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EFFECTS OF ANTILIPOLYTIC AGENTS ON GLUCOSE UTILIZATION BY ADIPOSE TISSUE

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Abstract—There is a biphasic response in the rate of glucose utilization when epididymal adipose tissue from fasted rats pretreated with lipolysis inhibitors, nicotinic acid or 5-carboxy-3-methylpyrazole (5C3MP), is incubated *in vitro*. Epididymal adipose tissue taken 10 min after administration of both agents, converted more glucose to CO_2 and incorporated more glucose into lipids. The reverse was seen when adipose tissues were incubated 1–6 hr after treatment. No changes in sensitivity to insulin or to norepinephrine were observed. Glucose utilization by diaphragm was not changed by the treatment with 5C3MP.

NICOTINIC acid and 5-carboxy-3-methylpyrazole (5C3MP) are powerful inhibitors of lipolytic activity in adipose tissue. Administered *in vivo*, these compounds cause a marked depression of plasma free fatty acids (FFA), which is followed by a secondary rise above the basal level (rebound).[1–3] Recently it has been observed that the pretreatment of rats with nicotinic acid or 5C3MP causes an increase in the lipolytic response to norepinephrine added to the medium in which epididymal adipose tissue, or isolated cells, were incubated.[4,5]

This latter effect seemed in some way related to the rise in plasma corticosterone elicited by antilipolytic drugs. It was, in fact, abolished by adrenalectomy and it was restored by the administration of corticosterone.[4] Glucocorticoids are known to depress the utilization of glucose by adipose tissue and, through a decreased reesterification, a greater release of FFA can be expected.[6]

The rebound phenomenon and the hypersensitivity of adipose tissue to lipolytic agents might, at least partially, be due to the action of glucocorticoids on adipose tissue.

However, there are evidences in contrast to this hypothesis, which show that nicotinic acid, added to the medium incubating adipose tissue, increases glucose utilization by this tissue.[7–9] Similar results were obtained when 5C3MP and glucose were injected simultaneously and glucose incorporation into adipose tissue triglycerides was measured few minutes later.[10]

Under these circumstances, only the direct effect of these compounds could be measured, while possible indirect effects were not detectable.

To ascertain whether indirect effects of antilipolytic agents on adipose tissue could be demonstrated, 5C3MP and nicotinic acid were given to intact animals and the changes in glucose metabolism were measured after different intervals in adipose tissue excised and incubated *in vitro*.

MATERIALS AND METHODS

Male Sprague–Dawley rats, weighing about 100 g were used throughout the study, except for adrenalectomized rats which weighed 150 g. The animals were fasted

overnight if not otherwise stated. The *in vivo* treatment consisted of intraperitoneal administration of 5-carboxy-3-methyl-pyrazole (5C3MP) 7·5 mg/kg, b.w. or nicotinic acid 50 mg/kg, b.w.

At different intervals after the administration of antilipolytic drugs the animals were killed, epididymal adipose tissue rapidly excised, minced, pooled and 200 mg placed in a flask with 2 ml of medium. The composition of the medium was as follows: Krebs–Ringer phosphate, with half the amount of Ca^{2+}, pH 7·4, containing 3 % of fatty acid-free bovine serum albumin prepared according to Chen,[11] EDTA 10^{-2} mM, glucose 1 mM containing 0·025 μCi of [U-^{14}C]glucose.

Insulin 100 μUnits/ml, or norepinephrine 0·25 μg/ml was added to some flasks. The incubation was carried out for 2 hr at 37° in a metabolic shaker and was stopped by the addition of 0·5 ml of 0·5 N H_2SO_4. The flasks were then shaken for an additional 90 min at room temperature to aid in the trapping of $^{14}CO_2$ by 0·2 ml of 10% NaOH which moistened a folded filter paper suspended from the cup of the incubation vial. Finally the filter paper was transferred to counting vials containing 10 ml of Gordon's mixture as a liquid scintillator.[12]

Adipose tissue was separated from the incubation medium by filtration through a piece of nylon stocking, blotted dried and weighed. Lipids were extracted with 21 ml of 2:1 chloroform–methanol. The chloroform phase was separated by the addition of 7 ml of 0·004 N HCl.

An aliquot of 12 ml of the chloroform phase was transferred to counting vials and evaporated, after which 15 ml of Bray's mixture were added; the vials were transferred to a Beckman liquid scintillation spectrometer for measurement of radioactivity. A second chloroform aliquot, 0·5 ml, was used to measure triglyceride content according to the method of Van Handel and Zilversmit.[13] Since the concentration of triglyceride was reasonably constant, the results will here be referred to by tissue weight. Hemi-diaphragms were incubated in a medium without albumin, following the same procedure used for adipose tissue. Glycogen was extracted according to the method of Van Handel[14] and counted in vials with Gordon's mixture.

Chemicals. 5-Carboxy-3-methylpyrazole (5C3MP) was obtained from Upjohn Co., Kalamazoo, Mich. Norepinephrine bitartrate was a gift from Recordati Co; pork insulin (glucagon-free) was a gift of Novo Research Institute, Copenhagen. Serum albumin, fraction V, was purchased from Pentex; [U-^{14}C]glucose (2·9 μCi/mM) from Radiochemical Centre, Amersham.

RESULTS

Results in Table 1 show the time-course effect that a pretreatment with 5C3MP (7·5 mg/kg, i.p.) elicited on *in vitro* glucose utilization by adipose tissue. A biphasic effect was observed. Within the first 10 min after 5C3MP treatment, glucose oxidation and its incorporation into lipids was slightly increased, while 60 and 120 min after treatment, glucose utilization was significantly depressed. Both $^{14}CO_2$ formation and [U-^{14}C]glucose incorporation into lipids followed the same trend. Experiment carried out at intermediate intervals (30 and 45 min after the *in vivo* treatment) did not give consistent results.

The depression of [^{14}C]glucose utilization persisted even when the measurements

TABLE 1. EFFECT OF PRETREATMENT WITH 5-CARBOXY-3-METHYLPYRAZOLE (5C3MP) AND NICOTINIC ACID ON (U-^{14}C)GLUCOSE UTILIZATION BY RAT EPIDIDYMAL ADIPOSE TISSUE

Treatment (mg/kg i.p.)	Time*	*In vitro* [U-^{14}C]glucose incorporation (counts/min./100 mg adipose tissue)					
		CO_2			Lipids		
		C†	T‡	Δ§	C†	T‡	Δ§
5C3MP 7·5	10 min	1139 ± 35	1778 ± 28‖	+739	3522 ± 33	3824 ± 78**	+304
	60 min	1585 ± 52	1353 ± 56‖	−207	4436 ± 134	3556 ± 52‖	−880
	120 min	1587 ± 48	470 ± 61‖	−1117	4440 ± 130	2196 ± 80‖	−2244
	6 hr	1178 ± 29	1068 ± 84	−110	4314 ± 130	2599 ± 122‖	−1715
	18 hr	1221 ± 35	924 ± 19‖	−297	4776 ± 103	3876 ± 103‖	−900
5C3MP 50	20 min	1139 ± 35	1422 ± 95**	+283	3522 ± 33	4116 ± 88‖	−594
	120 min	1814 ± 39	842 ± 94‖	−972	5778 ± 234	4781 ± 142‖	−997
	18 hr	1221 ± 35	769 ± 24‖	−460	4776 ± 103	3374 ± 129‖	−1402
Nicotinic acid 50	120 min	1885 ± 53	1409 ± 61‖	−476	4140 ± 110	2780 ± 70‖	−1360

Each figure is the mean of at least four determinations (4 rats per group).
* Overnight fasted rats were treated intraperitoneally and sacrificed at intervals indicated under time.
† C = controls.
‡ T = treated.
§ Δ = difference between treated and controls.
‖ $P < 0.01$; ** $P < 0.05$ in respect of untreated controls.

were carried out 6 and 18 hr after 5C3MP treatment. 5C3MP, 50 mg/kg, and nicotinic acid, 50 mg/kg produced the same qualitative effects.

The addition of insulin or norepinephrine (Table 2) to the incubation medium stimulated glucose utilization to the same degree in tissue from control and 5C3MP-treated rats.

When 5C3MP (7·5 mg/kg) or nicotinic acid (50 mg/kg) was administered to fasted adrenalectomized rats, a significant depression occurred only in [U-^{14}C]glucose incorporation into lipids (Table 3), under these conditions insulin seemed to be more effective in promoting glucose oxidation.

The administration of 5C3MP, 50 mg/kg, did not affect glucose utilization by diaphragm (see Table 4).

TABLE 2. INSULIN AND NOREPINEPHRINE STIMULATION OF GLUCOSE UTILIZATION *in vitro* IN EPIDIDYMAL ADIPOSE TISSUE FROM RATS PRETREATED WITH 5C3MP

Treatment (mg/kg i.p.)	Additions to the medium (μUnits or μg/ml)	[U-^{14}C]glucose incorporation (counts/min/100 mg adipose tissue ± S.E.)			
		CO_2	Δ	lipids	Δ
Saline	Saline	1745 ± 71		5069 ± 234	
	Insulin 100	2147 ± 130†	+ 402	5932 ± 213*	+ 863
	Insulin 1000	2912 ± 120*	+ 1167	7331 ± 191*	+ 2262
5C3MP 7·5	Saline	877 ± 102		3100 ± 237	
	Insulin 100	1244 ± 47*	+ 367	3994 ± 50*	+894
	Insulin 1000	2323 ± 134*	+ 1446	5668 ± 184*	+ 2568
Saline	Saline	1162 ± 33		2078 ± 58	
	Norepinephrine 0·25	1868 ± 23*	+ 706	3455 ± 80*	+ 1377
5C3MP 7·5	Saline	512 ± 50		1080 ± 15	
	Norepinephrine 0·25	1242 ± 50*	+ 710	2625 ± 95*	+ 1545

Overnight fasted rats, were injected intraperitoneally with 5C3MP 120 min before sacrifice. Insulin was added to an incubation medium containing glucose 1 mM. Noradrenaline was added to an incubation medium containing glucose 1 mg/ml. The incubation time was 2 hr.

* $P < 0.01$.

† $P < 0.05$ in respect to controls.

Each figure is the average of four determinations (4 rats per group).

Δ = difference between saline and insulin or norepinephrine.

DISCUSSION

The time-course of the effect that the administration of an antilipolytic drug, nicotinic acid or 5C3MP, elicited on glucose utilization by epididymal adipose tissue, was studied.

The antilipolytic agents were given intraperitoneally to the intact rat, while glucose utilization was measured *in vitro*.

An insulin-like effect similar to that described by Froesch[10] was observed when intervals between the administration of 5C3MP or nicotinic acid were short (10–20 min). As the interval became longer, this effect was less evident and a marked depression in glucose utilization was observed 2, 6 and 18 hr after the administration of the

TABLE 3. EFFECT OF PRETREATMENT WITH 5C3MP OR NICOTINIC ACID ON [U-^{14}C]GLUCOSE UTILIZATION *in vitro* BY ADIPOSE TISSUE OBTAINED FROM ADRENALECTOMIZED (ADX) RATS

In vivo exp. conditions	Treatment (mg/kg i.p.)	[U-^{14}C]glucose incorporation (counts/min/100 mg adipose tissue ± S.E.)					
		CO_2			Lipids		
		Saline	Insulin (100 Units/ml)	Δ	Saline	Insulin (100 μUnits/ml)	Δ
Fasted		1800 ± 56	2472 ± 87	+ 672	4570 ± 181	5959 ± 79	+ 1339
Fasted	5C3MP 7·5	1943 ± 86	3005 ± 185	+ 1063	3410 ± 222*	5249 ± 200	+ 1839
Fasted	Nicotinic acid 50	2028 ± 234	3035 ± 219	+ 1007	3381 ± 264*	4646 ± 182	+ 1365

Rats were adrenalectomized (ADX) 3 days before the administration of 5C3MP or nicotinic acid and were sacrificed 120 min after the treatment. Each figure is the mean of four determinations (4 rats per group).

Δ is the difference between insuline or noradrenaline and saline.

* $P < 0.01$ in respect to controls.

TABLE 4. EFFECT OF 5C3MP TREATMENT ON GLUCOSE UTILIZATION BY RAT DIAPHRAGM *in vitro*

In vivo treatment		Glucose utilization (counts/min/100 mg diaphragm ± S.E.)			
		CO_2		Glycogen	
(mg/kg i.p.)	Time	Saline	Insulin (100 μUnits/ml)	Saline	Insulin (100 μUnits/ml)
Saline		460 ± 50	650 ± 100	1690 ± 110	3020 ± 630
5C3MP 50	30 min	440 ± 11	590 ± 60	2180 ± 200	3590 ± 190
5C3MP 50	120 min	610 ± 60	590 ± 50	1750 ± 90	3440 ± 350
5C3MP 50	18 hr	440 ± 30	490 ± 30	2140 ± 70	3720 ± 380

Each figure is the mean of four determinations (4 rats per group).

compound. The initial effect is probably due to a direct action of these compounds on adipose tissue since a similar effect was observed when the agents were added directly to the incubation medium.[6-8]

The mechanism responsible for the depressed glucose utilization is not yet clear. The rise in plasma corticosterone elicited by antilipolytic agents is unlikely to be the cause, since 5C3MP elicited this effect even in adrenalectomized rats.

These findings and the observation that pretreatment with 5C3MP did not alter the effect of norepinephrine on glucose utilization by adipose tissue, suggest that the depression of glucose utilization and the increase of lipolysis (hypersensitivity) in the adipose tissue are concomitant, but not related phenomena. In fact, the hypersensitivity to lipolytic agents was abolished in adrenalectomized rats and was restored by the administration of glucocorticoids.[4]

The observation that administration of 5C3MP did not alter the utilization of glucose in diaphragm muscle, indicates that the depression of glucose metabolism caused by antilipolytic compound is very likely confined to adipose tissue.

Therefore, by decreasing the rate of re-esterification of FFA and the utilization of glucose, adipose tissue could provide FFA and indirectly extra-glucose to peripheral tissues deprived of energy supplies after inhibition of lipolysis.

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