LIPOPHILIC DITHIOCARBAZATES AS POTENT UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION

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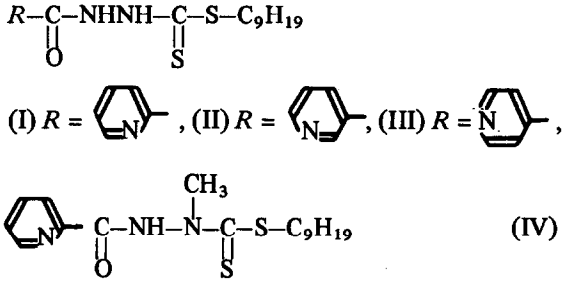
1. Introduction

Recently, Bäuerlein and Keihl [1] found that nonyl thiourea and nonyl thiouracil were inhibitors of oxidative phosphorylation, inhibiting the respiration in State 3 and stimulating respiration in State 4, in mitochondria. These effects were ascribed to the direct interaction of a nonphosphorylated high-energy intermediate $X\sim I$, with lipophilic thiourea or thiouracil, forming mixed disulfide complex [1]. Since these compounds contain the common structure –NH–CS–NH–, it is of interest to examine the activities of dithiocarbazates or dithiocarbamates, on mitochondrial functions, having a similar chemical structure, –NH–CS–S–.

We have studied the effects of some alkyl acyldithiocarbazates on mitochondria and found that they are potent uncouplers of oxidative phosphorylation. This paper deals with uncoupling activities of nonyl 3-picolinoyldithiocarbazate(PDTC-9; I), nonyl 3-nicotinoyldithiocarbazate(NDTC-9; II) and nonyl 3-isonicotinoyldithiocarbazate(IDTC-9; III).



Abbreviations: CCP, carbonyl cyanide phenylhydrazone; SF 6847, 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalononitrile

2. Materials and methods

Nonyl acyldithiocarbazates (I, II and III) and nonyl 2-methyl-3-picolinoyldithiocarbazate (methyl-PDTC-9, IV) were synthesized according to a method to be described [2]. SF 6847 and rotenone were kindly supplied by Sumitomo Chemical Industry, Osaka (Japan) and CCP was a gift of Dr P. G. Heytler, E.I. du Pont de Nemours and Co., Wilmington (USA). Other reagents were obtained from commercial sources and used without further purification.

Rat liver mitochondria were isolated according to the method of Hogeboom [3] as described by Myers and Slater [4]. The protein concentration of mitochondria was determined by the Biuret method [5] and from the maximum velocity of State 4 respiration stimulated by SF 6847 [6].

Oxygen uptake was monitored with a Galvani electrode as described by Utsumi et al. [7] at 25°C using medium, pH 7.2, containing 200 mM sucrose, 10 mM phosphate, 2 mM $MgCl_2$, 10 mM succinate and 1 mM EDTA, unless otherwise noted.

ATPase activity was determined by measuring medium pH-changes with a Hitachi-Horiba sensitive pH-meter, model F-7, according to the method of Bertina and Slater [8]. The medium contained 100 mM sucrose, 0.5 mM EDTA, 50 mM KCl and 10 mM Tris · HCl (pH 7.4). Before addition of uncoupler, mitochondria were incubated for 2 min with 2 mM ATP.

3. Results

Figure 1 shows the effects of PDTC-9 on the

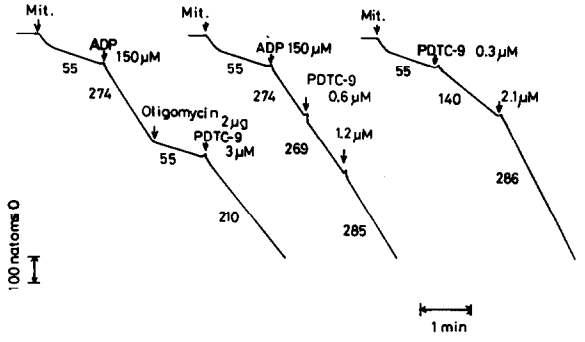


Fig.1. Effect of PDTC-9 on mitochondrial respiration using succinate as substrate. The numbers under the lines are respiration rates in natoms oxygen/min at 25°C. Final concentrations of reagents are indicated in the figure. Rotenone (1 μg) is present in the reaction mixture. Mitochondria, 1.9 mg protein in a total volume of 3.3 ml.

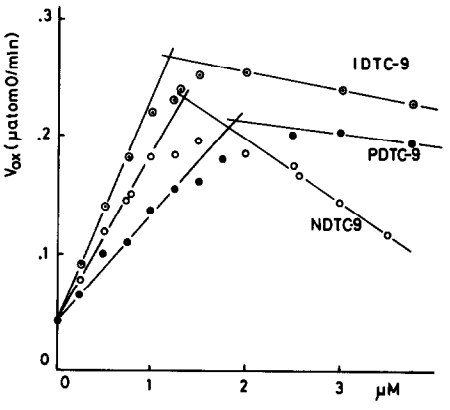


Fig.3. Titrations of State 4 mitochondria with nonyl acyldithiocarbazates at 25°C. The substrate was succinate and 1 μg rotenone was added to the reaction mixture before addition of uncoupler. Mitochondria, 0.98 mg, in total volume 2.0 ml. V_{ox}, rate of respiration.

respiration of rat liver mitochondria using succinate as substrate. PDTC-9, at 1–3 μM, releases oligomycin-inhibited respiration almost completely and stimulates State 4 respiration, but has little effect on the respiration in State 3. PDTC-9 also activates ATPase activity up to 20 μM (fig.2), but further addition of PDTC-9 causes an inhibition of this activity. Since these results are commonly observed in the uncoupling of oxidative phosphorylation of mitochondria [6,8,9], the effects of PDTC-9 on mitochondrial functions are regarded as due to its uncoupling activity. Other derivatives of nonyl acyldithiocarbazates, NDTC-9 and IDTC-9, exhibited essentially the same effects on mitochondria.

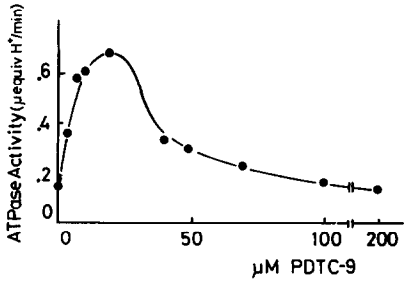


Fig.2. Effect of PDTC-9 on the mitochondrial ATPase activity. ATP hydrolysis was measured with a pH-meter (see Materials and methods). Final volume 3.2 ml, pH 7.4, 4 mg protien. The concentration of H^+ in the medium was standardized with oxalic acid.

In order to determine the potencies of the uncoupling activities of these compounds, titration of State 4 mitochondria was carried out. Figure 3 shows the titration curves of nonyl acyldithiocarbazates when succinate was used as a substrate. In all cases, successive addition of the uncoupler induces an almost linear increase in respiration, but further addition of the uncoupler causes the curve to bend before reaching a maximum rate (V_{max}). At still higher concentration of uncoupler, inhibition with respect to V_{max} occurs, especially in the case of NDTC-9.

The effectiveness of these uncouplers can be expressed as the slope of the linear portion of the titration curve at the lower concentrations of uncoupler, or as the concentration of uncoupler required for a maximal stimulation of the respiration (titration point) determined from the intersection of two straight lines of the initial ascending and the final descending parts of the titration curve (fig.3). Values of V_{max}, the slope of the titration curve and the concentration of uncoupler at the titration point (100% uncoupling concentration) are summarized in table 1. The values for CCP as a reference uncoupler are also listed. The results in table 1 indicate that uncoupling activities of nonyl acyldithiocarbazates are approximately the same as that of CCP, and the

Table 1
Uncoupling activities of nonyl acyldithiocarbazates[a]

Compound (ϕ)	V_{max} (natoms O/min)	Slope (atoms O/min·mol ϕ)	Titration point (μM)
PDTC-9	212	46	1.9
NDTC-9	235	74	1.3
IDTC-9	270	96	1.2
CCP	310	155	0.9

[a]Data listed in the table were obtained from fig.3

individual activity increases gradually in the order of PDTC-9, NDTC-9 and IDTC-9.

Figure 4 illustrates the effects of methyl-PDTC-9, an *N*-methyl derivative of PDTC-9, on the respiration of mitochondria. This compound has no effect up to 100 μM on State 4 respiration with succinate as substrate, but exhibits a partial inhibitory effect on State 3 respiration, decreasing the respiration to 60% of the initial State 3 respiration. This inhibition commences at 10 μM of methyl-PDTC-9, and the effect increases up to 100 μM. When methyl-PDTC-9 (final concentration, 100 μM) was added to mitochondria 1 min after the addition of ADP, State 3 respiration decreased gradually and finally reached the level of State 4 respiration. During the course of this inhibition, uncouplers PDTC-9, SF 6847 and CCP released the methyl-PDTC-9 inhibited respiration. Thus, the effect of methyl-PDTC-9 is similar to that of oligomycin.

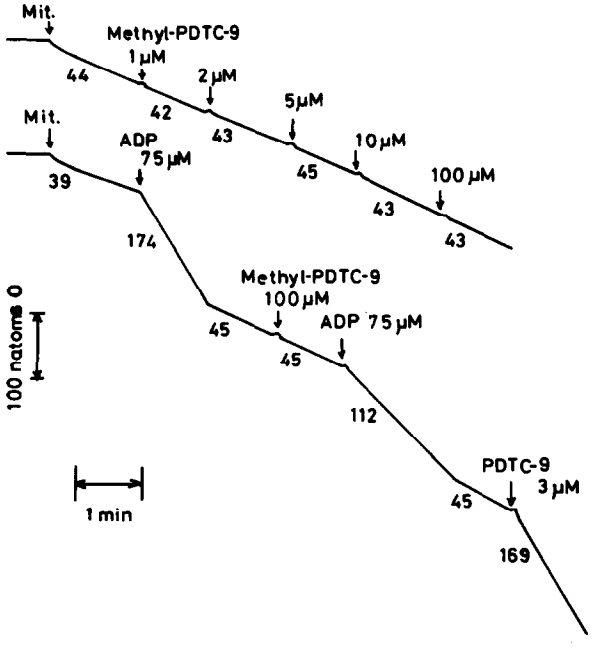
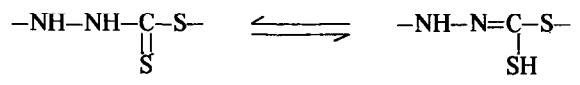


Fig.4. Effect of methyl-PDTC-9 on the coupled respiration of mitochondria using succinate as substrate. The numbers under the lines are the rate of respiration in natoms oxygen/min. Rotenone (1 μg) was added to the reaction mixture before addition of the reagent. Final concentration of the reagent is indicated in the figure. Mitochondria, 0.98 mg, volume of the reaction mixture, 2.0 ml, pH 7.2, temperature 25°C.

4. Discussion

From the results presented here, nonyl acyldithiocarbazates are found to be uncouplers of oxidative phosphorylation with a similar potency to CCP. The effect of these compounds on mitochondrial functions is common to other weakly acidic uncouplers, i.e., stimulating respiration in State 4 mitochondria and ATPase activity, releasing the oligomycin inhibited respiration, and inhibiting both the respiration and ATPase activity at higher concentrations.

It should be noted that methyl-PDTC-9 does not induce uncoupling up to 100 μM, but has an oligomycin-type activity. This compound exists only as the fixed structure of the thione form, while alkyl acyldithiocarbazates are present as thione–thiol tautomeric forms.



For the uncoupling activity, it is suggested that a structure capable of existing in thione–thiol tautomeric forms is essential. In view of the structural characteristics of this series of compounds, it is possible that alkyl acyldithiocarbazates act as sulfhydryl reagents, directly interacting with certain components of the oxidative phosphorylation system [1,9]. However, these lipophilic acyldithiocarbazates have ionizable group(s) in their chemical structures as commonly seen among most weakly acidic uncouplers, and it could also be presumed that these compounds exert their uncoupling effects through an enhancement of proton permeability across mitochondrial membranes.

References

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