Biochemical Pharmacology, Vol. 20, pp. 1201–1211. Pergamon Press, 1971. Printed in Great Britain

REDUCED LIPOLYTIC RESPONSE *IN VITRO* TO CATECHOLAMINES, ACTH AND CYCLIC ADENOSINE MONOPHOSPHATE IN BROWN FAT OF COLD-ACCLIMATIZED RATS

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(*Received* 20 *May* 1970; *accepted* 30 *October* 1970)

Abstract—The hormone-induced lipolysis and the effect of different antagonists have been studied in parallel in brown and white adipose tissue of normal and cold-acclimatized rats.

The results seem to indicate that brown adipose tissue of cold-acclimatized rats is not responsive to exogenous catecholamines and ACTH, while an increase in spontaneous lipolysis is observed. Moreover, the free fatty acids accumulate within the tissue, in contrast to epididymal fat where FFA are largely released in the incubation medium.

The absence of stimulation by adipokinetic agents is discussed in relation to the high level of FFA within the cells, which may produce a feedback control on lipolysis. Another possibility is that the absence of stimulation by adipokinetic agents depends on a deficiency of ATP, consequent to (a) a poor phosphorylating efficiency of the respiratory chain or, alternatively, (b) an energy dissipating mechanism operating in spite of a tightly coupled phosphorylating system, and represented by the FFA accumulated inside brown fat cells.

IN NEWBORN animals and hibernators the brown adipose tissue is believed to play a prominent role in heat production during exposure to cold or during arousal from hibernation.[1–7] A similar role has been proposed for the brown adipose tissue of the homeothermic animals exposed to cold and, in particular, in cold-acclimatized rats.[5,8–12]

The increase in heat production during cold adaptation, is sustained by a non-shivering thermogenesis related to an increased metabolic rate of the tissue. A greater oxygen uptake[10,12] is accompanied by an increased fatty acid oxidation.[8,13,14] Other adaptive changes of brown adipose tissue in cold exposed rats are represented by change in the mass,[10,12,15–17] composition[12,15–17] and temperature sensitivity.[18]

The response of brown fat in cold-acclimatized rats as regards hormone-stimulated lipolysis *in vitro*, was investigated taking into consideration, firstly, that the free fatty acids represent the main fuel for thermogenesis in this tissue;[8,13,14] secondly, that the regulation of brown fat thermogenesis is evidently mediated by the sympathetic nervous system,[19] and, finally, that, if brown adipose tissue is primarily a "thermogenic organ", cold adaptation would have to enhance the metabolic difference with the white adipose tissue.

The investigation was carried out using in parallel the interscapular brown fat and

the epididymal adipose tissue of the same rats acclimatized at 4° for a period of 3–4 months. Comparison with brown fat of normal rats was also carried out.

The results seem to indicate that brown adipose tissue of cold acclimatized rats is not responsive to exogenous catecholamines and ACTH, but its spontaneous lipolysis is increased. Moreover, brown fat apparently shows a decreased permeability to FFA as revealed by a reduced release from the tissue into the surrounding medium.

MATERIALS AND METHODS

Wistar rats having an initial weight of 150 ± 20 g were exposed at 4° for 3–4 months. They were maintained on a standard laboratory diet with water *ad lib.* up to the time of sacrifice. After light ether anaesthesia, epididymal (white) and interscapular (brown) fat pads were excised and immediately prepared for incubation. Interscapular brown fat was sliced and pooled, whereas samples of epididymal fat from different animals were simply randomized. Adipose tissue (100 ± 5 mg) was introduced in 2 ml of Ca^{2+} normal (2·7 mM) Krebs–Ringer bicarbonate buffer pH 7·2 containing 2·5% bovine albumin (fraction V, Sigma). After a pre-incubation of fat in the medium for 30 min at 37°, drugs were added and the assays incubated in a metabolic shaker at 37° for 150 min. Unless otherwise indicated the preincubation of the tissue was carried out at room atmosphere. At the end of incubation 0·1 ml of H_2SO_4 2·5 N was introduced in each assay for stopping the reaction. FFA were titrated in the incubation medium according to Dole[20] and glycerol according to Korn.[21] To test the intracellular content of FFA, the adipose tissue of each sample was resuspended in 2 ml of distilled water and homogenized with a Potter–Elvehjem homogenizer with teflon pestle. The homogenate was transferred directly into the extraction mixture for FFA titration according to Dole.[20]

Snake venom (phospholipase A) was dissolved in acetate buffer 0·1 M, pH 3·8 and purified by heating in boiling water for 10 min and by filtering. The samples were incubated with phospholipase A at 37° in a metabolic shaker for 30 min, before noradrenaline was added. Total lipids were extracted by chloroform–methanol (2:1) and weighed.

As regards the statistical evaluation in the *in vitro* experiments, we considered as significantly different only the differences having a $P < 0{\cdot}01$.

Prostaglandin E_1 was the gift of Dr. G. Persani (Upjohn, S.p.A., Milan, Italy). Digitoxin was purchased from Hoffman-La Roche (Basel, Switzerland). Noradrenaline bitartrate monohydrate from Recordati (Milano, Italy). Rotenone, 2,4-DNP and monoiodoacetic acid from British Drug Houses Ltd. (Poole, England). ACTH from Armour Pharmaceutical Co. (Chicago, U.S.A.). Nicotinic acid and sodium fluoride from Merck (Darmstadt, Germany). KCN and $NaAsO_2$ from C. Erba (Milan, Italy). Oligomycin (a mixture of oligomycin A and B) was supplied by Upjohn Co. (Kalamazoo, U.S.A.). Propranolol: 1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride from Imperial Chemical Industries (Wilmslow, England). D(-)INPEA: *N*-isopropyl-*p*-nitrophenylethanolamine hydrochloride from Selvi and C. (Milan, Italy). Phenoxybenzamine hydrochloride from Smith Kline and French (Philadelphia, U.S.A.). *Naja naja* venom (Phospholipase A) from Sigma (St. Louis, Missouri, U.S.A.). Dibutyryl cyclic 3′5′-AMP was the gift of Dr. M. Carissimi (Maggioni, Milan, Italy).

RESULTS

Spontaneous FFA and glycerol release. Unless otherwise indicated, lipolysis was expressed as FFA and glycerol released by a gram of fresh adipose tissue.

In brown adipose tissue of normal rats, the level of free fatty acids within the tissue (after incubation for 150 min) is greater than that in the incubation medium. In contrast, in white adipose tissue of the same animals, the FFA concentration is higher in the medium (Table 1).

TABLE 1. SPONTANEOUS FFA RELEASE IN BROWN AND WHITE FAT OF NORMAL AND COLD-ACCLIMATIZED RATS

	FFA (μequiv./g/150 min)			
	Normal rats		Acclimatized rats	
Site of titration	Brown fat	White fat	Brown fat	White fat
Tissue	12·60 ± 1·77	3·48 ± 0·75	26·19 ± 5·71	3·47 ± 0·53
Medium	7·65 ± 1·68	6·72 ± 0·13	15·49 ± 4·76	5·63 ± 1·05
Tissue/Medium	1·64	0·52	1·69	0·62

Rats acclimatized at 4° for 3 months. Samples of interscapular brown fat (100 + 5 mg) and of epididymal fat (100 ± 5 mg) were incubated in 2 ml of Ca^{2+} normal Krebs–Ringer bicarbonate buffer pH 7·2, containing 2·5% bovine albumin at 37° for 150 min in a metabolic shaker. Each value represents the mean ± S.E. of 10 assays.

Tables 1 and 2 show that after cold adaptation, the spontaneous lipolysis is about doubled in brown fat, whereas it remains unvaried in epididymal fat. The ratio of intratissue-FFA to medium-FFA was unaffected in both tissues (Tables 1 and 3).

Effect of noradrenaline, ACTH and dibutyryl cyclic 3′5′-AMP. Tables 4 and 5 show that in brown adipose tissue of normal and cold-acclimatized rats the effect of noradrenaline on FFA and glycerol release is very low in comparison to the effect in white tissue. Analogous results were obtained also when the fat was incubated in an atmosphere of 5% CO_2 and 95% O_2.

TABLE 2. SPONTANEOUS GLYCEROL RELEASE IN BROWN AND WHITE FAT OF NORMAL AND COLD-ACCLIMATIZED RATS

	Glycerol in the medium (μM/g/150 min)			
Animal group	Brown fat		White fat	
Normal rats	11·68 ± 1·22*	—	5·19 ± 0·73†	—
Acclimatized rats	16·93 ± 2·41*	(P > 0·02)	5·57 ± 1·48†	(P > 0·80)

Rats acclimatized at 4° for 3 months. Experimental conditions as in Table 1.
* Each value represents the mean ± S.E. of four assays.
† Each value represents the mean ± S.E. of five assays.

The lipid content of the brown adipose tissue is lower than that of the white one;[22,23] this difference is emphasized by cold-acclimatization.[17] To avoid a possible source of error due to this difference, the data of Table 4, indicating the lipolytic effect of increasing concentrations of noradrenaline, are expressed also as a ratio between FFA release per gram of lipid-free fresh tissue (Fig. 1) which corresponds[17] in the acclimatized rats to 20 per cent of the original wet weight in the case of epididymal fat pads, and to 60 per cent in the case of brown fat; in normal rats, the lipid-free fresh tissue corresponds to 20 per cent of the original wet weight in epididymal fat pads and to 30 per cent in brown fat. It is clear that noradrenaline, in contrast to the normal activation of lipolysis in epididymal fat of cold-adapted rats, is almost without effect in brown fat of the same animals. However, although reduced, a certain degree of stimulatory effect by noradrenaline is present in the brown fat of normal rats.

Figure 2 reports the FFA release from brown fat of cold adapted rats, followed during the time of incubation in the presence and absence of noradrenaline. The higher

TABLE 3. SPONTANEOUS FFA AND GLYCEROL RELEASE IN BROWN AND WHITE FAT OR COLD-ACCLIMATIZED RATS DURING 150 min OF INCUBATION

	Time of incubation (min)	FFA (μequiv./g)			Glycerol (μM/g)			
		Medium	Δ*	Tissue	Δ*	Tissue/ medium	Medium	Δ*
Brown tissue	0	—	—	12·73	—	—	1·66	
	150	11·24	+11·24	40·72	+27·99	2·49	12·30	+10·64
White tissue	0	2·16	—	2·60	—	—	—	
	150	9·25	+ 7·09	5·75	+ 3·15	0·44	5·23	+ 5·23

Rats acclimatized at 4° for 3 months. Experimental conditions as in Table 1. The reaction was stopped at 0 time and after 150 min of incubation by introducing in each assay 0·1 ml of 2·5 N H_2SO_4. Each value represents the mean of two assays of a single experiment.

* Δ = difference between FFA and glycerol content of the tissue and of the incubation medium, after and before an incubation period of 150 min.

TABLE 4. LIPOLYTIC EFFECT OF NORADRENALINE IN BROWN AND WHITE FAT OF NORMAL AND COLD-ACCLIMATIZED RATS

Drugs in the medium M. conc.	FFA (μequiv./g/150 min) (tissue + medium)			
	Normal rats		Acclimatized rats	
	Brown fat	White fat	Brown fat	White fat
—	20·10 ± 3·39	8·64 ± 1·64	45·43 ± 10·16	9·10 ± 99·00
Noradrenaline 10^{-7}	23·98 ± 3·34	12·16 ± 1·87	47·73 ± 9·49	12·45 ± 1·42
Noradrenaline 10^{-6}	29·64 ± 2·71	34·77 ± 5·66	47·73 ± 6·68	24·40 ± 2·11
Noradrenaline 10^{-5}	40·24 ± 2·34	50·94 ± 3·24	54·93 ± 8·40	53·64 ± 1·51
Noradrenaline 10^{-4}	40·42 ± 2·23	43·34 ± 4·91	53·17 ± 11·90	54·64 ± 1·93

Rats acclimatized at 4° for 3 months. Experimental conditions as in Table 1. Noradrenaline was added to the brown and to the white fat after a pre-incubation period of 30 min at 37° without stirring. Each value represents the mean ± S.E. of five assays.

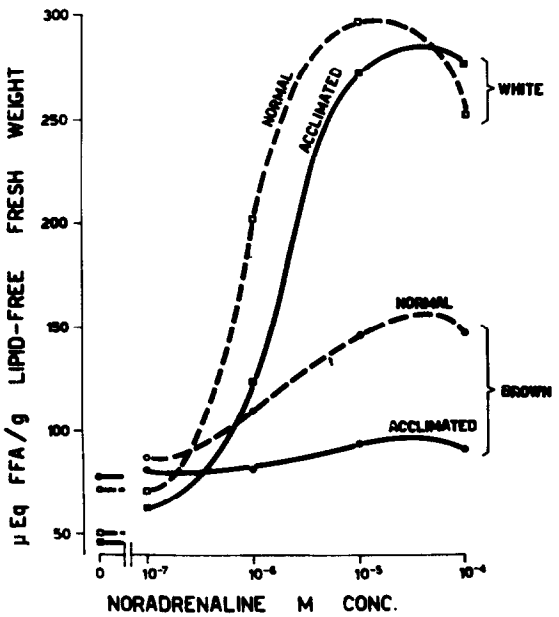

FIG. 1. Log concentration–response curves for noradrenaline-stimulated lipolysis in brown and white adipose tissue of normal (---) and cold-acclimatized (—) rats. Rats acclimatized at 4° for 3 months. Experimental conditions as in Table 1. Abscissa: molar conc. of noradrenaline in the incubation medium of adipose tissue (log scale). Ordinate: free fatty acid (tissue + medium) referred to a gram of lipid-free fresh tissue which corresponds to 20% of the original wet weight in the case of epididymal fat, to 30% in brown fat of normal rats and to 60% in brown fat of cold-acclimatized rats. Each value represents the mean of six different experiments.

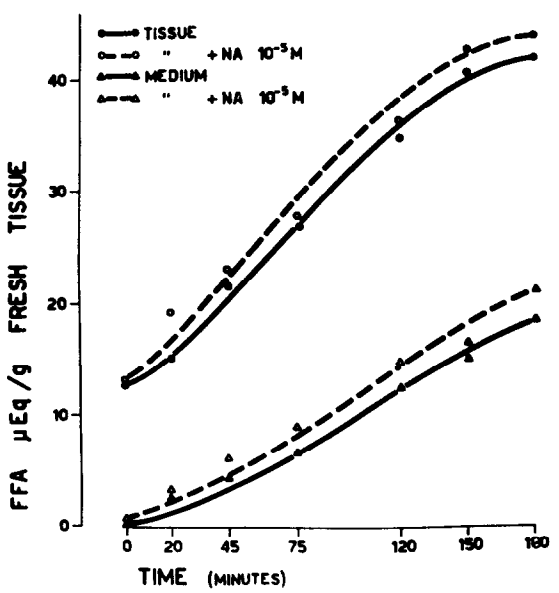

FIG. 2. Kinetic of the FFA release in the medium and inside brown adipose tissue of cold-acclimatized rats. Rats acclimatized at 4° for 3 months. Experimental conditions as in Table 1. Abscissa: incubation time. Ordinate: FFA conc. in the medium (▲, △) and inside the tissue (●, ○) in the absence (—) and in presence (---) of noradrenaline (NA) 10^{-5} M.

TABLE 5. EFFECT OF NORADRENALINE ON GLYCEROL RELEASE IN THE INCUBATION MEDIUM OF BROWN AND WHITE FAT OF NORMAL AND COLD-ACCLIMATIZED RATS

Drugs in the medium M conc.	Glycerol in the medium (μM/g/150 min)			
	Normal rats		Acclimatized rats	
	Brown fat	White fat	Brown fat	White fat
—	11·22 ± 1·58	4·89 ± 0·95	16·13 ± 1·93	5·26 ± 0·83
Noradrenaline 10^{-7}	16·20 ± 1·88	4·88 ± 0·93	16·12 ± 3·22	7·72 ± 1·43
Noradrenaline 10^{-6}	16·52 ± 1·35	14·80 ± 1·85	16·47 ± 2·80	11·50 ± 1·85
Noradrenaline 10^{-5}	17·77 ± 1·41	22·05 ± 1·28	18·82 ± 3·25	24·40 ± 1·08
Noradrenaline 10^{-4}	19·02 ± 1·91	21·60 ± 1·14	18·30 ± 2·00	23·80 ± 1·48

Rats acclimatized at 4° for 3 months. Experimental conditions as in Table 1. Noradrenaline was added to the brown and to the white fat after a pre-incubation period of 30 min at 37° without stirring. Each value represents the mean ± S.E. of five to ten assays.

curves represent the FFA level within the tissue, and the lower ones represent the FFA level in the medium. They clearly show that a higher concentration of FFA is present within the tissue and that noradrenaline does not produce a significant stimulant effect.

Thus, the results obtained by determining FFA release during all the time of incubation, confirm those obtained at a fixed time, and reported in Tables 1 and 4.

Like in the case of noradrenaline, also the ACTH effect (Table 6) in brown fat after cold adaptation, was scarcely evident (+12% FFA in tissue + medium, in the presence of ACTH 1 I.U./ml). In contrast, ACTH was very active on the epididymal fat of the same animals (+383% FFA in tissue + medium, with ACTH 1 U.I./ml).

Dibutyryl cyclic 3′5′-AMP showed a similar behaviour (Table 7).

Effect of phospholipase A. Taking into consideration the higher concentration of FFA always present within brown tissue, as compared with that in the incubation

TABLE 6. REPRESENTATIVE EXPERIMENT SHOWING THE EFFECT OF ACTH ON LIPOLYSIS IN BROWN AND WHITE FAT OF COLD-ACCLIMATIZED RATS

Drugs in the medium M conc.	FFA (μequiv./g/150 min)		Glycerol in the medium (μM/g/150 min)
	Medium	Tissue	
Brown tissue			
—	14·06	33·46	12·93
ACTH 0·2 I.U./ml	19·91	30·15	13·15
ACTH 1·0 I.U./ml	25·62	31·69	14·18
White tissue			
—	7·54	2·25	1·98
ACTH 0·2 I.U./ml	27·88	4·89	13·24
ACTH 1·0 I.U./ml	40·52	6·53	15·60

Rats acclimatized at 4° for 3 months. Experimental conditions as in Table 1. ACTH was added to the brown and to the white fat after a pre-incubation period of 30 min at 37° without stirring. Each value represents the mean of two assays.

TABLE 7. EFFECT OF DIBUTYRYL CYCLIC 3'-5'-AMP (CAMP-DB) ON LIPOLYSIS IN BROWN AND WHITE FAT OF COLD-ACCLIMATIZED RATS

Drugs in the medium M conc.	FFA (μequiv./g/150 min)		Glycerol in the medium (μM/g/150 min)
	Medium	Tissue	
Brown tissue			
—	13·97 ± 0·79*	30·48 ± 2·11*	13·22 ± 0·67*
CAMP-DB 5×10^{-3}	22·66 ± 1·17† (P < 0·001)	33·43 ± 3·67† (P > 0·40)	18·15 ± 1·33† (P < 0·01)
White tissue			
—	6·15 ± 0·41‡	2·18 ± 0·45‡	2·68 ± 0·85‡
CAMP-DB 5×10^{-3}	33·66 ± 3·94‡ (P < 0·001)	15·76 ± 4·59‡ (P < 0·01)	14·80 ± 1·61‡ (P < 0·001)

Experimental conditions as in Table 1. CAMP-DB was added to the assays after pre-incubation of the fat in the medium at 37° for 10 min, without stirring. Each value represents the mean ± S.E. of (*) nine assays, of (†) six assays, and of (‡) four assays.

TABLE 8. EFFECT OF PHOSPHOLIPASE A ON LIPOLYSIS IN BROWN FAT OF COLD-ACCLIMATIZED RATS

Drugs in the medium	FFA (μequiv./g/150 min)			Glycerol(μM/g/150 min)
	Tissue	Medium	Tissue + Medium	Medium
—	8·39 ± 2·39	2·93 ± 0·17	11·32 ± 0·77	9·38 ± 0·50 —
Phospholipase A 90 μg/ml	6·55 ± 0·85	7·64 ± 0·82	14·19 ± 0·65 (P > 0·20)	11·47 ± 0·38 (P > 0·01)
Noradrenaline 2×10^{-5} M	9·68 ± 0·53	6·38 ± 0·55	16·06 ± 0·81 (P > 0·05)	11·85 ± 0·52 (P > 0·01)
Phospholipase + Noradrenaline	6·79 ± 0·29	9·74 ± 0·58	16·53 ± 0·62 (P > 0·02)	11·69 ± 0·71 (P > 0·02)

Rats acclimatized at 4° for 4 months. Experimental conditions as in Table 1. Phospholipase A 7·30 μg/g protein. Adipose tissue was pre-incubated with phospholipase A at 37° in a metabolic shaker for 30 min without stirring. Each value represents the mean ± S.E. of four to five assays.

medium, we tried to modify the permeability of cell membrane by pre-incubating brown fat of cold-acclimatized rats in the presence of phospholypase A. The treatment did not significantly increase (P > 0·01) the response of the tissue to noradrenaline (Table 8).

Effect of drugs normally acting as inhibitors of lipolysis in white adipose tissue. In a further attempt to investigate the nature of the lipolytic process in brown fat of cold-acclimatized rats, we tested the effect of different drugs normally exerting inhibitory effect on catecholamine-stimulated lipolysis.[24,25] *Alpha* and *beta* antiadrenergic drugs (phenoxybenzamine, propranolol, INPEA, Table 9), prostaglandin E_1, digitoxin, nicotinic acid, insulin, glucose (Table 10), oxidative phosphorylation and glycolysis inhibitors (Table 11), were all ineffective against the spontaneous FFA and glycerol release in brown adipose tissue of cold-adapted rats.

Finally, $NaAsO_2$ and KCN were also tested, and they were found the only drugs showing some antagonistic activity against the basal lipolysis (Table 11). However, their widespread and relatively aspecific inhibitory effect on metabolic pathways, renders their action scarcely significant.

TABLE 9. EFFECT OF *alpha* AND *beta* ANTIADRENERGIC DRUGS ON SPONTANEOUS LIPOLYSIS IN BROWN FAT OF COLD-ACCLIMATIZED RATS

Drugs in the medium M conc.	FFA (μequiv./g/150 min)		Glycerol in the medium (μM/g/150 min)
	Tissue	Medium	
—	34·18	15·63	13·15
D(-) INPEA 10^{-6}	32·50	17·52	14·92
INPEA 10^{-5}	39·20	15·97	12·15
INPEA 10^{-4}	39·40	14·26	16·04
Propranolol 10^{-6}	35·69	17·10	14·77
Propranolol 10^{-5}	37·57	16·82	12·27
Propranolol 10^{-4}	36·83	14·26	14·47
—	36·85	13·41	—
Phenoxybenzamine 10^{-3}	33·15	11·96	—

Rats acclimatized at 4° for 4 months. Experimental conditions as in Table 1. Brown adipose tissue was pre-incubated for 30 min in the medium at 37° without stirring, before adding the drugs. Each value represents the mean of two assays.

TABLE 10. EFFECT OF PROSTAGLANDIN E_1, DIGITOXIN, NICOTINIC ACID, INSULIN AND GLUCOSE ON SPONTANEOUS LIPOLYSIS IN BROWN FAT OF COLD-ACCLIMATIZED RATS

Drugs in the medium M conc.	FFA (μequiv./g/150 min)		Glycerol in the medium (μM/g/150 min)
	Tissue	Medium	
—	33·46	14·06	12·93
Prostaglandin E_1 10^{-5}	31·89	13·06	13·26
Digitoxin 10^{-3}	28·54	16·44	—
—	37·36	18·58	13·74
Nicotinic acid 10^{-4}	36·57	20·17	13·74
Insulin 0·1 U/ml	34·64	15·97	16·56
Glucose 10^{-2}	37·10	17·91	—
Insulin + Glucose	39·57	17·25	—

Rats acclimatized at 4° for 4 months. Experimental conditions as in Table 1. Brown adipose tissue was pre-incubated for 30 min in the medium at 37° without stirring, before adding drugs. Each value represents the mean of two assays.

DISCUSSION

The results described seem to indicate that brown adipose tissue of cold-acclimatized rats is scarcely responsive to exogenous catecholamines, ACTH and dibutyryl cyclic 3',5'-AMP. Moreover, the FFA accumulate within the tissue, in contrast with epididymal fat where FFA are largely released in the incubation medium. Analogous differences between brown and white fat of normal mice were found by Napolitano *et al.*[26] A diminished rate of FFA release from brown fat in comparison with that from white fat, was also demonstrated by Wertheimer, Hamosh and Shafir[27] and by Bizzi *et al.*[28] in adult rats, and by Dawkins and Hull[8] in newborn rabbits.

TABLE 11. EFFECT OF OXIDATIVE PHOSPHORYLATION AND GLYCOLYSIS INHIBITORS, OF $NaAsO_2$ AND KCN ON SPONTANEOUS LIPOLYSIS IN BROWN FAT OF COLD-ACCLIMATIZED RATS

Drugs in the medium M conc.	FFA μequiv./g/150 min		Glycerol in the medium (μM/g/150 min)
	Tissue	Medium	
—	34·18	15·63	13·15
Rotenone 10^{-7}	32·81	14·75	13·76
Rotenone 10^{-6}	32·67	15·04	17·15
Rotenone 10^{-5}	33·33	13·86	15·00
Rotenone 10^{-4}	35·44	13·58	11·35
Oligomycin 10^{-7}	35·00	13·19	12·32
Oligomycin 10^{-6}	35·10	11·24	10·36
Oligomycin 10^{-5}	33·90	9·90	9·22
Oligomycin 10^{-4}	34·75	9·31	9·54
2,4-DNP 10^{-3}	30·61	15·31	14·88
—	25·97	10·23	8·60
NaF 4×10^{-2}	24·37	14·66	7·20
Iodoacetate 10^{-3}	20·94	9·75	9·80
—	36·86	13·41	—
$NaAsO_2$ 10^{-2}	18·03(−51%)	7·75(−42%)	—
KCN 10^{-2}	24·89(−32%)	9·74(−27%)	—

Rats acclimatized at 0° for 4 months. Experimental conditions as in Table 1. Brown adipose tissue was pre-incubated for 30 min in the incubation medium at 37° without stirring, before adding the drugs. Each value represents the mean of two assays.

The FFA accumulation within brown adipose tissue could be interpreted with a different permeability to FFA of brown fat cell, or with a different mechanism of FFA release from tissue into the surrounding medium. A carrier mechanism for release of FFA from the cell into the medium, or, alternatively, the existence of separate "spaces" or compartments in the adipose cell, into which release of FFA takes place, were postulated by Bally *et al.*[29] Multicompartmented pools of FFA were also indicated by Vaughan *et al.*[30] A difference between brown and white adipose tissue of normal mice on the mechanism of passage of FFA out of the cell was also proposed by Napolitano *et al.*[26]

As regards the absence of stimulation by adipokinetic agents, several possibilities may be put forward. One of these could be the high level of FFA within the cells, producing a feedback control on lipolysis. Evidence that the lipolytic process in the fat cells is regulated in part by the intracellular concentration of free fatty acids, was provided by Bally *et al.*[29] and by Rodbell.[31]

The absence of stimulation by adipokinetic agents could also be dependent on a deficiency of ATP, since energy was shown to be necessary for hormone-stimulated lipolysis.[25,32]

The lack of ATP could depend, in brown fat of cold-acclimatized rats, on two possibilities: (a) a poor phosphorylating efficiency of the respiratory chain or, (b) an energy dissipating mechanism operating in spite of a tightly coupled phosphorylating system.

Brown fat mitochondria from adult untreated animals are able to carry out oxidative phosphorylation in an apparently normal way.[33–36] In contrast, those obtained from

cold-acclimatized rats and from newborn rabbits, exhibited low P:O ratios.[34] Accordingly, in brown fat mitochondria of newborn guinea pigs, the respiratory chain-linked phosphorylation is partially uncoupled.[35] Moreover, in white adipose cells too, at least a portion of the respiration is not linked in an obligatory way to phosphorylation.[37] This portion of nonphosphorylating electron flow to oxygen, could be emphasized in brown fat (mostly after cold-adaptation) in view of thermogenesis.

On the other hand, ATP is necessary for the activation of FFA as metabolic substrate, as well as for their re-esterification to triglycerides.[38,39] Brown adipose tissue possess significant amounts of glycero-kinase.[40] An elevated degree of FFA re-esterification could mask the hormone lipolytic effect. It is thus conceivable that the FFA which accumulate inside brown fat cells of cold-acclimatized rats, represent an energy dissipating mechanism. The correlation of the FFA re-esterification process with the noradrenaline-stimulated non-shivering thermogenesis in brown fat, was firstly indicated by Ball[14] and by Dawkins and Hull,[8] considering FFA acting as an "overall ATPase". More recently, Prusiner *et al.*[36] proposed that the acyl-CoA hydrolase activity contributed to the degradation of ATP formed, thus preventing ATP to exert the normal respiratory control.

To conclude, independently from the intimate mechanism of action, the high level of FFA in brown adipose tissue cells, would allow a store of the physiological substrate for thermogenesis.

Acknowledgements—The authors would like to thank Dr. A. Bruni for his continued interest in this work. The valuable technical assistance of Mr. G. F. Daniel is gratefully acknowledged.

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