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Involvement of Reactive Oxygen Species in Capsaicinoid-induced Apoptosis in Transformed Cells

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Some varieties of sweet pepper accumulate non-pungent isosters of capsaicin, a type of compounds exemplified by capsiate. The only structural difference between capsaicin and capsiate is the link between the vanillyl and the acyl moieties, via an amide bond in the former and via an ester bond in the latter. By flow cytometry analyses we have determined that *nor*-dihydrocapsiate, a simplified analogue of capsiate, is a pro-oxidant compound that induces apoptosis in the Jurkat tumor cell line. The nuclear DNA fragmentation induced by *nor*-dihydrocapsiate is preceded by an increase in the production of reactive oxygen species and by a subsequent disruption of mitochondria trans-membrane potential. Capsiate-induced apoptosis is initiated at the S phase of the cell cycle and is mediated by a caspase-3-dependent pathway. The accumulation of intracellular reactive oxygen species in capsiate-treated cells is greatly prevented by the presence of ferricyanide, suggesting that capsiates target a cellular redox system distinct from the one involved in the mitochondrial electron-chain transport. Methylation of the phenolic hydroxyl of nor-dihydrocapsiate completely abrogated the ability to induce reactive oxygen species and apoptosis, highlighting the relevance of the presence of a free phenolic hydroxyl for the pro-oxidant properties of capsaicinoids.

Keywords: Capsiate; Capsaicin; ROS; VR1; Apoptosis

INTRODUCTION

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide, CPS), the pungent principle of hot pepper (*Capsicum* sp. vv.), and resiniferatoxin (RTX), a complex diterpenoid isolated from certain succulent African euphorbias, are the archetypal vanilloids. Both natural compounds are agonists of the vanilloid receptor type 1 (VR1), which is a non selective cation channel receptor expressed in several nerve tissues including peripheral sensory neurons.[1] VR1 is a nociceptive receptor that integrates several types of noxious stimuli to the central nervous system, evoking protective reflexes.[2,3] CPS and possibly other vanilloids as well, are endowed with a pleiotropic pattern of biological activities, some of which are mediated by the activation of cellular targets different from VR1. While natural vanilloids have been used as templates for the development of VR1-agonists and antagonists with improved clinical potential, there has been so far little attempt to dissect VR1-activity from other potentially useful applications of these compounds.[2,3]

We and others have shown that CPS, RTX and synthetic phorboid-homovanillates induce apoptosis in different tumor cell lines.[4–6] Moreover, CPS can behave like a quinone analogue inhibiting the plasma membrane NADH oxidase (PMOR).[7–9] The PMOR is a cellular system that transfers electrons from cytoplasmic NADH, putatively via coenzyme Q_{10} (CoQ), to external electron acceptors (oxygen, ferricyanide, transferrin and ascorbate).[8] This system plays an important regulatory role in the internal redox equilibrium in response to external stimuli,[10] and PMOR inhibitors can affect this redox

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balance, with an overall shift toward a pro-oxidative status. This might in turn lead to disruption of the transmembrane mitochondria potential ($\Delta\psi_m$), activation of executor caspases, and eventually apoptosis.[5,9]

Analogues of CPS devoid of VR1 activity but retaining the ability of the natural product to inhibit the PMOR system and induce apoptosis have far-reaching potential therapeutic applications. In this context, we have investigated the biological profile of capsiates, a class of non-pungent constituents of sweet pepper showing high structural homology to CPS.[11] Capsiate (*E*-vanillyl-8-methyl-6-nonenoate) is the archetypal member of this class of compounds. The remarkable difference between the sensory properties of capsaicin and capsiate is solely due to the way their vanillyl and the acyl moieties are linked, via amide bond in the former and via ester bond in the latter. In natural capsaicinoids, pungency and activation of VR1 are related,[12,13] but different structure–activity relationships exist with regard to their pro-oxidant properties.[14] This observation was fully confirmed by our finding that capsiates, though lacking VR1 agonist activity, are still potent inducers of apoptosis in tumoral cells.[15]

To shed light on capsiate-induced apoptosis, we have investigated its mechanistic details, obtaining evidence that *nor*-dihydrocapsiate (CPT), a synthetic analogue of natural capsiate has pro-oxidant activity in Jurkat cells, inducing $\Delta\psi_m$ dissipation and activating the executor caspase-3. In addition to these observations, we present evidence that the phenolic hydroxyl of capsiates plays a key role in ROS generation and the induction of apoptosis.

MATERIALS AND METHODS

Cell Lines and Reagents

Jurkat cells were maintained in exponential growth in RPMI-1640 medium (Bio-Whittaker, VerViers, Belgium). The culture media were supplemented with 10% heat inactivated FCS, 2 mM L-glutamine and the antibiotics penicillin and streptomycin (Gibco, Paisley, Scotland). The cell permeable paninhibitor of caspases zVAD-fmk was from Bachem (Bubendorf, Switzerland). FITC-12-deoxy-2-uridine triphosphate (FITC-dUTP) and terminal deoxynucleotidyltransferase (TdT) were from Roche (Mannheim, Germany). All other reagents not further specified were from Sigma Chemical Co. (St. Louis, MO, USA). The synthesis of *nor*-dihydrocapsiate (CPS) has been described elsewhere.[15]

Synthesis of *O*-methyl-nordihydrocapsiate (mCPT)

To a solution of 3,4-dimethoxybenzyl alcohol (292 µl, 338 mg, 2.0 mMol) in dry CH_2Cl_2 (5 ml), nonanoic acid (353 µl, 320 mg, 1 molEq.), dicyclohexylcarbodiimide (416 mg, 2.0 mMol, 1 molEq) and dimethylaminopyridine (10 mg) were added. After stirring 5 h at room temperature, the reaction was worked up by dilution with ether (10 ml), filtration and washing with sat. $NaHCO_3$ and brine. After removal of the solvent, the residue was purified by column chromatography (5 g silica gel, petroleum ether–EtOAc 8:2 as eluant) to afford 505 mg (79%) *O*-methyl-nordihydrocapsiate as a white powder. Mp 109°C, IR ν_{max}: 3432, 1736, 1518, 1466, 1267, 1242m 1161, 1030 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.93 (1H, br d,$J = 8$ Hz), 6.91 (1H, br s), 6.84 (1H, d,$J = 8$ Hz), 5.04 (2H, s), 3.88 (3H, s), 3.87 (3H, s), 2.33 (2H, t,$J = 7.4$ Hz), 1.63 (2H, m), ca. 1.27 (8H, m), 0.87 (3H, t,$J = 6.9$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.3 (s), 148.7 (s), 148.6 (s), 128.5 (s), 120.8 (d), 111.4 (d), 110.6 (d), 65.7 (t), 55.5 (q), 55.4 (q), 34.7 (t), 32.2 (t), 29.6 (t), 29.5 (t), 25.3 (t), 23.0 (t), 22.9 (t), 14.4 (q); CI-MS (isobutane), m/z: 309 $(M + H)^+$($C_{18}H_{28}O_4$ $+H)^+$ (100).

Determination of Mitochondrial Transmembrane Potential and ROS Generation

To study the superoxide anion generation (ROS), cells (10^6/ml) were incubated in phosphate buffered saline (PBS) with 2 µM dihydroethidine (HE) (red fluorescent after oxidation) for 20 min at 37°C, followed by analysis on an Epics XL Analyzer (Coulter, Hialeah, FL, USA). To measure the mitochondrial transmembrane potential ($\Delta\psi_m$) the cells were incubated in PBS with 20 nM $DiOC_6(3)$ (green fluorescence) (Molecular Probes, Eugene, OR, USA) for 20 min at 37°C and then analysed by flow cytometry.

Determination of Nuclear DNA Loss and Cell Cycle Analysis

The percentage of cells undergoing chromatinolysis (subdiploid cells) was determined after ethanol fixation (70%, for 24 h at 4°C). The cells were then washed twice with PBS containing 4% glucose and subjected to RNA digestion (RNAse-A, 50 U/ml) and PI (20 µg/ml) staining in PBS for 1 h at RT, and analysed by cytofluorimetry. Under these conditions, low molecular weight DNA leaks from the ethanol-fixed apoptotic cells and the subsequent staining allows the determination of the percentage of subdiploid cells (sub-G_0/G_1 fraction).

Detection of DNA Strand Breaks by the TUNEL Method

Cells (1×10^6) were fixed in 4% paraformaldehyde in PBS for 24 h at 4°C, washed twice in PBS and permeabilized in 0.1% sodium citrate containing

0.1% Triton X-100 for 20 min. Fixed cells were washed three times in PBS and resuspended in a final volume of 50 μl of TUNEL buffer (0.3 nmol FITC-dUTP, 3 nmol dATP, 50 nmol $CoCl_2$, 5 U TdT, 200 mM potassium cacodylate, 250 μg/ml BSA and 25 mM Tris–HCl pH 6.6). The cells were incubated for 1 h at 37°C and then washed twice in PBS and analysed by flow cytometry. To determine both DNA strand breaks and cell cycle, TUNEL stained cells were counterstained with PI and treated with RNAse as described above prior to cytofluorimetric analysis. In this method, fixation in formaldehyde prevents extraction of low molecular weight DNA from apoptotic cells and thus the cell cycle distribution estimates both apoptotic and non-apoptotic cells.

Determination of Caspase-3 Activity

Jurkat cells (3×10^6) were washed with PBS and incubated for 30 min on ice with 100 μl of lysis buffer (10 mM Tris–HCl, 10 mM $NaH_2PO_4/NaHPO_4$, pH 7.5, 130 mM NaCl, 1% Triton®-X100 and 10 mM sodium pyrophosphate). Cell lysates were spun, the supernatants collected and the protein concentrations determined by the Bradford method. For each reaction, 30 μg of protein from cell lysates were added to 1 ml of freshly prepared protease assay buffer (20 mM HEPES pH 7.5, 10% glycerol, 2 mM DTT) containing 20 μM of Ac-DEVD-AMC (Sigma Chemical Co.). Reaction mixtures in the absence of cellular extracts were used as negative controls (fluorescence background). Reaction mixtures were incubated for 1 h at 37°C and the AMC liberated from Ac-DEVD-AMC was measured using a spectrofluorometer (Hitachi F-2500 model, Hitachi Ltd., Tokyo, Japan) with an excitation wavelength of 380 nm and an emission wavelength range of 400–550 nm. Data were collected as the integral of relative fluorescence intensity minus the background fluorescence.

RESULTS

The capacity of natural vanilloids to generate ROS and to induce apoptosis in the Jurkat cell line has been investigated. The chemical structures of the compounds used in this study are portrayed in Fig. 1; Capsaicin, CPS (1), *nor*-dihydrocapsiate, CPT (2); and methyl-capsiate, mCPT (3).

Nor-dihydrocapsiate Induces ROS in Jurkat Cells

We have shown previously that CPS and other vanilloids induce ROS in different tumor cell lines and in activated T cells.[5,6,9] Since CPS and CPT show

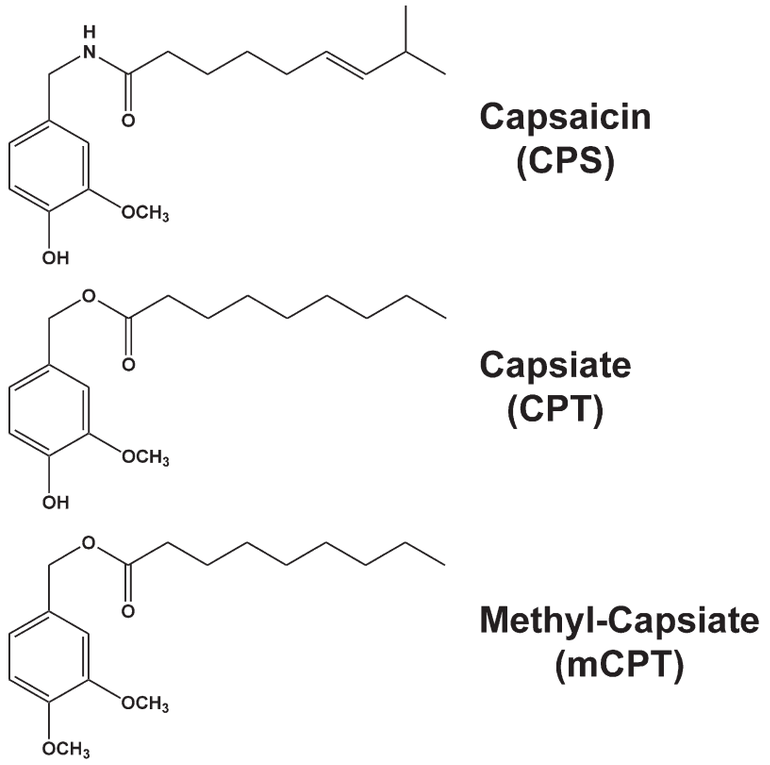

FIGURE 1 Chemical structures of capsaicin (CPS), capsiate (CPT) and methyl-capsiate (mCPT).

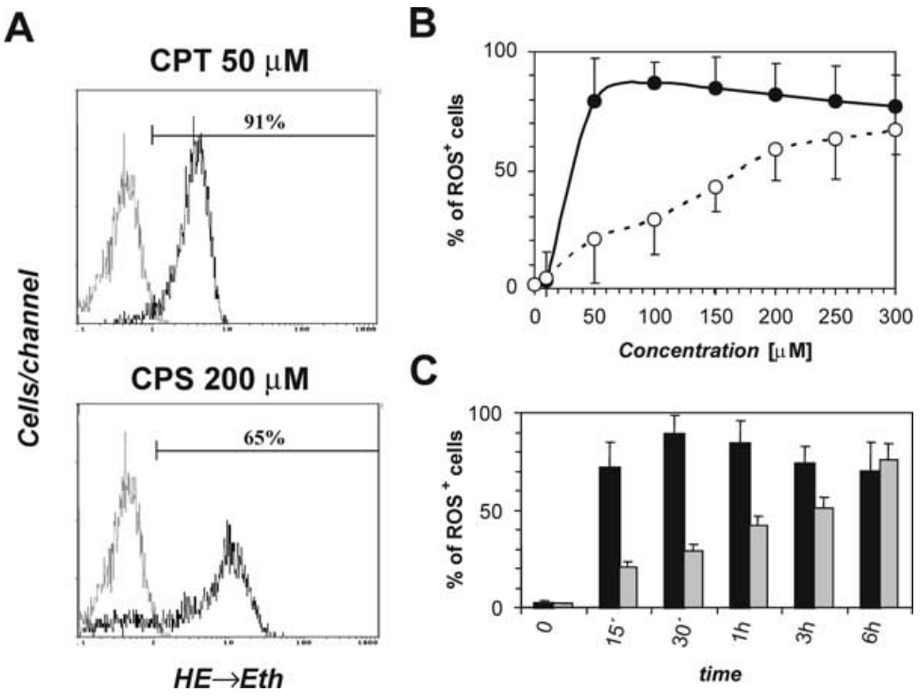

FIGURE 2 Reactive oxygen generation by capsaicinoids in Jurkat cells. (A) Jurkat cells were treated with either CPT or CPS at the indicated concentrations. After 4 h treatment, cells were collected ROS generation detected by flow cytofluorimetry. Results are representative of five independent experiments. (B) Dose response generation of ROS in capsaicinoids treated cells. Jurkat cells were treated with the indicated concentrations of CPT (filled circles) or CPS (open circles) for 4 h. The percentages of ROS-positive cells are given at the *y*-axis. (C) Kinetics of capsaicinoids-induced ROS generation. Jurkat cells were treated with either CPT (50 μM) (black bars) or CPS (200 μM) (gray bars) at different times and the generation of intracellular ROS was measured as in A. Values are means ± SE of three independent experiments.

a complete identity at the vanilloid moiety, we were interested to study the effects of CPT in the generation of intracellular ROS in the Jurkat cell line. We treated the cells with either CPT (50 μM) or CPS (200 μM) for 4 h. Then the treated cells were incubated with HE for 20 min. Intracellular ROS generated by vanilloid treatment results in the oxidation of HE that become red fluorescent, which is easily detected by flow cytometry (exc 488 nm; em 620 nm). The number of cells with high levels of intracellular ROS is represented as the percentage of fluorescent cells (91 and 65% for CPT and CPS, respectively) (Fig. 2A). The increase of ROS in both CPT and CPS-treated cells was dose and time dependent (Fig. 2B, C). It is remarkable that compared to CPS, the analogue CPT was more efficient in inducing intracellular ROS. Thus, CPT-treatment resulted in a rapid induction of ROS (within 15 min) with the lower concentration tested (50 μM), while in CPS-treated cells a similar percentage of ROS^+ cells was detected with doses of 200 μM and only after 6 h of treatment.

Next, to evaluate the source of ROS in CPT-treated cells we performed a simple experiment for detection of ROS in the presence of either ferricyanide (1 mM), an external electron acceptor, or rotenone (200 μM), a specific inhibitor of the complex I of the mitochondria respiratory chain. In Fig. 3, we show that both compounds were able to inhibit CPT-induced generation of intracellular ROS, but ferricyanide was clearly more efficient than rotenone, suggesting that intracellular ROS generated by CPT derived from both mitochondrial and extra-mitochondrial sources.

Induction of Apoptosis by Natural Capsaicinoids in Jurkat Cells

In a previous report, we demonstrated that CPS-induced apoptosis was preceded by a disruption of the $\Delta\Psi_m$.[5] Thus, to analyse whether these events were also induced by the non-pungent capsaicinoid, CPT, we treated Jurkat cells with either CPT (50 μM) or CPS (200 μM) for 6 h, and $\Delta\Psi_m$ dissipation detected by flow cytometry using $DiOC_6(3)$ (green fluorescent), a cationic probe that accumulates in the mitochondria as a function of its potential. As shown in Fig. 4A, untreated Jurkat cells present a high $\Delta\Psi_m$ that was diminished by treatment with CPT and CPS (71 and 42% of the cells showing $\Delta\Psi_m$ dissipation, respectively). An immediate consequence of the $\Delta\Psi_m$ breakdown can be the release to the cytosol of proapoptotic factors (cytochrome *c* and AIF). Then, cytosolic cytochrome *c* binds and

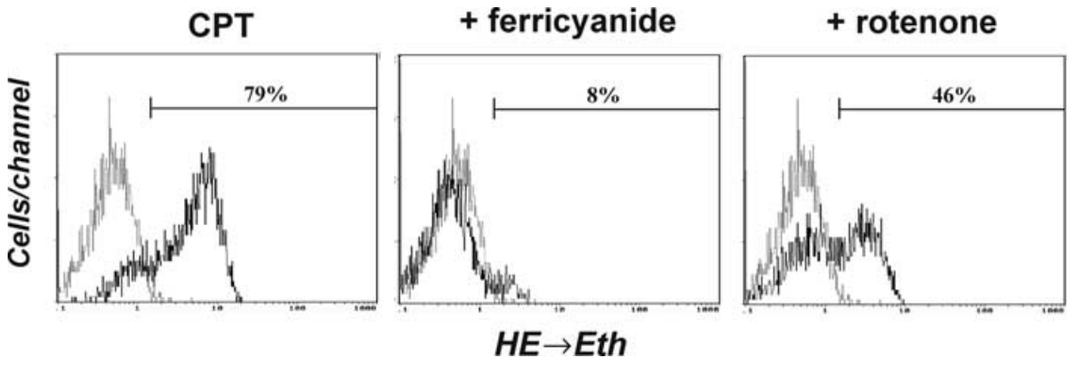

FIGURE 3 The ROS generation in capsiate-treated cells is inhibited by extracellular electron acceptor donors. Jurkat cells were preincubated with either rotenone (200 μM) or ferricyanide (1 mM) for 15 min and then incubated with CPT (50 μM) for 4 h at 37°C. ROS generation was measured as in Fig. 2. Results are representative of three independent experiments.

activates Apaf-1/caspase-9, allowing the activation of the effector protease caspase-3.[16] Thus, to study the activation of this caspase in a direct way, we incubated the cells with different doses of either CPT or CPS for 6 h, a time at which the $\Delta\Psi_m$ dissipation is evident (Fig. 4A), and determined the activity of caspase-3 by a fluorometric highly specific and sensitive method. Figure 4B shows that the treatment of Jurkat cells with CPT leads to the activation of this caspase in a dose-dependent manner. As expected, CPT was more efficient than CPS in the activation of caspase-3.

Since the activation of effector caspases orchestrate the biochemical execution of programmed cell death in a number of experimental systems,[17] we investigated the capacity of CPT to induce caspase-dependent DNA fragmentation in Jurkat cells. The percentage of apoptotic cells was determined 12 h

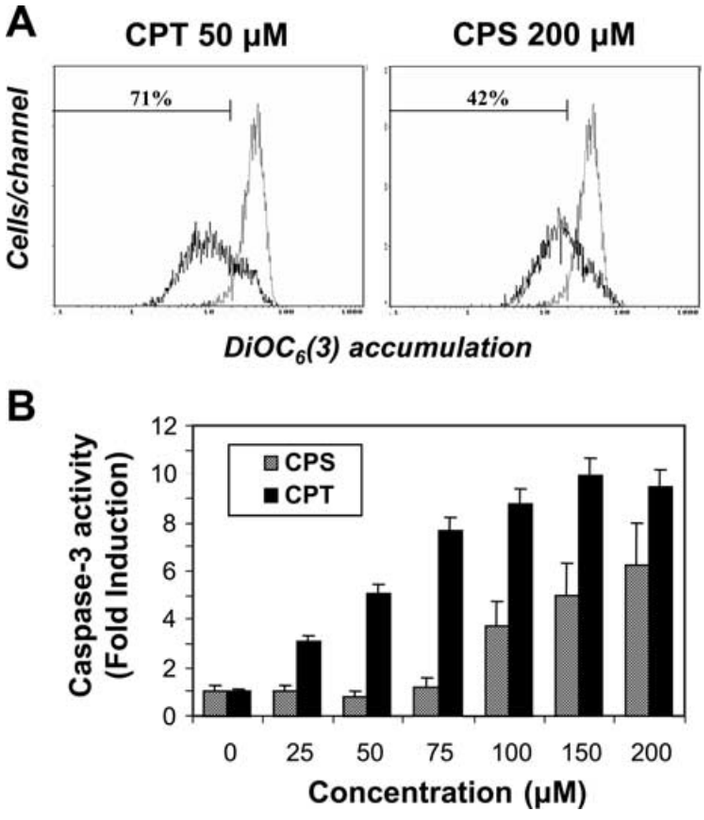

FIGURE 4 Effects of capsaicinoids on $\Delta\psi_m$ dissipation and caspase-3 activation. (A) Jurkat cells were treated with either CPT (50 μM) or CPS (200 μM) for 6 h and the $\Delta\psi_m$ measured as described in the "Material and Methods" section. The $\Delta\psi_m$ of untreated cells was used as control. (B) Capsaicinoids activate caspase-3. Jurkat cells were treated with CPT and CPS at the indicated concentrations for 6 h, then the cells were lysed and the caspase-3 activity measured by spectrofluorimetry and expressed as fold induction over untreated cells. Values are means ± SE of three independent experiments.

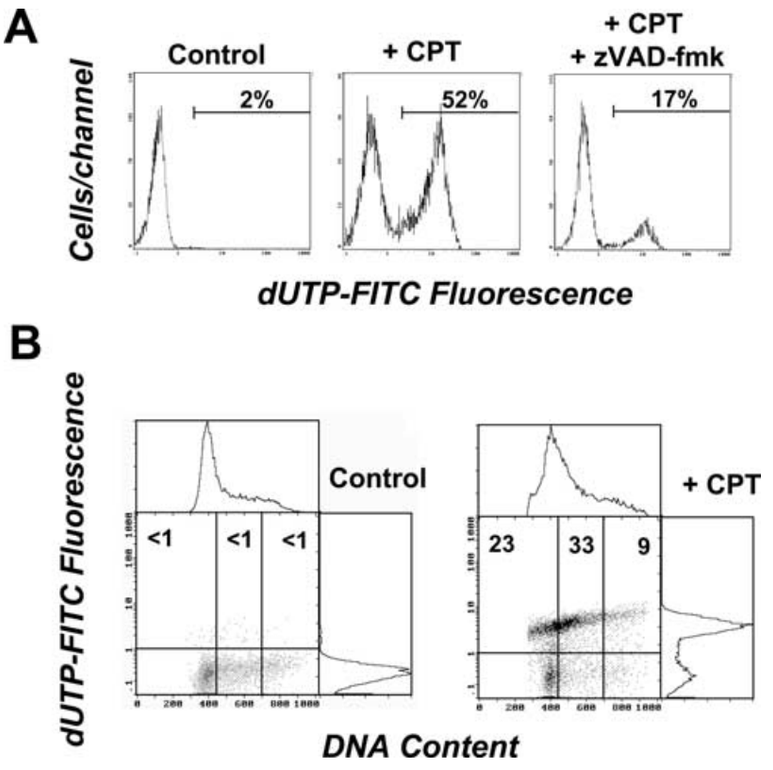

FIGURE 5 Induction of apoptosis by CPT in Jurkat cells. (A) Detection of DNA strand breaks by the TUNEL method. Jurkat cells were treated with CPT (50 μM) in the presence or absence of the pancaspase inhibitor zVAD-fmk (20 μM) for 12 h and the percentage of apoptotic cells was determined by the TUNEL method. Results are representative of three independent experiments. (B) Cell cycle dependence for apoptosis induced by Capsiate. Jurkat cells were treated with Capsiate (100 μM) for 18 h. The cell cycle phase (*X*-axis, G_0/G_1, S and G_2/M separated by vertical lines) and the DNA strand breaks (*Y*-axis) were analysed by flow cytometry. Results are representative of three independent experiments.

after CPT treatment by the TUNEL method and Fig. 5A reveals that apoptosis induced by this capsaicinoid was greatly inhibited by the pancaspase inhibitor zVAD-fmk. Next, to examine whether CPT-induced apoptosis in Jurkat cells is dependent on a specific phase of the cell cycle, we performed double staining experiments with PI and FITC-dUTP as described in the "Materials and Methods" section. In this way, the phase of the cell cycle in which DNA fragmentation occurs can be established. Most of the CPT-induced DNA fragmentation occurs in the G_0/G_1 and in the S phases of the cell cycle in Jurkat cells treated for 12 h with CPT (Fig. 5B). Taken together, these results strongly suggest that capsiate induces apoptosis in Jurkat cells through mechanisms similar to that previously described for other vanilloids.[6,9]

The OH-phenolic Group is Responsible for the Pro-oxidant Activity of Capsiate

To demonstrate that the vanilloid moiety of capsaicinoids is a functional quinone analogue, we generate a new compound in which the OH-group was chemically modified by methylation (methyl-capsiate) (Fig. 1). Then we tested this compound for its capacity to induce ROS and apoptosis in Jurkat cells. After treatment for 6 h, ROS generation and $\Delta\Psi_m$ dissipation were detected by double staining experiments. Untreated cells were used to establish control values, considering these cells to have a high $\Delta\Psi_m$ ($DiOC_6(3)^{high}$) and low levels of intracellular ROS ($HE^- \rightarrow Eth^{low}$) (Fig. 6A). CPT treatment of Jurkat cells leads to an increase in the percentage of ($HE^- \rightarrow Eth^{high}$) cells, reflecting ROS hypergeneration (70%) which was followed by a loss in the mitochondria transmembrane potential (28% of $DiOC_6(3)^{low}$ cells). In contrast, methyl-CPT did not induce changes in the percentage of cells with high levels of intracellular ROS ($HE^- \rightarrow Eth^{low}$). This result correlates well with the lack of apoptotic activity in methyl-capsiate compared with CPT, measured as the percentage of hypodiploid cells induced by these compounds in Jurkat cells (Fig. 6B).

DISCUSSION

The generation of ROS in eukaryotic cells is mainly under mitochondrial control, and involves

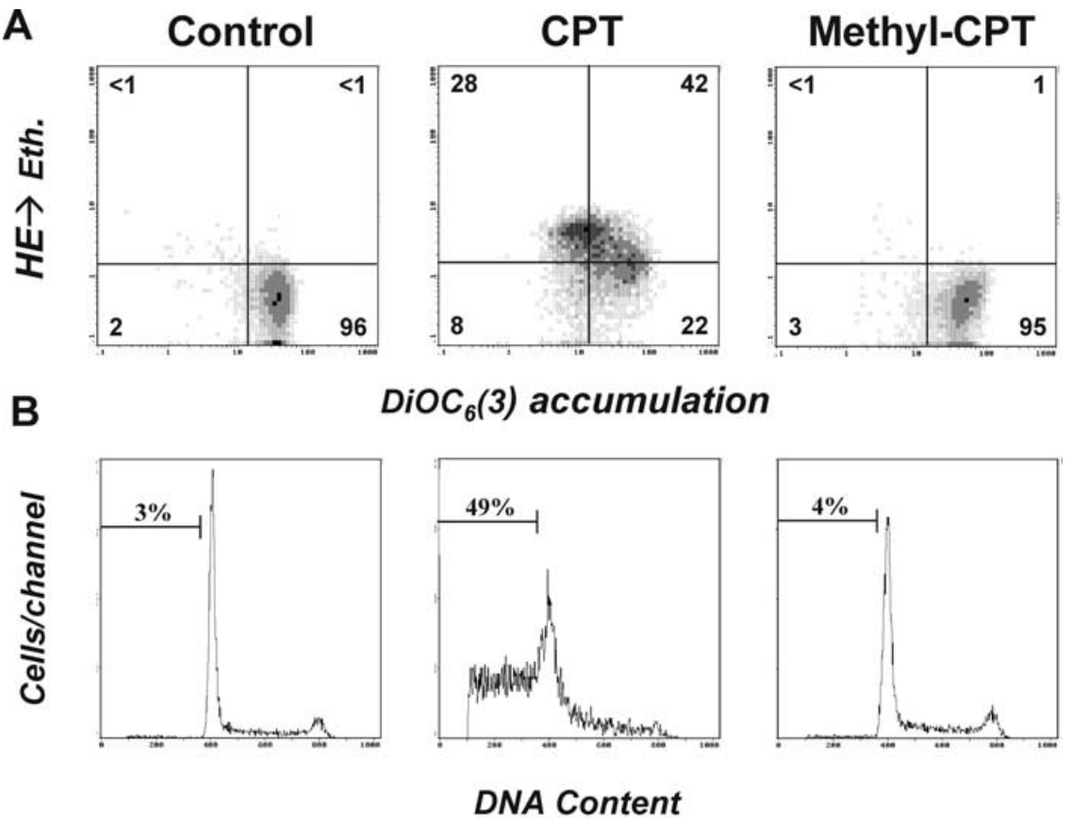

FIGURE 6 Methyl-capsiate does not induce ROS, $\Delta\Psi_m$ dissipation and apoptosis in Jurkat cells. (A) Jurkat cells were treated with either CPT or mCPT (50 μM). After 6-h treatment, cells were collected and the $\Delta\Psi_m$ disruption and ROS generation simultaneous detected by flow cytofluorimetry. The number in the corners represent the percentage of cells obtained in biparametric histograms delimited by four compartments, namely, (i) bottom-right that correspond to normal cells that have $\Delta\Psi_m^{high}$ indicated by $DiOC_6(3)^{high}$ staining and low levels of ROS indicated by $(HE- \rightarrow Eth)^{low}$; (ii) $\Delta\Psi_m^{low}/DiOC_6(3)^{low}$ and $(HE- \rightarrow Eth)^{low}$ (bottom-left); (iii) ROS generating cells $(HE- \rightarrow Eth)^{high}$ with $\Delta\Psi_m^{high}/DiOC_6(3)^{high}$ (top-right) and (iv) ROS generating cells $(HE- \rightarrow Eth)^{high}$ with $\Delta\Psi_m^{low}/DiOC_6(3)^{low}$ (preapoptotic cells, top-left compartment). (B) Jurkat cells were treated with CPT or mCPT for 16 and the percentages of hypodiploid cells were measured by cell cycle analyses. Results are representative of three independent experiments.

the production of hydrogen peroxide, hydroxyl radicals and superoxide anions. However, the plasma membrane of all cells tested contains a further electron transport chain, apparently essential to control cell growth and differentiation.[18] This PMOR system has been found to be upregulated in cells lacking an intact mitochondrial electron transport chain (ρ^0), in tumor cells, in senescent cells, and in lymphocytes from insulin-dependents diabetes mellitus patients.[19–21] It seems that at least one function of this redox system is to regenerate NAD^+ from NADH accumulated in the glycolytic production of ATP, increased in tumor cells.[22] In doing so, the PMOR transfers electrons from cytoplasmic NADH to external electron acceptors via coenzyme Q_{10} (CoQ). This redox system is apparently essential in cells whose mitochondria are functionality impaired or insufficient for cell survival.[23,24]

The constituents of the PMOR system have long remained elusive, but the cDNA for a tumor-associated plasma membrane NADH oxidase (tNOX) has recently been cloned and characterized.[25] Furthermore, the binding domains of capsaicin and adenine nucleotide (NADH) were identified by site directed mutagenesis.[26] The NADH oxidase activity of tNOX is inhibited by the vanilloid CPS.[25] Since CPT also inhibit the NADH-oxidase activity of the PMOR system (Baker, MA & Lawen, A, unpublished results), it is possible that NOX-inhibition by vanilloids in Jurkat cells results in the redirection of the normal electron flow in the plasma membrane complex, favoring the one-electron processes that generates superoxides and other aggressive oxidants (OH^-, H_2O_2) instead of H_2O. This could explain the protective effect of ferricyanide that might capture the redirected electrons and reduce Fe^{3+} to Fe^{2+}, preventing ROS-accumulation in capsiate-treated cells. The excess of ROS induced by capsiate might next oxidize the thiol groups of the mitochondrial permeability transition pores[27,28] causing the free distribution of solutes at both sides of the inner mitochondrial membrane and, eventually, activating the apoptotic-executor

caspase-3. In addition, a partial inhibition of the mitochondrial NADH-oxidase and a complete inhibition of NOX at the PMOR system can also induce an increase of the $NADH/NAD^+$ and ADP/ATP balances, which have been demonstrated to induce mitochondrial permeability transition.[27]

The PMOR may be an important target for anticancer drugs, since its upregulation could be a mechanism by which cancer cells can tolerate the high levels of ROS formed due to their increased metabolic rate. Moreover, cancer cells also have a low superoxide-dismutase activity, and are thus especially sensitive to drugs that interfere with the regulation of intracellular ROS. This in turn leads to free-radical damage to mitochondrial membranes, with release of cytochrome c and, eventually, apoptosis.[29]

The amide bond of CPS is replaced in by an ester bond in capsiates, a structural modification leading to a complete loss of pungency and lack of vanilloid activity over VR1.[15] The phenolic hydroxyl of capsaicin is essential for VR1 binding and pungency,[30] and the results presented here demonstrate that this phenolic hydroxyl is also required for its function as quinone analogue and ROS generation. The structure–activity relationships governing the pro-oxidant activity of capsaicinoids are largely unknown. However, it is important to point out that oxygen to nitrogen replacement at the benzyl position increases pro-oxidant activity and shuts down affinity for VR1. This, while marvelously testifying the relevance of isosterism in biology, also shows that dissection of VR1 binding and ROS induction can be easily accomplished, opening up new avenues of exploitation for capsaicinoids.

The increased capacity of ROS-induction attained by oxygen to nitrogen isosteric substitution in capsaicinoids is apparently surprising, since the close similarity of the acyl moiety translates into similar membrane permeability. On the other hand, several explanations can be put forward on electronic bases. Thus, the ethereal ester oxygen of capsiates might be a better electron donor toward oxygen centered *p*-radical compared to the amide nitrogen of capsaicin. Furthermore, the *p*-hydroxybenzyl ester moiety of capsiates can easily generate quinone methides via a phenylogous β-elimination reaction, a type of reaction impossible in capsaicin on account of the poor leaving group properties of the amide nitrogen.

The antitumoral activity of CPS has been documented "in vivo" both in prevention and in treatment experiments.[31] However, CPS is unsuitable for systemic use, because its affinity for the VR1 translates into severe side effects.[32] Systemic administration of capsiates was instead well tolerated in animals[33] as well as in humans,[34] and these compounds showed remarkable chemopreventive activity in the two-stage mouse skin carcinogenesis assay.[15] These observations make capsiates interesting leads for the development of new anticancer drugs, and we have provided evidence that interaction with the electron transport system seemingly underlies their oncological potential.

Acknowledgements

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