conditions the vasodilator action of taurine was enhanced by increasing the calcium concentrations.

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Identification of arachidonate metabolites in normal, benign and malignant human mammary tissues

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Most malignant tumours, including human mammary carcinomas, produce more prostaglandin-like material (PG-lm) than do the tissues in which they arise (see Bennett 1979, 1982). This finding contributes to the conclusion that PGs may play important roles in tumour growth and spread. Breast tumour prostaglandins have been tentatively characterized using paper chromatography (Bennett et al 1977). We now report formal identification of various arachidonate metabolites, and quantitation of PGD_2, PGE_2, $PGF_{2\alpha}$ and the PGI_2 hydrolysis product 6-keto-$PGF_{1\alpha}$, using gas chromatography-mass spectrometry.

Materials and methods

Tissue from 12 cancers, 3 benign lesions (fibroadenomas) and 4 specimens of macroscopically normal tissue were collected from patients undergoing breast surgery. Each sample were trimmed of fat, cut into small pieces and washed in Krebs solution. The methods were as described by Hensby et al (1982). In brief, weighed samples were homogenized in Krebs solution, extracted into chloroform, and purified using chromatography on LH20 and Amberlite XAD-2 columns and silica gel thin layer plates. The chloroform extraction method gives recoveries of about 70% with PGE, PGF and PGA compounds. Recoveries on subsequent purification were about 60–80%.

Chemical derivatization of the prostaglandin residues produced *O*-methyloximes which were converted into the corresponding methyl esters and then into trimethylsilyl ethers.

Samples were analysed by gas chromatography mass-spectrometry (g.c.–m.s.) using a Riber 10-10C mass spectrometer, and a Jirdel 31 gas chromatograph equipped with a 12·5 metre fused-silica capillary column (Hewlett Packard, SE30). Helium was used as the carrier gas at a flow rate of 2 ml min^{-1}, with a column temperature of 210–260 °C. The mass spectrometer was operated at 70 eV electron energy and an electron multiplier setting of 2200 V.

The samples were assessed qualitatively by full spectral scans for various eicosanoids. Quantitative g.c.-m.s. of PGD_2, PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ by selective ion monitoring was carried out on 18 extracted samples with added deuterated standards.

Results and discussion

The results of the qualitative g.c.-m.s. on 4 tumours and 2 normal tissues are shown in Table 1. Only compounds formed from arachidonate metabolism were detected. If metabolites of eicosatrienoic acid or eicosapentaenoic acid were present, their recovered amounts were less than the limit of detection which was approximately 20 ng g^{-1}. All extracts contained arachidonic acid, TxB_2, 6-keto-$PGF_{1\alpha}$, 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$ and 15-keto-13,14-dihydro-TxB_2. Using a less sensitive m.s. (Finnigan 9600) 12-HETE was detected in all breast tissues studied (6

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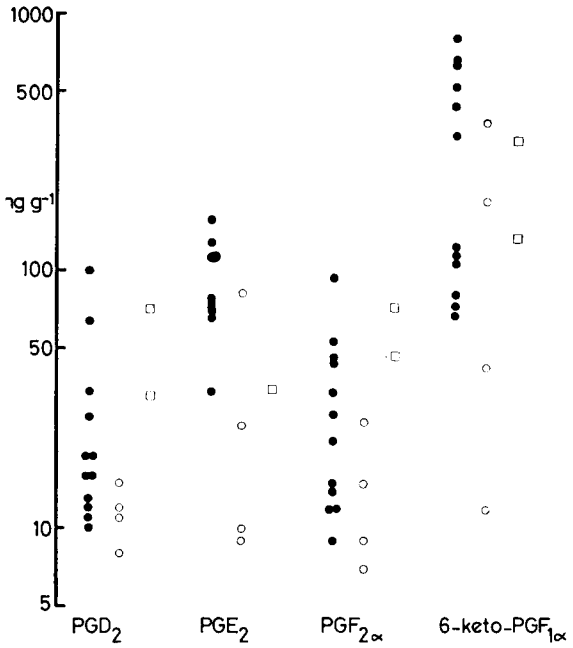

FIG. 1. Quantitative g.c.-m.s. of breast carcinomas (●), normal tissue (○) and fibroadenomas (□). Results are ng g^{-1} wet tissue, on a log scale.

carcinomas, 3 benign tumours and 2 specimens of normal tissue). 12-HETE was not looked for with the Riber m.s.

15-Hydroxy-dehydrogenase and Δ^{13}-reductase enzymes were present in extracts as indicated by the formation of the 13,14-dihydro derivatives of 6,15-diketo-$PGF_{1\alpha}$ and 15-keto-TxB_2.

The quantitative results on 18 samples are shown in Fig. 1. 6-keto-$PGF_{1\alpha}$ was present in greatest amounts in 16 extracts, and PGE_2 was most abundant in the other 2 (1 tumour, 1 normal tissue). In the 4 cases where normal tissue and carcinoma from the same specimen were studied, the tumour yielded more 6-keto-$PGF_{1\alpha}$ than did the normal tissue; in 3 of these cases tumour PGE_2 was also higher. Substantial amounts of prostaglandins can be formed by breast tissues, particularly carcinomas. Studies by various groups including Bennett et al (1977, 1979) and Rolland et al (1980) indicate that prostaglandins are important in breast cancer. Studies with B-16 amelanotic melanoma in mice implicate thromboxane formation as deleterious and prostacyclin formation as beneficial (Honn et al 1981). As more

Table 1. Qualitative g.c.-m.s. of extracts from malignant tumours (M) and normal breast tissue (N). The pairs in parentheses are from the same specimen. + and − denote the presence and absence of a prostaglandin (detection limit about 20 ng g^{-1}). In the above abbreviations, di-k represents di-keto, dihyd represents dihydro, and arach acid is arachidonic acid. 12-HETE was detected in all breast tissues studied with another mass spectrometer (see text), but was not looked for with the Riber m.s.

	M	M	(M	N)	(M	N)
PGE_2	+	+	+	+	−	−
PGD_2	+	+	+	−	−	−
PGF_2	−	−	−	−	−	−
6-Keto-$PGF_{1\alpha}$	+	+	+	+	+	+
6,15-Dik-$PGF_{1\alpha}$	+	+	+	+	+	+
6,-15-Dik-13,14-dihyd-$PGF_{1\alpha}$	+	+	+	+	+	+
TxB_2	+	+	+	+	+	+
15-Keto-13,14-dihyd-TxB_2	+	+	+	+	+	+
Arach acid	+	+	+	+	+	+

results are produced we shall be able to determine whether any particular eicosanoids are important in human mammary cancer.

However, at the moment our results should be treated cautiously: the measurements have been made on tumour stimulated to synthesize prostaglandins artificially from precursors released during homogenization, and we do not know the relative contributions by the various cell types present in the tumours.

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