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Expression of the transient receptor potential vanilloid 1 (TRPV1) in LNCaP and PC-3 prostate cancer cells and in human prostate tissue

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Abstract

Vanilloid receptor subtype-1 (TRPV1), the founding member of the vanilloid receptor-like transient receptor potential channel family, is a non-selective cation channel that responds to noxious stimuli such as low pH, painful heat and irritants.

In the present study, we show, as means of reverse transcriptase-polymerase chain reaction and Western blot analysis, that the vanilloid TRPV1 receptor is expressed in the prostate epithelial cell lines PC-3 and LNCaP as well as in human prostate tissue. The kinetic parameters inferred from [^{125}I]-resiniferatoxin binding were in concordance with data of TRPV1 receptors expressed in other tissues. The contribution of the endogenously expressed TRPV1 channel to intracellular calcium concentration increase in the prostate cells was studied by measuring changes in Fura-2 fluorescence by fluorescence microscopy. Addition of capsaicin, (*R*)-methanandamide and resiniferatoxin to prostate cells induced a dose-dependent increase in the intracellular calcium concentration that was reversed by the vanilloid TRPV1 receptor antagonist capsazepine. These results indicate that the vanilloid TRPV1 receptor is expressed and functionally active in human prostate cells.

Keywords: Vanilloids; Capsaicin; TRPV1; Prostate

1. Introduction

The transient receptor potential vanilloid (TRPV) receptors belong to the transient receptor potential superfamily of cation-selective ion channels with diverse functions ranging from tactile, thermal and taste sensing to osmolality regulation (Huang, 2004; Moran et al., 2004). Vanilloid channels have an oligomeric structure formed by subunits having six transmembrane segments with a pore domain formed by the fifth and sixth transmembrane regions and intracellular N- and C-termini (Ferrer-Montiel et al., 2004). Among the six TRPV members (TRPV1-6), the vanilloid receptor subtype-1, or TRPV1, is gaining increasing interest as a molecular integrator of physical and chemical noxious stimuli. The vanilloid TRPV1 receptor was originally described as a receptor for capsaicin, the pungent ingredient of hot chili peppers, and its ultrapotent analog from *Euphorbia resinifera*, resiniferatoxin, in primary sensory neurons where its activation elicits a sensation of burning pain (Caterina et al., 1997). It has also been described that the vanilloid TRPV1 receptor may be activated by protons, high temperatures and endogenous pro-inflammatory substances (Cortright and Szallasi, 2004) as well as by anandamide, *N*-arachidonoyldopamine and some lipoxygenase products (Huang et al., 2002; Van der Stelt and Di Marzo, 2004), which have been proposed to be endovanilloids (Van der Stelt and Di Marzo, 2004). In sensory neurons, activation of the vanilloid TRPV1 receptor by capsaicin, heat or low pH is thought to lead to an influx of calcium and sodium ions through the integral channel,

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causing a depolarisation of the cell and eventually leading to the sensation of pain (Moran et al., 2004).

The expression of the vanilloid TRPV1 receptor is not restricted to sensory neurons. TRPV1 has also been found in the kidney (Sanchez et al., 2001), gastrointestinal tract (Ward et al., 2003), urinary tract (Avelino et al., 2002; Birder et al., 2001; Ost et al., 2002), and epidermal keratinocytes (Denda et al., 2001; Stander et al., 2004). In the urinary bladder and skin, TRPV1 has not only been found in sensory afferent fibers that innervate the tissue but also in the epithelial cells (Birder et al., 2001; Denda et al., 2001; Stander et al., 2004) The discovery of non-neuronal tissues expressing the vanilloid TRPV1 receptor suggests that the role of this channel is not limited to the perception of noxious stimuli alone (Caterina, 2003).

The present study was undertaken to analyse the expression and functionality of the vanilloid TRPV1 receptor in human prostate cells and tissue. We show here that TRPV1 is expressed in LNCaP and PC-3 prostate epithelial cells as well as in human prostate tissue. Capsaicin, the endogenous cannabinoid analogue (*R*)-methanandamide and resiniferatoxin induced an increase in intracellular calcium concentration that was blunted by pre-treatment with the prototypical functional vanilloid TRPV1 receptor antagonist capsazepine. The expression of other TRPV family members has been previously demonstrated in the prostate cancer cell line LNCaP (Bödding et al., 2003; Vanden Abeele et al., 2003). Hence, this cell line is useful for studying the function and co-expression of the transient receptor potential family.

2. Materials and methods

2.1. Cell cultures

Human prostatic carcinoma (PC-3) and lymph node carcinoma of the prostate (LNCaP) cells were purchased from American Type Culture Collection (ATCC CRL-1435 and CRL-1740, respectively) (Rockville, MD, USA). Cells were routinely grown in RPMI 1640 medium supplemented with 100 IU/ml penicillin G sodium, 100 μg/ml sptreptomycin sulfate and 0.25 μg/ml amphotericin B (Invitrogen, Paisley, UK) and either 10% foetal calf serum for PC-3 cells or 5% foetal calf serum for LNCaP cells. One day prior to the experiments, 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.

2.2. Human prostate tissue

Tissue specimens were obtained from 55- to 75-year-old Benign Prostate Hyperplasia (BPH) patients undergoing open prostatectomy. The experimental protocol conformed to the Declaration of Helsinki and was approved by the corresponding ethics committee.

2.3. Isolation of total RNA, reverse transcriptase polymerase chain reaction (RT-PCR) and DNA electrophoresis

Total RNA was isolated from human prostate cells using the Ultraspec™ RNA isolation system (Biotecx Labs, Houston, TX, USA) according to the manufacturer's instructions. After extraction with chloroform, RNA was precipitated from the aqueous phase by addition of isopropyl alcohol, washed with ethanol and dissolved in diethyl pyrocarbonate-treated water. For RT analysis, 2 μg of RNA were reverse-transcribed using the M-MLV Reverse transcriptase kit (Life Technologies, Carlsbad, CA, USA) containing reverse transcriptase supplemented with 10 nM dithiotreitol, 40 U ribonuclease inhibitor (Promega, Madison, WI, USA) and 0.5 mM of deoxyribonucleotides. Four microliters of the RT reaction were then PCR-amplified with specific primers for VR1: sense 5′-GCT CAG CCC GAG GAA GTT T-3′, and antisense 5′-ACT CTT GAA GAC CTC AGC GTC C-3′ (Hayes et al., 2000). The PCR conditions included an initial 5-min denaturation step at 94 °C, followed by 32 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. The PCR products were separated by electrophoresis and visualized in 2% agarose gels. The corresponding bands were cut off from the gel, eluted, and automatically sequenced with an ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA). The quality and amount of cDNA prepared from each sample were verified by amplification of β-actin using the following primers: sense: 5′-AGA AGG ATT CCT ATG TGG GCG-3′; antisense: 5′-CAT GTC GTC CCA GTT GGT GAC-3′.

2.4. Western blot analysis

Cultured PC-3 and LNCaP cells were pooled together and lysed in a lysis buffer (50 mM Tris–HCl, pH 7.4, 5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM EGTA, 10 mM 2-mercaptoethanol) containing protease inhibitors (5 μg/ml leupeptin, 5 μg/ml aprotinin, 10 μg/ml soybean trypsin inhibitor and 1 mM phenylmethylsulfonyl fluoride) and disrupted by two freeze–thaw cycles and sonication. Western blot analysis were performed as previously described (Diaz-Laviada et al., 1991) with minor modifications. Twenty micrograms of cell lysates was resolved on a 10% acrylamide gel and the proteins were then transferred to a nitrocellulose membrane. The membrane was blocked with 3% fat-free milk and incubated with anti-TRPV1 (Sta. Cruz Biotechnology, Sta. Cruz, CA, USA) (1:1000 dilution) overnight at 4 °C. After washing, the membranes were incubated for 1 h with a secondary horseradish peroxidase-conjugated antibody and developed with Enhanced Chemiluminiscence (ECL) substrate (Amersham, UK).

2.5. [^{125}I]RTX binding assays

Human prostate tissues were homogenized with a Polytron PT-20 tissue homogenizer (Kinematica, AG, Lucerne, Switzerland) at maximal speed in 25 mM triethanolamine–HCl (TEA–HCl) buffer, pH 7.5, supplemented with 0.25 mM sucrose, 0.5 mM EDTA, and 0.1 mM PMSF at 4 °C. After centrifugation at 3500 × *g* for 5 min at 4 °C, the supernatants were once again centrifuged at 100,000 × *g* for 30 min at 4 °C. The resulting pellets were washed with 25 mM TEA–HCl buffer pH 7.5, and centrifuged at 100,000 × *g* for another 30 min. The pellets were homogenized by using a glass homogenizer in 25 mM TEA–HCl, pH 7.5, containing 0.1 mM PMSF, 5 μg/ml soybean trypsin inhibitor, 1 μg/ml leupeptin and 1 μg/ml aprotinin, and stored at −80 °C until the day of assay. Protein content was estimated using a protein assay kit (Bio-Rad, Hercules, CA). The same protocol was followed for prostate cells except that in the homogenization step the cells were disrupted by sonication instead.

The receptor binding assay was performed according to a modification of the method described by Szallasi et al. (1993). Briefly, membrane suspensions (50 μg of protein) were incubated at 37 °C for 60 min with 50 pM [^{125}I]-resiniferatoxin (2200 Ci/mmol, Perkin-Elmer, Wesley, MA, USA), in the presence or absence of increasing concentrations of the competitive non-radioactive resiniferatoxin (Sigma, St. Louis, MO, USA), (*R*)-methanandamide or capsaicin (Tocris, Bristol, UK), in a final volume of 0.25 ml of assay buffer (10 mM HEPES, pH 7.4, 5 mM KCl, 5.8 mM NaCl, 0.75 mM $CaCl_2$, 2 mM $MgCl_2$, 320 mM sucrose, 0.25 mg/ml BSA acid free). The reaction was stopped by chilling the assay mixture in an ice-cold water bath and adding 2 mg/ml of bovine α_1-acid glycoprotein in 25 μl of buffer. Bound and free [^{125}I]-resiniferatoxin were separated by pelleting the membranes in a Beckman TJ-6 centrifuge. The supernatant was removed by aspiration and the pellet was washed twice with ice cold assay buffer and carefully dried. The radioactivity of the pellet was measured in a gamma counter (1282 CompuGamma CS, Wallac, Turku, Finland). Nonspecific binding was defined as the binding in the presence of 1 μM non-radioactive resiniferatoxin.

2.6. Measurement of intracellular calcium concentration [Ca^{2+}]$_i$

PC-3 and LNCaP cells were plated on 35-cm^2 plates (20,000 cells per plate) containing round glass coverslips. Before assay, the cells were washed with 2 ml RPMI supplemented with 5% BSA. Measurements of intracellular [Ca^{2+}] with the fluorescent calcium indicator Fura-2 was achieved following a modified protocol of Gryenkiewicz et al. (1985). Briefly, the cells were incubated with minimum essential medium (MEM) containing 1 mM Fura2-AM at 37 °C for 1 h. Afterwards, the medium containing Fura-2 was removed and the cells were incubated in fresh MEM medium for 20 min at 37 °C. Fluorescence emission after the addition of different TRPV1 receptor agonists or antagonists was measured at 510 nm with excitation at 340 nm and 380 nm and was recorded using a fluorescent microscope Wallac/Perkin-Elmer (Gaithersburg, MD) Concord system incorporating a SpectraMaster multiwavelength controller and temperature-controlled stage (Melville, NY). The fluorescence ratio (340/380) was converted to $[Ca^{2+}]_i$ according to the Gryenkiewicz equation (Gryenkiewicz et al., 1985); R_{max} and R_{min} were determined using 20 μM ionomycin and 20 mM EGTA, respectively.

2.7. Statistical analysis

All data are presented as the mean ± S.D. of the number of experiments indicated. The statistical comparisons among groups were carried out with a *t*-test and the difference was considered to be statistically significant when the *P* value was < 0.05.

3. Results

3.1. Expression of the vanilloid TRPV1 receptor in prostate cells and prostate tissue

The human prostate epithelial cell lines LNCaP and PC-3 were used to study the expression of the vanilloid TRPV1 receptor channel in prostate cells. LNCaP is a lymph node

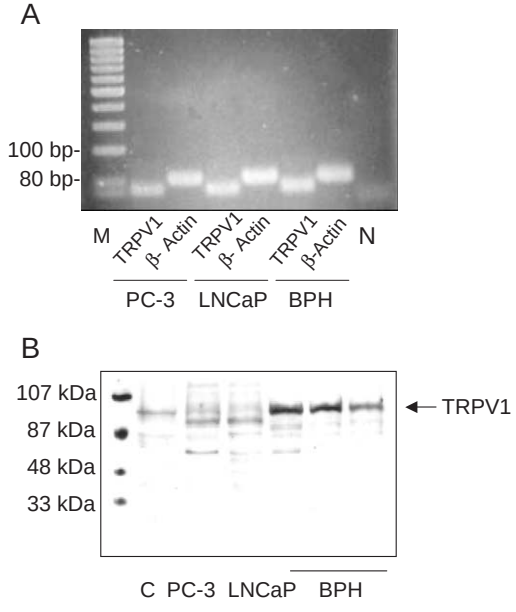

Fig. 1. Expression of the vanilloid TRPV1 receptor in the human prostate. (A) Identification, by RT-PCR, of TRPV1 transcripts in RNA extracted from PC-3 cells, LNCaP cells and prostate tissue from Benign Prostate Hyperplasia (BPH) patient. β-actin was used as the housekeeping gene. (B) Identification of the TRPV1 protein by Western blotting. Rat cerebellum, C, was used as a positive control. The electrophoretic mobility of the markers is shown on the left. The figure shows a representative gel and a representative blot of three other independent experiments.

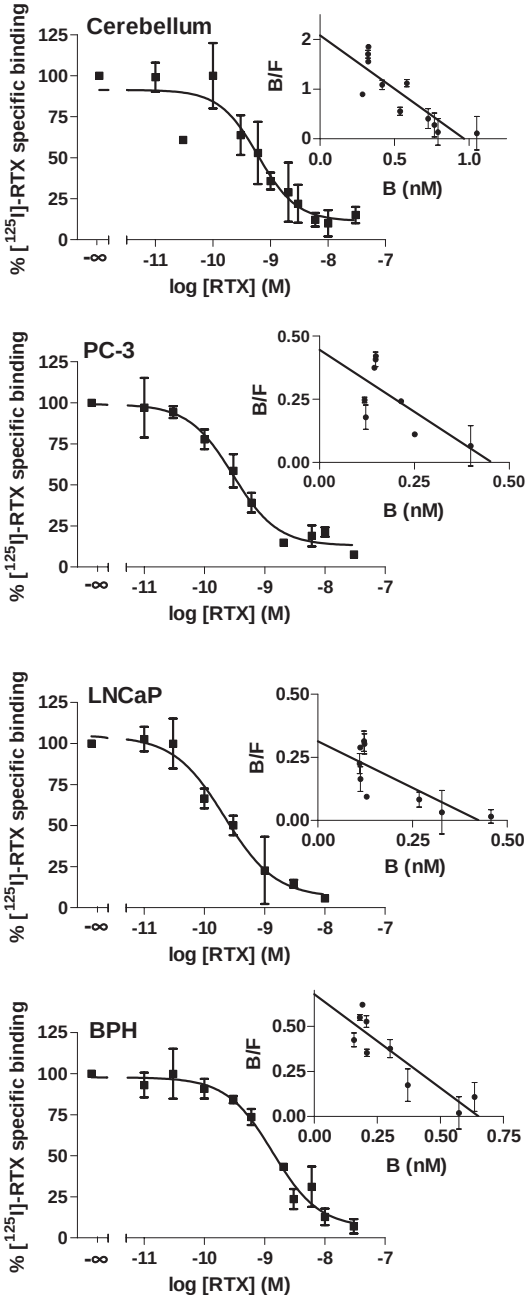

Fig. 2. Inhibition of specific [^{125}I]-resiniferatoxin (500 pM) binding to membrane preparations by non-radioactive resiniferatoxin. Binding of [^{125}I]-resiniferatoxin to membrane preparations from rat cerebellum, PC-3 cells, LNCaP cells, and prostate tissue from Benign Prostate Hyperplasia (BPH) patient was determined according to Materials and methods. Non-specific binding was determined in the presence of 1 μM non-radioactive resiniferatoxin (RTX). The insert shows the Scatchard analysis of the binding data. Each point represents the mean value ± S.D. of triplicate assays of a single experiment and are presented as a percentage of the specific binding obtained in the absence of non-radioactive RTX. Two additional experiments gave similar results.

prostate cancer cell line which responds to androgen stimulation for growth and hence represents an early and treatable cancer. The PC-3 cell line is a human prostatic adenocarcinoma metastatic to bone and is androgen-insensitive. It represents the androgen-refractory phase of advanced prostate cancer. As shown in Fig. 1, both cell lines as well as human prostate tissue obtained from radical prostactectomy of 55–75-year-old patients, expressed detectable levels of TRPV1 transcripts of ~72 bp (Fig. 1A), which was the expected size of the fragment comprised between the primers of the human TRPV1 DNA (GeneBank accession number GI22122001). Nucleotide sequence analysis of the TRPV1 PCR products from prostate cell lines and human prostate tissue revealed that the resulting sequence was identical to the expected fragment of human TRPV1 (data not shown).

Western blot analysis indicated that the TRPV1 protein was expressed in PC-3 cells, LNCaP cells and in human prostate from three different patients (Fig. 1B). As previously shown, the TRPV1 cDNA contains an open reading frame of 2514 nucleotides that encodes a protein of 838 amino acids with a predicted relative molecular mass of 95,000 (M_r 95 kDa) (Caterina et al., 1997).

The antibodies recognized TRPV1 proteins with molecular masses of 94.58 kDa and 103 kDa which are in concordance with the calculated relative molecular mass of human TRPV1. The two observed bands could well correspond to different glycosylated forms, as it has been previously documented that the TRPV1 monomer may be present in two forms: a non-glycosylated form of 95 kDa and a glycosylated form of 114 kDa (Kedei et al., 2001). The 103 kDa band was also expressed in rat cerebellum, which was used as a positive control (Mezey et al., 2000; Sanchez et al., 2001). Both bands were displaced by incubation with the antibody and the immunogenic peptide.

3.2. Characterization of [^{125}I]-resiniferatoxin receptor binding

The binding parameters of the vanilloid TRPV1 receptor expressed in prostate cells and human prostate tissue were

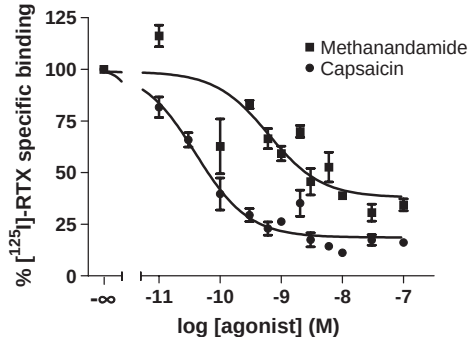

Fig. 3. Displacement of specific [^{125}I]-resiniferatoxin binding to prostate membrane preparations by capsaicin and by (*R*)-methanandamide. Specific binding of [^{125}I]-resiniferatoxin to membrane preparations from Benign Prostate Hyperplasia (BPH) was determined in the presence of increasing concentrations of capsaicin (circles) or (*R*)-methanandamide (squares). Each point represents the mean value ± S.D. of triplicate assays of a single experiment and are presented as percentage of specific binding obtained in the absence of non-radioactive RTX. One additional experiment gave similar results.

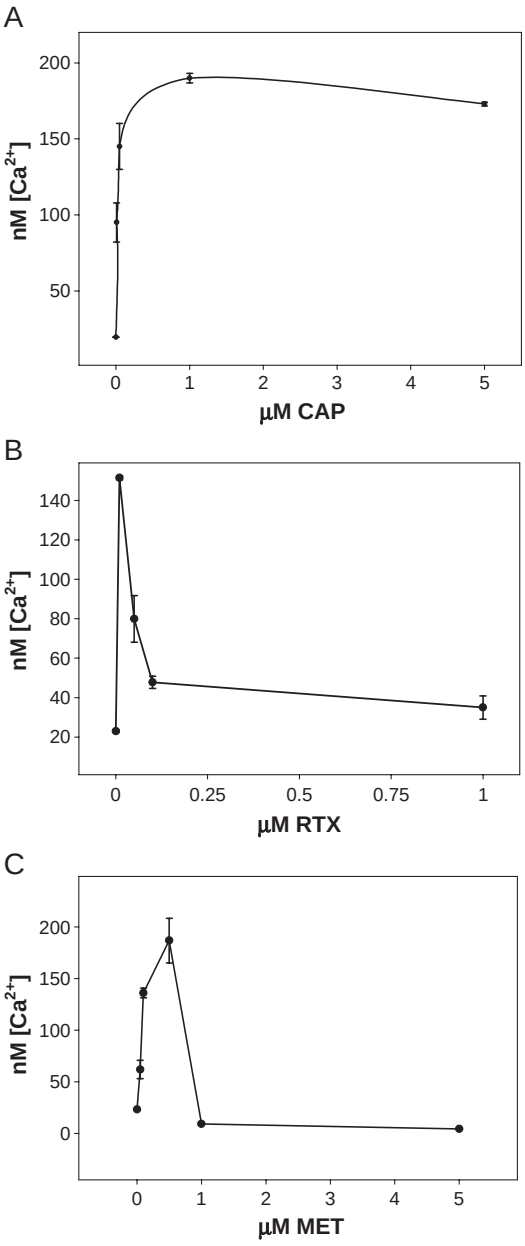

Fig. 4. Induction of a concentration-dependent increase in $[Ca^{2+}]_i$ by vanilloid agonists in LNCaP cells. The intracellular calcium concentration ($[Ca^{2+}]_i$) was monitored using Fura-2 in LNCaP cells before and after addition of vanilloid agonists. After the experiment, maximum and minimum fluorescence was monitored for calibration according to "experimental procedures" and the $[Ca^{2+}]_i$ was calculated with the Grynkiewicz equation. Data are given as the mean±SD of four experiments; (A) increasing doses of capsaicin (CAP); (B) increasing doses of resiniferatoxin (RTX); (C) increasing doses of (*R*)-methanandamide (MET).

determined by competitive inhibition of specific [^{125}I]-resiniferatoxin binding by non-radiactive resiniferatoxin. Both prostate cells and prostate tissue showed high levels of specific vanilloid binding sites, as shown in Fig. 2. Non-radioactive resiniferatoxin inhibited the specific binding of 500 pM [^{125}I]-resiniferatoxin with IC_{50} values of 606±83 pM for cerebellum, 296±43 pM for PC-3 cells, 216±37 pM for LNCaP cells and 1036±155 pM for prostate tissue (Fig. 2). The calculated Scatchard transformation of the binding data indicated that the binding parameters were comparable to those observed in rat cerebellum used as a positive control (Fig. 2, insert) and were in the same range as the kinetic parameters observed in different monkey brain areas (Szabo et al., 2002). The maximal binding capacities inferred from Scatchard analysis of three independent experiments were: 483±95 fmol/mg protein for cerebellum, 297±99 fmol/mg protein for PC-3 cells, 301.7±89 fmol /mg protein for LNCaP cells and 306±26 fmol/mg protein for prostate BPH tissue. The K_d values obtained were: 463±73, 1078±81, 1313±125, and 1643±182 pM, respectively.

As shown in Fig. 3, specific [^{125}I]-resiniferatoxin binding was displaced by the vanilloid TRPV1 receptor agonist capsaicin and by the anandamide analogue (*R*)-methanandamide, which displaced the specific binding at a higher IC_{50} than capsaicin.

3.3. Vanilloids induce an increase in $[Ca^{2+}]_i$ in LNCaP and PC-3 cells

To investigate the effects of vanilloid stimulation on intracellular $[Ca^{2+}]$ we used the Ca^{2+} fluorescent indicator

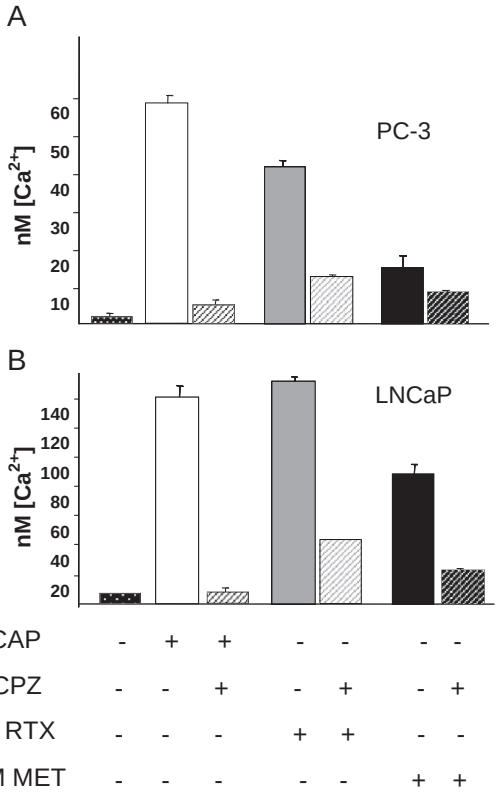

Fig. 5. The vanilloid antagonist capsazepine inhibits the vanilloid-induced $[Ca^{2+}]_i$ increase in prostate cells. $[Ca^{2+}]_i$ was monitored in PC-3 and LNCaP cells as described in Fig. 3. Experiments were performed after addition of capsaicin (CAP), resiniferatoxin (RTX) or (*R*)-methanandamide (MET) in the presence or absence of capsazepine (CPZ), which was added 1 min prior to the agonists. The figure shows the mean±S.D. of three different experiments. (A) PC-3 cells; (B) LNCaP cells.

Fura-2, which measures cytoplasmic Ca^{2+} levels. As shown in Fig. 4A, in LNCaP cells, the vanilloid TRPV1 receptor agonist capsaicin induced a dose-dependent increase in $[Ca^{2+}]_i$ with an apparent full effect occurring at about 1 μM. Doses as low as 5 nM capsaicin were able to increase the $[Ca^{2+}]_i$ from 22 to 90 nM whereas 1 μM capsaicin increased it to 190 nM (Fig. 4A). The potent vanilloid TRPV1 receptor agonist resiniferatoxin also increased $[Ca^{2+}]_i$ in these cells, evoking the maximal response at 10 nM (Fig. 4B). We then tested the ability of (*R*)-methanandamide, a stable anandamide analogue which has been shown to activate recombinant vanilloid receptors (Ralevic et al., 2001; Ross et al., 2001), to increase $[Ca^{2+}]_i$. MET exerted a similar although less potent effect, the maximal increase being observed at 0.5 μM (Fig. 4C).

The competitive vanilloid TRPV1 receptor antagonist capsazepine (1 μM) inhibited the capsaicin (5 μM)-, resiniferatoxin (10 nM)- and (*R*)-methanandamide (0.1 μM)-induced Ca^{2+} responses in both LNCaP and PC-3 cells (Fig. 5). This finding indicates the involvement of the vanilloid TRPV1 receptor in the intracellular calcium increase evoked by all three agonists in these cells.

4. Discussion

In this study we show, by means of three different techniques (i.e. RT-PCR, Western blot and binding studies), that the vanilloid TRPV1 receptor is expressed in prostate LNCaP and PC-3 cells as well as in human prostate hyperplasic tissue. RT-PCR using specific primers detected a band with the estimated size of the transcript, that was sequenced and proved to correspond to the expected TRPV1 fragment. The apparent molecular weight of the protein detected by Western blot was in concordance with that described in other cell types (Caterina et al., 1997; Someya et al., 2004). Iodo-resiniferatoxin (I-resiniferatoxin) has been reported to be a potent and selective vanilloid TRPV1 receptor antagonist which binds with high affinity to TRPV1. I-resiniferatoxin can be radiolabeled to a high specific activity and thus is a powerful probe to detect and pharmacologically characterize the vanilloid TRPV1 receptor (Wahl et al., 2001). Using a [^{125}I]-resiniferatoxin binding assay, we have detected specific vanilloid binding sites in the prostate PC-3 and LNCaP cells as well as in human prostate tissue. The K_d values obtained from Scatchard analysis of inhibition experiments are in good agreement with previous reports in other species and tissues using [^{3}H]-resiniferatoxin (Szabo et al., 2002). Prostate tissue revealed a higher density of binding sites than cultured cells, which could well correspond to the different cell types forming prostate tissue. In prostate tissue, vanilloid receptors might be expressed not only in epithelial cells but in the innervating sensory neurones and in the recently found prostate interstitial cells as well (Van der Aa et al., 2003). Expression of the TRPV1 mRNA in rat prostate and in one human sample has also been very recently observed in a serial study performed in rat and male genitourinary tract by Stein et al. (2004) which is in good agreement with our results of protein expression in prostate tissue and cells.

Previous reports have shown that vanilloids and some endocannabinoids exhibit anti-cancer properties acting through the vanilloid TRPV1 receptor, in other human cancer cells (Maccarrone et al., 2000; Contassot et al., 2004). Other authors as well as our research group have demonstrated that several cannabinoids like THC and anandamide also exert anti-proliferative effects in prostate tumoral cells (Ruiz et al., 1999; Melck et al., 2000; Mimault et al., 2003). The fact that prostate cancer cells express vanilloid TRPV1 receptors could explain some of the cannabinoid receptor-independent effects in these cells and might have relevance in cannabinoid-induced prostate cell death.

One of the major findings in our study is the detection of functionally active vanilloid TRPV1 receptor sites in human prostate tissue and human prostate epithelial cells. TRPV1 receptors expressed in prostate cells were functionally active as both vanilloid TRPV1 receptor agonists, capsaicin and resiniferatoxin, induced a dose-dependent increase in the intracellular calcium concentration that was abolished by the vanilloid TRPV1 receptor antagonist capsazepine. On the other hand, the anandamide analogue, (*R*)-methanandamide, which has been described to be a partial agonist at vanilloid TRPV1 receptors (Ralevic et al., 2001; Ross et al., 2001), was also able to activate the vanilloid TRPV1 receptors expressed in prostate cells, inducing an increase in $[Ca^{2+}]_i$ that was abolished by the TRPV1 receptor antagonist capsazepine. Both resiniferatoxin and (*R*)-methanandamide at high doses were unable to induce an increase in $[Ca^{2+}]_i$. This result might be due to a receptor desensitisation since resiniferatoxin, at doses over 1 nM, has been reported to cause desensitisation of sensory neurons and inhibition of calcium uptake by isolated neurons (Acs et al., 1997). On the other hand, a vanilloid receptor loss after treatment with resiniferatoxin has been described and associated with a long-term desensitisation phenomena (Szallasi and Blumberg, 1992). In this sense, we have observed that a 1-h treatment of LNCaP cells with 100 nM resiniferatoxin, or 1 μM (*R*)-methanandamide, induced a decrease in TRPV1 receptor levels, as measured by Western blot (data not shown). These observations could explain the atypical dose–response curves produced by resiniferatoxin and (*R*)-methanandamide in LNCaP cells.

In conclusion, the present study is the first to report the expression of functionally active vanilloid TRPV1 receptors in prostate epithelial cells . Further studies will be necessary to elucidate the physiological role of these receptors in prostate functionality. The expression of TRPM8 (Zhang and Barritt, 2004) and TRPV6 (Bödding et al., 2003) has been previously demonstrated in prostate LNCaP cells. The fact that both “cold”- and “hot”-activated transient receptor

potential channels are co-expressed in the same cell line provides a good experimental model for studying the functions and/or expression of transient receptor potential family members.

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