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The relationship between functional vasodilatation in adipose tissue and prostaglandin

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Summary

1. Earlier it had been found that during fat mobilization there was an increased blood flow in the adipose tissue and the tissue contained a vasodilator substance.

2. Extract of an activated fat pad contained 3 to 25 times as much activity as the contralateral resting fat pad.

3. The following findings suggest that the vasodilator substance is prostaglandin E_2:

(*a*) It caused contractions of the guinea-pig ileum which were not reduced by mepyramine, but were reduced by atropine.

(*b*) It caused a prolonged vasodilator response when injected close-arterially to the epigastric fat pad.

(*c*) It was eluted from a silicic acid column by a solvent system which is known to elute prostaglandins of the E series but not those of the F series.

(*d*) Its indices of discrimination were similar to those of prostaglandin E's when assayed on three different pharmacological preparations.

(*e*) On thin-layer chromatography it behaved more like prostaglandin E_2 than E_1.

4. Neither prostaglandin E_1 nor prostaglandin E_2 inhibited the release of free fatty acids from the rabbit epigastric fat pad by $ACTH^{1-24}$.

5. It seems likely that prostaglandin E_2 is responsible for the vasodilatation accompanying fat mobilization from adipose tissue.

Introduction

The blood flow in many tissues increases when the tissue becomes active. In skeletal muscle or exocrine glands, where activity is intermittent, the vasodilatation occurring during activity is pronounced and short lasting. In endocrine glands, on the other hand, where activity is continuous over long periods, the accompanying vasodilatation is moderate and prolonged (Holzbauer & Vogt, 1957; Staehelin, Barthe & Desaulles, 1965). In an earlier paper we showed that activation of rabbit adipose tissue by close arterial injection of lipolytic hormones resembles that in endocrine glands in being prolonged and accompanied by a prolonged increase in blood flow (Lewis & Matthews, 1968). More recently we were able to show that the functional vasodilatation in adipose tissue is accompanied by the release or formation of a vasodilator substance (Lewis & Matthews, 1970). In the present

experiments we have examined the nature of the substance. A preliminary account of this work was presented earlier (Lewis & Matthews, 1969).

Methods

Infusion experiments. Lipolytic agents were infused close-arterially to the epigastric adipose tissue as described earlier (Lewis & Matthews, 1970).

Extraction of adipose tissue. The method of Samuelsson (1963) was used to extract prostaglandin-like activity from the fat tissue.

Both epigastric fat pads were removed from the rabbit immediately after death. The contralateral resting fat pad was dissected out in the same way as the infused activated pad. The tissues were cut into small pieces in 50 ml of ice-cold ethanol, and usually stored at −10° C overnight. The mixture was then homogenized using an Ultra-Turrax homogenizer at room temperature. The homogenate was centrifuged at 3,000 rev/min for 10 min, the supernatant removed, a further 70 ml of 80% ethanol was added and the mixture centrifuged again. The combined supernatants were evaporated to 5–10 ml in a rotary evaporator at 40° C, acidified to pH 2 and extracted three times with three volumes of diethyl ether. The final ether phases were combined and dried using the "cold finger" technique at reduced pressure, before subjecting the product to silicic acid chromatography.

Silicic acid chromatography. This technique was used for the separation and identification of the active principle in extracts of adipose tissue according to the method described by Samuelsson (1963). The silicic acid used was SIL-R Sigma. The eluted fractions were dried, redissolved in saline and tested on isolated guinea-pig ileum, rabbit jejunum and fat-pad blood flow.

Thin-layer chromatography. This technique was used to identify which of the prostaglandin E (PGE) series the active principle most resembled. The method was that described by Green & Samuelsson (1964) using their AII system consisting of ethyl acetate, acetic acid, methanol, trimethylpentane and water.

Detection of the material by spraying the dried plates with a 10% solution of phosphomolybdic acid in ethanol, followed by heating for 15 min at 85° C as described by Green & Samuelsson, could be used only for the reference prostaglandin, since the amount of activity in the fat pad was too small to detect this way. Instead, the plates were divided into sections, corresponding to the position of the reference substances, and the gel extracted into 2 ml methanol. After centrifuging at 3,000 rev/min for 5 min, the residue was again washed with 2 ml methanol and centrifuged. The combined supernatants were dried, taken up in 2 ml water and extracted three times with three volumes of diethyl ether at pH 2. The combined ether phases were dried and redissolved in 1 ml saline for biological assay.

Biological assays. Samples were assayed on (1) the isolated guinea-pig ileum or (2) the isolated rabbit jejunum suspended in oxygenated Tyrode's solution at 34° C, or (3) for vasodilator activity by close arterial injection to the epigastric adipose tissue as described by Lewis & Matthews (1968).

In vitro *release of free fatty acids.* Rabbits weighing 3 kg were killed by a blow on the back of the head and the epigastric adipose tissue was removed and washed in Krebs-Ringer phosphate medium. The fat tissue was blotted dry and 200 mg portions placed in Erlenmeyer flasks together with 2 ml Krebs-Ringer phosphate

containing 4 g/100 ml bovine plasma albumin (Armour). Each flask was covered with aluminium foil to stop evaporation and then incubated in the presence of a lipolytic agent either alone or with a prostaglandin for 3 h in a Mickle shaker at 37° C. After removal from the shaker the fat tissue was removed and the flask corked and placed in the refrigerator (4° C) overnight. Free fatty acids (FFA) were estimated colorimetrically the following day by Duncombe's method (1964).

Results

In earlier experiments it was found that alcohol and acid ether extracts of epigastric adipose tissue excised during a period of fat mobilization contained a vasodilator substance. When tested on isolated smooth muscle preparations the extracts

TABLE 1. *Alcohol and acid–ether extracts of stimulated and resting epigastric fat depots*

Stimulated	Resting	Stimulus
35	—	Porcine growth hormone 5 μg
60	—	Glucagon 5 μg
30	—	Glucagon 10 μg
80	—	ACTH 1 μg/min for 15 min
60	10	ACTH 1 μg/min for 15 min
250	10	ACTH 1 μg/min for 15 min
100	20	ACTH 1 μg/min for 15 min
15	5	ACTH 1 μg/min for 15 min
130	40	ACTH 1 μg/min for 15 min

Activity is expressed as ng/g of prostaglandin E_1.

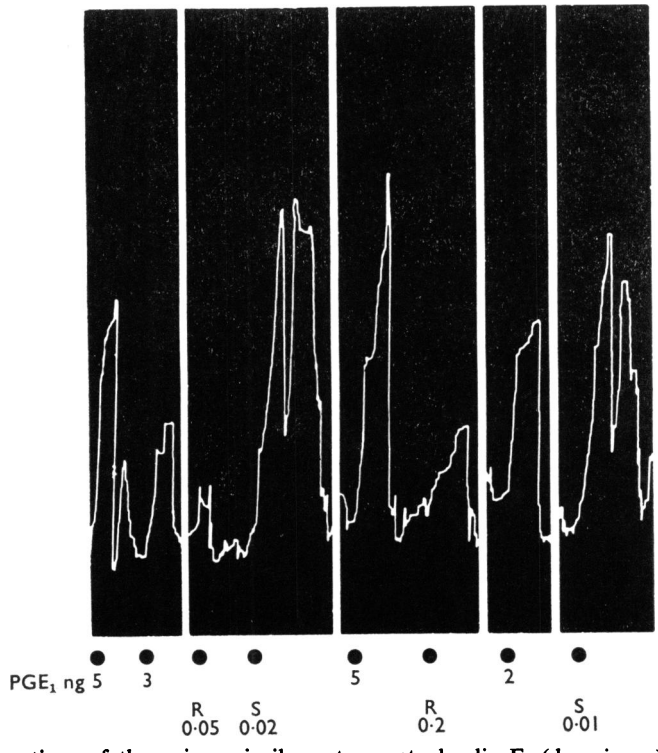

FIG. 1. Contractions of the guinea-pig ileum to prostaglandin E_1 (dose in ng) and to extracts of a resting fat pad (R) and of a fat pad activated by close arterial injection of a lipolytic agent (S) (dose in ml).

were found to be particularly active on tissues which respond well to prostaglandins of the E series, for example guinea-pig ileum, rabbit jejunum. Table 1 shows the results of nine experiments in which the activity was estimated, in equivalents of prostaglandin E_1, in extracts of epigastric fat pads excised during injection or infusion of various fat mobilizing substances. In the last five experiments in which ACTH 1 μg/min was infused close-arterially, the activity was also estimated in the contralateral resting fat pads. In each experiment the activity was considerably greater in the activated fat tissue. Figure 1 illustrates an experiment in which the smooth muscle stimulating activity of an extract of stimulated adipose tissue was considerably more active than that of a similar extract of the contralateral resting adipose tissue.

The responses of the guinea-pig ileum to the extract were not reduced by mepyramine 10^{-7} g/ml but, like those to prostaglandin E_1, were reduced by atropine 5×10^{-8} g/ml. Furthermore, like the prostaglandins of the E series, the extract was not active in contracting the isolated uterus from a rat in oestrus but caused a

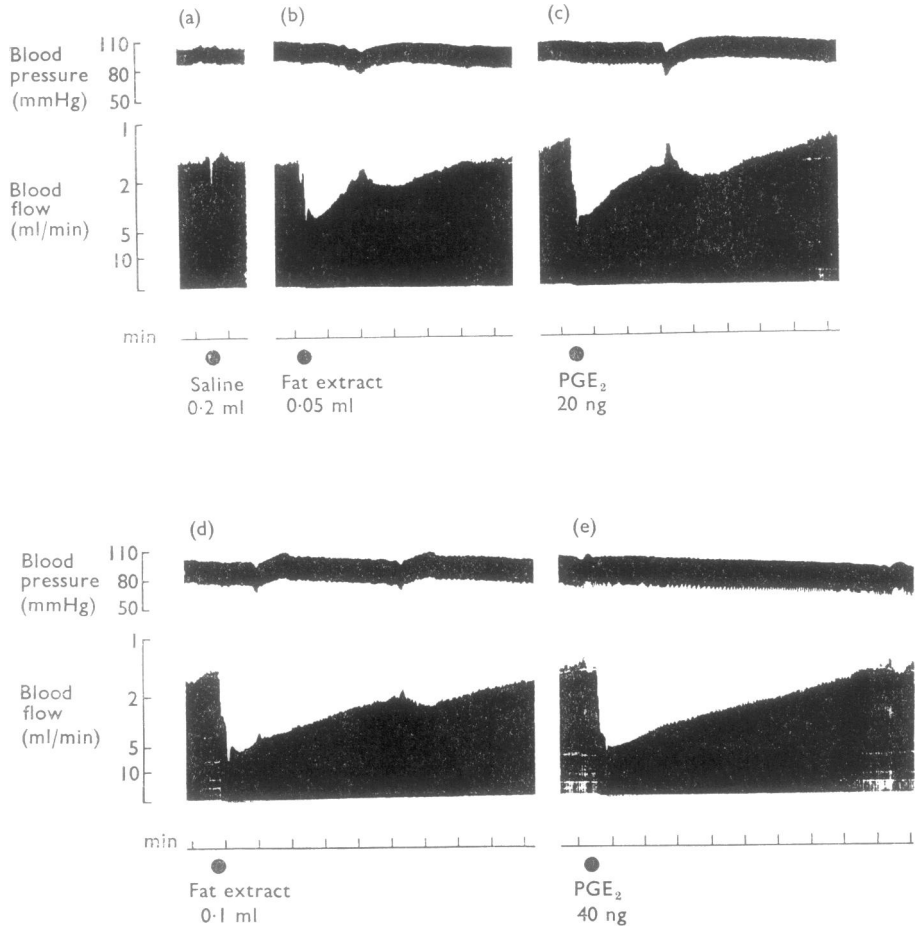

FIG. 2. Continuous record of arterial blood pressure (1 mmHg≡1·333 mbar) (upper record) and blood flow (lower record) through the epigastric adipose tissue of a rabbit 3 kg, anaesthetized with urethane. Close arterial injections were made at (a) saline 0·2 ml, at (b) fat pad extract 0·05 ml, at (c) PGE_2 20 ng, at (d) fat pad extract 0·1 ml and at (e) PGE_2 40 ng.

prolonged vasodilatation when injected close-arterially to the epigastric adipose tissue. Figure 2 illustrates this vasodilator activity of the extract compared with that of prostaglandin PGE_2.

Samuelsson (1963) has described a method for the separation of prostaglandins of the E and F series using silicic acid chromatography. When the extract was passed through the column, biological assay of the eluate revealed that it behaved more like the E series than the F series. Figure 3 shows the results of three experiments in which PGE_1 or PGF_1 or an extract of stimulated adipose tissue was passed through the column and the eluates assayed biologically on the isolated guinea-pig ileum. Although the peak of activity eluted from the column after chromatography of the fat pad extract was not in exactly the same fractions as prostaglandin E_1, it was

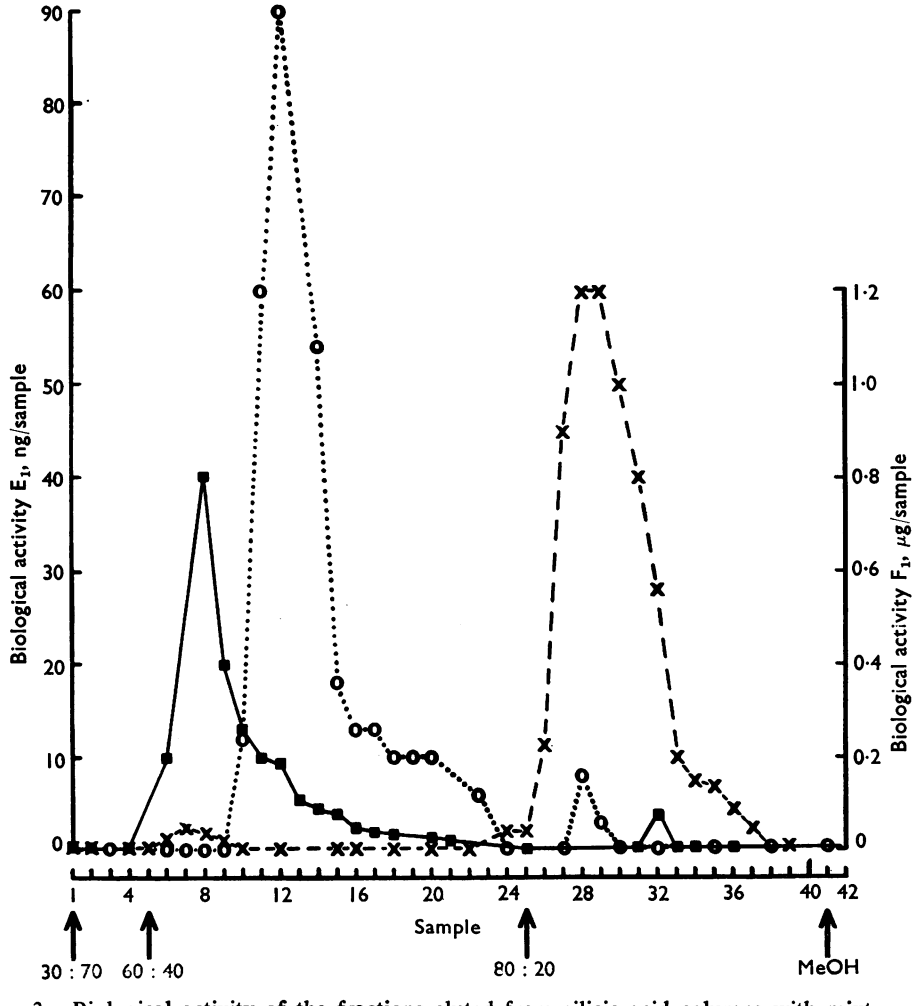

FIG. 3. Biological activity of the fractions eluted from silicic acid columns with mixtures of ethyl acetate and benzene. Three separate columns were run; extract of rabbit epigastric adipose tissue (■——■), solution of PGE_1 (○····○), and solution of PGF_1 (×– – –×). PGE_1 and the active principle in the extract of adipose tissues were eluted with a mixture of ethyl acetate:benzene 60:40, while PGF_1 was not eluted until the proportion of ethyl acetate was increased to 80:20.

eluted by the same solvent and its elution resembled more a prostaglandin of the E series than one of the F series.

The active principle which was separated on silicic acid chromatography was compared with a representative of each of the prostaglandin series E, F and A on several pharmacological preparations. In all these comparisons, the active principle behaved like a prostaglandin E. Table 2 shows the results of a comparison between the extract, PGE_1, PGF_1 and PGA_1 on three different pharmacological preparations. The results have been expressed as indices of discrimination as defined by Gaddum (1955). Although the indices for extract/PGE_1 on the different preparations are not unity as might be expected if the active principle was PGE_1, they are sufficiently near to suggest strongly that the active principle is a prostaglandin of the E series.

A final comparison of the active principle and two representatives of the E series, PGE_1 and PGE_2, was made using thin-layer chromatography. Green & Samuelsson (1964) have described a method of thin-layer chromatography on silica gel impregnated with silver nitrate which separates derivatives that differ from each other by the number of unsaturated bonds in the molecule. In the present experiments it was possible to detect PGE_1 and PGE_2 by the staining technique described by Green & Samuelsson. They were found to have R_F values of 0·90 and 0·68 respec-

TABLE 2. *Indices of discrimination*

	Rabbit jejunum / Guinea-pig ileum	Guinea-pig ileum / Fat pad vdn.	Fat pad vdn. / Rabbit jejunum
Extract/PGE_1	1/3	1/1	1/3
Extract/$PGF_{1\alpha}$	50	5	250
Extract/PGA_1	2	12	23

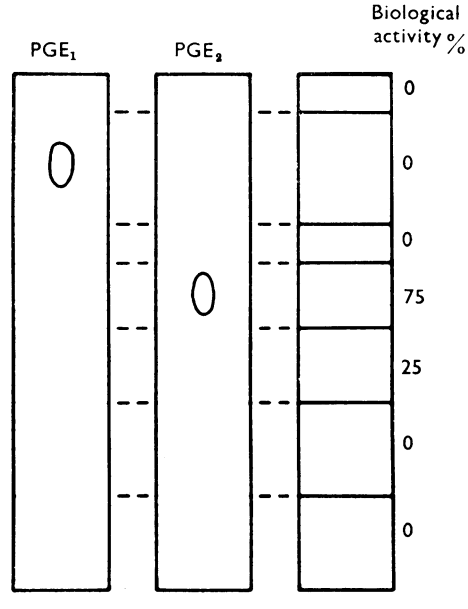

FIG. 4. Diagrammatic representation of the thin-layer chromatography of PGE_1, PGE_2 and extract of adipose tissue. The three principles were run simultaneously in system AII. PGE_1 and PGE_2 were identified by the staining technique and activity of the extract by the biological activity of the eluted fractions.

tively. The active principle of the extract obtained after silicic acid chromatography was subjected to thin-layer chromatography in the same way as the standards but instead of staining the silica gel was cut into sections which corresponded to the areas which stained for PGE_1 and PGE_2 and eluted with methanol. The R_F value of the majority of the biological activity eluted from the sections corresponded closely to PGE_2. The experiment of Fig. 4 is a typical example.

Effect of prostaglandin on FFA release

In the present experiment, the effect of PGE_1 and PGE_2 on the net increase in FFA concentration in the medium induced by ACTH in rabbit epigastric adipose tissue *in vitro* has been examined. As previously found *in vivo* (Lewis & Matthews, 1968), catecholamines caused no increase in FFA in the medium whereas $ACTH^{1-24}$, 1–10 μg/ml, caused a considerable lipolysis.

Table 3 shows the FFA released in experiments in which $ACTH^{1-24}$ 1 μg/ml was incubated with rabbit adipose tissue alone and in the presence of various concentrations of PGE_1 and PGE_2. The prostaglandins did not significantly alter the release of FFA.

Discussion

The activity of adipose tissue resembles that of an endocrine gland in being gradual and prolonged rather than the short intermittent bursts of strong activity which occur in exocrine glands or skeletal muscle. In our earlier investigations (Lewis & Matthews 1968, 1970), we were able to show that during the sustained lipolysis induced by close arterial infusion of fat mobilizing substances into the rabbit epigastric adipose tissue there was an accompanying vasodilatation. The present experiments show that during stimulation of adipose tissue, a substance which is indistinguishable from prostaglandin E_2, a potent vasodilator which when injected produces prolonged vasodilatation is formed or released in the tissue. This substance might be the mediator of functional vasodilatation in adipose tissue.

Shaw & Ramwell (1968) have already shown the presence of prostaglandins (PGE_1, PGE_2 and $PGF_{1\alpha}$) in rat epididymal adipose tissue. They found an increase when the fat was incubated *in vitro* with lipolytic hormones and on stimulation of

TABLE 3. *Effect of prostaglandins PGE_1 and PGE_2 on ACTH-induced lipolysis* in vitro

Incubation mixture	No. of expts	Increase in FFA (mequiv/l.)
ACTH 1 μg/ml	8	385±103
+E_1 0·01	4	370±112
+E_1 0·1	4	365±67
+E_1 1	4	291±89
+E_1 10	3	525±62
+E_1 30	2	605
+E_2 0·01	3	357±51
E_2 0·1	3	267±31
E_2 1	3	248±48
E_2 10	2	470
E_2 30	1	665

Doses of prostaglandins are given in μg/ml.

the epididymal nerve. They suggest that the function of the prostaglandins is to control the release of FFA by inhibition, so constituting a physiological feedback mechanism. However, the reports on the effect of prostaglandins in inhibiting the release of FFA are confusing. It has been reported that PGE_1 inhibits lipolysis in adipose tissue taken from fed rats and man when the tissues are incubated *in vitro* (Steinberg, Vaughan, Nestel & Bergström, 1963 ; Bergström & Carlson, 1965) and in fasted dogs *in vivo* (Bergström, Carlson & Oro, 1964). We had found earlier (Lewis & Matthews, 1968) that PGE_1 appeared to cause a reduction in the concentration of FFA in the venous blood coming from the adipose tissue after close arterial injection of growth hormone to the epigastric fat pad in rabbits. However, this might have been the result of the increase in blood flow following the injection of PGE_1 diluting the plasma FFA rather than reduction of FFA release. In contrast to the studies which indicate that prostaglandins inhibit lipolysis, Bergström, Carlson, Ekelund & Oro (1965), Bergström, Carlson & Oro (1966) and Carlson, Ekelund & Oro (1968) have shown that infusion of PGE_1 into man, fasting or fed, or into dogs, both anaesthetized and unanaesthetized, more frequently causes an increase in plasma FFA levels than a decrease. Furthermore, in the present experiments, no inhibition of lipolysis could be demonstrated when rabbit epigastric adipose tissue was incubated *in vitro* with lipolytic hormones.

Prostaglandins have not only been found to occur in many tissues (Bergström & Samuelsson, 1965), but their release from these tissues has been observed during functional activity. Ramwell, Shaw & Kucharski (1965) detected the release of prostaglandins from the rat diaphragm on stimulation of the phrenic nerve. Further, Ramwell & Shaw (1966) detected prostaglandins (mainly $PGF_{2\alpha}$) in the superfusate of the cat sensory cortex during afferent nerve and transcollosal stimulation and a mixture of PGE_1 and PGF_1 in the fluid surrounding the frog's spinal cord during stimulation.

The function of the released prostaglandins is unknown but the possibility exists that they account for the local vasodilatation which occurs in nervous tissue during activity.

In addition, Davies, Horton & Withrington (1967) found a prostaglandin which they concluded was PGE_2 released into the blood perfusing the isolated dog spleen after stimulation of the splenic nerves. These authors, however, did not report any observations on changes in blood flow during nerve stimulation.

It is possible that a prostaglandin is present in endocrine glands as well as adipose tissue and there too might mediate the vasodilatation accompanying activity. An increase in adrenal blood flow has been described by Holzbauer & Vogt (1957) after administration of ACTH, but these authors as well as Staehelin *et al.* (1965) found that the vasodilatation was not linked to corticosterone secretion. The latter workers, however, observed a parallelism between increased blood flow and output of ascorbic acid. Maier & Staehelin (1968) more recently have suggested that, as well as causing steroidogenesis, ACTH might cleave the tetraenoic acids—the precursors of the prostaglandins—which are present in the adrenals and this would lead to prostaglandin formation. Furthermore, Ramwell, Shaw, Douglas & Poisner (1966) have shown that prostaglandins are released during catecholamine secretion from the adrenal glands although it is not yet clear if there is an increased medullary blood flow during the release.

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