Endocannabinoids in endocrine and related tumours

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Abstract

The 'endocannabinoid system', comprising the cannabinoid CB1 and CB2 receptors, their endogenous ligands, endocannabinoids and the enzymes that regulate their biosynthesis and degradation, has drawn a great deal of scientist attention during the last two decades. The endocannabinoid system is involved in a broad range of functions and in a growing number of physiopathological conditions. Indeed, recent evidence indicates that endocannabinoids influence the intracellular events controlling the proliferation of numerous types of endocrine and related cancer cells, thereby leading to both *in vitro* and *in vivo* antitumour effects. In particular, they are able to inhibit cell growth, invasion and metastasis of thyroid, breast and prostate tumours. The chief events of endocannabinoids in cancer cell proliferation are reported highlighting the correspondent signalling involved in tumour processes: regulation of adenylyl cyclase, cyclic AMP-protein kinase-A pathway and MEK-extracellular signal-regulated kinase signalling cascade.

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Introduction

Up to date since the isolation and characterisation of the psychoactive component of *Cannabis sativa*, Δ^9-tetrahydrocannabinol (Δ^9-THC), about 60 different plant terpeno-phenols more or less related to THC have been isolated and defined cannabinoids. They include cannabidiol (CBD), cannabinol, cannabigerol and cannabichromene. The discovery of these principles stimulated the generation of a whole range of synthetic analogues that included not only compounds structurally similar to phytocannabinoids, but also analogues with different chemical structures, including classic and non-classic cannabinoids and aminoalkylindoles (Howlett *et al*. 2002) as well as the subsequently discovered endogenous arachidonic acid derivatives or endocannabinoids. The discovery of this family of endogenous cannabinoids (Devane *et al*. 1992, Mechoulam *et al*. 1995, Sugiura *et al*. 1995) has focused much attention on cannabinoids and their pharmacological properties during the last few years (Di Marzo *et al*. 2004). The best-known endogenous cannabimimetics are *N*-arachidonoyl-ethanolamine (AEA also called anandamide), another arachidonate derivate, 2-arachidonoyl-glycerol (2-AG) and an ether-type endocannabinoid, 2-arachidonoyl-glyceryl ether (Noladin ether) (Devane *et al*. 1992,

Mechoulam *et al*.1995, Sugiura *et al*. 1995, 2002, Hanus *et al*. 2001). Moreover, compounds called 'endocannabinoid-like' are present in human, rat and mouse brain where they might inhibit the degradation of AEA or 2-AG and, consequently, increase their activity (Mechoulam *et al*. 2002). So far, *N*-palmitoylethanolamine (PEA), *N*-oleoylethanolamine and *N*-stearoylethanolamine exhibit this endocannabinoid-like activity (Di Marzo 1998, Maccarrone & Finazzi-Agrò 2002).

Two different cannabinoid receptors (CBs) have been identified so far and cloned from mammalian tissues: CB1, or central receptor (Matsuda *et al*. 1990) and CB2, or peripheral receptor (Munro *et al*. 1993). Whereas the CB1 is preferentially expressed in the central nervous system (Matsuda *et al*. 1990), the CB2 has been described as the predominant form expressed by peripheral immune cells (Munro *et al*. 1993, Galiegue *et al*. 1995). An ever increasing number of reports and a lot of pharmacological evidence suggest that endocannabinoids might exert their biological effects also through non-CB1/CB2 receptors which, however, have not yet been cloned except for transient receptor potential vanilloid type 1 (TRPV1), the TRPV1 ion channel, which is activated by various lipids including anandamide (Begg *et al*. 2005).

Endocannabinoids show variable selectivity for the two receptors (McAllister & Glass 2002, Mechoulam *et al.* 2002). Both the CB1 and CB2 genes encode a seven-transmembrane domain protein belonging to the $G_{i/o}$-protein-coupled receptor family (Munro *et al.* 1993). CB1 receptors were found to efficiently couple and activate both G_i and G_o, whereas CB2 only G_o, also showing an agonist-selective G-protein signalling (Glass & Northup 1999).

The CB1 receptor is known to be coupled with the inhibition of adenylyl cyclase, inhibition of voltage-dependent Ca^{++} channels and activation of G-protein regulated inwardly rectifying K^+ currents (Howlett 1995, Porter & Felder 2001). Furthermore, the CB1 receptor has been shown to regulate different members of mitogen-activated protein kinase (MAPK), such as extracellular signal-regulated kinase (ERK; Bouaboula *et al.* 1995, Pertwee *et al.* 1997), c-Jun N-terminal kinase (Liu *et al.* 2000, Rueda *et al.* 2000), p-38 (Galve-Roperh *et al.* 2000, Rueda *et al.* 2000) and p42/44 (Bouaboula *et al.* 1995). It is also reported that the CB1 receptor activates phosphatidylinositol-3 kinase (PI3K), which in turn mediates tyrosine phosphorylation, activation of Raf and may also signal via phosphokinase B (PKB) in an SR141716-sensitive manner (Gomez del Pulgar *et al.* 2002*a,b*). It was shown that anandamide, via the CB1 receptor, increases the tyrosine protein phosphorylation of several proteins including focal adhesion kinase (FAK) in normal neurons of the rat hippocampus, by inhibiting adenylyl cyclase and phosphokinase A (PKA; Derkinderen *et al.* 1996). In addition, the CB1 receptor regulates the sphingolipid metabolism, leading to enhanced ceramide levels by either activating sphingomyelin hydrolysis (Sanchez *et al.* 1998, 2001) or increasing ceramide synthesis *de novo* (Gomez del Pulgar *et al.* 2002*a,b*).

CB2 receptors, similar to CB1, through their ability to couple to $G_{i/o}$, can inhibit adenylyl cyclase and activate MAP kinase and Krox-24 pathways through a phosphokinase C (PKC)-dependent activation of MAPK (Bouaboula *et al.* 1996). However, in contrast to CB1, CB2 receptors do not seem to modulate ion channels directly (Felder *et al.* 1995). Evidence suggests the involvement of the CB2 receptor in the activation of the PI3K/PKB pathway, which in turn induces the translocation of Raf-1 to the membrane and phosphorylation of p42/p44 MAP kinase (Sanchez *et al.* 2003*a,b*). It is also suggested that a cannabinoid-mediated reduction of MAP kinase may inhibit interleukin-2 (IL-2) production in mouse splenocytes and contribute a mechanism for immunosuppression by cannabinoids (Kaplan *et al.* 2003). Although it was not determined that the CB subtype was involved in mediating this response, it is likely to be CB2-mediated as this is the most abundantly expressed cannabinoid receptor subtype in the immune system (Parolaro *et al.* 2002). Collected evidence suggests that different structural classes of CB agonists have the unique ability to activate different signalling cascades which, in turn, influences agonist efficacy.

In the central nervous system, endocannabinoids act as modulator compounds as well as neurotransmitters (MacDonald & Vaughan 2001, Wilson & Nicoll 2002); in the peripheral and neural tissues, they have been shown to modulate as paracrine or autocrine mediators, protein and nuclear factors involved in cell proliferation, differentiation and apoptosis. These data suggest that the endocannabinoid signalling system could be involved, among other effects, in the control of cell survival, death and neoplastic transformation (Guzman *et al.* 2001, Bifulco & Di Marzo 2002, Bifulco *et al.* 2006).

The fundamental aspects of tumorigenesis widely accepted are deregulation of cell survival pathways and resistance to apoptosis. The aberrant growth and survival of tumour cells is dependent upon a small number of highly activated signalling pathways, the inhibition of which elicits potent growth inhibitory or apoptotic responses in tumour cells. Accordingly, there is a considerable interest in therapeutics that can modulate survival signalling pathways and target cancer cells for death.

Accumulated evidence indicates that CBs could be an important target for the treatment of cancer due to their ability to regulate signalling pathways critical for cell growth and survival. Several studies have produced exciting new leads in the search for anticancer treatments with cannabinoid-related drugs. Natural, THC, synthetic, HU210, WIN-55,212-2 and endogenous, 2-AG, AEA cannabinoids are nowadays known to control various cancer types by modulating tumour growth, apoptosis, migration and blood supply to tumours (Bifulco & Di Marzo 2002, Guzman *et al.* 2002). In this review, we have tried to summarise the importance of CB expression and modulation to induce antitumour effects.

Cannabinoids and breast cancer

Breast cancer is the number one cause of cancer in women (Glass *et al.* 2007). For the past few years, there has been an increasing interest in the development of agents targeted against molecular pathways considered to be involved in the process of malignant transformation or tumour progression. However, it is well

known that many of the signalling molecules required for normal mammary gland development and lactation are also involved in breast carcinogenesis, including those activated downstream of the oestrogen receptor (ER) and the human epidermal growth factor receptor family (EGFR and erbB; Visvader & Lindeman 2003). There are two main pathways involved in the tumour phenotype: the Ras/Raf–MAPK ERK1/2 pathway, and the PI3K/AKT pathway. Together, these pathways regulate cell survival, proliferation, growth and motility. Ras signalling is often enhanced in breast cancers, due to the increased expression of erbB receptors, signalling intermediates and/or Ras proteins themselves (Malaney & Daly 2001). An important Ras effector pathway resulting in mitogenic signalling is the Raf/MEK/Erk cascade, which influences multiple end points including increased transcription of cyclin D1 (Coleman *et al*. 2004). Similarly, oestrogens and progestins also activate cytoplasmic signalling pathways including Src/Ras/Erk signalling (Edwards 2005). There is also some evidence of cross-talk between the ErbB family of receptors and ER signalling in breast cancer. The overexpression of EGFR and heregulin receptor 2 (HER2) with the subsequent downstream activation of MAPKs is implicated in the mechanisms responsible for resistance to hormonal treatment during prolonged endocrine therapy or by long-term oestrogen deprivation

(Nicholson *et al*. 2004). Targeted therapies against all the above-mentioned pathways have recently become one of the most active and promising areas of development in oncology. Therefore, new drugs affecting multiple points along these pathways are increasingly needed.

For instance, cannabinoids modulate MAPK/ERK and PI3K/AKT survival pathways, which have a prominent role in the control of cell fate (Guzman 2003). In 1998, De Petrocellis *et al*. demonstrated for the first time the antimitogenic action of CB1 receptor stimulation in human breast cancer cell lines, EFM-19 and MCF-7, known to express oestrogen and prolactin (PRL) receptors and proliferate in response to the treatment with steroid or lactogenic hormones (Clevenger *et al*. 1995). Anandamide inhibited the expression of PRL receptor, induced downregulation of the breast cancer associated antigen (*brca1*) gene product and the high-affinity neurotrophin receptors *trk* (Melck *et al*. 1999*a,b*; Fig. 1). The dose-dependent antiproliferative effect was proportional to the degree of hormone dependency of breast cancer cell lines. The mechanism involved in such an effect has been ascribed to the inhibition of adenylyl cyclase, cyclic AMP (cAMP) protein kinase-A (PKA) pathway and, consequently, to the activation of MAPK (Fig. 1). Cannabinoids prevent the inhibition of RAF1 (caused by PKA-induced Raf-phosphorylation) and induce a prolonged activation of the RAF1-MEK-ERK
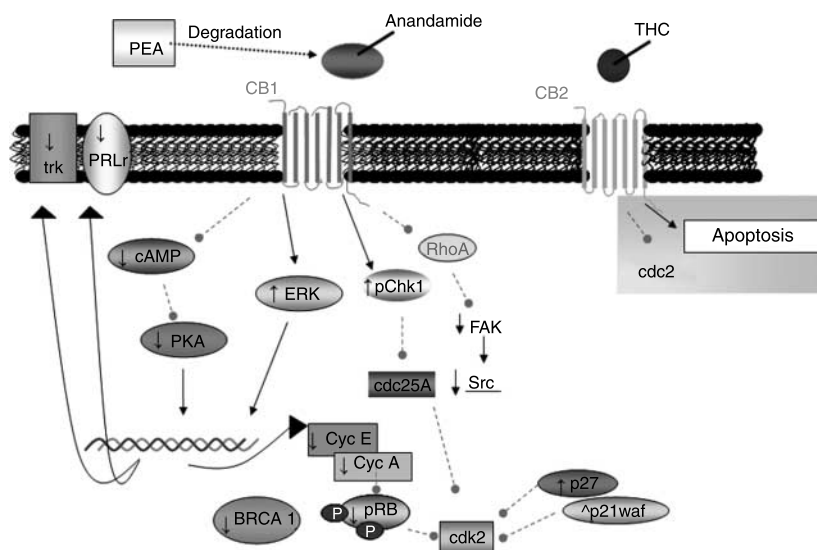

Figure 1 Schematic of signalling pathways associated with cannabinoid receptor activation induced by its agonists. Upon receptor binding, cannabinoid receptor agonists inhibit cell proliferation through inhibition of cAMP-dependent protein kinase. Cannabinoid receptor agonists activate ERK cascade leading to the downregulation of PRL receptor and trk levels. Anandamide induces cyclin kinase inhibitor p27/KIP1 and p21waf with the modulation of cell cycle regulatory molecules CycA/and CycE/cdk2. Furthermore, it activates a cell cycle checkpoint though Chk1 activation and Cdc25A proteolysis determining a cell cycle arrest. The proposed mechanisms are based on the available literature and are cell-specific, and not all pathways are triggered simultaneously.

cascade (Melck *et al.* 1999*a*,*b*), leading to the down-regulation of the PRL receptor and *trk* levels. Moreover, compounds like palmitoylethanolamide (PEA) might act as 'entourage' substance for AEA, enhancing cannabinoid biological actions. Di Marzo *et al.* (2001) reported that chronic treatment with PEA enhances the AEA-induced inhibition of cell proliferation through decreased expression of fatty acid amide hydrolase (FAAH), the enzyme chiefly responsible for AEA degradation. Similar results, obtained with arvanil, a more stable AEA analogue (Melck *et al.* 1999*a*,*b*), and HU210, which cannot be hydrolysed by FAAH, suggested that PEA could also enhance the vanilloid receptor (VR1)-mediated effects of AEA on calcium influx into cells (De Petrocellis *et al.* 2000, 2002).

The cell cycle machinery was deregulated at multiple levels in breast cancer (Caldon *et al.* 2006). Cyclins, Cdk and Cdk inhibitors, have been extensively studied as cell cycle regulators in breast cancer cells, as putative mammary oncogenes or tumour suppressor genes and as potential markers of therapeutic response or outcome. We reported that anandamide arrests the proliferation of human breast cancer cells MDA-MB-231 in the S phase of the cell cycle as a consequence of the specific loss in Cdk2 activity, upregulation of p21waf and a reduced formation of the active complex cyclin E/Cdk2 kinase (Laezza *et al.* 2006). Recently, it has been demonstrated that the checkpoint kinase Chk1 mediates both intra-S and G2 phase checkpoints by targeting the Cdc25A phosphatase to proteolysis following DNA damage, being also periodically activated in every S phase of the unperturbed cell cycle (Sorensen *et al.* 2003, Uto *et al.* 2004). It has been observed that anandamide activates a cell cycle checkpoint, through Chk1 activation and Cdc25A proteolysis, thereby preventing Cdk2 activation by dephosphorylation on critical inhibitory residues (Thr14/Tyr15), which arrests cells in S phase. This entails that the endocannabinoid system could be involved in the regulation of cell cycle, the main process controlling cell fate. Moreover, this could be of great medical interest, since it has been proposed that DNA damage checkpoints might become activated during the early stages of tumorigenesis leading to cell cycle blockade or apoptosis and could act as a barrier against genomic instability and tumour progression (Bartkova *et al.* 2005).

Δ^9-THC was reported to reduce human breast cancer cell proliferation by blocking the progression of cell cycle in G2/M phase via the downregulation of Cdc2 and by inducing apoptosis. In this case, the effects were mediated by CB2 receptors (Fig. 1). However, CB2-selective antagonists significantly but not totally prevented such effects, pointing to the existence of CB receptor-independent mechanism (Caffarel *et al.* 2006). In contrast, a previous paper (McKallip *et al.* 2005) demonstrated that human breast cancer cell lines, MCF-7 and MDA-MB-231, and the carcinoma induced in mice by mouse mammary 4T1 cells injection, are resistant to the Δ^9-THC-induced cytotoxicity. Furthermore, mice exposure to Δ^9-THC led to significantly elevated 4T1 tumour growth and metastasis, probably due to inhibition of the specific antitumour immune response. Indeed, the well-known immunosuppressive properties of cannabinoids, through CB2 receptors, have to be taken in regard, since they may compromise antitumour immune responses (Klein 2005). In contrast, McKallip reported that the breast cancer cell lines express low levels of CB1/CB2 receptors, hypothesising that the degree of sensitivity of a tumour to Δ^9-THC may be related to the level of CB1/CB2 expression and that Δ^9-THC exposure may lead to increased growth and metastasis of tumours with low or no expression of CBs (McKallip *et al.* 2005). It is an unsurprising data that different clones of the same cell lines showed very variable levels of receptors as well as different responsivity to hormones and growth factors; moreover, the CB receptor expression could be modulated, at least in part, by culture conditions and the number of subculturing passages, even in the absence of specific ligands (Melck *et al.* 2000). In addition, 4T1 cells express high levels of VR1, and this could be a very interesting data because these breast cancer cells may be more sensitive to AEA, a potent agonist for the VR1 rather than Δ^9-THC (Melck *et al.* 1999*a*,*b*, Zygmunt *et al.* 2000). Caffarel *et al.* (2006) reported a correlation between CB2 expression and the histologic grade of human breast tumours. The overexpression of the growth factor receptors and the ER negative receptor status has been linked to a poor prognosis and a more aggressive breast tumour phenotype. CB2 expression was reported to be higher in those tumours with poor prognosis and predicted low response to conventional therapies, for instance estrogen receptor-negative (ER–) and progesterone receptor-negative (PR–) tumours, which are weakly responsive to the adjuvant tamoxifen (Glass *et al.* 2007). Noteworthy, the main limitation of the possible future use of Δ^9-THC in cancer therapy might be represented by its psychotropic properties. An alternative could be represented by the non-psychotropic CBD, which has recently become a highly attractive therapeutic entity for a plethora of pharmacological positive effects not limited to cancer. It was reported to inhibit breast cancer growth both *in vitro* and *in vivo* in xenograft

tumours, inducing apoptosis via direct or indirect activation of CB2 and/or VR1 and increasing intracellular calcium and reactive oxygen species (Ligresti *et al*. 2006). The antitumour mechanism of action is somewhat puzzling, since the modulation of a distinct signalling pathway has not been identified. CBD has a very low affinity for both CB1 and CB2 receptors, in some models being antagonist at CB1 receptors (Mechoulam *et al*. 2007). A very recent paper reported that CBD was able to inhibit the invasiveness of highly malignant breast cancer cells through the inhibition, at the promoter level, of Id-1 an inhibitor of basic helix-loop-helix transcription factors strongly involved in tumour progression (McAllister *et al*. 2007).

The CB1 receptor signalling has been reported to be involved in metastatic processes. Indeed, anandamide inhibited breast cancer cell migration, downregulating FAK and Src phosphorylation/activation (Grimaldi *et al*. 2006; Fig. 1). All these effects correlated with an inhibitory effect on breast cancer metastasis *in vivo*, since anandamide reduced the formation of lung metastatic nodules in mice, and were all attenuated by the CB1 receptor antagonist SR141716. CB1 receptors might be a target for therapeutic strategies not only to slow down the growth of breast carcinoma but also to inhibit its metastatic diffusion *in vivo*. Considering the antitumour properties of the CB agonists, it could be expected that CB antagonists/inverse agonists like SR141716 (rimonabant, Acomplia, Sanofi-Aventis) introduced in the clinic as anti-obesity drug, if used alone, could instead enhance proliferation of normal and malignant cells leading to cancer. Collected data excluded this possibility, reporting rather that not only agonists to CBs but also antagonists, when used alone, are able to inhibit tumour growth (Bifulco *et al*. 2004, 2007*a*,*b*, Pisanti *et al*. 2006) or induce apoptosis in cancer cells (Derocq *et al*. 1998, Powles *et al*. 2005). Indeed, we recently provided evidence of antiproliferative effect exerted by the CB1 cannabinoid antagonist SR141716 in breast cancer cells (Sarnataro *et al*. 2006). We reported that rimonabant exerts antitumour effects on breast cancer *in vitro*, through G1/S phase arrest and *in vivo* in xenograft tumours, providing a new mechanism of action for this drug. Rimonabant, at nanomolar concentrations, inhibits human breast cancer cell proliferation, being more effective in highly invasive metastatic cells, depending on both the presence and the different expression levels of the CB1 receptor and the ER status. The molecular mechanism at the basis of rimonabant function implicates an inhibition of downstream ERK1/2 signalling inside lipid rafts/caveolae. The antiproliferative effect requires lipid rafts integrity

and the presence of CB1 receptor in lipid rafts, previously reported to be highly localised in this compartment and regulated in its trafficking by agonist binding (Sarnataro *et al*. 2005). Interestingly, lipid rafts and caveolin 1, a protein enriched in rafts, play a critical role in breast tumour growth and metastasis (Sloan *et al*. 2004, Williams *et al*. 2004). Perturbation of lipid rafts/caveolae may represent a useful antitumoural tool to control CB1 signalling in breast cancer (Sarnataro *et al*. 2006).

Cannabinoids and prostatic cancer

Prostate cancer is the most commonly diagnosed malignancy in men and the second leading cause of cancer death in males (Society American Cancer 2005). Most early tumours are androgen-dependent, thus depriving the tumour of androgens via surgical or medical castration (Gnanapragasam *et al*. 2003) has proven to have significant effects at the initial stages of prostate cancer. Despite the early efficacy of androgen ablation, advanced prostate cancer is resilient to such treatments and eventually relapses into a hormone refractory (androgen-independent) disease, with devastating results on morbidity and mortality rates (Isaacs 1994, Lara *et al*. 2004). In spite of being insensitive to hormone-withdrawal therapy, a majority of these tumours continue to express the androgen receptor (AR) and androgen-regulated genes like prostate-specific antigen (PSA), indicating that the AR pathway is active (Denmeade *et al*. 2003).

The AR activity seems to be tightly regulated by the activation of distinct growth factor cascades that can induce the AR modifications, including phosphorylation and acetylation or changes in interactions of AR with cofactors (Culig *et al*. 2004, Taplin & Balk 2004) such as EGFR, insulin-like growth factor-I (IGF-I), keratinocyte growth factor, IL-6 and oncostatin M. IGF-I, which is produced by prostatic stromal cells in response to androgen stimulation, works in a paracrine manner by stimulating the surrounding prostatic epithelial cells, resulting in an increased proliferation (Moschos & Mantzoros 2002, Garrison & Kyprianou 2004). The proliferation of prostate cancer cells is stimulated by an activated IGF-I signalling pathway (Stattin *et al*. 2004). The primary cell survival pathway activated by IGF-I is the PI3/Akt signalling pathway. The binding of the IGF-I ligand to the IGF-I receptor (IGF-IR) results in the activation of phosphoinositol-3 kinase (PI3) that further activates the Akt pathway, resulting in the phosphorylation (deactivation) of the proapoptotic Bad protein and effectively blocking apoptosis (Moschos & Mantzoros 2002).

IGF-I also induces the activation of the MAPK pathway via the Ras protein, deactivating the downstream target Bad protein and, leading to cell survival and proliferation (Moschos & Mantzoros 2002). Fibroblast growth factors (FGFs) play a significant role in the development of prostate cancer; FGF-2 acts as a mitogen for prostatic stromal cells, exerts its effect mainly in an autocrine manner (Ropiquet *et al*. 1999, Garrison & Kyprianou 2004) and also contributes to angiogenesis (Mydlo *et al*. 1988). In contrast, FGF-7 acts in a paracrine manner as a mitogen for prostatic epithelial cells (Ittman & Mansukhani 1997). FGF-8 is thought to play a role in carcinogenesis due to its overexpression in prostate cancer cells. Once activated, the FGFRs target the downstream MAPK pathway, resulting in cell survival, proliferation and angiogenesis (Tsang & Dawid 2004, Yamada *et al*. 2004).

Transforming growth factor-β (TGF-β) is released from prostatic stromal cells and works in a paracrine manner, inhibiting prostatic epithelial cell growth and inducing apoptosis (Wu *et al*. 2001, Bhowmick *et al*. 2004). SMAD proteins, primary intracellular effectors of TGF-β signalling, trigger the activation of a series of transcription factors that dictate the proliferative and/or apoptotic outcomes of the cells (Bello-DeOcampo & Tindall 2003). The SMAD-activated transcription factors downregulate the transcription of the cell survival factor Bcl-2 (Guo & Kyprianou 1999). Further, the cell cycle is effectively halted by the increased expression of the cyclin-dependent kinase inhibitor p27Kip1 (Guo & Kyprianou 1999). Transcription activated by the TGF-β/SMAD signalling pathway leads to an increased expression of IGF binding protein-3 (IGFBP-3), the primary binding protein involved in sequestering IGF-I (Nickerson *et al*. 1997, Motyl & Gajewska 2004). Finally, the activated SMAD also has an effect on cytosol, activating the apoptosis initiation factor caspase-1 (Guo & Kyprianou 1999).

The progression of prostate cancer is dependent on angiogenesis, mediated primarily via the increased expression of vascular endothelial growth factor (VEGF). Once VEGF is released, it binds to VEGF receptors on adjacent endothelial cells and induces a series of cell survival and mitogenic pathways, primarily through the PI3/Akt pathway and the Ras-mediated MAP kinase pathway. VEGF may also exert its action by positively feeding back on the Src protein in the cytosol, maintaining the VEGF promoting stimulus. Thus, Src, hypoxia-inducible factor 1α, and signal transducer/activator of transcription-3 act to regulate cell survival (Semenza 2003).

Several prostatic intraepithelial neoplasia and invasive prostatic cancer show an increased expression of EGFR tyrosine kinase, EGF and TGF-α (Kim *et al*. 1999). Moreover, androgen-independent human prostate cancer cell lines, PC3 and DU145, overexpress EGFR, which, through selective interaction with autocrine- and paracrine-secreted EGF and TGF-α, promotes cell proliferation. In these models, androgen and EGF downregulate p27kip, inhibitor of the cyclin-dependent protein kinase (Ye *et al*. 1999). Activated EGFR may induce the stimulation of distinct mitotic cascades, including Shc, MAPK, PI3K/Akt, nuclear factor-kappa B (NF-κB) and phospholipase Cγ (PCγ) signalling pathways, which participate in the stimulation of proliferation, survival, motility and invasion of PC cells (Mimeault *et al*. 2003*a*,*b*, Torring *et al*. 2003, Bonaccorsi *et al*. 2004, Mimeault *et al*. 2006).

Recent investigations also revealed that the EGF–EGFR signalling elements could play a pivotal role during different stages of PC progression by modulating several other signalling pathways including AR, hedgehog and Wnt/β-catenin cascades (Mimeault *et al*. 2003*a*,*b*, 2006, Torring *et al*. 2003). EGF may induce the activation of AR synergistically in the presence of low androgen levels or in the absence of androgens in a cell type-dependent manner (Culig *et al*. 1994, Orio *et al*. 2002, Gregory *et al*. 2004, Festuccia *et al*. 2005). It has also been reported that EGF may induce the AR nuclear translocation and enhance the growth of the CWR22R 2152 cell subline (Festuccia *et al*. 2005).

In prostate tumour cells, an upregulated expression of hedgehog signalling components also appears to occur. In particular, the enhanced expression level of sonic hedgehog ligand, SHH, in PC cells may lead to the activation of the GLI-1 transcription factor. This results in the expression of numerous tumorigenic genes, including cyclin D1 and c-Myc, which participate in the sustained growth of PC cells (Fan *et al*. 2004, Karhadkar *et al*. 2004, Olsen *et al*. 2004, Sanchez *et al*. 2004).

Several Wnt ligands are expressed at significant levels in prostatic stromal cells, androgen-dependent and -independent PC cell lines and tumoural tissues (Chen *et al*. 2004, Zhu *et al*. 2004). Wnt1 and β-catenin are also highly expressed in the metastatic LNCaP, DU145 and PC3 cells (Chen *et al*. 2004). It has been reported that Wnt3a induces AR transcriptional activity in the absence or in the presence of low concentrations of androgens, at least in part, through an increase in the cytosolic and nuclear β-catenin levels in AR-positive CWR22Rv1 and LNCaP cells. This effect was accompanied by an enhanced rate of cell growth (Verras *et al*. 2004).

It has been reported that the PC cell lines, including LNCaP, CWR22Rv1, DU145 and PC3 cells, express the receptor IL-6R, showing a high affinity for IL-6 (Okamoto *et al*. 1997, Culig *et al*. 2005). Moreover, IL-6 is also secreted by highly metastatic CWR22Rv1, DU145 and PC3 cells, while LNCaP cells did not produce a significant IL-6 level. The treatment with the exogenous IL-6 of diverse PC cells has revealed that this cytokine may modulate AR activity. It has been reported that IL-6 may enhance AR activity in AR-transfected DU145 and PC3 cells as well as AR-mutant LNCaP cells synergistically in the presence of low levels of androgen and/or in a ligand-independent manner (Yang *et al*. 2003, Culig *et al*. 2005). Similarly, it has also been reported that the IL-6-type cytokine, oncostatin M, may induce in a paracrine fashion, the activation of AR and growth stimulation in DU145-AR and CWR22Rv1 cells (Godoy-Tundidor *et al*. 2005).

A molecular dissection of the deregulation of growth factor signalling pathways in prostate tumorigenesis may provide promising new therapeutic targets for prostate cancer. We report here the emerging findings providing evidence that cannabinoids should be considered effective agents for the treatment of prostate cancer.

Exposure of PC3 cells, both to THC and to R-(+)-methanandamide (MET) stimulated the PI3K/PKB pathways, via CB1/CB2 activation, which increased phosphorylation of PKB, induced translocation of Raf1 to the membrane and phosphorylation of p44/42 Erk-kinase (Sanchez *et al*. 2003*a*,*b*). The treatment with AEA at micromolar concentration for 48 h (Mimeault *et al*. 2003*a*,*b*) results in the inhibition of EGF-induced proliferation of DU145 and PC3 cells as well as androgen-stimulated LNCaP, via G1 arrest, and down-regulated EGFR levels (Fig. 2). Both phenomena were CB1-mediated. A similar growth arrest and receptor modulation was also reported for PRL and nerve growth factor-stimulated DU145 (De Petrocellis *et al*. 1998, Melck *et al*. 2000), via the same AEA-modulated signal transduction pathways described in breast cancer cells (Melck *et al*. 2000). Importantly, a longer incubation time (5–6 days) with AEA was able to induce massive apoptotic effects, via cellular ceramide accumulation, CB1/CB2-mediated, in DU145 and PC3, whereas in LNCaP cells AEA did not exert similar effects (Fig. 2). Intriguingly, 4 days of treatment with MET or exogenous cannabinoids, at submicromolar concentrations, increased the proliferation rate of LNCap cells and the expression of AR; long-lasting incubation periods led to differentiation (Sanchez *et al*. 2003*a*,*b*). Met-induced mitogenic effect seems PKC, rather than cAMP-pathway dependent; furthermore, in this cellular model the androgen receptor expression is CB1- and, partially, CB2-mediated (Sanchez *et al*. 2003*a*,*b*, Sarfaraz *et al*. 2005, 2006). In a recent study,
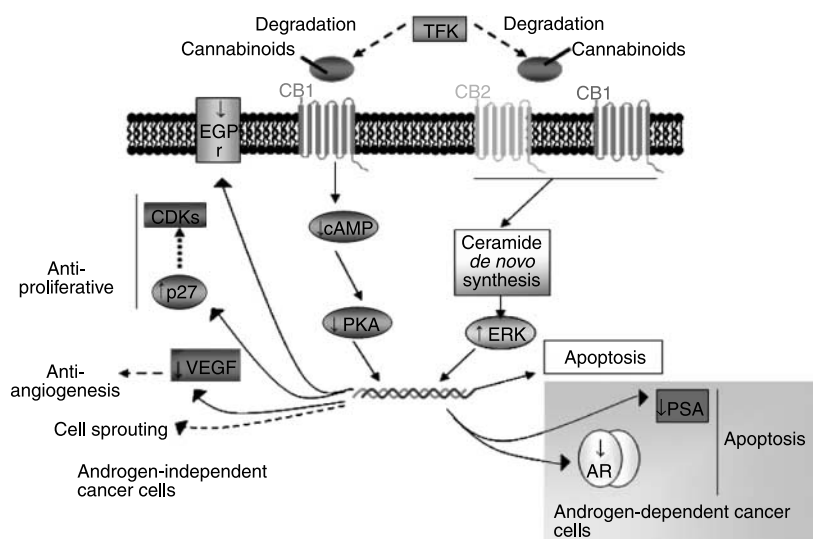

Figure 2 Signalling pathways associated with cannabinoid receptor activation induced by its agonists in prostate cancer cells. Upon receptor binding, cannabinoids inhibit cell proliferation and invasion through activation of ERK1/2, induction of p27kip and inhibition of cell cycle regulator molecules CDKs and inhibition of VEGF expression in androgen-independent cancer cells. In a recent study, cannabinoids treatment decreased AR expression and PSA levels in androgen-dependent cancer cells via cAMP-dependent protein kinase. Moreover, antiproliferative and apoptotic effects of endogenous cannabinoid anandamide in human prostate cancer cell lines were found to be mediated through the downregulation of epidermal growth factor receptor (EGFR) and accumulation of ceramide. The proposed mechanisms are based on the available literature and are cell-specific, and not all pathways are triggered simultaneously.

WIN-55,212-2 treatment significantly decreased LNCaP cell viability and AR expression in a dose- (micromolar) and time-dependent manner, with maximal effect at 72 h (Sarfaraz *et al*. 2005); concomitantly, the authors showed a decrease in the intracellular as well as the secreted levels of the PSA, a glycoprotein androgen-receptor regulated (Henttu *et al*. 1990, Montgomery *et al*. 1992, Lee *et al*. 1995) and, presently the most accepted marker of assessment of prostate cancer progression (Stamey *et al*. 1987; Fig. 2). Their results showed that treatment of LNCaP with WIN-55,212-2 also inhibits VEGF protein expression, a ubiquitous cytokine that plays a key role in angiogenesis (Blazquez *et al*. 2003). Finally, 2AG inhibits the invasion of androgen-independent prostate cancer cells PC3 and DU145 through CB1-dependent inhibition of adenylyl cyclase and decreased activity of PKA (Nithipatikom *et al*. 2004; Fig. 2). Recently, Sarfaraz *et al*. (2006) showed that treatment of human prostate cancer LNCaP cells with CB agonist WIN-55,212-2 caused an arrest of the cells in the G0/G1 phase of the cell cycle, sustained by the activation of ERK1/2, induction of p27/KIP1 and inhibition of cyclin D1 (Fig. 2). G0/G1 arrest upregulated the Bax/BCl-2 ratio and activated caspases resulting in an induction of apoptosis. The blocking of both cannabinoid receptors CB1 and CB2 by their specific antagonist resulted in the inhibition of ERK1/2 activation. The inhibition of ERK1/2 signalling by the ERK1/2 inhibitor PD98059 reversed the distribution of cells in the G1 phase of the cell cycle and also decreased the percentage of apoptotic cells when compared with WIN-55,212-2 treatment alone. The ERK1/2 inhibitor also reversed the effects of WIN-55,212-2 on p27/KIP1 and cyclin D1 proteins operative in the G1 phase of the cell cycle and Bcl-2, an important pro-apoptotic protein. Similar results were observed when ERK1/2 was silenced using siRNA. Moreover, WIN-55,212-2 treatment of the cells resulted in a dose-dependent decrease in protein expression of cyclin D1, cyclin D2 and cyclin E, as well as cdk2, cdk4 and cdk6. Downregulation of cdk4/6 has been shown to be associated with a decrease in the expression of retinoblastoma (pRb) tumour suppressor protein, a key regulator of the G1/S phase transition in the cell cycle (24, 25). The authors have observed that the treatment with this agonist resulted in a decrease in the protein expression of pRb and its molecular partner, the transcriptional factor E2F. Because the activity of E2F is known to be dependent on its heterodimeric association with members of the DP family of proteins, they also evaluated the effect of WIN-55,212-2 treatment on both the members of DP family viz. DP-1 and DP-2. WIN-55,212-2 caused a dose-dependent decrease in the protein expression of DP-1 and DP-2. Finally, the authors suggested that the CB agonist should be considered an effective agent for the treatment of prostate cancer but this hypothesis must be supported by *in vivo* experiments.

Cannabinoids and thyroid cancers

Thyroid cancer is the most common endocrine malignancy. It is characterised by genetic alterations resulting in a dysregulation of cell growth and death. Alterations in key signalling effectors seem to be the hallmark of distinct forms of thyroid neoplasia. The overexpression and/or uncontrolled activation of receptor tyrosine kinases, downstream signalling molecules and the inhibition of programmed cell death (apoptosis) have all been demonstrated to occur in thyroid cancer. Several compounds presently being tested in preclinical and clinical studies target intracellular molecules involved in these processes. These agents are not tumour-specific, as these pathways are active in normal and malignant cells, but are thought to be tumour-selective because the cancers demonstrate higher levels of pathway activation, making more sensitive than normal cells at lower concentrations (Braga-Basaria & Ringel 2003, Kondo *et al*. 2006). Ras activation is central to the pathogenesis of some thyroid cancers, and it can occur through mutations in the genes encoding Ras or through activation of upstream regulators. In thyroid carcinoma, activating mutations of ras genes (N-, K- or H-ras) can be found in as many as 30% of cases (Mizukami *et al*. 1988). Ras is a small GTP-binding protein (G-protein) regularly expressed in normal thyroid cells, and its protein product is involved in several important functions, including proliferation, differentiation and cell survival. In thyroid cancer, the overactivation of Ras may occur through activating mutations in the ras gene or by the over-activation of receptor tyrosine kinase receptors. Mutations in the gene encoding Ras can result in the expression of Ras proteins that are constitutively bound to GTP, i.e. once they are activated they are not able to be turned off (Lemoine *et al*. 1989, Namba *et al*. 1990, Karga *et al*. 1991). In addition to activating mutations, Ras overactivation can occur secondary to receptor overactivation. The enhanced signalling of receptor tyrosine kinases is a common event in thyroid cancer, particularly papillary thyroid cancer. For these reasons, Ras is a reasonable molecular target to consider for novel forms of thyroid cancer therapy. Moreover, overexpression of receptor tyrosine kinases, including FGF, EGF, hepatocyte growth factor (c-Met), VEGF, insulin and IGF-I receptors are commonly identified in thyroid cancers. Several of these receptors are common to many

cancers and they are expressed at very low levels in non-neoplastic tissues and may be associated with angiogenesis or progression, making them excellent therapeutic targets. It is known that the formation of new blood vessels is a crucial step in determining tumour expansion and is greatly dependent on proangiogenic factors that are produced in a paracrine fashion by tumour cells undergoing hypoxia or mechanical compression. Several growth factors are involved in the process of angiogenesis in malignant tumours; among them, VEGF appears to be the most prominent, being the functional of stimulating vascular proliferation and permeability, and inducing metastasis. Significantly increased levels of VEGF have recently been demonstrated in the serum of patients with well-differentiated metastatic thyroid tumours when compared with lower levels found in patients considered to be in a complete remission (Tuttle *et al.* 2002). In patients with other malignancies, a worse prognosis was observed in those who expressed higher levels of VEGF in their tumours, probably due to an increased vessel formation and development of metastasis (Huang *et al.* 2001, Klein *et al.* 2001, Lennard *et al.* 2001). The molecular alterations identified in this disease represent targets for early clinical trials that are aimed at tailoring multimodal approaches to the treatment of thyroid cancers. Recently, it has been described that endo/cannabinoids are able to affect the activity or expression of these molecules. In a recent paper, Bifulco *et al.* (2001) showed the effect of 2-methyl-arachidonyl-2-fluoro-ethylamide (Met-F-AEA), a stable analogue of

the endocannabinoid anandamide, on a rat thyroid epithelial cell line (FRTL-5) transformed by the K-ras oncogene (KiMol), and on epithelial tumours derived from these cells. Met-F-AEA induced a dose-dependent (IC50 = 5 μM) arrest of the cell cycle of these cells at the G0/G1 phase, associated with a significant reduction of cells in the S and G2/M phase. The antiproliferative effect was accompanied by a striking reduction in the p21ras activity (Fig. 3). All these effects were attenuated significantly by SR141716. The Met-F-AEA cytostatic action was significantly smaller in non-transformed FRTL-5 cells than in KiMol cells. The treatment with Met-F-AEA exerted opposite effects on the expression of CB1 receptors in KiMol and FRTL-5 cells, with a strong upregulation in the former case and a suppression in non-transformed cells. The authors evaluated the Met-F-AEA effect in a nude mouse xenograft model, where K-ras-transformed (KiMol) cells were implanted subcutaneously. The treatment with Met-F-AEA induced a drastic reduction in the tumour volume. This effect was inhibited by the CB1 receptor antagonist SR141716 and was accompanied by a strong reduction in the K-ras activity. The decrease in tumour volume induced by Met-F-AEA was accompanied by a strong upregulation of CB1 receptor mRNA and protein when compared with vehicle-treated tumours. Similarly, KiMol cells treated with Met-F-AEA expressed significantly more CB1 receptors, and this effect was abolished by SR141716. Cell immunofluorescence studies with both permeabilised and non-permeabilised cells showed that
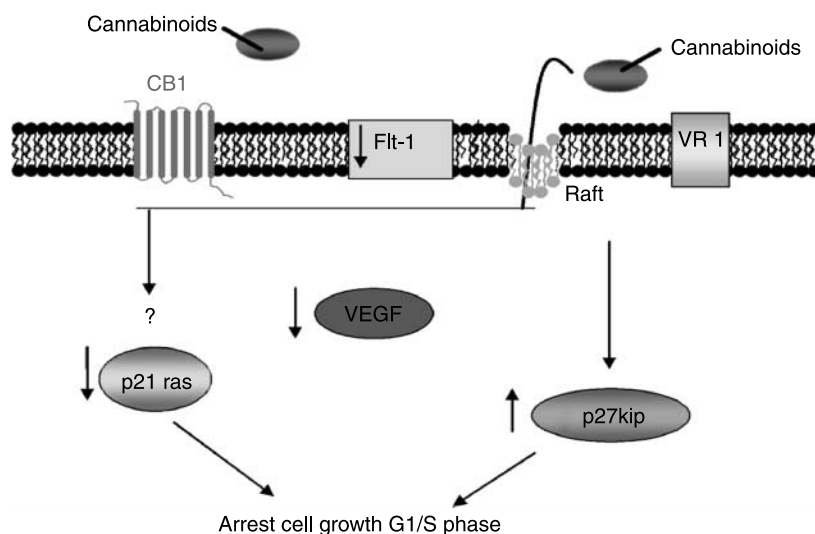

Figure 3 The antiproliferative effect of endocannabinoids in thyroid cancer cells. Upon receptor binding, endo/cannabinoids inhibit cell proliferation through the induction of p27kip1 inducing an G1/S arrest. Met-F-AEA reduces the expression of VEGF receptors (Flt-1)/VEGFR-1 resulting in an inhibition of VEGF signalling and consequently in an inhibition of cell invasion. In cells transformed with K-ras oncogene, the agonist of CB1 receptor inhibits the ras activity.

Met-F-AEA increased the levels of CB1 receptors on both the cell membrane and the cytosol. By contrast, non-transformed FRTL-5 cells treated with Met-F-AEA exhibited less CB1 receptors than vehicle-treated cells in both the cell membrane and the cytosol. In accordance with this opposite regulation of CB1 receptor expression in transformed versus healthy cells, the proliferation of KiMol cells treated with Met-F-AEA was significantly more strongly inhibited by the cannabimimetic substance (up to 70% inhibition) than the response of FRTL-5 cells, which barely reached statistical significance after 3 days (up to 28% inhibition). Afterwards, Portella *et al.* (2003) studied whether cancer growth *in vivo* would be limited by CBs when the tumour is already established and growing. Therefore, they investigated the possible tumour growth inhibitory effect of intratumoural administrations of Met-F-AEA and the possibility that this compound, by acting at CB1 receptors, also interferes with angiogenesis and metastatic processes. In order to evaluate the effects of this compound on already established tumours, the authors s.c. injected 45 nude mice with K-ras-transformed FRTL-5 cells (KiMol), which are able to induce the growth of undifferentiated carcinomas when injected s.c. into athymic mice. Twenty days later, when tumours were clearly detectable, saline solution containing Met-F-AEA was injected in the peritumoural area on days 2 and 5 of a 7-day cycle for 4 weeks. The Met-F-AEA treatment induced a drastic reduction in the tumour volume with respect to the vehicle control-treated mice. Subsequently, it has been observed that this compound inhibited angiogenesis by affecting the expression of VEGF. In addition, Met-F-AEA treatment also reduced the expression of one of the VEGF receptors (Flt-1/VEGFR-1) in tumours, thus indicating that this treatment was very likely to result in a strong inhibition of VEGF signalling and, hence, tumour angiogenesis (Fig. 3). These inhibitory effects of Met-F-AEA were attenuated by the selective CB1 receptor antagonist, SR141716, thus strongly suggesting the involvement of CB1 receptors in the anti-VEGF action of the compound. The addition of Met-F-AEA to KiMol cells was also able to significantly decrease VEGF and VEGF receptor (Flt-1/VEGFR-1) expression. They also founded that Met-F-AEA treatment of tumours and KiMol cells increased p27(kip1) levels (Fig. 3), and that this effect was attenuated by the selective CB1 receptor antagonist, SR141716. The cyclin-dependent kinase inhibitor p27(kip1) is another protein suggested to play a role as a proangiogenic factor and is under the negative control of the ras oncogene in proliferating human thyroid cells. Moreover, it has been described the effect of Met-F-AEA on metastatic processes, comparing the antiproliferative action of this compound on two other cell lines derived from a rat thyroid carcinoma (TK-6 cells) or its lung metastasis (MPTK-6 cells). A 4-day treatment with Met-F-AEA was able to inhibit the proliferation of both neoplastic thyroid cell lines. The growth of metastasis-derived cells was inhibited more efficaciously than that of primary thyroid carcinoma-derived cells, and this was accompanied by a stronger upregulation of CB1 receptor levels in MPTK-6 cells than in TK-6, together with a stronger downregulation of VEGF receptor levels in MPTK-6 than in TK-6 cells. Finally, the authors tested the effects of Met-F-AEA *in vivo* on the induction of metastatic foci in mice lungs after intra-paw injection of the highly metastatic 3LL cells. A dramatic inhibitory effect of Met-F-AEA was observed against lung nodules induced by 3LL cells. The metastatic growth inhibitory effect was blocked by the CB1 receptor antagonist SR141716. In conclusion, the local administration of the stable anandamide analogue and cannabinoid CB1 receptor agonist, Met-F-AEA, blocked the growth of an already established rat thyroid carcinoma in athymic mice, underlining that this strong anticancer effect might be due at least in part to inhibition of angiogenesis, because it was accompanied by blockade of VEGF signalling and overexpression of p27(kip1; Fig. 3). Furthermore, Met-F-AEA, by acting at CB1 receptors, more efficaciously inhibited the proliferation of metastasis-derived than primary tumour-derived rat thyroid cancer cells and counteracts the formation of metastatic loci in an *in vivo* model of metastasis. Anandamide-based drugs may be efficacious for the inhibition of K-ras-induced epithelial cancer cell growth *in vivo* through the activation of CB1 receptors, inhibition of p21ras activity (Fig. 3) and blockade of the cell cycle. This strong anticancer effect might be due at least in part to inhibition of angiogenesis because it was accompanied by the blockade of VEGF signalling (Fig. 3).

Effect of cannabinoids on other endocrine tumours

Studies on the effects of cannabinoids on other types of endocrine tumour have been performed.

Recently, it has been demonstrated that agonists of cannabinoids receptors modulate insulin release in RIN m5F rat insulinoma β cells (De Petrocellis *et al.* 2007). In particular, the CB1 agonist arachidonoyl-chloro-ethanolamide (ACEA) and the CB2 agonist JWH133, elevated $Ca(2+)$, in a way sensitive to the inhibitor of phosphoinositide-specific phospholipase C (PI-PLC), U73122, and independently from extracellular $Ca(2+)$. ACEA, but not JWH133, significantly inhibited the effect on $Ca(2+)$ of bombesin, which acts via

G(q/11)- and PI-PLC-coupled receptors in insulinoma cells. Anandamide and *N*-arachidonoyldopamine, which also activate TRPV1 receptors expressed in RIN m5F cells, elevated Ca(2+) in the presence of extracellular Ca(2+) in a way sensitive to both CB1 and TRPV1 antagonists. These results suggest that, in RIN m5F cells, CB(1) receptors are coupled to PI-PLC-mediated mobilisation of Ca(2+).

Pheochromocytoma is a rare catecholamine-secreting tumour derived from chromaffin cells. It has been found that rat adrenal pheochromocytoma PC-12 cells contain the endocannabinoids anandamide and oleamide, together with the enzyme responsible for their degradation, FAAH and the proposed biosynthetic precursors for arachidonoylethanolamide and related acylethanolamides, the *N*-acyl-phosphatidylethanolamines (Bisogno *et al*. 1998). Moreover, several studies have reported that anandamide induces apoptosis in PC-12 cells triggering JNK and p38 MAPK pathways. The activation of p38 MAPK/JNK was accompanied by the release of cytochrome *c* from the mitochondria and caspase activation, suggesting that anandamide triggers a mitochondrial-dependent apoptotic pathway (Sarker *et al*. 2000, 2003). Also, the synthetic cannabinoid recptor CB1 agonist CP55,940 induced apoptosis in PC12 cells, also inducing NF-κB. However, the elevation in NF-κB activity was not demonstrated an integral part of the apoptotic signalling cascade in PC12 cells, because its inhibition was not related to the reduction of TUNEL-positive cells (Erlandsson *et al*. 2002).

A potentially important role of the endocannabinoid system in pituitary pathophysiology has been studied extensively (Pagotto *et al*. 2006). Normal human pituitary gland and pituitary adenomas have been reported to express CB type 1 and synthesise endogenous cannabinoids. CB1 was present in adrenocorticotrophin (ACTH)-, PRL- and growth hormone (GH)-producing cells, whereas no immunoreactivity was found in luteinizing hormone-, follicle-stimulating hormone- and thyrotrophin-positive cells. CB1 was detected in acromegaly-associated pituitary adenomas, Cushing's adenomas and prolactinomas, whereas a faint or no expression was found in non-functioning pituitary adenomas. The content of endocannabinoids in pituitary tumours was higher than that in normal human pituitary. In particular, prolactinomas showed the highest level of AEA, followed by acromegaly-associated pituitary tumours and corticotropinomas, where the 2-AG content was also increased. Moreover, the endocannabinoid content in pituitary adenomas was shown to be correlated with the presence of CB1, by being elevated in the acromegaly-associated pituitary adenomas, Cushing's adenomas and prolactinomas, which were the tumours positive for CB1, and lower in non-functioning adenomas, which are characterised by a low or absent CB1 expression (Pagotto *et al*. 2001). The existence of an auto/paracrine cannabinoid loop in pituitary adenomas that may have an important role in modulating hormone overproduction could be postulated. Natural or synthetic cannabinoids have been shown to affect hormonal pituitary release in several *in vivo* and *in vitro* rodent models (Fernandez-Ruiz

Table 1 The main trophic actions of endo/cannabinoids

Tumour (cell type)	Endocannabinoid	Anticancer effect	Mechanism of action	References
Human breast cancer cell lines (MCF7; EFM-19)	AEA, 2-AG, HU210	+	Inhibition of the mitogen-induced stimulation of the G0/G1-S phase	De Petrocellis *et al*. (1998) and Melck *et al*. (2000)
Androgen independent prostate cancer cells (PC3, DU145)	AEA R-(+)-MET	+	Inhibition of mitogen-induced proliferation, G1 arrest	De Petrocellis *et al*. (1998), Melck *et al*. (2000) and Mimeault *et al*. (2003a,b)
Androgen dependent prostate cancer cells (LNCap)	AEA R-(+)-MET (at micromolar concentration)		Inhibition of mitogen-induced proliferation, G1 arrest	De Petrocellis *et al*. (1998), Melck *et al*. (2000) and Mimeault *et al*. (2003a,b)
Androgen dependent prostate cancer cells (LNCap)	WIN-55,212-2 (micromolar concentration)	+	Dose- and time-dependent induction of apoptosis; decreased expression of AR and PSA	Sarfaraz *et al*. (2005)
K-ras transformed FRTL-5 thyroid cells (KiMol)	Met-F-AEA	+	*In vivo*, inhibited growth of tumours induced in nude mice	Bifulco *et al*. (2001)

et al. 1997, Jackson & Murphy 1997). To attribute a functional significance to CB1, primary tumour cell cultures were stimulated with cannabinoids in the presence and absence of physiological stimulants. The cannabinoid agonist WIN-55,212-2 (1 μM) inhibited GH secretion in most of the acromegaly-associated pituitary tumours tested, and this effect was generally reversed by the specific CB1 antagonist SR 141716, suggesting that cannabinoids are able to directly influence basal GH secretion through CB1 activation. Interestingly, WIN-55,212-2 was able to suppress the stimulatory effect on GH release produced by GH-releasing hormone, but not that caused by growth hormone-releasing peptide (GHRP). In all corticotropinomas tested, WIN-55,212-2 alone was not able to influence basal ACTH secretion, but together with CRF had an additive effect on ACTH release that was specifically blocked by SR 141716A, thereby indicating a CB1-mediated effect (Pagotto *et al.* 2001). Cannabinoids can modulate PRL secretion (Pagotto *et al.* 2006), but it is still controversial whether this is a direct pituitary action or an indirect activation of central neurotransmitters. Nevertheless, in the single case studied by Pagotto *et al.* (2001), WIN-55,212-2 was able to inhibit basal PRL secretion.

Conclusion

It is extremely important today to identify new targets for drug development, either for cancers that are insensitive to the present therapies, as substitutes for common toxic chemotherapeutic regimens or to adjuvant other treatments improving efficacy and avoiding recurrence and resistance. In this frame, the case of endocannabinoid-related drugs appears intrinsically interesting and not sufficiently explored, especially with regard to the mechanistic insights into the triggered cellular events. A summary of the main trophic actions of endo/cannabinoids via their receptor in modulating tumour cell proliferation is shown in Table 1. During the last few years, it has become evident that multiple mechanisms of action, not solely limited to the CNS, are involved in the endocannabinoid-mediated control of cell proliferation. In this review, we have tried to summarise the importance of endo/CB expression and modulation to interfere with tumour growth. There is compelling evidence that endo/cannabinoids may regulate the growth and spread of normal and neoplastic tissues. An accepted notion is that endocannabinoid system very often induces opposite effects in normal versus neoplastic cells in important physiological processes, such as proliferation and migration (Guzman *et al.* 2001). This apparent paradox could be explained on the basis of CB receptors coupling

efficiency to different subsets of G-proteins, able to activate different downstream pathways. However, there is sill much to learn about this topic. In this review, we focused our attention on endocrine and related cancers, first because the endocannabinoid system seems to be directly involved in the control of neuroendocrine function, also through a direct effect on peripheral target endocrine organs and second because initial studies of endocannabinoid control of cell proliferation were performed on endocrine cancer cells. Agonists of endo/CBs seem to be effective drugs with antiproliferative activity in breast, prostate and thyroid cancers *in vitro* and *in vivo*, simultaneously affecting multiple signalling pathways and biological processes that have been implicated in the development of the malignant phenotype and are downstream endocrine receptors stimulation. An increasingly detailed knowledge of those cell signalling pathways involved in malignancy provides a sound basis for the development of drugs aimed at selected components of the pathways. Obviously, the modulation of a single target that simultaneously inhibits multiple critical pathways is an intriguing anticancer strategy. The inhibitory effect of endo/cannabinoids on tumour growth could be dependent on the differential localisation and expression of different receptor subtypes and on the signal transduction mechanisms activated following the binding of specific agonists. Further studies may clarify whether CBs stimulation could uncouple endocrine receptors from their downstream signalling, thereby providing a useful perturbation of hormonal-dependent cancers. Collected evidence suggests a strong connection between endocannabinoid system biology and lipid rafts (Sarnataro *et al.* 2005, 2006). In this context, it should be very interesting characterise the role of lipid rafts/caveolae in CB receptors signalling and interplay with endocrine receptors, since these compartments could represent a cellular device for intracellular trafficking, as well as a favourable platform to regulate intracellular signalling. Furthermore, in view of the recent evidence that endocannabinoid-induced cell arrest may occur via both receptor-dependent and -independent mechanisms, we venture to suggest that the clarification of the role of endocannabinoid and its receptors in cancer development may hold great promise for the treatment of patients affected by endocrine and related malignancies. In sum, CB1 receptors represent a promising endocrine tumour drug target for several reasons: 1) this is due to the ubiquity of these receptors expressed in a large variety of endocrine cells; 2) cannabinoids selectively affect tumour cells more than their non-transformed counterparts that might even be protected from cell death and 3) a large number of ligands have been generated by

introducing several modifications in the structure of the lead compounds, some of them with high affinity and selectivity and lack of adverse psychotropic effects. It appears clear that the documented antitumour activity of the endo/cannabinoids, intrinsically interesting but not sufficiently explored, needs a deeper knowledge especially in regard to the mechanistic insights into the triggered cellular events and to their safe translation into the clinical setting.

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