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Homogenates of rat placenta contain a factor(s) which inhibits uterine arachidonic acid metabolism

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The main arachidonic acid (AA) metabolite of vascular tissue is prostacyclin (PGI_2) (Bunting, Gryglewski, Moncada & Vane, 1976). Despite rich vascularisation the rat placenta does not produce PGI_2 (Williams, Dembinsak-Kiec, Zmuda & Gryglewski, 1978). We have therefore investigated placental tissue to determine whether it contains a PG synthetase inhibitor.

A 25% (w/v) homogenate of rat placentae was prepared in distilled water (pH 7). After centrifugation at 3000 *g* for 30 min the supernatant was ultracentrifuged (100,000 *g* for 60 minutes). The microsomal supernatant (PIF-M) was passed through silicic acid or XAD-2, and then lyophylised. Samples (1–8 mg in 0.05–0.4 ml) in distilled water of PIF-M were incubated with 1 ml samples of decidual or myometrial microsomes (protein concentration 1–2 mg/ml) in 0.1 M. Tris buffer (pH 8) with co-factors (Williams & Downing, 1977). The reaction was terminated with citric acid (0.25 ml 2 M). Products were extracted with 2 × 2 volumes of ether and separated by thin-layer chromatography (TLC), using a solvent system of chloroform:methanol:acetic acid:water (90:9:1:0.65). Similar volumes of boiled PIF-M served as controls. Areas of radioactivity were detected by TLC scanning. After scraping and extraction with methanol the absolute activity was determined by liquid scintillation counting.

PIF-M produced a dose dependent inhibition of PG-synthesis by decidual microsomes, $ID_{50} = 2.95 \pm 0.62$ mg (mean ± s.e. mean, $n = 4$). Inhibition of PG synthesis by rabbit kidney medulla and ram seminal vesicle microsomes was much lower 47% and 32% inhibition per 8 mg PIF-M respectively. PIF-M was found to be unstable in acid solution (pH 3) or when heated at 80°C for 10 minutes. PIF-M was non-dialysable and was precpitated by 10% w/v $(NH_4)_2SO_4$. Incubation of PIF-M with the non-selective, proteolytic enzyme mixture, 'Pronase', 2 mg/ml for 2 h at 37°C resulted in the complete loss of inhibitory activity.

When studying conversion of radiolabelled AA errors can result from dilution by cold precursor giving apparent inhibition of conversion of the labelled substrate. In the present investigations we have precluded this possibility by passage of the placental microsomal supernatant through silicic acid or XAD-2, which eliminated 99% of added [1-^{14}C]-AA. The possibility still remained that PIF-M contained a phospholipase enzyme which could cleave AA from phospholipid contained in the uterine microsomes. This source or error was removed because (1) lipids were removed from uterine microsomes by washing with an acetone-pentane mixture (Wallach & Daniels, 1971) but this did not result in loss of inhibitory activity from PIF-M. (2) No decrease in inhibitory potency of PIF-M was noted when the uterine microsomes were pre-incubated with the phospholipase A_2 antagonist, mepacrine (500 μg/ml).

Human placentae have also been found to contain a PIF-M. The experiments suggest that the placenta contains a protein or polypeptide which even in this impure form is a potent inhibitor of uterine arachidonic acid metabolism.

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The action of salmon calcitonin on indomethacin-induced gastric ulceration in the rat

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We have recently demonstrated that salmon calcitonin inhibited the development of indomethacin-induced gastric ulceration in the mouse when administered s.c. but not when administered intragastrally (Bates, Buckley & Strettle, 1979). Calcitonin has been shown to inhibit stress-induced gastric ulceration when administered intragastrally in several species (Barlet, 1974; Barlet & Bates, 1974; Hotz, Goebell, Minne & Ziegler, 1974), including the rat (Bates & Barlet, 1974).

We have now investigated the action of salmon calcitonin upon indomethacin-induced gastric ulceration in the rat.

Gastric ulceration was induced over a 5 h period, in starved (24 h) Sprague–Dawley rats, by the administration of indomethacin 40 mg/kg i.p. (Djahanguiri, 1969). The excised stomachs were washed through with 5 ml of distilled water and the pH measured. The ulcers were stained using a modification of the method of Robert & Nezamis (1964). Plasma calcium concentrations were measured using a Corning 940 calcium analyser.

Salmon calcitonin (0.1 MRC u/kg–100 MRC u/kg) caused an inhibition of the development of gastric ulceration when administered either s.c. or intragastrally (Figure 1). Subcutaneously administered calcitonin caused a greater inhibition of ulceration than did intragastral administration (Figure 1). Intragastral administration of 500 MRC u/kg salmon calcitonin failed to give a statistically significant inhibition of gastric ulceration. All s.c. doses of salmon calcitonin (0.1 MRC u/kg–100 MRC u/kg) caused statistically significant decreases in plasma calcium concentrations. This parameter was unaffected after intragastral administration of the hormone. Neither mode of administration caused statistically significant changes in gastric pH.

Thus it is possible to conclude that indomethacin-induced gastric ulceration in the rat, in contrast to the mouse, is sensitive to the antiulcerogenic actions of intragastrally administered calcitonin. In addition,

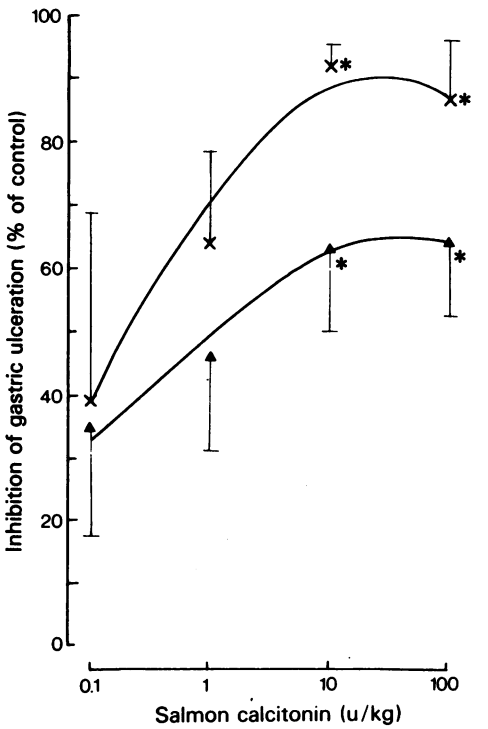

Figure 1. Effect of administration of salmon calcitonin (0.1 MRC u/kg–100 MRC u/kg) by s.c. (×——×) or intragastral (▲——▲) routes on indomethacin-induced gastric ulceration in the rat. Discrete areas larger than 1 mm in any cross-sectional dimension when viewed from the mucosal surface were scored as ulcers and the total number of such areas for each stomach was summed. Salmon calcitonin was administered in a vehicle containing 1 mg/ml bovine serum albumin in either 154 mM saline (s.c.) or distilled water (intragastral). Controls received vehicle only. Results are expressed as mean and s.e. of mean. Each value is the mean of 6 determinations. Statistically significant differences are presented between controls and calcitonin treated animals at the $P < 0.05$ level (*) (Mann-Whitney U test).