Metabolism and incorporation in the tissues of arachidonic acid in normal and essential fatty acid deficient rats

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The metabolism of arachidonic acid (AA) was studied after i.v. injection of [1-^{14}C]-AA into rats. The urinary excretion of radioactive material was determined in both normal and essential fatty acid (EFA) deficient rats. The highest amounts were excreted on the first day. After 15 days there was still a certain amount of radioactivity present in the urine. The overall excretion of radioactivity was considerably lower in EFA-deficient rats. There was a difference between normal and EFA-deficient rats in the time in which the excretion of the radioactivity was reduced to 50% in both the period of 1–4 and 5–15 days.

Radioactive metabolites were isolated from the urine by Amberlite chromatography and TLC. $^{14}CO_2$ production was measured in normal animals. Highest formation occurs in the first hour. At the end of the second hour, the amount of $^{14}CO_2$ rapidly diminishes. Incorporation of AA in the tissues was determined after the injection of 92.5 μg AA containing 18.5 μCi [1-^{14}C]-AA into normal and EFA-deficient rats. At different times, the animals were sacrificed and ultra-thin total body slices were prepared. Of the latter, autoradiograms were made by long-term exposure. After 5 min, the highest level of radioactivity is seen in the following organs:

Normal: brown fat, perispinal fat, liver, heart muscle, kidney, adrenal. The distribution in the liver was not uniform.
EFA-deficient: similar

After 30 min
Normal: liver
EFA-deficient: same as 5 min;

After 24 h
Normal: radioactivity had nearly disappeared, small amount present in liver
EFA-deficient: same as 30 min, small decrease.

These results indicate that EFA-deficient rats retain the injected AA longer than normal animals. In how far a relationship exists between the rapidity of incorporation of AA and prostaglandin metabolism in the tissues is unknown. A higher sensitivity of EFA-deficient rats to certain effects of prostaglandins has been reported (Bonta & Parnham, 1979). This fact may eventually establish a link with the enhanced incorporation of AA into certain tissues.

Reference

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Effects of exogenous arachidonic acid on the anti-inflammatory actions of dexamethasone in the rat

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It has been postulated that the anti-inflammatory action of steroids may be due to inhibition of arachidonic acid production from phospholipids (Hong & Levine, 1976). As arachidonic acid is the precursor not only of the proinflammatory prostaglandins but also of the chemotactic lipid 12-L-hydroxy-5, 8, 10, 14-eicosa-tetraenoic acid (Turner, Tainer & Lynn, 1975) the proposal offers an explanation for the effects of anti-inflammatory steroids on the cellular as well as the exudative phase of inflammation. We have tested this hypothesis by investigating the effects of exogenous arachidonic acid on the inhibition by dexamethasone of carrageenin-induced oedema and carboxymethylcellulose-induced cell emigration in the rat.

Oedema was produced in the left hind paw of female PVG/c rats by subplantar injection of 0.1 ml 1% (w/v) carrageenin in saline. Arachidonic acid was given with the carrageenin. Paw volumes were measured by mercury displacement. Cell emigration was induced in male PVG/c rats by injecting 5 ml of 2% (w/v) carboxymethylcellulose into a dorsal air pouch (Ishikawa, Mori & Tsurufuji, 1969). Exudate fluid was diluted with heparin-saline and cells (predominantly polymorphs) were counted using a haemocytometer. Dexamethasone was given orally 1 h (cell emigration) or 2.5 h (oedema) before induction of inflammation.

Arachidonic acid (1 mg/kg) increased carrageenin

induced oedema measured at 1 h by 118% ($P < 0.01$) but had no effect on oedema measured at 4 hours. Dexamethasone (1 and 5 mg/kg) inhibited the 1 h response by 44% ($P < 0.01$) and 59% ($P < 0.01$) respectively, but in the presence of arachidonic acid inhibition was prevented and oedema was enhanced by 60% ($P < 0.01$) and 92% ($P < 0.01$) respectively. These results are in general agreement with those of Lewis, Nelson & Sugrue (1975).

A different profile was seen when oedema was measured after 4 hours. Dexamethasone (1 and 5 mg/kg) inhibited oedema by 62% ($P < 0.01$) and 88% ($P < 0.01$) respectively. Arachidonic acid (1 mg/kg) had no effect on inhibition by dexamethasone (1 mg/kg) but reduced inhibition by dexamethasone (5 mg/kg) to 24% ($P < 0.01$).

Arachidonic acid (1 mg/kg) did not affect the accumulation of cells in the carboxymethylcellulose pouch at any time up to 5 h after injection of carboxymethylcellulose. Dexamethasone (0.25 mg/kg) inhibited the 5 h response by 78% ($2.21 \pm 0.22 \times 10^7$; control $9.67 \pm 0.96 \times 10^7$, $P < 0.01$) and this was not altered by arachidonic acid ($1.3 \pm 0.16 \times 10^7$; control $7.81 \pm 1.15 \times 10^7$, $P < 0.01$). Similar results were obtained with dexamethasone (5 mg/kg).

The results suggest that while the effects of dexamethasone on oedema may be ascribed to inhibition of arachidonic acid generation, cell emigration and its inhibition by dexamethasone are independent of arachidonic acid.

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U-46619, a selective thromboxane A_2-like agonist?

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Instability and the necessity for biosynthetic preparation complicate the study of the biological actions of prostaglandin endoperoxides (PGG_2 and PGH_2) and thromboxane A_2 (TXA_2). Stable analogues of PGH_2 have been synthesised; these resemble the natural compound in contracting rabbit aorta and aggregating human platelets (Malmsten, 1976). However, TXA_2 also has these actions (Hamberg, Svensson & Samuelson, 1975), and in view of these similarities we have carried out a comparison of the effects of PGH_2, TXA_2 and the stable PGH_2 analogue, U-46619 (Bundy, 1975), on a range of isolated smooth muscle preparations.

Guinea-pig ileum (Horton & Main, 1963), guinea-pig lung strip (Lulich, Mitchell & Sparrow, 1976), guinea-pig fundus (Vane, 1957), rabbit aortic strip (Furchgott & Bhadrakom, 1953), dog and cat iris sphincter muscle (van Alphen & Angel, 1975) and dog saphenous vein (Humphrey, 1978) were suspended for cascade superfusion (Vane, 1964). Preparations were superfused at 10 ml/min with oxygenated modified Krebs solution (Apperley, Humphrey & Levy, 1976) at 37°C containing indomethacin (2.8×10^{-6} mol/l), phenoxybenzamine (7×10^{-7} mol/l) and atropine (4×10^7 mol/l). PGH_2 was prepared by the method of Gorman, Sun, Miller & Johnson (1977). TXA_2 was prepared by incubating PGH_2 with indomethacin-treated sheep platelet microsomes at 0°C for 15–120 seconds. PGH_2 (15–1500 ng) contracted all preparations, with a threshold dose of <15 ng on dog iris and 15–50 ng on the other preparations. Incubation of PGH_2 with platelet microsomes potentiated activity on rabbit aorta, dog saphenous vein and guinea-pig lung strip; although threshold doses on these preparations were unchanged, dose-effect curves became steeper. On the other preparations, incubation with platelet microsomes reduced the potency of PGH_2; threshold doses increased 3–30 times and dose-effect curves became shallower. These changes in biological activity were presumed to be due to TXA_2 formation, since they could be prevented by treatment of the platelet microsomes with imidazole (300 μg/ml), a selective thromboxane synthetase inhibitor (Moncada, Bunting, Mullane, Thorogood, Vane, Raz & Needleman, 1977). U-46619 (10 ng–