The distribution and mobilisation of arachidonic acid in fat cell ghosts and its modification by glucocorticoids

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Rabbit isolated fat cells have previously been shown to synthesize and release prostaglandins. During lipolysis glucocorticoids increased the tissue/medium ratio of prostaglandins (Chang, Lewis & Piper, 1977). In the present study fat cell ghosts were used as a simpler system in which to investigate the mechanism and site of action of steroids. The ghosts were prepared from isolated fat cells by lysis in hypotonic medium and resealing in hypertonic Krebs solution (Rodbell, 1967). Prostaglandins were extracted and separated by t.l.c. using ethyl acetate:acetic acid:isooctane:water (100:20:50:100). Stimulation with $ACTH_{1-24}$ released [^{14}C]-prostaglandins from ghosts which had been incubated with [^{14}C]-arachidonic acid (AA) (52 μCi/μmol), showing that prostaglandin synthesizing enzymes are present in ghosts. The percentage conversion of AA to prostaglandins E_2 and $F_{2\alpha}$ was 0.82 ± 0.44, 1.37 ± 0.6 (n = 4) respectively. Synthesis of prostaglandins from endogenous AA was estimated by radioimmunoassay and found to be prostaglandin $F_{2\alpha}$, 10.3 ± 4.4, prostaglandin E_2, 9.8 ± 4.4 ng/mg lipid.

To identify the AA pool within the ghosts, a whole lipid extraction was carried out (Bligh & Dyer, 1959) and neutral and phospholipid fractions separated by silicic acid chromatography (Hirsch & Ahrens, 1958). The fractions were then separated into their subclasses by t.l.c. in chloroform : methanol : water (65:25:4) for phospholipids and in petroleum ether (40–60°):diethyl ether:acetic acid (80:20:1) for neutral lipids (Lepage, 1967) and their fatty acid content analysed by gas liquid chromatography. The ratio of phospholipid to neutral lipid was approximately 2:1, 89.9% of the AA being present in phospholipids, 8.5% in neutral lipids and 1.6% unbound. However, when ghosts are incubated with [^{14}C]-AA, uptake occurred non-specifically: 20% in phospholipid, 10% in neutral lipids and 70% remained free.

Since AA must be released before being converted to prostaglandins, the effects of a number of compounds known to stimulate lipase activity were investigated. $ACTH_{1-24}$ (1 μg/ml), dibutyryl cAMP (0.35 mg/ml), bradykinin (4 μg/ml) and theophylline (18 ng/ml) were all found to induce the release of arachidonic acid.

Ghosts were incubated with [^{14}C]-AA and, in some experiments, hydrocortisone (50 μg/ml) or dexamethasone (10 μg/ml) were added to the incubation medium. In the presence of steroid, the incorporation of AA into the neutral lipids was enhanced while that into the phospholipids was reduced. When the release of bound [^{14}C]-AA was stimulated with $ACTH_{1-24}$ in the presence of steroids, release from the phospholipids was inhibited while that from the neutral lipids was stimulated. The same effect was found on release of endogenous arachidonic acid.

Thus, glucocorticoids appear to have two actions in fat cell ghosts: inhibition of mobilisation of AA from phospholipids while stimulating its release from triglycerides. The activities of phospholipases and fatty acyl transferases are influenced by membrane charge density and surface pressure and interaction of glucocorticoids with the membrane may inhibit these enzymes. Chang, Lewis & Piper (1977) have shown that steroids do not inhibit synthesis of prostaglandins in fat cells, which suggests that when they are stimulated with ACTH, triglycerides are the major source of AA for prostaglandin synthesis.

We wish to thank the Medical Research Council for grants.

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Alterations in prostaglandin E_1-induced blood flow changes in granulation tissue

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As granulation tissue develops, the increased vascular permeability response to local prostaglandin (PG)E becomes more pronounced (Chang & Tsurufuji, 1976) and the response to other inflammatory mediators also changes (Chang & Tsurufuji, 1976; Hurley, Edwards & Ham, 1970). We have, thus, studied PGE_1-induced changes in blood flow at different stages of granuloma development.

Carrageenin-soaked polyether sponges, with indwelling cannulae, were implanted (s.c., 2 sponges/rat)