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Advances in Brief

5(*S*)-Hydroxy-6,8,11,14-*E*,*Z*,*Z*,*Z*-eicosatetraenoate Stimulates PC3 Cell Signaling and Growth by a Receptor-dependent Mechanism[1]

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Abstract

5(*S*)-Hydroxy-6,8,11,14-*E*,*Z*,*Z*,*Z*-eicosatetraenoate (5-HETE) causes PC3 cells to grow by an unknown mechanism. We find that it also induces the cells to activate extracellular signal-regulated kinases and Akt. Pertussis toxin inhibits both responses. 5-HETE, 5-oxo-6,8,11,14-*E*,*Z*,*Z*,*Z*-eicosatetraenoate, and 5-oxo-15-hydroxy-eicosatetraenoate are known to stimulate leukocytes by a receptor coupled to pertussis toxin-sensitive G proteins. Their respective relative potencies in leukocytes are 1, 10, and 3. In PC3 cells, however, these values are 10, 1, and 0. PC3 cells, we propose, express a non-leukocyte-type, G protein-coupled, 5-HETE receptor. This novel receptor and the extracellular signal-regulated kinase and Akt pathways it recruits may contribute to the progression of prostate adenocarcinoma.

Introduction

Cyclooxygenase-2 may aid the progression of colorectal cancer by forming prostaglandins that bind to parent cell GPCRs[3] to induce proliferation (1). A similar scenario may apply to adenocarcinoma of the prostate. This cancer and its PC3 cell line make a 5-Lox metabolite, 5-HETE. Moreover, 5-Lox inhibitors arrest PC3 cell growth; 5-HETE reverses this effect. As proposed by several groups (2–6), 5-HETE may be a survival factor for prostate cancer, although its mechanism of action is unknown. 5-HETE is known to activate leukocytes by a putative GPCR coupled to PT-sensitive G proteins (7–9). Indeed, Hosoi *et al.* (10) have identified a gene encoding a GPCR that mediates responses to 5-HETE, couples to PT-sensitive G proteins, and, based on these and other data, may be the leukocyte 5-HETE receptor. 5-HETE might stimulate PC3 cells to grow through this leukocyte-type GPCR (5, 9, 10). Here we initiate studies testing this notion.

Materials and Methods

Cells, Buffers, and Eicosanoids. Human prostate PC3 cells were from American Type Culture Collection. PMN preparations (>95% PMNs, <5 platelets/100 PMNs, no erythrocytes) were isolated from human blood (7). We synthesized all eicosanoids (8), but some studies used 5-HETE from Cayman Chemical Co.

Proliferation. PC3 cells were seeded on 96-well plates at 10^3 cells/well in 200 μl of RPMI 1640/10% FCS, exposed to AA861 or MK886 (Biomol) for 20 min, incubated (± an eicosanoid) for 1–3 days, and assayed with the CellTiter96 AQueous One Solution Cell Proliferation Assay reagent (Promega).

ERK and Akt. PC3 cells (5×10^5) were seeded on 60-mm plates in 3 ml of RPMI 1640/10% FCS. At 24 h, cells were washed in serum-free RPMI 1640, serum-starved for 18 h in 2 ml of RPMI 1640, treated with an eicosanoid for 1–60 min, and harvested in lysis buffer (100 mM NaCl, 2 mM EDTA, 0.1% SDS, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 50 mM NaF, and 1 mM sodium vanadate). Lysates were sonicated, centrifuged (4°C, 12,000 × *g*, 10 min), and assayed for protein (BCA Protein Assay; Pierce) using BSA standards. For ERKs, proteins (60 μg) were resolved on 12% SDS-PAGE and transferred to polyvinylidene difluoride membranes (New England Nuclear Life Science Products). Blots were incubated for 18 h with mouse antibody to ERKs phosphorylated on Ser and Thr in their TEY regulatory sequence (Santa Cruz Biotechnology) and for 1 h with antimouse IgG-horseradish peroxidase antibody (Santa Cruz Biotechnology). Alternatively, proteins (25 μg) were processed as described above, and blots were probed with ERK1/2 antibody (Upstate Biotechnology) for 2 h and antibody to rabbit IgG (Sigma Chemical Co.) for 1 h. PMNs (5×10^5) were incubated in 100 μl of buffer (37°C) for 20 min, challenged for 2–40 min, transferred to 250 μl of lysis buffer (7), and analyzed as described above. ERKs were visualized by chemiluminescence (Pierce SuperSignal). For Akt, PC3 cells were processed as described for ERKs. Proteins (40 μg) were resolved on 10% SDS-PAGE, and Western blots were probed with antibody to Akt phosphorylated on Ser-473 or to Akt, reacted with secondary antibody, and visualized (PhosphoPlus Akt Antibody Kits; Cell Signaling Technologies). Band densities were quantified with ImageQuaNT Version 4.2 (Molecular Dynamics).

Results and Discussion

In a series of experiments, we observed that 5-HETE induced PC3 cells to grow; two 5-HETE synthesis inhibitors, MK886 and AA861, arrested growth; and 5-HETE reversed the arrested state (Fig. 1). These data agree with those from previous studies (Refs. 3–6, but see Ref. 11). Results with other eicosanoids, however, proved critical for interpreting the effect of 5-HETE. 5-OxoETE was weaker than 5-HETE, whereas 5-oxo-15-OH-ETE and 15(*S*)-hydroxy-6,8,11,13-*Z*,*Z*,*Z*,*E*-ETE did not stimulate growth or rescue cells from 5-Lox blockade. In contrast, the four ETEs bind to the PMN putative 5-HETE GPCR and stimulate PMN function with relative potencies of 1, 10, 3, and 0, respectively (9). The cloned GPCR likewise mediates responses to 5-oxoETE more effectively than to 5-HETE and thereby resembles the PMN GPCR (10). Thus, either PC3 cells do not use the latter receptors to recognize ETEs or, alternatively, the relative activity of the ETEs varies with the type of response evaluated. That is, experiments on PMNs and the cloned receptor looked at responses developing over minutes. The 3-day study of PC3 cell growth could distort structure-activity relations if ETEs are differentially inactivated during incubation. We addressed this issue in the following experiments.

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[3] The abbreviations used are: GPCR, G protein-coupled receptor; 5-Lox, 5-lipoxygenase; PMN, polymorphonuclear neutrophil; ETE, eicosatetraenoate, 5-HETE, 5(*S*)-hydroxy-6,8,11,14-*E*,*Z*,*Z*,*Z*-ETE; 5-oxoETE, 5-oxo-6,8,11,14-*E*,*Z*,*Z*,*Z*-ETE; 5-oxo-15-OH-ETE, 5-oxo-15(*S*)-hydroxy-6,8,11,13-*E*,*Z*,*Z*,*E*-ETE; LTB_4, leukotriene B_4; ERK, extracellular signal-regulated kinase; PT, pertussis toxin.

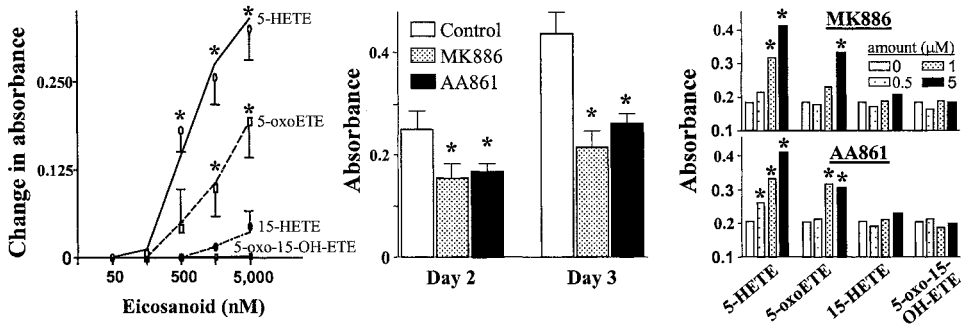

Fig. 1. Effect of ETEs and 5-Lox inhibitors on PC3 cell growth. *Left panel*, 10^3 cells in 200 μl of RPMI/10% FCS were stimulated with an ETE for 3 days and assayed with CellTiter solution. Data are the mean ± SE ($N = 4$) absorbances (450 nm) of cell cultures after correcting for that of unstimulated cell cultures. *Center panel*, cells treated with 20 μM MK886 or AA861 were grown for 2–3 days. Data are the means ± SE ($N \geq 5$) absorbances for cell cultures minus that of cell-free cultures. *Right panels*, cells were treated with 20 μM MK886 (*top panel*) or AA861 (*bottom panel*) and an ETE for 3 days. Data are mean absorbances of challenged cultures minus that of cultures treated with a 5-Lox inhibitor but no eicosanoid ($N = 4$; SEs were <20% of the means). *Asterisks* indicate values below ($P < 0.05$, Student's paired *t* test) those of control cultures.

5-HETE stimulated PC3 cells to phosphorylate ERK1/2 and Akt, as detected in Western blots probed with phospho-specific antibodies (Fig. 2). Because blots probed with antibody to total (i.e., phosphorylated plus unphosphorylated species) ERK1/2 or Akt had no such change (data not shown), and because phosphorylation at the antibody-defined sites raises the activity of these kinases, Fig. 2 data imply that 5-HETE induces ERK and Akt activation. ERK and Akt phosphorylation responses developed within 1 min, peaked at 5–10 min, and tended to persist for ≥60 min (ERK) or returned to baseline by 40 min (Akt). 5-HETE was more potent than 5-oxoETE, whereas 5-oxo-15-OH-ETE, 15(*S*)-hydroxy-6,8,11,13-*Z*,*Z*,*Z*,*E*-ETE, 8(*S*),12(*S*)-dihydroxy-5,9,11,13-*Z*,*E*,*Z*,*E*-ETE, and LTB_4 were inactive (Fig. 2). 5-HETE, 5-oxoETE, and 5-oxo-15-OH-ETE likewise stimulated PMNs to activate ERKs; responses began by 1 min, peaked at 5–10 min, and declined thereafter. However, PMNs showed 5-fold rises in ERK phosphorylation at 70, 800, and 250 nM of the ETEs; these values were 500, 50, and >5000 nM for PC3 cells (Table 1). ERK activation potencies thus rank as 5-oxoETE > 5-oxo-15-OH-ETE > 5-HETE in PMNs and as 5-HETE > 5-oxoETE ≫ 5-oxo-15-OH-ETE in PC3 cells. The ETEs have the former potency profile in eliciting other responses in PMNs, eosinophils, and monocytes (7–9) but have the latter profile in inducing PC3 cells to activate Akt (Fig. 2) and grow (Fig. 1). Thus, the difference in ETE potencies

Table 1 *Relative potencies of three eicosanoids in stimulating ERK1 phosphorylation in PC3 cells and PMNs*

Cells were challenged for 2, 5, 10, 20, and 40 min and analyzed for phospho-ERKs by Western blotting as described in Fig 2. Optimal response magnitude means (4–7 experiments) were plotted against ETE concentrations. ETE levels causing 5-fold rises in phosphorylation of the ERK1 were extrapolated.

	Cell type	
Eicosanoid	PC3 cells[a]	PMNs[a]
5-HETE	50	800
5-oxoETE	500	70
5-oxo-15-OH-ETE	5,000	250

[a] Extrapolated concentration (nM) of eicosanoid that stimulates a 5-fold rise in ERK1 phosphorylation. Extrapolations for ERK2 gave virtually identical results.

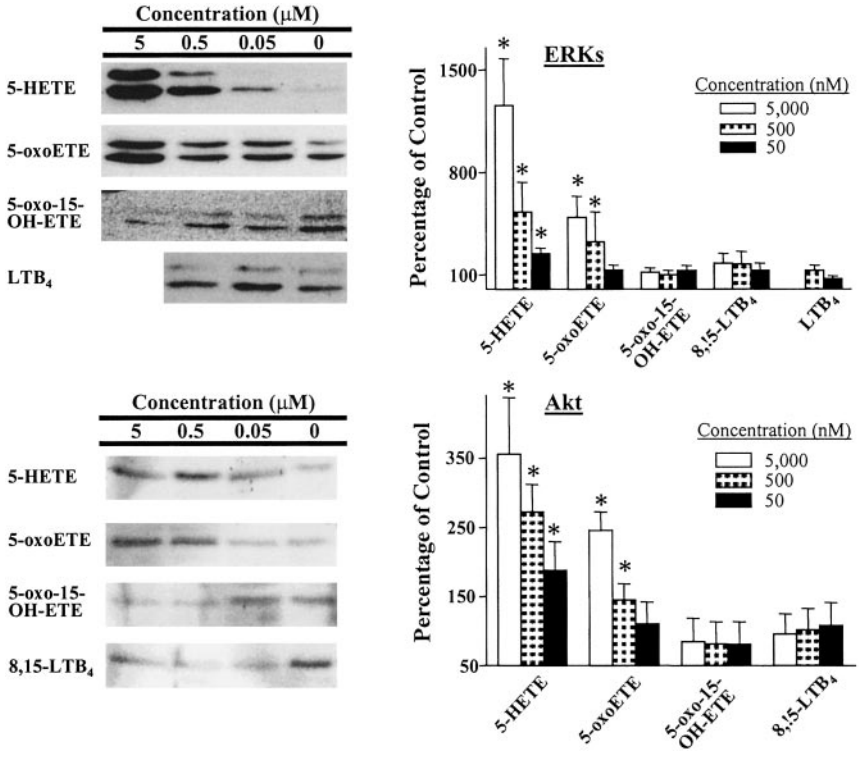

Fig. 2. PC3 cell ERK and Akt responses. Cell cultures were stimulated for 10 min and processed as described in "Materials and Methods." *Left panels*, Western blots of cells treated with eicosanoids and probed for phospho-ERKs (*top panels*) or Akt (*bottom panels*). *Right panels*, relative density (percentage of control cultures) of phosphorylated ERK1/2 (*top panel*) or Akt (*bottom panel*) in stimulated cells. Data are representative of five experiments (*left panels*) or are the means ± SE of four to eight experiments (*right panels*). *Asterisks* indicate values above ($P < 0.05$, Student's paired *t* test) those of unstimulated cell cultures.

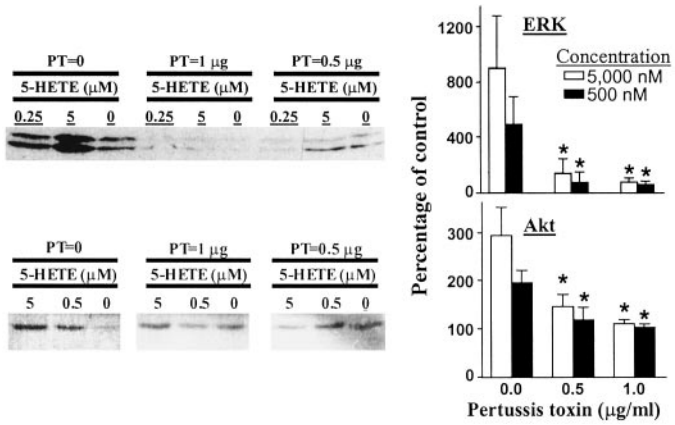

Fig. 3. Effect of PT on PC3 cells. Cells incubated for 18 h in serum-free media ± PT were exposed to 5-HETE for 10 min and processed as described in the Fig. 2 legend. *Top panels*, Western blots of ERKs (*left three panels*) or mean percentage rises in ERK1 + ERK2 band densities relative to control (*right panel*). *Bottom panels*, Western blots of Akt (*left three panels*) or mean percentage rises in Akt band density relative to control (*right panel*). Data are absorbances of cell cultures after correcting for the absorbance of cell-free cultures and representative (*left*) or means ± SE (*right*) of ≥4 experiments. *Asterisks* indicate values below ($P < 0.05$, Student's paired-*t* test) those of PT-untreated cell cultures.

between PC3 cells and leukocytes occurs with ERK and Akt as well as growth responses and apparently reflects nonidentical 5-HETE recognition systems.

PT blocked the ERK and Akt response of PC3 cells to 5-HETE (Fig. 3). Because this inhibition did not occur if 10 nM phorbol myristate acetate was the stimulus (data not shown), PT did not alter GPCR-independent responses. Given the specificity of the toxin, this result provides the first evidence that the PC3 cell recognition system for 5-HETE, like that in PMNs or the cloned receptor, involves a GPCR and PT-sensitive G proteins. We propose that 5-HETE activates target cells through leukocyte and PC3 cell types of GPCR, a situation resembling LTB_4, for which two GPCRs with different affinities for LTB_4 analogues exist (12). Because LTB_4 does not stimulate PC3 cells (Fig. 3) or rescue them from 5-Lox blockade (3), and 5-HETE does not bind to LTB_4 GPCRs (12), the latter receptors are not responsible for the effects seen here.

5-HETE may be a pro-growth autocoid (2–6) or, when made by other tissue [*e.g.*, bone (13)], an attracting hormone for prostate adenocarcinoma. ERKs and Akt may also contribute to this cancer (14, 15). 5-HETE analogues stimulate PC3 cells to activate the latter kinases by a PT-sensitive mechanism and, with the same potency profile, induce the cells to grow. A novel, non-leukocyte-type GPCR coupled to PT-sensitive G proteins, ERKs, and Akt may mediate this growth response. It may likewise be involved in the growth and spread of human prostate and other cancers that make 5-HETE and die in response to 5-Lox blockade (16).

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