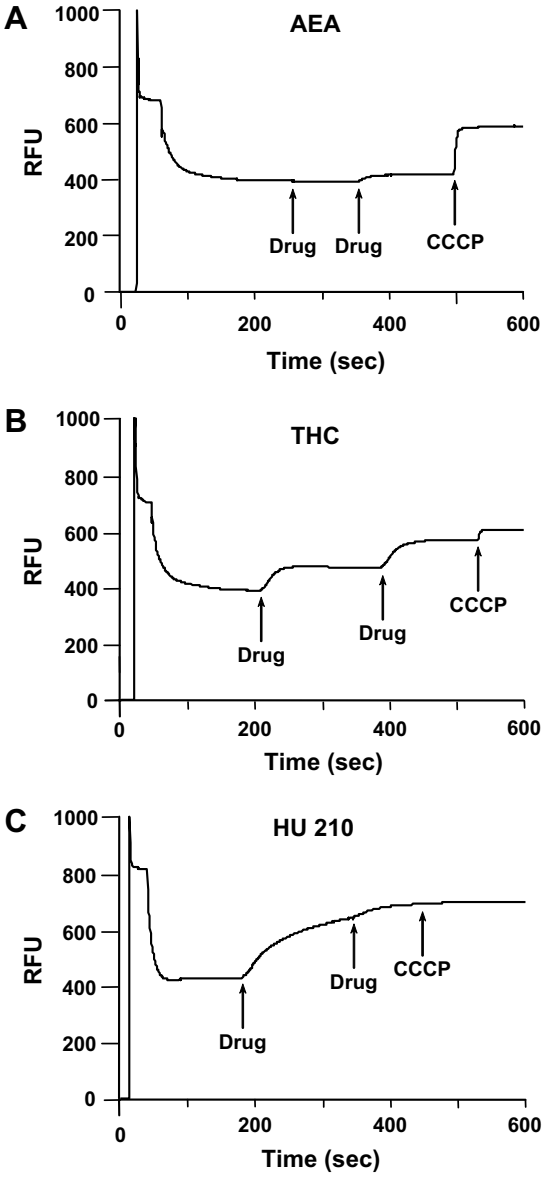

Fig. 2. Fluorimeter traces (A–C) of Rhodamine 123 fluorescence before and after subsequent additions of the various cannabinoid receptor agonists to isolated rat heart mitochondria. The word "Drug" on the figures indicates the sequential addition of 2×10 μM (20 μM f.c.) of the respective cannabinoid receptor agonist. CCCP indicates the addition of the uncoupler CCCP.

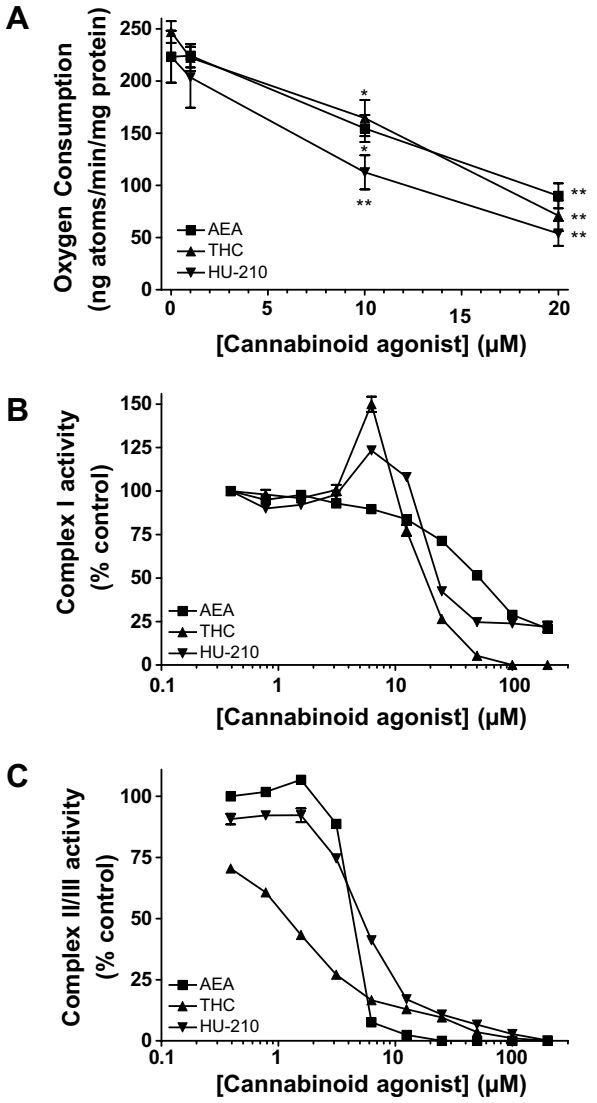

Fig. 3. (A) Mitochondrial oxygen consumption versus drug concentration for AEA, THC, and HU 210. The asterisk symbol (*) indicates statistical significance relative to control values at $P < 0.05$. (B) Mitochondrial complex I activity versus drug concentration for AEA, THC, and HU 210. (C) Mitochondrial complex II–III activity versus drug concentration for AEA, THC, and HU 210.

than with either AEA or THC, with no apparent residual membrane potential being left at the end of the incubation period, as indicated by the lack of increase in fluorescence on addition of CCCP.

Cannabinoid receptor agonists inhibit mitochondrial oxygen consumption

Fig. 3A shows plots of mitochondrial oxygen consumption versus cannabinoid concentration for AEA, THC, and HU 210. All three cannabinoid receptor agonists caused a concentration dependent decrease in mitochondrial oxygen consumption, with HU 210 having a statistically significantly greater effect on oxygen consumption than AEA or THC.

Cannabinoid receptor agonists cause biphasic changes in mitochondrial complex I activity

Fig. 3B shows plots of mitochondrial complex I activity versus cannabinoid concentration for AEA, THC, and HU 210. Whilst AEA, THC, and HU 210 causes no significant change in mitochondrial complex I activity below a concentration of 3.125 μM, both THC and HU 210 caused a significant increase in mitochondrial complex I activity at 6.25 μM (THC), and 6.25 and 12.5 μM (HU 210) which was then followed by a significant decrease in complex I

activity. At concentrations above 12.5 μM, all three cannabinoid receptor agonists caused concentration dependent decreases in mitochondrial complex I activity, with AEA demonstrating less potency as compared to THC and HU 210.

Cannabinoid receptor agonists cause biphasic changes in miochondrial complex II–III activity

Fig. 3C shows plots of mitochondrial complex II–III activity versus cannabinoid concentration for AEA, THC, and HU 210. AEA caused a small but significant increase in mitochondrial complex II–III activity at 1.56 μM followed by a concentration dependent rapid decrease in mitochondrial complex II–III activity. THC caused a significant decrease in complex II–III activity compared to control even at the lowest concentration used in these studies (0.39 μM). HU 210 caused a concentration dependent decrease in complex II–III activity from 1.56 μM upwards.

Cannabinoid receptor agonists can increase mitochondrial hydrogen peroxide production

Fig. 4 shows mitochondrial hydrogen peroxide production in the presence of various concentrations of the cannabinoid receptor agonists. AEA (Fig. 4B) did not cause any significant change in mitochondrial hydrogen peroxide production. In contrast, both THC (Fig. 4B) and HU 210 (Fig. 4C) caused a concentration dependent increase in mitochondrial hydrogen peroxide production.

Cannabinoid receptor agonists cause changes in H460 cell Janus green staining

Fig. 5 (supplementary material) shows plots of Janus green staining (measured as OD 595 nm) in H460 cells exposed to a range of concentrations of AEA, THC, and HU 210 for 2 h. Fig. 5A shows that with exposure to AEA at concentrations of 10, 20, and 50 μM there was a significant increase in OD 595 nm, whilst at the higher concentration of 100 μM, there was a significant decease in OD 595 compared to the vehicle control (to which the equivalent volume of ethanol was added). Fig. 5B shows that following 2 h of exposure to THC, there was a small but significant increase in OD 595 in the presence of 10 μM THC, with 20 and 50 μM THC causing no significant change in OD 595, and that with 100 μM THC, there was a significant decrease in OD 595 compared to control. Fig. 5C shows that following 2 h of exposure to HU 210 there was a significant decrease in OD 595 at all concentrations of HU 210 used.

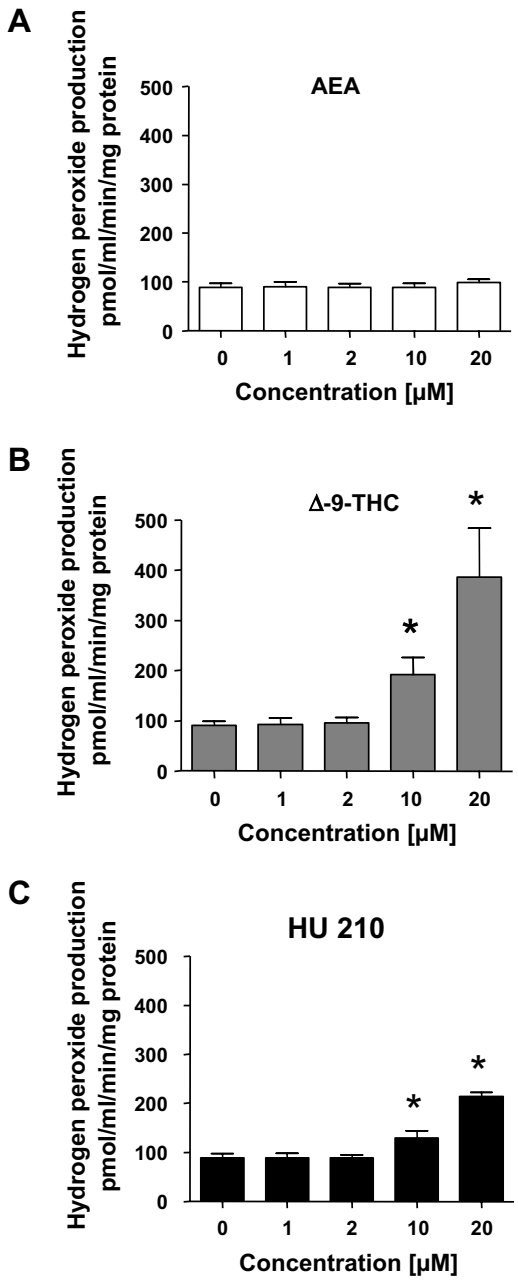

Fig. 4. A graph of mitochondrial hydrogen peroxide production versus drug concentration for AEA, THC, and HU 210. The asterisk symbol (*) indicates statistical significance relative to control values at $P < 0.05$.

Discussion

Janus green is only taken up by live cells, staining both proteins and DNA, and its uptake correlates linearly with cell numbers over a broad range of cell densities [6]. Therefore, the increases in OD 595 seen at lower concentrations of AEA and THC are suggestive of an increase in cell number through cell division, which was not an apparent feature of the digital images. These increases in OD 595 are more likely to be due to increases in DNA synthesis and subsequent synthesis of pro-apoptotic proteins, as it has been demonstrated that the protein synthesis inhibitor cycloheximide can block apoptosis (induced by the mitochondrial complex IV inhibitor cyanide) in cultured neurons [8]. Taken together with the time-lapse microscopy data, the Janus green

data obtained with higher concentrations of AEA or THC and with all concentrations of HU 210 suggest that the decreases in OD 595 are due to detachment of cells from the surface of the cell culture dish.

The decrease in mitochondrial oxygen consumption observed is consistent with AEA, THC, and HU 210 being inhibitors of the mitochondrial respiratory chain. Earlier studies reported that a variety of cannabinoids when administered to rats *in vivo* caused differential decreases in the synthesis of RNA, DNA, and protein synthesis [9], the latter 2 processes being ATP dependent. However, in further studies, which measured oxygen consumption in L1210 murine leukemia cells the authors concluded that there was no relationship between the inhibition of oxygen consumption and the inhibition of DNA synthesis they had previously observed [10].

In another study, THC also caused a stimulation of oxygen consumption at low concentrations of THC which the authors commented "vanishes" in the presence of 50 μg THC per mg protein [11]. Biphasic, concentration dependent changes in ATPase activity and mitochondrial swelling were also observed, and these effects were suggested to be due to THC interacting with "protein-phospholipid receptors in the mitochondrial membrane" [11]. Previous studies using liver mitochondria reported that THC acted as an uncoupler, and caused mitochondrial swelling and release of matrix enzymes, and concluded that the effects of THC on mixed phospholipid micelles may have been due to the "destabilisation" of the mitochondrial phospholipid cardiolipin [12]. Any interactions of cannabinoids with cardiolipin are potentially important, as, modulation of the oxidation state of cytochrome *c* can cause its release from cardiolipin in the inner membrane, to the intermembrane space, thus raising the free cytochrome *c* concentration, which favours type II (mitochondrial) apoptosis [13].

One earlier study reported that THC can decrease mitochondrial NADH oxidase activity [14], which is only a partial and inaccurate measurement of mitochondrial complex I activity. However, the rotenone-sensitive mitochondrial complex I activity assay used in this present study which was developed in approximately the same year [15] and modified several years afterwards [16] is much more specific and provides an accurate measure of mitochondrial complex I activity, especially when used with highly purified mitochondria.

The concentration dependency of the anti-mitochondrial effects of the cannabinoids needs to be carefully considered in relation to the likelihood of such concentrations being achieved *in vivo* after drug administration. Pharmacokinetic modelling of THC administration in a pig model [17] indicates that intravenous administration of a dose of 200 μg/kg THC (a dose that would induce psychoactive effects in humans) produced peak tissue levels ranging from 0.4 ng/g in bile to 1888 ng/g in lung. This approximates to a maximal tissue concentration of 6 μM, and is within the concentration range that might modulate mitochondrial function *in vivo*. The present data relate to acute effects and it is possible that repeated exposure to cannabinoids might produce more marked changes in mitochondrial function. In addition, the heart mitochondria used in our experiments have the highest activities of mitochondrial complexes of any tissue and it is likely that much lower concentrations of cannabinoids would be needed to produce significant effects on mitochondria in other tissues such as brain, kidney, and liver [7].

In situ tissue levels of the endocannabinoids in humans are unknown, but in animal models they are known to change significantly in response to pathophysiological challenge. For example, in a rat model of focal stroke, AEA concentrations in the affected cerebral hemisphere were reported to reach 3 μM [18], which is possibly within the effective concentration range to affect mitochondrial function *in vivo*.

It has also been shown that mitochondrial enzyme activities in the brain exhibit distinct developmental profiles [19,20], and are decreased in ageing and in neurodegenerative diseases [21], all of which may leave the brain selectively vulnerable to the effects of THC.

The increases in complex I activity at lower concentrations of both AEA and THC may be due to changes in membrane fluidity, as V_{max} and/or K_m can be altered due to changes in membrane fluidity [22], suggesting that cannabinoids may be physiological/pathophysiological modulators of mitochondrial function.

Furthermore, these data also allow the drawing of parallels to data obtained over the last two decades with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a contaminant of a "designer" drug which causes Parkinsonism in relatively young people [23], through the inhibition of mitochondrial complex I activity [18].

In conclusion, our data strongly suggest that cannabinoids may be beneficial for cancer chemotherapy. However without careful monitoring of plasma tissue levels, cannabinoids may also be harmful in individuals with a genetic predisposition to mitochondrial disease and/or undeveloped, aged or dysfunctional mitochondria, such as has been observed in a wide range of neurological [21] and psychiatric disorders including schizophrenia [24].

Acknowledgments

We thank the Medical Research Council (UK) and the School of Molecular Medical Sciences M.Sc. program in Oncology and the European Union for financial support. We also thank Mr. Ian Ward for his excellent technical assistance with time-lapse microscopy.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.09.107.

References

[1] R.G. Pertwee, Pharmacological actions of cannabinoids, Handb. Exp. Pharmacol. (2005) 1–51.

[2] Y. Sun, S.P. Alexander, D.A. Kendall, A.J. Bennett, Cannabinoids and PPARalpha signalling, Biochem. Soc. Trans. 34 (2006) 1095–1097.

[3] K. Mackie, N. Stella, Cannabinoid receptors and endocannabinoids: evidence for new players, AAPS J. 8 (2006) E298–E306.

[4] T.A. Sarafian, N. Habib, M. Oldham, N. Seeram, R.P. Lee, L. Lin, D.P. Tashkin, M.D. Roth, Inhaled marijuana smoke disrupts mitochondrial energetics in pulmonary epithelial cells in vivo, Am. J. Physiol. Lung Cell. Mol. Physiol. 290 (2006) L1202–L1209.

[5] A. Athanasiou, P.A. Smith, S. Vakilpour, N.M. Kumaran, A.E. Turner, D. Bagiokou, R. Layfield, D.E. Ray, A.D. Westwell, S.P. Alexander, D.A. Kendall, D.N. Lobo, S.A. Watson, A. Lophatanon, K.A. Muir, D.A. Guo, T.E. Bates, Vanilloid receptor agonists and antagonists are mitochondrial inhibitors: how vanilloids cause non-vanilloid receptor mediated cell death, Biochem. Biophys. Res. Commun. 354 (2007) 50–55.

[6] G. Raspotnig, G. Fauler, A. Jantscher, W. Windischhofer, K. Schachl, H.J. Leis, Colorimetric determination of cell numbers by Janus green staining, Anal. Biochem. 275 (1999) 74–83.

[7] E. Daley, D. Wilkie, A. Loesch, I.P. Hargreaves, D.A. Kendall, G.J. Pilkington, T.E. Bates, Chlorimipramine: a novel anticancer agent with a mitochondrial target, Biochem. Biophys. Res. Commun. 328 (2005) 623–632.

[8] M.S. Jensen, B. Ahlemeyer, A. Ravati, P. Thakur, H.D. Mennel, J. Krieglstein, Preconditioning-induced protection against cyanide-induced neurotoxicity is mediated by preserving mitochondrial function, Neurochem. Int. 40 (2002) 285–293.

[9] A.N. Tucker, M.A. Friedman, Effects of cannabinoids on L1210 murine leukemia I. Inhibition of DNA synthesis, Res. Commun. Chem. Pathol. Pharmacol. 17 (1977) 703–714.

[10] A.N. Tucker, M.A. Friedman, Effects of cannabinoids on L1210 murine leukemia III. Inhibition of respiration, Res. Commun. Chem. Pathol. Pharmacol. 23 (1979) 327–332.

[11] T. Bino, A. Chari-Bitron, A. Shahar, Biochemical effects and morphological changes in rat liver mitochondria exposed to 1-tetrahydrocannabinol, Biochim. Biophys. Acta 288 (1972) 195–202.

[12] J.M. Mahoney, R.A. Harris, Effect of 9-tetrahydrocannabinol on mitochondrial processes, Biochem. Pharmacol. 21 (1972) 1217–1226.

[13] M. Ott, B. Zhivotovsky, S. Orrenius, Role of cardiolipin in cytochrome *c* release from mitochondria, Cell Death. Differ. (2007) [Epub ahead of print].

[14] A. Bartova, M.K. Birmingham, Effect of delta-9-tetrahydrocannabinol on mitochondrial NADH-oxidase activity, J. Biol. Chem. 251 (1976) 5002–5006.

[15] C.I. Ragan, NADH-ubiquinone oxidoreductase, Biochim. Biophys. Acta 456 (1976) 249–290.

[16] T.E. Bates, S.J. Heales, S.E. Davies, P. Boakye, J.B. Clark, Effects of 1-methyl-4-phenylpyridinium on isolated rat brain mitochondria: evidence for a primary involvement of energy depletion, J. Neurochem. 63 (1994) 640–648.

[17] B. Brunet, C. Doucet, N. Venisse, T. Hauet, W. Hebrard, Y. Papet, G. Mauco, P. Mura, Validation of Large White Pig as an animal model for the study of cannabinoids metabolism: application to the study of THC distribution in tissues, Forensic Sci. Int. 161 (2006) 169–174.

[18] S. Muthian, D.J. Rademacher, C.T. Roelke, G.J. Gross, C.J. Hillard, Anandamide content is increased and CB1 cannabinoid receptor blockade is protective during transient, focal cerebral ischemia, Neuroscience 129 (2004) 743–750.

[19] A. Almeida, K.J. Brooks, I. Sammut, J. Keelan, G.P. Davey, J.B. Clark, T.E. Bates, Postnatal development of the complexes of the electron transport chain in synaptic mitochondria from rat brain, Dev. Neurosci. 17 (1995) 212–218.

[20] T.E. Bates, A. Almeida, S.J. Heales, J.B. Clark, Postnatal development of the complexes of the electron transport chain in isolated rat brain mitochondria, Dev. Neurosci. 16 (1994) 321–327.

[21] V. Calabrese, G. Scapagnini, A.M. Giuffrida Stella, T.E. Bates, J.B. Clark, Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity, Neurochem. Res. 26 (2001) 739–764.

[22] S.P. Patel, S.S. Katyare, Insulin-status-dependent modulation of FoF1-ATPase activity in rat liver mitochondria, Lipids 41 (2006) 695–703.

[23] J.W. Langston, P. Ballard, J.W. Tetrud, I. Irwin, Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis, Science 219 (1983) 979–980.

[24] L. Martorell, T. Segues, G. Folch, J. Valero, J. Joven, A. Labad, E. Vilella, New variants in the mitochondrial genomes of schizophrenic patients, Eur. J. Hum. Genet. 14 (2006) 520–528.