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EFFECT OF ANTIMYCIN A ON HORMONE-STIMULATED LIPOLYSIS *IN VITRO*

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Abstract—The effect of antimycin A on hormone-stimulated lipolysis *in vitro* was investigated using noradrenaline, cyclic 3′,5′-AMP and theophylline. The titration curve of antimycin against noradrenaline-stimulated lipolysis was sigmoidal, like the behaviour of antimycin on the respiratory chain of mitochondria. Antimycin A was found to antagonize the lipolytic effect of both cyclic 3′,5′-AMP and theophylline. The range of active concentrations against the different drugs, was very similar. These data confirm that hormone-induced lipolysis needs ATP, generated by oxidative phosphorylation, both before and after cyclic 3′,5′-AMP synthesis.

THREE inhibitors of oxidative phosphorylation, rotenone, 2,4-dinitrophenol and oligomycin, were shown to antagonize hormone-induced lipolysis in adipose tissue.[1–4] Oligomycin was found to inhibit the hormonal activation of lipolysis also in brown adipose cells.[5] The effect of these inhibitors suggested that the lipolytic process requires a continuous supply of energy. To check further on this interpretation, investigations on the effect of antimycin A, another metabolic inhibitor,[6,7] were carried out. This inhibitor interacts with the respiratory chain between cytochromes b and c_1[7–11] and thus in a site different from that of the aforementioned drugs.

The data in this report show that antimycin A is also a potent antagonist of hormone-stimulated lipolysis. The inhibition exerted by antimycin not only against noradrenaline, but also against theophylline and cyclic 3′,5′-AMP, further demonstrates that ATP is required in the process after the synthesis of cyclic AMP.

MATERIALS AND METHODS

Fed Wistar rats (200–250 g) were used. Samples of epididymal adipose tissue (100 ± 5 mg) were placed in 2 ml of Krebs–Ringer bicarbonate buffer pH 7·2 containing 2·5% bovine albumin (fraction V, Sigma) and pre-incubated at 37° for 30 min. Antimycin A was dissolved in ethanol and added to the incubation medium before introducing the fat. The same volume of ethanol (50 μl) was introduced in the control samples. After the pre-incubation period, noradrenaline, theophylline or dibutyryl cyclic 3′,5′-AMP were added, and the assays incubated at 37° for 150 min in a metabolic shaker. At the end of the incubation the reaction was stopped by addition of 0·25N H_2SO_4 (0·1 ml/assay). After centrifugation for 20 min at 5000 *g*, the free fatty acids were determined in the incubation medium according to Dole[12] and glycerol according to Korn.[13]

Noradrenaline bitartrate monohydrate was from Recordati (Milan, Italy), theophylline from C. Erba (Milano, Italy), antimycin A type III from Sigma (St. Louis,

TABLE 1. INHIBITORY EFFECT OF ANTIMYCIN ON NORADRENALINE-STIMULATED LIPOLYSIS IN RAT ADIPOSE TISSUE *in vitro*

Drugs in the medium (M)	Δ FFA * (μequiv./g/150 min)		P	Δ Glycerol * (μM/g/150 min)		P
NA 2×10^{-7}	3·92 ± 0·47	—	—	1·46 ± 0·04	—	—
NA 2×10^{-7} + antimycin 10^{-5}	2·15 ± 0·31	(−45%)	<0·05	1·74 ± 0·24	—	>0·30
NA 2×10^{-7} + antimycin 10^{-4}	2·00 ± 0·33	(−49%)	<0·05	0·98 ± 0·16	—	>0·05
NA 2×10^{-6}	20·29 ± 0·76	—	—	16·55 ± 0·25	—	—
NA 2×10^{-6} + antimycin 10^{-5}	11·85 ± 1·19	(−41%)	<0·01	11·96 ± 0·35	(−28%)	<0·01
NA 2×10^{-6} + antimycin 10^{-4}	3·54 ± 0·21	(−82%)	<0·001	1·86 ± 0·12	(−89%)	<0·001
NA 2×10^{-5}	32·54 ± 1·35	—	—	17·16 ± 0·49	—	—
NA 2×10^{-5} + antimycin 10^{-5}	17·64 ± 1·70	(−46%)	<0·01	7·41 ± 0·49	(−57%)	<0·01
NA 2×10^{-5} + antimycin 10^{-4}	6·40 ± 1·05	(−80%)	<0·001	2·67 ± 0·25	(−84%)	<0·005

Epididymal fat (100 ± 5 mg) was incubated for 150 min at 37° in 2 ml of Krebs–Ringer bicarbonate buffer pH 7·2 containing 2·5% bovine albumin. NA = noradrenaline. Antimycin 5×10^{-6} corresponds to 5 nmoles/mg protein of adipose tissue. Each value represents the mean ± S.E. of four to five experiments.

* FFA and glycerol absolute increase from control (fat incubated without noradrenaline) in the incubation medium.

TABLE 2. INHIBITORY EFFECT OF ANTIMYCIN ON THEOPHYLLINE-STIMULATED LIPOLYSIS IN RAT ADIPOSE TISSUE *in vitro*

Drugs in the medium (M)	Δ FFA * (μequiv./g/150 min)		P	Δ Glycerol * (μM/g/150 min)		P
Theophylline 2×10^{-3}	23·31 ± 3·64	—	—	7·54 ± 0·75	—	—
Theophylline 2×10^{-3} + Antimycin 10^{-5}	7·19 ± 0·30	(−69%)	<0·05	2·32 ± 0·30	(−69%)	<0·01
Theophylline 2×10^{-3} + Antimycin 10^{-4}	0·62 ± 0·22	(−97%)	<0·001	0·61 ± 0·08	(−92%)	<0·01
Theophylline 5×10^{-3}	31·49 ± 2·36	—	—	11·47 ± 0·25	—	—
Theophylline 5×10^{-3} + Antimycin 10^{-5}	10·75 ± 0·56	(−66%)	<0·001	3·07 ± 0·25	(−73%)	<0·01
Theophylline 5×10^{-3} + Antimycin 10^{-4}	1·63 ± 0·47	(−95%)	<0·001	1·02 ± 0·04	(−91%)	<0·01

Experimental conditions as in Table 1. Antimycin 10^{-5} M corresponds to 10 nmoles/mg protein of adipose tissue. Each value represents the mean ± S.E. of three to four experiments.

* FFA and glycerol absolute increase from control (fat incubated without theophylline) in the incubation medium.

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TABLE 3. INHIBITORY EFFECT OF ANTIMYCIN ON DIBUTYRYL CYCLIC 3′,5′-AMP-STIMULATED LIPOLYSIS IN RAT ADIPOSE TISSUE *in vitro*

Drugs in the medium (M)	Δ FFA * (μequiv./g/150 min)		P	Δ Glycerol * (μM/g/150 min)		P
CAMP-DB 2×10^{-3}	23·95 ± 1·12	—	—	11·94 ± 0·71	—	—
CAMP-DB 2×10^{-3} + Antimycin 10^{-5}	10·26 ± 0·77	(−57%)	<0·001	5·14 ± 0·49	(−57%)	<0·005
CAMP-DB 2×10^{-3} + Antimycin 10^{-4}	4·03 ± 0·14	(−83%)	<0·001	1·67 ± 0·14	(−86%)	<0·001
CAMP-DB 5×10^{-3}	29·55 ± 0·93	—	—	14·61 ± 0·68	—	—
CAMP-DB 5×10^{-3} + Antimycin 10^{-5}	11·74 ± 0·30	(−60%)	<0·001	16·05 ± 0·39	(−58%)	<0·001
CAMP-DB 5×10^{-3} + Antimycin 10^{-4}	6·21 ± 0·44	(−79%)	<0·001	2·78 ± 0·19	(−81%)	<0·001

Experimental conditions as in Table 1. Antimycin 10^{-5} M corresponds to 10 nmoles/mg protein of adipose tissue. Each value represents the mean ± S.E. of three experiments.

* FFA and glycerol absolute increase from control (fat incubated without CAMP-DB) in the incubation medium.

CAMP-DB = Dibutyryl cyclic 3′,5′-adenosine-monophosphate.

Missouri, USA). $^6NC_2'$-dibutyryl cyclic 3',5'-adenosine-monophosphate was a generous gift of Dr. M. Carissimi (Maggioni, Milano, Italy).

RESULTS

Effect of antimycin A on the basal and on noradrenaline-stimulated lipolysis

The free fatty acids (FFA) and glycerol release induced by noradrenaline from adipose tissue, were both inhibited by antimycin (Table 1). The glycerol release inhibition indicates that the effect of the metabolic inhibitor is due to a real inhibition of lipolysis.

TABLE 4. CONCENTRATIONS OF ANTIMYCIN REDUCING TO 50 per cent THE EFFECT OF SOME LIPOLYTIC DRUG

Lipolytic drug	Antimycin ED_{50}
Noradrenaline	$8{\cdot}55\ (\pm 0{\cdot}16) \times 10^{-6}$ M
Dibutyryl cyclic 3',5'-AMP	$4{\cdot}25\ (\pm 1{\cdot}25) \times 10^{-6}$ M
Theophylline	$2{\cdot}00\ (\pm 0{\cdot}20) \times 10^{-6}$ M

The ED_{50} values were calculated from the dose–effect curves of noradrenaline, dibutyryl cyclic 3',5'-AMP and theophylline, in the absence and in presence of different concentrations of antimycin.

A 50 per cent inhibitory effect was exerted by antimycin at $8{\cdot}55 \times 10^{-6}$ M (Table 4) corresponding to 14 nmoles/mg of adipose tissue protein. However, these quantitative data are only indicative, because of the presence of bovine albumin in the incubation medium of adipose tissue (25 mg bovine serum albumin/mg tissue protein). In fact, it has been reported that antimycin inhibition on respiratory chain may be reversed by addition of serum albumin.[14] Another result to be considered is the sigmoidal shape of the inhibition curve of antimycin in contrast with the hyperbolic curve of rotenone (Fig. 1). This is in accordance with the behaviour of the antimycin inhibition curve on mitochondrial respiratory chain[15–17] and agrees with the assumption that the inhibitory effect on hormone-stimulated lipolysis induced by these drugs, is dependent on their action at mitochondrial level.

The similar effect of antimycin against increasing concentrations of noradrenaline (2×10^{-7} M, 2×10^{-6} M and 2×10^{-5} M) indicated that the antagonism is of a non-competitive type.

Finally, the basal FFA and glycerol release (from untreated adipose tissue) was not significantly affected by antimycin.

Effect of antimycin A on lipolysis induced by theophylline and by dibutyryl cyclic 3',5'-adenosine-monophosphate

The inhibitory effect of antimycin was evidentiated also against theophylline (Table 2) and dibutyryl cyclic 3',5'-AMP stimulated FFA and glycerol release (Table 3). This suggests that the site of action of antimycin is located after the synthesis of cyclic AMP by adenyl-cyclase. The ED_{50} of antimycin was $4{\cdot}25 \times 10^{-6}$ M against dibutyryl cyclic 3',5'-AMP, and $2{\cdot}0 \times 10^{-6}$ M against theophylline (Table 4).

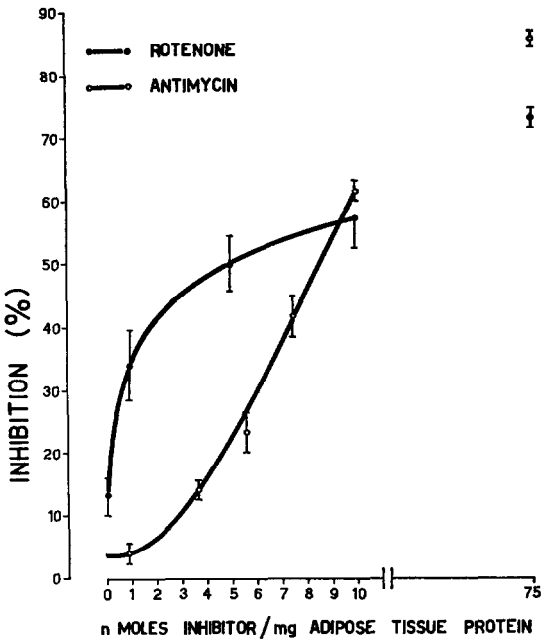

FIG. 1. The inhibition of antimycin A and of rotenone on the noradrenaline-stimulated lipolysis in rat epididymal fat *in vitro*. Experimental conditions as in Table 1. Each point represents the mean ± S.E. of four to six assays.

DISCUSSION

Antimycin A showed an antagonistic effect against lipolysis stimulated by noradrenaline, by theophylline and by dibutyryl cyclic 3′,5′-AMP in rat epididymal fat *in vitro*.

Like antimycin, other inhibitors of oxidative phosphorylation, rotenone, 2,4-dinitrophenol and oligomycin, were shown to inhibit hormone-stimulated lipolysis in adipose tissue.[1–5] These inhibitors are known to act on oxidative phosphorylation at different levels and by different mechanisms:[18,19] 2,4-DNP is an uncoupling agent, oligomycin interferes with energy transfer reactions, rotenone inhibits the oxidation of NADH on the substrate side of cytochrome b and antimycin interferes with respiratory chain between cytochromes b and c_1.[6–11] These differences clearly suggest that the way in which oxidative phosphorylation is affected by these inhibitors is not determinant for their effect on lipolysis, thus indicating that the overall process is needed. However, as yet, we have not sufficient data demonstrating whether the end product ATP is necessary, or if some intermediate rich in energy could be utilized. Investigations are being carried out along this line.

The inhibition by antimycin on the theophylline and dibutyryl cyclic 3′,5′-AMP-induced lipolysis, indicates that the energy is required not only in the cyclic AMP formation by adenylcyclase system,[20] but also at some further step of the process. This conclusion agrees with data obtained by Rizack[21,22] showing that in a cell-free lipolytic enzyme system from adipose tissue the simultaneous presence of 3′,5′-AMP and of ATP is necessary to stimulate the hormone-sensitive lipolytic activity. These

data are, finally, in complete accordance with the recent finding of Kuo and Greengard,[23] demonstrating the presence in adipose tissue of a cyclic AMP-dependent protein kinase, which requires ATP to phosphorylate a still unknown substrate.

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