Enhancement of androgen receptor expression induced by (R)-methanandamide in prostate LNCaP cells

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Abstract **It has been recently shown that cannabinoids may regulate the growth of many cell types. In the present work we examined the effect of the anandamide analogue (R)-methanandamide (MET) on androgen-dependent prostate LNCaP cell growth. We found that 0.1 μM MET had a mitogenic effect measured by [3H]thymidine incorporation into DNA. The effect exerted by MET was blocked by the cannabinoid receptor antagonists SR141716 (SR1) and SR144528 (SR2) as well as by the phosphoinositide 3-kinase (PI3K) inhibitor LY294002, suggesting an involvement of cannabinoid receptors and the PI3K pathway in the mechanism of MET action. MET treatment of LNCaP cells also induced an up-regulation of androgen receptor expression that was blocked by the two cannabinoid receptor antagonists SR1 and SR2. These results show for the first time that cannabinoids may modify androgen receptor expression in an androgen-dependent cell line and by this mechanism could regulate prostate cell growth.**
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Key words: Cannabinoids; (R)-methanandamide;
Androgen receptor; LNCaP cells; Prostate

1. Introduction

The principal psychoactive ingredient of marijuana is Δ^9-tetrahydrocannabinol (THC), which exerts many of its biological effects through binding to specific cannabinoid receptors expressed in cellular membranes. Recently, endogenous substances that bind to the same receptors as *Cannabis sativa*-derived compounds have been identified and they have been called endocannabinoids [1,2], arachidonoylethanolamide (anandamide) being the first endocannabinoid to be isolated [3]. Cannabinoid receptors described to date include two cloned G protein-coupled receptors named CB_1 and CB_2 as well as one non-selective cation channel known as vanilloid receptor subtype 1 [4,5]. Many intracellular transduction systems may be triggered by cannabinoids upon receptor binding. CB_1 and CB_2 are negatively coupled to adenylyl cyclase through $G_{i/o}$ proteins in many cells and tissues inducing a general inhibition of the cAMP/protein kinase A (PKA) signalling pathway [6,7]. Activation of the mitogen-activated protein kinase (MAPK) cascade by cannabinoids has also been demonstrated both in vitro [6,8] and in vivo [9], suggesting a role for can-nabinoids in the regulation of cellular fate [10]. In fact, cannabinoid agonists have been shown to induce the death of some transformed neuronal and non-neuronal cells while exert a protective action in normal neurons [11–14]. However, at sub-micromolar concentrations, cannabinoids may stimulate proliferation of lymphocytes [15] and prostate PC-3 cells [16] and recent evidence suggests that the CB_2 cannabinoid receptor may be a novel oncogene [17].

Prostate cell proliferation is regulated by many extracellular and intracellular factors including steroid hormones, neuropeptides and growth factors [18]. In men, the primary circulating androgen is testosterone secreted by the testes under hypothalamic–pituitary regulation and it is required for normal growth and functional activities of the prostate. In the prostate, testosterone is converted to the more potent 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase [19]. The actions of both testosterone and DHT are mediated by the intracellular androgen receptor that binds to androgen-responsive elements in genes to regulate gene transcription [20]. Tumoral transformation results from a multi-step process which is mainly under androgenic control and therefore inhibition of androgen action is one of the standard therapies for metastatic prostate cancer. Most prostate cancers initially respond to hormonal therapy, but after a limited period of relapse, cancer recurs as an androgen-independent disease [21]. LNCaP is an androgen-responsive cell line derived from a metastatic lesion of the lymph nodes of a patient with confirmed diagnosis of prostate cancer. It has been widely used to study the regulation of the androgen receptor which contains a mutation in the ligand binding domain [22,23]. Androgen receptor is up-regulated upon DHT treatment in LNCaP cells with the involvement of MAPK activation and the phosphoinositide 3-kinase (PI3K)/Akt pathway [24]. We have previously demonstrated that cannabinoids may activate the PI3K and MAPK cascade in prostate cells [16]. The present work was undertaken to study the role of cannabinoids in the regulation of LNCaP cell growth and the involvement of the androgen receptor in the effect of cannabinoids. We show here that (R)-methanandamide (MET) at nanomolar concentrations stimulates the DNA synthesis in LNCaP cells via a cannabinoid receptor-dependent mechanism.

2. Materials and methods

2.1. Materials
MET, HU-210 and JWH-015 were purchased from Tocris (Bristol, UK). THC and DHT were from Sigma (St. Louis, MO, USA). Can-

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nabinoid receptor antagonists SR141716 (SR1) and SR144528 (SR2) were a kind gift from Sanofi Recherche (Montpellier, France). Enzyme inhibitors LY294002, PD98059, 8-bromoadenosine 3′,5′-cyclic monophosphothioate (BrA), 8-bromoadenosine 3′,5′-cyclic monophosphothioate Rp isomer (BrARpI) and bisindolylmaleimide I (BIM) were from Alexis Corporation (San Diego, CA, USA). SB203580 was from Tocris. Anti-human androgen receptor monoclonal antibody was from Becton Dickinson Biosciences (San Diego, CA, USA). Other agents were from Sigma.

2.2. Cell cultures

Human prostate LNCaP cells were purchased from American Type Culture Collection (ATCC CRL 1740) (Rockville, MD, USA). They were routinely grown in RPMI 1640 medium supplemented with 5% fetal calf serum. For experiments, cell passages between 3 and 10 were used. Cells were seeded at 30 000 cells/cm^2 and grown for 2 days. Twenty-four hours before the experiment, the serum-containing medium was removed and a chemically defined medium consisting of RPMI 1640 supplemented with 5 μg/ml insulin, 5 μg/ml transferrin and 5 ng/ml sodium selenite was added.

2.3. [^{3}H]Thymidine incorporation

Cells were treated with different concentrations of MET according to the experiment. DNA synthesis was determined by pulsing the cells with [^{3}H]thymidine (1 μCi/well) for the last 16 h of the culture period. After extensive washing, cells were incubated with 10% trichloroacetic acid for 15 min at 4°C and then neutralized with 1 N NaOH for 1 h. Radioactivity incorporated was monitored by liquid scintillation.

2.4. Western blot

Cultured cells were treated according to the experimental conditions, lysed into lysis buffer (50 mM Tris–HCl, pH 7.4, 5 mM EDTA, 1 mM EGTA, 10 mM 2-mercaptoethanol containing 5 μg/ml leupeptin, 5 μg/ml aprotinin, 10 μg/ml soybean trypsin inhibitor and 1 mM phenylmethylsulfonyl fluoride) and disrupted by sonica-
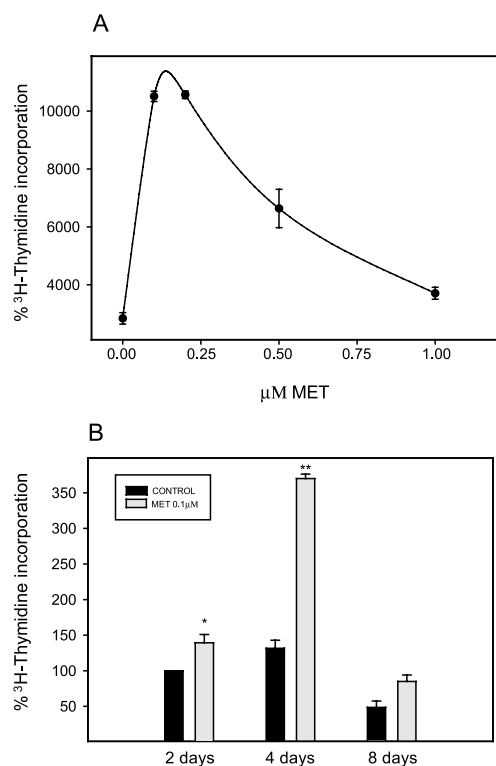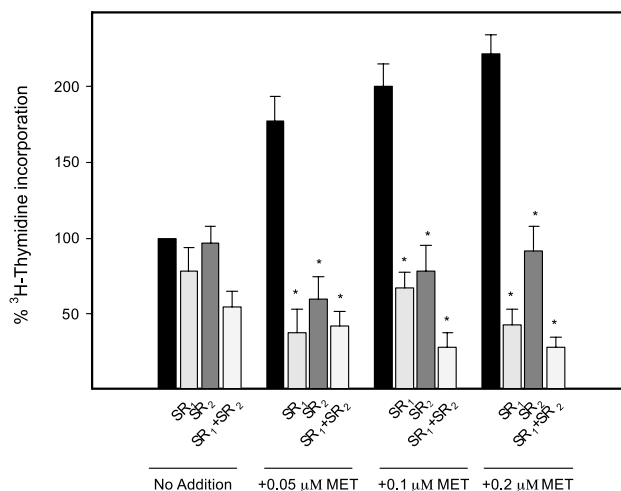

Fig. 2. Effect of cannabinoid receptor antagonists on the mitogenic response induced by MET in LNCaP cells. Cells were treated with different doses of MET in the presence or absence of 0.5 μM SR1, 0.5 μM SR2 or both together for 2 days. Data are means ± S.D. of two different experiments performed in triplicate and are expressed as percent over control. *$P < 0.001$ compared with the corresponding MET treatment.

tion. 20 μg of cellular lysates were loaded into acrylamide gels and proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis after which they were electrophoretically transferred to a nitrocellulose membrane. Membranes were blocked with 3% fat-free milk and incubated with anti-human androgen receptor (1:500 dilution) overnight at 4°C. Then, membranes were incubated for 1 h with a secondary horseradish peroxidase-conjugated antibody and developed with ECL luminescent substrate (Amersham, UK).

3. Results

3.1. Effect of cannabinoids on LNCaP cell proliferation

The effect of cannabinoids on cellular proliferation was studied by [^{3}H]thymidine incorporation into DNA using the anandamide analogue MET which possesses a higher potency and metabolic stability than anandamide [25]. Addition of increasing MET concentrations to cultured LNCaP cells induced a mitogenic effect that peaked at 0.1–0.2 μM (Fig. 1A). Doses of MET over 1 μM induced cellular death (data not shown). The mitogenic effect induced by MET was maximal at 4 days treatment, after which cell growth stopped and cells began to differentiate (Fig. 1B and data not shown).

In order to evaluate the involvement of cannabinoid receptors in the response induced by MET, the growth test was performed in the absence or presence of the CB$_1$ receptor antagonist SR1 and the CB$_2$ receptor antagonist SR2. We have previously demonstrated by Western blot that LNCaP cells express both CB$_1$ and CB$_2$ cannabinoid receptors [16]. As shown in Fig. 2, pre-incubation of LNCaP cell with either SR1, SR2 or both antagonists together blocked the proliferative response induced by MET. Moreover, the cannabinoid receptor antagonists alone or in combination with MET reduced the [^{3}H]thymidine incorporation into DNA to below the control values (Fig. 2), which is in concordance with the inverse agonist properties proposed for these compounds [26,27].

We next tested the ability of other cannabinoid agonists to induce cellular proliferation of LNCaP prostate cells. THC, which is an agonist for both CB$_1$ and CB$_2$, HU-210 which is

Fig. 1. Effect of MET on LNCaP cell proliferation. (A) Cells were incubated with different doses of MET during 4 days after which [^{3}H]thymidine incorporation into DNA was measured by liquid scintillation. (B) Cells were incubated with 0.1 μM MET for 2, 4 or 8 days. Data are means ± S.D. of four different experiments performed in triplicate and are expressed as percent over control. *$P < 0.1$; **$P < 0.005$ compared with the corresponding controls.
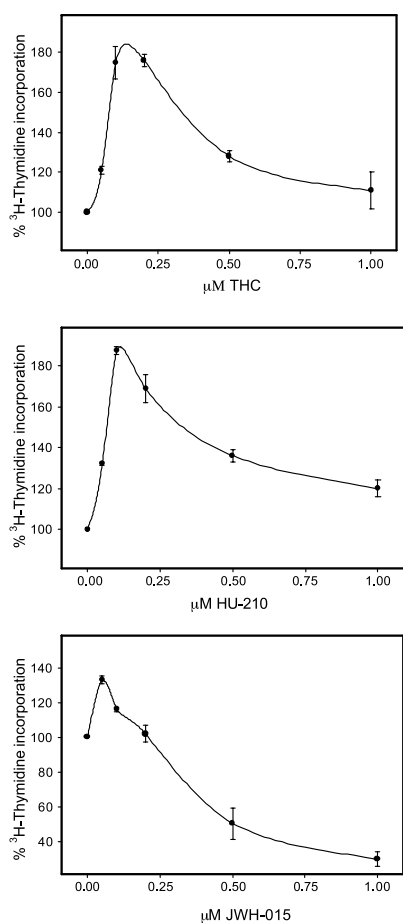
Fig. 3. Effect of exogenous cannabinoids on LNCaP cell proliferation. Cells were incubated with different doses of THC, HU-210 and JWH-015 for 2 days after which [3H]thymidine incorporation into DNA was measured by liquid scintillation. Data are means ± S.D. of two different experiments performed in triplicate and are expressed as percent over control.

more potent than THC, and JWH-015, which is a CB_2 agonist [28], also exerted a mitogenic effect that was maximal at 0.1 μM concentration (Fig. 3). The CB_2 agonist JWH-015 inhibited cellular growth at doses over 0.5 μM, which is lower than THC, HU-210 and MET, providing the basis for future in-

vestigations studying the role of CB_2 in the antiproliferative response of high doses of cannabinoids.

3.2. Effect of various enzyme inhibitors on the proliferative response induced by MET

To investigate the mechanism whereby MET induces cellular proliferation we used various inhibitors of several intracellular transduction pathways (Table 1). The different inhibitors, when added alone, had no effect on cell viability with the exception of SB203580, a highly selective inhibitor of MAPK, which increased cell proliferation, suggesting that in LNCaP cells, the p38 MAPK pathway may probably be activated in basal conditions.

We have previously demonstrated that cannabinoids activate the MAPK cascade in prostate PC-3 cells via PI3K activation [16,29] and therefore we investigated whether these intracellular pathways were also activated in LNCaP cells. The mitogenic effect elicited by MET was blocked by the addition of LY294002, an inhibitor of PI3K, and by the addition of PD98059, a selective inhibitor of the extracellular signal-regulated kinase kinase (Table 1). These results suggest that MET activates both intracellular pathways in LNCaP cells and in these ways exerts its mitogenic effect.

It has previously been shown that cannabinoids activate the stress-related p38 MAPK in some cells [30]. So, we tested the ability of the p38 inhibitor SB203580 to block the pro-survival effect of MET in LNCaP cells. Addition of 50 μM SB203580 to cultured cells did not significantly block the effect of MET (Table 1) suggesting that the mitogenic effect of MET is independent of this pathway. Similar results were obtained with the PKA activator BrA or the PKA inhibitor BrARpI. None of them affected the mitogenic effect induced by MET (Table 1). This was not the case for the PKC inhibitor BIM which totally blocked the pro-survival effect of MET (Table 1).

3.3. Effect of MET on the expression of the androgen receptor in LNCaP cells

The LNCaP cell line depends on androgens for growth. Androgens, through binding to the androgen receptor expressed in these cells, promote cellular proliferation. Therefore, we next tested the effect of MET on androgen receptor expression determined by Western blotting. MET at 0.1 μM concentration increased the expression of androgen receptor

Table 1
Effect of different enzyme inhibitors on the mitogenic action of MET in LNCaP cells

	[3H]Thymidine incorporation (cpm)	%
No addition	78 072 ± 16 679	100
0.1 μM MET	164 443 ± 17 209[#]	210 ± 22
10 μM LY294002	85 861 ± 15 098	109 ± 19
0.1 μM MET+10 μM LY294002	79 104 ± 14 437**	101 ± 10
0.1 μM PD98059	78 682 ± 8 960	100 ± 11.4
0.1 μM MET+0.1 μM PD98059	92 020 ± 6 483*	117 ± 8.3
50 μM SB203580	126 905 ± 16 374	162 ± 20
0.1 μM MET+50 μM SB203580	137 371 ± 1 702	175 ± 2
50 μM BrA	79 845 ± 3 998	102 ± 5
0.1 μM MET+50 μM BrA	154 130 ± 42 591	197 ± 54
50 μM BrARpI	96 457 ± 19 487	123 ± 24
0.1 μM MET+50 μM BrARpI	141 078 ± 10 370	180 ± 13
2 μM BIM	78 553 ± 14 337	100 ± 18
0.1 μM MET+2 μM BIM	79 147 ± 28 791**	101 ± 13

Cells were pre-incubated with the different agents for 10 min and then incubated with 0.1 μM MET for 4 days, after which [3H]thymidine incorporation into DNA was measured by liquid scintillation. Data are means ± S.D. of two different experiments performed in triplicate. *$P < 0.05$; **$P < 0.001$ vs. 0.1 μM MET; [#]$P < 0.0001$ vs. no addition as assessed by the paired Student's t-test.
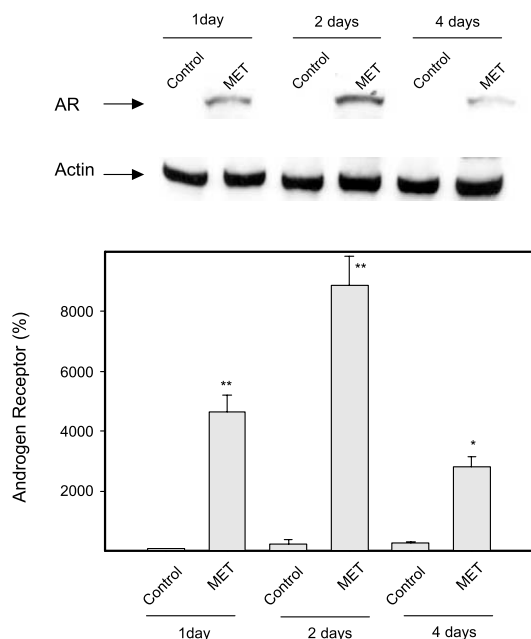
Fig. 4. Effect of MET on androgen receptor expression in LNCaP cells. Cells were treated with 0.1 μM MET for different days and androgen receptor expression was determined by Western blot. The figure shows a representative image of three independent experiments. Actin expression was also determined as a control. Densitometric analysis of three independent experiments, expressed as percent over control at 1 day, is represented in the histogram. *$P < 0.01$; **$P < 0.0005$ compared with the corresponding controls.

in LNCaP cells which was maximal at 2 days treatment (Fig. 4). The fact that the maximal induced expression of the androgen receptor was earlier than the maximal MET-induced mitogenic effect, which was at 4 days, suggests that the mitogenic effect induced by MET could be mediated by the increase in the androgen receptor.

We then examined the involvement of the cannabinoid receptors in the action of MET. Treatment of cells with the CB_1 antagonist SR1 reversed the effect produced by MET at 2 and 4 days treatment (Fig. 5A). As shown in Fig. 5B, the effect induced by MET was dose-dependent and was inhibited by SR1 at the three doses studied. Treatment with the CB_2 antagonist SR2 also reversed the androgen receptor induction by MET at 2 days (Fig. 6A) and low doses of treatment (Fig. 6B). However, the CB_2 antagonist did not significantly block the MET-induced androgen receptor up-regulation at 4 days or at 5 μM MET (Fig. 6).

4. Discussion

It has been recently shown that endocannabinoids may regulate cellular fate [10,31]. Depending on cell type and cannabinoid concentration, cannabinoids may induce either cell death or cell growth. At doses greater than micromolar, cannabinoids inhibit the growth of prostate cells [32–34] whereas at submicromolar concentrations, cannabinoids activate mitogenic signal transduction pathways [16]. Here, we show that the endocannabinoid anandamide analogue MET, at submicromolar concentration, had a mitogenic effect in prostate LNCaP cells and increased the expression of the androgen receptor in these cells. The maximal effect produced by MET was observed at 0.1–0.2 μM and at 4 days of treatment.

At longer incubation periods, cell growth was reduced and cells began to differentiate. The effect of MET was mediated by the cannabinoid receptors CB_1 and CB_2 and the activation of the PI3K and MAPK pathways. We have previously demonstrated that cannabinoid receptors activate the MAPK cascade through stimulation of the PI3K/Akt pathway in prostate cells [16]. Here, we show that the activation of this pathways leads to the stimulation of prostate LNCaP cell growth.

Cannabinoid receptors are coupled to adenylyl cyclase through G proteins. In most cells, cannabinoids inhibit adenylyl cyclase although under certain conditions they may stimulate the enzyme. However, the observed MET-induced mitogenic effect in LNCaP cells was independent of the cAMP pathway, since neither PKA activator nor PKA inhibitor affected the effect induced by MET. On the other hand, the fact
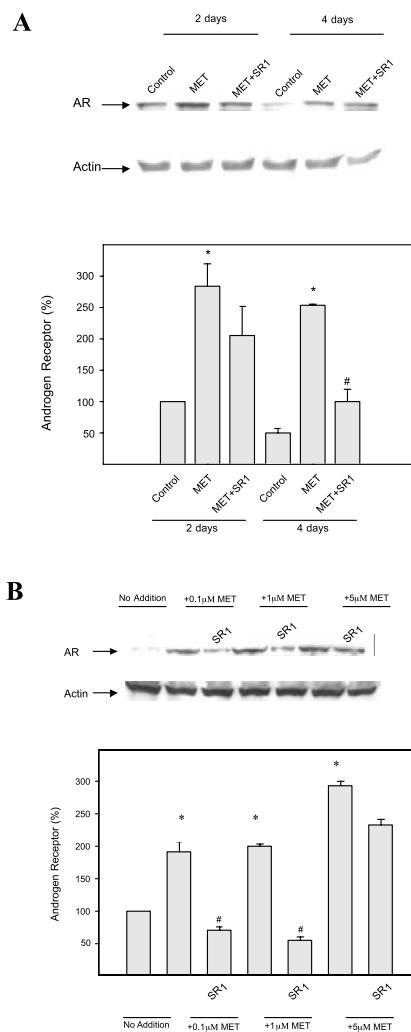

Fig. 5. Inhibition of the MET-induced up-regulation of the androgen receptor in LNCaP cells by CB_1 cannabinoid antagonist. (A) Cells were treated with 0.1 μM MET for different days in the presence or absence of 0.5 μM SR1. (B) Cells were treated with different doses of MET in the presence or absence of 0.5 μM SR1 for 2 days. The figure shows a representative image of two independent experiments. Actin expression was also determined as a control. Densitometric analysis of two independent experiments, expressed as percent over control at 2 days, is represented in the histogram. *$P < 0.005$ compared with the corresponding control; #$P < 0.05$ compared with MET treatment.
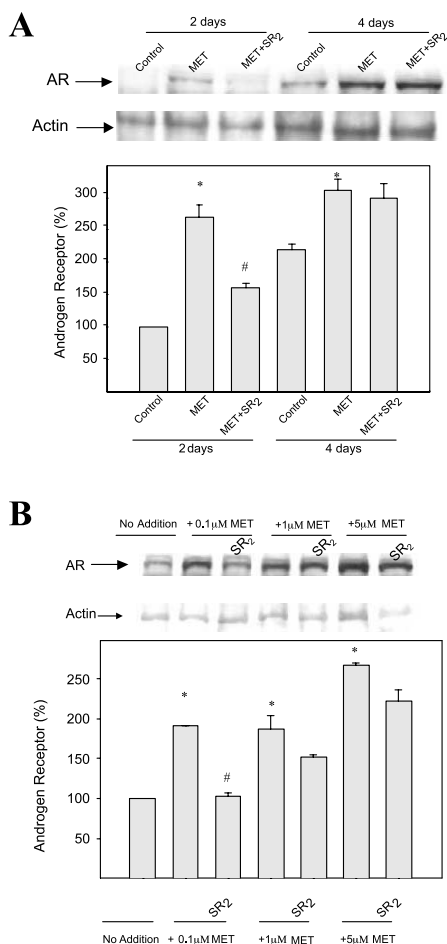
A

2 days   4 days

AR →

Actin →

300
250
200
150
100
50

Androgen Receptor (%)

Control MET MET+SR₂ Control MET MET+SR₂

2 days   4 days

B

No Addition   +0.1µM MET   +1µM MET   +5µM MET

AR →

Actin →

250
200
150
100
50

Androgen Receptor (%)

No Addition   + 0.1µM MET   +1µM MET   +5µM MET

Fig. 6. Inhibition of the (R)-methanandamide induced up-regulation of the androgen receptor in LNCaP cells by CB₂ cannabinoid antagonist. (A) cells were treated with 0.1 µM MET for different days in the presence or absence of 0.5 µM SR144528. (B) Cells were treated with different doses of MET in the presence or absence of 0.5 µM SR144528 for 2 days. Figure shows a representative image of two independent experiments. Actin expression was also determined as a control. Densitometric analysis of two independent experiments expressed in % over control at 2 days, is represented in the histogram. (*, $p < 0.005$ compared with their corresponding control; #, $p < 0.05$ compared with MET treatment.)

that the protein kinase C (PKC) inhibitor BIM blocked the effect of MET points to an involvement of PKC in the mitogenic response induced by cannabinoids in prostate cells. This pathway will be further explored in our laboratory.

MET also induced an increase in the androgen receptor expression that was mediated by the CB₁ receptor under all conditions studied and partially by CB₂ receptor activation. Although the CB₂ antagonist was able to reverse the effect induced by MET at low doses of MET (0.1 µM) and short periods of incubation (2 days), the up-regulation of androgen receptor at higher MET concentrations or longer times seems to be CB₂-independent. In fact, the mitogenic response induced by the CB₂ agonist JWH-015 was less prominent than the response induced by the other agonists tested (Fig. 3).

All these results are in agreement with previous reports showing that androgen receptor expression is regulated by the PI3K/Akt pathway in LNCaP cells [24]. Therefore, one possible mechanism for MET up-regulation of androgen re-

ceptor could be the stimulation of the PI3K/Akt pathway. The enhancement of androgen receptor levels could explain the proliferative effect of MET, since LNCaP cells depend on androgens for growth. One interesting point is the fact that some androgens like DHT or the synthetic androgen R1881 exert a peculiar biphasic dose-dependent influence on the proliferation of LNCaP cells [35] similar to the action of cannabinoids. DHT induces an up-regulation of the androgen receptor in these cells even at micromolar concentrations [24] that inhibit cell growth [35], which is in concordance with our results. Therefore, in prostate LNCaP cells, cannabinoids, acting via cannabinoid receptors and the PI3K pathway, could modulate cellular growth through the regulation of androgen receptor levels.

References

[1] Maccarrone, M. and Finazzi-Agro, A. (2002) Vitamins Hormones 65, 225–255.
[2] Sugiura, T. and Waku, K. (2002) J. Biochem. 132, 7–12.
[3] Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A. and Mechoulam, R. (1992) Science 258, 1946–1949.
[4] Howlett, A.C., Barth, F., Bonner, T.I., Cabral, G., Casellas, P., Devane, W.A., Felder, C.C., Herkenham, M., Mackie, K., Martin, B.R., Mechoulam, R. and Pertwee, R.G. (2002) Pharmacol. Rev. 54, 161–202.
[5] Lutz, B. (2002) Prostaglandins Leukotr. Essent. Fatty Acids 66, 123–142.
[6] McAllister, S. and Glass, M. (2002) Prostaglandins Leukotr. Essent. Fatty Acids 66, 161–171.
[7] Kaminski, N.E. (1998) J. Neuroimmunol. 83, 124–132.
[8] Bouaboula, M., Poinot-Chazel, C., Bourrie, B., Canat, X., Calandra, B., Rinaldi-Carmona, M., Le Fur, G. and Casellas, P. (1995) Biochem. J. 312, 637–641.
[9] Valjent, E., Pages, C., Rogard, M., Besson, M., Maldonado, R. and Caboche, J. (2001) Eur. J. Neurosci. 14, 342–352.
[10] Guzmán, M., Sánchez, C. and Galve-Roperh, I. (2001) J. Mol. Med. 78, 613–625.
[11] Sarker, K.P., Obara, S., Nakata, M., Kitajima, I. and Maruyama, I. (2000) FEBS Lett. 472, 39–44.
[12] Campbell, V.A. (2001) Neuropharmacology 40, 702–709.
[13] De Petrocellis, L., Melck, D., Palmisano, A., Bisogno, T., Laezza, C., Bifulco, M. and Di Marzo, V. (1998) Proc. Natl. Acad. Sci. USA 95, 8375–8380.
[14] Galve-Roperh, I., Sanchez, C., Cortes, M.L., Gomez del Pulgar, T., Izquierdo, M. and Guzmán, M. (2000) Nat. Med. 6, 313–318.
[15] Derocq, J.M., Ségui, M., Marchand, J., Le Fur, G. and Casellas, P. (1995) FEBS Lett. 369, 177–182.
[16] Sánchez, M.G., Ruiz-Llorente, L., Sánchez, A.M. and Díaz-Laviada, I. (2003) Cell. Signal. 15, 851–859.
[17] Jorda, M.A., Rayman, N., Valk, P., De Wee, E. and Delwel, R. (2003) Ann. NY Acad. Sci. 996, 10–16.
[18] Marker, P.C., Donjacour, A.A., Dahiya, R. and Cunha, G.R. (2003) Dev. Biol. 253, 165–174.
[19] Jin, Y. and Penning, T.M. (2001) Best Pract. Res. Clin. Endocrinol. Metab. 15, 79–94.
[20] Lee, D.K. and Chang, C. (2003) J. Steroid Biochem. Mol. Biol. 84, 41–49.
[21] Debes, J.D. and Tindall, D.J. (2002) Cancer Lett. 187, 1–7.
[22] Suzuki, H., Ueda, T., Ichikawa, T. and Ito, H. (2003) Endocr. Relat. Cancer 10, 209–216.
[23] Veldscholte, J., Berrevoets, C.A., Ris-Stalpers, C., Kuiper, G.G.J.M., Jenster, G., Trapman, J., Brinkmann, A.O. and Mulder, E. (1992) J. Steroid Biochem. Mol. Biol. 41, 665–669.

[24] Manin, M., Baron, S., Goossens, K., Beaudoin, C., Jean, C., Veyssiere, G., Verhoeven, G. and Morel, L. (2002) Biochem. J. 366, 729–736.
[25] Abadji, V., Lin, S., Taha, G., Griffin, G., Stevenson, L.A., Pertwee, R.G. and Makriyannis, A. (1994) J. Med. Chem. 37, 1889–1893.
[26] Meschler, J.P., Kraichely, D.M., Wilken, G.H. and Howlet, A.C. (2000) Biochem. Pharmacol. 60, 1315–1323.
[27] Rhee, M.H. and Kim, S.K. (2002) J. Vet. Sci. 3, 179–184.
[28] Pertwee, R.G. and Ross, R.A. (2002) Prostaglandins Leukotr. Essent. Fatty Acids 66, 101–121.
[29] Velasco, M.L., Ruiz, L., Sánchez, M.G. and Díaz-Laviada, I. (2001) Eur. J. Biochem. 268, 531–535.
[30] Derkinderen, P., Ledent, C., Parmentier, M. and Girault, J. (2001) J. Neurochem. 77, 957–960.
[31] Guzmán, M., Sánchez, C. and Galve-Roperh, I. (2002) Pharmacol. Ther. 95, 175–184.
[32] Ruiz, L., Miguel, A. and Díaz-Laviada, I. (1999) FEBS Lett. 458, 400–404.
[33] Melck, D., de Petrocellis, L., Orlando, P., Bisogno, T., Laezza, C., Bifulco, M. and Di Marzo, V. (2000) Endocrinology 141, 118–126.
[34] Mimeault, M., Pommery, N., Wattez, N., Bailly, C. and Hénichart, J. (2003) Prostate 56, 1–12.
[35] Hofman, K., Swinnen, J.V., Verhoeven, G. and Heyns, W. (2001) Biochem. Biophys. Res. Commun. 283, 97–101.