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DIFFERENT RESPONSES OF WHITE AND BROWN ADIPOSE TISSUE TO DRUGS AFFECTING LIPOLYSIS*

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Abstract—5 Carboxyl-3 methylpyrazole (5C3MP) and nicotinic acid are very effective in lowering FFA in plasma and white adipose tissue but not in brown adipose tissue under conditions of FFA mobilization obtained by fasting and cold and by the administration of norepinephrine, theophylline and ACTH. Similar results were obtained also *in vitro* except that in this case the effect of theophylline on brown adipose tissue was also slightly inhibited by 5C3MP. PGE_1 inhibited the effect of norepinephrine and theophylline on white but not on brown adipose tissue. The results obtained indicate that *in vivo* the level of FFA in plasma depends more on the lipolysis occurring in the white than in the brown adipose tissue.

SEVERAL studies indicate that the brown and the white adipose tissues should be considered separate entities. Morphological evidences supporting this statement are well known[1–4] while more recently a number of biochemical differences have been reported. For instance the brown adipose tissue contains in respect to the white adipose tissue, more mithochondria,[5, 6] cytochromes,[5–7] coenzyme Q,[5] glycerokinase,[8] glycogen[5] but less triglycerides.[2] A rich network of adrenergic terminals[9] may explain the higher content of norepinephrine[10–12] in the brown than in the white adipose tissue. This difference underlines the characteristic functions of these two kinds of adipose tissue. The white adipose tissue is considered an active store of triglycerides to be hydrolyzed for providing energy to other tissues,[13] while the brown adipose tissue seems to be primarily devoted to the production of heat in situ[5, 14, 15] through a local utilization of triglycerides as shown also by the high oxygen consumption.[5, 16]

In contrast to the biochemical knowledge, very little is known about the response of the brown adipose tissue to drugs affecting lipid mobilization. In this paper the effect of 5 carboxyl-3-methyl pyrazole, nicotinic acid and prostaglandin (PGE_1) on brown and white adipose tissue has been compared *in vivo* and *in vitro* both in normal conditions and after lipolysis stimulation.

METHODS

Male Sprague–Dawley rats of the average body weight of 150 ± 10 g were used through all the experiments.

Details of the various experimental conditions are referred in the text and under the tables.

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At the end of the treatment plasma was collected, epididymal (white) and interscapular (brown) adipose tissues were rapidly excised and frozen before performing the chemical analysis.

The *in vitro* incubations were performed according to the method previously described.[17] The medium was Krebs–Ringer bicarbonate containing 3% albumine at pH 7·4.

The determinations of FFA were carried out according to Dole[18] with minor modifications. Washings with 0·05% H_2SO_4, according to Trout,[19] were adopted in order to avoid interferences of the drugs during the titration.

Drugs used were 5 carboxyl-3-methylpyrazole and prostaglandin, PGE_1 (kindly obtained from Upjohn Co., Kalamazoo, Mich., U.S.A.), nicotinic acid (C. Erba, Milan), theophylline and norepinephrine bitartrate (Recordati, Milan). ACTH in a depot-form (Ormonoterapia Richter, Milan). For the studies *in vitro* a synthetic ACTH was used (Sinacthen^R obtained from Ciba, Milan).

RESULTS

Table 1 reports the effect of inhibitors of lipolysis, such as 5 carboxyl-3-methyl pyrazole (5C3MP) and nicotinic acid on plasma FFA of fasted rats.

The marked lowering of plasma FFA is paralleled by a decrease of FFA in the white adipose tissue without any effect on the brown adipose tissue.

TABLE 1. EFFECT OF 5 CARBOXYL 3 METHYLPYRAZOLE (5C3MP) AND NICOTINIC ACID ON THE LEVEL OF FREE FATTY ACIDS (FFA) OF PLASMA, WHITE AND BROWN ADIPOSE TISSUE OF RATS

Treatment (mg/kg i.v.)	Plasma FFA (μEquiv/l. ± S.E.)	Adipose tissues (FFA μEquiv/g + S.E.) white	brown
Controls fasted	779 ± 68	10·3 ± 0·4	9·9 ± 0·7
5C3MP 5 (30′)	386 ± 40*	5·6 ± 0·4*	9·3 ± 0·9
5C3MP 15 (30′)	220 ± 50*	3·2 ± 0·3*	8·8 ± 0·7
5C3MP 50 (30′)	259 ± 32*	2·3 ± 0·3*	10·8 ± 0·1
Controls fasted	895 ± 92	7·1 ± 0·5	12·0 ± 1·2
5C3MP 30 (30′)	299 ± 35*	2·5 ± 0·1*	11·0 ± 0·7
5C3MP 30 (60′)	324 ± 55*	3·4 ± 0·2*	10·2 ± 1·2
5C3MP 30 (120′)	378 ± 35*	4·4 ± 0·4*	10·8 ± 0·8
Controls fasted	702 ± 37	9·1 ± 0·7	13·5 ± 1·2
Nicotinic acid 10 (30′)	163 ± 10*	2·6 ± 0·4*	11·5 ± 0·3
Nicotinic acid 30 (30′)	299 ± 33*	2·5 ± 0·3*	11·0 ± 0·7

Animals were fasted overnight. Each value is the average of at least 5 determinations.

Numbers in parenthesis indicate the time between treatment and sacrifice of the animals.

* $P < 0·01$ in respect to controls.

This difference is evident at various doses of the drugs and at various times after drug administration. It should be noticed that fasting increases FFA in the white adipose tissue but shows only a little effect, if any, on brown adipose tissue. Three lipolytic agents such as norepinephrine, ACTH and theophylline, increase the FFA in plasma and in the white adipose tissue while they are almost completely inactive on the brown adipose tissue with the possible exception of theophylline. Table 2

shows these data and demonstrates that 5C3MP blocks the effect of lipolytic agents on plasma and white adipose tissue but that it is without effect on the brown adipose tissue in the various experimental conditions.

TABLE 2. EFFECT OF 5 CARBOXY-3-METHYLPYRAZOLE (5C3MP) AND PGE_1 ON THE LEVEL OF FFA OF PLASMA, WHITE AND BROWN ADIPOSE TISSUE OF RATS TREATED WITH LIPOLYTIC AGENTS

Treatment (mg/kg)	Plasma FFA (μEquiv/l. ± S.E.)	Adipose tissue (FFA μEquiv/g ± S.E.) white	brown
Controls	405 ± 19	4·7 ± 0·6	8·9 ± 0·7
Theophylline 150 (30′)	854 ± 56	9·6 ± 0·7	13·9 ± 0·7
Theophylline 150 (30′) + 5C3MP 30 i.v. (30′)	263 ± 29*	2·6 ± 0·1*	12·9 ± 0·8
Controls	213 ± 33	3·0 ± 0·1	8·53 ± 0·3
Theophylline 150 (60′)	660 ± 87	7·4 ± 0·6	11·0 ± 0·3
Theophylline 150 (60′) + 5C3MP 30 i.v. (60′)	294 ± 26*	3·1 ± 0·2*	11·9 ± 1·1
Controls	290 ± 24	3·0 ± 0·1	6·5 ± 0·6
Theophylline 150 (30′)	612 ± 23	11·0 ± 0·8	8·8 ± 0·6
Theophylline 150 (30′) + PGE_1 0·14 infusion (30′)	343 ± 26*	5·6 ± 0·5*	7·2 ± 0·3
Controls	225 ± 28	4·5 ± 0·5	11·2 ± 1·0
Norepinephrine 1 s.c. (60′)	893 ± 34	10·5 ± 0·5	12·2 ± 0·2
Norepinephrine 1 s.c. (60′) + 5C3MP 7·5 i.p. (60′)	438 ± 14*	3·5 ± 0·2*	10·5 ± 0·3
Controls	225 ± 52	2·9 ± 0·3	8·7 ± 0·4
ACTH 200 UI/kg s.c. (120′)	690 ± 106	5·8 ± 0·7	9·4 ± 0·7
ACTH 200 UI/kg s.c. (120′) + 5C3MP 30 i.v. (60′)	224 ± 35*	3·5 ± 0·5*	8·5 ± 0·5
ACTH 200 UI/kg s.c. (120′) + 5C3MP 30 i.v. (120′)	193 ± 32*	2·1 ± 0·3*	8·5 ± 0·5

Numbers in parenthesis indicate the time between treatment and sacrifice of the animals. Each value is the average of at least 5 determinations.
Animals were fasted only at the beginning of the experiment.
* $P < 0·01$ in respect to controls treated with lipolytic agent.

Also when PGE_1 has been infused the concentration of FFA was decreased in the white but not in the brown adipose tissue.

In another experiment fasted animals were exposed to cold since it is known that this condition affects the physiological activity of the brown adipose tissue.[1, 20] Again 5C3MP depressed FFA in plasma and white adipose tissue, without influencing the level of FFA in the brown adipose tissue (see Table 3).

In a last series of experiments, white and brown adipose tissues were incubated *in vitro* in presence of lipolytic agents and/or inhibitors of lipolysis. Table 4 summarizes the results obtained.

It is evident that ACTH at concentrations able to markedly stimulate lipolysis in the white adipose tissue was without effect on the brown adipose tissue. On the contrary norepinephrine and theophylline, at high doses, were very effective on both adipose tissues.

TABLE 3. EFFECT OF 5 CARBOXYL-3 METHYLPYRAZOLE (5C3MP) ON PLASMA FFA AND GLYCEROL AND ON WHITE AND BROWN ADIPOSE TISSUE FFA OF RATS SUBMITTED TO COLD

Treatment	Plasma FFA (μEquiv/l. $\pm$ S.E.)	Adipose tissue (FFA μEquiv/g $\pm$ S.E.) white	brown
Controls fasted	610 $\pm$ 48	9·7 $\pm$ 0·9	11·5 $\pm$ 0·3
5C3MP	159 $\pm$ 15*	5·8 $\pm$ 0·9*	10·5 $\pm$ 0·9
Controls fasted + cold	718 $\pm$ 57	11·5 $\pm$ 0·8	10·7 $\pm$ 0·7
Fasted + Cold + 5C3MP	140 $\pm$ 20*	3·8 $\pm$ 0·3*	10·9 $\pm$ 0·6

Animals were fasted for 31 hr and then submitted to cold (4°) for 4 hr.
Treatment with 5C3MP was given intravenously (30 mg/kg) 30 min before determinations.
Each value is the average of at least 5 determinations.
* $P < 0.01$ in respect to controls.

TABLE 4. EFFECT OF VARIOUS DRUGS ON THE LIPOLYSIS OF WHITE AND BROWN ADIPOSE TISSUES INCUBATED *IN VITRO*

Additions (μg/ml)	Tissue	Release of FFA (μEquiv/g/hr) Saline	Norepinephrine (μg/ml)* 0·5	Theophylline (μg/ml)† 75	250	ACTH (μg/ml) 0·05
Saline	white	2·9 $\pm$ 0·7	13·2 $\pm$ 0·7	8·1 $\pm$ 0·5	9·6 $\pm$ 0·1	24·0 $\pm$ 1·0
Saline	brown	8·6 $\pm$ 1·2	13·5 $\pm$ 0·8	7·7 $\pm$ 0·3	13·5 $\pm$ 0·6	8·8 $\pm$ 0·2
5C3MP 20	white	1·8 $\pm$ 0·3‡	7·7 $\pm$ 0·5‡	3·2 $\pm$ 0·2‡	4·5‡ $\pm$ 0·3	16·0 $\pm$ 2·0‡
5C3MP 20	brown	8·1 $\pm$ 0·1	12·6 $\pm$ 0·5	5·2 $\pm$ 0·3‡	10·8 $\pm$ 0·4‡	7·7 $\pm$ 0·6
Nicotinate 20	white	—	—	—	3·9 $\pm$ 0·1‡	—
Nicotinate 20	brown	—	—	—	12·3 $\pm$ 0·6	—
PGE_1 1	white	—	6·7 $\pm$ 1‡	—	4·3 $\pm$ 0·3‡	—
PGE_1 1	brown	—	12·9 $\pm$ 0·3	—	10·5 $\pm$ 0·7‡	—

* As tartrate salt.
† as theophylline monohydrate.
‡ $P < 0.01$ in respect to relative controls (saline).

5C3MP was again effective on the white adipose tissue but it did not show activity on the brown adipose tissue with the exception of the theophylline induced stimulation. Also nicotinic acid and PGE_1 were more effective on white than on brown adipose tissue in inhibiting the lipolysis activated by theophylline.

DISCUSSION

The content of FFA in the intrascapular brown adipose tissue in rats was higher than in the epididymal white adipose tissue.

Experimental conditions such as fasting and cold, and lipolytic agents, such as norepinephrine and ACTH, known to increase mobilization of FFA, enhanced the concentration of FFA in the "white" without changing the level of FFA in the brown adipose tissue.

Different results were obtained when the adipose tissues were incubated *in vitro*. In this condition only ACTH failed to raise FFA in brown adipose tissue, while norepinephrine and theophylline were moderately effective.

The discrepancy between *in vivo* and *in vitro* studies with the brown adipose tissue may be only apparent because it is conceivable that in the two conditions the relative degree of release, local utilization and re-esterification of FFA may have a different quantitative importance for the net result.

The possibility of a different concentration of the lipolytic agents *in vivo* and *in vitro* at the level of the adipose cells must also be taken into consideration.

Another difference between the white and brown adipose tissue is evident by using various drugs inhibiting lipolysis.

In this paper nicotinic acid,[21] 5C3MP[17, 22] and PGE_1[23, 24] were invariably able to lower FFA in the white adipose tissue without affecting the brown adipose tissue. In particular the effect of 5C3MP was studied *in vivo* on the increase of lipid mobilization induced by fasting, cold, norepinephrine, ACTH and theophylline and *in vitro* on the stimulation of lipolysis induced by the three lipolytic agents. The effect of nicotinic acid was investigated on fasting *in vivo* and on theophylline *in vitro*. The effect of PGE_1 was investigated on theophylline *in vivo* and on norepinephrine and theophylline *in vitro*. The results obtained at different durations and concentrations indicated always a good parallelism between decrease of FFA in plasma and in white adipose tissue but almost no effect on the brown adipose tissue. These results confirm, from a pharmacological point of view, that the level of plasma FFA depends on the white but not on the brown adipose tissue.

Because their point of attack is largely unknown, it is very difficult at the present time to establish why the drugs inhibit lipid mobilization on the white but not on the brown adipose tissue. It may however be stressed that the brown adipose tissue contains a high level of norepinephrine[10–12] and presumably a high level of cyclic 3,5 AMP. It may be relevant in this respect to mention that nicotinic acid, 5C3MP and PGE_1 are inactive also on the white adipose tissue in presence of high concentrations of cyclic 3,5 AMP (dibutyril ester) (unpublished results).

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