Differential prostaglandin production by microsomal fractions of rat pregnant uterus

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Crude homogenates of decidual tissue from rat pregnant uteri synthesize prostaglandin (PG) F as estimated by parallel bioassay (Williams, Sneddon & Harney, 1974). As recent work has shown the increasing complexity of arachidonic acid metabolism we have developed a radiometric assay to allow more detailed analysis of products formed by the uterus.

Pregnant rats were killed (day 22 of pregnancy) and 20% homogenates of deciduum and myometrium prepared in Tris/acetate buffer (pH 8) containing hydroquinone 0.5 mM and reduced glutathione 2 mM. Microsomes were prepared by ultracentrifugation and samples incubated with 1 ml aliquots of buffer containing arachidonic acid (99 nmol) and [^{14}C]-arachidonic acid (3.5 nmol) for 30 min at 37°C. Pilot experiments indicated these conditions were optimal for decidual tissue with regard to substrate and co-factor concentrations, pH and incubation time. Samples boiled before incubation served as controls. After solvent extraction residues were subjected to thin-layer chromatography (TLC) using the solvent chloroform 90 : methanol 9 : acetic acid 1 : water 0.65 by volume. The radioactivity in consecutive 0.5 cm bands of each zone was then estimated by liquid scintillation counting. Authentic $PGF_{2\alpha}$, PGE_2, [^{14}C]-6-oxo $PGF_{1\alpha}$ (prepared as described by Cottee, Flower, Moncada, Salmon & Vane, 1977) and arachidonic acid were used as markers.

With decidual microsomes 23% of radioactivity on the TLC plate was converted to products other than monohydroxy acids (range 14–50%, 15 experiments). 50% of this radioactivity had an R_F value similar to PGE_2, 25% ran as PGD_2 while 20% had a mobility equivalent to $PGF_{2\alpha}$. Myometrial substrate conversion was lower, 6% (range 2–15%, 15 experiments) and all the radioactivity was located in the PGE_2 zone. However, in this solvent system PGE_2 and 6-oxo $PGF_{1\alpha}$ have similar R_F values. Therefore the zones of radioactivity corresponding to PGE_2 were removed, eluted and rechromatographed using solvent systems devised to separate these two substances (Cottee *et al.*, 1977). Using these TLC systems we found that approximately 12% of radioactivity in the decidual PGE zone behaved as 6-oxo $PGF_{1\alpha}$ and 70% as PGE_2. However 84% of myometrial radioactivity appeared to be 6-oxo $PGF_{1\alpha}$ and only 6% PGE_2.

Thus decidual microsomes produce PGE_2 as the major product whereas myometrial preparations synthesize predominantly 6-oxo $PGF_{1\alpha}$. Further investigation is needed to determine whether this myometrial PG production plays any part in regulating uterine function during pregnancy.

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Accumulation of platelets at acute inflammatory sites

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The involvement of blood platelets in acute inflammatory reactions is a topic of some interest. One experimental approach has been the use of animals made thrombocytopenic by treatment with anti-platelet sera. Some workers have reported that such animals either show diminished responsiveness to reactions such as the active Arthus and Shwartzman (Margaretten & McKay, 1969, 1971) or a decreased appearance of platelet-like cells into developing inflammatory exudates (Smith, Walker, Ford-Hutchinson & Penington, 1976). In other animal models of acute inflammation, such as hind paw oedemas, it has been reported that platelet-like bodies occur in the swollen paw (Rédei & Kelemen, 1969) but that the development of the oedema is not affected by pre-treatment of the animals with anti-platelet sera (Ubatuba, Harvey & Ferreira, 1975).

We have examined the distribution of platelets and erythrocytes in and around implanted inert sponges in the rat. Female albino Wistar rats (150–200 g) received either homologous platelets labelled with ^{51}Cr (sodium chromate) according to the method of Radegran (1976) or homologous erythrocytes labelled with ^{51}Cr according to the method of Gray & Sterling (1950). Eighteen hours after labelling, the animals were subjected to an inflammatory insult, either subdermal implantation of 3 to 6 saline soaked sponges or subdermal incisions without sponge implantation. The rats were killed after various time intervals and the sponges, the skin around the sponges or incision, skin from other areas and blood samples

were removed for determination of radioactive content.

The results of the experiments showed that the labelled platelets appeared only in the sponge exudates to a minor degree but that they preferentially accumulated in the vascular bed around the implanted sponges and in the other areas of skin examined. It is suggested that if platelets have a role in the early phases of the development of inflammatory reactions then this is intravascular rather than extravascular.

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Estimation of plasma nicotine by combined capillary column gas chromatography–mass spectrometry

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A quantitative method for the determination of nicotine by combined capillary column gas chromatography–mass spectrometry has been developed. The initial extraction of nicotine, from plasma, used a modification of the method of Feyerabend, Levitt & Russell (1975). This modification allowed the addition of the quinoline internal standard direct to the plasma, prior to extraction.

The nicotine was finally extracted into benzene and injected (0.8 µl) into a gas chromatograph fitted with a 20 m (0.3 mm internal diameter) glass capillary column coated with SP1000. The initial column temperature was 140°C and this was increased at 6°C/min to a final temperature of 180°C.

A V.G. Micromass 12B2 mass spectrometer was directly coupled via a 0.15 mm internal diameter glass capillary restriction to the gas chromatograph. The mass spectrometer operating conditions were: interface 250°C, ion source 200°C, ionizing potential 70 eV, accelerating voltage 4 KV, and source pressure 10^{-5} mmHg.

The most abundant ion in the nicotine spectrum (mass to charge ratio (m/e) 84) and the molecular ion of quinoline (m/e 129) were measured by selective ion monitoring. The accelerating voltage was switched between 2.6 and 4 kV to bring these ions into focus.

The ratio of the m/e 84 and m/e 129 peak heights was linearly related to nicotine concentration over the range 5–100 ng nicotine/ml plasma.

This method has been applied to the study of the pharmacokinetics of nicotine in man and experimental animals.

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