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METABOLIC INHIBITORS AND CATECHOLAMINE-STIMULATED LIPOLYSIS

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Abstract—The effects of varying dose and time in the antagonism between oxidative phosphorylation inhibitors (rotenone, oligomycin, 2,4-DNP) and norepinephrine-stimulated lipolysis *in vitro* were determined. A comparison was made between the kinetics of antagonism of this group of compounds with that of some glycolysis inhibitors (mono- iodoacetic acid, sodium fluoride). The results led to the following conclusions. (a) The antagonism by oxidative phosphorylation inhibitors is a non-competitive one. In the experimental conditions used, pD'_2 values were: rotenone = 5, oligomycin = 4·8 and 2,4-DNP = 2·7. (b) Both oxidative phosphorylation and glycolysis seem to be implicated in the norepinephrine-stimulated lipolysis. However, the results gave no indicationof the relative importance of the two metabolic pathways in the process studied.

INHIBITORS of oxidative phosphorylation were found to antagonize strongly the free fatty acid release stimulated by norepinephrine from rat adipose tissue *in vitro*.[1, 2] This finding disagrees with data previously reported by Mosinger, in which only a small effect on epinephrine-induced lipolysis was observed with substances that inhibit oxidative processes or uncouple oxidative phosphorylation. On the other hand, the same author found a strong antagonistic effect of glycolysis inhibitors on the lipomobilizing action of epinephrine.[3]

This paper reports the investigations made on the type of antagonism exerted on lipolysis by oxidative phosphorylation inhibitors (rotenone, oligomycin and 2,4-dinitrophenol). Secondly, the antagonistic action of glycolysis inhibitors (monoiodoacetic acid and sodium fluoride) was studied from a kinetic point of view and compared with that of oxidative phosphorylation inhibitors, in order to determine the relative importance of these metabolic processes in the catecholamine-stimulated lipolysis.

MATERIALS AND METHODS

Sprague–Dawley male rats (200 ± 30 g) were used. Samples of epididymal fat (100 ± 10 mg) from normal animals were incubated in 2 ml of Krebs–Ringer bicarbonate buffer pH 7·2 containing 3 per cent bovine albumin (fraction V Sigma), at 37° in a metabolic shaker for varying intervals of time. Norepinephrine and the metabolic inhibitors were added to the incubation vessels before introduction of the fat. Ascorbic acid (200 μg/ml) was added to all tests in order to delay the oxidation of norepinephrine. Oligomycin, rotenone and 2,4-dinitrophenol (2,4-DNP) were dissolved in absolute ethanol. The same volume of ethanol (0·05 ml) was introduced in the control samples.

At the end of incubation, the medium was immediately acidified with H_2SO_4 2·5 N (0·1 ml/essay). After centrifugation for 20 min at 5000 *g*, the free fatty acids (FFA) were titrated in 1 ml of the incubation medium according to Dole's method.[4] The adipose tissue of each sample was resuspended in 2 ml of 9‰ NaCl and homogenized with an Elvejhem–Potter homogenizer with glass pestel. The homogenate was transferred directly into the extraction mixture for FFA titration according to Dole.[4] Glycerol released into the incubation medium was determined using Korn's method.[5]

Norepinephrine bitartrate monohydrate was obtained from Recordati (Milano, Italy,) rotenone, 2,4-DNP and monoiodoacetic acid from British Drug Houses Ltd. (Poole, England) and sodium fluoride from Merck (Darmstadt, Germany). Oligomycin (a mixture of oligomycin A and B) was supplied by the Upjohn Co. (Kalamazoo, U.S.A.).

RESULTS

Effect of metabolic inhibitors on the free fatty acid and glycerol mobilization from adipose tissue induced by norepinephrine

The free fatty acid and the glycerol release in the incubation medium under the influence of norepinephrine, are both antagonized by inhibitors of oxidative phosphorylation and of glycolysis (Table 1). This suggests that the antagonistic action of

TABLE 1. EFFECT OF OXIDATIVE PHOSPHORYLATION AND GLYCOLYSIS INHIBITORS ON THE FREE FATTY ACID AND GLYCEROL RELEASE BY NOREPINEPHRINE FROM ADIPOSE TISSUE *in vitro*

Drugs added to the incubation medium Molar conc.		Δ FFA* μEquiv/g fresh tissue/150 min	Δ Glycerol* μM/g fresh tissue/150 min
Norepinephrine 2×10^{-5}		35·01 ± 2·48	19·91 ± 0·50
Norepinephrine 2×10^{-5}	+ rotenone 10^{-5}	12.02 ± 1·38	7·38 ± 0·40
Norepinephrine 2×10^{-5}	+ oligomycin 10^{-5}	12·89 ± 0·60	6·30 ± 1·31
Norepinephrine 2×10^{-5}	+ 2,4-DNP 10^{-3}	11·16 ± 2·15	6·87 ± 0·25
Norepinephrine 2×10^{-5}		31·39 ± 1·41	19·54 ± 0·47
Norepinephrine 2×10^{-5}	+ monoiodoacetic acid 2×10^{-3}M	7·20 ± 0·86	3·52 ± 0·20
Norepinephrine 2×10^{-5}	+ NaF 4×10^{-2}M	9·45 ± 0·94	3·57 ± 0·26

Rat epididymal fat (100 ± 10 mg) was incubated in 2 ml of Krebs–Ringer bicarbonate buffer pH 7·2 containing 3 per cent bovine albumin, at 37° for 150 min. Ascorbic acid was not added to the medium.

* Free fatty acid and glycerol increase from control (fat incubated without norepinephrine) in the incubation medium. Each value represents the mean ± S.E. of 4–6 determinations.

metabolic inhibitors is due to a real inhibition of lipolysis rather than to an interference with the re-esterification of free fatty acids via glycerophosphate.

Antagonistic behaviour of rotenone, oligomycin and 2,4-dinitrophenol on the norepinephrine-stimulated lipolysis in vitro

From the log dose-action curves of norepinephrine in the presence of different concentrations of rotenone, oligomycin and 2,4-DNP (Figs. 1–3) it is evident that the inhibition by these drugs is a non-competitive one. In the experimental conditions

used the pD_2'* values are as follows: rotenone = 5 ± 0·2; oligomycin = 4·8 ± 0·3; 2,4-DNP = 2·7. It must be pointed out that the action of inhibitors used is antagonized to some extent by ascorbic acid. However, the presence of this drug permitted us to evaluate better the pattern of the ascending branch of log dose–action curves and, consequently, the type of antagonism.

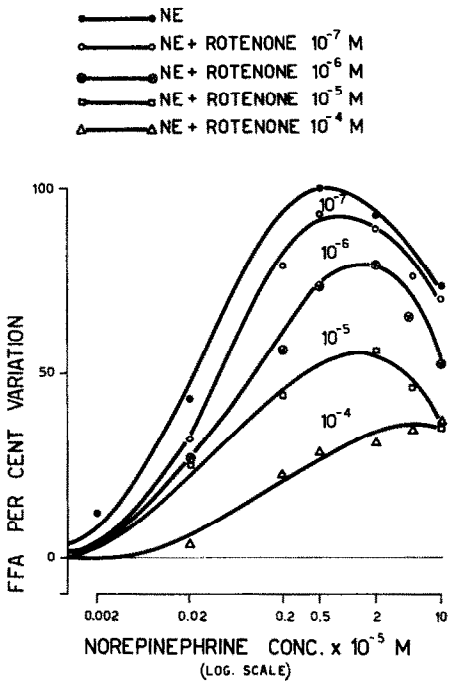

FIG. 1. Dose–action curves for the antagonism between norepinephrine and rotenone on lipolysis *in vitro*. Abscissa: molar concentration of norepinephrine (NE) in the medium. Ordinate: FFA per cent relative increase in the incubation medium. The FFA increase from control, induced by norepinephrine 0·5 × 10^{-5}M (24·98 ± 1·47 μEquiv/g fresh tissue per 90 min), was taken as 100 per cent of the effect. Each point represents the mean of four determinations.

Rat epididymal fat (100 ± 10 mg) was incubated in 2 ml of Krebs–Ringer bicarbonate buffer pH 7·2, containing 3 per cent bovine albumin and ascorbic acid 200 μg/ml, at 37° for 90 min.

Kinetic of the basal and norepinephrine-stimulated lipolysis and of inhibition exerted thereon by oxidative phosphorylation inhibitors

In Figs. 4 and 5 the FFA release in the medium is plotted as a function of incubation time in the absence and in the presence of norepinephrine and of rotenone or oligomycin. The inhibition rate is lower in the first phase of incubation (45–60 min) reaching its maximum when the norepinephrine action is approaching the plateau, after 120–150 min. The basal FFA release (without addition of norepinephrine) is appreciably inhibited only after the first 75–90 min.

* pD_2'—The negative logarithm of the molar concentration of antagonist that reduces the effect of the agonist to 50 per cent.[6]

The antagonistic action of rotenone was investigated against two concentrations of norepinephrine (0·2 and 2 × 10^{-5}M). The inhibition rate is independent from the norepinephrine concentration in the medium.

The kinetic of FFA release was also determined inside the adipose tissue. Here too, the inhibitory effect of oligomycin on the norepinephrine-stimulated lipolysis increases with time, showing a pattern similar to that of FFA release in the medium (Fig. 6).

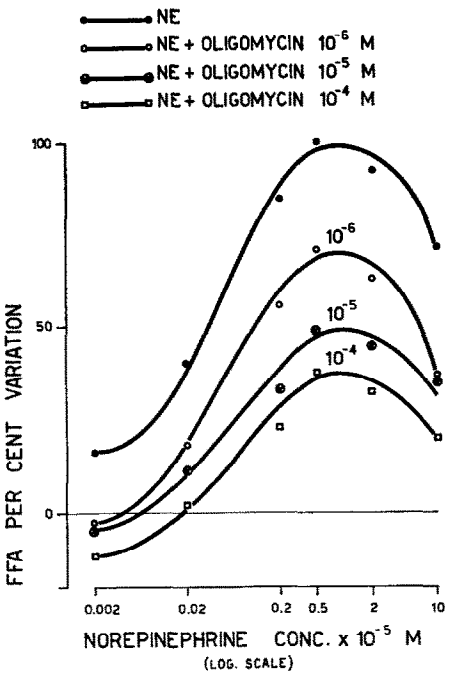

FIG. 2. Dose–action curves for the antagonism between norepinephrine and oligomycin on lipolysis *in vitro*. Abscissa: molar concentration of norepinphrine (NE) in the medium. Ordinate: FFA per cent relative increase in the incubation medium after 90 min of incubation. The FFA increase from control induced by norepinephrine 0·5 × 10^{-5}M was taken as 100 per cent of the effect. Each point represents the mean of four determinations. Experimental conditions as in Fig. 1.

Kinetic of the inhibition exerted on lipolysis by monoiodoacetic acid and sodium fluoride

The time–action curves of the inhibition by monoiodoacetic acid and sodium fluoride on the basal and norepinephrine-stimulated lipolysis show similar behaviour to that of oxidative phosphorylation inhibitors (Figs. 7 and 8). In this case too the inhibition rate is independent from the norepinephrine concentration in the medium.

DISCUSSION

The experiments described show that oxidative processes exert an important role in the norepinephrine-stimulated lipolysis *in vitro*. Rotenone,[7, 8] oligomycin[9, 10]

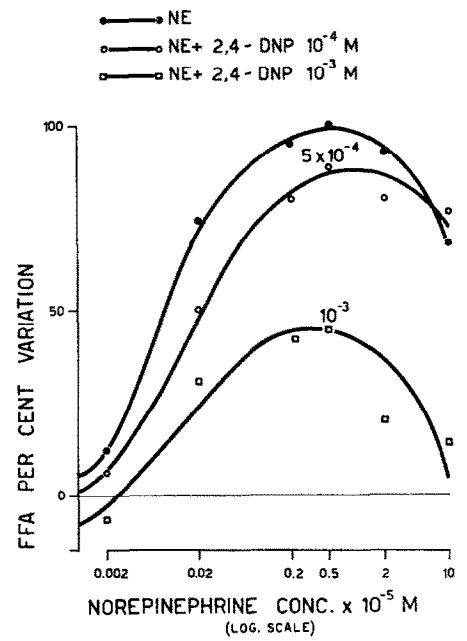

FIG. 3. Dose–action curves for the antagonism between norepinephrine and 2,4-dinitrophenol (2,4-DNP) on lipolysis *in vitro*. Abscissa: molar concentration of norepinephrine (NE) in the medium. Ordinate: FFA per cent relative increase in the incubation medium after 90 min of incubation. The FFA increase from control induced by norepinephrine $0{\cdot}5 \times 10^{-5}$M was taken as 100 per cent of the effect. Each point represents the mean of four determinations. Experimental conditions as in Fig. 1.

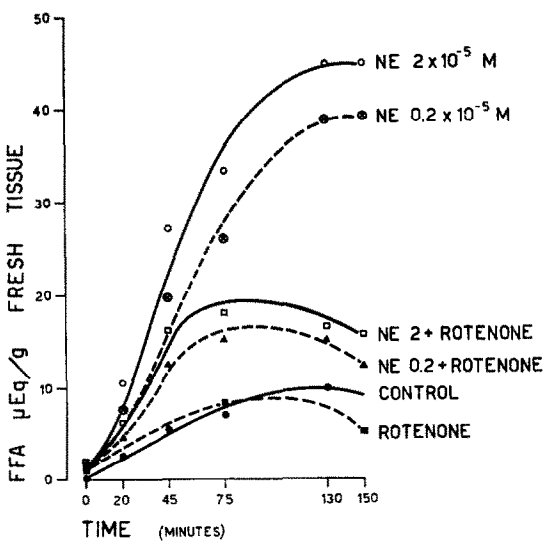

FIG. 4. Kinetic of the FFA release in the medium of rat epididymal fat incubated in the absence (control) and in presence of norepinephrine (NE, 2 and $0{\cdot}2 \times 10^{-5}$M) and of inhibition exerted thereon by rotenone 5×10^{-5}M.

Abscissa: incubation time. Ordinate: FFA concentration in the medium (μEquiv/g fresh tissue). Experimental conditions as in Fig. 1.

and 2,4-DNP[11] have different sites and mechanisms of action on oxidative phosphorylation. The fact that these differences do not influence the antagonistic action against norepinephrine is particularly interesting. The action of 2,4-DNP which permits and even stimulates respiration, but inhibits lipolysis, seems to indicate that the lipolytic process depends not only on respiration, but requires a continuous supply of energy.

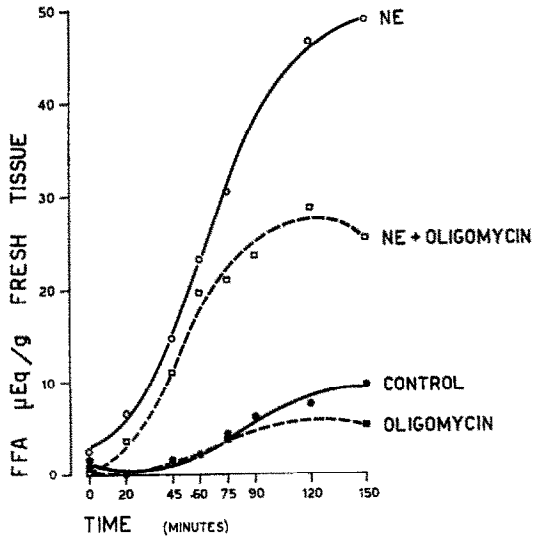

FIG. 5. Kinetic of the FFA release in the medium of rat epididymal fat incubated in the absence (control) and in the presence of norepinephrine (NE, 2×10^{-5}M) and inhibition exerted thereon by oligomycin 10^{-5}M.
Abscissa: incubation time. Ordinate: FFA concentration in the medium (μEquiv/g fresh tissue). Experimental conditions as in Fig. 1.

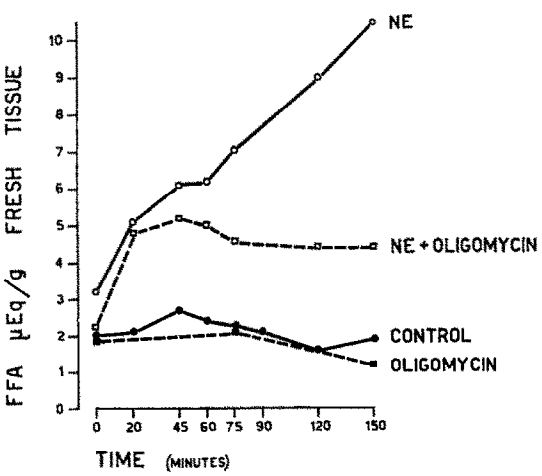

FIG. 6. Kinetic of the FFA release inside rat epididymal fat incubated in the absence (control) and in the presence of norepinephrine (NE, 2×10^{-5}M) and inhibition exerted thereon by oligomycin 10^{-5}M.
Abscissa: incubation time. Ordinate: FFA concentration in the incubated fat (μEquiv/g fresh tissue). Experimental conditions as in Fig. 1.

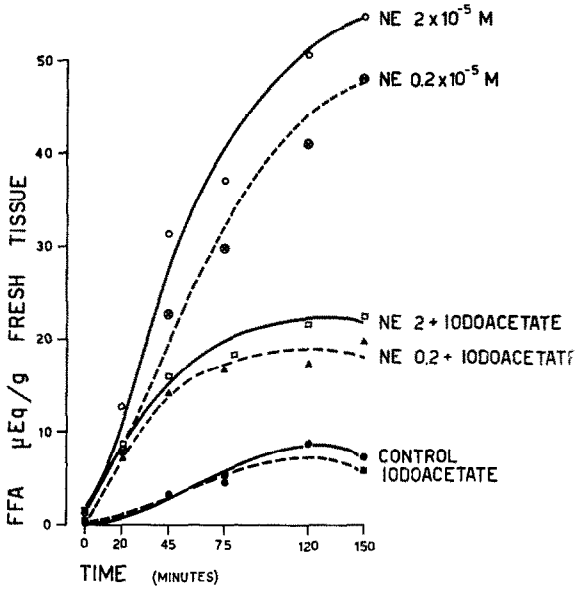

FIG. 7.

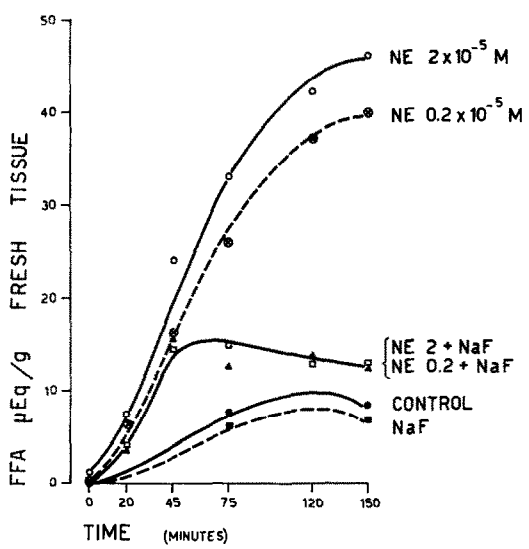

FIG. 8.

FIGS. 7 and 8. Kinetic of the FFA release in the medium of rat epididymal fat incubated in the absence (control) and in presence of norepinephrine (NE, 2 and 0·2 × 10^{-5}M) and inhibition exerted thereon by glycolysis inhibitors.

Upper graph: sodium iodoacetate 10^{-3}M. Lower graph: sodium fluoride 4 × 10^{-2}M. Abscissa: incubation time: Ordinate: FFA concentration in the medium. Experimental conditions as in Fig.1.

Mosinger's results regarding the influence of oxidative phosphorylation on epinephrine-induced lipolysis are not in agreement with ours, however the data on glycolysis agreed.[3] We therefore investigated if the differences were due to varying experimental conditions (in particular incubation time) by comparing the kinetics of the norepinephrine-stimulated lipolysis under both the influence of oxidative phosphorylation and glycolysis inhibitors. A different behaviour of time–action curves in the antagonistic action of these two classes of compounds could, moreover, have discriminated the importance of glycolysis and oxidative phosphorylation in successive stages of the lipolytic process. But the kinetic of the inhibitory activity appeared similar in the two cases. Thus, our results point out the importance of oxidative phosphorylation as well as of glycolysis in the process studied, but the relative significance of the two metabolic pathways as regards the norepinephrine-stimulated lipolysis still remains unsolved.

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