Studies of Prostaglandins in Rat Mammary Tumors Induced by 7,12-Dimethylbenz(a)anthracene

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INTRODUCTION

Release of prostaglandins has been observed in several types of cancers (6, 7, 16, 20). Mouse fibrosarcoma reportedly (20) synthesizes and secretes PGE2. Certain experimental tumors of ascite cells and sarcoma also reportedly produce abnormal levels of prostaglandins (16). Prostaglandins have hormone-like properties (9) and are known to play a role in lactogenesis (15). Mammary tumors are responsive to treatment with hormones (13), but the role of these compounds is not known. Reported here is a comparison of the endogenous concentration and synthesis of prostaglandins in the mammary glands of normal female rats and tumors induced in this gland by the intravenous injection of DMBA.

MATERIALS AND METHODS

Induction of Rat Mammary Tumors. Mammary tumors were induced in female Sprague-Dawley rats, 125 to 150 g, by a modification of the procedure described by Huggins et al. (3). A lipid emulsion (0.2 ml) of 1% (w/v) DMBA was injected into the caudal vein of animals at ages of 50, 53, and 56 days. Fifty mg of DMBA were dissolved in 1.5 ml of corn oil, and the mixture was warmed and then stirred with a Vortex mixer until a clear solution was obtained. The final emulsion was obtained by mixing 3.5 ml of fresh rat serum with this solution on a Vortex mixer.

Prostaglandin reference standards, PGE1 and PGE2 (95% pure) and arachidonic acid were obtained from the Lipids Preparation Laboratory of The Hormel Institute. Radioactive arachidonic acid (5,8,11,14-eicosatetraenoic acid-1,14-C) specific activity, 55 mCi/mnmole, was obtained from Dhom Products, Ltd., North Hollywood, Calif.

Radioimmunoassay of Tissue Prostaglandins. Radioimmunoassay was assayed by a modification of the procedure described by Lapidus et al. (10). The tissue (1 g) was homogenized in 10 ml of 0.2 M Tris-HCl buffer, of 1 mM glutathione, adjusted to a final pH of 8 with HCl. The homogenates were incubated with the radioactive substrates in an atmosphere of air for 1 hr at 37° with shaking. The reaction was stopped by adjusting the pH of the mixture to 3 with HCl. The tissue was removed by either filtration or centrifugation, and the tissue-free solution was extracted with 3 volumes of ethyl acetate. The combined ethyl acetate extracts were reduced in volume by evaporation to approximately 20 ml and extracted twice with 10 ml of 0.2 M phosphate buffer, dichloromethane, and ethanol as described in the biosynthesis section (9, 13). The radioimmuno-
RESULTS

Virtually all of the animals given injections of DMBA developed mammary tumors. The tumors ranged in size from 1 to 4 cm in diameter approximately 3 months after the last injection, when they were harvested. The major component of both tumor and normal mammary gland tissue prostaglandins was the E₂ type, as shown by a combination of silicic acid and argentation TLC. The presence of PGF's was also detected in some mammary tumors, but the irregularity of their occurrence did not warrant their quantitative estimation. The results of the radioimmunoassay (Table 1) showed that the concentration of PGE₂, for which the assay was specific, was higher in the tumor than in normal mammary gland tissue, without exception. Determination of PGE content, on the basis of absorption at 278 nm after alkali addition, also showed that the mammary tumors had higher levels of PGE compared with the mammary glands from the control animals. In estimating the endogenous content of prostaglandins in animal tissues, some synthesis could occur from incubation in buffer. The relative amounts of the prostaglandins also may be influenced by the conditions under which the tissues are handled (11). However, in this study, control and tumor tissues were treated identically to minimize the influence of these factors. Moreover, little endogenous synthesis appeared to take place with these tissues, because the quantity of prostaglandins detected in accessory experiments by homogenization of the sample in alcohol was essentially the same as that obtained in buffer.

Experiments on the conversion of labeled fatty acid substrates to prostaglandins (Table 2) showed that the tumor tissue contained the prostaglandin synthetase system. The labeled prostaglandins were not identified specifically in these experiments by argentation-TLC, but it was assumed that 5,8,11-eicosatrienoic acid-1-'⁴C and 5,8,11,14-eicosatetraenoic acid-1-'⁴C (arachidonic acid— gave PGE₁, PGF₁, and PGE₂, PGF₂, respectively. Comparison of the values in Table 2 showed that the tumor tissue was generally more active in the conversion of fatty acids to prostaglandins than normal mammary gland tissue.

DISCUSSION

The effects of DMBA on the DNA of the mammary glands have been implicated in the induction of mammary tumors in rats (3, 4, 5). The change in the cellular phosphoprotein content may be one reflection of this genetic aberration of other physiological states (18, 19). Another feature of mammary tumors is their hormonal response insofar as the role of other hormones in mammary carcinomas induced by DMBA. Prostaglandins have been regarded by some investigators as having hormone-like properties (9), and release of prostaglandins in considerable amount has been reported in a number of tumors (6, 7, 16, 20).

The higher levels of PGE₂ in the mammary gland tumors may be related to the higher rate of biosynthesis of prostaglandins, as indicated by the results herein. It also may be pertinent that the concentration of arachidonic acid, the precursor of PGE₂, is much higher in the lipids of mammary tumor tissue than in those of normal mammary gland [Tan et al. (The Hormel Institute), unpublished studies]. Whether the presence of prostaglandins is only an indication of tissue damage (17) or whether prostaglandins are functionally involved in tumor growth, is a subject that

### Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control mammary glands (ng PGE₂/g dried tissue)</th>
<th>Mammary tumors (ng PGE₂/g dried tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>215</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>210</td>
</tr>
<tr>
<td>4</td>
<td>79</td>
<td>170</td>
</tr>
<tr>
<td>5</td>
<td>93</td>
<td>250</td>
</tr>
<tr>
<td>6</td>
<td>63</td>
<td>290</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Type of mammary tissue</th>
<th>Substrate</th>
<th>Product</th>
<th>% conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9,11,14-Eicosatrienoic acid-1-'⁴C</td>
<td>PGE₂</td>
<td>0.67</td>
</tr>
<tr>
<td>Control</td>
<td>8,11,14-Eicosatrienoic acid-1-'⁴C</td>
<td>PGF₁α</td>
<td>0.66</td>
</tr>
<tr>
<td>Control</td>
<td>5,8,11,14-Eicosatetraenoic acid-1-'⁴C</td>
<td>PGE₂</td>
<td>0.61</td>
</tr>
<tr>
<td>Control</td>
<td>5,8,11,14-Eicosatetraenoic acid-1-'⁴C</td>
<td>PGF₁α</td>
<td>0.23</td>
</tr>
<tr>
<td>Tumor</td>
<td>8,11,14-Eicosatrienoic acid-1-'⁴C</td>
<td>PGE₂</td>
<td>1.34</td>
</tr>
<tr>
<td>Tumor</td>
<td>8,11,14-Eicosatrienoic acid-1-'⁴C</td>
<td>PGF₁α</td>
<td>0.89</td>
</tr>
<tr>
<td>Tumor</td>
<td>5,8,11,14-Eicosatetraenoic acid-1-'⁴C</td>
<td>PGE₂</td>
<td>1.92</td>
</tr>
<tr>
<td>Tumor</td>
<td>5,8,11,14-Eicosatetraenoic acid-1-'⁴C</td>
<td>PGF₁α</td>
<td>0.46</td>
</tr>
</tbody>
</table>
requires further study. Cyclic 3',5'-AMP and PGE\textsubscript{1} enhanced contact inhibition of growth and decreased cell motility in cell cultures (8). Since PGE\textsubscript{1} elevates cyclic AMP levels, there is the possibility that prostaglandins are produced as part of the physiological defense mechanism against tumor growth.

ACKNOWLEDGMENTS

Dr. John E. Pike of the Upjohn Co. kindly provided the crystalline prostaglandins. Dr. Howard W. Sprecher kindly supplied the 8,11,14-eicosatrienoic acid-1\textsuperscript{14}C.

REFERENCES


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