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

Joseph T. O’Flaherty,2 LeAnn C. Rogers, Brad A. Chadwell, John S. Owen, Anurada Rao, Scott D. Cramer, and Larry W. Daniel

Departments of Medicine (J. T. O. and L. C. R.), Cancer Biology (A. R. and S. D. C.), and Biochemistry (J. S. O. and L. W. D.), Wake Forest University School of Medicine and Department of Biology (L. C. L.), Wake Forest University, Winston-Salem, North Carolina 27156

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 GPCRs3 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 extracellular signal-regulated kinase; PT, pertussis toxin.

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-OxoEET was weaker than 5-HETE, whereas 5-oxo-15-Oh-EET and 15(S)-hydroxy-6,8,11,13-Z,Z,Z,E-EET 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-oxoEET 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.
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 LTB4 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 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.

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>PC3 cells</th>
<th>PMNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HETE</td>
<td>50</td>
<td>800</td>
</tr>
<tr>
<td>5-oxoETE</td>
<td>500</td>
<td>70</td>
</tr>
<tr>
<td>5-oxo-15-OH-ETE</td>
<td>5,000</td>
<td>250</td>
</tr>
</tbody>
</table>

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

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...
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 LTB4 for which two GPCRs with different affinities for LTB4 analogues exist (12). Because LTB4 does not stimulate PC3 cells (Fig. 3) or rescue them from 5-Lox blockade (3), and 5-HETE does not bind to LTB4 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).

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

Joseph T. O’Flaherty, LeAnn C. Rogers, Brad A. Chadwell, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/23/6817

Cited articles
This article cites 16 articles, 7 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/23/6817.full.html#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/62/23/6817.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.