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.

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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)

into male Wistar rats (170–250 g) as described previously (Bonta, Adolfs & Parnham, 1978). Subsequently, (see Table 1) the left ventricle was catheterized via the carotid artery, under urethane anaesthesia. Intra-ventricular injections of radioactively-labelled plastic microspheres (15 ± 5 (s.d.) μm dia, 20,000–150,000 microspheres/0.1 ml saline) were given 15 min before and 1.5 min and 15 min after injections of 0.5 ml PGE_1 (200 ng/ml) into one sponge and 0.5 ml saline into the other. Each microsphere injection involved a different isotope. Animals were killed with sodium pentobarbitone 15 min later and dissected into various organs and tissues which were counted in a Packard γ-scintillation counter (Johnston & Saxena, 1978). Blood flow was expressed as percent distribution of cardiac output (C.O.)/100 g tissue (see Table 1).

PGE_1 increased blood flow to granulomata, most markedly 15 min after injection. This effect became more pronounced on later days (Table 1a). The response to PGE_1 on day 5 was somewhat greater than that shown in Table 1a, since absolute flow in saline-treated granulomata also increased slightly (from a baseline of 8.9 ± 0.9% C.O./100 g (± s.e. mean) to 12.5 ± 1.9% C.O./100 g after 15 min, $P>0.05$), probably due to diffusion of PGE_1 from the other sponge. On day 10, PGE_1 also caused a detectable increase in flow to the skin covering the granulomata (Table 1b), but not to other organs.

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Table 1 Blood flow changes induced by prostaglandin E_1 following injection into carageenin-impregnated sponges at different times after sponge implantation

	Time after PGE_1 injection (min)	*Change in percent distribution of C.O./100 g tissue* day 5§ (n = 4)	day 7 (n = 7)	day 10 (n = 5)
a *Granulomata*	−15.0	−0.84 ± 2.26	−0.40 ± 1.30	−0.16 ± 0.78
	1.5	−0.90 ± 1.62	3.89 ± 1.52*	7.00 ± 1.21**
	15.0	4.47 ± 1.84	8.03 ± 2.11**	15.58 ± 4.07**
b *Skin covering granulomata*	−15.0	−0.04 ± 1.80	−0.01 ± 0.82	−0.16 ± 0.72
	1.5	0.23 ± 0.97	1.38 ± 1.12	5.71 ± 5.55
	15.0	0.40 ± 1.84	0.15 ± 0.92	2.98 ± 1.07*

Values shown are means ± s.e. mean calculated by subtraction of values (percent distribution of C.O./100 g tissue) for saline-treated tissues from those for PGE_1-treated tissues in the same animal. In each case percent distribution of C.O./100 g tissue was determined as: radioactivity in tissue ÷ radioactivity in whole body × 100 ÷ 100 g tissue.

§Time after sponge implantation (day 1) on which surgical procedures and drug treatment were carried out. Significance of differences from baseline (Mann-Whitney U test, one-tailed): *$P<0.05$; **$P<0.01$.