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RELATIONSHIP BETWEEN LIPOLYSIS AND STORAGE OF CORTICOSTERONE IN ADIPOSE TISSUE

A. BIZZI, A. M. TACCONI and S. GARATTINI

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milano, Italy

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Abstract—Corticosterone added *in vitro* accumulates in the epididymal adipose tissue in an amount proportional to its concentration in the medium, and inversely proportional to the rate of lipolysis. This effect requires the presence of albumin in the medium. Determinations of partition coefficients between buffer phosphate and oil indicate that corticosterone is retained in the water phase when albumin is added. The simultaneous presence of albumin and FFA further decreases the entry of corticosterone in the oil phase.

These results are interpreted in terms of the degree of binding of corticosterone to albumin.

IT HAS been well established that glucocorticoids exhibit a so-called permissive role on lipolytic activity in adipose tissue.[1,2] The mechanism by which glucocorticoids promote lipolysis in adipose tissue seems to be at least partially related to an impaired utilization of glucose by adipose tissue[3,4] with a consequent decrease in the rate of fatty acid esterification. Although a direct effect on lipolysis has also been reported[5] the site of action of glucocorticoids is not yet known. Whether it is necessary for these compounds to enter the cells in adipose tissue in order to elicit their effects, or whether their permissive role is secondary to a release of other hormones or to some metabolic changes occurring in cells other than adipose tissue has not been established.

This investigation was designed to study the entry of corticosterone into rat epididymal adipose tissue, and to determine the relation between the accumulation of corticosterone in such tissue and the rate of lipolysis.

MATERIALS AND METHODS

Male, Sprague–Dawley rats weighing about 200 g, were used.

Incubation of adipose tissue

Epididymal adipose tissue obtained from unfasted or overnight fasted rats (as indicated under results) was chopped, pooled and distributed (250 mg) in flasks containing Krebs–Ringer phosphate pH 7·4 with one half of the recommended Ca^{2+} and 3% bovine serum albumin (fract V, Pentex).

Corticosterone, glucose and noradrenaline bitartrate were added from concentrated stock solutions to provide the final concentrations reported in the results.

The flasks were incubated at 37° for 2 hr with gentle shaking. At the end of the incubation period, adipose tissue and medium were immediately separated by filtration.

Adipose tissue fragments were carefully washed with incubation medium, blotted dry on filter paper and weighed. Determinations of corticosterone and triglycerides were made in adipose tissue; FFA were measured in the medium.

Preloading of adipose tissue with corticosterone

Epididymal adipose tissue, chopped and pooled was incubated as previously described. The concentration of corticosterone was 6 μg/ml; the ratio between the weight of adipose tissue (250 mg) and the volume of the incubation medium was 1:16.

At the end of incubation period, adipose tissue was separated from the medium by filtration. Adipose tissue was carefully washed with medium free of corticosterone, blotted dry and distributed (250 mg) in flasks containing 4 ml of medium free of corticosterone. The second incubation was carried out at 37° for periods varying from 5 to 120 min.

At the end of the incubation, adipose tissue was treated as described in the previous section.

Partition coefficient between oil and aqueous phase

Three kinds of aqueous phases were used:
(1) Krebs phosphate Ringer pH 7·4
(2) Krebs phosphate Ringer pH 7·4 + 3% albumin
(3) Krebs phosphate Ringer pH 7·4 + 3% albumin + sodium oleate (5 or 20 μequiv.).
Corticosterone 6 μg/ml was added to these media.

Ten ml of these media were shaken with 10 ml of arachis oil. Oil and aqueous phases were then separated by centrifugation. Corticosterone was measured both in oil and in aqueous phases.

Chemical methods

Determinations of corticosterone were carried out according to the method of Frankel *et al.*[6]

Triglycerides were measured according to the procedure described by Van Handel *et al.*[7] and glycerol according to Wieland.[8]

FFA were determined by the method of Dole[9] with minor modifications, i.e. a concentrated solution of sodium hydroxide was diluted in ethanol and the alcoholic solution of bromothymol blue was diluted with heptane. These modifications permitted the titration to be made in one step.

RESULTS

Epididymal adipose tissue was incubated in a medium containing increasing amounts of corticosterone.

Results reported in Table 1 indicate that the extent of accumulation of corticosterone was related to the amount of the compound present in the medium.

This relation was irrespective of the experimental conditions. In fact it occurred when adipose tissue was obtained from unfasted or overnight-fasted rats, and even when lipolytic activity was stimulated by noradrenaline.

However, the extent of the accumulation differed with experimental conditions. In

TABLE 1. ACCUMULATION OF CORTICOSTERONE IN THE EPIDIDYMAL ADIPOSE TISSUE UNDER CONDITION OF BASAL OR NORADRENALINE STIMULATED LIPOLYSIS

Expt.	Experimental condition	CS added to the medium (μg/ml)	Saline			Noradrenaline 0·25 (μg/ml)		
			FFA (μequiv./g ± S.E.)	CS (μg/g ± S.E.)	TG (mg/g ± S.E.)	FFA (μequiv./g ± S.E.)	CS (μg/g± S.E.)	TG (mg/g ± S.E.)
1	Unfasted	0·5	0·51 ± 0·05	0·78 ± 0·03	620 ± 10	12·8 ± 0·6	0·56 ± 0·06*	620 ± 40
		1	0·40 ± 0·08	1·64 ± 0·06	640 ± 4	13·9 ± 0·4	0·88 ± 0·01†	670 ± 20
		2	0·46 ± 0·13	2·94 ± 0·08	640 ± 20	13·4 ± 1·2	1·80 ± 0·05†	660 ± 10
		4	0·98 ± 0·19	4·91 ± 0·51	670 ± 20	15·5 ± 0·4	2·83 ± 0·07†	650 ± 30
		6	0·52 ± 0·13	11·95 ± 0·52	650 ± 20	16·7 ± 0·7	5·84 ± 0·18†	660 ± 20
2	Fasted overnight	0·5	8·9 ± 1·4	0·39 ± 0·06	580 ± 40	21·2 ± 0·8	0·15 ± 0·05*	540 ± 10
		1	8·8 ± 0·3	0·69 ± 0·05	560 ± 4	25·6 ± 0·6	0·42 ± 0·02†	550 ± 10
		2	9·2 ± 0·4	1·68 ± 0·10	490 ± 20	21·7 ± 0·7	1·15 ± 0·08†	540 ± 70
		4	9·4 ± 0·5	3·84 ± 0·20	640 ± 30	21·1 ± 0·6	2·43 ± 0·03†	600 ± 40
		6	9·3 ± 0·8	5·31 ± 0·31	580 ± 30	20·5 ± 1·1	4·04 ± 0·50†	580 ± 20

Unfasted rats had free access to the food till the sacrifice. Corticosterone and noradrenaline bitartrate were added to the medium. Corticosterone (CS) and triglycerides (TG) were measured in adipose tissue; free fatty acids (FFA) in the medium. Each figure is the mean of four determinations.

* $P < 0·01$ with respect to saline.

† $< 0·05$ with respect to saline.

fact, it was significantly decreased when noradrenaline was present in the medium (see Table 1) or when adipose tissue from fasted rats was compared to adipose tissue from fed rats (see Table 2).

TABLE 2. ACCUMULATION OF CORTICOSTERONE IN ADIPOSE TISSUE FROM UNFASTED AND OVERNIGHT FASTED RATS

Experimental conditions	CS added to the medium (μg/ml)	FFA (μequiv./g $\pm$ S.E.)	CS (μg/g $\pm$ S.E.)
Unfasted	4	1·84 $\pm$ 0·30	7·14 $\pm$ 0·28
	6	1·30 $\pm$ 0·30	10·31 $\pm$ 0·33
Fasted	4	6·84 $\pm$ 0·13	5·85 $\pm$ 0·16*
	6	7·54 $\pm$ 0·56	8·52 $\pm$ 0·23*

Unfasted rats had free access to the food until the sacrifice. Free fatty acids (FFA) were measured in the medium, corticosterone (CS) in the adipose tissue.
* $P < 0·01$ with respect to unfasted with the same concentration of CS.

On the contrary, the accumulation of the corticoid was slightly increased when the release of FFA was reduced by adding glucose to the incubation medium (see Table 3). These data suggested the occurrence of an inverse relationship between the release of FFA from adipose tissue and corticosterone accumulation in the same tissue.

In fact, when concentrations of corticosterone in adipose tissue (in this case the corticosterone concentration in the medium was 6 μg/ml) were plotted against the amounts of FFA released by this tissue, a negative significant ($P < 0·01$) linear relationship was obtained (Fig. 1).

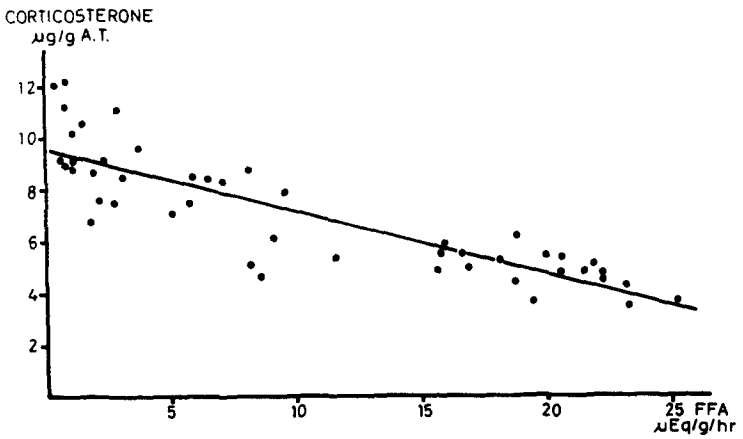

FIG. 1. Relationship between lipolysis and corticosterone in the adipose tissue (corticosterone 6 μg/ml). Data obtained from different experiments (fasted and unfasted animals).

$$y = 9·59 - 0·24\ x$$
$$r = -0·86$$
$$P < 0·01$$

TABLE 3. ACCUMULATION OF CORTICOSTERONE IN THE EPIDIDYMAL ADIPOSE TISSUE FROM FASTED RATS IN PRESENCE OR IN ABSENCE OF GLUCOSE

CS added to the medium (μg/ml)	Saline			Glucose (2 μmoles/ml)		
	FFA (μequiv./g $\pm$ S.E.)	CS (μg/g $\pm$ S.E.)	TG (mg/g $\pm$ S.E.)	FFA (μequiv./g $\pm$ S.E.)	CS (μg/g $\pm$ S.E.)	TG (mg/g $\pm$ S.E.)
0·5	5·2 $\pm$ 0·1	0·65 $\pm$ 0·04	640 $\pm$ 20	1·4 $\pm$ 0·2	0·86 $\pm$ 0·05*	609 $\pm$ 19
1	5·9 $\pm$ 0·2	1·31 $\pm$ 0·10	580 $\pm$ 10	1·5 $\pm$ 0·4	1·38 $\pm$ 0·06	610 $\pm$ 50
2	6·0 $\pm$ 0·4	2·37 $\pm$ 0·05	610 $\pm$ 20	0·9 $\pm$ 0·2	3·01 $\pm$ 0·19*	676 $\pm$ 34
4	5·6 $\pm$ 0·2	4·29 $\pm$ 0·10	600 $\pm$ 10	1·9 $\pm$ 0·8	5·07 $\pm$ 0·27*	644 $\pm$ 15
6	6·6 $\pm$ 0·3	8·58 $\pm$ 0·11	630 $\pm$ 10	3·2 $\pm$ 0·2	10·99 $\pm$ 0·50*	611 $\pm$ 22

Rats were fasted overnight. Corticosterone and glucose were added to the medium. Free fatty acids (FFA) were measured in the medium; corticosterone (CS) and triglycerides (TG) in adipose tissue. Each figure is the mean of four determinations.

* $P < 0.05$ with respect to saline.

TABLE 4. RETENTION OF CORTICOSTERONE IN THE ADIPOSE TISSUE PRELOADED WITH CORTICOSTERONE AND INCUBATED IN PRESENCE OF SALINE OR NORADRENALINE

Incubation time (min)	Saline			Noradrenaline 0·25 μg/ml		
	FFA (μequiv./g ± S.E.)	CS (μg/g ± S.E.)	TG (mg/g ± S.E.)	FFA (μequiv./g ± S.E.)	CS (μg/g ± S.E.)	TG (mg/g ± S.E.)
0	—	8·87 ± 0·17	580 ± 28	—	8·87 ± 0·17	580 ± 28
5	0·29 ± 0·18	3·84 ± 0·15	600 ± 26	0·91 ± 0·32	3·27 ± 0·28	620 ± 20
15	0·22 ± 0·12	2·18 ± 0·15	611 ± 25	3·80 ± 0·19	2·19 ± 0·18	590 ± 17
30	0·74 ± 0·11	1·79 ± 0·18	613 ± 18	5·24 ± 0·66	1·49 ± 0·15*	616 ± 17
0	—	6·59 ± 0·28	518 ± 10		6·59 ± 0·28	518 ± 10
30	0·73 ± 0·21	1·71 ± 0·14	595 ± 25	5·57 ± 0·20	1·01 ± 0·18*	641 ± 57
60	1·05 ± 0·09	1·28 ± 0·12	586 ± 55	9·66 ± 0·58	0·76 ± 0·10*	665 ± 50
120	1·16 ± 0·13	1·11 ± 0·05	602 ± 9	21·27 ± 1·52	0·37 ± 0·10*	600 ± 20

Epididymal adipose tissue obtained from unfasted rats was preloaded with corticosterone (6 μg/ml) by a preincubation for 120 min and it was subsequently incubated in a corticosterone free medium for the times indicated in the table. Free fatty acids (FFA) were measured in the medium; corticosterone (CS) and triglycerides (TG) in the adipose tissue.

* $P < 0.05$ in respect to saline.

TABLE 5. ACCUMULATION OF CORTICOSTERONE IN EPIDIDYMAL ADIPOSE TISSUE INCUBATED WITH AND WITHOUT ALBUMINE

Experimental conditions	CS added to the medium (μg/ml)	Saline			Noradrenaline 0·25 μg/ml × 2		
		FFA (μequiv./g)	Glycerol (μM/g ± S.E.)	CS (μg/g)	FFA (μequiv./g)	Glycerol (μM/g ± S.E.)	CS (μg/g)
Buffer phosphate	6	0	3·7 ± 0·2	13·0 ± 0·6	0	7·9 ± 0·1	14·6 ± 0·5
Buffer phosphate + albumine 3%	6	1·36	3·0 ± 0·2	8·7 ± 0·2	15·3 ± 0·4	11·8 ± 0·5	4·2 ± 0·6*

Epididymal adipose tissue taken from unfasted rats was incubated for 2 hr. Noradrenaline and corticosterone (CS) were added to the medium. FFA and glycerol were measured in the medium, CS in the adipose tissue at the end of incubation. Each figure is the mean of four determinations.

* $P < 0·01$ with respect to saline.

TABLE 6. PARTITION OF CORTICOSTERONE BETWEEN OIL AND BUFFER PHOSPHATE IN PRESENCE OF ALBUMINE AND FFA

Expt. No.	Group	Additions to the buffer			Corticosterone (μg/10 ml ± S.E.)		% Total Recovery	Partition coefficient Oil–water
		Corticosterone (μg)	Albumine (%)	FFA (μequiv.)	Buffer	Oil		
1		10	—	—	2·75 ± 0·01	6·2 ± 0·18	89	2·25
		20	—	—	5·34 ± 0·30	11·7 ± 0·35	85	2·19
		10	3	—	4·91 ± 0·28	4·1 ± 0·18	90	0·84
		20	3	—	9·13 ± 0·31	8·6 ± 0·21	89	0·94
2	a	10	—	—	2·28 ± 0·06	7·09 ± 0·24	94	3·12
	b	10	3	5	6·80 ± 0·29	2·93 ± 0·17	96	0·44*
	c	10	3	20	7·37 ± 0·10	2·52 ± 0·10	99	0·34†
	d	10	3	20	8·00 ± 0·11	2·12 ± 0·00	101	0·26*
3		10	—	—	2·84 ± 0·09	7·25 ± 0·18	100	2·55
		10	—	20	2·84 ± 0·11	7·25 ± 0·28	100	2·55

Corticosterone was added to 10 ml of Krebs–Ringer Phosphate pH 7·4 with half the amount of Ca^{2+}. Ten ml of buffer was subsequently shaken with 10 ml of arachis oil for 10 min. The oil and aqueous phases were then separated by centrifugation and the determinations were carried out in both phases.

In experiment Nos. 2 and 3 the phases were centrifuged at 13,000 rev/min in a Servall centrifuge. Figures are the mean of four determinations.

* $P < 0·01$ in group d versus group b (expt. 2).

† $P < 0·05$ in group c versus group b (expt. 2).

Table 4 shows that part of the observed difference may be due to the fact that the degree of corticosterone retention in adipose tissue was affected by the rate of lipolysis.

When corticosterone-preloaded tissue was incubated in a medium containing noradrenaline, less compound was usually left in the tissue than when the same preloaded adipose tissue was incubated in a medium without noradrenaline. In order to establish if the different rates in corticosterone accumulation were due to medium composition (albumin, FFA) or to changes occurring in the tissue during stimulation of lipolysis, epididymal adipose tissue from unfasted rats was incubated in an albumin free medium. In this case FFA are not released in the medium and therefore glycerol release was measured in order to have an indication of the rate of lipolysis.

Data reported in Table 5 show that the presence of albumin reduced the accumulation of corticosterone in the adipose tissue and that the rate of lipolysis did not affect the entry of corticosterone when albumin was absent.

Also the partition coefficient of corticosterone between oil and Krebs–Ringer phosphate was considerably decreased by the addition of albumin (Table 6).

When albumin and FFA were added simultaneously, the coefficient was further decreased (FFA without albumin were unable to change the partition coefficient of corticosterone).

DISCUSSION

The data presented in this paper show that corticosterone added *in vitro* accumulates in the epididymal adipose tissue. The rate of accumulation was directly related to the concentration of corticosterone in the medium, but it was inversely related to the concentration of FFA in the medium.

This effect was observed in tissues from fasted and unfasted rats and it occurred irrespective of the experimental conditions, which are known to change the rate of lipolysis (i.e. noradrenaline and glucose). The retention of corticosterone in adipose tissue, preloaded with the hormone, was also reduced when the rate of FFA release was increased by noradrenaline.

Further experiments established that the critical factor in reducing the accumulation of corticosterone in adipose tissue was albumin. In fact, when the degree of lipolysis was similar, more corticosterone was present in the adipose tissue incubated with an albumin free medium. Conversely, when no albumin was present in the incubation medium, accumulation of corticosterone in the adipose tissue was unmodified even when the lipolysis was doubled.

Similar results were obtained when the partition coefficient of corticosterone between oil and medium was studied. It was shown that the presence of albumin reduced the passage of corticosterone in the oil and this effect was enhanced by the simultaneous presence of FFA although the latter were inactive.

These findings suggest that binding may occur between corticosterone and albumin, which would be enhanced by the presence of lipophilic groups such as formed when FFA bind to albumin. This binding of corticosterone to albumin would therefore compete for the entry of the hormone both in the oil phase or in the adipose tissue.

The rate of lipolysis would therefore change the accumulation of corticosterone in the adipose tissue by affecting its binding to albumin.

It is interesting to recall that, in contrast, other drugs such as phenobarbital[10] and diphenylhydantoin[11] accumulate in the adipose tissue to an extent directly related to

lipolysis. At present it is difficult to speculate on the possible physiological significance of the mechanism for the accumulation of corticosterone in the adipose tissue *in vitro*. The knowledge of the factors affecting storage of drugs in the adipose tissue may however be important for understanding kinetic aspects in the distribution of drugs in the whole body.

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