Biochemical Pharmacology, Vol. 27, pp. 1409–1412.

0006-2952/78/0515-1409/$02.00/0

COMMENTARY

INTERACTIONS OF ANTI-INFLAMMATORY STEROIDS WITH PG SYSTEM IN ADIPOSE TISSUE

G. P. LEWIS and PRISCILLA J. PIPER

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London, WC2A 3PN, U.K.

The release of prostaglandins from adipose tissue of various species occurs in response to activation by lipolytic agents [3] and electrical stimulation [18].

Non-steroid anti-inflammatory drugs interfere with the prostaglandin system of tissues by inhibiting cyclo-oxygenase and thereby preventing the synthesis (and release) of all the products of metabolism of arachidonic acid which occur subsequent to the action of this enzyme(s) [23, 19, 2]. Glucocorticoids also inhibit the release of prostaglandins from various tissues including adipose tissue [15], lung, blood vessels [11], rheumatoid synovial explants and mouse fibrosarcoma cells in culture [20, 12] but these drugs do not inhibit the cyclo-oxygenase enzyme system [15, 9]. Therefore, although the overall effect of the two types of drug on release of prostaglandins is similar, anti-inflammatory steroids influence the prostaglandin system by mechanisms which differ from that of the non-steroid anti-inflammatory drugs.

This commentary will discuss the mechanism of interaction of steroids with the prostaglandin system in adipose tissue. Although fat is a rather specialised tissue, our findings in adipose tissue may also be relevant to some other tissues.

Rabbit adipose tissue in vivo

Lipolysis in rabbit blood-perfused subcutaneous adipose tissue may be stimulated by lipolytic agents such as ACTH and is accompanied by a functional vasodilatation which is prostaglandin-mediated, probably by prostaglandin E_2 [3, 13]. During lipolysis prostaglandin is formed within the fat tissue and its preliminary identification as prostaglandin E_2 has been confirmed by mass spectrometry [7]. When fat tissue is stimulated supramaximally with a lipolytic agent, small amounts of prostaglandin-like material are released into the venous blood draining the fat pad [14]. Since the functional vasodilatation and prostaglandin formation in the fat may be inhibited by non-steroid anti-inflammatory drugs which leave lipolysis unchanged [2], it seems that prostaglandins are synthesised within the fat cells and then act on the blood vessels to cause the functional vasodilatation.

When the anti-inflammatory steroids, hydrocortisone, prednisolone or betamethasone are infused into subcutaneous adipose tissue before the lipolytic stimulus, lipolysis is again unaltered while the vasodilatation is inhibited. However, the mechanism of action of the steroids differs from that of aspirin-like drugs since although the vasodilatation is blocked, the formation of prostaglandins within the fat is unchanged [15]. One possible explanation of these results was that steroids act in some way to prevent the release of prostaglandins, which have been synthesised in the fat without interfering with their synthesis.

Rabbit adipose tissue in vitro

Experiments were carried out in chopped subcutaneous adipose tissue from rabbits in an attempt to clarify the actions of glucocorticoids which had been observed *in vivo* [5]. Chopped fat was incubated in Krebs solution, lipolysis was induced by ACTH and monitored by release of glycerol into the incubation medium. The concentrations of prostaglandins formed in the fat tissue (T), and released into the Krebs solution (M) were measured and expressed as a ratio (T/M). In the presence of steroid and non-steroid anti-inflammatory drugs this ratio is altered in different ways. Indomethacin prevents the formation of prostaglandins in the fat and therefore their release into the medium. However, in the presence of steroids, whilst the release of prostaglandins into the medium is inhibited, the content in the fat tissue increases to well above control levels (Fig. 1). Similar

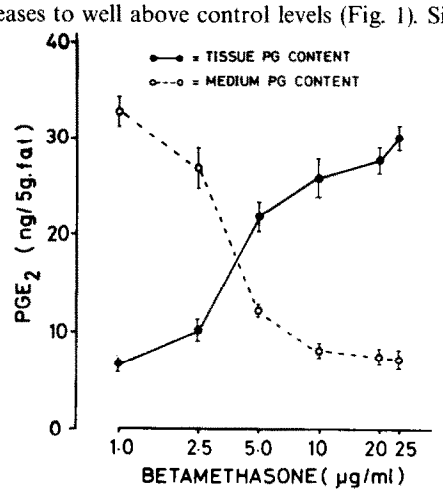

Fig. 1. Effect of betamethasone on the prostaglandin content of chopped fat tissue ●——● and supernatant (medium) O----O. Incubations were carried out for 2 hr in the presence of $ACTH_{1-24}$ 0.1 μg/ml and varying concentrations of betamethasone. Prostaglandin concentration was estimated by radioimmunoassay. Each point is the mean of 4–6 experiments. Vertical bars show S.E. mean. (From Chang, Lewis and Piper (1977a) with permission).

results have been obtained in isolated fat cells [5] and together these results support the hypothesis that steroids act to prevent the release but not the synthesis of prostaglandins in adipose tissue.

Human adipose tissue in vitro

Abdominal subcutaneous adipose tissue was removed from patients undergoing major abdominal surgery. Since ACTH is not a potent lipolytic agent in human fat, lipolysis was induced in the chopped tissue with adrenaline [6]. Adrenaline was shown to induce prostaglandin formation in the fat tissue during lipolysis just as ACTH had done in rabbit fat tissue. As in the experiments with rabbit fat, prostaglandins were estimated in terms of prostaglandin E_2 by radioimmunoassay. However, incubation of microsomes from human fat with arachidonic acid lead to the formation of prostaglandins E_2, $F_2\alpha$, 6-keto-$F_{1\alpha}$ and thromboxane B_2 [6] which suggests that either the fat tissue or the blood vessels supplying it may form a number of prostaglandins and related substances during stimulation. In the presence of steroid and non-steroid anti-inflammatory drugs the tissue/medium ratio of prostaglandin concentrations vary as in the rabbit adipose tissue, again showing that steroids cause the retention of prostaglandins within the fat. The lipolytic action of adrenaline is inhibited by the β-adrenoceptor antagonist, propranolol. However, the formation of prostaglandins is not affected by propranolol and would therefore appear to be mediated by a different mechanism.

Mechanism of action of steroids

The experiments described show that whereas non-steroid anti-inflammatory drugs reduce the total amount of prostaglandin formed in fat during lipolysis, anti-inflammatory steroids increase the tissue/medium ratio but do not reduce the total formation.

Several theories have been put forward to explain the prevention of prostaglandin release by glucocorticoids. Steroids are known to stabilize some cell membranes [24] and this may be the mechanism by which steroids cause the retention of prostaglandins within fat cells, i.e. act on the membrane in some way to prevent their release. If the cell membrane is damaged and can no longer prevent diffusion of materials, steroids no longer prevent the release of prostaglandins, whereas inhibitors of prostaglandin synthesis are still effective. The retention of prostaglandins within cells depends on the balance of substrate availability and metabolising enzymes and the latter have been shown to be virtually absent in fat [5]. It is widely accepted that steroids do not act on the enzymes which synthesise prostaglandins although there are some reports which suggest that steroids do affect prostaglandin synthesis [10, 20]. An alternative explanation for the accumulation of prostaglandins in fat in the presence of steroids could be that these drugs influence the uptake of prostaglandins into the fat tissue. However, when [^{3}H]prostaglandin E_2 was incubated with rabbit adipose tissue *in vitro*, about 30 per cent was taken up into the fat and the presence of hydrocortisone or betamethasone did not alter the prostaglandin uptake [5].

Anti-inflammatory steroids as well as non-steroid anti-inflammatory drugs inhibit the release of prostaglandins and thromboxanes from lung tissue stimulated by anaphylasix [11] or by rabbit aorta contracting substance releasing factor (RCS-RF) [17], or by slow reacting substance of anaphylaxis (SRS-A) [8]. In addition, they also inhibit release of prostaglandins from blood vessels constricted with noradrenaline [11]. Gryglewski and co-workers [11] however, showed that there was a fundamental difference between the action of the corticosteroids and the aspirin-like drugs. They found that the inhibitory action of the steroids, but not that of indomethacin was reversed by arachidonic acid. Since arachidonic acid is normally cleaved from membrane phospholipids during their hydrolysis by phospholipase A Gryglewski *et al.* suggested that the corticosteroids interfere with the activity of this enzyme thereby preventing the supply of the endogenous arachidonic acid substrate for the microsomal cyclo-oxygenase. Steroids may affect the intercompartmental barriers between enzymes and substrate and influence reactions without any direct effect on the enzymes involved, and evidence exists to show that although steroids might affect the action of phospholipase, it is not phospholipase A itself that is inhibited but an earlier step in its activation [1, 4].

However, prevention of availability of arachidonic acid from phospholipids cannot be the only action of steroids in adipose tissue since when arachidonic acid was incubated with chopped fat during steroid block, although 70–80 per cent was taken up into the fat, it did not reverse the action of steroids [5]. Similar results were found *in vivo* showing that, after steroid treatment, arachidonic acid does not restore the prostaglandin mediated vasodilatation which accompanies lipolysis [14].

When uptake of [^{14}C]arachidonic acid by rabbit chopped adipose tissue *in vitro* was studied [5], it was found that although 70–80 per cent of the arachidonic acid was taken up by the fat, during lipolysis only 0.2–0.5 per cent was converted to prostaglandins. Therefore it appeared that the exogenous arachidonic acid did not enter the pool from which the endogenous substrate is converted to prostaglandins during lipolysis; it may perhaps be necessary for arachidonic acid to be incorporated into phospholipids prior to conversion into prostaglandisn. If this were true also in the guinea-pig lungs, it would be difficult to explain the reversal of the activity of steroids by arachidonic acid in the experiments of Gryglewski *et al.* since it occurred immediately after the administration of arachidonic acid to the preparation.

If it is assumed that steroids prevent the hydrolysis of phospholipids in fat cells as in lung tissue, then there must be an alternative pathway for the synthesis of prostaglandins since prostaglandins accumulate in fat tissue in the presence of steroids. A possible source of arachidonic acid is the triglycerides [16] which are metabolised to free fatty acids and glycerol during lipolysis. Although the triglycerides give rise to free fatty acids, there is little evidence to date that these include more than a small proportion of arachidonic acid, but it is known that triglycerides contain linoleic acid which can be converted into arachidonic acid by an enzyme system which is widely distributed. Therefore, if triglycerides can provide substrate for prostaglandin synthesis, there are then two possible

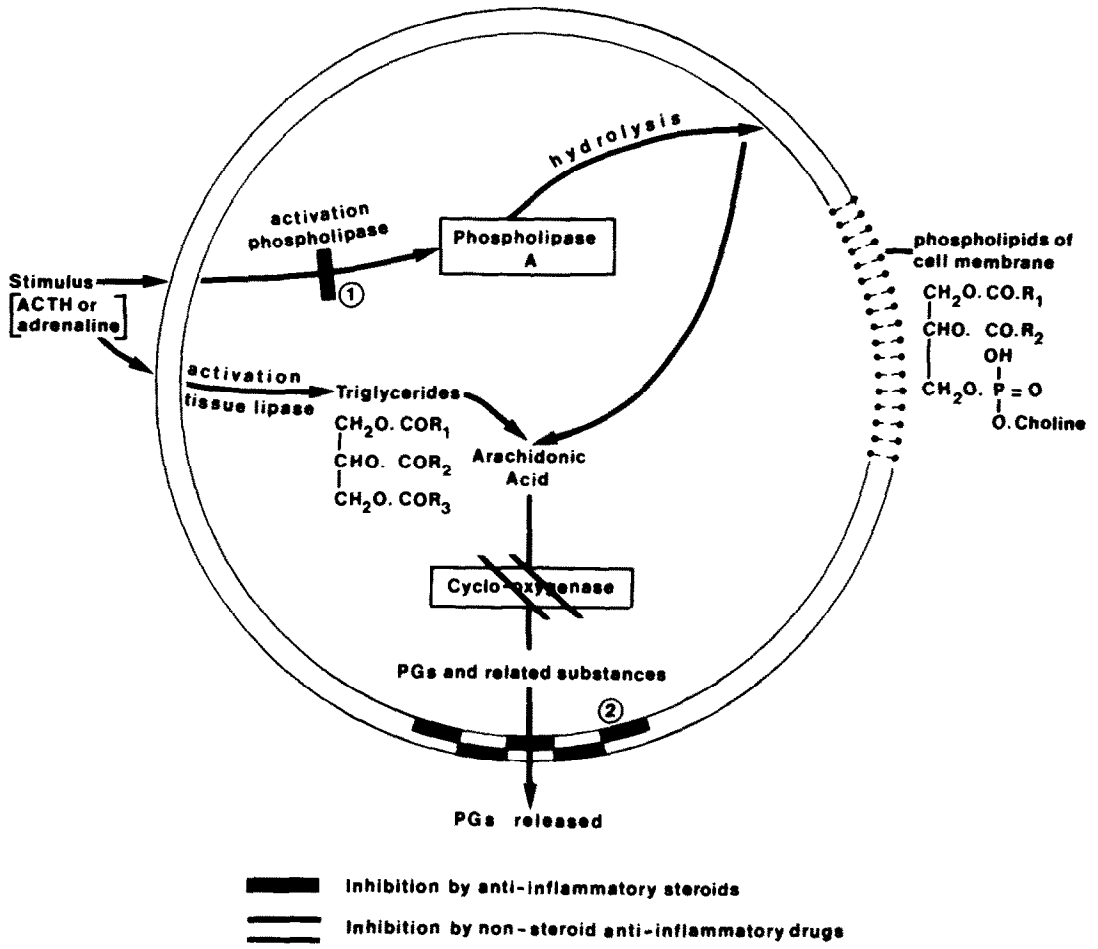

Fig. 2. Two possible pathways of arachidonic acid release. Activation of phospholipase A causes hydrolysis of membrane phospholipids and consequent release of arachidonic acid. Secondly, activation of tissue lipase causes release of free fatty acids among which could be arachidonic acid or its possible precursor, linoleic acid. Non-steroid anti-inflammatory drugs prevent the conversion of arachidonic acid to prostaglandins by cyclo-oxygenase. Anti-inflammatory steroids either inhibit the activation of phospholipase A or in the case of an alternative source of arachidonic acid, inhibit release of prostaglandins after their formation.

pathways for the formation of prostaglandins in adipose tissue as shown in Fig. 2. This Figure also shows the hypothetical sites of action of steroid and non-steroid anti-inflammatory drugs.

The finding that propranolol, while preventing lipolysis, does not reduce prostaglandin formation in adipose tissue, casts some doubt on the likelihood of triglycerides being the alternative source of arachidonic acid. Corticosteroids themselves have been shown to induce the release of free fatty acids from triglyceride stores in lymphocytes [22] and to cause accumulation of free fatty acids in these cells [21]. If steroids have a similar effect in adipose tissue and arachidonic acid is among the accumulated fatty acids, this might explain the continued synthesis of prostaglandins in the presence of steroids. The steroids do not, however, cause retention of the fatty acids themselves in adipose tissue.

Whatever the second source of substrate, the existance of two pathways for the synthesis of prostaglandins in adipose tissue would provide an explanation for the action of steroids in this tissue. It is possible that in certain tissues the second pathway for synthesis of prostaglandins is insignificant but that when the "phospholipase A pathway" is blocked by steroids, synthesis is diverted to the "triglyceride pathway". The effect of glucocorticoids which we have observed in human and rabbit subcutaneous adipose tissue might, therefore, be one of the factors involved in the mechanism of their anti-inflammatory activity.

REFERENCES

1. G. J. Blackwell, R. J. Flower, F. P. Nijkamp and J. R. Vane, *Br. J. Pharmac.* **59**, 441P (1977).
2. B. Bowery and G. P. Lewis, *Br. J. Pharmac.* **47**, 305 (1973).
3. N. G. Bowery, G. P. Lewis and J. Matthews, *Br. J. Pharmac.* **40**, (1970).
4. S. Brain, G. P. Lewis and B. J. R. Whittle, *Br. J. Pharmac.* **59**, 440P (1977).
5. J. Chang, G. P. Lewis and P. J. Piper, *Br. J. Pharmac.* **59**, 425 (1977).
6. J. Chang, G. P. Lewis and P. J. Piper, *Br. J. Pharmac.* **61**, 446P (1977).

7. C. Dalton and W. C. Hope, *Prostaglandins* **6**, 227 (1974).
8. D. M. Engineer, P. J. Piper and P. Sirois, *Br. J. Pharmac.* **59**, 444P (1977).
9. R. J. Flower, R. J. Gryglewski, K. Herbaczynska-Cedro and J. R. Vane, *Nature, Lond.* **238**, 104 (1972).
10. M. W. Greaves and W. J. McDonald Gibson, *Br. J. Pharmac.* **46**, 172 (1972).
11. R. J. Gryglewski, B. Panezenko, R. Korbut, L. Grodzinska and A. Ocetkiewicz, *Prostaglandins* **10**, 343 (1975).
12. F. Kantrowitz, D. R. Robinson, M. B. McGuire and L. Levine, *Nature, Lond.* **258**, 737 (1975).
13. G. P. Lewis and J. Matthews, *J. Physiol. Lond.* **207**, 15 (1970).
14. G. P. Lewis and P. J. Piper (Unpublished).
15. G. P. Lewis and P. J. Piper, *Nature, Lond.* **254**, 308 (1975).
16. G. P. Lewis and P. J. Piper, in *Perspectives in Inflammation. Future trends and developments* (Eds G. P. Velo and D. A. Willoughby). p. 519. Piccin Medical Books, Padua, London (1977).
17. F. P. Nijkamp, R. J. Flower, S. Moncada and J. R. Vane, *Nature, Lond.* **263**, 479 (1976).
18. J. E. Shaw and P. Ramwell, *J. Biol. Chem.* **243**, 1498 (1968).
19. W. L. Smith and W. E. M. Lands, *J. Biol. Chem.* **246**, 7600 (1971).
20. A. H. Tashjian, E. F. Voelkel, J. McDonough and L. Levine, *Nature, Lond.* **256**, 739 (1975).
21. R. W. Turnell and A. F. Burton, *Molecul. Cell. Biochem.* **9**, 175 (1975).
22. R. W. Turnell, L. H. Clarke and A. F. Burton, *Cancer Res.* **33**, 302 (1973).
23. J. R. Vane, *Nature, New Biol.* **231**, 232 (1971).
24. G. Weissman and J. T. Dingle, *Expl cell Res.* **25**, 207 (1961).