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Mitochondrial protonophoric activity induced by a thyromimetic fatty acid analogue

Orit Hermesh, Bella Kalderon, Benjamin Berman, Jacob Bar-Tana *

Department of Human Nutrition and Metabolism, Faculty of Medicine, Hebrew University Medical School, P.O. Box 12272, Jerusalem 91120, Israel

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Abstract

Calcium-dependent uncoupling of liver mitochondrial oxidative phosphorylation by a non-metabolizable long chain fatty acyl analogue was compared with uncoupling induced by in vivo thyroid hormone treatment. β,β′-Methyl-substituted hexadecane α,ω-dioic acid (Medica 16) is reported here to induce a saturable 20–30% decrease in liver mitochondrial $\Delta\Psi$, ΔpH and protonmotive force which proceeds in the presence of added Ca^{2+} to cyclosporin A-sensitive mitochondrial permeabilization. Ca^{2+}-dependent uncoupling by Medica 16 was accompanied by atractylate-enhanced, bongkrekic-inhibited activation of mitochondrial Ca^{2+} efflux. The direct mitochondrial effect exerted in vitro by Medica 16 is similar to that induced by in vivo thyroid hormone treatment. Hence, the thyromimetic protonophoric activity of Medica 16 and the uncoupling activity of TH converge onto components of the mitochondrial permeabilization transition pore.

Keywords: Mitochondria; Uncoupling; Fatty acid; Thyroid hormone

1. Introduction

The mitochondrial uncoupling activity of long chain fatty acids (LCFA) results in increase in mitochondrial state 4 respiration with a concomitant decrease in P/O ratio (reviewed in [1]). This activity reported both in isolated mitochondria and cells is of importance in modulating the efficiency of energy expenditure under variable physiological conditions [2] as well as in promoting mitochondrial permeability transition (MPT) (reviewed in [3,4]) under conditions of ischemia-reperfusion damage [5].

Medica 16 is a long chain fatty acyl analogue consisting of β,β′-methyl-substituted hexadecane α,ω-dicarboxylic acid (HOOC-CH_2-$C(CH_3)_2$-$(CH_2)_{10}$-$C(CH_3)_2$-CH_2-COOH) [6]. Since the ω-carboxyl substitution of Medica 16 interferes with its esterification into lipids while the methyl substitutions exclude its β-oxidation, this fatty acyl analogue may serve as a model compound for modulatory effects exerted by LCFA independent of their role as substrates. Specifically, Medica 16 may be used for analyzing the intrinsic uncoupling activity of LCFA on oxidative

Abbreviations: ANT, adenine nucleotide translocase; CSA, cyclosporin A; LCFA, long chain fatty acids; Medica 16, β,β′-methyl-substituted hexadecane α,ω-dioic acid; MPT, mitochondrial permeability transition; pmf, protonmotive force; TH, thyroid hormone

* Corresponding author. Fax: +972 (2) 6431105;
E-mail: bartanaj@cc.huji.ac.il

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phosphorylation under conditions where uncoupling is not compromised by respiration of the putative uncoupler. Medica 16 has indeed been recently reported by us to induce in liver cells as well as in liver and heart mitochondria a pronounced but saturable decrease in mitochondrial protonmotive force (pmf) with a concomitant increase in state 4 respiration [7,8]. This uncoupling activity of Medica 16 was found to be partially suppressed by atractyloside [7], in line with recent studies indicating that the protonophoric activity of LCFA may be accounted for by inward spontaneous flip-flop of the undissociated fatty acid followed by adenine nucleotide translocase (ANT)-catalyzed efflux of the fatty acid anion [9–12].

The mitochondrial uncoupling activity of LCFA or fatty acyl analogues is similar in many respects to that induced by treatment with thyroid hormones (TH) (reviewed in [13]). Indeed, respiration is increased while mitochondrial membrane potential is decreased in liver cells incubated in the presence of added long chain fatty acyl analogues [7] or derived from TH-treated animals [14,15]. Furthermore, in vivo treatment with either Medica 16 or TH results in a pronounced increase in respiration accompanied by decrease in liver phosphate and redox potentials [16]. Hence, analyzing the protonophoric mode of action of Medica 16 as compared with TH may point to shared mitochondrial elements affected by both treatment modes.

Studies concerned with the protonophoric activity of LCFA, fatty acyl analogues or TH were usually performed in Ca^{2+}-free medium in order to avoid Ca^{2+}-dependent activation of mitochondrial oxidation of the fatty acid substrate (reviewed in [17]) as well as Ca^{2+}-dependent activation of the MPT pore [18–21]. However, Ca^{2+} involvement in the protonophoric activity of LCFA or TH could be of particular interest in light of recent findings indicating that the channel activity of mitochondrial ANT [22] as well as that of the low conductance MPT pore [23] might be reversibly modulated by Ca^{2+}. The non-β-oxidizable Medica 16 combined with gating of the MPT pore by cyclosporin A (CSA) [24] makes it possible to analyze the protonophoric activity of LCFA or TH in the presence of added Ca^{2+} and to dissect steps leading from low to high mitochondrial conductance.

2. Experimental procedures

Male albino rats weighing 150–180 g were fed a standard Purina laboratory chow diet (Ralston-Purina, St. Louis, MO). Hyperthyroidism was induced by daily injection of L-T_3 (50 μg/100 g BW in 0.05 N NaOH in saline) for 5 days. Control animals were injected with the vehicle alone. Hypothyroidism was induced by adding 0.025% w/v methimazole to the drinking water for 4 weeks.

Rat liver mitochondria were prepared by standard methods in a medium containing 250 mM sucrose, 5 mM Tris (pH 7.4), and 1 mM EGTA. Mitochondrial pellets were washed twice in a medium containing 250 mM sucrose and 1 mM [3-(*N*-morpholino)-propanesulfonic acid]-Na^+ buffer, pH 7.4, to remove EGTA. The washed pellets were dissolved in the same medium at a final protein concentration of 50 mg protein/ml and kept in ice for not more than 4 h.

Mitochondrial membrane potential (Δψ) and proton gradient (ΔpH) were measured as described by [25] using $^{86}Rb^+$ (in the presence of valinomycin) and [^{14}C]acetate, respectively. Total, extra- and intramitochondrial spaces were measured by following the distribution of ^3H_2O together with either [^{14}C]sucrose or [^{14}C]inulin [25]. Inulin, with a molecular mass of 5200 Da, does not penetrate the MPT pore and allows for measuring mitochondrial space under conditions of MPT [26]. A calibration factor for the inulin space was derived by correlating the sucrose and inulin spaces of mitochondria incubated in the presence of EGTA and variable sucrose concentrations in the range of 50–250 mM, as previously described [25]. For mitochondrial Δψ and ΔpH measurements, 2–2.5 mg mitochondrial protein were incubated at 30°C for 7 min in 1.5 ml plastic vials containing 1 ml reaction mixture composed of 250 mM sucrose, 5 mM HEPES (pH 7.2), 5 mM succinate, 5 μM rotenone, 0.6 μg/ml oligomycin, 320 pmol valinomycin/mg mitochondrial protein, 0.5 μCi $^{86}Rb^+$ (0.16 μCi/nmol), 0.36 μCi [^{14}C]acetate (0.05 μCi/mmol), 2 μCi ^3H_2O, either 0.45 μCi [^{14}C]sucrose (0.01 μCi/nmol) or 0.6 μCi [^{14}C]inulin (0.13 μCi/mmol). 20 nmol Ca^{2+}/mg mitochondrial protein, 1 mM EGTA or 1 μg CSA/mg mitochondrial protein were added where indicated. After incubation, the mitochondrial suspension was transferred to a 1.5 ml Eppendorf microfuge tube containing 150 μl

silicon oil (approx. 0.99–1.05 density) and centrifuged at 12 000×*g* for 2 min at 4°C. Aliquots of the mitochondrial and mitochondrial-free fractions were transferred to 1.5 N $HClO_4$, the perchloric acid extracts were centrifuged, and aliquots of the perchloric acid supernatants were counted in Quicksafe A scintillation fluid. Δψ, ΔpH and pmf were calculated as previously described [25].

Mitochondrial Ca^{2+} influx and efflux were measured by following the free Ca^{2+} concentration in the incubation medium using a Ca^{2+} electrode (ORION Ca^{2+} electrode, Model 93-20) calibrated as described by Bers [27]. Ca^{2+} influx was measured at 30°C in 1 ml incubation medium containing 250 mM sucrose, 5 mM HEPES (pH 7.4), 10 mM succinate, 5 μM rotenone, 0.8–1.25 mg mitochondrial protein/ml reaction mixture, 20 nmol Ca^{2+}/mg mitochondrial protein, and 1–2 μg CSA/mg mitochondrial protein. For Ca^{2+} efflux determinations, mitochondria were first preloaded for 2 min by Ca^{2+} at 30°C in an incubation medium containing 250 mM sucrose, 5 mM HEPES (pH 7.4), 10 mM succinate, 5 μM rotenone, 0.8–1.25 mg mitochondrial protein/ml reaction mixture, 20 nmol Ca^{2+}/mg mitochondrial protein and 1–2 μg of CSA/mg mitochondrial protein. Following Ca^{2+} loading, Ca^{2+} efflux was measured in the presence of added 2 nmol ruthenium red/mg mitochondrial protein.

Mitochondrial swelling was spectrophotometrically measured by determining the apparent absorbance at 540 nm in 1 ml incubation medium containing 250 mM sucrose, 5 mM HEPES (pH 7.2), 10 mM succinate, 5 μm rotenone, 0.6 μg/ml oligomycin, 0.35–0.75 mg of mitochondrial protein/ml reaction mixture, 20 nmol Ca^{2+}/mg mitochondrial protein, and EGTA, CSA or ruthenium red as indicated.

Statistical analysis for the comparison of two groups was performed by Student's *t*-test.

3. Results

3.1. Ca^{2+} dependence of the protonophoric activity of Medica 16

The effect of Medica 16 on proton gradient, membrane potential and mitochondrial pmf in isolated liver mitochondria was evaluated in the absence and presence of added Ca^{2+}. As shown in Fig. 1A, and in line with our previous results [7], adding Medica 16 to mitochondria respiring on succinate in the absence of Ca^{2+} (added EGTA) resulted in a saturable, concentration dependent, 20–30% decrease of mitochondrial ΔpH, Δψ and pmf. The EC_{50} for the uncoupling effect of Medica 16 amounted to

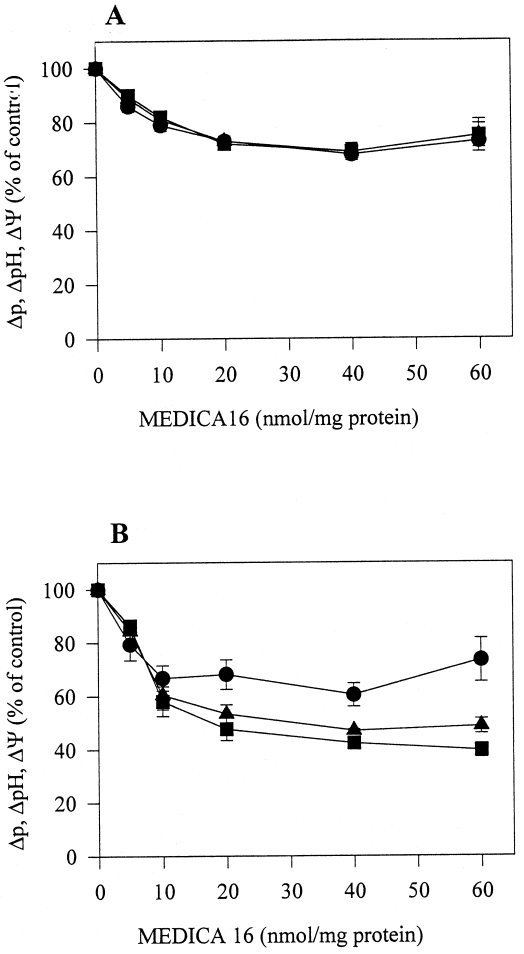

Fig. 1. Medica 16-induced decrease in mitochondrial proton gradient, membrane potential and protonmotive force. Mitochondrial ΔpH (●), Δψ (■) and Δ*p* (▲) were measured as described in Section 2 in liver mitochondria incubated in the presence of 20 nmole Ca^{2+}/mg mitochondrial protein and Medica 16 as indicated. Values are expressed as percent of basal respective values (100%) measured in the absence of added Medica 16. Each value is the mean ± S.E. of three independent experiments. (A) In the presence of 1 mM EGTA. Basal values amounted to 69 ± 6, 171 ± 3 and 242 ± 5 mV for ΔpH, Δψ and Δ*p*, respectively. (B) In the presence of 1 μg CSA/mg mitochondrial protein. Basal values amounted to 62 ± 4 mV, 165 ± 5 mV and 227 ± 8 mV for ΔpH, Δψ and Δ*p*, respectively.

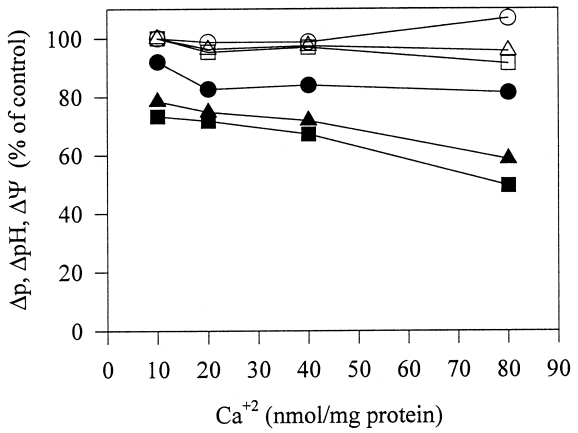

Fig. 2. Ca^{2+} dependence of the protonophoric activity of Medica 16. Mitochondrial ΔpH (●,○), Δψ (■,□) and Δ*p* (▲,△) were measured as described in Section 2 in liver mitochondria incubated in the presence (closed symbols) or absence (open symbols) of 20 nmol Medica 16/mg mitochondrial protein, 1 μg CSA/mg mitochondrial protein and added Ca^{2+} as indicated. Values are expressed as percent of basal respective values (100%) measured in the absence of Medica 16 and in the presence of 10 nmol Ca^{2+}/mg protein. Basal values amounted to 54, 171 and 225 mV for ΔpH, Δψ and Δ*p*, respectively. Means of two independent experiments.

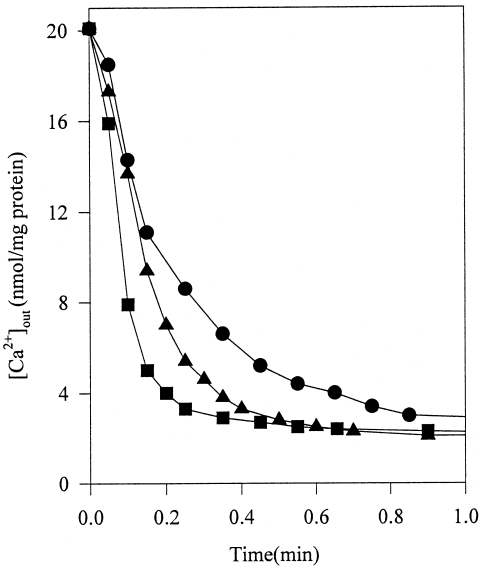

Fig. 3. Inhibition of mitochondrial Ca^{2+} influx by Medica 16. Mitochondrial Ca^{2+} influx was determined as described in Section 2 in the absence (■) and presence of 10 (▲) or 20 (●) nmol Medica 16/mg mitochondrial protein. One representative experiment out of four.

11.5 nmol/mg protein. Adding Ca^{2+} (20 nmol/mg mitochondrial protein) while gating the MPT pore by CSA resulted in amplifying the protonophoric activity of Medica 16, amounting to 50–60% decrease in mitochondrial pmf under conditions of saturation with Medica 16 (Fig. 1B). The EC_{50} for Medica 16 remained unaffected by added Ca^{2+}. These results were further confirmed by measuring the protonophoric activity of saturating Medica 16 in rat liver mitochondria preloaded with increasing concentrations of Ca^{2+} under conditions of gating the MPT pore by CSA (Fig. 2). Mitochondrial ΔpH was slightly increased and Δψ and pmf remained essentially unaffected by increasing Ca^{2+} concentrations in the absence of added Medica 16. Increase in mitochondrial ΔpH could reflect Ca^{2+} accumulation by energized mitochondria at the expense of H^+ extrusion. Increasing Ca^{2+} concentrations in the presence of added Medica 16 resulted, however, in a pronounced decrease in mitochondrial Δψ and pmf as function of added Ca^{2+}. The Medica 16/Ca^{2+} interplay may therefore indicate that Ca^{2+} is involved in the protonophoric activity of Medica 16, and that the uncoupling activity observed in its absence (Fig. 1A) could be accounted for by endogenous mitochondrial Ca^{2+}.

Table 1
The effect of TH treatment on mitochondrial proton gradient, membrane potential and protonmotive force

	EGTA			CSA		
	ΔpH	Δψ	Δ*p*	ΔpH	Δψ	Δ*p*
Euthyroid	62.8 ± 5.5	175.5 ± 4.3	238.3 ± 9.6	58.3 ± 3.7	143.5 ± 14.3	201.8 ± 17.9
Hyperthyroid	50.8 ± 5.0	147.5 ± 6.3*	198.3 ± 1.6*	50.0 ± 7.3	107.0 ± 18.1	157.0 ± 23.5

Mitochondrial ΔpH, Δψ and Δ*p* in mV were measured as described in Section 2 in mitochondria isolated from euthyroid or hyperthyroid rats and incubated in the presence of 20 nmol Ca^{2+}/mg mitochondrial protein, and either 1 mM EGTA or 1 μg CSA/mg mitochondrial protein as indicated. Each value is the mean ± S.E. of three independent experiments.
*Significantly different from the respective euthyroid value, $P < 0.05$.

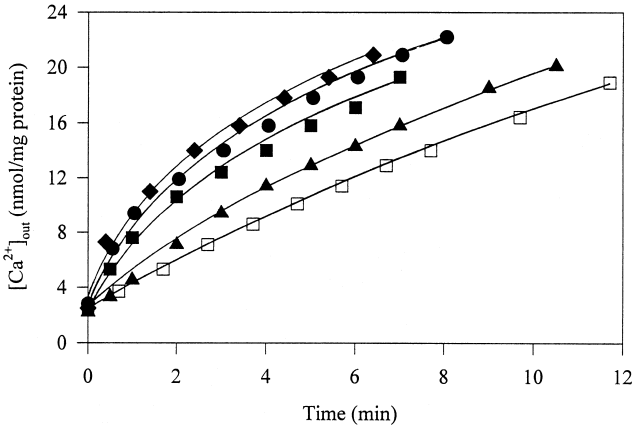

Fig. 4. Activation of mitochondrial Ca^{2+} efflux by Medica 16. Mitochondrial Ca^{2+} efflux was determined as described in Section 2 in the absence (open symbols) and presence (closed symbols) of 20 nmol Medica 16/mg mitochondrial protein. □,■, no further additions; ▲, 16 nmol bongkrekic acid/mg mitochondrial protein; ●, 30 or ♦, 50 nmol atractylate/mg mitochondrial protein. One representative experiment out of four.

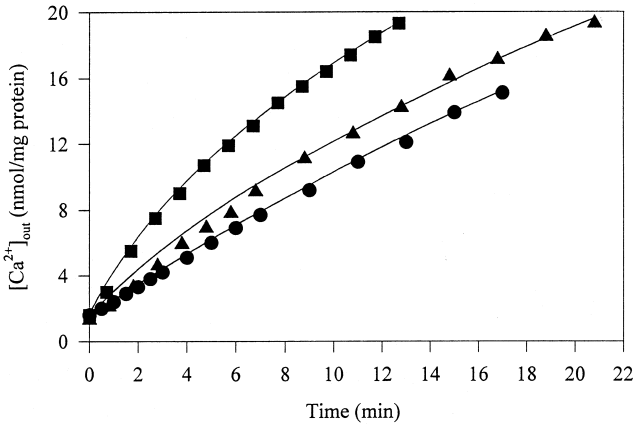

Fig. 5. Mitochondrial Ca^{2+} efflux in eu-, hyper- and hypothyroid rats. Mitochondrial Ca^{2+} efflux was determined as described in Section 2 in mitochondria derived from euthyroid (▲), hyperthyroid (■) or hypothyroid (●) rats. One representative experiment out of four.

The Medica 16/Ca^{2+} interplay was further analyzed in terms of effects exerted by Medica 16 on mitochondrial Ca^{2+} influx and efflux under conditions of gating the MPT pore by CSA. Mitochondrial Ca^{2+} influx catalyzed by the Ca^{2+} uniporter and driven by mitochondrial pmf (reviewed in [28]), was indeed inhibited by added Medica 16 (Fig. 3), thus presumably reflecting the consequence of its protonophoric activity. Liver mitochondrial Ca^{2+} efflux catalyzed by the Na-independent ($Ca^{2+}/2H^{+}$ exchange) transporter [28] was expected to be similarly inhibited by Medica 16 as a result of its uncoupling activity. However, as shown in Fig. 4, mitochondrial Ca^{2+} efflux was pronouncedly activated by added Medica 16, being further modulated by the conformation of ANT. Promoting the C (cytosolic) or M (matrix) ANT conformations by atractylate or bongkrekic acid respectively [29], resulted in activation or inhibition of Medica 16-induced Ca^{2+} efflux, respectively. Mitochondrial Ca^{2+} efflux in the absence of added Medica 16 remained unaffected by atractylate or bongkrekic acid thus indicating that ANT was specifically involved in Ca^{2+} efflux induced by Medica 16.

3.2. *Ca^{2+} dependence of the uncoupling activity of thyroid hormones*

The mitochondrial uncoupling activity of TH as function of Ca^{2+} was evaluated in liver mitochondria derived from hyperthyroid as compared with euthyroid animals. As shown in Table 1, in vivo TH treatment resulted in 15% decrease in mitochondrial $\Delta\psi$ and pmf in the absence of added Ca^{2+} (added

Table 2
Mitochondrial permeabilization transition by Medica 16 or by in vivo TH treatment

	ΔpH	Δψ	Δ*p*
Euthyroid	55.7 ± 4.3	135.3 ± 16.4	191.0 ± 19.9
Euthyroid+Medica 16	21.0 ± 7.4*	28.3 ± 11.9*	49.3 ± 18.9*
Hyperthyroid	30.7 ± 1.2*	25.3 ± 2.6*	56.0 ± 3.6*

Mitochondrial ΔpH, Δψ and Δ*p* in mV were measured as described in Section 2 in mitochondria isolated from euthyroid or hyperthyroid rats and incubated in the presence of 20 nmol Ca^{2+}/mg mitochondrial protein. 10 nmol Medica 16/mg mitochondrial protein were added as indicated. Each value is the mean ± S.E. of three independent experiments.
*Significantly different from the respective euthyroid value, $P < 0.05$.

EGTA). Adding Ca^{2+} to hyperthyroid mitochondria while gating the MPT pore by CSA resulted in further decrease in $\Delta\psi$ and pmf, thus indicating that in vivo TH treatment acted synergistically with mitochondrial Ca^{2+} in mitochondrial uncoupling.

Similarly to the protonophoric activity of Medica 16, the uncoupling activity induced by in vivo TH treatment was characterized by activation of Ca^{2+} efflux in hyperthyroid mitochondria and its suppression in hypothyroid mitochondria under conditions of gating the MPT pore by CSA (Fig. 5).

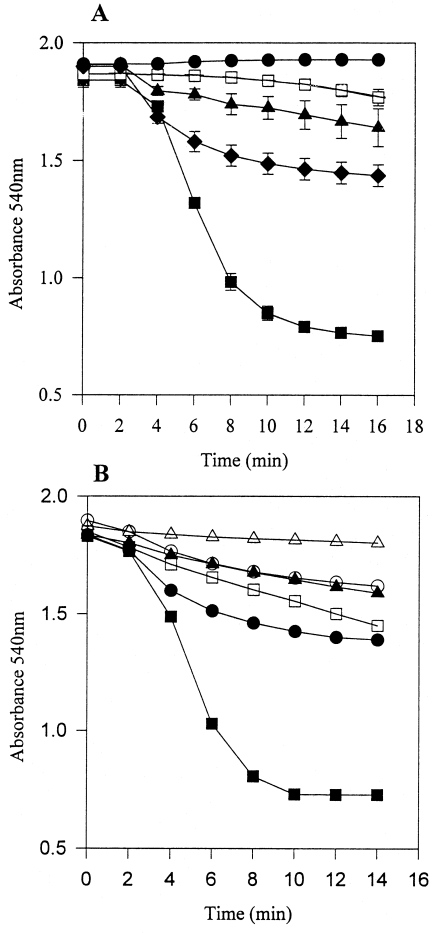

Fig. 6. Induction of Ca^{2+}-dependent mitochondrial swelling by Medica 16 (A) or by in vivo thyroid hormone treatment (B). (A) Mitochondrial swelling was determined at 30°C as described in Section 2 in the absence (open symbols) and presence (closed symbols) of 10 nmol Medica 16/mg mitochondrial protein. □,■, no further additions; ♦, 2 nmol ruthenium red/mg mitochondrial protein; ▲, 1 mM EGTA; ●, 1 μg CSA/mg mitochondrial protein. Each value is the mean ± S.E. of three independent experiments. (B) Mitochondrial swelling was determined at 35°C as described in Section 2 in mitochondria isolated from euthyroid (open symbols) or hyperthyroid (closed symbols) rats. □,■, no further additions; △,▲, 1 mM EGTA; ○,●, 1 μg CSA/mg mitochondrial protein. One representative experiment out of five.

3.3. *Mitochondrial permeability transition by Medica 16 and TH*

In the presence of added Ca^{2+} and in the absence of CSA, uncoupling of oxidative phosphorylation by added Medica 16 or by in vivo TH treatment resulted in mitochondrial swelling and collapse of mitochondrial pmf (Table 2, Fig. 6). Ca^{2+}-dependent collapse of mitochondrial pmf could be partially prevented by added CSA, or by abrogating mitochondrial Ca^{2+} cycling by ruthenium red (Fig. 6). Swelling resulted in 30–35% increase in mitochondrial volume (not shown).

4. Discussion

Replacing LCFA by the non-β-oxidizable Medica 16 makes it possible to analyze the role of Ca^{2+} in the protonophoric activity of LCFA independently of their mitochondrial oxidation. In the absence of Ca^{2+} (added EGTA) Medica 16 induces a saturable 20–30% decrease in mitochondrial pmf (Fig. 1A) with a concomitant pronounced decrease of mitochondrial pH (8). This protonophoric activity may be ascribed to inward flip-flop of the undissociated acid followed by ANT-mediated efflux of the fatty acyl anion [9], as previously verified in reconstituted ANT systems [10], ANT-deficient yeast mutants [30], by using ANT inhibitors [20,31] or azido fatty acids [32]. The inward flip-flop of the dicarboxylic Medica 16 requires the β,β′-substituted methyl groups for shielding the ω-carboxyl function [8,33,34]. Complementing the protonophoric activity of Medica 16 by added Ca^{2+} results in transition to CSA-sensitive mitochondrial permeabilization catalyzed by the MPT pore (Fig. 6A) in line with previous reports of Ca^{2+}-dependent activation of the MPT pore by LCFA [20]. As ANT constitutes an integral component of the MPT pore and since the ANT/MPT pore may be reversibly converted by Ca^{2+} into a channel [22,35–39], the effect of added Medica 16/Ca^{2+} may be ascribed to ANT transition from a low to a high conductance channel, resulting in mitochondrial perme-

abilization. Mitochondrial permeabilization by Medica 16/Ca^{2+} is only partially inhibited by CSA (Fig. 1B and 2) since gating of the MPT pore by CSA is competed by Ca^{2+} [40–42]. The transition from the physiological low conductance ANT to high conductance mitochondrial permeabilization state may therefore be visualized as a continuous sequel where the extent of transition may be modulated by the interplay between LCFA, Ca^{2+}, ANT openers (e.g., cyclophilin D) and ANT blockers (e.g., CSA-like factors). Transition could be mediated by recruiting ANT/MPT pores of a given conductance or by modulating the conductance level of given pores.

Mitochondrial permeabilization by Medica 16/Ca^{2+} is accompanied by activation of mitochondrial Ca^{2+} efflux. Since the Na^{+}-independent mitochondrial Ca^{2+} transporter is energy- and ΔpH-dependent [28], uncoupling by Medica 16 makes it unlikely that this transporter accounts for Medica 16-induced Ca^{2+} efflux. Alternatively, as Medica 16-induced Ca^{2+} efflux is modulated by ANT inhibitors (Fig. 4), and since main characteristics of the ANT channel are identical with those of the MPT pore (e.g., Ca^{2+} dependence, inhibition by bongkrekic acid or ADP and activation by atractylate), Ca^{2+} efflux is proposed to involve the ANT/MPT pore which may allow for efflux of both, the LCFA (or Medica 16) anion as well as Ca^{2+}. Efflux of Ca^{2+} and the fatty acyl anion through the ANT/MPT pore could perhaps reflect efflux of the LCFA-Ca^{2+} or Medica 16-Ca^{2+} complex.

Characteristics of mitochondrial uncoupling by in vivo TH treatment appear to be very similar to the protonophoric activity of Medica 16. Uncoupling by both is synergistic with added Ca^{2+} (Fig. 2, Table 1), mitochondrial Ca^{2+} efflux is activated by both (Figs. 4 and 5), and the effect exerted by both is modulated by ANT inhibitors (Fig. 4, [1,7,8,31,43]), thus indicating that both effectors may converge onto the ANT/MPT pore. Moreover, hypothyroidism has been recently reported to induce resistance to MPT in liver mitochondria [44]. However, and in spite of the apparent convergence of both effectors onto the same or related mitochondrial elements, the two effectors differ with respect to their specific mode of action. In contrast to TH treatment, the mitochondrial protonophoric activity of Medica 16 was not observed in mitochondria derived from rats treated for 6 days with 0.25% (w/w) of Medica 16 and studied in vitro in the absence of added Medica 16 [7], thus indicating that the Medica 16 effect requires its direct interaction with mitochondria while uncoupling by in vivo TH treatment is maintained and preserved in mitochondria derived from TH-treated rats and in the absence of TH added to the mitochondrial preparation. Hence, uncoupling by TH may reflect its transcriptional activity while that of Medica 16 results from its mitochondrial cycling. Uncoupling by TH could result from changing the proportion of ANT units within the MPT pore complex due to induction of ANT by TH [45]. Alternatively, uncoupling by TH could result from TH induction of other MPT components.

Treatment with TH results in 50% increase in total body energy expenditure [46]. A similar but less pronounced calorigenic activity has been reported for Medica 16, amounting to 25% increase in total body oxygen consumption [47]. However, since Medica 16 is not transported into skeletal or cardiac muscles (H. Bdeir, in preparation), its calorigenic activity is mainly due to its liver activity, and the lower increase in total body energy expenditure induced by Medica 16 treatment as compared with TH may be accounted for by its limited tissue specificity. Indeed, liver phosphate and redox potentials taken as indicators for coupling of mitochondrial oxidative phosphorylation under in vivo conditions were pronouncedly decreased by both TH or Medica 16 treatment [48], thus indicating that Medica 16 may be realized as a tissue specific thyromimetic effector.

References

[1] L. Wojtczak, P. Schönfeld, Effect of fatty acids on energy coupling processes in mitochondria, Biochim. Biophys. Acta 1183 (1993) 41–57.
[2] D.F.S. Rolfe, G.C. Brown, Cellular energy utilization and molecular origin of standard metabolic rate in mammals, Physiol. Rev. 77 (1997) 731–758.
[3] P. Bernardi, D.R. Broekemeier, J. Pfeiffer, Recent progress on regulation of the mitochondrial permeability transition pore, a cyclosporin-sensitive pore in the inner mitochondrial membrane, J. Bioenerg. Biomembr. 26 (1994) 509–517.
[4] M. Zorrati, I. Szabo, The mitochondrial permeability transition, Biochim. Biophys. Acta 1241 (1995) 139–176.
[5] A.P. Halestrap, P.M. Kerr, S. Javadov, K.Y. Woodfield, Elucidating the molecular mechanism of the permeability

transition pore and its role in reperfusion injury of the heart, Biochim. Biophys. Acta 1366 (1998) 79–94.
[6] J. Bar-Tana, G. Kahn-Rose, M. Srebnik, Inhibition of lipid synthesis by β,β′-tetramethyl-substituted C14-C22 α,ω dicarboxylic acids in the rat in vivo, J. Biol. Chem. 260 (1985) 8404–8410.
[7] O. Hermesh, B. Kalderon, J. Bar-Tana, Mitochondrial uncoupling by long chain fatty acyl analogue, J. Biol. Chem. 273 (1998) 3937–3942.
[8] L. Wojtczak, M.R. Wieckowski, P. Schönfeld, Protonophoric activity of fatty acid analogs and derivatives in inner mitochondrial membrane: a further argument for fatty acid cycling model, Arch. Biochem. Biophys. 357 (1998) 76–84.
[9] V.P. Skulachev, Fatty acid circuit as a physiological mechanism of uncoupling of oxidative phosphorylation, FEBS Lett. 294 (1991) 158–162.
[10] N. Brustovetsky, M. Klingenberg, The reconstituted ATP/ADP carrier can mediate H^+ transport by free fatty acids which is further stimulated by mersalyl, J. Biol. Chem. 269 (1994) 27329–27336.
[11] P. Schönfeld, R. Bohnensack, Fatty acid-promoted mitochondrial permeability transition by membrane depolarization and binding to the ADP/ATP carrier, FEBS Lett. 420 (1997) 167–170.
[12] P. Jezek, H. Engstova, M. Zackova, A.E. Vercesi, A.D.T. Costa, P. Arruda, K.D. Garlid, Fatty acid cycling mechanism and mitochondrial uncoupling proteins, Biochim. Biophys. Acta 1365 (1998) 319–327.
[13] S. Soboll, Thyroid hormone action on mitochondrial energy transfer, Biochim. Biophys. Acta 1144 (1993) 1–16.
[14] M.E. Harper, M.D. Brand, The quantitative contributions of mitochondrial proton leak and ATP turnover reactions to the changed respiration rates of hepatocytes from rats of different thyroid status, J. Biol. Chem. 268 (1993) 14850–14860.
[15] V. Bobyleva, T.L. Pazienza, R. Maseroli, A. Tomasi, S. Salvioli, S. Cossarizza, C. Franceschi, V.P. Skulachev, Decrease in mitochondrial energy coupling by thyroid hormones: a physiological effect rather than a pathological hyperthyroidism consequence, FEBS Lett. 430 (1998) 409–413.
[16] B. Kalderon, R. Hertz, J. Bar-Tana, Effect of thyroid hormone treatment on redox and phosphate potentials in rat liver, Endocrinology 131 (1992) 400–407.
[17] J.G. McCormack, A.P. Halestrap, R.M. Denton, Role of calcium ion in regulation of mammalian intramitochondrial metabolism, Physiol. Rev. 70 (1990) 391–425.
[18] D.R. Hunter, R.A. Haworth, J.H. Southard, Relationship between configuration, function, and permeability in calcium-treated mitochondria, J. Biol. Chem. 251 (1976) 5069–5077.
[19] K.M. Broekemeier, D.R. Pfeiffer, Inhibition of the mitochondrial permeability transition by cyclosporin A during long time frame experiments: relationship between pore opening and the activity of mitochondrial phospholipases, Biochemistry 34 (1995) 16440–16449.
[20] M.R. Wieckowski, L. Wojtczak, Fatty acid-induced uncoupling of oxidative phosphorylation is partly due to opening of the mitochondrial permeability transition pore, FEBS Lett. 423 (1998) 339–342.
[21] B. Kalderon, O. Hermesh, J. Bar-Tana, Mitochondrial permeability transition is induced by in vivo thyroid hormone treatment, Endocrinology 136 (1995) 3552–3556.
[22] N. Brustovetsky, M. Klingenberg, Mitochondrial ADP/ATP carrier can be reversibly converted into a large channel by Ca^{2+}, Biochemistry 35 (1996) 8483–8488.
[23] F. Ichas, J.-P. Mazat, From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high-conductance state, Biochim. Biophys. Acta 1366 (1998) 33–50.
[24] A.P. Halestrap, A.M. Davidson, Inhibition of Ca^{2+}-induced large-amplitude swelling of liver and heart mitochondria by cyclosporin is probably caused by the inhibitor binding to mitochondrial-matrix-peptidyl-prolyl cis-trans isomerase and preventing it interacting with the adenine nucleotide translocase, Biochem. J. 268 (1990) 153–160.
[25] H. Rotenberg, The measurement of membrane potentials and ΔpH in cells, organelles, and vesicles, Methods Enzymol. 55 (1979) 547–569.
[26] U. Igbavboa, D.R. Pfeiffer, Transient induction of the mitochondrial permeability transition by uncoupler plus a Ca^{2+}-specific chelator, Biochim. Biophys. Acta 1059 (1991) 339–347.
[27] D.M.A. Bers, A simple method for the accurate determination of free [Ca] in Ca-EGTA solutions, Am. J. Physiol. 242 (1982) C404–C408.
[28] T.E. Gunter, K.K. Gunter, S.-S. Sheu, C.F. Gavin, Mitochondrial calcium transport: physiological and pathological relevance, Am. J. Physiol. 267 (1994) C313–C339.
[29] K. Le Quoc, D. Le quoc, Involvement of the ADP/ATP carrier in calcium-induced perturbations of the mitochondrial inner membrane permeability: importance of the orientation of the nucleotide binding site, Arch. Biochem. Biophys. 265 (1988) 249–257.
[30] P. Polcic, L. Sabova, J. Kolarov, Fatty acids induced uncoupling of *Saccharomyces cerevisiae* mitochondria requires an intact ADP/ATP carrier, FEBS Lett. 412 (1997) 207–210.
[31] A.Yu. Andreyev, T.O. Bondareva, V.I. Dedukhova, E.N. Mokhova, V.P. Skulachev, L.M. Tsofina, N.I. Volkov, T.V. Vygodina, The ATP/ADP-antiporter is involved in the uncoupling effect of fatty acids on mitochondria, Eur. J. Biochem. 182 (1989) 585–592.
[32] P. Schönfeld, P. Jezek, E.A. Belyaeva, J. Borecky, V.S. Slyshenkov, M.R. Wieckowski, L. Wojtczak, Photomodification of mitochondrial proteins by azido fatty acids and its effect on mitochondrial energetics. Further evidence for the role of ADP/ATP carrier in fatty-acid-mediated uncoupling, Eur. J. Biochem. 240 (1996) 387–393.
[33] O.V. Markova, D.I. Bondarenko, V.N. Samartsev, The anion-carrier mediated uncoupling effect of dicarboxylic fatty acids in liver mitochondria depends on the position of the second carboxyl group, Biochemistry (Mosc.) 64 (1999) 565–570.

[34] L. Wojtczak, M.R. Wieckowski, The mechanisms of fatty acid-induced proton permeability of the inner mitochondrial membrane, J. Bioenerg. Biomembr. 31 (1999) 447–455.
[35] A. Ruck, M. Dolder, T. Wallimann, D. Brdickzka, Reconstituted adenine nucleotide translocase forms a channel for small molecules comparable to the mitochondrial permeability transition pore, FEBS Lett. 426 (1998) 97–101.
[36] Yu.V. Evtodienko, V.V. Teplova, J. Kahavaja, N.E. Saris, The Ca^{2+}-induced permeability transition pore is involved in Ca^{2+}-induced mitochondrial oscillations. A study on permeabilised Ehrlich ascites tumor cells, Cell Calcium 15 (1994) 142–152.
[37] F. Ichas, L.S. Jouaville, J.-P. Mazat, Mitochondria are excitable organelles capable of generating and conveying electrical and calcium signals, Cell 89 (1997) 1145–1153.
[38] T. Minamikawa, D.A. Williams, D.N. Bowser, P. Nagley, Mitochondrial permeability transition and swelling can occur reversibly without inducing cell death in intact human cells, Exp. Cell Res. 246 (1999) 26–37.
[39] V. Petronilli, G. Miotto, M. Canton, M. Brini, R. Colonna, P. Bernardi, F. DiLisa, Transient and long-lasting opening of the mitochondrial permeability transition pore can be monitored directly in intact cells by changes in mitochondrial calcium fluorescence, Biophys. J. 76 (1999) 725–734.
[40] S.A. Novgorodov, T.I. Gudz, Y.M. Milgrom, G.P. Brierley, The permeability transition in heart mitochondria is regulated synergistically by ADP and cyclosporin A, J. Biol. Chem. 267 (1992) 16274–16282.
[41] M. Crompton, L. Andreeva, On the interaction of Ca^{2+} and cyclosporin A with a mitochondrial inner membrane pore: a study using cobaltammine complex inhibitors of the Ca^{2+} uniporter, Biochem. J. 302 (1994) 181–185.
[42] C.P. Connern, A.P. Halestrap, Recruitment of mitochondrial cyclophilin to the mitochondrial inner membrane under conditions of oxidative stress that enhance the opening of a calcium-sensitive non-specific channel, Biochem. J. 302 (1994) 321–324.
[43] P. Schönfeld, M.R. Wieckowski, L. Wojtczak, Thyroid hormone-induced expression of the ADP/ATP carrier and its effect on fatty acid induced uncoupling of oxidative phosphorylation, FEBS Lett. 416 (1997) 19–22.
[44] E. Chavez, M. Franco, H. Reyes-Vivas, C. Zazueta, J. Ramirez, R. Carrillo, Hypothyroidism renders liver mitochondria resistant to the opening of membrane permeability transition pore, Biochim. Biophys. Acta 1407 (1998) 243–248.
[45] K. Luciakova, B.D. Nelson, Transcript levels for nuclear-encoded mammalian mitochondrial respiratory-chain components are regulated by thyroid hormone in an uncoordinated fashion, Eur. J. Biochem. 207 (1992) 247–251.
[46] T.H. Oppenheimer, H.L. Schwartz, J.T. Lane, M.P. Thompson, Functional relationship of thyroid hormone-induced lipogenesis, lipolysis and thermogenesis in the rat, J. Clin. Invest. 87 (1991) 125–132.
[47] N. Mayorek, B. Kalderon, E. Itach, J. Bar-Tana, Sensitization to insulin induced by β,β′-methyl-substituted hexadecanedioic acid (MEDICA 16) in obese Zucker rats in vivo, Diabetes 46 (1997) 1958–1964.
[48] B. Kalderon, R. Hertz, J. Bar-Tana, Tissue selective modulation of redox and phosphate potentials by β,β′-methyl-substituted hexadecanedioic acid, Endocrinology 131 (1992) 1629–1635.