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Vanilloid receptor agonists and antagonists are mitochondrial inhibitors: How vanilloids cause non-vanilloid receptor mediated cell death

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Abstract

Time-lapse photomicroscopy of human H460 lung cancer cells demonstrated of the transient receptor potential V1 (TRPV1) channel agonists, (*E*)-capsaicin and resiniferatoxin, and the TRPV1 antagonists, capsazepine, and SB366791, were able to bring about morphological changes characteristic of apoptosis and/or necrosis. Immunoblot analysis identified immunoreactivity for the transient receptor potential V1 (TRPV1) channel in rat brain samples, but not in rat heart mitochondria or in H460 cells. In isolated rat heart mitochondria, all four ligands caused concentration-dependent decreases in oxygen consumption and mitochondrial membrane potential. (*E*)-Capsaicin and capsazepine evoked concentration-dependent increases and decreases, respectively, in mitochondrial hydrogen peroxide production, whilst resiniferatoxin and SB366791 were without significant effect. These data support the hypothesis that (*E*)-capsaicin, resiniferatoxin, capsazepine, and SB366791 are all mitochondrial inhibitors, able to activate apoptosis and/or necrosis via non-receptor mediated mechanisms, and also support the use of TRPV1 ligands as anti-cancer agents.

Keywords: TRPV1; Vanilloid; Capsaicin; Resiniferatoxin; Capsazepine; SB366791; Apoptosis; Necrosis; Mitochondria; Cell death; Chemotherapy; Cancer

Ligands of the transient receptor potential V1 channel (TRPV1; also known as the vanilloid receptor) have been shown to have anti-proliferative and/or pro-apoptotic effects both *in vitro*, and in animal models *in vivo* [1]. However, the molecular mechanisms involved in the anti-cancer actions of vanilloid receptor agonists, such as capsaicin and antagonists, such as capsazepine, are complex, and their targets and the molecular mechanisms by which they initiate cancer cell death are incompletely understood. This is in part due to the complexity of the TRP channel family and the expanding list of ligands which act as agonists and/or antagonists [2]. In addition, recent studies have shown that vanilloid receptors are present on a more diverse range of cells than at first suspected, such as thymocytes [3]. Furthermore, vanilloid receptors may be transiently expressed

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on certain cell types in response to physiological stimuli [4]. The finding that vanilloid compounds may be ligands for other receptor types such as cannabinoid receptors [5], and conversely, that molecules such as polyamines [6] and endogenous cannabinoids, like anandamide [7], are suggested to be vanilloid receptor agonists has served to confound attempts to understand how vanilloid ligands cause cell death. Data from many studies using vanilloid ligands are made more difficult to interpret, as many of the cell types investigated have not been demonstrated to express functional vanilloid receptors, leading to the suggestion that the involvement of other, non-vanilloid receptor mediated mechanisms. There are indications that mitochondria could be involved in capsaicin-induced cell death, although some of these studies did not include the study of vanilloid receptor antagonists [8,9]. Recent studies have demonstrated that the tricyclic antidepressant chlorimipramine has tumour selective, pro-apoptotic effects in a variety of human cancer glioma cell lines, an action due to its ability to bind to mitochondrial complex III [10]. The aim of this study was, therefore, to investigate whether vanilloid agonists and antagonists also have pro-apoptotic and/or pro-necrotic actions on cancer cells, and if so, to further clarify their mechanism of action using highly purified preparations of isolated mitochondria.

Materials and methods

Cells and chemicals. H460 cells (a human non-small cell lung cancer line) were obtained from the American Type Culture Collection, Manassas, VA. Drugs were dissolved in 100% ethanol at a stock concentration of 10 mM. All chemicals used were of the highest grade available and were from Sigma Chemical Company, Merck Biosciences, Tocris, or InVitrogen. For all vanilloid receptor ligands used, the diluent (ethanol) was never present at >1.0%, and control culture flasks with H460 cells or mitochondrial incubations having the same concentration of diluent showed no statistically significant effects.

Time-lapse photomicroscopy of H460 cells. H460 cells were grown on Iwaki 35 mm glass based dishes to 60–70% confluency maintained in a water jacketed thermostatically controlled incubator at 37 °C in 95% O_2/5% CO_2 in RPMI 1640 with 10% foetal calf serum (FCS) and then treated with the drugs. Digital images were captured every 1 min for 2 h using a Leica DMIRE2 microscope and a Hamamatsu ORCA II BT 1024 CCD camera.

Isolation of rat heart mitochondria. Rat heart mitochondria was prepared from male 250 g Lister rats as previously described [11].

Measurement of mitochondrial oxygen consumption. Rat heart mitochondrial oxygen consumption was measured poloragraphically at 37 °C with 10 mM malate + 10 mM glutamate as previously described [12].

Measurement of mitochondrial membrane potential. Mitochondrial membrane potential was measured fluorimetrically (using rhodamine 123) as previously described [10] using either (1) 10 mM glutamate + 10 mM malate or (2) 10 mM succinate + 1 μM rotenone (to block complex I). Mitochondrial hydrogen peroxide production was measured fluorimetrically (using amplex red) at 37 °C in a Hitachi F2500 fluorimeter as previously described [13]. Fluorimeters were calibrated daily using fresh hydrogen peroxide solutions of known (spectrophotometrically determined) concentration.

SDS–PAGE and immunoblot analysis. H460 cells and isolated rat heart mitochondria were re-suspended in lysis buffer, then electrophoresed through 10% polyacrylamide gels and electroblotted to nitrocellulose membranes according to standard procedures [14]. TRPV1 receptor protein was detected with rabbit anti-TRPV1 polyclonal antibodies (Tocris Bioscience, Bristol, UK, product number 2233) according to the suppliers' instructions. Bound anti-TRPV1 antibodies were detected with goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (Dako, Ely, UK, code number P0048) and visualised by enhanced chemiluminescence (ECL).

Protein assay. Protein concentrations were determined using a microplate Lowry assay with bovine serum albumin used as a concentration standard [10].

Statistical analysis. All experiments were repeated $n = 3$–8 times. Statistical analysis was performed using Student's t-tests. Significance was attributed when $P < 0.05$.

Results

TRPV1 ligands induce morphological features of apoptosis and necrosis in H460 cells

Figs. 1A and B show single images taken at time = 0 and time = 2 h in the time-lapse microscope in the presence of 50 μM (*E*)-capsaicin or 50 μM resiniferatoxin, respectively. There was a general loss of cells in the fields of view (due to their detaching from the growing surface of the cell culture

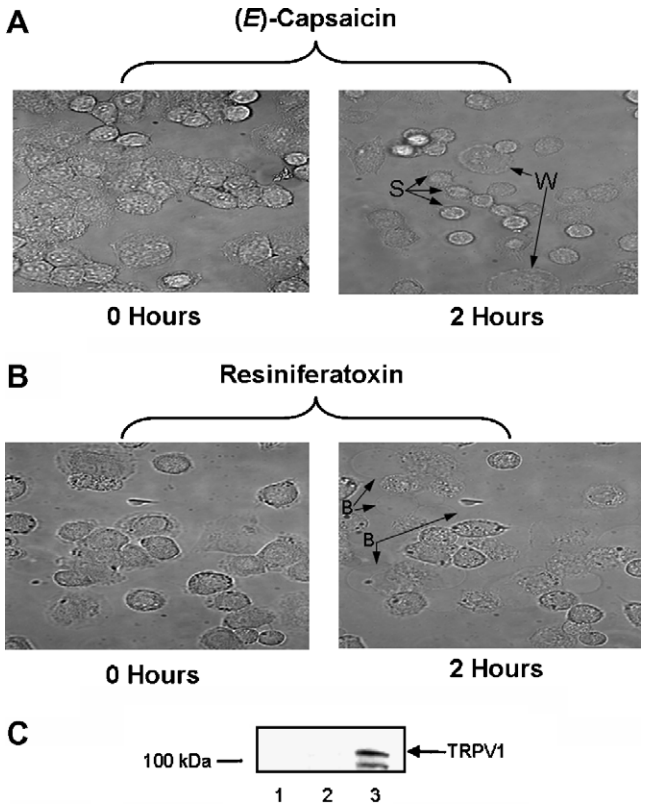

Fig. 1. Photomicroscopy of H460 cells at time = 0 and time = 2 h in the presence of (A) 50 μM (*E*)-capsaicin or (B) 50 μM resiniferatoxin. Rounding of some individual cells is indicated by an S on the images, swelling of other individual cells is indicated by a W on the images, "ballooning out" of the cytoplasm in some cells exposed to resiniferatoxin is indicated by a B on the images. (C) Shows an immunoblot of isolated rat heart mitochondria (lane 1), H460 cells (lane 2), and rat brain homogenate (lane 3). The arrow TRPV1 indicates immunoreactivity, corresponding to the molecular weight indicated by the supplier.