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EFFECT OF L8027, A NEW POTENT INHIBITOR OF PROSTAGLANDIN BIOSYNTHESIS, ON THE METABOLISM AND RESPONSE TO GLUCAGON OF RAT ADIPOSE TISSUE

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Abstract—L8027, a new potent inhibitor of prostaglandin biosynthesis, has been found to increase rat adipose tissue lipolysis *in vitro*. In contrast with acute *in vivo* injection, chronic administration of L8027 to rats enhanced both glucagon-induced and exercise-induced lipid mobilization without significantly affecting blood glucose, plasma insulin and plasma glucagon. These data support the concept that endogenous prostaglandins may be concerned in the regulation of hormone-induced lipolysis and that these compounds may modulate the action of lipolytic hormones by a negative feed-back mechanism.

PROSTAGLANDIN E_1 (PGE_1) is a potent inhibitor of hormone stimulated lipolysis *in vitro* in the adipose tissue of the rat,[1–4] rabbit,[5,6] bird[7–9] and man.[10,11] Since it has been demonstrated that lipolysis, caused by hormones and by nerve stimulation, is accompanied by the release of significant quantities of prostaglandins into the incubation medium,[12–14] it has been suggested that the local release of prostaglandins during lipolysis may modulate the action of the original stimulus by a negative feed-back mechanism.[15,16] Such a concept is supported by the findings of Illiano and Cuatrecasas[17] who demonstrated that blocking endogenous prostaglandin biosynthesis with indomethacin or competitively antagonizing the action of prostaglandins by specific inhibitors, such as 7-oxa-13 prostynoic acid or SC-19220, a dibenzoazepine hydrazide, resulted in enhanced adrenaline- or ACTH-induced lipolysis. The aim of the present study was to investigate the effect of L8027, a new potent inhibitor of prostaglandin biosynthesis[18] on basal, glucagon-stimulated and exercise-stimulated adipose tissue lipolysis in rats. The results of both *in vitro* and *in vivo* experiments, support the concept of Illiano and Cuatrecasas[17] that endogenous prostaglandins may be concerned with the regulation of hormone-induced lipolysis.

MATERIALS AND METHODS

L8027, a pyridyl indolyl ketone (Fig. 1) was obtained from Labaz, S.A. 1120 Bruxelles; recrystallized monocomponent glucagon was obtained from Novo Industri A.S. Copenhagen and prostaglandin E_1 from Upjohn, Kalamazoo. All reagents used were of analytical grade.

Male albino rats of the Wistar strain were used in these studies. Unless otherwise

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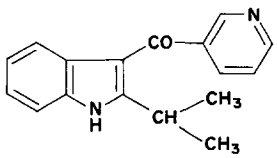

FIG. 1. Formula of L8027.

stated they were fed on pellets (Hesby, Liège) and fasted overnight prior to experiments. Water was given *ad libitum*. Animals were killed by a blow on the head and blood samples immediately obtained by cardiac puncture using heparinized syringes. Blood glucose was determined using a glucose oxidase method.[19] The plasma concentration of free fatty acids (FFA) was determined according to the method of Dole and Meinertz.[20] For hormone determinations, 10% Trasylol® was added to the blood samples. Plasma insulin was assayed in duplicate according to the method of Quabbe,[21] using rat insulin as standard. Plasma glucagon was determined in duplicate using a radioimmunoassay technique[22] specific for pancreatic glucagon.* Epididymal fat pads were removed as rapidly as possible after the death of the animal; pieces weighing 100–110 mg were incubated in a modified[23] Krebs–Ringer bicarbonate buffer[24] in an atmosphere of 95% O_2 and 5% CO_2. The incubation medium was enriched with glucose (100 mg%) and crystalline bovine albumin (4%, Armour Co). In some experiments, the incubation medium was supplemented with appropriate concentrations of PGE_1, L8027 or glucagon. For this purpose, an appropriate volume of PGE_1 solution containing 500 μg per ml of 95% ethanol was added to the experimental incubation medium. Similarly, L8027 was added from a stock solution containing 17·6 mg per ml of pure ethanol and glucagon from a stock solution containing 100 μg per ml of glycine buffer (pH 8·8). Glycerol in the medium was measured enzymatically,[25] FFA by microtitration[20] and glucose using an autoanalyzer.[26] In some experiments, the glycerol and FFA content of adipose tissue prior to incubation was determined on homogenates of tissue using the same methods.

In some experiments, rats were pretreated with L8027. In acute experiments, L8027 (10 mg/ml in ethanol) was injected intraperitoneally, 1 hr before sacrifice at a dose of 10 mg/kg body wt; control rats received an appropriate volume of solvent alone. In chronic experiments, L8027 was suspended in 5% gum arabic at a concentration of 0·5 per cent and given at a dose of 50 mg/kg body wt per day for 15 days by gastric intubation (a fresh suspension was prepared every 2 days); control rats received similar volumes of gum arabic only. The final dose was given on the morning of the experiment, 2 hr before sacrifice. In one experiment, treated and control animals were submitted to standardized muscular exercise consisting of a 60 min forced-swim in tepid water following a procedure described previously.[27,28]

RESULTS

Inhibition of glucagon-induced lipolysis by PGE_1. As shown in Fig. 2, PGE_1 was a potent inhibitor of glucagon-induced lipolysis; 0·05 μg/ml of PGE_1 caused a 50 per cent inhibition of the lipolysis due to 0·01 μg/ml of glucagon.

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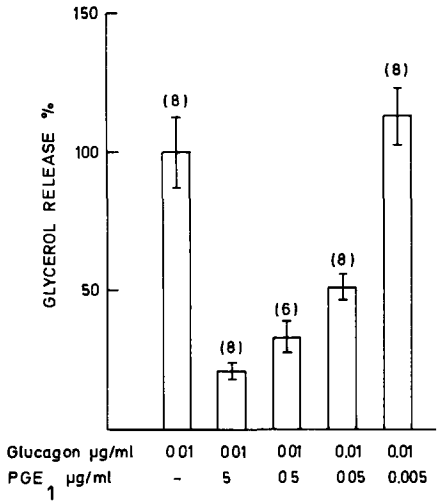

FIG 2. Effect of prostaglandin PGE_1 on the glucagon-induced glycerol release from rat epididymal adipose tissue incubated *in vitro*. Results are expressed in % of the glycerol release obtained in the presence of 0·010 μg/ml of glucagon. The asterisk indicates a P value less than 0·01. Results are given as mean ± S.E.M. n = number of determinations.

Effect of L8027 on basal lipolysis in vitro. As illustrated in Table 1, L8027 exerted a significant stimulatory effect on basal lipolysis. Concentrations of 10^{-4} and 10^{-3} M were active and for these two concentrations the effect was dose-related.

Effect of acute in vivo *administration L8027 on basal and glucagon-stimulated lipolysis.* When administered to rats at a dose of 1 mg/100 g body wt intraperitoneally 1 hr before sacrifice, L8027 did not affect significantly basal or glucagon-stimulated glycerol release from epididymal adipose tissue *in vitro*. In the absence of glucagon, basal glycerol release was 5·83 ± 0·66 (n = 8) and 5·69 ± 0·74 (n = 8) μM/g/2 hr in control and in L8027 injected rats, respectively. The corresponding values in the presence of 1 μg/ml glucagon were 17·19 ± 2·00 (n = 8) in controls and 16·26 ± 2·19 (n = 8) μM/g/2 hr in L8027 injected animals.

Effect of chronic administration of L8027 on body weight, blood glucose, plasma FFA, insulin and glucagon and on adipose tissue metabolism of rats. As illustrated in Fig. 3, the gain in weight of rats treated with L8027 by mouth for 15 days was not significantly affected. Similarly, blood glucose, plasma FFA, plasma insulin, plasma

TABLE 1. EFFECT OF L8027 ON *in vitro* ADIPOSE TISSUE LIPOLYSIS

	Glycerol release (μmoles/g/2 hr)	Statistical comparison with control
Control	6·84 ± 0·53 (7)	—
L8027 10^{-7} M	6·50 ± 0·75 (7)	NS
L8027 10^{-6} M	6·91 ± 0·70 (7)	NS
L8027 10^{-5} M	7·62 ± 0·57 (7)	NS
L8027 10^{-4} M	8·78 ± 0·33 (6)	$P < 0·02$
L8027 10^{-3} M	12·78 ± 0·9 (9)	$P < 0·01$

Results are expressed as mean ± S.E.M. The number of determinations is indicated in parentheses. Significance was determined by Student's *t*-test. NS = not significant.

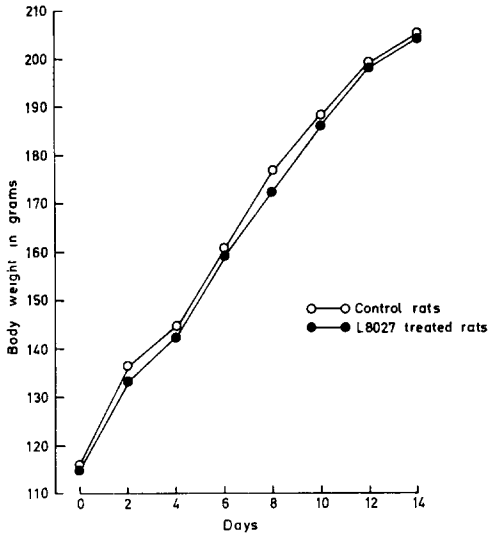

FIG 3. Body weight of controls and chronically L8027 treated rats.

glucagon and adipose tissue glycerol and FFA content were not significantly different from controls. In 7 controls and 7 L8027 treated rats, the following values were obtained respectively: blood glucose 67 ± 2·8 and 72·3 ± 2·4 mg %; plasma FFA 975 ± 89 and 918 ± 111 μEq $^{0}/_{00}$; plasma insulin 17·5 ± 1·2 and 17·1 ± 1·2 μU/ml; plasma glucagon 56·8 ± 31·0 and 73·9 ± 19·3 pg/ml; adipose tissue glycerol content 8·04 ± 0·53 and 8·30 ± 1·09 μmol/g and adipose tissue FFA content 7·14 ± 0·95 and 6·96 ± 1·21 μEq/g. Basal glycerol and FFA release from adipose tissue incubated *in vitro* were comparable in controls and L8027 treated rats, but glucose uptake was significantly reduced in the latter (Table 2). Although glucagon stimulated glycerol release was significantly greater in adipose tissue from L8027-treated rats, the increase in FFA release was not significant (Table 3).

Effect of chronic administration of L8027 on blood glucose, plasma FFA, insulin and glucagon and on adipose tissue metabolism of exercised-rats. In rats submitted to a 60 min forced-swim, 15 days pretreatment with L8027 did not affect blood glucose,

TABLE 2. EFFECT OF CHRONIC ADMINISTRATION OF L8027 ON BASAL GLYCEROL RELEASE, FFA RELEASE AND GLUCOSE UPTAKE BY EPIDIDYMAL ADIPOSE TISSUE

	Control rats	L8027 treated rats	Statistical Comparison
Glycerol release (μmoles/g/2 hr)	7·75 ± 0·89 (9)	6·36 ± 0·71 (9)	NS
FFA release (μEq/g/2 hr)	5·07 ± 1·00 (9)	7·07 ± 0·92 (9)	NS
Glucose uptake (mg/g/2 hr)	3·28 ± 0·24 (9)	2·11 ± 0·16 (9)	$P < 0·01$

Results are expressed as mean ± S.E.M. One piece of epididymal adipose tissue was taken from each rat. The number of animals is indicated in parentheses. Treated rats received L8027 per os. (50 mg/kg for 15 days, see text); control rats received solvent alone. Significance was determined by Student's *t*-test. NS = not significant.

TABLE 3. EFFECT OF CHRONIC ADMINISTRATION OF L8027 ON GLUCAGON-INDUCED LIPOLYSIS *in vitro*

	'Glucagon effect' 0·5 μg/ml	
	Control rats	L8027 treated rats
Glycerol release (μmoles/g/2 hr)	+2·83 ± 1·19 (13) (P < 0·05)	+8·03 ± 1·73 (14) (P < 0·01)
	P < 0·05	
FFA release (μEq/g/2 hr)	+3 85 ± 0·99 (15) (P < 0·01)	+6·16 ± 2·15 (14) (P < 0·01)
	NS	

Results are expressed as 'glucagon effect' i.e. change in glycerol or FFA release induced by glucagon on epididymal adipose tissue originating from the same animal (paired comparison). Treated rats received L8027 per os. (50 mg/kg for 15 days, see text); control rats received solvent alone. The number of animals is indicated in parentheses Significance was determined by Student's *t*-test. NS = not significant.

TABLE 4. EFFECT OF CHRONIC ADMINISTRATION OF L8027 ON BLOOD GLUCOSE, PLASMA FFA, INSULIN AND GLUCAGON AND ON GLYCEROL AND FFA CONTENT OF ADIPOSE TISSUE IN RATS SUBMITTED TO A 60 min-FORCED SWIM

	Control rats	L8027 treated rats	Statistical comparison
Blood glucose (mg %)	82·3 ± 6·5 (8)	83·8 ± 2·5 (7)	NS
Plasma FFA (μEq °/₀₀)	1200 ± 91 (8)	1312 ± 153 (8)	NS
Plasma insulin (μU/ml)	17·9 ± 2·4 (8)	19·3 ± 1·8 (8)	NS
Plasma glucagon (pg/ml)	166 ± 28 (7)	132 ± 23 (8)	NS
Adipose tissue glycerol content (μmoles/g)	8·30 ± 0·31 (8)	9 71 ± 0·51 (8)	P < 0·05
Adipose tissue FFA content (μEq/g)	13·89 ± 1·05 (8)	14·87 ± 0·85 (8)	NS

Results are expressed as mean ± S.E.M. The number of rats is indicated in parentheses. Treated rats received L8027 per os. (50 mg/kg for 15 days, see text); control rats received solvent alone. All animals were submitted to a 60 min-forced swim. Significance was determined by Student's *t*-test. NS = not significant.

TABLE 5. EFFECT OF CHRONIC ADMINISTRATION OF L8027 ON GLYCEROL RELEASE, FFA RELEASE AND GLUCOSE UPTAKE BY ADIPOSE TISSUE OF RATS SUBMITTED TO A 60 min-FORCED SWIM

	Control rats	L8027 treated rats	Statistical comparison
Glycerol release (μmoles/g/2 hr)	4·87 ± 0·59	8·53 ± 0·68	P < 0·01
FFA release (μEq/g/2 hr)	2·09 ± 0·46	5·59 ± 0·88	P < 0·01
Glucose uptake (mg/g/2 hr)	2·22 ± 0·15	1·59 ± 0·17	P < 0·01

Results are expressed as mean ± S.E.M. Two pieces of epididymal adipose tissue were taken from each rat. Eight animals were thus studied in each series, n = 16. Treated rats received L8027 per os. (50 mg/kg for 15 days, see text); control rats received solvent alone. All animals were submitted to a 60 min-forced swim. Significance was determined by Student's *t*-test.

plasma FFA, plasma insulin or plasma glucagon. Adipose tissue glycerol content was slightly (+17 per cent) but significantly increased (Table 4). As shown in Table 5, glycerol and FFA release was significantly increased and glucose uptake significantly

reduced when epididymal fat pads from L8027-treated exercised rats were incubated *in vitro*.

DISCUSSION

The present findings support the concept[17] that endogenous prostaglandins may be involved in the regulation of hormone-induced lipolysis. As reported by Deby *et al.*,[18] L8027, a newly synthesized anti-inflammatory compound, has been found to inhibit markedly the synthesis of prostaglandins from tritiated arachidonid acid by an homogenate of sheep seminal vesicles. In this system, L8027 at a concentration of 0·014 mM exerted a 50 per cent inhibition of prostaglandin biosynthesis, while concentrations of 0·95 mM and 0·80 mM of phenylbutazone and acetylsalicylic acid respectively are necessary to induce a similar inhibition. Therefore, this compound appears at least 50 times more active than phenylbutazone and aspirin with respect to the inhibition of prostaglandin biosynthesis. Like indomethacin, another inhibitor of prostaglandin synthesis,[29,30,31] L8027 added *in vitro* stimulates lipolysis in the absence of exogenous lipolytic agents. If, in fact, this effect is linked to the inhibitory action of this compound on PG biosynthesis, such an observation may support the assertion of Illiano and Cuatrecasas[17] that prostaglandins may "assist in the fine regulation of lipolytic processes in normal, basal conditions, and that their function is not restricted to special circumstances (hormone-induced lipolysis) requiring dramatic feed-back controls". Chronic administration of L8027 increases the *in vitro* glucagon-induced lipolysis. This finding is comparable to the observation that indomethacin enhances both adrenaline- and ACTH-induced lipolysis[17] and again suggests that endogenous prostaglandins may locally regulate hormone-induced lipolysis, evoked here by glucagon. This observation is confirmed by the original finding that chronic treatment with L8027 also induces stimulation of exercise-induced lipolysis. It must be stressed that, under these conditions of chronic administration of L8027, basal lipolysis does not appear to be significantly affected. Muscular exercise in rats is a condition in which the mobilization of free fatty acids from the adipose tissue stores results both from stimulation of lipolysis and inhibition of FFA (re) esterification.[28,32] It is associated with several hormonal changes including rises in plasma corticosterone,[27] glucagon[28,33] and catecholamines[34] and diminution of insulin production.[27,35] The present data clearly indicate that both glycerol and FFA release from adipose tissue of rats submitted to muscular exercise are markedly increased by previous chronic administration of L8027, these metabolic changes being associated with a decrease in glucose uptake, a phenomenon which is an exaggeration of a process which already occurs in muscular exercise itself.[28,32] The stimulation of lipolysis in L8027-treated exercised rats is reflected by the significant rise in adipose tissue glycerol content and the tendency to a rise in blood FFA (although the latter is not statistically significant). It is tempting to consider that these effects may be due to an inhibition of prostaglandin biosynthesis within the adipose tissue with a subsequent suppression or reduction of a naturally occurring mechanism inhibiting lipolysis and leading to enhancement of exercise-induced mobilization of lipids.

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