FEBS Letters 375 (1995) 206–210 FEBS 16282

Nature of respiratory stimulation in hyperthyroidism: the redox behaviour of cytochrome *c*

I. Schmehl, S. Luvisetto*, M. Canton, F. Gennari, G.F. Azzone

CNR Unit for the Study of Biomembranes and Department of Biomedical Sciences, University of Padova, via Trieste 75, 35131-Padova, Italy

Received 1 September 1995; revised version received 2 October 1995

Abstract Hyperthyroid mitochondria show an increased K_m and V_{max} in the high affinity phase of cytochrome oxidase kinetics. During inhibitor titrations, cytochrome *c* shows a different redox behaviour in hyperthyroid with respect to protonophore-treated euthyroid mitochondria. The observations are discussed in terms of a different regulation of electron input and output into the respiratory chain during slip and leak types of uncoupling. In hyperthyroid mitochondria during inhibitor titrations, the pattern of the relationship between uncoupler-induced extra-respiration and membrane potential is highly non-linear. The complex nature of the respiratory stimulation in hyperthyroid mitochondria is discussed.

Key words: Hyperthyroidism; Cytochrome *c*; Cytochrome oxidase; Mitochondrion; Uncoupling

1. Introduction

Thyroid hormones are the most significant factors in regulating energy transformations in mammals by both short term (say few hours) and long term (say few days) effects (for reviews see [1–3]). In vivo, thyroid hormones are involved in setting the basal metabolic rate in many target tissues, such as liver, heart, kidney and brain. Corresponding changes in respiratory activity occur in mitochondria isolated from tissues of hypo- and hyperthyroid animals. The nature of the respiratory stimulation in mitochondria isolated from hyperthyroid rats is the object of the present work.

In a preceding paper [4] we have obtained evidence that the respiratory stimulation in hyperthyroidism was linked to an uncoupling of the proton pumps mainly at the level of cytochrome oxidase. Alterations of cytochrome oxidase are compatible with the high sensitivity of electron transport and proton pumping to variations of distance, milieu, or electric fields and with the thyroid hormone-dependent regulation of cytochromes subunit synthesis [5,6].

At low ionic strength cytochrome oxidase exhibits a non-hyperbolic kinetics during oxidation of cytochrome *c* with two distinct kinetic phases, high- and low-affinity, and characteristic maximal turnover number (V_{max}) and K_m [7]. We have found that both the K_m and V_{max} values of the high-affinity low-turnover phase are increased in hyperthyroid with respect to euthyroid RLM.

Alterations of cytochrome oxidase activity should be accompanied by a modified redox behaviour of cytochrome *c*, direct electron donor to the oxidase complex. In fact, we have found a markedly different redox behaviour of cytochrome *c* during inhibitor titrations in euthyroid RLM, in euthyroid RLM partly uncoupled with FCCP and in BSA-supplemented hyperthyroid RLM.

By analyzing the pattern of the uncoupler-induced extra-respiration as a function of the membrane potential during inhibitor titrations, Brand et al. [8] have suggested a distinction between leak and slip types of uncoupling. Application of this analysis to BSA-supplemented hyperthyroid RLM provides a pattern consistent with the thyroid hormone-induced enhancement of a slip type of uncoupling.

2. Materials and methods

Hyperthyroidism was induced as described in [4]. RLM was prepared according to standard procedures [9]. The standard incubation medium contained: 0.2 M sucrose, 30 mM MOPS/Tris, 5 mM succinate/Tris, 0.2 mM EGTA/Tris, 5 μM rotenone, 1 μg/mg oligomycin, catalase, pH 7.4, T 25°C. T3 and FCCP was purchased from Sigma (St. Louis, MO). Other reagents were of maximal purity commercial grade. The electrical potential gradient and the respiratory rate was determined as essentially described in [4]. In the respiratory inhibitor titrations the same relationships were obtained with malonate, antimycin or myxothiazol. Spectrophotometric studies were performed as described in [10]. The 100% of oxidation of the cytochrome *c* was taken as the absorbance after addition of excess malonate (5 mM) to respiring RLM, while the 100% of reduction was assigned as the absorbance after exhaustion of oxygen [10]. Steady-state activity of cytochrome *c* oxidase was determined polarographically in intact RLM at 25°C in the standard incubation medium (at low ionic strength) by measuring the rate of oxygen consumption upon addition of variable amount of TMPD (0–200 μM) in the presence of 2 mM ascorbate. The concentration of cytochrome oxidase was determined from the reduced-minus-oxidized difference spectra at the wavelength couple 605–630 nm, using a millimolar extinction coefficient of 27 cm^{-1}. V_{max} and K_m values were obtained for both phases in the absence and in the presence of excess of FCCP, as described in [11].

3. Results

3.1. The cytochrome oxidase kinetics

The average cytochrome oxidase concentration of hyperthyroid RLM was about 150% of that of euthyroid RLM, 0.18 and 0.12 nmol/mgprot respectively. The values for the V_{max} and K_m of the two phases are summarized in Table 1. In the high-affinity phase, both V_{max} and V_{max}(U) (where U stands for uncoupled with excess FCCP) were slightly increased in hyperthyroid RLM. In hyperthyroid as compared to euthyroid

*Corresponding author. Fax: (39) (49) 827 6049.
E-mail: siro@cribi1.bio.unipd.it

Abbreviations: $\Delta\psi$, transmembrane electrical potential; J_o, rate of respiration; BSA, bovine serum albumin; BSA-Hyper, BSA-supplemented hyperthyroid mitochondria; DNP, 2,4-dinitrophenol; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)-phenylhydrazone; FCCP-Eu, FCCP-supplemented euthyroid mitochondria; Eu, euthyroid mitochondria; RLM, rat liver mitochondria; T3, 3,3,5-triiodo-L-thyronine.

SSDI 0014-5793(95)01211-7

Table 1
Effect of hyperthyroidism upon cytochrome oxidase kinetics[a]

	Parameter measured	Mitochondrial prepn.	
		Control ($n = 5$)	Hyperthyroid ($n = 3$)
High-affinity	V_{max} (s^{-1})	4.4	5.2
	K_m ($\times 10^{-7}$ M)	2.4	4.5
	V_{max} (U) (s^{-1})	6.6	8.3
	K_m (U) ($\times 10^{-7}$ M)	4.6	16.3
Low-affinity	V_{max} (s^{-1})	130	136
	K_m ($\times 10^{-6}$ M)	13.3	13.6
	V_{max} (U) (s^{-1})	256	288
	K_m (U) ($\times 10^{-6}$ M)	15.9	15.85

[a] Conditions are as described in section 2. The measured parameters are expressed per nmol heme-aa_3. (U) represents the kinetic values determined by TMPD titrations in the presence of excess of FCCP (150 nmol/mg).

RLM, the K_m and K_m(U) values were, respectively, 2 and 3 times higher. In the low-affinity phase only a slight increase of the K_m was observed in uncoupled hyperthyroid mitochondria.

3.2. Inhibitor titrations

Fig. 1 shows the malonate titrations of the respiratory rate (panel A), the membrane potential (panel B) and the % reduction of cytochrome *c* (panel C), in euthyroid RLM, in FCCP-partly uncoupled euthyroid RLM, and in BSA-supplemented hyperthyroid RLM, from now on denoted as Eu, FCCP-Eu and BSA-Hyper RLM, respectively. The concentration of FCCP (15 pmol/mg) was so selected to provide the same initial respiratory rate in Eu as in BSA-Hyper RLM. Supplementation with BSA in hyperthyroid RLM was required to abolish the portion of fatty acids-induced stimulation of the respiration [4]. Panel A indicates that the malonate-induced gradual inhibition of the respiration was identical in FCCP-Eu and in BSA-Hyper RLM. On the other hand, the short initial constancy of the membrane potential was followed by a drastic decline in FCCP-Eu RLM and only by a slight diminution in BSA-Hyper RLM (panel B). Panel C shows that cytochrome *c* was considerably more oxidized in FCCP-Eu RLM and became almost completely oxidized already at 50% respiratory inhibition, while in BSA-Hyper RLM the oxidation of cytochrome *c* occurred parallel to the inhibition of the respiration. In Eu RLM an intermediate behaviour was observed for all the measured parameters.

Three other types of relationships have been analyzed during the respiratory inhibitor titrations. Fig. 2A shows that the classical non-linear relationship between respiration and membrane potential observed in Eu RLM became more linear in the FCCP-Eu RLM, due to the protonophore-induced ohmic increase of the membrane proton conductance [12], and more markedly biphasic in BSA-Hyper RLM. A larger extent of linearization occurred at higher FCCP concentrations (results not shown). The hyperthyroidism-induced increase of biphasicity, attributed to increased slip [4,13], was further enhanced by the presence of BSA due to removal of free fatty acids (see [4,14,15]). Fig. 2B shows that, in all cases, addition of malonate caused a gradual diminution of the extent of cytochrome *c* reduction while the level of the membrane potential remained largely unaffected. Only at higher malonate concentrations there was a marked depression of the membrane potential. The only difference was a shift of the relationship toward higher values of membrane potential and of % reduction of cytochrome *c* in BSA-Hyper and toward lower values in FCCP-Eu compared to Eu RLM. Fig. 2C shows that in Eu RLM the inhibition of the respiration was accompanied by an almost proportional oxidation of cytochrome *c*. Addition of FCCP resulted in an increased respiration and a decreased % of cytochrome *c* reduction and transformed the respiration–cytochrome *c* relationship from proportional into biphasic. The higher the concentration of FCCP the more marked was the biphasicity (data not shown). The same results were obtained in DNP-supplemented RLM. On the contrary, in BSA-Hyper RLM the same respiratory stimulation was accompanied by an increased % of cytochrome *c* reduction and the relationship became strictly proportional.

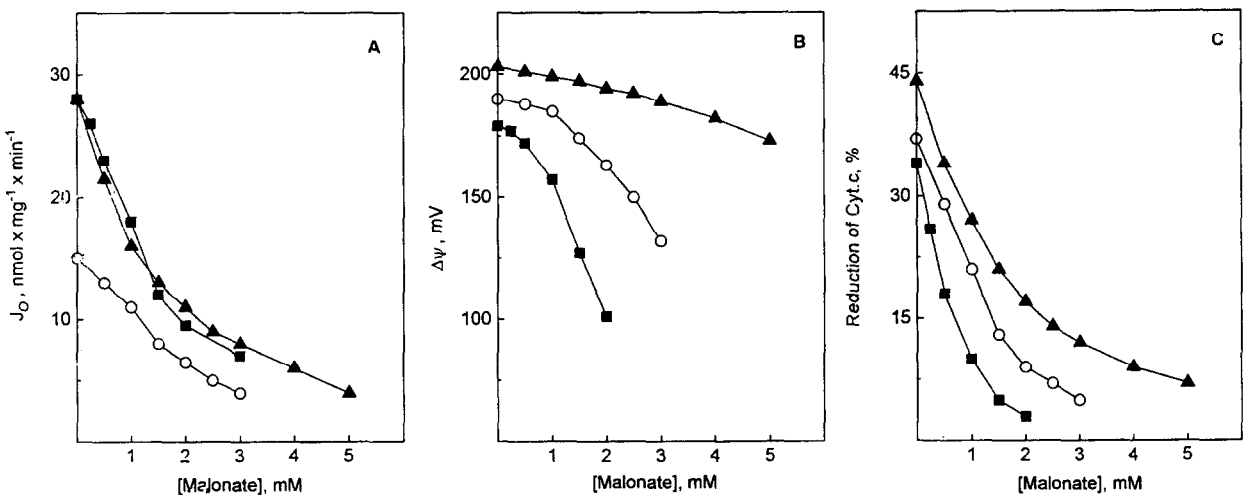

Fig. 1. Effect of increasing malonate concentrations on respiration (A), membrane potential (B) and % of cytochrome *c* reduction (C) in Eu, FCCP-Eu, and BSA-Hyper RLM. Eu RLM (2 mg/ml) were incubated in the absence (○) or in the presence of 15 pmol/mg FCCP (■), Hyper RLM (2 mg/ml) in the presence of 0.1% BSA (▲). After 2 min of incubation, succinate (5 mM) and increasing amounts of malonate (0–5 mM) were added and the oxygen consumption and membrane potential values were measured. The % reduction of cytochrome *c* was determined in parallel samples: after reaching anaerobiosis, an excess of oxygen was added and the response of cytochrome *c* at 421–407 nm was titrated by increasing amounts of malonate.

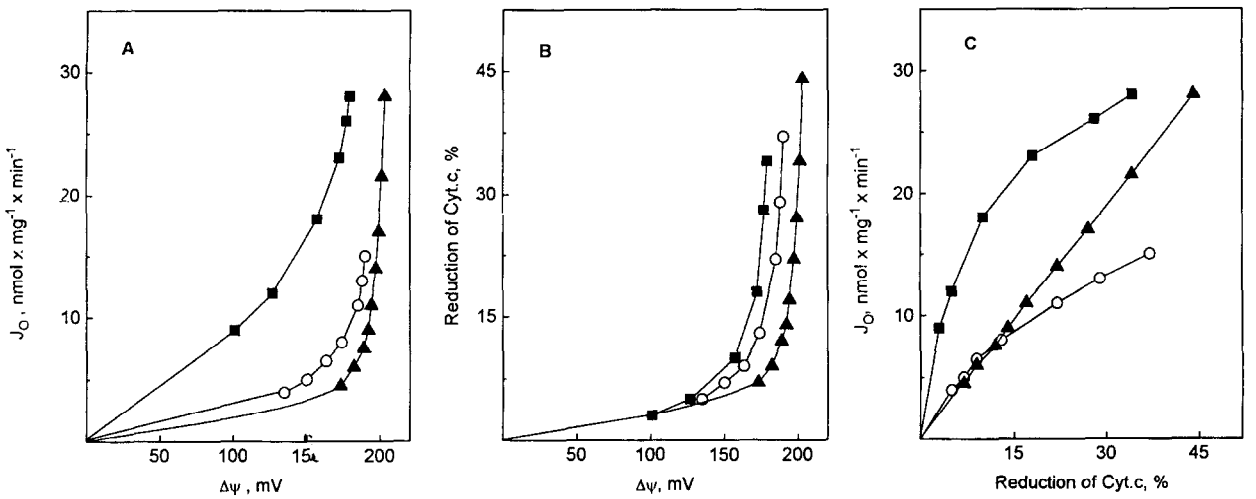

Fig. 2. Relationships between respiration (panel A) or % of cytochrome *c* reduction (panel B) and membrane potential and between respiration and % of cytochrome *c* reduction (panel C) during inhibitor titrations in Eu, FCCP-Eu, and BSA-Hyper RLM. Same conditions and experimental procedures as in Fig. 1.

3.3. Relationship between DNP-induced extra respiration and membrane potential in BSA-Hyper and FCCP-Eu mitochondria

From the patterns of the relationships between uncoupler-induced extra respiration and membrane potential during inhibitor titrations, Brand et al. [8] have suggested a distinction between leak and slip types of uncoupling in the resting respiration, i.e. leak-dependent if the pattern is linear and slip-dependent if the pattern is non-linear [8]. Fig. 3A shows the relationships between respirations and membrane potentials under four conditions. The first two were those of the BSA-Hyper and of the FCCP-Eu mitochondria. Both BSA-Hyper and FCCP-Eu mitochondria were then treated with a minimal amount of DNP. In BSA-Hyper mitochondria the relationships remained highly biphasic even in the presence of DNP. In FCCP-Eu mitochondria the relationship was exponential in the absence, and tended to linearity in the presence, of DNP. Fig. 3B shows the relationship between the DNP-induced extra respiration, ΔJ_o/[DNP], and the membrane potential as derived from the data in panel A. From this relationship the dependence of the proton pump stoichiometric ratio, H^+/O ratio (n), on the membrane potential may be estimated. According to Brand et al. [8], if the plot of the DNP-induced extra-respiration versus $\Delta\psi$ is linear, or non-linear, the H^+/O ratio of the resting respiration does not vary, or varies, with the $\Delta\psi$. The inset of Fig. 3B shows the elaboration of the experimental data indicating the presumable change of the pump stoichiometry vs. $\Delta\psi$. This calculation has already been used to estimate the change of the stoichiometry as a function of $\Delta\psi$ in mitochondria incubated at different temperature [16]. Following Brand et al. [8] (see also [16]), the relationship between ΔJ_o/[DNP] and $\Delta\psi$ is:

$$(\Delta J_o/[DNP])_{\Delta\psi} = (L^{DNP}/[DNP]) \times (1/n)_{\Delta\psi} \times \Delta\psi \quad (1)$$

where the ratio L^{DNP}/[DNP], i.e. the DNP induced proton conductance divided by the DNP concentration, is constant and is not affected by the membrane potential, while the terms $(1/n)_{\Delta\psi}$ denotes the $\Delta\psi$-dependence of the stoichiometric ratio. To perform the calculation of the dependence of n from $\Delta\psi$, we have assumed a pump stoichiometry of 6 at low membrane potential, namely at 100 mV. The value of the ratio L^{DNP}/[DNP] has been then calculated by inserting into Equation (1) the values of the ΔJ_o/[DNP] at 100 mV, and of the pump stoichiometry equal to 6. This allows, by inserting into equation (1) the experimental values of the $\Delta\psi J_o$/[DNP] at each $\Delta\psi$ and the calculated value of L^{DNP}/[DNP], to calculate the value of the pump stoichiometry at each membrane potential. The inset of Fig. 3B shows that the changes of the ΔJ_o/[DNP] correspond to a decrease of the pump stoichiometry in BSA-Hyper mitochondria from 6 to 5.2 in the 120–180 mV range, and from 5.2 to 2.2 in the 180–200 mV range. In FCCP-Eu mitochondria the stoichiometry was almost constant in the 100–150 mV range and was slightly decreased from 6 to 4.8 in the 150–170 mV range.

4. Discussion

Horrum et al. [17] have reported in hyperthyroid RLM an increased content and reduction level for most of the cytochromes and also an enhanced activity for the succinic dehydrogenase. In accord with these observations, during uncoupler titrations we have found a higher percent of cytochrome *c* reduction in hyperthyroid (data not shown) with respect to euthyroid RLM [10] at each membrane potential, indicating an increased level of substrate oxidation in the formers. Furthermore, in our hyperthyroid RLM the average cytochrome oxidase content was about 50% increased compared to that of the euthyroid RLM. The kinetic analysis of cytochrome oxidase and the comparison of the thermodynamic behaviour of the FCCP-Eu and the BSA-Hyper RLM during inhibitor titrations shed some light on the nature of the respiratory stimulation of hyperthyroid RLM.

4.1. Alteration of cytochrome oxidase activity

The changes of the kinetic parameters reported in Table 1 confirm the existence of an alteration of the cytochrome oxidase activity in RLM isolated from hyperthyroid rats. Our kinetic data, on one side, resemble the pattern reported by Sone

and Nicholls [18] after heat treatment in cytochrome oxidase proteoliposomes, where also a decrease of the proton pump stoichiometry was observed. On the other side, they are partly in accord and partly in contrast with those of Paradies et al. [19] obtained in heart RLM at high ionic strength, where only a unique kinetic phase is detectable. These authors found unchanged the cytochrome oxidase content, and reported an unaltered K_m value and an increased V_{max} in hyperthyroid with respect to euthyroid RLM during oxidation of variable concentrations of cytochrome *c*. The enhancement of the cytochrome oxidase activity was attributed to an increased content of cardiolipids in hyperthyroid heart RLM.

Morgan and Wikstrom [20] have pointed out that, in RLM the total amount of cytochrome *c* is constant so any biphasic kinetics observed in intact RLM with TMPD as substrate may not be related to the kinetics of cytochrome *c* binding but it may arise from the intrinsic properties of the enzyme or from an interaction of TMPD with the oxidase.

Whatever the molecular explanation, the marked increase of the K_m values in our hyperthyroid RLM suggests the presence of alterations at the level of cytochrome oxidase. It has been widely demonstrated that isoforms of the cytochrome oxidase containing modified subunits have different kinetic parameters. Thyroid hormones, regulating the synthesis of certain subunits [5,6], may influence the assembly and the final structure of the oxidase complex. Changes in the lipid environment, in surface charge, or in pH also determined alterations of the kinetics of cytochrome *c* oxidation. A common property of these factors is the ability to induce conformational changes in the enzyme complex, which in turn may influence either the binding of cytochrome *c* or the kinetics of the internal electron transfer. Brand et al. [21] have reported no permanent functional alterations induced by hyperthyroidism of the reconstituted oxidase. However, conformational changes may be lost during the isolation and reconstitution of the enzyme into liposomes. Recent data obtained in vitro [22] indicate that thyroid hormones can bind directly to cytochrome oxidase and induce conformational changes in the complex.

4.2. *The behaviour of cytochrome* c *in inhibitor titrations*

Under conditions of limiting electron supply a striking discrepancy is observed between the behaviours of FCCP-Eu and BSA-Hyper RLM. While the two systems show the same stimulation of respiration, their initial levels of membrane potential and of cytochrome *c* reduction are markedly different. Moreover, the identical inhibition of the respiratory rate is accompanied by a markedly diverse decline of the membrane potential and of the reduction level of cytochrome *c* (Fig. 1), leading to completely different patterns in the relationships between respiration and membrane potential or respiration and cytochrome *c* reduction. The linear pattern of the classical respiration-membrane potential relationships (Fig. 2A) in FCCP-Eu RLM has been attributed to the ohmicity of the FCCP-induced membrane proton conductance [12] while the non-linearity in BSA-Hyper RLM has been interpreted as due to a potential-dependent slip in the cytochrome oxidase [4].

The non-linearity of the flow–force relationship of BSA-Hyper RLM may be attributed to non-ohmic proton leaks

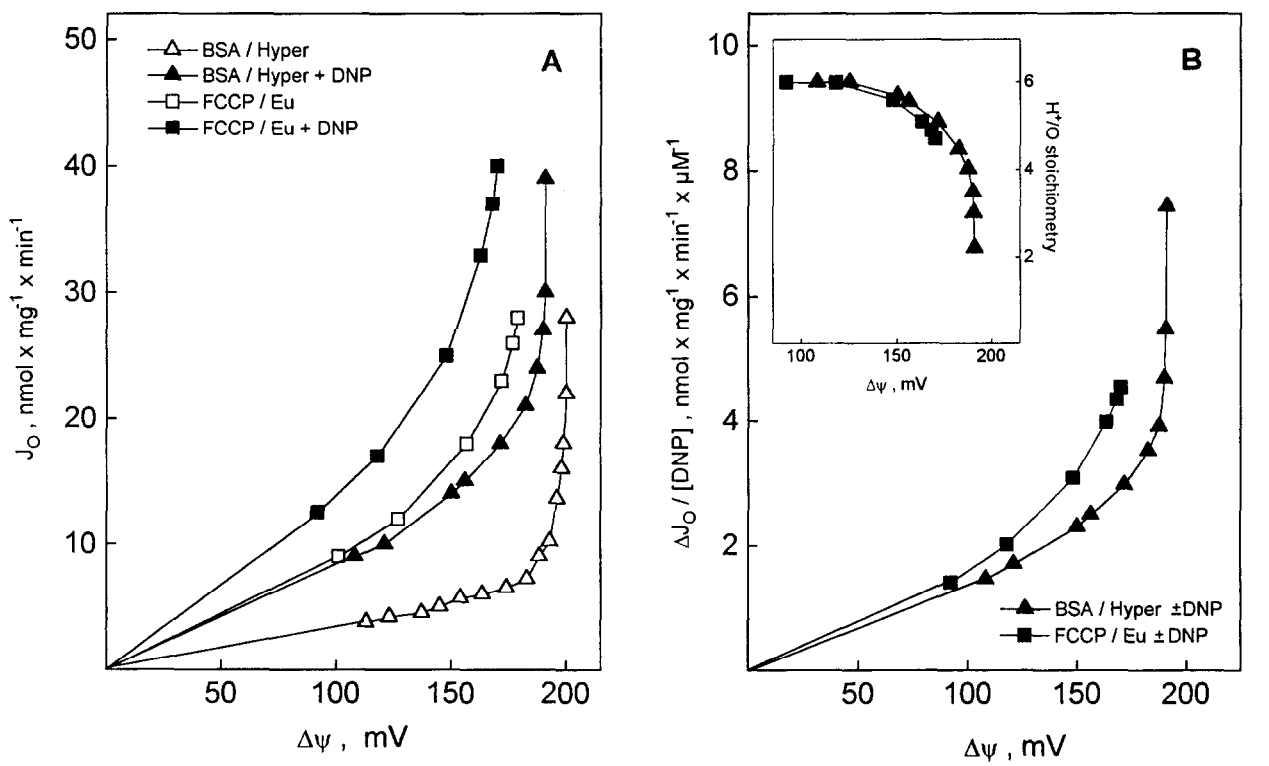

Fig. 3. Relationships between respiration (panel A) or DNP-induced extra respiration (panel B) and $\Delta\psi$ in BSA-Hyper and FCCP-Eu RLM. Panel A: BSA-Hyper and FCCP-Eu RLM were incubated at 25°C with increasing amounts of malonate in the absence (BSA-Hyper, △; FCCP-Eu, □) or in the presence of DNP (3.5 μM; ▲, ■). After 2 min incubation, succinate (5 mM) was added and the rate of respiration and the membrane potential measured. Panel B: Data from panel A. The ΔJ_O was calculated as the difference of the respiratory rate, at each membrane potential, of the BSA-Hyper and FCCP-Eu RLM ± DNP. Inset: Estimation of the dependence of the H^+/O ratio on the membrane potential in BSA-Hyper and FCCP-Eu RLM. See text for more details.

[23–25] rather than to slip in the redox proton pumps. An increase of non-ohmic leaks is observed for the H^+ leak either mediated by thermogenin in brown fat mitochondria (for review see [26–28]), or induced by incubation at high temperatures in rat liver mitochondria [16,29], or present as a partial contribution to the respiration in non-phosphorylating yeast mitochondria [30], and in other bioenergetic membranes (for review see [24,25]). According to Brand et al. [8], both in the presence of ohmic or of non-ohmic leaks the differential respiration is linearly related to the membrane potential and this linearity indicates that the stoichiometric ratio remains almost constant. Application of the same approach used in the case of Fig. 3 indicates that the high temperature-induced increase of non-ohmic leak gives rise neither to a biphasic differential respiration nor to a variation of the stoichiometric ratio [16]. The decrease of the pump stoichiometry in BSA-Hyper RLM (Fig. 3B) thus supports the view that the stimulation of the respiration of BSA-Hyper RLM is related to proton pump rather than membrane leaks processes.

The results of Fig. 2 together with those of Fig. 3 indicate that at high membrane potential the measured respiratory rate exceeds that required to compensate for the energy dissipation via leaks, this being the reason why a large part of the respiration can be inhibited without affecting the membrane potential [31,32]. Interestingly, also an extensive oxidation of cytochrome *c* occurs in each system without significant depression of membrane potential (Fig. 2B). The most feasible interpretation for the biphasic patterns of Fig. 2B is that the electron carriers of the respiratory chain act as a buffer of electrons during the inhibitor titration and the cytochromes readjust their redox equilibria when the electron supply becomes restricted. This results in a facilitation of the intermolecular electron-transfer, and consequently, in a net oxidation of cytochrome *c* accompanied by only a slight diminution of the membrane potential.

Consider now the relationships between respiration and % of cytochrome *c* reduction (Fig. 2C). The linearity of this relationship in BSA-Hyper RLM is presumably due to the relatively high membrane potential which, in a slip type of uncoupling, initially is not affected by the respiratory inhibition, and prevents an extensive oxidation of the cytochrome *c*. On the other hand, the biphasicity of the relationship in FCCP-Eu RLM is presumably due to the marked lowering of the membrane potential which, in case of a leak type uncoupling, accompanies the inhibition of the respiration, and favours a large oxidation of cytochrome *c*. The intermediate pattern obtained for all kind of relationships in euthyroid RLM indicates the participation of both types of uncoupling processes to the resting respiration.

In conclusion, the increased rate of substrate oxidation and the high level of the membrane potential determine a higher ratio between the rates of input and output of electrons into cytochrome *c* in BSA-Hyper with respect to FCCP-Eu RLM. While the increase of respiration and the redox behaviour of cytochrome *c* in FCCP-Eu RLM are consequences of a leak-type of uncoupling, it is more likely that the equivalent respiratory stimulation in BSA-Hyper RLM is due to an increased substrate oxidation accompanied by a slip-type of uncoupling for three reasons: (a) the marked biphasicity of the flow-force relationships and of the uncoupler-induced extra respiration, (b) the alterations of the kinetic parameters of the cytochrome oxidase, and (c) the redox behaviour of cytochrome *c*.

Acknowledgements: This work was supported in part by grants from the Consiglio Nazionale delle Ricerche (Target Project Invecchiamento), MURST (Ministero della Università e della Ricerca Scientifica e Tecnologica), Regione Veneto (Project 355/01/93), and by a fellowship of the University of Padova to I.S. We wish to acknowledge Prof. M.K.F. Wikstrom for helpful discussions and suggestions during the preparation of this manuscript and L. Pregnolato for expert technical assistance.

References

[1] Oppenheimer, J.H., Schwartz, H.L., Mariash, C.N., Kinlaw, W.B., Wong, N.C.W. and Freake H.C. (1987) Endocr. Rev. 8, 288–308.
[2] Hoek, J.B. (1992) in: Molecular Mechanisms in Bioenergetics (Ernster, L., Ed.) pp. 421–461, Elsevier, Amsterdam.
[3] Soboll, S. (1993) Biochim. Biophys. Acta 1144, 1–16.
[4] Luvisetto, S., Schmehl, I., Intravaia, E., Conti, E. and Azzone, G.F. (1992) J. Biol. Chem. 267, 15348–15355.
[5] Van Italie, C.M. (1990) Endocrinology 127, 55–62.
[6] Nelson, B.D., Luciakova, K., Ronggui, L. and Betina, S. (1995) Biochim. Biophys. Acta 1271, 85–91.
[7] Cooper, C.E. (1990) Biochim. Biophys. Acta 1017, 187–203.
[8] Brand, M.D., Chien, L.-F. and Diolez, P. (1994) Biochem. J. 297, 27–29.
[9] Massari, S., Frigeri, L. and Azzone, G.F. (1972) J. Membr. Biol. 9, 57–70.
[10] Azzone, G.F., Schmehl, I., Luvisetto, S. and Canton, M. (1994) Biochim. Biophys. Acta 1187.
[11] Sinjorgo, K.M.C., Meijling, J.H. and Muijsers, A.O. (1984) Biochim. Biophys. Acta 767, 48–56.
[12] Benz, R. and McLaughlin, S.G.A. (1983) Biophys. J. 41, 381–398.
[13] Luvisetto, S., Schmehl, I., Conti, E., Intravaia, E. and Azzone, G.F. (1991) FEBS Lett. 291, 17–20.
[14] Diamant, S., Gorin, E. and Shafrir, E. (1972) Eur. J. Biochem. 26, 553–559.
[15] Gutknecht, J. (1988) J. Membrane Biol. 106, 83–93.
[16] Canton, M., Luvisetto, S., Schmehl, I. and Azzone, G.F. (1995) Biochem. J. 310, 477–481.
[17] Horrum, M.A., Tobin, R.B. and Ecklund, R.E. (1985) Mol. Cell. Endocrinology 41, 163–169.
[18] Sone, N. and Nicholls, P. (1984) Biochemistry 23, 6550–6554.
[19] Paradies, G., Ruggiero, F.M., Petrosillo, G. and Quagliariello, E. (1994) Biochim. Biophys. Acta 1225, 165–170.
[20] Morgan, J.E. and Wikstrom, M. (1991) Biochemistry 30, 948–958.
[21] Brand, M.D., Steverding, D., Kadenbach, B., Stevenson, P.M. and Hafner, R.P. (1992) Eur. J. Biochem. 206, 775–781.
[22] Goglia, F., Lanni, A., Barth, J. and Kadenbach, B. (1994) FEBS Lett. 346, 295–298.
[23] Garlid, K.D., Beavis, A.D. and Ratkje, S.K. (1989) Biochim. Biophys. Acta 976, 109–120.
[24] Murphy, M.P. (1989) Biochim. Biophys. Acta 977, 123–141.
[25] Brown, G.C. (1992) FASEB J. 6, 2961–2965.
[26] Nicholls, D.G. and Locke, R.M. (1984) Physiol. Rev. 64, 1–64.
[27] Nedergaard, J. and Cannon, B. (1984) in: Bioenergetics (Ernster, L., Ed.) pp. 291–314, Elsevier, Amsterdam.
[28] Skulachev, V.P. (1988) Bioenergetics, Springer-Verlag, Berlin.
[29] Brown, G.C. and Brand, M.D. (1986) Biochem. J. 234, 75–81.
[30] Ouhabi, R., Rigoulet, M., Lavie, J.-L. and Guerin, B. (1991) Biochim. Biophys. Acta 1060, 293–298.
[31] Pietrobon, D., Azzone, G.F. and Walz, D. (1981) Eur. J. Biochem. 117, 389–394.
[32] Pietrobon, D., Zoratti, M. and Azzone, G.F. (1983) Biochim. Biophys. Acta 932, 306–324.